PHARMACOGENETICS OF GLAUCOMA:

THE ROLE OF BETA2 ADRENORECEPTOR AND PROSTANOID (FP) RECEPTOR POLYMORPHISMS IN THE RESPONSE TO TOPICAL TIMOLOL AND LATANOPROST IN MALAYSIAN POPULATION

Liza Sharmini Binti Ahmad Tajudin

UCL

PhD

I, Liza Sharmini binti Ahmad Tajudin confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Liza Sharmini Ahmad Tajudin

Abstract

Personalised medicine has been area of interest for decades especially in treatment of chronic diseases, such as glaucoma. Topical pressure lowering medication is an effective mode of treatment. Variation in responsiveness to these medications is well recognised. In this thesis, the possible role of genetics in governing the responsiveness to topical Timolol XE 0.5% and latanoprost 0.005% was studied. The possible role of beta2 adrenoreceptor gene (*ADRB2)* and prostaglandin FP receptor gene (*PTGFR*) as susceptibility genes for glaucoma was also explored.

A prospective observational cohort study of 97 and 86 glaucoma patients (POAG and NTG) treated with topical timolol monotherapy and topical latanoprost respectively was conducted. Intraocular pressure (IOP) was measured at baseline, 1, 3, 6 and 12 months post-treatment. Venesection was conducted on glaucoma patients and 190 unrelated age/ethnicity-matched controls. High purity genomic DNA was extracted and subjected for multiplex PCR to detect polymorphism at specific codons within *ADRB2.* Direct sequencing was used to screen the *PTGFR* covering 3000bp upstream from 5'UTR and 1000bp downstream from 3'UTR.

Topical timolol and latanoprost provided good pressure lowering effect with mean IOP reduction from baseline of 5.4(5.1) mmHg and 7.1(4.2) mmHg respectively. Higher baseline IOP was found in patients with -47CC and 79CC of *ADRB2.* There was no significant association between *ADRB2* and responsiveness to topical timolol. 79G and -20T were found to increase the susceptibility to glaucoma 1.9-fold (95%CI 1.0, 3.6) and 1.7-fold (95% CI 1.1, 2.7) respectively.

Certain *PTGFR* polymorphisms appeared to confer protective effects against glaucoma. The minor alleles of rs11162505, rs554185 and rs551253 reduced the susceptibility to glaucoma significantly. rs686262GG was associated with 6.3-fold (95%CI 1.3, 31.0) risk of poor response to topical latanoprost.

Therefore, *ADRB2* and *PTGFR* are potential pharmacodynamic genes for the responsiveness to topical timolol and latanoprost. *ADRB2* and *PTGFR* may also act as susceptibility genes for glaucoma.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

ACG	**Angle Closure Glaucoma**
ADRB1	**Beta 1 adrenoreceptor**
ADRB2	**Beta 2 adrenoreceptor**
ADRB2	**Beta 2 adrenoreceptor gene**
AGIS	**Advanced Glaucoma Intervention Study**
ANOVA	**Analysis of Variance**
Apo E	**Apolipoprotein E**
APRRE	**Acute-Phase Reactant Regulatory Elements**
ARMD	**Age Related Macular Degeneration**
ATP	**Adenosine Triphosphate**
BAC	**Benzylkonium Chloride**
BUP	**Beta Upstream Peptide**
BMI	**Body Mass Index**
cAMP	**Cyclic Adenosine Monophosphate**
CCT	**Central Corneal Thickness**
CH	**Corneal Hysteresis**
CHW	**Chinese Hamster Fibroblast**
CI	**Confidence interval**
CIGTS	**Collaborative Initial Glaucoma Treatment Study**
CME	**Cystoids Macular Edema**
CNTGS	**Collaborative Normal Tension Glaucoma Syndrome**
CRE	**Cyclic Adenosine Monophosphate Response Element**
CRTH	**Chemo-attractant Receptor Homologous Molecules**
COX	**Cyclooxygenase**

CYP	**Cytochrome P450**
ddH_2O	**Double deionised water**
DNA	**Deoxyribonucleic Acid**
dNTPs	**Deoxynucleoside Triphosphate**
EDTA	**Ethyldiaminetetraacetic acid**
EGPS	**European Glaucoma Progression Study**
EM	**Extensive metaboliser**
EMGT	**Early Manifest Glaucoma Treatment**
EVP	**Episcleral Venous Pressure**
GAT	**Goldmann Applanation Tonometry**
GDP	**Guanosine Diphosphate**
GFS	**Gel Forming Solution**
GPCR	**G Protein-Coupled Receptor**
GRKs	**G Protein-Coupled Receptor Kinases**
GTP	**Guanosine Triphosphate**
GWAS	**Genome Wide Association Study**
HASM	**Human Airway Smooth Muscle cells**
HFA	**Humphrey Visual Field Analysis**
HLM	**Human Lung Mast cells**
HM	**Homozygous Mutant**
HT	**Heterozygous**
5-HT_3	**Serotonin**
HW	**Homozygous Wild**
HWE	**Hardy Weinberg Equilibrium**
IOP	**Intraocular Pressure**

IOPG	**Intraocular Pressure Goldmann**
IOP_G	**Goldmann-correlated Intraocular Pressure**
JOAG	**Juvenile Open Angle Glaucoma**
5'-LC	**5'-Leader Cistron**
LCI	**Lower Confidence Interval**
LD	**Linkage Disequilibrium**
LIID	**Latanoprost-Induced Iris Darkening**
LLA	**Linear-by-Linear Association**
LOH	**Loss Of Heterozygosity**
LOXL1	**Lysyl Oxidase-Like protein 1**
MAF	**Minor Allele Frequency**
MD	**Mean Deviation**
$MgCl_2$	**Magnesium Chloride**
MMP	**Matrix Metalloproteinase**
mRNA	**Messenger Ribonucleic**
MSI	**Microsatellite Instability**
MYOC	**Myocilin**
NAT-1	**N-acetyltransferase 1 gene**
NAT-2	**N-acetyltransferase 2 gene**
NTG	**Normal Tension Glaucoma**
OAG	**Open Angle Glaucoma**
OHT	**Ocular Hypertension**
OHTS	**Ocular Hypertension Treatment Study**
OPTN	**Optineurin**
OR	**Odd Ratio**

PACG	**Primary Angle Closure Glaucoma**
PAWS	**Predictive Analytic Software Programme**
PCR	**Polymerase Chain Reaction**
POAG	**Primary Open Angle Glaucoma**
PM	**Poor Metaboliser**
PSD	**Pattern Standard Deviation**
PTGFR	**Prostaglandin F2α receptor**
PTGFR	**Prostaglandin F2α receptor gene**
QOL	**Quality of Life**
QTL	**Quantitative Trait Locus**
RM ANOVA	**Regression Measure Analysis Of Variance**
SD	**Standard Deviation**
SE	**Standard Error**
SNP	**Single nucleotide polymorphism**
TBE	**Tris-Base, Borate and EDTA**
TIGR	**Trabecular meshwork inducible glucocorticoid responsive**
TNF	**Tumour Necrosis Factor**
TPMT	**Thiopurine Methytransferase**
UCI	**Upper Confidence Interval**
UTR	**Untranslated Region**
UM	**Ultra-rapid Metaboliser**
VCDR	**Vertical Cup to Disc Ratio**
WDR36	**WD40-repeat 36**
WHO	**World Health Organization**
XFG	**Pseudo-exfoliation glaucoma**

Acknowledgement

First and foremost, I would like to express my sincere gratitude to my supervisor, Professor Dr Paul J Foster, who has supported me throughout my PhD with his patience and knowledge whilst allowing me the room to work on my own. Dr Anand Viswanathan, my co-supervisor who has provided me with his guidance and advice. My deepest gratitude to Professor Rusli Ismail, my second co-supervisor, who has shared with me his knowledge and expertise in the field of pharmacogenetics.

My genetic laboratory work was conducted in two continents; Asia and Europe, which gave me the opportunity not only to learn about molecular genetics but also exposed me to various different laboratory techniques and clever innovation. I would like to thank Mr Nizam Zahary, who has helped me with the basic molecular laboratory work at the initial stage of this research project. He tried very hard not to laugh when my eppendorf tubes were overheated or when I ran gel electrophoresis without the PCR product!

I would like to thank Professor Shoami Bhattacharya for allowing me to use his laboratory facilities at the Institute of Ophthalmology. His thoughtful advice and guidance is of a great value to me. My deepest gratitude goes to Naushin Waseem, who is not only a genius in 'trouble shooting', even through long distance communication, but also a great teacher and a big shoulder to cry on. To Amna, Sabrina, Anna, Becky, Christina and Bev, thank you for being a good listener and for the lively lunch break sessions. To Sancy, thank you for being a true friend. Last but not least, I would like to thank Mr Lee Pak Sang, for being a great problem solver especially on the technical aspect.

My laboratory findings will not be meaningful without the statistical analysis guidance from Dr Khor Chieun Chen and Ms Karoline Kuchenbacker. To CC Khor, thank you for answering all my silly questions and for the step by step guide on statistical analysis. This research project was made possible by research grant funding from Ministry of Science, Technology and Innovation (MOSTI) and Ministry of Higher Education (MOHE), Malaysia.

To my husband, Zilfalil Alwi, thank you for your understanding and patience. To my children, Zahim, Annisa and Aliya, you are my pillar of strength and aspiration. To my loving mum and my belated father, who passed away during my thesis writing, thank you for instilling the 'competitive' spirit in me. And to my parents in law, thank you for looking after my children whenever I am away.

CHAPTER 1

Introduction

Chapter 1

1.1 The eye

The eye is an important sensory organ that acts as a window in the complex physiology of seeing. Light passes through the transparent cornea, the outermost avascular structure that refracts light to the lens. The amount of light entering the eye is regulated by the iris through its opening, the pupil. The pupil dilates in the dark or dim surroundings and constricts in bright surroundings. Light is refracted by the lens to the retina. The crystalline lens is a transparent structure behind the pupil, held in place by suspensory ligaments and zonules.

The light then reaches the retina. The retina acts in the manner of film in the camera, by capturing images, colours, objects and other beautiful sights. The retina contains specialized light-sensitive cells known as rods and cones. There are 110 to 125 million rods, which facilitate vision in dim light. Cones number approximately 6 to 7 million, are mainly concentrated in the macula, and are responsible for vision in bright surroundings and capturing colours. The macula lutea surrounds the depressed, yellowish spot known as the fovea. The macula is the most sensitive part of the retina, capturing images directly and sending them to the brain through the optic nerve. The optic nerve, which contains 1.5 million nerve fibres, acts as an electrical cable sending information to the brain. In diseases such as glaucoma, wherein the nerve fibres are damaged, the information sent to the brain is affected. The inadequate information results in certain patterns of visual field defects that reflect the area of affected nerve fibres. Each nerve fibre is responsible for sending information from a specific area of the visual field.

Figure 1.1: Cut section of the eye illustrating important anatomical structures
(Adapted from http://www.eyephysiciansoflakewood.com/anatomy-of-the-eye.php)

1.2 Glaucoma

The word *glaucoma* originates from the Greek word 'glaukos', meaning blue, green, and cloudy, and was described in the literature as early as 400 BC. It was later described as 'migraine of the eye' and 'headache of the pupil' in the 13th century. In the 18th and 19th centuries, it was thought to be a disease of the vitreous humour, arthritis, and iritis, a theory disproven by Helmholtz in 1851. He found that the changes in the optic nerve. Based on clinical observation, glaucoma understanding evolved. Donder in 1862 observed that glaucoma is a chronic disease with elevated pressure but without signs of inflammation causing fixed dilated pupil at the end stage (von Graefe, 1862). Later, glaucoma was defined as optic neuropthy resulted from numerous risk factors (Drance, 1973).

Glaucoma is a complex disease with many underlying mechanisms which has a relationship with intraocular pressure (IOP). Elevation of IOP, which exerts mechanical pressure, was believed to cause direct damage to the retinal nerve fibre layer. Initially, increased IOP was thought of as a causative factor in glaucoma, but recently has been regarded as a risk factor, indeed the only modifiable risk factor. The new understanding of glaucoma came from observation of those with normal pressures who developed glaucomatous cup disc changes with typical patterns of visual field defects (Sjögren, 1946; Drance, 1972; Leighton and Philips, 1972). This subtype of glaucoma is then named as normal tension glaucoma (NTG). The changes of optic nerve head with normal pressure challenged the pressure-dependent theory, which perhaps only applies to cases of elevated IOP. Current theories identify two mechanisms responsible for glaucomatous damage to the optic nerve head: pressure-dependent or pressure-independent.

A pressure-independent mechanism with specific focus on retinal ganglion cell death may be a more relevant postulation (Caprioli, 2007). Pressure-independent mechanisms encompass various postulations, including impaired microcirculation of the optic nerve head, excitotoxicity (Kaushik et al, 2003), abnormal glial-neuronal interactions (Tezel and Wax, 2000), neurotrophin starvation (Schuettauf et al, 2002), defective endogenous protection (Caprioli et al, 2007), and autoimmunity (Maruyama et al, 2000). Glaucoma may result from various predisposing factors and interaction of pressure and pressure independent mechanisms. For example genetic predisposing factors, initiating factors such as pressure changes, promoting factors such as changes in the laminar structure, and sustaining factors such as excitatory mechanisms may be

responsible for glaucoma (Caprioli, 2007). Thus, glaucoma is not a single disease with one direct cause, as a specific organism causes a specific infectious disease.

Glaucoma represents multiple conditions with a final common pathway leading to optic nerve damage and visual field loss. Glaucomatous optic neuropathies can result from various causative factors and is commonly classified as open-angle glaucoma (OAG) or angle-closure glaucoma (ACG), according to the angle configuration. These classifications are further divided according to causative aetiology: primary, or those without any explanatory cause and secondary, those associated with systemic and other ocular pathologies. Glaucoma is also classified according to age of onset; congenital, juvenile and adult onset.

However, glaucoma is not well defined. The variable definitions of glaucoma not only create problems in detection and accurate assessments of prevalence, but also cause confusion when analyzing the type of glaucoma. The first population-based study to identify glaucoma without specific reference to IOP, using visual field, and optic disc anatomy to define glaucoma was carried out in south Wales (Hollows and Graham, 1966). Later, IOP was specifically identified as a modifiable risk factor and not a criterion for glaucoma diagnosis (Sommer, 1989). There is no absolute level where IOPs above or below are strongly associated with development of or protection against glaucoma. In addition, there are factors such as age; diurnal variation, central corneal thickness (CCT), and the type of tonometer employed that affect the accuracy of IOP measurements. Defining glaucoma solely based on IOP will lead to over- and underestimation of incidence and prevalence of glaucoma. However, IOP has

remained part of the definition of glaucoma in many population-based studies (Dielemans et al, 1994; Klein et al, 1992).

Most studies define glaucoma based on vertical cup-to-disc ratio (VCDR) and visual field changes (Klein et al, 1992; Leske 2007; Tielsch et al, 1991; Mitchell et al, 1996). Dandona et al (2000) defined their subjects as definite primary open angle glaucoma (POAG) on the basis of VCDR and previously obtained visual field data and suspected cases included those with suspicious optic disc damage but without definitive visual field loss. The Visual Impairment Project (VIP) adopted definite, probable and possible diagnoses as defined by a panel of experts (Weih et al, 2001). The variable definitions and lack of consensus in various population-based studies resulted in certain subjects being diagnosed as having glaucoma in one study and the same subject being labelled as suspect, a probable case, or worse, considered a non-glaucoma subject in another study. Currently, a group of experts in glaucoma research defines glaucoma based on structural and functional evidence of glaucomatous optic neuropathy, mainly relying on VCDR and visual field changes (Foster et al, 2002).

1.2.1 Prevalence of glaucoma

Glaucoma was ranked by the World Health Organization (WHO) as the third leading cause of vision loss in 1990 and is responsible for 13% of all blindness, affecting approximately 5 million people (Thylefors and Négrel, 1994). In the most recent WHO report, glaucoma has moved to second place as the leading cause of global blindness (Resnikoff S et al, 2004). It is estimated that approximately 1 in 200 of the general population over the age of 40 and more than 5% of those older than 75 were affected by glaucoma in 1985 (Gibson et al, 1985). By the year 2000, glaucoma was

estimated to affect approximately 66.8 million people worldwide, with 6.7 million suffering from bilateral blindness (Quigley, 1996). The estimation was based on 111 published glaucoma prevalence studies in 7 stratified populations with a clear definition of glaucoma, random selection of samples, and large sample sizes.

Ten years later, using different criteria, Quigley and Broman (2006) predicted that 60.5 million people will be affected by glaucoma in 2010 and estimated a further increase to 79.6 million in 2020. This prediction was not based entirely on the previous 111 publications on glaucoma prevalence used in the 1996 study (Quigley, 1996) but utilized a new set of criteria emphasizing population-based studies and a more definitive definition of glaucoma. It is also estimated that 8.4 million people will be bilaterally blind due to primary glaucoma in 2010, with 11.1 million will be affected by 2020. Based on a meta-analysis of 8 population-based studies including the Baltimore Eye Study, Barbados Eye Study, Beaver Dam Eye Study, Blue Mountains Eye Study, Kongwa Eye Project, Proyecto Vision Evaluation Research, Rotterdam Study, and the Melbourne Visual Impairment Project, the prevalence of glaucoma has been extrapolated to affect 3.36 million Americans by 2020 (Eye Disease Prevalence Research Group, 2004). The prevalence of glaucoma is increasing but only half of the sufferers are likely to be known to the health system in developed countries (Coffey et al, 1993). This number is estimated to be lower in developing countries. Furthermore, glaucoma is responsible for irreversible blindness. Unlike cataracts, for which timely surgery and intraocular lens implantation can reverse vision loss, the burden of glaucoma is more devastating if appropriate measures are not taken. With the growing aged population, glaucoma is disease of longevity.

OAG is the most common type of glaucoma affecting people in all parts of the world. Quigley and Broman (2006) predicted that the mean prevalence of OAG is 1.96% among persons over 40 in 2010. The prevalence of OAG based on persons 40 years and older is reported to be 1–8% in Africa and higher among those in West Africa (7–9%), 2–3% in Australia, 1–4% in Asia, and 1–3% in Europe (Leske, 2007; Weih et al, 2001; Foster et al, 2000).

ACG is more likely to affect people in Asia and the Pacific region, with 80% of them in developing countries (Thylefors and Negrel, 1994). Based on the Quigley and Broman (2006) estimation, half of the blindness caused by glaucoma was in Asia in 2000. In 2010, it was predicted that 86.5% of ACG will be in Asia with 46.5% of the cases in China. The visual loss in ACG is more profound than in OAG (Foster and Johnson, 2001). Although the estimated impact of ACG in 2010 was lower (15.7 million) compared to OAG (44.7 million), the frequency of ACG-blindness is almost equal to that of OAG due to the greater morbidity of ACG (Quigley and Broman, 2006). Although great emphasis has been given to ACG, OAG is still the most common glaucoma in Asia (Quigley and Broman, 2006; Foster and Johnson, 2001). The largest absolute number of people affected by both OAG and ACG is in Asia, followed by Europe and India. Africa has the highest frequency of glaucoma in the adult population. Asia, including East and Southeast Asia, is the most populous continent in the world. China is home to 1/5 of the world population, with a recorded glaucoma prevalence ranging between 3.0 and 3.8% among those 40 years old and older (He et al, 2006; Foster et al, 2000). Thus, it is no surprise that the incidence of glaucoma is high in this part of the world.

The 1994 World Health Organization (WHO) model estimated that 2.7 million people were affected by secondary glaucoma and congenital glaucoma affects 300,000 children (Thylefors and Negrel, 1994). As most other prevalence models are based on populations more than 40 years old, congenital glaucoma is frequently overlooked. The WHO model divided the data into congenital, OAG, ACG, and secondary glaucoma; IOP was also used as one of the diagnosis criteria. This conflict has resulted in different prevalence and estimation outcomes; nonetheless, these studies provide us with useful data on the importance of prevention of blindness in glaucoma.

1.2.2 Glaucoma in Malaysia

Figure 1.2: Map of Malaysia

Malaysia, a Southeast Asian country consists of a peninsula bordering Thailand and the northern third of the island of Borneo. The strategic location of peninsular Malaysia, surrounded by the Straits of Malacca and the South China Sea, attracted Chinese and Indian explorers, Arab traders, Christian missionaries, Portuguese crusaders, and traders from other parts of the world. Malaysia was a British colony in

the 18th and 19th centuries. During the British era, immigrants from Southern China and Southern India were brought in to increase the work force in the rubber and tin mining industries. Most immigrants stayed on after Malaysia gained independence in 1957, enriching the Malaysian population with multi-ethnic Asian cultures and beliefs. Based on 2004 statistics, the population is comprised of 50.4% Malays, 23.7% Chinese, 11% indigenous people, 7.1% Indians and 7.8% others. The total population of Malaysia is 25,715,819 with 31.4% aged 0–14 years, 63.6% aged 15–64, and only 5% are 65 and older (July 2010 estimation from Department of Statistics, Malaysia). Malaysia has a relatively young population and exhibits a broad-based pyramidal population distribution.

Since Malaysia gained independence from Great Britain in 1957, the country has progressed from exporter of raw materials (especially rubber, tin, and petroleum) to manufacturing, service, and tourism. The escalation of economic strength has not only improved the quality of life but also the healthcare system, which has increased life expectancy. In general, Malaysia has a two-tier health care system comprised of a government-run universal health system run by the Ministry of Health and supplemented by university hospitals run by the Ministry of Higher Education and a private healthcare system. The system is the legacy of British colonization but has undergone many transformations to meet the needs of Malaysians (Ismail and Rohaizat, 2002).

There are 143 government hospitals and 209 private hospitals with 52,938 beds (Planning and Development Division, Ministry of Health, Malaysia, 2009). In

addition, there are 802 health clinics, 1927 community clinics, 95 maternal and child health clinics, and 193 mobile health clinics run by the Ministry of Health, Malaysia. There are 6371 registered private medical clinics and 1435 private dental clinics. The infant mortality rate, an important marker of the effectiveness of a health system, is ranked 123rd in the world, with 6.3 deaths per 1000 live births (Department of Statistics, Malaysia). The life expectancy at birth is 73.3 years and is slightly longer for females (76.2 years). The over-50 population has increased drastically from 3.8% in 1998 to 4.6% in 2010 (Department of Statistics, Malaysia). Age-related diseases including glaucoma and age-related macular degeneration (ARMD) have become increasingly important. ARMD was unknown in Malaysia in the late 1970s and on the rise lately.

Glaucoma was ranked as the fifth major cause of blindness and visual impairment based on the National Eye Survey conducted in 1996 (Zainal M et al, 2002). However, the survey was conducted in the respondents' homes with the aid of torch lights and a direct ophthalmoscope, without appropriate facilities such as slit lamps, tonometry, gonioscopic, and visual field assessments required for accurate diagnosis of glaucoma. Thus, underestimation of disease prevalence is quite likely. In addition, the response rate was low. However, this survey provides an important baseline on which to build a strategy for blindness prevention. A cross-sectional study in 3 small villages in the Sepang district of Selangor, Malaysia, found that glaucoma was the third (4.4%) most common cause of ocular disease among selected individuals aged 40 years and above (Reddy et al, 2006). However, eye examinations were successfully conducted in only 159 participants out of 341 eligible respondents. Seven cases of glaucoma were detected, with 5 cases newly diagnosed. In spite of the small number

of newly detected cases, 1 had visual impairment and 1 was already blind due to glaucoma (Reddy et al, 2006). Based on a retrospective 5-year survey of patients attending the ophthalmology clinic at Hospital Kuala Lumpur, the largest hospital in Malaysia, POAG was found as the most common type of glaucoma, followed by primary angle closure glaucoma (PACG) (Sharif and Selvarajah, 1997). This finding did not reflect true prevalence and cannot be extrapolated to the Malaysian population at large. Moreover, this data should be analysed with care in light of the lessons learned in Singapore. Based on hospital admission, Wong et al (2000) found that PACG was the most common type of glaucoma among Chinese in Singapore, but this was not reflected in a population-based study (Foster et al, 2000). However, the Sharif and Selvarajah (1997) study provides the hospital incidence of a common type of glaucoma in Malaysia. Unfortunately, there have been no population-based studies to determine the prevalence of glaucoma in Malaysia. The need for a prevalence study is timely, as a majority of glaucoma cases present at the advanced stage of disease even at their initial presentation to the hospital (Chieng et al, 2005).

Predicting the prevalence of glaucoma is a challenge due to Malaysia's ethnic diversity. However, the prevalence data from available population-based studies from countries with similar ethnicities (table 1.1) may help in predicting the possible impact of glaucoma in the Malaysian population. Based on the 2000 Malaysian National Census (the next national census is due in 2010), there are 5,419,185 people over the age of 40 (Table 1.2) residing in Malaysia. The recently published Singapore-Malay Eye Study (SiMES) is important in predicting the prevalence of glaucoma in Malays especially in the Malay Archipelago. SiMES reported the prevalence of glaucoma as 3.4% (Shen et al, 2008). Based on this prevalence, it is estimated that

88,306 of the Malay population in Malaysia are affected by glaucoma. However, this is likely to be a low estimate as the total number of Malays in Malaysia is much higher than in Singapore.

Similar to the Chinese in Singapore, a majority of Chinese in Malaysia originated from South China. If the prevalence of 3.5% is used (based on the mean of the prevalence from studies conducted in Tanjung Pagar, Singapore, and Guangzhou, China), the estimated number of Chinese in Malaysia affected by glaucoma is 64,426. The migration of Indians from South India, especially from Chennai and Kerala during the British era, led a majority of them to stay on after Malaysia gained independence. Based on the prevalence reported in Southern India (Ramakrishnan R et al, 2003), the estimated number of Indians affected by glaucoma in Malaysia is 11,209.

Overall, there is a greater likelihood of underestimation as the vast majority of indigenous people (Iban, Kadazan, Senoi, Negrito, etc.) are not included, nor are those categorized as 'other' in Malaysia. The indigenous or Bumiputra population contributed 9.2% of the total estimated Malaysian population aged 40 years old and older. There are 19 sub-ethnic groups of indigenous people in Peninsular Malaysia, mainly Negrito, Senoi, and aboriginal Malays. The aboriginal Malays differ from the Deutero-Malays that currently dominate Malaysia in their physical appearance and cultural practices. There are more than 30 sub-ethnic groups in Sabah and 50% of Sarawak consists of indigenous people. They speak different languages and dialects, practice traditional farming, and some still live as nomads in the deep tropical jungles

of Malaysia. Although many of them have embraced Islam and Christianity, many more still practice animism. There is little knowledge regarding their incidence of ocular disease. In spite of the possible under- and overestimation based on available predictive data, an alarming 163,941 people over 40 years old are predicted to be affected by glaucoma in Malaysia.

Future public health strategy should not only aim to provide better healthcare in urban areas, but should also reach out to the indigenous and socioeconomically disadvantaged people who live in the remote areas of Malaysia, especially East Malaysia, and effectively address their reluctance to accept modern medicine. Currently the ophthalmologist-to-population ratio is estimated to be 1:200,000. The exact number of ophthalmologists in private practice is not available. The ratio is more than adequate based on the WHO minimum recommendation but fails to reach the 1:50,000 target of Malaysia's Ministry of Health requirements. Furthermore, the 1:200,000 is not well distributed in Malaysia, especially East Malaysia. The ratio is estimated to reach 1:75,000 or even less in urban metropolitan areas such as Kuala Lumpur but the gap is greater in rural areas such as Sabah and Sarawak, East Malaysia. This disparity will create a huge gap in the quality of health services in Malaysia if the issue is not addressed appropriately.

Table 1.1: Prevalence of glaucoma in Southeast Asia based on studies conducted in Singapore, Southern China and Southern India

Population	Location	Author	Prevalence (95% Confidence Interval)		
			Overall^	POAG**	PACG**
Malay	**Singapore**	Shen et al, 2008*	3.4% (3.3-3.5)	2.5% (2.4-2.6)	0.12% (0.10-0.14)
Chinese	**Tg Pagar, Singapore**	Foster et al, 2000*	3.2% (2.3-4.1)	2.4% (1.6-3.2)	1.5% (0.8-2.1)
Chinese	**Guangzhou, Southern China**	He et al, 2006#	3.8% (2.8-4.8)	2.1% (1.4-2.8)	1.5% (0.8-2.1)
Indian	**Chennai, Southern India**	Vijaya et al, 2005* (urban)		3.51% (3.04-3.98)	0.88% (0.60-1.16)
Indian	**Aravind, Southern India**	Ramakrishnan et al, 2003*	2.6% (2.2-3.0)	1.7% (1.3-2.1)	0.5% (0.3-0.7)

* based on population aged ≥ 40 years
# based on population aged ≥ 50 years
^age-and –sex standardised prevalence of all type of glaucoma
**POAG and PACG specific prevalence without considering other type of glaucoma (e.g. pseudoexfoliation glaucoma)
POAG: primary open angle glaucoma, PACG: primary angle closure glaucoma

Table 1.2: Distribution of the Malaysian population according to ethnicity is based on the 2000 National Census*

	ETHNICITY															
Age group	**Malay**			**Bumiputra (Aboriginal)**			**Chinese**			**Indian**			**Other**			**Total**
	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	
40-44	343802	340211	**684013**	70118	65459	**135577**	224837	212926	**437763**	63377	62518	**125895**	8578	7110	**15688**	**1398936**
45-49	274857	266831	**541688**	51806	46847	**98653**	193646	177745	**371391**	48654	48806	**97460**	6149	4699	**10848**	**1120040**
50-54	214540	203069	**417609**	38714	36403	**75117**	166043	148002	**314045**	37818	36172	**73990**	3870	3488	**7358**	**888119**
55-59	142024	137904	**279928**	31198	27667	**58865**	114175	102360	**216535**	19829	20091	**39920**	2797	2544	**5341**	**600589**
60-64	124156	132252	**256408**	23064	23425	**46489**	100201	94085	**194286**	17058	19872	**36930**	2458	1849	**4307**	**538420**
65-69	71802	81292	**153094**	16866	18472	**35338**	58928	62997	**121925**	11279	13831	**25110**	1497	1309	**2806**	**338273**
70-74	60362	67568	**127930**	12010	11269	**23279**	40716	46881	**87597**	7932	7942	**15874**	1237	1139	**2376**	**257056**
>75	61602	74949	**136551**	12592	12485	**25077**	39475	57724	**97199**	7344	8603	**15947**	1590	1388	**2978**	**277752**
Total	1293145	1304076	**2597221**	256368	183413	**498395**	938021	902720	**1840741**	213291	217835	**431126**	28176	23526	**51702**	**5419185**

*This information was provided by the Department of Statistics, Malaysia

1.3 Genetic basis of primary open-angle glaucoma

A definitive genetic basis, or even a specific mode of inheritance, is not well established for glaucoma, but genetics remains a potential mechanism for understanding the pathogenesis of glaucoma. A genetic basis for this cause of optic neuropathy and blindness was observed as early as 1869 (von Graefe, 1869), followed by observations by Duke-Elder (1941) who described autosomal dominant inheritance in a family with glaucoma. Since then, more observations and studies were conducted in selected families (Posner and Schlossman, 1949; Becker et al, 1960). In addition to the autosomal dominant inheritance described in 1941, autosomal recessive inheritance has also been described (Probert, 1952). Juvenile-onset open angle glaucoma (JOAG) is commonly described as a product of autosomal dominant inheritance but the mode of inheritance in POAG (adult-onset) is inconclusive (Nemesure et al, 2001). A majority of glaucoma pedigrees do not show a simple Mendelian pattern of inheritance. POAG has been described as oligogenic, polygenic, and even multifactorial, and is regarded as a complex disease. However, most studies are clinic-based and therefore subject to selection bias, ambiguity in assessing family history, and non-standardised diagnoses of glaucoma, which perhaps account for the inconclusive mode of inheritance (McNaught et al, 2000; Nemesure et al, 2001). Incomplete penetrance of glaucoma further complicates the genotype-phenotype association.

Epidemiological studies have determined that a family history of glaucoma increases the risk of glaucoma between 13% and 60% (Tielsch et al, 1994; Francois, 1966; Shin et al, 1977). The Baltimore Eye Survey, the Barbados Eye Study (BES) and the Vision Impairment Project (VIP) found a significant association with first-degree relatives with glaucoma (Leske et al, 1995; Weih et al, 2001, Tielsch et al, 1994). The VIP study estimated a 3-fold increased risk of glaucoma in those with a family history of the disease (Weih et al, 2001). History of

glaucoma among siblings provides a stronger association than does parental history (Leske et al, 1995; Wolfs et al, 1998), suggesting the possible interaction of similar genetic inheritance and environmental exposure. However, the accuracy of this association has been challenged by possible biases while obtaining information regarding family history. Furthermore, 27% of previously diagnosed POAG patients were unaware of their positive family history (McNaught et al, 2000). The Glaucoma Inheritance Study in Tasmania (GIST), which involved more than 1042 glaucoma cases and their unaffected relatives, found that 60% of people with glaucoma have a positive family history (Green et al, 2007). A familial aggregation study, part of the population-based Rotterdam Eye Study, conducted thorough ophthalmological examination in all family members with glaucoma and found that the lifetime risk of glaucoma in the relatives of glaucoma patients was 22% or 10-fold greater than the risk to those without family members with glaucoma (Wolfs et al, 1998). The family members demonstrated higher cup-to-disc ratios and IOP; however, there was no definitive mode of inheritance in this complex disease.

A possible candidate gene, Myocilin (MYOC), was identified in a linkage analysis study (Stone et al, 1997). MYOC was previously known as the trabecular meshwork inducible glucocorticoid responsive (TIGR) gene, located at chromosome 1q23 (GLC1A). Variations in the MYOC gene are population specific; founder effects have been reported and not all mutations result in abnormal Myocilin protein, which is abundant in ocular tissue (Hewitt et al, 2008; Zhou et al, 2008). However, the variations in MYOC were only found in 2% to 4% of glaucoma patients and more frequently in juvenile open-angle glaucoma (JOAG) (Fingert et al, 1999; Sripriya et al, 2004). To date, more than 70 mutations of the MYOC gene have been found to be associated with glaucoma (Hewitt et al, 2008). The GIST found a possible association of MYOC with the staging or severity of glaucoma (Craig et al, 2001; Mackey et

al, 2003). The Gln368Stop mutation is associated with mild cases, Thr377Met and Gly252Arg are associated with intermediate or moderate cases, and Pro370Leu is more common in advanced-stage glaucoma (Craig et al, 2001; Mackey et al, 2003).

The Optineurin (OPTN) gene, located at chromosome 10p15-p14 (GLC1E) was identified in a linkage analysis of a large British family with normal tension glaucoma (NTG) (Sarfarazi et al, 1998). OPTN mutations are found in only 16.7% of hereditary POAG and are more associated with NTG (Rezaie et al, 2002). The Glu50Lys mutation is rare but was found to be associated with OAG. Unlike MYOC, minimal variation of OPTN was found to be associated with glaucoma (Fuse et al, 2004; Alward et al, 2003; Mukhopadhyay et al, 2005). A combination of OPTN variants with certain TNF-α variant have been reported in Japanese POAG patients and are associated with a severe phenotype (Funayama et al, 2004).

In 2005, a sequence variant of WD40-repeat 36 (WDR36), located at the GLC1G locus on chromosome 5q22, was reported to be responsible for glaucoma (Monemi et al, 2005). The frequency of WDR36 variants in glaucoma patients was lower than the frequency of MYOC and OPTN gene variants, estimated between 1.6 and 17% (Hauser et al, 2006). Despite the lower frequency, variants of WDR36 were associated with a severe glaucoma phenotype, suggesting its role in disease susceptibility (Hauser et al, 2006). Apolipoprotein E (Apo E) gene was reported as a strong modifier gene for glaucoma. The polymorphism -219T>G in the promoter region of the Apo E gene was associated with increased susceptibility to optic nerve damage; interaction of -419A>T with -1000C>G increased susceptibility to elevated IOP (Copin et al, 2002). Interestingly, Apo E gene variants also interact with MYOC gene variants (Copin et al, 2002).

Other genes that may be associated with glaucoma include optic atrophy 1 (OPA1), tumour protein p53, tumour necrosis factor (TNF), IL-1, noelin 2 (OLM 2), and cytochrome P450 1B1 (CYP 1B1) (Allingham et al, 2008). Glaucoma is, therefore, a heterogeneous disease produced by complex interactions between genetics and the environment. There is also evidence of gene-gene interactions in glaucoma. Overexpression of OPTN causes up-regulation of endogenous MYOC in ocular tissue (Park et al, 2007). Funayama et al (2006) found significant association of synonymous single nucleotide polymorphism (SNP) in OLM 2 (317G>A and 1281C>T) and OPTN 412G>A. Another association was found with OLM 2 317G>A and 678G>A, and OPTN 603T>A, suggesting a polygenic aetiology.

The advancement of molecular genetics, particularly with the introduction of genome-wide association studies (GWAS), will reveal more genes as potential candidates. The identification of Lysyl Oxidase-like protein 1 (LOXL1) was a great breakthrough in the GWAS quest for glaucoma genes (Thorleifsson et al, 2007). Pseudo-exfoliation glaucoma (XFG) is the most common secondary OAG, where fibrillar material is deposited on the ocular surfaces, especially on the trabecular meshwork, lens capsule, and papillary margin (Ritch, 1994). Deposition of this material at the trabecular meshwork clogs aqueous outflow, resulting in IOP elevation; XFG is more resistant than POAG to medical treatment and is further complicated by lens-related problems. XFG and pseudoexfoliation syndrome (XFS) is most frequent among Caucasians of European descent (Jonasson et al, 2003; Hirvela et al, 1994). The likelihood of genetic transmission was based on the identification of families with XFG or XFS (Allingham et al, 2001).

Three LOXL1 SNPs, rs1048661, rs3825942, and rs2165241, are associated with pseudo-exfoliation glaucoma and pseudo-exfoliation syndrome in many populations (Lemmelä et al, 2009; Ozaki et al, 2008; Aragon-Martin et al, 2008). The GWAS association of intronic SNP rs2165241 was statistically significant but lost its significance after genotyping of non-synonymous SNPs rs3825942 and rs1048661. Exonic rs3825942 and rs1048661 are in strong linkage disequilibrium and the significance of rs2165241 is due to the effective tagging to those haplotypes (Thorleifsson et al, 2007). There was no association of LOXL1 with POAG and PACG (Chakrabarti et al, 2008; Liu et al, 2008). Thus, the quest for understanding the genetics of glaucoma continues.

1.4 Intraocular pressure

Intraocular pressure (IOP), which is determined by the rate of aqueous humour production, drainage resistance, and episcleral venous pressure, represents the ocular pressure. IOP was first equated by Goldmann and Schmidt (1957) and later known as IOPG:

$$IOPG = EVP + Ap/Ad$$

EVP: episcleral venous pressure
Ap : rate of aqueous production
Ad : rate of aqueous drainage

The ciliary body and its processes are responsible for aqueous humour production through several possible mechanisms including active secretion, passive diffusion, and ultra-filtration. The aqueous humour drains through 2 outflow mechanisms: pressure-dependent trabecular outflow and pressure-independent uveoscleral outflow. The majority of drainage occurs via trabecular outflow and only 20% occurs via uveoscleral outflow, based on perfusion studies

with radio-labelled albumin. In the normal eye, the rate of aqueous humour production is almost equal to the drainage rate; 2.5 to 2.8 µL/min.

The episcleral veins are connected to the central circulation by a valveless system that creates a direct correlation between central venous pressure and IOP. An increase in central venous pressure such as occurs in superior vena cava obstruction, Sturge-Weber syndrome, and cortico-cavernous fistula causes a direct increase in episcleral venous pressure and subsequent increase of IOP (Greenfield, 2000; Jorgensen and Guthoff, 1988). In addition, 80% of aqueous humour outflow occurs through the Schlemm canal (conventional outflow) via a passive mechanism, which is dependent on the gradient of IOP and episcleral venous pressure (Mäepea and Bill, 1989). The rate of aqueous humour production has a lesser effect on IOP, only exerting its influence at higher IOPs. Episcleral venous pressure plays an important role in IOP. IOP is measured by tonometry with the main purpose of obtaining an accurate IOP measurement with minimal disturbance to the eye. Manometry is an invasive technique causing major disturbance to the eye but provides accurate IOP measurement. Due to the invasiveness of the technique, it is more popular as a laboratory technique and is an ideal reference pressure for tonometer (Eisenberg et al, 1998). Tonometry is divided into force and pressure tonometry; force tonometer includes applanation and indentation tonometer.

Adopting the principle that the pressure inside a dry, thin-walled sphere is proportional to the force applied to its surface, the Goldmann applanation tonometer (GAT) has been accepted as the gold standard in measuring IOP for 5 decades (Goldmann and Schmidt, 1957). Applanation force is the force required to cancel the force induced by sclera rigidity and tear

film surface tension to produce a circular area of cornea flattening 3.06 mm in diameter. Therefore, the applanation force is directly proportional to the IOP in accordance with the Imbert-Fick principle (Pressure = force/area) based on an assumed central corneal thickness (CCT) of 500 µm. Based on the Imbert-Fick principle, the applanation pressure can be manipulated by altering the force or area. The applanation pressure can be measured by applying sufficient force to flatten a fixed area (fixed-area tonometer) or measuring the area flattened by a fixed force (fixed-force tonometer). Although it is the gold standard, the GAT is far from perfect; factors affecting IOP measurements with the GAT are further discussed in 1.4.1.

1.4.1 Factors affecting intraocular pressure

In spite of being the gold standard and the most widely accepted tool for IOP measurement, GAT is far from perfect. Many factors affect its accuracy but physiological and systemic factors also give rise to variations in IOP measurements. These factors are important for proper interpretation of IOP. The applanation principle of GAT uses the corneal surface as the plane of IOP measurement. Thus, physiological changes or abnormalities of the corneal surface affect the IOP measurement. Goldmann and Schmidt (1957) acknowledged the possibility of CCT variation affecting the accuracy of GAT. At the time, it was assumed that CCT was constant with minimal inter-individual variability. However, thinner CCT requires less applanation force, resulting in possible underestimation of IOP, while more applanation force is required for thicker corneas. Therefore, CCT is an important factor in providing an accurate diagnosis of glaucoma.

In 1975, by cannulation of the anterior chamber (manometry) during cataract surgery, Ehlers et al found that every 10 µm increase in CCT created a 0.71 mmHg inaccuracy in the IOP measurement. The impact of CCT on accuracy of IOP was acknowledged, and ultrasonic pachymetry in healthy normal subjects showed that the IOP error was 0.16 to 0.67 mmHg for every 10 µm difference in CCT (Shimmyo et al, 2003; Doughty and Zaman, 2000; Saleh et al, 2006; Wolfs et al, 1997). CCT exhibits diurnal fluctuations with evidence of thickening during sleep and thinning in the afternoon (Fujita, 1980; Feldman et al, 1978). In spite of the great interest in the inaccuracy of GAT induced by CCT, there is still no specific acceptable nomogram for adjustment of IOP measurements according to CCT. Furthermore, there is no difference in IOP measurements by intra-cameral cannulation and applanation tonometry with increasing CCT (Feltgen et al, 2001).

Orsenggo and Pye (1999) suggested that, to obtain a true IOP (IOPT) from GAT (IOPG) mathematically, corneal biometry including corneal thickness, corneal elasticity or rigidity, anterior corneal radius, and area of applanation must be included as factor K.

$$IOPT = \frac{IOPG}{K},$$

$$\text{where } K = \frac{B_c + C_c + C}{B}$$

B_c : coefficient of IOPG for calibration of cornea
C_c : coefficient of IOPT for calibration of cornea
C : coefficient of IOPT for any cornea
B : coefficient of IOPG for any cornea

CCT is not the only important corneal biometry affecting the accuracy of IOP measurements. Currently, biomechanical properties of the cornea such as corneal hysteresis (CH), central

corneal power, axial length, and corneal curvature have also been implicated (Özcura et al, 2008; Francis et al, 2007; Kohllhass et al, 2006). Although refractive error is a risk factor for glaucoma, axial length is poorly correlated with IOP measurement (Özcura et al, 2008; Kohllhass et al, 2007). Similar weak correlations are observed with central corneal power and corneal curvature (Özcura et al, 2008; Francis et al, 2007). CH has been the main principle of ORA; unlike CCT, it is constant with no diurnal influence (Laiquzzaman et al, 2006; Kida et al, 2008). CCT and CH have been suggested as pressure-independent risk factors for glaucoma (Congdon et al, 2006).

IOP in humans is not consistently calculated by the Goldmann mathematical equation. Postural changes, stress, valsalva manoeuvre, exercise, alcohol or caffeine consumption, smoking, and marijuana use induce short-term changes that affect IOP (Linder et al, 1988; Pasquale and Kang, 2009). IOP in the supine position is 2–4 mmHg higher than IOP measured in a seated position and alarming 3-fold increase in complete gravity inversion (Galin et al, 1963; Weinreb et al, 1984). IOP increased 2-fold from baseline during a headstand posture (Sirsasana) during Yoga exercise (Baskaran et al, 2006). This phenomenon is believed to be due to rapidly increased choroidal vessel filling that increases the episcleral venous pressure (Linder et al, 1988, Aihara et al, 2003).

IOP is also affected by the valsalva manoeuvre, a physiological phenomenon occurring during coughing, lifting, vomiting, straining, and defecation. The sympathetic and parasympathetic autonomic nervous systems are stimulated during the straining and release phases of the valsalva manoeuvre. During the straining phase, intra-thoracic pressure builds, impinging on venous return and causing engorgement of the choroidal vessels. Subsequent

sclera rigidity causes an increase in episcleral venous pressure and intraocular pressure. To overcome the reduction of venous return, there is concomitant reduction of arterial pressure, cardiac output, and blood pressure. Reflex-mediated tachycardia and peripheral vasoconstriction then occur to overcome the drop in blood pressure. Reduced arterial pressure was believed to reduce choroidal filling (Booth et al, 1991). The physiological changes of IOP during the valsalva manoeuvre last for 15 to 30 minutes. Based on experimental valsalva manoeuvre, IOP increased during the straining phase (Lanigan et al, 1989). The arterial baroreceptor is stimulated during the release phase, increasing the cardiac output that later relieves the peripheral constriction, heart rate, and blood pressure, and leads to a drop in IOP. Thus straining (holding the breath) during tonometry measurement can produce an effect similar to that of the valsalva manoeuvre, and is a source of inaccuracy during IOP measurement (Whitacre and Stein, 1993). IOP is affected more by the valsalva manoeuvre than by psychological stress (Brody et al, 1998). The measurement of IOP is largely affected by an uncooperative patient.

There is conflicting evidence on the effect of exercise on IOP. Within 5 minutes of starting an exercise, an initial elevation of IOP is followed by a gradual decrease up to 60 minutes post exercise with no change in optic nerve perfusion due to the auto-regulatory mechanisms (Qureshi, 1995b; Movaffaghy et al, 1998). However, there is evidence of significant increases in pulsatile ocular blood flow (Price et al, 2003). Increased serum osmolarity is believed to be responsible for the acute post exercise drop in IOP (Stewart et al, 1970; Martin et al, 1999). The reduction in IOP was observed in healthy and glaucoma subjects with a greater drop and longer post exercise recovery in glaucoma subjects (Qureshi, 1995a). Acute IOP reduction is proportional to the degree and duration of exertion (Qureshi et al, 1996). However, strenuous exercises such as weight lifting and bench press exercise may have effects similar to those of

the valsalva manoeuvre (Vieira et al, 2008). A consistent IOP reduction was observed in sedentary non-glaucoma patients after constant prolonged exercise for 3 months but the effect was diminished in athletic patients (Lempert et al, 1967). Unfortunately, the effect is inconsistence; other researchers have observed elevated IOP, and others have observed no IOP changes (Era et al, 1993).

Nicotine, the primary active substance in cigarettes, is known to have detrimental effects on health. Cigarette smoking induces vasoconstriction and increases blood pressure (Tamaki et al, 2000). Its effect on IOP is not well established. It is believed that increased blood viscosity induced by smoking is responsible for IOP elevation (Yoshida et al, 2003). A significant elevation of IOP was observed in 50 healthy normotensive young male subjects after a month of smoking 2 cigarettes per day (Timothy and Nneli, 2007). Similarly, an acute increase in IOP was observed in both normal and glaucoma subjects 5 minutes after the last puff of a cigarette, but the pressure normalized after 15 to 30 minutes in normal subjects (Mehra et al, 1976). However, other studies have found no significant IOP difference between smokers, non-smokers, and ex-smokers (Bahna and Bjerkedahl, 1948; Morgan and Drance, 1975; Shephard et al, 1978). Furthermore, there is no evidence of an association between cigarette smoking and progression of ocular hypertension to glaucoma (Kang et al, 2003).

Moderate and heavy alcohol consumption may play a protective role in cataract development and age-related macular degeneration (Wang et al, 2008). Its impact on IOP and glaucoma is not well established. During the acute ingestion of alcohol, there is evidence of IOP reduction, which may be due to osmotic effects, suppression of anti-diuretic hormone, and inhibition of secretory cells in the ciliary processes (Houle and Grant, 1967; Peczon and

Grant, 1965). These mechanisms reduce water movement to the eye and aqueous humour production. The ocular hypotensive effect is dose-dependent. Consumption of one alcoholic beverage drink (10 grams) yielded no effect; one hour of hypotensive effect was achieved with 17 grams and up to 3 hours with 50 grams of alcohol (Obstbaum and Podos, 1973; Buckingham and Young, 1986; Houle and Grant, 1967). However, epidemiological studies that addressed the effect of alcohol on IOP yielded inconsistent results (Lin et al, 2005; Weih et al, 2001; Wu and Leske, 1997). The majority of the studies were based on questionnaires and interviews with non-standardized measures of alcohol consumption. Reports described elevated IOP with increased consumption, but others reported no effect or a hypotensive effect (Yoshida et al, 2003; Leske et al, 1996; Klein et al, 1992). An earlier study suggested an inverse relationship between alcohol consumption and elevated IOP (Seddon et al, 1983). The association between alcohol and IOP may be modified by sex with higher IOP in men than in women of Asian and Afro-Caribbean descent (Lin et al, 2005; Wu and Leske, 1997).

Helper and Frank introduced the potential role played by cannabinoid compounds (found in marijuana) in glaucoma management in 1971, based on a study in a small number of subjects. A marked 25% reduction of IOP from baseline was observed up to 3–4 hours after smoking (Helper et al, 1976). Green (1998) found that the IOP reduction was inconsistent and some patients failed to demonstrate any IOP changes. Nevertheless, marijuana and its main psychoactive substance, Δ9–tetrahydrocannabinol (THC) is a potential therapeutic drug for glaucoma management. The mechanism by which marijuana reduces pressure is poorly understood. The promise of its beneficial effects is limited by the risk of potentially devastating systemic and ocular side effects. Topical administration of Δ9-THC would be an ideal delivery method to minimize potential unwanted side effects, but the lipophilic nature of Δ9-THC makes this a challenge.

Wearing tight neckties has been found to elevate IOP by 2 mmHg in both normal and glaucoma subjects (Teng et al, 2003). There was no significant different between glaucoma and normal subjects. The elevation of IOP is postulated to be due to constriction of the jugular vein, which elevates episcleral venous pressure (Bigger, 1975). However, no persistent elevation of IOP is observed after extended wear of tight neckties and avoidance is unnecessary in glaucoma patients (Talty and O'Brien, 2005). Nevertheless, the accuracy of GAT may be affected in subjects wearing a tight necktie. Caffeine consumption (in coffee) has also been linked to elevated IOP (Chandrasekaran et al, 2005). Drinking 5 or more cups of coffee a day increases the risk of POAG, particularly in those with a positive family history of glaucoma (Kang et al, 2008).

Long-term factors that may be associates with IOP changes include age, sex, systemic blood pressure, refractive error, iris colour, obesity, and environmental factors such as cold climate. The prevalence of glaucoma is age-related. Although IOP is identified as the major modifying risk factor for glaucoma, the relationship between IOP and age is inconsistent and not linearly correlated. Increasing IOP and increasing age have been positively correlated in many longitudinal and large cross-sectional studies in Caucasians (Martin et al, 1985; Klein et al, 1992). Age was positively associated with IOP by univariate analysis but not by multivariate analysis in a cross-sectional study of Caucasians residing in Australia (Rochtchina et al, 2002). Age-related changes in IOP were positively correlated with changes in systolic blood pressure and body mass index (BMI) in cross-sectional and longitudinal studies (McLeod et al, 1990). Aging causes slight reduction of the outflow capacity at a rate of 3.2% per decade after the age of 10 (Brubaker, 1991). At the same time, age-related

changes lead to increased intra-orbital fat, which leads to increase episcleral venous pressure. In addition, age-related reduction of cells in the trabecular meshwork is believed to cause a net effect of IOP elevation with age (Alvarado et al, 1981, Gabelt et al, 2003; Brubaker et al, 1981).

IOP increases proportionally with age but decreases in Afro-Caribbean over 70 years old, partially due to selective mortality (Wu et al, 1997; Wu et al, 2006). An inverse relationship between IOP and advanced age was observed in Japanese, which may explain the higher prevalence of NTG in this population, well known for longevity (Nomura et al, 1999; Shiose, 1984; Shiose and Kawase, 1986; Shiose et al, 1991). A similar relationship was observed in other East Asian populations (Lin et al, 2005; Xu et al, 2005; Lee et al, 2002). Moreover, CCT decreases with age in East Asian populations, which perhaps further strengthens this interesting observation (Foster et al, 1998; Foster et al, 2003; Suzuki et al, 2005). The inverse relationship of IOP and age is observed in cross-sectional studies but demonstrated an exponential relationship in a Japanese longitudinal study (Nomura et al, 1999; Kawase et al, 2008).

The relationship between age and IOP is dependent on systemic blood pressure and BMI (Carel et al, 1984; Klein and Klein, 1981). Hypertension has been associated with IOP elevation in many population-based studies (Tielsch et al, 1995; Klein et al, 1992; Wu et al, 1997; Foster et al, 2003). Increased ciliary pressure induced by changes in hypertension lead to increase aqueous humour production, ultimately increasing the IOP (Shiose and Kawase, 1986; Bulpitt et al, 1975). Increased sympathetic tone and corticosteroid was also postulated to be involved, although the mechanism remains unknown (Carel et al, 1984). Both systolic

and diastolic blood pressures have been implicated. Positive associations between IOP and systolic blood pressure have been observed in many longitudinal and cross-sectional population-based studies (Hennis et al, 2003; Dielemans et al, 1995; Klein et al, 2005). The Beaver Dam Study reported that for every 10 mmHg increase in systolic blood pressure there was a 0.2 mmHg increase in IOP and a 0.4 mmHg increase in IOP for every 10 mmHg increase of diastolic pressure (Klein et al, 2005). The Barbados Eye Study reported a more marked influence of diastolic blood pressure on IOP (Wu and Leske, 1997). However, there was no significant difference between the effects of systolic and diastolic blood pressure. From another perspective, reducing blood pressure could have a protective effect in reducing IOP and reducing the risk of glaucoma. The Beaver Dam Eye Study found that a decrease in either systolic or diastolic blood pressure of 10 mmHg over 5 years is associated with a decrease in IOP (Klein et al, 2005).

Body mass index (BMI) has a significant influence on IOP. Obesity leads to increase intra-orbital fat, elevate episcleral venous pressure, increase blood viscosity and reduce outflow capacity (Shiose, 1984; Shiose and Kawase, 1986). IOP is directly correlated with BMI, even after controlling for confounding factors such as age, blood pressure, and presence of diabetes (Mori et al, 2000; Klein et al, 1992; Wu and Leske, 1997). The Beaver Dam Study found a direct exponential relationship between increasing IOP and BMI in Caucasians (Klein et al, 1992). Likewise, in cross-sectional studies of East Asian populations, reduced incidence of obesity and lower BMI was believed to be responsible for the inverse relationship between IOP and age (Nomura et al, 1999; Mori et al, 2000). After an 8 year longitudinal study in the same population, a linear relationship between IOP and BMI was observed, similar to observation in Caucasians (Nomura et al, 1999; Mori et al, 2000). These studies suggest the importance of weight control in preventing elevation of IOP.

Those with high BMI are at risk for systemic hypertension, diabetes mellitus, and heart diseases. Diabetes mellitus was also associated with changes in IOP in cross-sectional population-based and longitudinal studies (Hennis et al, 2003; Mitchell et al, 1997; Wu and Leske, 1997; Wu et al, 2006). However, after excluding subjects with cataracts, only a weak association was found in the Barbados Eye Study (Hennis et al, 2003). There is also evidence that argues against the possible association of diabetes and IOP (Bouzas et al, 1971; Armaly, 1967). Glycosylated haemoglobin level was exponentially associated with IOP (Klein et al, 1992). Thicker CCT in diabetes subjects, especially during hyperglycaemic episodes, may prompt false detection of high IOP (Bron et al, 1999; Sahin et al, 2009). Diabetes-related autonomic dysfunction and the osmotic gradient induced by elevated blood glucose may cause a fluid shift into the intraocular space, resulting in IOP elevation (Mapstone and Clark, 1985, Cristiansson, 1961). A more accepted theory is that fluid shifts from the intraocular space into the cells, resulting in the net reduction of IOP (Dielemans et al, 1994). Genetic predisposition has also been postulated based on the prevalence of diabetes in glaucoma patients with strong family history of glaucoma (Clark and Mapstone, 1986). The relationship between IOP and diabetes remains controversial.

Unlike the impact of age on IOP, sex exerts a lesser effect. Some studies have found that IOP is higher in women (Normura et al, 1999; Yoshida et al, 2003; Leske et al, 1997), and it has been suggested that the higher frequency of hypertension, obesity, and relative longevity in women may contribute to this effect (Memarzadeh et al, 2008). Other studies found higher IOP in men (Leske et al, 1994; Doshi et al, 2008). Most studies found no association of

gender with IOP and postulated selection bias as the probable explanation for any reported associations (Weih et al, 2001; Klein et al, 1992).

Refractive error, especially myopia, has been found to influence IOP measurement in case-control studies (David et al, 1985; Abdalla and Hamdi, 1970; Tomlinson and Philips, 1970). The Blue Mountain Eye Study, a population-based cross-sectional study, found a strong positive association between myopia and higher IOP (Mitchell et al, 1999). Similar findings were documented in Afro-Caribbean and Asian populations (Wu et al, 1999; Nomura et al, 2004; Xu et al, 2007; Kawase et al, 2008). There is an exponential relationship between increasing severity of myopia and progressive elevation of IOP in Caucasians (Wong et al, 2003). The relationship is believed to be due to the thinner CCT associated with myopia and affect IOP measurement. However, Nomura et al (2004) reported a significant exponential relationship of myopia severity and IOP even after adjusting for CCT and age. A contrasting report described an effect of myopia at a lower IOP with a weakening effect on increasing IOP (Grødum et al, 2001). Interestingly, hyperopia has also been associated with significant elevation of IOP, but the assertion requires further evaluation (Wong et al, 2003).

Iris colour has also been suggested to affect IOP measurement. Darker iris colour was associated with higher IOP in a Caucasian population. The Blue Mountain Eye Study found a modest association between iris colour and IOP, supporting the findings of other, smaller studies (Mitchell et al, 2003; Hiller et al, 1982; Weih et al, 2001). There was a significantly lower IOP in subjects with lighter iris in the Blue Mountain Eye Study (Mitchell et al, 2003). However, the reduction in IOP was not statistically significant in green and light brown iris in comparison to blue, grey, or green iris in both normal and glaucoma subjects in the

Melbourne Visual Impairment Project (Weih et al, 2001). Higher IOP is associated with darker iris (Memarzadeh et al, 2008, Semes et al, 2006), although there is no clear mechanism or explanation for this relationship.

1.4.2 Intraocular pressure fluctuation

Similar to blood pressure, IOP is also subject to circadian variation. Fluctuation of aqueous humour production is postulated to be responsible for IOP fluctuation, which is highest in the early morning, decreases throughout the day, and is lowest during sleep in normal individuals (Topper and Brubaker, 1985). Fluorophotometry was used to demonstrate that aqueous humour formation occurs through the day (late morning) in normal and glaucoma subjects (Friedenwald and Brubaker, 1955; Langley and Macdonald, 1952). Hormonal and neurological stimulation has also been implicated (Brubaker, 1991). However, by far the strongest evidence points to β-adrenergic stimulation (Larson and Brubaker, 1988). High levels of circulating catecholamine drive the production of aqueous humour during the day but the lack of this stimulation at night slows production (Armaly, 1963). However, sympathetic denervation fails to reduce aqueous humour production, suggesting that neurological stimulation may not be as important (Wentworth and Brubaker, 1981). On the other hand, there is no evidence of diurnal variations of outflow facility influencing episcleral venous pressure (Takeda and Azuma, 1978). Episcleral venous pressure remains constant throughout the day (Takeda and Azuma, 1978).

The IOP fluctuation can be divided into diurnal (day time) fluctuation, nocturnal (night time) fluctuation, 24 hour fluctuation and long term fluctuation. Similar to glaucoma, the major

problem in IOP fluctuation is inconsistency of definition. Some define fluctuation as variation in IOP during the diurnal period and the other includes nocturnal fluctuation as diurnal fluctuation (Singh and Shrivastava, 2009). For the purpose of discussion, diurnal fluctuation is defined as IOP fluctuation in the day time and nocturnal is exclusive for night time fluctuation. Long term fluctuation is defined as inter-visit IOP during long period of observation.

Based on the circadian curve, IOP fluctuation is further categorized into morning, day, night, and flat types (Zeimer, 1996). IOP fluctuation can also be divided into regular and irregular groups. Regular fluctuation refers to IOP fluctuation that is generally constant day-to-day and irregular refers to those with random pressure peaks (Zeimer, 1996). The commonest circadian curve is the morning type, with lowest IOP in the early morning, typically following with aqueous humour production (Wilensky, 1991). The range of diurnal fluctuation was found to be 2–3 times higher in glaucoma patients than in normal individuals (Drance, 1960; Drance, 1963; Hollows and Graham, 1966; Wilensky, 1991). Both normal and glaucoma subjects experience a maximal peak before noon (David et al, 1992; Saccà et al, 1998). Based on 690 diurnal curves, higher IOP fluctuation was seen in glaucoma and ocular hypertension subjects than in normal individuals (David et al, 1992). Higher fluctuation was observed in ocular hypertension than in open-angle glaucoma, suggesting that the higher the IOP, the greater the range of fluctuation (David et al, 1992).

CCT has been identified as a confounding factor for IOP measurement using GAT. Similar to IOP, circadian variation of CCT has been reported in glaucoma patients (Fogagnolo et al, 2006). Although the 24-hour CCT fluctuations were small, there was a significant difference

between the peak at 4 am and trough at 4 pm (Fogagnolo et al, 2006). It is logical to postulate a potential relationship between IOP and CCT fluctuation, although the nature of the relationship remains elusive. Fogagnolo et al (2006) found that CCT fluctuation does not significantly influence IOP fluctuation in glaucoma patients. Similar findings were observed in healthy young volunteers and aging subjects (Kida et al, 2006; Kida et al, 2008). Nocturnal peak CCT occurred a few hours earlier than IOP nocturnal peak in young healthy subjects (Kida et al, 2006). Based on the available evidence, IOP fluctuation is not affected by CCT fluctuation.

Nocturnal IOP is higher than diurnal IOP in habitual positions, with the difference of peak and trough as high as 8.2 (SD 1.4) mmHg (Liu et al, 1999). Similarly, nocturnal IOP is significantly higher than diurnal IOP but the difference is not as great as in normal subjects without ocular diseases. The effect of habitual position on IOP is partly explained by the increase in episcleral venous pressure and redistribution of body fluid in the recumbent position (Friberg et al, 1987). However, nocturnal fluctuations are also observed in seated healthy individuals (Liu et al, 2003). Physiologically lower blood pressure during sleep may be detrimental to the optic nerve due to significant reduction of ocular blood perfusion with higher IOP (Bagga et al, 2009; Pemp et al, 2008). Based on this postulation, prediction of disease progression would require a more accurate 24-hour IOP measurement rather than a single measurement during office hours. However, the impact of 24-hour fluctuation on glaucoma progression has not been studied prospectively.

The importance of IOP fluctuation measurement is debatable especially without proper clinical trials and unavailability of suitable portable devices for accurate measurement. Asrani

et al (2000) found a strong correlation between 24-hour IOP fluctuations with visual field progression. The accuracy of the home tonometry used in his study and poorly-defined visual field progression has been questioned. In contrast, Bengtsson and Heijl (2005) reviewed the large data set of the Malmo Ocular Hypertension Treatment Study and found that mean IOP, not its fluctuation, was strongly associated with glaucoma progression. IOP fluctuation is dependent on IOP level; there was an increase of 0.17 mmHg in IOP fluctuation for each 1 mmHg increase of mean IOP. In other words, mean IOP is reflective of IOP fluctuation. The debate further escalated with the outcome of post hoc analyses of many large prospective, multicentre randomized clinical trials.

The earlier post-hoc outcome of the Advanced Glaucoma Intervention Study (AGIS) found that long-term fluctuation over a minimum period of 3 years (between visits) is an independent risk factor for glaucoma progression (Nouri-Mahdavi et al, 2004). IOP fluctuation of $\geq$ 3 mmHg was significantly associated with visual field progression based on AGIS score. In a retrospective review of AGIS, long-term IOP fluctuation was observed at 3 months post intervention until there was evidence of worsening visual field or completion of follow-up, whichever came first (Caprioli and Coleman, 2008). Mean IOP and long-term IOP fluctuation were weakly associated. Greater IOP fluctuation was observed in those with low mean IOP, suggesting that fluctuation has a more detrimental effect in eyes with low mean IOP (Caprioli and Coleman, 2008).

In contrast, Early Manifest Glaucoma Treatment (EMGT) found that higher mean IOP is associated with higher IOP fluctuation (Bengtsson et al, 2007). The finding is similar to the earlier observation of 690 diurnal curves of glaucoma and normal subjects (David et al,

1992). IOP fluctuation is not an independent risk factor for visual field progression. EMGT predicted an 11% increased risk of glaucoma progression for every 1 mmHg increase in mean IOP (Bengtsson et al, 2007). IOP fluctuation is not an independent risk factor for development of glaucoma in untreated ocular hypertensive patients (Medeiros et al, 2008). Perhaps the most striking differences between many large randomized clinical trials are due to the type of glaucoma and severity of the disease. In advanced glaucoma (AGIS), the fluctuation of IOP causes stress and de-stress of the fragile optic nerve fibres, leading to further progression (Caprioli and Coleman, 2008). At the earlier stage of glaucoma, such as in EMGT and ocular hypertension, the optic nerve is more resistant to stress with a direct exponential relationship between IOP fluctuations and mean IOP (Caprioli and Coleman, 2008). Based on the current available evidence, both mean IOP and IOP fluctuation measurements are important in glaucoma management.

1.4.3 The importance of IOP reduction in glaucoma management

Currently, a diagnosis of glaucoma is independent of the IOP; nevertheless, IOP remains the only modifiable risk factor. Most available modes of glaucoma treatment including medical, laser, and surgical management, are aimed at IOP modification. There have been many large, randomized controlled trials of therapies for various types and severities of glaucoma over long periods of prospective observation (Heijl et al, 2002; The AGIS investigators, 2000; Musch et al, 2009; Collaborative Normal-Tension Glaucoma Study Group, 1998). Although controversial and neglected issues remain unaddressed, these trials have provided insight into the natural history of the disease and have revolutionized glaucoma management. Visual field progression was used as the endpoint of all these clinical trials, regardless of the type and severity of glaucoma. The definition of progression differed in each trial protocol.

The risk of glaucomatous optic neuropathy in ocular hypertensive (OHT) patients was reduced up to 50% with a 20% reduction of IOP in the Ocular Hypertensive Treatment Study (OHTS). OHTS studied the benefit of topical hypotensive treatment in a large, randomized controlled trial. Topical hypotensive treatment confers a 10% decreased risk of conversion for each mmHg of IOP reduction (Gordon et al, 2002). A meta-analysis of 9 clinical trials found a 14% reduction in the relative risk of conversion to glaucoma with each mmHg of IOP reduction in OHT patients (Peeters et al, 2010).

The Early Manifest Glaucoma Trial (EMGT), a randomized controlled trial in 255 patients with early glaucoma, was conducted to determine the effectiveness of aggressive treatment with laser trabeculoplasty and topical betaxolol (Leske et al, 1999). The study also studied the effectiveness of the proposed treatment in reducing pressure, identified predictors for progression, and described the natural history of newly diagnosed glaucoma. Pseudoexfoliative glaucoma and normal tension glaucoma patients were also included. Similar to the findings of the OHTS, ocular hypotensive treatment was effective in preventing further glaucomatous optic nerve damage with 10% risk reduction for each mmHg of IOP reduction from baseline (Heijl et al, 2002). Age and glaucoma severity were also identified as predictors for progression. However, increased incidence of cataract was reported following the treatment modalities, without a significant increase in cataract surgery.

The European Glaucoma Progression Study (EGPS) recruited OHT patients at lower IOP (22 to 29 mmHg) and tested the efficacy of topical dorzolamide (The European Glaucoma

Progression Study Group, 2002). However, topical dorzolamide exhibited poor effectiveness, having little impact on IOP reduction and glaucoma prevention. In fact, the untreated group showed a reduction in visual field progression (The European Glaucoma Progression Study Group, 2005). Outcomes were affected by a high drop-out rate due to failure to achieve the target IOP.

IOP reduction has been proven effective in open-angle glaucoma with higher than normal baseline IOP. Does IOP reduction benefit patients with normal tension glaucoma? The Collaborative Normal Tension Glaucoma Study (CNTGS) was designed to determine the possible benefit of IOP modification with topical treatment, laser therapy, and surgical intervention in normal tension glaucoma patients (CNTGS group, 1998a). Target IOP reduction was set at 30% from baseline in the treated group. The incidence of cataract was significantly higher in the treated group than in the untreated group. The benefit of IOP reduction was only significant after the impact of cataract was removed. The effects of cataract on visual field progression should be taken into account when assessing field changes. However, there were subsets of patients who did not progress or demonstrated slow progression even without treatment. Although the treatment group had better survival analysis outcomes in terms of progression, there were still cases that progressed in spite of achieving target pressure (CNTGS group, 1998b).

The merit of surgical versus medical treatment has been a matter of debate for decades (Migdal et al, 1994; Smith, 1972). The Collaborative Initial Glaucoma Treatment Study (CIGTS), a longitudinal randomized clinical trial addressed the issue of medical treatment versus surgical intervention as the first-line treatment for open-angle glaucoma including

pseudoexfoliation and pigmentary glaucoma (Musch et al, 1999). Both modes of treatment provide acceptable IOP reduction. However, trabeculectomy provided better and more consistent IOP reduction, slowing progression of optic nerve damage (Musch et al, 2009). The benefit of trabeculectomy was more pronounced in patients with more advanced visual field loss. Diabetes mellitus has a disadvantageous effect on the success of trabeculectomy (Musch et al, 2009). As expected, the incidence of cataract was greater in the surgical intervention group.

The Advanced Glaucoma Intervention Study (AGIS), a longitudinal randomized control trial, was designed to determine the effectiveness of aggressive treatment in advanced glaucoma patients (AGIS group, 1994). Caucasian and African Americans were recruited and randomized into argon laser trabeculoplasty-trabeculectomy-trabeculectomy (ATT) and trabeculectomy-argon laser trabeculoplasty-trabeculectomy (TAT) treatment groups. Argon laser trabeculoplasty or trabeculectomy was chosen as first-line management after medical treatment failure and subsequent treatment was dependent on reaching the target IOP. Both groups demonstrated post intervention IOP reduction and reduced progression of visual field defects (AGIS group, 2000). IOP reduction was significantly associated with slowed progression of visual field defects even after adjustment for cataract formation and diabetes (AGIS group, 2000).

Computer-simulated modelling of the US population demonstrated that glaucoma treatment was cost effective if diagnostic assessments are excluded and successful therapy is assumed (Rein et al, 2009). Treatment efficacy was based on relative risk of progression and reduction of visual field loss based on the model of the EMGT (Leske et al, 1999). The impact of

aggressive, more efficacious treatment and surgical intervention was based on the CIGTS (Musch et al, 1999). The incremental cost incurred by additional or earlier cataract surgery was also included. The model estimated that without treatment, 24.6% would have visual field progression of at least -16 dB at the peak of glaucoma impact (75 to 79 years old). On the other hand, there was evidence that glaucoma progressed in spite of achieving or exceeding the target IOP. Neuro-protective drugs may have better therapeutic potential than pressure-lowering drugs. Nevertheless, the importance of pressure-lowering treatment is without doubt beneficial, both clinically and economically.

1.4.4 Target IOP

IOP reduction is important in retarding the progression of glaucomatous damage but the question remains: how much IOP reduction is required to stabilize damage and to maintain adequate functional vision? The term 'target IOP' was introduced in 1950 but popularized by the committee of the American Academy of Ophthalmology (AAO) Preferred Practice Pattern guidelines for POAG in 1989, which was regarded as the 'holy grail' of glaucoma but is now under debate (American Academy of Ophthalmology, 1989; Singh et al, 2000). The outcomes of the AGIS and CIGTS supported the setting of a target IOP in prevention of glaucoma progression (Palmberg, 2002). The AGIS showed no net visual field progression in subjects whose IOP measurements remained below 18 mmHg throughout the study.

Target IOP is defined as the range of IOP sufficient to stop progressive pressure-induced optic neuropathy or to cause the rate of ganglion cell loss to be no greater than the age-dependent rate (Brubaker, 1996). Target IOP is dynamic and cannot be predetermined upon

initiation of therapy; it changes according to the disease course and depends on the individual response and the resistance of the optic nerve to pressure-related damage (Goldberg, 2003; Damji et al, 2003). The initial baseline IOP, amount of baseline damage or disease severity, rate of damage, family history, cost of treatment, ocular and systemic side effects, life expectancy, and health-related quality of life are taken into account in establishing target IOP (Jampel, 1997; Palmberg, 2000). Age and ethnicity are also considered. Even non-pressure related risk factors such as diabetes mellitus and migraine have been addressed (Anderson et al, 2003).

Establishing the target IOP is a challenge and must be customized to the individual patient. An important question is whether a percent reduction or a defined IOP value should be chosen as the target IOP (Hitchings and Tan, 2001). The OHTS defined the target IOP as a reduction of at least 20% from baseline. The treatment target was set at 30% from baseline in the CNTGS (CNTGS group, 1998a). The absolute threshold of ≤ 21 mmHg was defined as the target IOP in the Glaucoma Laser Trial and Moorfields Primary Treatment Trial, in which individual variation was not addressed (Hitchings and Tan, 2001; Migdal et al, 1994). A target IOP range accounts for individual variation and the dynamic nature of IOP and provides a working framework for defining therapeutic goals. Jampel (1997) introduced an algorithm that accounted for the initial IOP, optic nerve damage severity, and therapeutic burden. Optic nerve damage and therapeutic burden are ranked 0 to 3 from the normal disc and no effect on QOL to advanced optic disc damage and maximum effect on QOL: Target range = [Initial IOP * (1 - Initial IOP/100) – Z + Y] +/- 1 mmHg. Z referred to optic nerve damage severity and Y denotes the burden of therapy. The CIGTS used another algorithm: target IOP = (1 - [reference or baseline IOP + VF score]/100 X reference IOP (Jampel, 1997). Maximum target IOP level or percentage was adopted by many glaucoma guidelines, based

on the severity of optic disc damage (Terminology and Guidelines for Glaucoma, 2008; Asia Pacific Glaucoma Guideline, 2008).

Lower target IOP is recommended for those with advanced or severe optic disc damage and higher baseline IOP. Guideline manuals have been established to provide target IOPs according to glaucoma severity (Terminology and Guidelines for Glaucoma, 2003). Further reduction of the target IOP is needed in the presence of greater non-pressure risk factors. However, target IOP fails to address the issue of IOP fluctuation; instead, it is based on a single measurement taken during an office visit. The IOP and its effects on the optic nerve fluctuate throughout the day; therefore, selecting one measurement does not represent the entire patient scenario (Ziemer et al, 1991, Jampel, 1997). Even worse, in order to establish the target IOP, the patient must sustain additional optic nerve damage before that damage can be stabilized.

1.5 Topical pressure-lowering drugs

Glaucoma is an irreversible chronic disease; thus, management is a great challenge. The main goal of treatment is to prevent further nerve fibre damage. The present modes of treatment include pressure-lowering medications, laser treatment, and surgical interventions, all of which aim to reduce IOP. Diversion of aqueous humour outflow through an iatrogenic fistula to the sub-conjunctival space is the principle underlying glaucoma surgery. Surgical intervention provides sustainable constant IOP reduction but not without intra- and postoperative complications (Migdal et al, 1994; Musch et al, 2009). In fact, glaucoma surgery hastens cataract formation, which may necessitate further surgical intervention (Musch et al, 2009; AGIS group, 2000). Surgical equipment, a proper operating theatre, and

an experienced surgeon increase the cost of filtration surgery. The invasiveness of the procedure interrupts the natural defence mechanism of the eye, increasing the risk of infection. Laser treatment is less invasive and is associated with a lower risk of infection. Similar to the filtration surgery, it is a permanent procedure and does not require a high technology environment but requires an expensive high-maintenance laser machine. However, the pressure-lowering effect is insufficient and temporary (Glaucoma Laser Trial Research Group, 1995). Manipulation of aqueous humour production and outflow is the mainstay mechanism of topical pressure-lowering medications. Topical medications are widely available in industrialised nations, are non-invasive and easily transportable. In addition, they are relatively easy to apply without the need for special equipment or a high technology environment. Importantly, unlike surgical or laser treatment, it is non-permanent and easily discontinued if it is ineffective or produces unwanted side effects.

However, patient compliance is required to ensure maximum effectiveness of medical therapy, especially in long-term administration, and is even more challenging in asymptomatic disease at the early stage of glaucoma. The term compliance is inappropriate; instead, adherence and persistence provide a better description of patients' behaviour toward medication instillation. Adherence is a measure of the degree to which a patient obeys pharmacotherapy instruction over a defined period of time (Schwartz and Quigley, 2008). For example, if topical timolol is prescribed twice a day for a month but the patient only instilled 40 times, his/her adherence is 66%.

Persistence is defined as the time to discontinuation. Accurate assessment of adherence and persistence is a challenge, especially when most patients routinely overestimate their

adherence (Friedman et al, 2008; Friedman et al, 2007). Poor adherence and persistence are associated with drug cost, tolerability, difficulty in instillation, lack of education, forgetfulness, denial, schedule and travel issues (Tsai et al, 2003; Friedman et al, 2008). 'White coat adherence' is another issue, in which patients are at their best adherence during the 5 days prior to a follow up appointment, followed by a declining pattern until the next follow up approaches (Feinstein, 1990). Frequency of dosing and complexity of the regime also play important roles; poorer adherence is observed in those receiving adjunctive treatment (Nordstrom et al, 2005).

Patients' understanding of the importance of taking their medication, their satisfaction with the drug, tolerability, and cost are reflected in their persistence. Persistence ranges from 20% to 67% (Dasgupta et al, 2002; Spooner, 2002) and differs according to the class of pressure-lowering drugs. Latanoprost, a prostaglandin analogue, has demonstrated better persistence when compared to other drugs (Reardon et al, 2004; Schwartz et al, 2004). Combination pressure-lowering therapies have become more popular, such as adding a prostaglandin-β blocker, a carbonic anhydrase inhibitor-β blocker, or a pilocarpine-β blocker, with the aim to improve adherence and persistence.

In addition to the problems with adherence and persistence, drug-induced subclinical conjunctival inflammation and its possible association with the success of trabeculectomy is another controversial issue. Long-term and multiple treatments with topical anti-glaucoma drugs are believed to induce subclinical inflammation of the conjunctiva that may cause excessive scarring of the bleb and eventual trabeculectomy failure (Sherwood et al, 1989; Broadway et al, 1994a; Broadway et al, 1994b). The histological evidence is inconsistent.

Although there is evidence to suggest that pressure-lowering drugs induce inflammatory markers and macrophages, there is also evidence to the contrary (Sherwood et al, 1989; Broadway et al, 1994a; Baun et al, 1995; Liza-Sharmini et al, 2007). In addition, it is still not clear whether the active ingredient or the preservative exerts a more detrimental effect. Benzalkonium chloride, the most common preservative in topical anti-glaucoma medication, has been linked to elevation of inflammatory markers in tissue culture and animal models (De Saint Jean et al, 1999; Becquet et al, 1998). Preservative-free timolol induces less expression of interleukins and inflammatory markers (Baudouin et al, 2004). On the other hand, sympathomimetics induce significant conjunctival cell profile changes and are associated with poorer trabeculectomy outcome (Broadway et al, 1994a). In fact, discontinuation of sympathomimetics and steroid treatment reverses this silent effect of pressure-lowering medication (Broadway et al, 1996).

Commercially available topical pressure lowering drugs is illustrated in table 1.3. However, only topical β-adrenoreceptor antagonists and prostaglandin analogs are discussed in detail in this chapter.

Table 1.3: Commercially available topical anti-glaucoma drugs

Group	Mode of action	Drugs (concentration)	Dose	IOP reduction
Parasympathomimetics	Increased aqueous outflow	Pilocarpine (0.25-4%)	TID or QID	20-25%
Sympathomimetics	*Non-selective*			
	Decrease aqueous production and increase outflow	Epinephrine (0.25-2.0%)	TID	
		Dipiverfin (0.1%)	BD	
	Selective			
	Decrease aqueous production	Apraclonidine (0.5-1.0%)	BD or TID	20-25%
		Brimonidine (0.2%)	BD	
Carbonic anhydrase inhibitor	Decrease aqueous production	Dorzolamide (2%)	BD or TID	15-20%
		Brinzolamide (1%)		
β-adrenoreceptor antagonist	*Non-selective*			
	Decrease aqueous production	Timolol (0.1, 0.25, 0.5%)	BD	20-25%
		Levobunolol (0.25, 0.5%)		
		Timolol GFS (0.25, 0.5%)	Once a day	
	Selective			
	Decrease aqueous production	Betaxolol (0.25, 0.5%)	BD	15-20%
Prostaglandin analogue	Increased aqueous outflow	Latanoprost (0.005%)	Once a day	25-30%
		Travoprost (0.004%)		
		Unoprostone (0.12, 0.15%)		
		Bimatoprost (0.03%)	Once a day	25-30%

1.6 Topical β-adrenoreceptor antagonists

The potential benefit of systemic β-adrenoreceptor antagonists in lowering IOP was initially evaluated and intravenous propanolol was found to be the most effective (Philips et al, 1967). The profound corneal anaesthesia induced by propanolol, however, outweighs its potential utility. Intensive ophthalmic research eventually led to the introduction of topical timolol. In 1978, topical timolol revolutionized glaucoma management and remains until now as the first-line treatment for glaucoma.

Topical β-blocker acts predominantly by decreasing aqueous humour production without any effect on outflow capacity, despite the presence of beta-2 adrenoreceptor (ADRB2) in the trabecular meshwork (Coakes and Brubaker, 1978; Yablonski and Zimmerman, 1978; Sonntag et al, 1978). β-Blocker action is predominantly mediated by the abundant ADRB2 in the ciliary epithelium and ciliary body. Aqueous humour is produced by ciliary bodies through ultra-filtration and active secretion by the ciliary epithelium. The reversible β-blocker binding prevents binding of catecholamine that in turn prevents activation of intracellular adenylate cyclase and reduces the intracellular concentration of cyclic Adenosine Monophosphate (cAMP) at the ciliary body. Through an unknown mechanism, this process reduces aqueous humour production (Neufeld, 1979). The basal level of cAMP is maintained, as is the response to other transmitters. cAMP is an important second messenger in the intracellular cascade. Since the understanding of aqueous humour production is imprecise, the mechanism of action of topical β-blocker remains unknown.

β-blocker has a less potent effect on β1-adrenoreceptor in decreasing cAMP synthesis (Juzych and Zimmerman, 1997). Serotonin receptor, particularly 5-HT_{1A}, is abundant in the iris and ciliary body and has a similar molecular structure as ADRB2. Serotonin receptor, 5-

HT_{1A} is negatively coupled to adenyl-cyclase, decreases the intracellular cAMP and causes reduction in aqueous production (Osborne and Chidlow, 1996; Tobin and Osborne, 1989). Timolol demonstrated high affinity towards 5-HT_{1A} in the ciliary process of rabbits, which further supports the effect of timolol as a suppressor of aqueous humour production (Osborne and Chidlow, 1996).

Although the classic association of reduced cAMP synthesis and aqueous humour production is widely accepted, other evidence disputes this postulation. Schmitt et al (1980) found no association between decreased cAMP and the pressure-lowering effect of β-blockers on rabbits. Drugs that increase intracellular cAMP such as forskolin and cholera toxin also reduce the IOP, which contradicts the previous popular hypothesis (Caprioli et al, 1984). Another hypothesis postulated that the reduction of aqueous humour formation is achieved by direct inhibition of adrenergic stimulation of the secretory ciliary epithelium by endogenous epinephrine (Topper and Brubaker, 1985). Decreased ocular blood flow induced by β-blockers provides another alternative hypothesis. The effect of β-blockers on the vascular smooth muscle of the ciliary body inhibits vasodilatation and induces vasoconstriction of ciliary arterioles, which reduces capillary perfusion and stromal ultra-filtration (Vareilles et al, 1977). Reduction of aqueous humour production is an indirect consequence of decreases ocular blood flow (Watanabe and Chiou, 1983). There is also direct evidence that dopamine plays a role in ocular blood circulation. Haloperidol, a dopamine-blocking agent, reduces IOP.

For more than 3 decades, the topical β-blockers, particularly timolol, have been proven effective ocular hypotensive drugs in many types of glaucoma. Currently, 5 topical β-blockers are available worldwide: timolol maleate, betaxolol hydrochloride, levobunolol

hydrochloride, carteolol hydrochloride, and metipranolol (Table 1.5). In Malaysia, only timolol and betaxolol are widely available. Although the aqueous solution of timolol maleate is widely used, a gel-forming solution has been introduced and is well accepted. Gel-forming solution is prepared from purified *Pseudomonas elodea* cell wall and forms gel solution upon contact with mono-valent and divalent cations in tear film. This novel ophthalmic vehicle provides a similar pressure-lowering effect as the aqueous form with once-daily dosing (Shedden et al, 2001). It is thought to reduce the incidence of systemic adverse effects but has a higher reported incidence of transient blurring of vision (Dickstein et al, 2001; Stewart et al, 2001).

Table 1.4: Properties of topical β-blockers

Property	**Timolol**	**Betaxolol**	**Levobunolol**	**Carteolol**	**Metipranolol**
Concentrations (%)	0.25, 0.5	0.25, 0.5	0.25, 0.5	1.0	0.3
Preservatives	BAC# 0.01%	BAC# 0.01%	BAC# 0.004%	BAC# 0.005%	BAC# 0.004%
β-blocker potency*	4.7	1.0	14.6	10.0	1.8
Serum half life (hrs)	3–5	12–20	6	3–7	2
Cardio-selective	-	++	-	-	-
Intrinsic sympathomimetics	-	-	-	++	-
Ocular discomfort	++	+++	++	±	+
Systemic side effect					
Decreased heart rate	++	±	++	+	++
Respiratory impairment	++	±	++	+	++
Hyperlipidemia	+	?	?	-	?
Ocular perfusion	±	±	?	±	?

*β-blockade potency in comparison to propanolol, #BAC: benzalkonium chloride

1.6.1 Topical timolol

Topical timolol is a lipophilic, non-cardio-selective β antagonist without intrinsic sympathomimetics activity. It also lacks the ability to act as partial agonist and lacks membrane-stabilizing ability. Its chemical name is (-)-1-(*tert*-butylamino)-3-[(4-morpholino-1, 2, 5-thiadiazol-3-yl) oxy]-2- propanol maleate (1:1) (salt). The asymmetrical carbon atom in its structure forms a laevo-isomer (Figure 1.3). The optical rotation of timolol maleate is $[\alpha]_{405\ nm}^{25°}$ in 1.0N HCl (C = 5%) = –12.2° (–11.7° to –12.5°). with a molecular weight of 432.50. It is an enantiomer; D- and L-enantiomer are stereo-isomers that are non-super-imposable mirror images of each other.

Timolol maleate is a white crystalline powder soluble in water, methanol, and alcohol, with a pKa of approximately 9 in water at 25ºC. It is available as a sterile, isotonic, buffered aqueous solution with pH approximately 7.0 (range between 6.5 and 7.5) and osmolarity of 274–328 mOsm. There are also inactive ingredients such as monobasic and dibasic sodium phosphate, sodium hydroxide for pH adjustment, and water for injection. Benzalkonium chloride 0.01% is added as a preservative. Timolol maleate as the pure chemical is extremely stable to light and temperature, but the formulated topical form is less stable with a shelf life of 2 years.

Figure 1.3: Chemical structure and formula of timolol maleate

(Adapted from Zimmerman and Boger, 1979)

The ocular hypotensive effect of timolol is more profound when administered orally but significant effect is also achieved topically. Although the ocular hypotensive effect of topical timolol is achieved at a concentration as low as 0.008%, the optimal therapeutic dose is 0.25% and 0.5% twice daily in aqueous solution. The active ingredient in each millilitre of 0.25% of timolol maleate contains 2.5 mg of timolol (3.4 mg of timolol maleate) and each millilitre of 0.5% contains 5 mg of timolol (6.8 mg of timolol maleate). The concentration of timolol in the anterior chamber reaches 1–2 μM (8–100 ng/mL) after an hour of topical instillation, which is much higher than the minimum amount required to bind ADRB2 in the ciliary body (Philips et al, 1985). Thus, the pressure-lowering effect is achieved just 20 to 30 minutes after instillation with the peak seen 2 hours post instillation (Zimmerman and Kaufman, 1977). This is followed by further pressure reduction that is sustained up to 24 hours. Surprisingly, the half-life of topical timolol is just 1.5 hours (Schmitt et al, 1980).

Reversible binding of timolol to ocular melanin provides a reservoir for slow release of the active drug and is responsible for prolonging the pressure-lowering effect of timolol despite its short half-life. Animal studies have shown that timolol has a high affinity for and binds easily to melanin. Dark-pigmented rabbits demonstrated higher concentration of timolol maleate in the iris ciliary body when compared to albino rabbits, reducing the amount of active ingredient available for pharmacological action (Menon et al, 1989). Melanin near to the site of pharmacological action did not inactivate the active drug. Paradoxically, melanin competitively inhibits timolol. The net effect is that highly pigmented eyes require a higher concentration than less pigmented eyes, which is reflected in clinical observations in Asians and Africans (Ong et al, 2005; Otaleju and Ajayi, 1999; Katz and Berger, 1979).

Zimmerman and Kaufman (1977) reported the first 24-hour dose response to topical timolol 0.25% and 0.5% was similar. Maximum pressure reduction was reported to be nearly 40% in glaucoma patients treated with 0.5% topical timolol in short- and long-term dose-response studies (Zimmerman and Kaufmann, 1977; Boger et al, 1978; Lin et al, 1979). The pressure-lowering effect is best in daytime and poor at night, when aqueous humour production is reduced to less than half. In its early days, the effectiveness of timolol was compared only to topical pilocarpine and epinephrine, and was found to be significantly more effective with the added advantage of less frequent dosing (Boger et al, 1978; Zimmerman and Boger, 1979). The effectiveness of timolol has earned it status as the 'gold standard' used as the comparator for other new drugs that have flourished the glaucoma pharmaceutical market.

Topical timolol is effective in nearly all types of glaucoma including refractory glaucoma such as neovascular, aphakic, and uveitic glaucoma (Weber, 1981, Lin et al, 1979). Perhaps, due to its suppressive effect on aqueous humour production, it is also effective in angle-closure glaucoma (Lass and Pavan-Langston, 1979; Chew et al, 2004). Long-term treatment with timolol has been proven effective, but the effect is not sustained in more than half of patients after 5 years (Watson et al, 2001). Boger (1979) reported short-term escape and long-term drift phenomena in certain individuals. Up regulation of ADRB2 receptors in the iris and ciliary body is believed to be responsible for blunting the effect of timolol after short-term treatment (Boger, 1979). A meta-analysis comparing a wide range of topical anti-glaucoma drugs and prostaglandin analogue found that timolol is as effective as prostaglandin analogue in providing good IOP reduction at peak and trough (van de Valk et al, 2005). Prostaglandin analogues are slightly but not significantly better than timolol in pressure-lowering effectiveness.

Similar reduction of pressure by timolol was also reported in the contralateral, untreated eye (Dunham et al, 1994; Piltz et al, 2000). Absorption of timolol by the nasopharyngeal mucosa has raised concerns of potentially life-threatening side effects following topical administration (Passo et al, 1984; Diggory and Franks, 1997). The elderly and those with cardio-respiratory impairment are at risk, and prescribing timolol in these patients must be done with caution (Diggory et al, 1998; Diggory et al, 1994; Leier et al, 1986). Although timolol has some effects on hypoglycaemia and hyperlipidemia, the effect is minimal with low clinical importance (Coleman et al, 1990; Shorr et al, 1997). Decreased libido, depression, and hallucination are among the reported side effects of timolol (Lama, 2002). The introduction of gel-forming timolol solution has lessened its systemic effect (Shedden et al, 2001).

Timolol gel-forming solution (GFS) increases the viscosity of the drug, promotes ocular bioavailability, and facilitates ocular drug penetration. The prolongation of ocular contact depends on the gel formulation, which acts as a physical barrier to drainage or as a viscosity promoter. The gel in Timolol XE 0.5% promotes viscosity and bleb formation, which creates a temporary plug in the inner canthus and impedes timolol drainage through the punctum. Once-daily dosing of timolol GFS provides a similar pressure-lowering effect as timolol maleate in aqueous form with twice-daily instillation in glaucoma and ocular hypertensive patients (Roselund, 1996; Shedden et al, 2001). Plasma concentrations of timolol GFS are significantly lower than timolol ophthalmic solution, which perhaps explains the reduced systemic side effects associated with the gel solution (Shedden et al, 2001; Dickstein et al, 2001; Uusitalo et al, 2006). Blurred vision upon instillation of timolol in gel solution and ocular discomfort were reported in many patients (Shedden et al, 2001).

1.6.2 Beta 2 adrenoreceptor (ADRB2)

The metabolic and neuro-endocrine effects of adrenaline and nor-adrenaline are mediated by a class of membrane-bound proteins designated as the adrenergic receptor. The concept of an adrenergic receptor, which was previously regarded as a hypothetical structure, was introduced in 1948. Ahlquist (1948) disproved the concept of different epinephrine acting on specific receptors. There are actually different receptors that act specifically to produce different efferent organ effects. He coined the terms α- and β-receptor mediator to differentiate between excitatory and inhibitory functions. However, the action of the receptors in his experiment was epinephrine concentration-dependent.

Further observation and experiments (Lands, 1967; Lands, 1952) revealed that the β receptor was not a single entity; instead, catecholamine stimulation of the β receptor was dependant on the site of the effectors organ. Further research revealed 2 β-receptor subtypes: β1 and β2. β1 adrenoreceptor (ADRB1*)* are abundant in the myocardium and adipose tissue and bind nor-epinephrine and epinephrine with similar affinity. β2 adrenoreceptors (ADRB2) are found in the lung, liver, lymphocytes, bronchial smooth muscle, and vascular tissue, and have 10-fold greater affinity for epinephrine (Lands, 1967). Initially, the quantification of β adrenoreceptor was only possible using autoradiographic ligands *in vitro* (Spina et al, 1989). ADRB2 was found distributed through the airway smooth muscle and in other lung cell types including epithelial, endothelial, mast, and type II cells (Johnson, 1992). The invention of positron emission tomography has made *in vivo* quantification of β receptor possible using radio-ligands (IIC) CGP12177 (Ueki et al, 1993).

Since β-adrenergic agents play a significant role in IOP regulation, the presence of β adrenoreceptor in ocular cells is of physiological and clinical importance (Nathanson, 1980).

Radio-ligands and hormone-sensitive adenyl cyclase were used to demonstrate that ADRB2 is the major β-adrenoreceptor subtype (75–90%) in human ciliary processes, similar to its distribution in the human lung (Nathanson, 1981). The distribution of ADRB2 is similar in the eyes of rabbits and monkeys (Neufeld et al, 1978). Human iris, ciliary body, trabecular cells, and trabecular meshwork are also predominantly occupied by ADRB2 (Wax and Molinoff, 1987; Wax et al, 1989), further strengthening the possible importance of β adrenoreceptor in aqueous humour formation and as a pharmacological target in IOP control.

ADRB2 is a member of the 7-transmembrane receptor super-family; it is composed of a 7-helix membrane-spanning domain with 3 extracellular and 3 intracellular loops (Figure 1.2). The amino terminus is extracellular and the carboxyl terminus is intracellular. The ADRB2 structure shares significant homology with rhodopsin in terms of the relative orientation of the 7 transmembrane helices, but the second extracellular loop has a unique conformation. A Cys residue at position 341, immediately after the transmembrane 7 (TMVII) domain, is the site of palmitoylation. Palmitoylation is the formation of a reversible covalent attachment of fatty acids to cysteine that enhances protein hydrophobicity and functions in sub-cellular trafficking (Strosberg, 1993). Palmitoylation enhances the ability of agonist-bound ADRB2 to mediate adenylyl cyclase stimulation, promoting the insertion of several adjacent residues in the membrane (Moffet et al, 1993). Palmitoylation also anchors ADRB2 to the membrane and some consider it to be the fourth intra-cytoplasmic loop in the active conformation for G-protein coupling (Ligget, 1999). ADRB2 contains 2 disulfide bonds essential for ligands binding. Cys^{106} and Cys^{184} form one disulfide bond and the other is between Cys^{190} and Cys^{191}. The most important ligands-binding residue is Asp^{113} in TMIII. Hydrogen bonding between Ser^{204} and Ser^{207} in TMV is also essential for ligands binding. Asp^{79} and Asp^{130} in

TMII are important for signal transmission, as are Tyr^{316} and Asn^{312} in TMVII (Strosberg, 1993).

ADRB2 is also known as a G protein-coupled receptor (GPCR), a large family of transmembrane receptors that sense diverse extracellular molecular stimuli including odours, pheromones, hormones, neurotransmitters, and drugs, translating these signals into a cellular response. According to the nomenclature committee of the International Union of Pharmacology (NC-IUPHAR), GPCR are categorized into 4 groups; group 1 (also known as family A) is comprised of rhodopsin-like receptors; group 2 (family B) includes secretin-like receptors; group 3 (family C) includes GABA and metabotropic-glutamate receptors; and the last group includes the fizzled family receptors (Foord et al, 2005). ADRB2 is classified into group 1 for its structural similarity to rhodopsin; receptor phosphorylation activates the cAMP pathway to mediate cellular responses. The Human Genome Project has successfully sequenced most of the GPCRs. To date, the cellular or drug interaction functions of most of the 726 GPCRs encoded by the human genome have been characterized, but over 100 GPCRs, especially olfactory receptors, are uncharacterised 'orphans'. Orphan receptors are sequenced heptahelical receptors for which neither ligands nor cellular function has been identified (Lefkowitz RJ, 2007).

Ligands are signal-triggering molecules, which are specifically bound by target receptor proteins to induce a cellular response. Ligands can be an agonist or antagonist drug, which exhibits functional selectivity or ligands-induced differential signalling; different ligands can induce different receptor conformations (Urban et al, 2007). The strength of ligands depends on its affinity (ability of the drug to bind and the strength of ligands-receptor association) and efficacy (ability to induce biological response). Partial agonists are less efficacious than are

full agonists (Huber et al, 2008). Antagonists have no effect on basal receptor activity, also known as 'zero efficacy', but block access of other ligands. Inverse agonists inhibit basal activity. Basal activity refers to the resting state interaction with G-protein.

In the absence of ligands, basal receptor activity is determined by the equilibrium between the inactive (R) and active states (R*). The efficacy of ligands is determined by its ability to alter this equilibrium. The changes in the ADRB2 receptor are best illustrated by agonist binding. An agonist binds to the transmembrane segment and induces conformation changes (Hoffmann et al, 2008). Our understanding of these conformation changes and the molecular mechanism of cellular response have been facilitated by the formation of engineered crystalline structures of human ADRB2 (Rasmussen et al, 2007). Agonist-induced conformation changes involve both extracellular and intracellular loops. However, the rate at which these conformational changes occur differs between full and partial agonists (Ghanouni et al, 2001). The conformation changes induce internalization of the receptor and signal transduction via activation of α subunit heterotrimeric G protein (Gs) (Kobilka, 2007) that induces coupling with adenylate cyclase and guanosine triphosphate (GTP). Replacement of GTP with guanosine diphosphate (GDP) and catalysis by adenylate cyclase changes ATP to cAMP. Elevation of intracellular cAMP initiates receptor desensitization, which is characterised by phosphorylation, sequestration, and down-regulation of receptor number. Desensitization is an auto-regulatory process that prevents overstimulation. Phosphorylation of ADRB2 by the cAMP-independent kinase (βARK) or other related G protein-coupled receptor kinases (GRKs) results in β-arrestin binding and partial uncoupling of the agonist-occupied receptor from the Gs protein (Johnson, 1998; Fredholm et al, 2007). Sequestration takes place after prolonged agonist exposure (seconds to minutes); internalization of a portion of ADRB2 into the sub-cellular compartment makes the receptor unavailable for signal

transduction (Liggett, 1999). Longer agonist exposure (hours to days) decreases the total complement of ADRB2, which is a process known as receptor down-regulation.

1.6.3 Beta 2 adrenoreceptor gene (*ADRB2*)

Figure 1.4: Molecular and genetic structure of *ADRB2*
(Adapted from Johnson, 1998)

Human ADRB2 is encoded by an intronless gene of 2042 bp, located on the long arm of chromosome 5q31-32 (Kobilka et al, 1987). The protein is composed of 413 amino acids with a molecular mass of approximately 46,500 Daltons (Da). There are 12 single-nucleotide polymorphic sites in the coding region of *ADRB2*, but only 5 are non-synonymous. There are 2 non-synonymous SNPs at the N terminus; the 46A/G substitution generates an Arginine to Glycine (Arg16Gly) variant; a 79C/G substitution generates a Glutamine to Glutamate (Gln27Glu) variant. The remaining SNPs are located in the membrane-spanning domain:

100G/A (Val34Met), 491C/T (Thr164Ile) and 523C/A (Arg175Arg). Amino acid 164 is situated in the fourth transmembrane domain adjacent to Ser165, which is postulated to interact with β-OH of adrenergic ligands (Johnson, 1998). Based on the functional studies of ADRB2 *in vitro* and *in vivo*, only 46A/G, 79C/G, and 491C/T are thought to be important. Val34Met is rare and the SNP at 523 may be functionally significant based on its recent association with bronchodilator responsiveness in asthmatic patients (Silverman et al, 2003).

There are 8 additional SNPs within the 1.5 kb 5′-un-translated region (UTR), which is believed to contain the main transcriptional regulatory sequences for *ADRB2* ; -20 T/C, -47 T/C, -367 T/C, -468 C/G, -654 G/A, -1343 A/G, and -1429 T/A (Scott et al, 1999). In addition, -47T/C (Arg-19Cys), which is located within a short open reading frame also known as the Beta Upstream Peptide (BUP) or the 5′-leader cistron (LC), influences receptor expression at the translational level (Scott et al, 1999). The promoter region also contains a cAMP response element (CRE), 2 NFL-IL6 sites, 4 AP-2 sites, and a steroid-binding hexamer (Parola and Kobilka, 1994).

The impact of *ADRB2* polymorphisms on receptor expression, agonist and antagonist binding affinity, physical and functional coupling to Gs protein, receptor trafficking, and receptor regulation by agonists were studied *in vitro* and *in vivo* (Leineweber and Brodde, 2004). Polymorphic *ADRB2* had no effect on ligands binding or adenylyl cyclase activity in specialized COS-7 and Chinese hamster fibroblast (CHW) cells (Green et al, 1993). Moreover, Arg16Gly and Gln27Glu do not alter receptor function in human lung mast cells (HLM), human airway smooth muscle cells (HASM), and human lymphocytes (Leineweber et al, 2004). *Ex vivo* findings further reaffirmed that 46A/G, 79C/G, and 491C/T do not affect ADRB2 activity (Moore et al, 2000; Bruck et al, 2003a). This finding was also reflected on *in*

vivo cardiac and vasodilatory responses; there was no difference in heart rate and contractility between wild-type and variant (46A/G and 79C/G) ADRB2 (Bruck et al, 2003b). Based on the evidences, *ADRB2* polymorphisms may not alter the receptor function.

However, ADRB2 polymorphisms are important in agonist-induced receptor desensitization and down-regulation. Studies on transfected CHW cells and HASM with ADRB2 polymorphisms have shown that 46G (Gly16) enhanced agonist-promoted down-regulation in comparison to wild type (Green et al, 1994; Green et al, 1995). In contrast, 79G (Glu27) appears to protect against down-regulation. Based on the number of receptors and β_2-agonist-mediated cAMP formation in cultured HASM, 79G (Glu27) down-regulated to a much lesser extent than 79C (Gln27) (Green et al, 1994; Moore et al, 2000). The 79G (Glu27) variant demonstrated strong resistance towards agonist-promoted down-regulation. In a site-directed mutagenesis study, 46G79G (Gly16Glu27) demonstrated a 46G (Gly16)-dominant phenotype, but 46A79G (Arg16Glu27) was completely resistant to down-regulation (Green et al, 1994, Chong et al, 2000). However, 46A79G (Arg16Glu27) is extremely rare in the general population.

491T (Ile164) in CHW cells exhibited extensive signalling defects due to reduce agonist affinity (Green et al, 1993). Moreover, reduction of basal and agonist-induced activation of adenylyl cyclase and a right-shift of the agonist concentration-effect curve exhibited by 491T suggested impairment of receptor-G-protein interaction (Green et al, 1993). Thus, 491T is believed to play an important role in adenylyl cyclase coupling and G-protein interaction *in vivo*. There is also evidence to suggest that 491T induces a conformational alteration and decreases agonist activation. Agonist-competition binding studies found that 491T does not display high-affinity agonist binding in the absence of guanine nucleotides (Green et al,

1993). Transgenic mice expressing 491T in cardiomyocytes demonstrated lower basal and isoprenaline-stimulated adenylyl cyclase activity in comparison to 491C (Turki et al, 1996). Similarly, the 491C/T polymorphism demonstrated reduced receptor signalling in HLM, human adipose tissue, and lymphocytes in 3 cystic fibrosis patients (Büscher et al, 2000; Kay et al, 2003; Hoffstedt et al, 2001).

ADRB2 expression in COS-7 cells transfected with wild-type ADRB2 and a -47C polymorphic variant was determined by radio-ligands binding (McGraw et al, 1998). Quantitative ribonuclease protection assays showed that the wild-type and -47C variant mRNA levels were the same. The -47C (-19Cys 5′ LC) variant construct yielded a higher ADRB2 expression level. In HASM, the -47C yielded approximately 2-fold higher ADRB2 expression in comparison to -47T (Ligget, 1999). Thus, *in vitro*, *in vivo*, and *ex vivo* studies have shown that ADRB2 polymorphisms affect the expression, coupling, and desensitization of the ADRB2 receptor.

Some of these SNPs are in linkage disequilibrium (Scott et al, 1999; McGraw et al, 1998; Drysdale et al, 2000). Thus, subjects homozygous for 79CC (Glu27) are nearly always homozygous for 46GG (Gly16); the 46A79G (Arg16Glu27) haplotypes is extremely rare, occurring in less than 1% of the population. There is also tight linkage disequilibrium between 46G79G (Gly16Glu27) and -47T (Arg-19). Only 12 haplotypes have been detected out of 8192 statistically possible haplotypes of *ADRB2* (Drysdale et al, 2000) and a majority are uncommon. The 4 major haplotypes are -47T46G79G491T (Arg-19Gly16Glu27Thr164), -47C46A79C491T (Cys-19Arg16Gln27Thr164), and -47C46G79C491T (Cys-19Gly16Gln27Thr164). Linkage disequilibrium is the major confounding factor in the inconsistency of the effect of *ADRB2* SNPs in *vivo* and *in vitro* studies. Tantalizing results

from *in vitro* studies and the strong desensitization effect of ADRB2 in *in vivo* studies may not be easily translated into clinical application. To complicate matters further, the allele frequencies of reported *ADRB2* SNPs differ between populations (Table 1.5).

Table 1.5: Allele frequencies and phenotypes of *ADRB2* polymorphisms

Codon	Amino Acid	SNP	Caucasians	African	Asian	Latino	Phenotype
-20		T/C	NA	NA	NA	NA	Increased expression
-47	-19	C/T					Increased expression
	Arg		65.0**	79.0**	92.0**	82.4#	
	Cys		35.0	21.0	8.0	17.6	
46	16	A/G					
	Arg16		45.7*	48.8*	58.7*	57.9#	Increased
	Gly16		54.3	51.2	41.3	42.1	desensitization
79	27	C/G					
	Gln27		65.2*	79.3*	92.8*	NA	Reduced desensitization
	Glu27		34.8	20.7	7.2		
491	164	C/T					
	Thr164		96.0[@]	98.0[@]	99.0[@]	97.0[@]	Reduced coupling
	Ile164		4.0	2.0	1.0	3.0	

*Xie et al, 1999; #Litonjua et al, 2004; **McGraw et al, 1998; @Small et al, 2003
NA: not available

ADRB2 receptors are expressed and have diverse functions in various tissues; thus, polymorphisms of ADRB2 are postulated to play a role in various diseases, particularly cardio-respiratory disorders. The ADRB2 gene has been extensively studied in asthma. However, the possible role of ADRB2 gene polymorphisms in asthma pathogenesis remains inconclusive. A majority of the findings suggested that ADRB2 polymorphisms, alone or as haplotypes, may not influence asthma susceptibility, severity, or pathogenesis in various populations (Munakata et al, 2006; Weir et al, 1998; Tsai et al, 2006). Perhaps the interplay of genetic and environmental risk factors has more influence than genetics alone in a complex

disease such as asthma. ADRB2 polymorphisms are likely to influence responsiveness to β-2 agonist drugs.

Based on the evidence that ADRB2-mediated vasodilation is involved in physiological blood pressure regulation, *ADRB2* is a candidate gene for hypertension. Vascular response to ADRB2 stimulation is impaired in hypertensive patients and *in vivo* studies have demonstrated that ADRB2 affects vasodilation (Brodde, 2007). The findings from association studies in various populations remain controversial (Xie et al, 2000; Lee et al, 2004; Brodde, 2008). However, based on a meta-analysis, ADRB2 polymorphisms are not significantly important in the aetiology of hypertension (Hahntow et al, 2006).

Although β_1-adrenoreceptor is the predominant adrenergic receptor in the heart, ADRB2-mediated positive inotropic and chronotropic effect to a certain extent. ADRB2 is anti-apoptotic in murine and rat cardiomyocytes, suggesting that ADRB2 polymorphisms could confer a protective effect in cardiac failure (Bruck et al, 2003). However, the promising role of ADRB2 polymorphisms in animal studies has failed to translate effectively into humans (Brodde, 2008). The potential role of 46A/G and 79C/G in predicting survival in patients with cardiac failure remains unclear.

1.7 Topical prostaglandin analogue compounds

For 25 years, topical timolol maleate has been widely accepted as the treatment of choice for glaucoma. It is undoubtedly efficacious in almost all types of glaucoma. A lack of intolerable side effects in comparison to topical non-selective sympathomimetics and mitotic further contributed to the popularity of topical timolol. The quest for more potent agents began in the early 1980s. During the frenzy of interest in prostaglandin as possible ocular anti-

inflammatory effects and potential therapeutic role, prostaglandin was infused into cannulated experimental animal eyes and was found to cause ocular hypertension with breakdown of the blood-aqueous barrier (Bito et al, 1989a). Accidentally, the ocular hypotensive effect was achieved with a low concentration of topical prostaglandin with breakdown of the blood-aqueous barrier even without cannulation. Naturally occurring prostaglandins are relatively polar, hydrophilic molecules that poorly cross biological membranes due to their carboxylic acid moiety and several hydroxyl groups. Prostaglandin effects differ between species (Bito et al, 1989b). Different prostanoids have different side effects on the human eye, consistent with the reported multiplicity and low selectivity of naturally occurring prostaglandin for different subtypes of prostanoids (Woodward et al, 1997).

Figure 1.5: Chemical structure of major topical prostaglandin analogue pro-drugs and their hydrolyzed free acids.
(Adapted from Bean and Camras, 2008)

There was a major setback in the first experiment with topical prostaglandin in human volunteers using a high concentration (200 µg) tromethamine salt form of $PGF_{2\alpha}$, which resulted in severe ocular hyperaemia, ocular pain, and headache (Giuffre, 1985). Lower concentrations (up to 100-fold) were found to potentiate better ocular hypotensive effects with esterification of the prostaglandin carboxylic acid group, which is the basis of the pro-drug principle (Kerstetter et al, 1988). Esterification of the carboxylic acid reduces polarity and facilitates penetration of the prodrug through biological lipid membranes. The prodrug is then converted to free acids to activate the specific FP receptors once it crosses the corneal epithelium in the specific direction known as orthrorectified transport or the slow release system, which is ideal for chronic therapy in glaucoma and minimizes unwanted ocular and systemic side effects (Figure 1.5).

After more than 2 decades in search of a topical prostaglandin with an acceptable therapeutic index, Unoprostone (13, 14-dihydro-15-keto metabolite of $PGF_{2\alpha}$) with the trade name of Rescula (Ciba Vision, Duluth GA) was made commercially available in Japan. The drug failed to gain popularity worldwide due to lack of efficacy and the requirement for twice-daily administration. In 1996, latanoprost (13, 14-dihydro-17-phenyl-18, 19, 20-trinor-$PGF_{2\alpha}$-isopropyl ester) was marketed as Xalatan (Pfizer Inc, New York) and gained approval worldwide. Later, travoprost (Travatan; Alcon) and bimatoprost (Lumigan; Allergan) were introduced. Unlike latanoprost and travoprost, bimatoprost has been controversially known as prostamide owing to the presence of a C1 ethyl amide group that activates different receptors. Topical prostaglandin analogues are believed to achieve pressure-lowering effects by increasing uveoscleral outflow without any effect on aqueous humour production (Toris et al, 1993). However, their effect on the trabecular meshwork remains unclear (Oh et al, 2006; Johnson et al, 2010). There was no statistically significant difference in efficacy between the

3 commercially available topical prostaglandin analogues but there was a reported borderline increased incidence of ocular hyperaemia with bimatoprost (van de Valk et al, 2005; Zimmerman et al, 2009). Bimatoprost has the edge in effectiveness but with slightly more pronounced side effects (Cracknell and Grierson, 2009).

Topical prostaglandin analogues have changed the paradigm of glaucoma management, most notably with the declining rate of glaucoma surgeries in the late 1990s (Bateman et al, 2002). Currently, due to their efficacy and better tolerability, topical prostaglandin analogues are replacing topical timolol as the first-line drug of choice in glaucoma management (Holmstrom et al, 2006). It is amazing that low lipid solubility drugs in low concentrations can achieve such an impact in glaucoma management.

1.7.1 Topical latanoprost 0.005%

Topical prostaglandin analogue PhXA34 (13, 14-dihydro-17-phenyl-18, 19, 20-trinor-$PGF_{2\alpha}$-isopropyl ester) known as latanoprost, differs from the naturally occurring $PGF_{2\alpha}$, where C18 to C20 have been substituted by a benzene ring, the double bond between C13 and C14 has been saturated, and the carboxylic acid moiety on C1 has been esterified with isopropanol (Figure 1.7). The molecular weight of latanoprost is 432.6 and the hydrolysed compound (free acid) is 390.5 (Stjernschantz and Alm, 1996). The octanol-water partition coefficient is 4.3 at pH 7.4, with poor solubility in water. It is available as a colourless to slightly yellow oil solution in 0.005% concentration (50µg/ mL) preserved with 0.02% benzalkonium chloride. It is commercially available in 5 mL plastic bottles (2.5 mL latanoprost solution), which requires refrigeration to maintain a temperature of 2 to 8°C for unopened bottles. Once opened, bottles can be stored safely at room temperature for a maximum of 6 weeks.

Latanoprost can be prescribed as either evening or morning once-daily dosing but evening dosing is more efficacious (Alm and Stjernschantz, 1995).

Latanoprost is a selective FP receptor agonist with marginal spillover effect on other prostanoid receptors, resulting in fewer unwanted side effects. Naturally occurring $PGF_{2\alpha}$ has greater affinity than latanoprost for the FP receptor but also interacts with other prostaglandin receptors, which is partly responsible for side effects such as iritis and conjunctival hyperaemia (Astin and Stjernschantz, 1997). As a prodrug, it is relatively inactive until the hydrolyzation of the isopropyl ester to free acid in the cornea and plasma. Latanoprost in free acid form is measurable in the aqueous humour within 4 hours of instillation. Approximately 1% of topically applied latanoprost is absorbed into the eye, the majority being absorbed into the systemic circulation through either conjunctiva vessels and nasal mucosa or gastrointestinal tract absorption. The peak concentration is reached about 2 hours after topical instillation with the distribution volume of 0.16 ± 0.02 L/kg in humans (Sjöquist and Stjernschantz, 2002). The half-life of free acid in human plasma is about 17 minutes. Plasma levels of latanoprost acid were below the detection limit in patients treated with latanoprost for a year. Latanoprost is metabolized by β-oxidation in the liver; it is not metabolized in the cornea and is mainly excreted in the urine (88–98%).

Although the exact mechanism of action of latanoprost is still uncertain, FP receptor plays essential role and FP receptor-deficient mice do not exhibit any pressure-lowering effect (Ota et al, 2005; Crowston et al, 2004). Aqueous humour production is not significantly affected by latanoprost but the most consistent finding is a substantial increase in uveoscleral (pressure-insensitive) outflow; a less consistent finding is the role in trabecular (pressure-sensitive) outflow capacity (Toris et al, 2008; Lim et al, 2008; Johnson et al, 2010). There are

3 potential mechanisms by which latanoprost could increase uveoscleral drainage. These include: (1) Remodelling of extracellular matrix of the ciliary muscle and sclera causing permeability changes, (2) widening of the connective tissue-filled spaces among the ciliary muscle bundles, which may be caused by relaxation of the ciliary muscle and (3) changes in the shape of ciliary muscle cells as a result of altered actin and vinculin localization (Toris et al, 2008; Lindsey et al, 1997). The remodelling of extracellular matrix is believed to be responsible for sustaining the long-term pressure-lowering effect (Johnson et al, 2010).

Ciliary muscle relaxation is believed to be responsible for the initial reduction of IOP but the effect is not prominent in latanoprost. Remodelling of the extracellular matrix within the ciliary muscle and sclera is the most thoroughly understood and most accepted mechanism. Latanoprost stimulates induction of Matrix Metalloproteinases (MMPs) 1, 2, and 3 that cause dissolution of collagen types I and III within the connective tissue-filled spaces between the outer longitudinal muscles (Lütjen-Drecoll and Tamm, 1988). The mRNA of MMP 1, 2, 3, 11, 12, 14, 15, 16, 17, 19 and 24 were identified in human ciliary body and smooth muscle (Oh et al, 2006). Activity of MMP is regulated by family of extracellular inhibitory proteins; tissue inhibitors of metalloproteinases (TIMPs). Each TIMP regulates specific MMPs. In human ciliary body, only TIMP-1, TIMP-2, TIMP-3 and TIMP-4 are found (Murphy and Docherty, 1992). Animal experimental studies showed evidence of changes in ciliary muscle; the tissue spaces of the ciliary muscle were enlarged and organized into tube-like spaces covered by endothelial-like cells with close basement membrane contact, and contained myelinated nerve fibre bundles that resembled a lymphatic system in the choroid (Krebs and Krebs, 1988; Richter et al, 2003).

Latanoprost induced MMP 3, 9, 17, and TIMP-3, and down-regulated MMP 1, 2, 12, 14, 15, 16, and TIMP-4 in human ciliary body (Oh et al, 2006). Latanoprost acid induced concentration-dependent increases in MMP 1, 3, and 9 gene transcriptions and a concentration- and time-dependent increase in TIMP-1 but not TIMP-2 mRNA and protein in human ciliary muscle (Anthony et al, 2002). In general, MMP 1, 2, 3 and 9 and TIMP -1 seem to play important role in remodelling of extracellular matrix.

Cyclooxygenase (COX)-2 is also believed to play a role in the pressure-lowering effect of latanoprost (Sales et al, 2008). The mechanism of latanoprost-induced MMP secretion is through protein kinase C and extracellular signal-regulated protein kinase 1/2-dependent pathways (Chen et al, 2001; Anthony et al, 2002). Mitogen-activated protein kinase and tumour necrosis factor α-dependent signalling pathways may also be involved (Yousufzai et al, 2000). The vasodilatation effect of latanoprost, although minimal, is also postulated to play a role in facilitating uveoscleral outflow. Although increases aqueous outflow through non-conventional pathways seems to be responsible for the pressure-lowering effect of latanoprost, there are ongoing studies providing evidence of the possible role of trabecular meshwork outflow (Oh et al, 2006).

In spite of uncertainty in the mechanism of latanoprost action, latanoprost is a clinically proven efficacious topical anti-glaucoma drug. Its effectiveness has been observed in many populations. Hedman and Larsson (2002), based on mean diurnal IOP reduction, found that latanoprost is more effective than timolol in 8 different populations with greater reduction among Mexican and Asian populations. A meta-analysis involving 1256 glaucoma patients found that latanoprost is superior to timolol in long-term IOP control (Zhang et al, 2001). Latanoprost has the advantage of achieving IOP reduction during both day and night while

timolol has a minimal effect on nocturnal IOP (Larsson et al, 2002). In a long-term study, latanoprost sustained meaningful IOP reduction (Hedman et al, 2002). Latanoprost is not only effective in OAG but also in angle-closure glaucoma (Chew et al, 2004). In spite of its higher therapeutic index, there was a reported 18–25% non-responder rate (Scherer, 2002; Cheong et al, 2008; Camras and Hedmann, 2003). The definition of a responder varies according the predetermined cut-off point. Based on the US latanoprost study group, a greater proportion of patients classified as non-responders on any particular visit were responders on all other visits if treated with latanoprost rather than timolol (Camras and Hedmann, 2003). Among PG analogues, bimatoprost seems to be slightly superior in reducing pressure but not without a price (van der Valk et al, 2005). Bimatoprost has a higher incidence of conjunctival hyperaemia.

Although $PGF_{2\alpha}$ is responsible for stimulating bronchial hyper-responsiveness, respiratory impairment induced by latanoprost has not been reported (Hedner et al, 1997). Short half-life and rapid clearance of the active latanoprost acid minimizes unwanted systemic side effects (Sjöquist and Stjernschantz, 2002). Furthermore, latanoprost free acids that enter the systemic circulation do not permeate tight-junction cell membrane barriers such as the blood-brain barrier, minimizing the potential for central nervous system side effects. However, some nonspecific systemic side effects such as headache, flulike syndromes, upper respiratory tract infections, and musculoskeletal pain have been reported (Alm et al, 1995).

Latanoprost-induced ocular side effects are a major concern. Conjunctival hyperaemia is a common side effect with the incidence range between 5 to 15% (Stewart et al, 2003; Walters et al, 2004). The incidence is much higher in travoprost and bimatoprost (Honrubia et al, 2009; Walters et al, 2004). Conjunctival hyperaemia is generally mild and transient, and

commonly develops within 1 month of therapy initiation. Vasodilation induced by prostaglandin promotes the release of nitric oxide that may be responsible for conjunctival hyperaemia (Alm et al, 2008). The saturated double bond in C13 and C14 of latanoprost is partly responsible (Resul and Stjernschantz, 1993). Ocular irritation, burning sensation, and dry eye are also reported (Stewart et al, 2003). However, the most intriguing side effect is the ability of latanoprost to induce pigmentation in the iris, eyelid, and eyelashes. Latanoprost-induced iris darkening (LIID) was found in higher frequency in heterogeneous hazel irises and homogeneous gray and blue irises are less likely to develop LIID in Caucasians (Alm et al, 2008). Japanese and South East Asians, in spite of having homogeneous dark brown irises, were more likely to develop LIID (Chiba et al, 2004; Chou et al, 2005). During phase III of a latanoprost study, latanoprost was postulated to have the ability to promote iris melanocyte proliferation (Stjernschantz et al, 2002). However, there was no evidence of increases melanogenesis in tissue culture studies (Kashiwagi et al, 2002; Drago et al, 1999). Histopathological and morphometric studies found evidence of increased iris melanocyte in the stroma and redistribution of the melanocyte to the anterior iris stroma without a net increase in number (Cracknell and Grierson, 2009; Cracknell et al, 2003; Albert et al, 2008).

LIID is irreversible and causes cosmetic concerns, especially when it occurs in one eye, but has no incapacitating visual side effects. Hyperpigmentation, elongation, and thickening of the eyelashes (hypertrichosis) may cause the lashes to touch the spectacles or cause difficulty in topical drug instillation but is never a major concern (Johnstone, 1997; Shaikh and Bodla, 2006). Unlike LIID, hypertrichosis is reversible and disappears several weeks after discontinuation of treatment. Peri-ocular hyperpigmentation is also reported and is most likely due to accidental spillover during drug administration (Herndon et al, 2003). There were also reported cases of hypo-pigmentation (Herndon et al, 2003).

Disruption of the blood-aqueous barrier and posterior lens release of inflammatory mediators causes cystoids macular oedema (CME) following latanoprost treatment (Miyake et al, 1999). Latanoprost-induced CME may cause visual impairment but the incidence is uncommon in comparison to the frequency of pigmentation-induced side effects (Warwar et al, 1998). Prostaglandin at higher concentrations acts as an inflammatory mediator and anterior uveitis was reported following latanoprost treatment (Warwar et al, 1998). Reactivation of herpes simplex keratitis has been reported in 3 patients with a history of herpes simplex infection (Wand et al, 1999). In patients with a high risk of CME, anterior uveitis, and past history of herpes simplex, latanoprost is not recommended for glaucoma treatment.

1.7.2 Prostanoid receptors

Prostaglandins are lipid-derived autacoids generated by sequential metabolism of arachidonic acid by cyclooxygenase (COX) and prostaglandin synthase. Arachidonic acid is released from the cell membrane by phospholipase A_2 and oxidised by COX to PGG_2 followed by reduction to unstable endoperoxide PGH_2. PGH_2 acts as a substrate for prostaglandin synthase enzymes, which through enzyme specificity (PGE synthase, PGF synthase, PGD synthase, PGI synthase and TX synthase) are responsible for producing 5 principal bioactive prostaglandins (prostanoid receptor sub-family); PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2 (prostacyclin), and TXA_2 (thromboxane). The COX enzyme exists as 2 major isozymes. COX-1 is expressed constitutively in most cells and is a major source of prostanoid for physiological function, while COX-2 expression is inducible in response to inflammatory cytokines, stress, tumour promoter, and stimuli such as bacterial lipopolysaccharide (LPS). Prostaglandins are ubiquitously produced and act locally in an autocrine or juxtacrine function to produce a diverse set of pharmacological effects modulating many physiological systems and

influencing a broad array of diseases including cancer, inflammation, cardiovascular disease, and hypertension.

The physiological, pharmacological, and pathological effects of prostaglandin are mediated in part by G-protein coupled prostanoid receptors. There are 8 well-known prostanoid receptors: DP, EP1, EP2, EP3, EP4, FP, IP (prostacyclin), and TP (thromboxane A_2). Recently, another prostaglandin receptor was discovered; the chemo-attractant receptor homologous molecule expressed on Th2 cells (CRTH2) binds to PGD_2. Although it is more closely related to chemo-attractant receptors, it is identified as the ninth prostaglandin receptor. Individual prostanoid receptors share around 20% to 30% sequence identity and encode specific motifs common only to members of the subfamily (Hata, 2004). As GPCR is a classical example, coupling with the heterotrimeric G protein-mediated signal transduction pathway is required and the biological effect depends on ligands affinity, receptor expression profile, differential coupling to signal transduction pathways, and cellular expression. The existence of multiple receptors coupling to different signal transduction pathways for a given prostaglandin allows for synergistic antagonism between prostanoid receptors. Moreover, the structural similarities between prostanoid receptors may lead to activation of more than one prostanoid subfamily receptor; these characteristics complicate our understanding of the mechanism of action of each receptor.

As a member of GPCR, the structure of all prostanoid receptors consists of 7 putative α-helical membrane-spanning domains with an extracellular amino terminus, an intracellular carboxyl terminus, 3 extracellular loops, and 3 intracellular loops. The intracellular loops contain potential phosphorylation sites, which mediate receptor desensitization and internalization. Potential glycosylation sites are often associated with the amino terminus of

G-protein-coupled receptors. In the prostanoid receptor family, 28 amino acid residues are conserved in the molecular structure. Eight of these 28 amino acids are shared by other GPCR, mainly believed to maintain structure and/or function of receptors in the same group. The conserved Asp in the second transmembrane domain in the prostanoid subfamily is believed to be responsible in coupling ligands binding to activation of G proteins. Two Cys residues in the first and second extracellular loops are also conserved and believed to form a disulfide bond critical for stabilization of the receptor and ligands-binding conformations. The arginine in the seventh transmembrane domain is conserved in all prostanoid receptors and is the proposed carboxyl-binding site. There are also several conserved motifs.

Phylogenetic analysis of prostanoid receptors shows that the receptors segregate into 2 branches; one branch contains the DP, IP, EP_2, and EP_4 and the second branch contains the EP_1, EP_3, FP, and TP receptors. Based on their signal transduction and action, the prostanoid receptors can be divided into 3 categories; relaxant receptors, contractile receptors, and inhibitory receptors. The relaxant receptors include IP, DP, EP_2, and EP_4, which induce smooth muscle relaxation mediated by elevated intracellular cAMP. The contractile receptors include TP, FP, and EP_1, which induce smooth muscle contraction mediated by calcium mobilization. EP_3 is the only inhibitory receptor that mediates decreases in cAMP and inhibits smooth muscle relaxation. Structure homology between receptors in each category is up to 50%.

In spite of molecular structural similarities, the human prostanoid receptors are not encoded near each other in the genome. The genes encoding the human EP_1, EP_3, EP_4, FP, IP, and TP receptors were mapped to chromosome bands 19p13.1, 1p13.2, 5p13.1, 1p13.1, 19p13.3, and 19p13.3 (Duncan, 1995). The loci of EP_3 and FP receptor genes are in close proximity,

suggesting their evolution by gene duplication. Classically, all known members of the prostanoid receptor family have a short untranslated exon 1, an exon 2 with a large coding region, and exon 3 (Ogawa, 1995). Identification of additional exons encoding carboxyl-terminal tails in some of the prostanoid receptors and alternative splicing of these exons creates various receptor isoforms. Most of these isoforms have almost identical ligand-binding specificities in each receptor family. However, isoforms of EP_3 and TP receptors were found to couple with different G proteins and mediated different signalling pathways (Namba, 1993; Hirata, 1996). Basal promoter motifs for transcription such as TATA and CCAAT boxes have been identified in the 5′ flanking region of EP_3 and EP_4 but these motifs are absent in TP, IP, and FP receptors. Other receptor-specific motifs are important for their ligand-binding and signalling pathways. For example, the human TP receptor gene contains an SP-1 binding site, AP-2 consensus sequences, a phorbol ester response element (TRE), acute-phase reactant regulatory elements (APRRE), a c-myc binding motif, and a glucocorticoid response element (Nüsing, 1993).

1.7.3 Prostaglandin $F_{2\alpha}$ receptor (FP)

Figure 1.6: Molecular structure of the human and bovine FP receptor.
(Adapted from Anderson et al, 2001)

$PGF2_{\alpha}$ was first identified in the secretory endometrium during the menstrual cycle (Abel and Baird, 1980). The effect of $PGF2_{\alpha}$ on the human reproductive system has been widely studied including during parturition, menstruation, and reproductive organ carcinoma (Myatt and Lye, 2004). Later, it was found to play a role in renal physiology, cardiac hypertrophy, and IOP regulation. FP receptor is expressed in the corpus luteum, renal cells, ocular tissues, and

ventricular myocytes. Since the identification of prostaglandin known as ‘irin’ in the anterior chamber, researchers have been interested in the role of prostaglandin in various ocular pathologies (Ambache, 1957).

Immuno-fluorescence labelling revealed abundant FP receptor proteins in human ciliary epithelium, the circular portion of the ciliary body, the stromal and smooth muscle of the iris, corneal epithelium, conjunctival epithelium, and retinal cell layers except for the Muller cells and retinal pigmented epithelium (Schötzer-Schrehardt et al 2002). FP receptor proteins are also detected in the trabecular meshwork but in smaller amounts. FP receptor transcripts are absent in vessels, corneal stroma, conjunctival stroma, sclera and corneal endothelium but FP receptor protein is detected in monkey ocular tissue (Ocklind et al, 1996). In contrast to the discrepancy between mRNA expression and FP receptor protein distribution in monkey ocular tissue, there is no such discrepancy in humans (Schötzer-Schrehardt et al 2002). FP receptors are widely distributed in human ocular tissue, suggesting a functional role for this prostanoid receptor type in the eye (Mukhopadhyay et al, 2001). The interest in FP receptor increased with the discovery of a topical prostaglandin agonist, a potent pressure-lowering drug.

FP receptors in the ocular tissue are structurally similar to those in the corpus luteum. The FP receptor protein is 40,060 Da in humans and 40,983 Da in bovines. Bovine and human FP receptors share 86% homology. There are 359 amino acids in the open reading frame of human FP receptor (Figure 1.4). FP receptor has similar 7 transmembrane loops with an extracellular amino terminus and intracellular carboxyl terminus as in other prostanoid and GPCR receptors. The proposed specific binding site of FP receptor is at Arg291 and His81. Arg291 is believed to form a Schiff base with the carboxyl group (Sakamoto et al, 1995).

His81, located at the second transmembrane domain, is in close proximity with Arg291 and is believed to act as a hydrogen bond donor. Asn4 and Asn19 are postulated N-glycosylation sites (Sakamoto et al, 1994; Abramovitz et al, 1994). Two serine or threonine (Ser-144 and Thr-148) in the second intracellular loop and 4 in the carboxyl terminus (Thr-148, Ser-337, Ser-341, and Thr-353) have been suggested as potential sites for phosphorylation by protein kinase C (PKC). Cysteine residues (Cys-108 and Cys-186) in the first and second extracellular loops are likely to form a disulfide bond to stabilize the FP receptor protein structure. Three proline residues (Pro-170, Pro-264, and Pro-301) in transmembrane domains IV, VI, and VII are likely to form kinks in the α-helices and may be essential in the formation of the ligands binding pocket (Figure 1.6).

A common key prostaglandin-binding site was identified at the C1 carboxylic acid and C15 hydroxyl (Figure 1.6). C1 (first carbon) carboxylic acid of $PGF_{2\alpha}$ appears to form a Schiff base with an arginine in the seventh transmembrane domain (Anderson et al, 2001). C15 (15^{th} carbon) hydroxyl is important in prostaglandin metabolism; enzymatic dehydrogenation of C15 by 15-hydroxy-prostaglandin dehydrogenase leads to inactivation of prostaglandin. Binding affinity of prostaglandin to FP receptor is reduced when the C15 hydroxyl is changed to 15-keto $PGF_{2\alpha}$ or 15-methyl $PGF_{2\alpha}$. Attaching a phenyl group between C17 and C20, as occurs with FP receptor agonists such as latanoprost and fluprostenol, results in longer half lives *in vivo*. C9 and C11 are believed to play a role in the binding specificity of a subfamily of prostaglandins. However, alteration of C11 produced little change in binding affinity. Changing the hydroxyl group to ketone on C9 confers partial specificity by interacting with distinct amino acids of specific prostaglandin receptors (Oien et al, 1975). Alteration of the double bond between C5 and C6 dramatically decreased FP receptor binding. Binding of $PGF_{2\alpha}$ to the FP receptor leads to numerous intracellular effects: activation of trimeric G

proteins $G\alpha_q$ and $G\alpha_{11}$, small G-protein Rho, phospholipase C, inositol triphosphate (IP_3) generation and elevation of intracellular calcium concentration, and activation of phospholipase D and mitogen-activated protein kinase (Abramovitz et al, 1994; Pierce et al, 1999; Gusovsky, 1991; Davis et al, 1987; Chen et al, 1998). Low affinity of cross-reaction between several prostanoid receptors and FP receptor has been observed.

Figure 1.7: Chemical structure of $PGF_{2\alpha}$
(Adapted from Anderson et al, 20001)

1.7.4 FP receptor gene (*PTGFR*)

Human FP receptor is encoded by the $PGF_{2\alpha}$ gene known as *PTGFR*. It is located in the short arm of chromosome 1 (13.1) in close proximity to the GIPC2 gene (Betz et al, 1999). *PTGFR* consists of 4 exons and 3 introns spanning 43.3 kb (Vielhauer et al, 2004). The first exon is relatively short (165 bp) and comprises most of the 5′-untranslated region (5′-UTR). Intron 1 is approximately 1.3 kb in size and may contain part of the promoter region. The second exon (870 bp) consists of approximately 70 bp of untranslated region, encodes the remaining 5′-UTR, and the rest of the second exon is translated. The translated region continues up to Leu266 near the end of transmembrane VI. However, it is interrupted by the large second intron (4.3 kb) and the third intron (38.5 kb). A small third exon is approximately 70 bp in

size. The fourth exon is quite large, spanning 3344 bp, but only a small fragment is translated and the rest is the 3′-untranslated region (3′-UTR).

Figure 1.8: Human *PTGFR* based on Ensembl and Havana databases (http://www.ensembl.org/)

The promoter region of human *PTGFR*, spanning around 4106 bp including 2436 bp upstream from the ATG of exon 1, exon 1, intron 1, and part of exon 2, lacks canonical TATA- and CAAT-boxes (Zaragoza et al, 2004). However, there are 2 SP-1/GC elements located within 100 bp of the transcription start site at positions -114 and -86. It is believed that SP-1 functions as a tethering moiety to recruit the general transcription machinery to TATA-less promoters. Several GATA and AP-1 sites are found throughout the human FP promoter. There is also a STAT site at position -950 and a cAMP response element (CRE) at position -2124. There are also possible repressor and enhancer regions, which may be important in physiological regulation.

Unlike the EP receptor, there is no reported FP receptor subtype. However, 2 alternative spliced isoforms of FP receptors, designated as FPA and FPB, were cloned from human corpus luteum, placenta, uterus, heart, and ocular tissue (Pierce et al, 1996; Vielhauer et al, 2004). The isoforms have almost identical structures except for the carboxyl tail. FPA has another 46 amino acids beyond the 9 shared residues, while FPB terminates after only 1 amino acid (Pierce et al, 1996). The FPB isoform is produced by splicing out a putative 3.2 kb intron sequence that is retained in the FPA isoform. Although the 2 isoforms have indistinguishable radio-ligand binding activity, they differ in functional coupling to phosphatidylinositol hydrolysis.

Genetic variation of *PTGFR* is not as widely studied as it is in *ADRB2*. Loss of heterozygosity (LOH) at chromosome 1p31.1, where *PTGFR* is located, was associated with sporadic breast cancer. *PTGFR* is therefore implicated as a possible candidate gene for breast cancer (Sossey-Alaoui et al, 2001). Dystocia is a condition associated with prolonged or dysfunctional labour and cephalopelvic disproportion. There is an association of *PTGFR* with dystocia in animals but not in humans (Algovik et al, 1999). Recently, FP receptor was found to be essential in IOP regulation. A study on PTGFR knockout mice found that FP receptor signalling is important in IOP reduction by latanoprost treatment (Crowston et al, 2004). Based on the response of healthy volunteers to short-term latanoprost treatment, *PTGFR* gene polymorphisms have a possible role in the pressure-lowering effect of latanoprost in humans (Sakurai et al, 2007). However, the role of *PTGFR* in glaucoma has not yet been evaluated.

1.8 Pharmacogenetics

Individual variation in drug response poses a significant clinical problem, ranging from failure to respond to a drug to life threatening adverse drug reactions. The causative factors are probably genetic, physiological, pathophysiological, and environmental. Genetic factors are likely to play an important role in controlling drug absorption, distribution, metabolism, and drug-drug interactions. However, the influence of genetic mechanisms is almost certainly interrelated with the action of other factors. It is estimated that genetics is responsible for 15–30% of variation in drug responsiveness (Evan and McLeod, 2003). However, in certain drugs, genetic factors could contribute up to 95% of variation. The study of pharmacogenetics aims to understand how genetic variations contribute to variations in response to medicines. The interest in pharmacogenetics began in the 1950s, strengthened by family and twin studies in the 1960s and 70s, extended by biochemical studies in the 1970s, and further escalated by molecular studies in the 1980s (Evans and Relling, 2004).

Genetic variations may influence drug action by affecting its pharmacokinetics, which includes absorption, distribution, metabolism, and excretion, or its pharmacodynamic properties (what the drug does to the body), which involves target receptors, enzyme targets, and disease modifiers. Genetic polymorphisms are naturally occurring variations that may not cause disease but are responsible in altering the products they encode and have a reported frequency of more than 1% of the population (Ford, 1940). The variations in all genes are believed to cause different individuals or populations to express different forms of protein gene products, including those responsible for metabolizing the drug or the site of drug action. Genes encoding drug transporters were also identified as potential factors causing alteration in drug response (Evans and McLeod, 2003).

The variation of drug response can be divided into Gaussian variation and monogenic (all-or-none) variation. The initial understanding of pharmacogenetics is based on monogenic variation; the impact of a single gene product may lead to all-or-none responsiveness (Kalow, 1997). Gaussian variation is a mathematically calculated variation in the form of median effective or lethal dose of a drug (ED_{50} or LD_{50}) and determined mainly by environmental factors but with hereditary elements (Vesell, 1992; Trevan, 1927). The principle is based on a distribution curve of the frequency of response to a standard drug dose in a group of individuals. A majority of the known drugs demonstrate a unimodal distribution similar to a bell-shaped or Gaussian curve (Turner et al, 2001). Gaussian distribution represents the effect of multifactorial determinants by interaction of genetic and environmental factors without any single factor having a discernibly large effect on the response. Thus, it is more difficult to identify the effects of individual genes. Bimodal distribution is due to separate subpopulations with distinctly different drug responses suggesting that a single factor, possibly segregation of alleles at a single genetic locus, has a large effect on drug response (Murphy, 1964). Responders and non-responders to a certain drug may be represented as a bimodal distribution curve (McLaren and Moroi, 2003).

Pharmacogenetics is potentially important in customizing or personalizing medication. Tailoring the medication according to the predicted response, minimizing the side effects, and maximizing the expected drug response is ideal to promote compliance and persistency of medication especially in chronic diseases. ‘Candidate gene’ or ‘candidate pathway’ approaches have been adopted to predict the disposition or response to a given drug. So far, polymorphisms are the most studied genetic variations. Polymorphisms can be homozygous or heterozygous, depending on how many copies of a variant or wild-type allele are present. Based on the balanced polymorphism concept, a double dose of a variant allele (homozygous

mutant) may exert a detrimental effect but a single copy may increase fitness (heterozygous mutant) (Ford, 1940). The best example is in sickle cell disease; homozygous individuals have a poor survival rate and die at a young age due to disease-related complications but heterozygous individuals are better able to survive malaria (Kalow, 1997).

The impact of polymorphisms of cytochrome P450 (CYP) enzymes and thiopurine methyltransferase (TPMT) are the most established and well studied (Idle and Smith, 1979). Cytochrome P450s are a multi-gene family of enzymes found predominantly in the liver, the most important site for metabolic elimination of most drugs. Cytochrome P450 CYP2D6 (known as debrisoquine hydroxylase), CYP2C9, and CYP2C19 are among the most studied cytochrome P450s and affect the metabolism of 20–30% of clinically used drugs (Kirchheiner et al, 2004; Kirchheiner and Brockmoller, 2005).

Polymorphisms in CYP2D6 result in different metabolic capacities for antidepressants, anti-hypertensives such as β-blockers, and antipsychotic drugs. Some mutations in CYP2D6 result in complete loss of enzyme activity and severely compromises drug metabolism; this is known as the 'poor metaboliser (PMs)' phenotype. Other mutations or duplications of CYP2D6 produce increased metabolic capacity; individuals with such variation are known as ultra-rapid metabolisers (UMs). Those with wild-type levels of activity are known as extensive metabolisers (EMs). PMs require low doses of a drug or higher doses if it is a prodrug, while UMs and EMs require higher doses or a more frequent dose administration regime. An individual can be a PM of one drug and EM of another. There is evidence of racial influence in phenotypic of CYP2D6. It is believed due to the effect of selective breeding rather than direct racial influence. CYP2D6 PMs were found in 6–10% of

Caucasians, fewer in African populations (5%), and even fewer in Asians (less than 1%) (Kalow, 1991; Marez et al, 1997; Masimirembwa et al, 1993).

Another important example is the 'isoniazid acetylation polymorphism', which was observed in tuberculosis patients treated with isoniazid (Bönicke and Reif, 1953). A high incidence of peripheral neuropathy was caused by slow clearance of isoniazid compound and patients were phenotypic as slow or rapid acetylators (Evans, 1989). Based on family studies, the slow acetylator phenotype was found to be inherited as an autosomal recessive trait (Grant, 1993). Slow acetylators of isoniazid were homozygous for N-acetyltransferase functional gene (*NAT1* and *NAT2)*. Rapid acetylators were either homozygous or heterozygous for the wild type gene (Grant, 1993). Genetic polymorphism of thiopurine methyltransferase (TPMT), which is responsible for metabolism of anti-tumour agent 6-mercaptopurine and 6-thioguanine, is associated with difficulty in achieving an effective dose in childhood leukaemia.

However, the concept of single gene effects are now somewhat outdated in the pharmacogenetics field. The drug-response phenotype is typically not governed by a single gene (monogenic trait) but by multiple genes (polygenic) that has spawned the term 'pharmacogenomics'. The effects of most drugs are determined by many proteins and composite genetic polymorphisms in multiple genes coupled with non genetics factors are postulated to be responsible in drug response. For example; serotonin ($5\text{-}HT_3$) antagonist tropisetrone, a CYP2D6 substrate if given to patient with high enzyme activity due to gene duplication will not achieve effective drug concentrations. Inability to achieve effective drug concentration is not entirely due to the CYP2D6 polymorphism but may be due to other factors influencing the entire pathway before reaching the target organ or tissue. As $5\text{-}HT_3$

antagonist is also a p-glycoprotein (p-gp) substrate, the level of p-gp expression will affect ability of HT_3 antagonist to transfer from blood to the brain. Once the drug reach the 5-HT_3 receptor, the magnitude of response will depend on the drug concentration, neurotransmitter concentration in the synaptic cleft and genetic polymorphisms of the receptor. Moreover, serotonin concentration is further influenced by proteins involved in biosynthesis, transport and catabolism. Thus, the pharmacogenetics analysis of poor response to 5-HT_3 antagonist should include all of these candidate genes that involved in the pathway of this drug before reaching the target tissue.

1.8.1 Pharmacogenetics of topical pressure lowering medications

Pharmacogenetics studies have been conducted with various systemic drugs but minimal emphasis has been given to topical ophthalmic drugs. The initial observation was on topical phenylephrine 4% and homatropine 4%: longer duration was needed to achieve full mydriasis in dark-skinned Africans than in Caucasians (Emiru, 1971). Variation of response to topical anti-glaucoma drugs has been observed in various populations. Higher concentrations of topical timolol are required in African-Americans to achieve the same pressure-lowering effect seen in Caucasians at lower concentrations (Katz and Berger, 1979). Iris pigmentation was implicated in the difference of response (Otalegu and Ajayi, 1999). Latanoprost has a more significant effect in Latinos and Asians than in other populations (Hedmann and Larsson, 2002). The influence of ethnicity was also implicated in the variation of response (Matthew, 1995).

The term ‘ethnicity’ and ‘race’ is rather complex, overlapping and often confusing. Debate on the concepts and terminology on ethnicity and race in health research is still on-going (Bhopal and Rankin, 1999). The concept of ‘race’ as describing genetically different human

population is scientifically weak (Bhopal, 1998). It is deemed inappropriate and outdated. 'Race' is believed to provide social origins rather than its biological basis. 'Ethnicity' is used to describe a social grouping of people with similar culture and belief including language, religion, diet, marital customs and other factors related ancestry (Senior and Bhopal, 1994; Bhopal, 1997). Thus, ethnicity is more suitable to describe the prevalence of disease and susceptibility to certain disease in health research (Bhopal and Rankin, 1999).

The main question was 'does genetics govern the response to topical pressure lowering medication in glaucoma patients?' Several pharmacogenetics studies of common topical pressure lowering drugs have been conducted in several populations. Genotyping of 210 glaucoma patients for *ADRB1*, *ADRB2*, *ADRB3*, and *CYP2D6* in the Marshfield Clinic Personalized Medicine Research Project found that Gln27Glu of *ADRB2* was associated with meaningful pressure-lowering effectiveness of topical timolol (McCarty et al, 2008). CYP2D6 polymorphisms have no influence on the effectiveness of topical β-blocker (McCarty et al, 2008). Polymorphisms of *PTGFR* in the promoter and exon 1 were associated with response to latanoprost in normal volunteers (Sakurai et al, 2008). However, the study was conducted on normal volunteers and the response to latanoprost may differ in glaucoma patients. Furthermore, responsiveness was defined as a predetermined IOP percentage cut-off point; the study results, therefore, may not represent the actual clinical scenario and are subject to statistical manipulation. In addition, the presence and frequency of genetic polymorphisms tend to differ between populations.

Can the *ADRB2* and *PTGFR* polymorphisms reported in the previous studies be replicated in Malaysian population? Does the pressure lowering effect of topical Timolol XE 0.5% and latanoprost 0.005% in Malaysian population differ from other reported studies? Another

equally important question was whether *ADRB2* and *PTGFR* polymorphisms play a role in susceptibility to glaucoma in Malaysian population? A prospective observational cohort with 12 months follow-up period was conducted. Blood was taken for genetic screening of *ADRB2* and *PTGFR*. The main objective of this research project was to determine the association of *ADRB2* and responsiveness of Malaysian glaucoma patients to topical timolol XE 0.5% and the association of *PTGFR* and responsiveness of glaucoma patients to topical latanoprost 0.005%. In addition, we aimed to determine the possible association of *ADRB2* and *PTGFR* in glaucoma susceptibility in Malaysians.

CHAPTER 2

Pressure lowering effect of topical timolol and latanoprost

Chapter 2

2.1 Objectives

To determine pressure lowering effect of topical timolol XE 0.5% and topical latanoprost 0.005% among open angle glaucoma patients in Malaysia

2.2 Material and methods

2.2.1 Patients selection

A prospective observational cohort study was conducted involving newly diagnosed primary open angle glaucoma (POAG), normal tension glaucoma (NTG) and ocular hypertension (OHT) patients seen in the eye clinic of two main hospitals in Kelantan, Malaysia. Hospital Universiti Sains Malaysia and Hospital Raja Perempuan Zainab II provide tertiary eye care in Kelantan state of Malaysia. Ethical approval was obtained from Research and Ethical Committee Universiti Sains Malaysia, ethical committee of Hospital Raja Perempuan Zainab II and London School of Hygiene and Tropical Medicine. Written consent was obtained from all recruited subjects.

Recruitment of subjects was conducted between May 2007 and August 2008. The selected subjects were patients diagnosed with POAG and NTG were prescribed with topical antiglaucoma treatment as first line management. Any potential participants with a history of intraocular surgery, especially glaucoma filtrating surgery, were excluded. The diagnosis was based on visual field assessment, vertical cup to disc ratio, angle structure morphology and intraocular pressure measurement. Visual field assessment was based on two consecutive, reliable and reproducible tests using a Humphrey Visual Field Analyzer 30-2 programme. Visual field test was repeated on a subsequent visit if it was not reliable on the initial visit.

Reliability of visual field assessment was based on false negative and false positive of less than 33% and less than 50% of fixation loss. Glaucomatous field loss was defined as a Glaucoma Hemifield Test graded outside normal limits and a cluster of three contiguous depressed points at the 5% level on pattern deviation plot (Foster et al, 2000). Defects crossing the horizontal midline and those respecting the vertical midline (i.e. with a "neurological" pattern), and those consistent with another ocular (e.g. retinitis pigmentosa or extensive laser marks) or systemic disorder that could explain the visual field defect were considered non-glaucomatous, and were excluded.

Goldmann two-mirror gonioscopic with contact fluid (Vislube, USA) was conducted to assess the angle morphology. Modified Shaffer angle classification was used to describe the status of the angle. Exclusion criteria include the presence of any signs that may indicate appositional contact between the peripheral iris and posterior trabecular meshwork, abnormal vessels, presence of pseudoexfoliation material or pigmentation, abnormal insertion of the iris and peripheral anterior synechiae. Slitlamp biomicroscopy examination (Haag-Straig, Germany) was then performed to evaluate the status of the anterior segment and to exclude signs of secondary glaucoma. For example the presence of pseudoexfoliation material, pigment deposition, iris atrophy was among the criteria for exclusion.

Optic nerve head assessment was conducted through dilated pupil with the aid of Volk 90 dioptre lens (Volk, USA). Vertical cup to disc ratio (VCDR) of more or equal to 0.7 was selected as the cut off point for diagnosis of glaucoma. The cut off point of VCDR was adopted from other studies based on 97.5^{th} percentile VCDR distribution in various Asian populations (Foster et al, 1996; Foster et al, 2000). Other optic nerve head signs related to glaucoma including disc haemorrhage, presence of peripapillary atrophy, the characteristic of

the vessel entering the disc, neuroretinal rim status, baring of lamina cribrosa and also other retinal changes such as vein occlusion, diabetic retinopathy changes were also sought. Any patient with suspicious characteristics of the optic disc that is not clearly associated with glaucoma was excluded. Posterior segment photograph (Kowa, Japan) was also obtained for baseline documentation.

Goldmann applanation tonometry (GAT) measurement was conducted between 8 am to 12pm in clinic setting. Hourly IOP phasing was conducted between 8 am to 5pm for confirmation diagnosis of normal tension glaucoma (NTG). IOP was measured in sitting position. Topical anaesthesia Benoxinate 0.4% (Novartis, Switzerland) was applied prior to the procedure. The corneal was then stained with fluorescein using fluorescein strip (Chauvin Pharmaceutical, England). IOP was taken with gentle or no pressure on the upper eyelid. Baseline IOP was defined as IOP taken for the first time by primary investigator (LS) either the highest recorded IOP during office phasing, or prior to commencing of glaucoma medication.

The diagnosis of glaucoma was based on the criteria fulfilling the category 1 described by Foster et al (2000). Category 1 is defined as VCDR or asymmetry VCDR ≥0.7 or the neuroretinal rim width, or asymmetry ≥0.2, combined with definite evidence of a glaucomatous visual field defect. In cases with advanced glaucoma with VCDR ≥0.85 in whom reliable visual field testing was not possible, diagnosis was based on structural changes only. NTG was defined as evidence of glaucomatous optic neuropathy with median of 10 IOP reading (based on hourly phasing) ≤21mmHg and none of the IOP reading exceeded 24mmHg (Collaborative Normal Tension Glaucoma Study, 1998). In cases when both eyes were eligible, only the right eye was selected for analysis.

Central corneal thickness (CCT) was also measured in all participants using non contact specular microscopy (Specular Microscopy 2000P, Japan). Anterior segment photograph with special focus on the conjunctiva, iris and eyelashes was also taken as baseline data before instillation of topical latanoprost 0.005%. Systemic co-morbidities such as hypertension, diabetes mellitus, hyperlipidemia, cardiovascular disease and cerebrovascular accident were also documented based on medical record review. If the recruited patient was treated outside the hospital, the local general practitioner who was treating the recruited patient was contacted to ascertain the diagnoses of systemic illnesses. Hypertension was defined as systolic blood pressure of ≥140mmHg and diastolic blood pressure≥ 90mmHg in subjects more than 50 years old (Chobanian et al, 2003). In the presence of type 1 or type 2 diabetes mellitus and renal disease, hypertension was defined as blood pressure of 130/80mmHg. Diabetes mellitus was defined as fasting blood sugar more or equal to 7.0mmol and 2 hours plasma glucose level post oral glucose tolerance test of 11.1mmol (WHO, 1999). Hyperlipidemia was defined as elevated fasting serum cholesterol and/or triglyceride level with or without the levels of HLDL and LDL. Fasting serum cholesterol of more than 5.2mmol and triglyceride of more than 2.0mmol was considered elevated, which was obtained retrospectively from the initial diagnosis of hyperlipidemia prior to initiation of lipid lowering medication. All systemic medications prescribed to the subjects at the time of recruitment were also documented.

In view of the recognised variation in genetic polymorphisms attributable to ethnicity, eligible subjects were asked to confirm a negative history of any possible interracial marriage for at least three generations. A pedigree chart was also drawn to help rule out any possibility of family history of glaucoma as well as to rule out the possibility of consanguineous marriages. Subjects with a medical contraindication for beta-blocker treatment such as

respiratory and cardiac disorders were excluded from the treatment with topical timolol. The possible systemic side effects following topical timolol may aggravate an existing systemic disorder that may affect compliance or cause discontinuation of the topical timolol. Similarly, those who had already experienced allergic reactions to topical timolol or latanoprost were also excluded. The recruited subjects and family members were given a full explanation of the study purpose and duration, prior to being asked to consent for this research.

2.2.2 Initiation of treatment

After the confirmation of diagnosis by the primary investigator (LS), the selected patients were assigned to either monotherapy with topical timolol XE 0.5% once in the morning (Project B) or topical latanoprost 0.005% at night (Project P). Patients who were already on topical timolol XE 0.5% monotherapy but failed to achieve target IOP, or if there was evidence of progression were also recruited and assigned to Project P for adjunctive treatment with topical latanoprost. There was no randomization of treatment as the comparison of the effectiveness between the two drugs was not part of the objective of this study.

Detailed explanation of the mode of action of the treatment, possible side effects and adverse effects, risks and benefits of the study was given to the subjects and their family members. Proper storage of the drug was also explained. The importance of compliance was also emphasized. A demonstration of proper drop instillation technique was also given by the primary investigator (LS) and a nurse. Double DOT technique was applied in this study, which includes punctual occlusion technique (Digital Occlusion Technique) and eyelid closure for at least 3 minutes (Do not Open Technique). The subjects who were planned to be treated with topical latanoprost as an adjunctive therapy were advised to wait for at least 5 minutes in between topical instillation. If any of the recruited patients expressed

unwillingness or inability to self medication, the family members were asked to appoint at least 2 relatives responsible for the instillation of topical drug. Subsequent clinic appointment was then given if the appointed member of the family was not present during the briefing. All patients were also reminded to bring along all the empty bottles during each visit.

2.2.3 Follow up visits

2.2.3.1 Visit 1

All recruited patients were scheduled for visit 1 at 1 month after commencement of treatment. The empty bottles were weighted to ensure adherence. Recruited patients were then questioned regarding their adherence to treatment and the presence of symptoms suggestive of side effects. Subjects were allowed to terminate their participation if they developed intolerable ocular or systemic side effects. They were also allowed to terminate their participation in this research project at anytime for whatever reason without affecting their clinical management.

Anterior segment photographs were taken to document the changes in the eyelashes, injected conjunctiva and colour of the iris on subjects in Project P. The assessment of the conjunctival, eyelashes and latanoprost induced iris pigmentation was done by two optometrists (BH and AS). The assessment was based entirely on printed anterior segment photographs and was conducted at different times by the two independent assessors. A third assessor (WJ) was also included in cases when there was conflicting assessment outcome.

Slitlamp biomicroscopy (Haag Strait, Germany) examination was conducted to rule out any possible inflammation associated with topical latanoprost as well as for detection and grading of conjunctival hyperaemia. Serial photographs were taken for most patients. Conjunctival

hyperaemia was graded 0 to 4 (Stewart et al, 2003) (Table 2.1). The evaluation was conducted in appropriate brightness. An optometrist (WJ) who was masked to the diagnosis and treatment protocol was responsible in the grading of the conjunctival hyperaemia. The grading was based on the average score of primary investigator (LS) and the optometrist (WJ).

Table 2.1: Grading of conjunctival hyperaemia induced by topical latanoprost*

Grade	Classification	Clinical Signs
0	None	No visible vessel dilation
1	Minimal	Barely noticeable regional vessel dilatation
2	Mild	Fairly obvious diffuse vessel dilatation giving light pinkish hue
3	Moderate	Obvious diffuse vessel dilatation giving moderate pinkish hue
4	Severe	Very obvious diffuse vessel dilation giving deep pinkish hue

*Based on the grading by Stewart et al (2003).

Target IOP was calculated and customised according to individual subjects based on severity or staging of glaucoma, type of glaucoma, age, life expectancy and the presence of other risk factors. IOP was measured between 9 am to 12 pm in sitting position by primary investigator (LS). However, in cases where the pressure was still elevated and failed to reach target IOP, other medication was either added or switched or a decision was made to proceed to filtrating surgery. Once there was alteration in management protocol, the patient was automatically classified as poor responder and further follow up according to the study protocol was discontinued. The subject with good IOP control was prescribed with the respective topical antiglaucoma drugs for another 2 months and further appointment was given for Visit 2.

2.2.3.2 Subsequent visits

The subsequent visits were scheduled at 3 months (Visit 2), 6 months (Visit 3) and 12 months (Visit 4) post commencement of treatment. Similar routine examination and documentation of the parameters was conducted during each visit. Humphrey visual field 30-2 test was conducted at 6 months and 12 months post commencement of treatment. Whenever the subjects failed to achieve target IOP or demonstrated visual field progression, they were discontinued from the treatment protocol and scheduled for routine eye clinic glaucoma follow up. However, the available clinical data (up to the date of discontinuation) was kept for phenotype and genotype association analysis.

At Visit 4, the mean IOP reduction was calculated based on:

$$\text{Mean IOP reduction} = \text{Baseline IOP} - \frac{[\text{summation of IOP at subsequent visits}]}{\text{Number of visits}}$$

For example:

$$\text{Mean IOP reduction (Subject 1)} = \text{Baseline IOP} - \frac{[\text{IOP at Visit 1+Visit 2+Visit 3+ Visit 4}]}{4}$$

$$\text{Mean IOP reduction (Subject 2)*} = \text{Baseline IOP} - \frac{[\text{IOP at Visit 1 + Visit 2}]}{2}$$

*Subject 2 was discontinued at Visit 2 due to poor IOP control

Due to the different in the effectiveness between the topical timolol and latanoprost in Malaysian population, two different cut off points were selected. The cut off point of percentage of IOP reduction was selected based on the mean percentage of IOP reduction of each medication. The patients were then categorised as:

Project B:

Good responder: percentage of IOP reduction more or equal to 20% from baseline

Poor responder: percentage of IOP reduction less than 20% from baseline

Project P:

Good responder: percentage of IOP reduction more or equal to 25% from baseline

Poor responder: percentage of IOP reduction less than 25% from baseline

2.2.4 Statistical analysis

All the available data was then analyzed in Statistical Package for Social Science (SPSS) Predictive Analytic Software programme for Windows (PASW) version 18.0. Double entry was done to avoid any possible error. The acquired data was analyzed using SPSS or PASW version 18.0 and Stata SE version 11.0.

Pearson chi-square and student t-test were used to analyse the difference of demographic data between racial groups in both projects and treatment modalities in Project P. Independent t-test was used to determine the difference between the clinical parameters with racial groups and treatment modalities. For comparison of clinical parameters of different type of glaucoma (POAG and NTG), student t-test was used. The possible confounders for IOP measurement was detected using multiple linear regression analysis. Repeated Measure Analysis of Variance (RM ANOVA) was used to analyse the pressure lowering effect of topical timolol XE 0.5% and topical latanoprost 0.005% at 1, 3, 6 and 12 months. RM ANOVA within and between subject effect was conducted in condition where certain parameters were found to affect IOP measurement. Further analysis of between subject effects was done using multiple paired t-tests with Bonferroni correction. Multiple paired t-tests with Bonferroni correction

was considered significant at 0.005, based on 0.05 divided by the possible number of different pairing. Otherwise in all other analysis, level of significant was based on p-value less than 0.05.

2.3 Results

2.3.1 Topical timolol XE 0.5% monotherapy (Project B)

2.3.1.1 Demographic data

A total of 97 open angle glaucoma patients were recruited with 60 POAG, and 37 NTG. The ethnic distribution reflected the distribution of the local population (table 2.2). There was almost twice the number of men compared to women recruited in this study. Mean age of recruited subjects was 64.1 SD 9.2 years old. Hypertension and hyperlipidemia were the most common systemic co-morbidities observed among the glaucoma subjects. Calcium channel blocker (34.5%) was the most common first line drug for hypertensive treatment, followed by angiotensin converting enzyme inhibitor (23.6%), beta blockers (18.2%) and alpha-agonist (18.2%). All hyperlipidemia patients were on Statin drugs.

Table 2.2: Demographic data of the recruited glaucoma patients

Characteristic	Number (%) N=97
Ethnicity	
Malay	66 (68.0)
Chinese	31 (32.0)
Sex	
Male	62 (63.9)
Female	35 (36.1)
Type of glaucoma	
POAG	60 (61.9)
NTG	37 (37.1)
Systemic co-morbidity	
Hypertension	55 (56.7)
Diabetes mellitus	38 (39.2)
Hyperlipidemia	43 (44.3)
Cardiovascular diseases	11 (11.3)
Cerebrovascular accident	3 (3.1)

POAG: primary open angle glaucoma, NTG: normal tension glaucoma, OHT: ocular hypertension

2.3.1.2 Baseline clinical characteristics of glaucoma patients

Mean baseline IOP in the recruited patients was 22.7 SD 5.7 mmHg, with POAG patients demonstrated statistically significantly higher mean IOP. Based on mean deviation of Humphrey Visual field, majority of the cases were moderate to advanced glaucoma. The severity of glaucoma is based on Hodapp-Parrish and Anderson classification. CCT was statistically significant thinner in POAG as compared to NTG.

Table 2.3: Comparison of clinical characteristics between POAG and NTG patients

Clinical characteristics	n	Mean (SD)	p-value*
Baseline IOP(mmHg)	60	26.1 (4.4)	
POAG	37	17.2 (2.4)	**<0.001**
NTG	97	22.7 (5.7)	
Total			
MD (dB)			
POAG	58	-14.07 (9.42)	
NTG	37	- 7.50 (5.99)	**<0.001**
Total	95	-11.51 (8.82)	
PSD (dB)			
POAG	58	6.88 (2.95)	
NTG	37	4.70 (2.53)	**<0.001**
Total	95	6.03 (2.98)	
VCDR			
POAG	60	0.81 (0.07)	
NTG	37	0.78 (0.04)	**0.040**
Total	97	0.80 (0.06)	
CCT (µm)			
POAG	60	500 (34.2)	
NTG	37	524 (30.3)	**0.001**
Total	97	510 (35.1)	

*P<0.05 is considered statistically significant based on student t-test

IOP: intraocular pressure, POAG: primary open angle glaucoma, NTG: normal tension glaucoma, OHT: ocular hypertension, MD: mean deviation, PSD: pattern standard deviation, VCDR: vertical cup to disc ratio, CCT: central corneal thickness

2.3.1.3: Comparison of demographic and clinical characteristics between Malay and Chinese glaucoma patients

Although Malays presented with more advanced diseases and thinner CCT measurement but there was no statistically significant difference in glaucoma parameters between Malay and Chinese patients except for pattern standard deviation of visual field. Malays were slightly older than Chinese but this was not statistical significant.

Table 2.4: Distribution of demographic and clinical characteristics of glaucoma patients according to ethnicity

	Malay N=66	**Chinese N=31**	**p-value**
Age (years)			
Mean (SD)	64.8 (9.3)	62.8 (8.9)	0.327
Sex			
Male	43	19	0.717*
Female	23	12	
Type of glaucoma			
POAG	43	17	0.330*
NTG	23	14	
Baseline IOP (mmHg)			
Mean (SD)	23.1 (5.8)	21.8 (5.5)	0.283
MD (dB)			
Mean (SD)	-12.09 (9.04)	-10.30 (8.37)	0.356
PSD (dB)			
Mean (SD)	6.50 (5.07)	5.07 (2.53)	**0.027**
VCDR			
Mean (SD)	0.80(0.07)	0.79(0.05)	0.480
CCT (µm)			
Mean (SD)	505 (36)	517 (31)	0.125

p <0.05 is considered statistically significant based on t-test and Pearson chi-square test*

IOP: intraocular pressure, POAG: primary open angle glaucoma, NTG: normal tension glaucoma, OHT: ocular hypertension, MD: mean deviation, PSD: pattern standard deviation, VCDR: vertical cup to disc ratio, CCT: central corneal thickness

2.3.1.4 Factors affecting baseline IOP

Univariate linear regression analysis was conducted to identify the possible variables affecting baseline IOP measurement. Age, sex, race, type of glaucoma, central corneal thickness and systemic co-morbidities including hyperlipidemia were identified as possible variable affecting baseline IOP measurement. Hyperlipidemia was found to have higher baseline IOP measurement. NTG was associated with linearly lower IOP compared to POAG.

Table 2.5: Univariate linear regression analysis on factors affecting baseline IOP

	Coefficient value	**SE**	**t**	**95 % CI**	**p-value#**
Age (years)	0.008	0.044	0.13	- 0.08, 0.10	0.851
Sex	-0.242	0.847	-0.29	- 1.93, 1.44	0.776
Ethnicity	-0.508	0.830	-0.62	- 2.16, 1.14	0.540
Type of glaucoma	-8.441	0.869	-9.72	-10.17, -6.72	**<0.001**
CCT	-0.002	0.012	-0.17	- 0.03, 0.02	0.865
Systemic hypertension	-1.576	1.141	-1.38	- 3.85, 0.69	0.171
Diabetes mellitus	0.008	1.015	0.02	- 2.01, 2.03	0.994
Hyperlipidemia	3.666	1.111	3.30	1.46, 5.87	**0.001**
Cardiovascular diseases	-0.184	1.354	-0.01	- 2.88, 2.51	0.892
Cerebrovascular disease	-0.942	2.357	-0.03	- 5.67, 3.74	0.690

# $p < 0.05$ is considered statistically significant based on simple linear regression analysis
POAG: primary open angle glaucoma, NTG: normal tension glaucoma, OHT: ocular hypertension, CCT: central corneal thickness

2.3.1.5 Mean IOP reduction during 12 months treatment with topical Timolol XE 0.5%

Overall mean IOP reduction was 5.4 SD 5.1 mmHg, based on the different between the baseline IOP and summation of IOP divided by the number of visits. The mean percentage of IOP reduction was 22.9 SD 18.1%. At visit 4 (12 months after starting treatment with topical timolol) only 50 patients were still on treatment. Topical Timolol XE 0.5% was discontinued in two patients due to shortness of breath and chest discomfort. One patient developed progression that required surgical intervention. Additional medication was required in majority of the patients due to inability to achieve target pressure (table 2.7). The highest number of 'drop-out' from study protocol was recorded at 6 months post treatment.

Table 2.6: Mean IOP reduction and percentage of reduction from baseline at 1, 3, 6 and 12 months follow-up

IOP	n	Mean (SD) mmHg	Percentage of reduction from baseline (SD)
Baseline	97	22.7 (5.7)	-
Visit 1 (1 month)	97	17.3 (4.7)	22.19 (17.76)
Visit 2 (3 months)	85	16.4 (3.8)	24.08 (16.54)
Visit 3 (6 months)	63	15.8 (3.5)	23.88 (17.79)
Visit 4 (12 months)	50	14.8 (3.5)	27.79 (18.72)

Table 2.7: Percentage and reason of 'drop-out' from study protocol

Follow up	n#	Treatment discontinued	Reasons	Additional treatment required	Reasons
Baseline N=97	0 (0%)	-	-	-	-
Visit 1 (1 month) N=97	0 (0%)	-	-	-	-
Visit 2 (3 months) N=85	12 (12.4%)	2	Side effect	10	Failed to reach target pressure
Visit 3 (6 months) N=63	22 (25.9%)	-	-	22	Failed to reach target pressure
Visit 4 (12 months) N=50	7 (11.1%)	-	-	7	Progression (1) Failed to reach target pressure (6)

The number and percentage of 'drop-out' from the last visit. Percentage is calculated from the number of 'drop-out' out of the total number of patients from the last visit e.g. the number of 'drop-out' at 3 months follow up was 12. Total number of patients at last visit was 97. Percentage of 'drop-out'=12/97x 100

2.3.1.6 Repeated measures Analysis of Variance (RM ANOVA) of IOP at 1, 3, 6 and 12 months post-treatment with topical Timolol XE 0.5% monotherapy

There was statistically significant pressure lowering effect of topical Timolol XE 0.5% over 12 months treatment period based on RM ANOVA (Figure 2.1). Multiple paired t-tests were then conducted, significant IOP reduction was observed between baseline and 1 month, baseline and 3 months, baseline and 6 months and baseline and 12 months post treatment (Table 2.8). However, there was no significant difference of IOP reduction between subsequent visits.

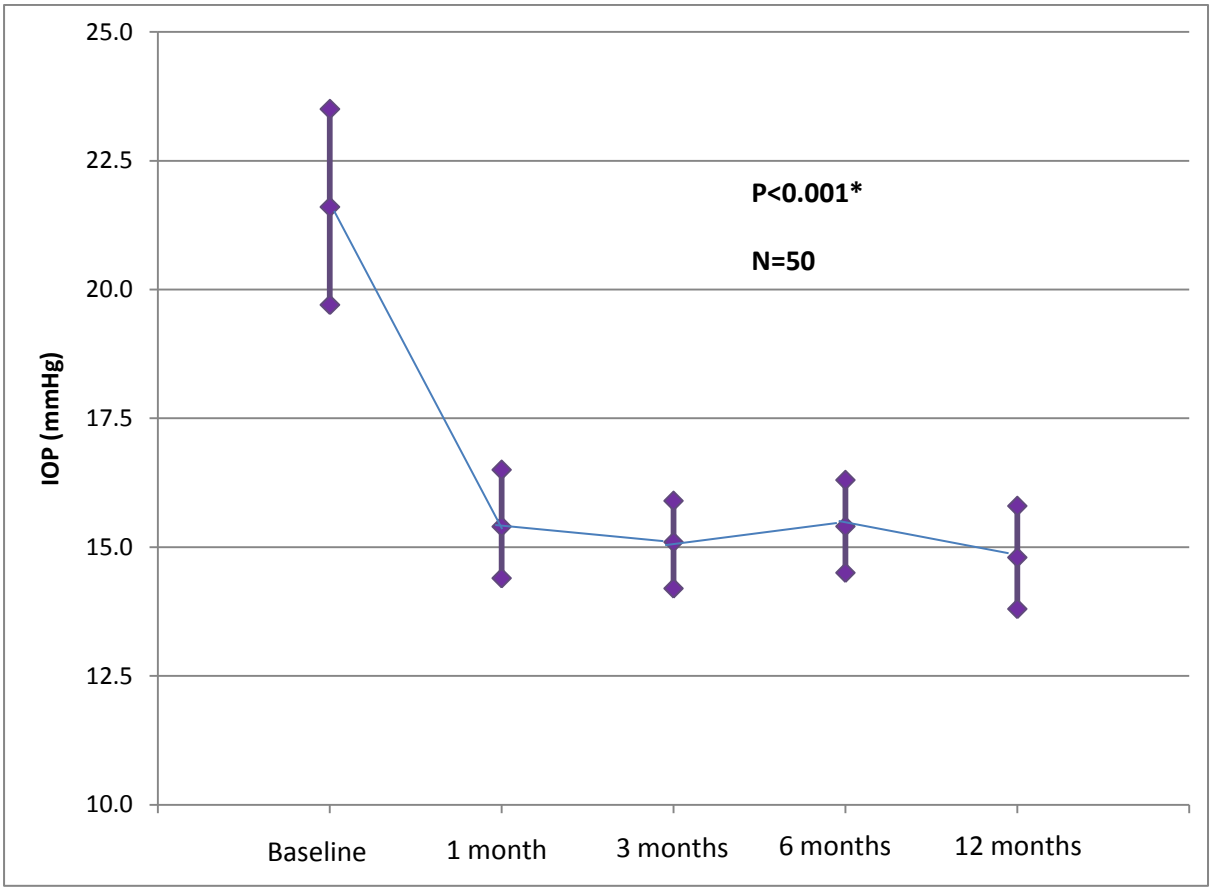


Figure 2.1: Pattern of pressure lowering effect of topical Timolol XE 0.5% monotherapy at baseline, 1 month, 3 months, 6months and 12 months post-treatment

*p< 0.05 is considered statistically significant based on RM ANOVA

Table 2.8: Mean IOP difference between follow –up visits

Time pairing#	Number of observation (n)	Mean IOP difference (SE) (mmHg)	95% CI of mean IOP difference	p-value*
Visit 1-Visit 2	97	5.4 (0.5)	4.4, 6.5	**<0.001**
Visit 1-Visit 3	85	6.1 (0.6)	5.0, 7.2	**<0.001**
Visit 1-Visit 4	63	5.9 (0.7)	4.5, 7.3	**<0.001**
Visit 1-Visit 5	50	6.8 (0.9)	5.0, 8.6	**<0.001**
Visit 2-Visit 3	85	0.4 (0.4)	-0.7, 0.7	0.975
Visit 2-Visit 4	60	0.2 (0.4)	-0.7, 1.0	0.681
Visit 2-Visit 5	51	0.6 (0.5)	-0.3, 1.6	0.197
Visit 3-Visit 4	63	-0.4 (0.3)	-1.1, 0.2	0.200
Visit 3-Visit 5	50	0.3 (0.5)	-0.8, 1.3	0.623
Visit 4-Visit 5	50	0.6 (0.4)	-0.3, 1.4	0.197

Visit 1 is baseline IOP, Visit 2 is IOP at 1 month, Visit 3 is IOP at 3 months, Visit 4 is IOP at 6 months and Visit 5 is IOP at 12 months post treatment with topical Timolol XE 0.5% monotherapy. SE: standard error

*$p<0.005$ is considered statistically significant based on multiple paired t-tests with Bonferroni correction

2.3.1.7 Mean IOP reduction at baseline and 1 month, 3 months, 6 months and 12 months post-treatment with between and within subject effects

Based on the findings of linear regression analysis, type of glaucoma and hyperlipidemia status are significantly associated with baseline IOP measurements, RM ANOVA within and between subjects was further analyzed. RM ANOVA within and between subjects effect was only conducted on 50 subjects that have completed 12 months of IOP measurement. There was a statistically significant difference between different type of glaucoma and IOP reduction over 12 months (Figure 2.2).

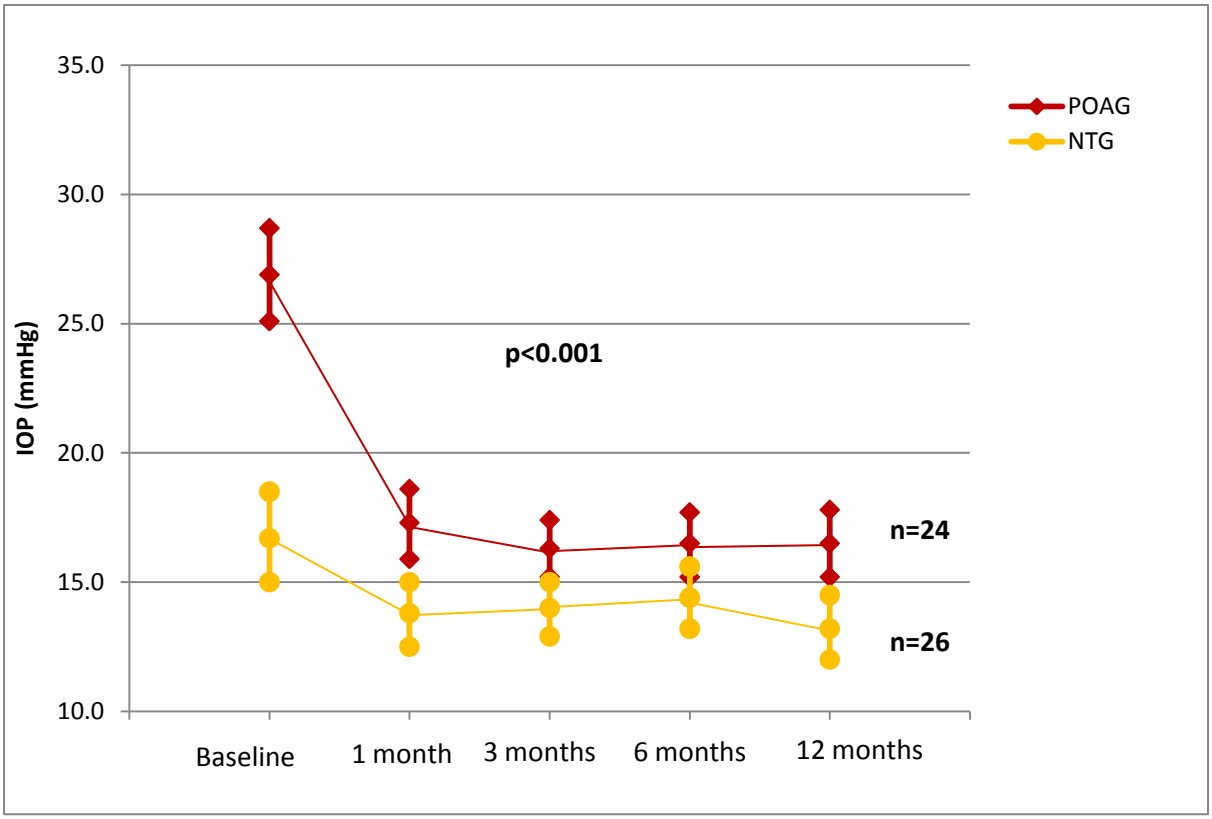


Figure 2.2: Pattern of pressure lowering effect of topical Timolol XE 0.5% monotherapy at baseline, 1 month, 3 months, 6 months and 12 months in POAG and NTG patients

*p <0.05 is considered significant based on RM ANOVA

POAG: primary open angle glaucoma, NTG: normal tension glaucoma, OHT: ocular hypertension.

Multiple paired t-tests with Bonferroni correction was conducted on POAG and NTG patients. There was significant mean IOP difference between baseline and follow up visits (visit 2, 3, 4 and 5) in both POAG and NTG patients (Table 2.9).

Table 2.9: Mean IOP difference between follow up visits of POAG and NTG patients

Comparison#	POAG N=60		NTG N=37	
	Mean IOP difference (95% CI) mmHg	p-value*	Mean IOP difference (95% CI) mmHg	p-value*
Visit 1-Visit 2	7.0 (5.4,8.5)	**<0.001**	2.0 (2.3, 3.7)	**<0.001**
Visit 1-Visit 3	8.5 (7.0,10.0)	**<0.001**	2.8 (1.8, 3.6)	**<0.001**
Visit 1-Visit 4	9.2 (7.0, 11.3)	**<0.001**	2.5 (1.6, 3.4)	**<0.001**
Visit 1-Visit 5	10.3 (7.2, 13.5)	**<0.001**	3.5 (2.6, 4.4)	**<0.001**
Visit 2-Visit 3	0.1 (-1.1,1.3)	0.813	-0.2 (-0.8, 0.5)	0.606
Visit 2-Visit 4	0.6 (-0.9, 2.1)	0.394	-0.3 (-1.1, 0.6)	0.495
Visit 2-Visit 5	0.7 (-1.1, 2.5)	0.416	0.5 (-0.4, 1.5)	0.271
Visit 3-Visit 4	-0.8 (-1.7, 0.2)	0.105	-0.1 (-1.1, 0.9)	0.849
Visit 3-Visit 5	-0.3 (-2.2, 1.7)	0.789	0.7 (-0.4, 1.8)	0.190
Visit 4-Visit 5	-0.1 (-1.7, 1.6)	0.918	1.2 (0.4, 2.0)	0.006

*p-value less than 0.005 based on multiple paired t-tests with Bonferroni correction
Visit 1 is baseline IOP, Visit 2 is IOP at 1 month, Visit 3 is IOP at 3 months, Visit 4 is IOP at 6 months and Visit 5 is IOP at 12 months post-treatment with topical timolol XE 0.5% monotherapy

Hyperlipidemia was found to linearly affect the baseline IOP. Patients with hyperlipidemia (treated with "Statin" drugs) demonstrated lower baseline IOP, better and more stable IOP reduction. However, there was no statistical significant difference in mean IOP at each follow up between patients with hyperlipidemia and non-hyperlipidemia patients except for baseline IOP (Table 2.10). None of the patients were diagnosed as hyperlipidemia post topical timolol treatment. Mean IOP reduction from baseline of glaucoma patients who received concomitant treatment with oral calcium channel blockers and beta blockers were 6.5(3.7) mmHg and 4.3(3.8) mmHg. There was no significant different of mean IOP reduction between those treated with oral beta blockers and non oral beta blockers (p=0.394, student t-test).

Table 2.10: Comparison of mean IOP at follow up visits between hyperlipidemia and non-hyperlipidemia glaucoma patients

Visits	Mean IOP (SD) (mmHg)		p-value*
	Hyperlipidemia N=43	Non-hyperlipidemia N=54	
Visit 1 (Baseline)	20.6 (4.5)	24.3 (6.1)	**0.001**
Visit 2 (1 month)	16.7 (5.0)	17.7 (4.4)	0.282
Visit 3 (3 months)	15.7 (3.6)	16.9 (3.9)	0.138
Visit 4 (6 months)	15.3 (3.1)	16.3 (3.7)	0.225
Visit 5 (12 months)	14.0 (3.3)	15.5 (3.7)	0.137

*P<0.05 is considered significant based on independent t-test

2.3.1.8 Responsiveness and side effects to topical Timolol XE 0.5% monotherapy

A "good responder" was defined by IOP reduction ≥ 20% reduction from baseline at the last IOP reading. Based on this definition, slightly more than half of all cases were good responders. However, if the criteria of good responder are adjusted at 10%, 15%, 20%, 25%, 30%, 35%, 40% and 45%, there was different percentage of responder rate according to definition provided (table 2.11). Eleven subjects responded very well to timolol with more than 45% pressure reduction. On the other hand, 20 subjects responded poorly with pressure reduction less than 10% from baseline.

Two subjects (subject 50 and 81) requested to drop out of the study due to chest discomfort and shortness of breath. There was no complaint of blurry vision or other ocular related side effects.

Table 2.11: Percentage of good and poor responder according to different definition of responsiveness to topical Timolol XE 0.5%

Definition of responder	Good responder n (%)	Poor responder n (%)
≥10%	77 (77.4)	20 (20.6)
≥15%	71 (73.2)	26 (26.8)
≥20%	56 (57.7)	41 (42.3)
≥25%	43 (44.3)	54 (55.7)
≥30%	31 (32.0)	66 (68.0)
≥35%	22 (22.7)	75 (77.3)
≥40%	17 (17.5)	80 (82.5)
≥45%	11 (11.3)	86 (88.7)

2.3.2 Topical latanoprost 0.005% (Project P)

2.3.2.1 Demographic data of recruited glaucoma patients

A total of 86 glaucoma patients were recruited with 64 of them diagnosed as POAG and 22 NTG. Over two thirds of them were Malays (67.4%); the remainder were Chinese. There was also preponderance of men (69.8%). The mean age of the recruited patients was 66.9 SD 9.2 years old. Hypertension remained the most common systemic co-morbidities among the recruited patients, followed by hypercholesterolemia and diabetes mellitus. Slightly more than half of them received topical latanoprost 0.005% as adjunctive treatment to topical timolol XE 0.5%.

Table 2.12: Demographic data of recruited glaucoma patients

Characteristic N=86	N (%)
Sex	
Male	60 (69.8)
Female	26 (32.2)
Ethnicity	
Malays	58 (67.4)
Chinese	28 (32.6)
Type of glaucoma	
POAG	64 (74.4)
NTG	22 (25.6)
Treatment modalities	
Monotherapy	39 (45.3)
Adjunctive	47 (54.7)
Systemic co-morbidities	
Hypertension	55 (64.0)
Diabetes Mellitus	34 (39.5)
Hypercholesterolemia	38 (44.2)
Cardiovascular diseases	11 (12.8)

POAG: primary open angle glaucoma, NTG: normal tension glaucoma, OHT: ocular hypertension

2.3.2.2 Distribution of demographic data according to ethnicity and treatment modalities

There was no significant difference between demographic data and ethnicity. Similar observation was seen in different treatment modalities but there was significant higher number of patients with POAG treated with topical latanoprost as adjunctive therapy (Table 2.13).

Table 2.13: Comparison of demographic data according to ethnicity and treatment modalities

Characteristic	Ethnicity			Treatment modalities		
	Malay N=58	**Chinese N=28**	**p-value**	**Monotherapy N=39**	**Adjunctive N=47**	**p-value**
Mean age (SD)	67.7 (9.5)	65.8 (8.4)	0.379#	65.8 (10.6)	68.1 (7.7)	0.252#
Sex (%)						
Male	44	16	0.077	28	32	0.709
Female	14	12		11	15	
Ethnicity						
Malay	---	---	---	29	29	0.212
Chinese				10	18	
Type of glaucoma						
POAG	40	24	0.095	20	44	**<0.001**
NTG	18	4		19	3	
Treatment						
Monotherapy	29	10	0.212	---	---	---
Adjunctive	29	18				

P< 0.05 is considered significant based on Pearson chi-square test and # independent t-test
POAG: primary open angle glaucoma, NTG: normal tension glaucoma, OHT: ocular hypertension

2.3.2.3 Comparison of baseline clinical characteristics according to type of glaucoma, ethnicity and treatment modalities

Based on both mean deviation (MD) and pattern standard deviation (PSD) of Humphrey Visual Field Analyzer (HFA), majority of recruited glaucoma patients have moderate to severe glaucoma. HFA assessment was only conducted on 82 subjects. Four patients were unable to perform accurate reliable HFA test and diagnosis was made based on other available data (Table 2.14). There was a statistically significant difference between type of glaucoma and optic nerve function but not structural parameter (VCDR) (Table 2.14).

Table 2.14: Comparison of mean age and baseline clinical characteristic between POAG and NTG patients

Clinical characteristics	n	Mean (SD)	t-statistic(df)	p-value*
Age (year)				
POAG	64	68.7 (8.1)	3.05 (84)	**0.003**
NTG	22	62.1 (10.5)		
Total	86	67.1 (9.2)		
Baseline IOP (mmHg)				
POAG	64	23.8 (3.6)	6.61 (84)	**<0.001**
NTG	22	18.3 (2.8)		
Total	86	22.4 (4.1)		
HFA MD (dB)				
POAG	61	-12.36 (8.45)	2.25 (80)	**0.027**
NTG	21	- 7.68 (7.46)		
Total	82	-11.6 (8.42)		
HFA PSD (dB)				
POAG	61	7.01 (3.25)	2.51 (80)	**0.014**
NTG	21	5.06 (2.50)		
Total	82	6.51 (3.18)		
VCDR				
POAG	64	0.79 (0.08)	0.38 (84)	0.704
NTG	22	0.78 (0.08)		
Total	86	0.79 (0.08)		
CCT (µm)				
POAG	64	504 (42)	2.04 (84)	**0.045**
NTG	22	523 (27)		

Total	86	509 (40)

*p< 0.05 is considered statistically significant based on independent t- test

IOP: intraocular pressure, POAG: primary open angle glaucoma, NTG: normal tension glaucoma, HFA: Humphrey visual field analysis, MD: mean deviation, PSD: pattern standard deviation, VCDR: vertical cup to disc ratio, CCT: central corneal thickness

There was statistically significant difference in VCDR between the two major ethnic groups in Malaysia (Table 2.15). Malays demonstrated significant more advanced structural damage than Chinese. Glaucoma patients who were treated with topical latanoprost as adjunctive therapy have significant thinner CCT and more advanced visual field defect based on MD of HFA (Table 2.16).

Table 2.15: Comparison of baseline clinical characteristics between Malays and Chinese

Clinical characteristics	**Ethnics (n) N=86**	**Mean (SD)**	**95% CI for difference**	**t-statistic (df)**	**p-value***
Baseline IOP (mmHg)	Malay (62) Chinese (28)	22.2 (4.4) 22.9 (3.6)	-2.6, 1.2	-0.73 (84)	0.465
VCDR	Malay (62) Chinese (28)	0.80 (0.07) 0.76 (0.07)	0.01, 0.07	2.29 (84)	**0.025***
HFA MD (dB)	Malay (54) Chinese (28)	-12.21 (8.79) - 9.12 (7.37)	-6.96, 0.77	-1.59 (80)	0.115
HFA PSD (dB)	Malay(54) Chinese (28)	6.84 (3.27) 5.87 (2.96)	-0.50, 2.44	1.32 (80)	0.191
CCT (µm)	Malay (62) Chinese (28)	508 (41) 511 (37)	-20, 16	-0.33 (84)	0.816

*p<0.05 is considered statistically significant based on independent t-test

IOP: intraocular pressure, POAG: primary open angle glaucoma, NTG: normal tension glaucoma, HFA: Humphrey visual field analysis, MD: mean deviation, PSD: pattern standard deviation, VCDR: vertical cup to disc ratio, CCT: central corneal thickness

Table 2.16: Comparison of baseline clinical characteristics between monotherapy and adjunctive therapy of topical latanoprost

Clinical characteristics	Treatment modalities (n) N=86	Mean (SD)	95% CI for difference	t-statistic (df)	p-value*
Baseline IOP (mmHg)	Monotherapy (39)	22.6 (4.1)	-3.2, 0.3	-1.69 (84)	0.655
	Adjunctive (47)	23.1 (4.1)			
VCDR	Monotherapy (39)	0.77 (0.07)	-0.07, 0.00	-2.02 (84)	0.152
	Adjunctive (47)	0.80 (0.08)			
HFA MD (dB)	Monotherapy (38)	- 8.36 (6.28)	1.66, 8.77	2.92 (80)	**<0.001**
	Adjunctive (44)	- 13.57 (9.31)			
HFA PSD (dB)	Monotherapy (38)	6.28 (3.03)	-1.83, 0.98	-0.60 (80)	0.822
	Adjunctive (44)	6.71 (3.33)			
CCT (µm)	Monotherapy (39)	522 (37)	7.29, 39.98	2.88 (84)	**0.005**
	Adjunctive (47)	498 (39)			

*p< 0.05 is considered statistically significant based on independent t-test

IOP: intraocular pressure, POAG: primary open angle glaucoma, NTG: normal tension glaucoma, HFA: Humphrey visual field analysis, MD: mean deviation, PSD: pattern standard deviation, VCDR: vertical cup to disc ratio, CCT: central corneal thickness

2.3.2.4 Factors affecting baseline IOP

Simple linear regression analysis was conducted to assess the possible predictors that may affect IOP measurement. Type of glaucoma was identified as significant predictor affecting baseline IOP measurement (Table 2.17).

Table 2.17: Univariate linear regression analysis on factors affecting baseline IOP

Baseline IOP	Coefficient	SE	t-stat	95 % confident interval	p-value#
Age	0.20	0.05	1.83	- 0.01, 0.18	0.071
Sex	0.03	0.98	0.23	-1.72, 2.17	0.821
Race	0.08	0.96	-- 0.73	-1.20, 2.60	0.465
Type of glaucoma	-0.64	1.00	-6.09	-8.07, -4.09	<**0.001**
CCT	-0.09	0.01	-0.96	- 0.03, 0.01	0.339
Treatment modalities	-0.15	0.91	-1.34	3.04, 0.50	0.185
Systemic hypertension	-0.02	0.90	-0.17	-1.93, 1.64	0.870
Diabetes mellitus	-0.08	0.85	-0.76	- 2.33, 1.05	0.452
Hyperlipidemia	0.16	0.90-	1.50	-0.48, 3.13	0.138
Cardiovascular diseases	-0.03	1.15 --	-0.31	-3.65, 1.94	0.760

#p-value less than 0.05 is considered statistically significant. POAG: primary open angle glaucoma, NTG: normal tension glaucoma, CCT: central corneal thickness

2.3.2.5 Mean IOP reduction of 12 months treatment with topical latanoprost 0.005%

In general there was more than a 30% IOP reduction from baseline at most of the follow up visits. Overall mean IOP reduction was 6.9 SD 4.0 mmHg, based on the different between the baseline IOP and summation of IOP divided by the number of visits. The mean percentage of IOP reduction was 26.7%, SD 19.3%. The mean IOP reduction (mean percentage of reduction) for patients treated with topical latanoprost 0.005% monotherapy was 7.1 SD 3.2mmHg (29.2 SD 14.5%) and adjunctive group was 6.8 SD 4.6 mmHg (24.7 SD 22.4%) (p=0.724; independent t-test). At the end of 12 months treatment, only 65 patients were still on topical latanoprost 0.005% treatment (table 2.18). There was no significant difference of mean IOP reduction or mean percentage of IOP reduction from baseline between monotherapy and adjunctive therapy (Table 2.19).

Table 2.18: Mean IOP and percentage of IOP reduction from baseline

Visits	n	Mean (SD) mmHg	Percentage of reduction from baseline (SD)
Baseline	86	22.4 (4.1)	-
Visit 1 (1 month)	86	15.7 (3.1)	28.86 (13.40)
Visit 2 (3 months)	81	15.3 (4.3)	30.86 (19.26)
Visit 3 (6 months)	74	15.0 (3.0)	31.93 (13.03)
Visit 4 (12 months)	65	14.8 (2.7)	31.39 (15.16)

Table 2.19: Comparison of mean IOP and percentage of IOP reduction from baseline between monotherapy and adjunctive therapy of topical latanoprost

	Mean IOP reduction from baseline (mmHg)			Mean percentage of IOP reduction from baseline		
	Monotherapy n Mean (SD)	Adjunctive n Mean (SD)	p-value*	Monotherapy n % (SD)	Adjunctive n % (SD)	p-value*
Baseline	39 ---	47 ---		39 ---	47 ---	
1 month	39 6.9 (5.0)	47 6.8 (3.9)	0.891	39 28.86 (13.73)	47 28.87 (13.27)	0.997
3 months	37 8.1 (5.2)	44 6.6 (6.1)	0.269	37 34.37 (13.09)	44 27.90 (22.96)	0.135
6 months	35 7.8 (4.9)	39 7.4 (4.2)	0.683	35 33.06 (11.42)	39 30.92 (14.40)	0.484
12 months	32 6.8 (3.8)	33 6.8 (4.6)	0.919	32 32.68 (14.41)	33 30.15 (15.98)	0.506

*$p < 0.05$ is considered significant based on independent t-test

2.3.2.6 Repeated measures Analysis of Variance (RM ANOVA) of IOP at 1, 3, 6 and 12 months treatment with topical latanoprost 0.005%

RM ANOVA analyzed only 65 patients who completed 12 months follow up. There was a statistical significant IOP reduction over 12 months follow up (Figure 2.3). Further analysis was conducted using multiple paired t-tests with Bonferroni correction on the 10 possible pairing of the difference between visits (Table 2.20). P-value of 0.005 was deemed statistically significant. Multiple paired t-tests were conducted on the actual number of recruited patient according to follow up visit. There was significant pressure lowering effect between baseline and 1 month, baseline and 3 months, baseline and 6 months, and baseline and 12 months post treatment. However, there was no significant different between the subsequent follow up visits pairing.

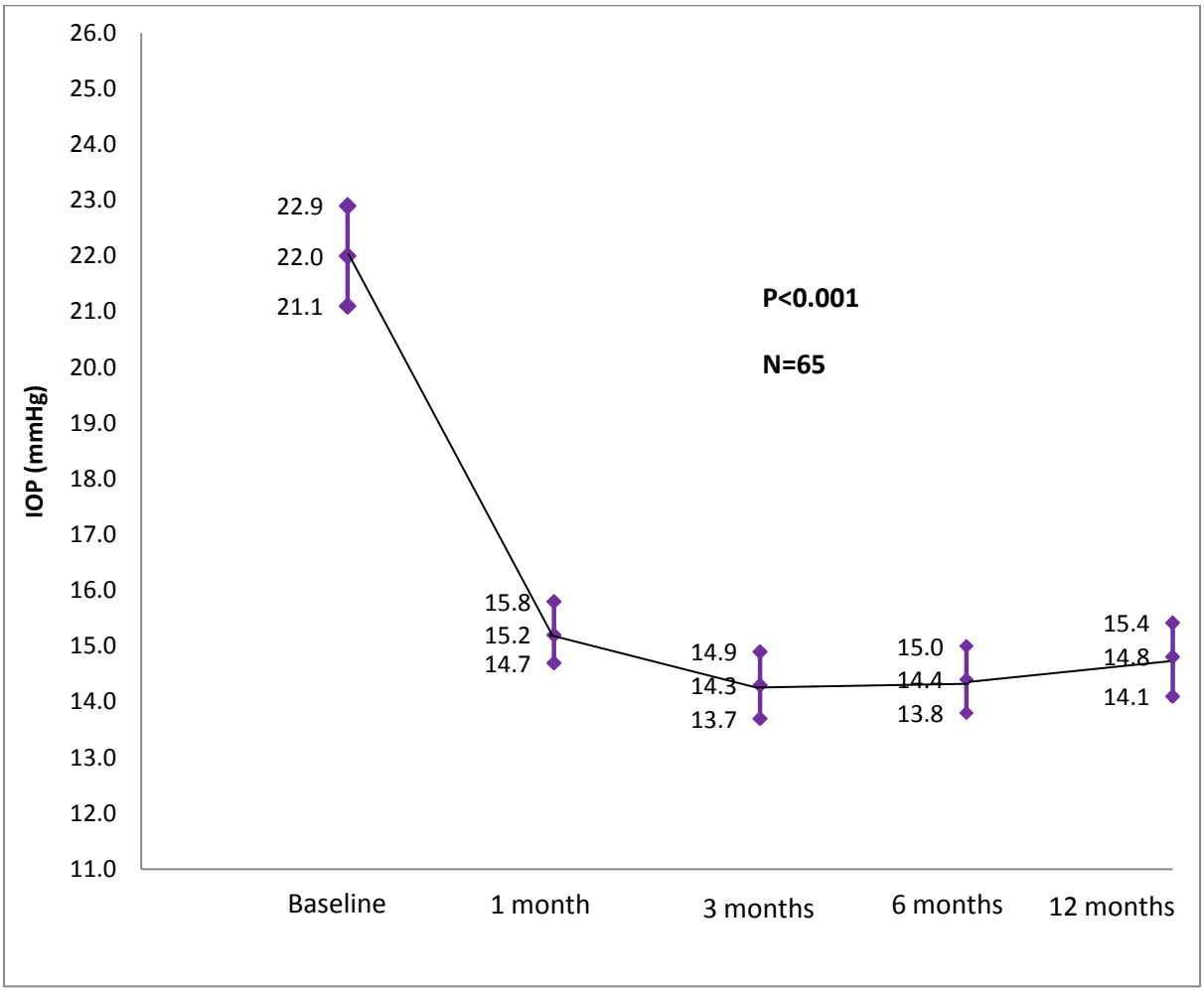


Figure 2.3: Pattern of pressure lowering effect of topical latanoprost 0.005% at baseline, 1 month, 3 months, 6months and 12 months post-treatment

*p< 0.05 is considered statistically significant based on RM ANOVA

Table 2.20: Comparison of mean IOP difference at follow-up visits

Time pairing#	Mean IOP difference (SD) (mmHg)	95% CI	t-statistic(df)	p-value*
Baseline-Visit 1	6.7 (3.7)	6.9, 7.5	17.04 (85)	**<0.001**
Baseline-Visit 2	7.2 (4.9)	6.1, 8.2	13.19 (80)	**<0.001**
Baseline-Visit 3	7.4 (3.6)	6.5, 8.2	17.46 (73)	**<0.001**
Baseline-Visit 4	7.2 (4.0)	6.2, 8.2	14.46 (64)	**<0.001**
Visit 1-Visit 2	0.3 (3.9)	-0.6, 1.1	0.60 (84)	0.554
Visit 1-Visit 3	0.2 (3.4)	-0.6, 1.0	0.58 (73)	0.564
Visit 1-Visit 4	0.4 (3.1)	-0.3, 1.2	1.16 (64)	0.249
Visit 2-Visit 3	-0.4 (2.8)	-1.1, 0.2	-1.25 (73)	0.216
Visit 2-Visit 4	-0.4 (2.8)	-1.1, 0.3	-1.23 (64)	0.222
Visit 3-Visit 4	-0.4 (3.0)	-1.1, 0.4	-1.83 (64)	0.305

*p< 0.005 based on multiple paired t-tests with Bonferroni correction
Visit 1 is baseline IOP, Visit 2 is IOP at 1 month, Visit 3 is IOP at 3 months, Visit 4 is IOP at 6 months and Visit 5 is IOP at 12 months post-treatment with topical latanoprost 0.005%

2.3.2.7 Mean IOP reduction at baseline and 1 month, 3 months, 6 months and 12 months post-treatment with between and within subject effects

Type of glaucoma was identified as the significant factor associated with IOP measurement based on multivariate analysis. Thus, type of glaucoma was included as between the subject factor in RM ANOVA analysis (figure 2.4). Although, treatment modality was not found to affect the IOP measurement but it was included out of clinical interest. However, there was no significant different in pressure lowering effect of topical latanoprost between monotherapy and adjunctive therapy over a year of follow up (p=0.775; RM ANOVA).

There was a statistically significant difference between the type of glaucoma and pattern of pressure lowering effect of topical latanoprost 0.005% (figure 2.4). The significant reduction was seen between baseline IOP and subsequent IOP measurement in both POAG and NTG (table 2.21).

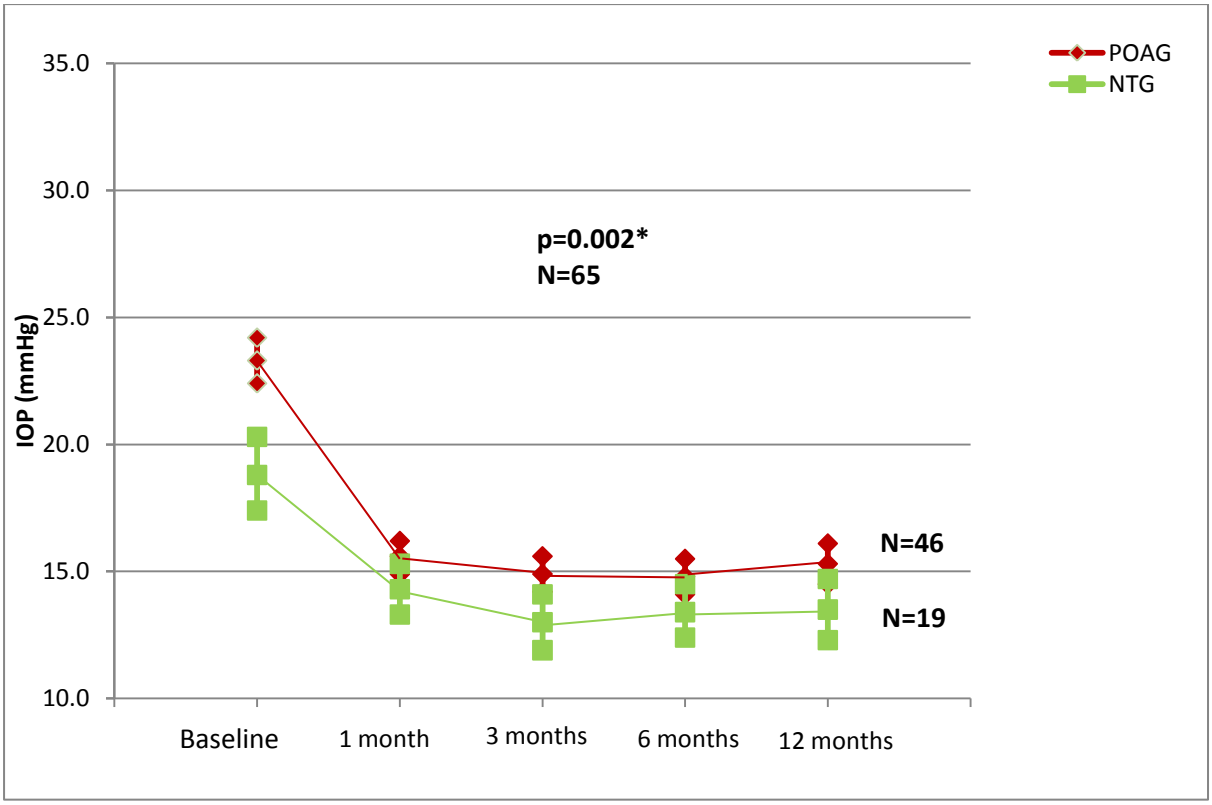


Figure 2.4: Pattern of pressure lowering effect of topical latanoprost in POAG and NTG patients

P<0.05 is considered statistically significant based on RM ANOVA

Table 2.21: Mean IOP difference at follow-up visits in POAG and NTG patients

Comparison#	POAG			NTG		
	Mean IOP difference (95% CI)	t-stat (df)	p-value*	Mean IOP difference (95% CI)	t-stat (df)	p-value*
Visit 1-Visit 2	7.6 (6.7, 8.5)	17.0 (63)	<**0.001**	4.1 (3.0, 5.3)	7.7 (21)	<**0.001**
Visit 1-Visit 3	7.6 (6.3, 9.0)	11.3 (60)	<**0.001**	5.8 (4.3, 7.2)	8.4 (19)	<**0.001**
Visit 1-Visit 4	8.0 (7.0, 9.0)	15.9 (54)	<**0.001**	5.4 (4.2, 6.6)	9.7 (18)	<**0.001**
Visit 1-Visit 5	8.0 (6.7, 9.2)	13.0 (45)	<**0.001**	5.4 (3.8, 6.9)	7.4 (18)	<**0.001**
Visit 2-Visit 3	-0.1 (-1.2, 1.0)	-0.2 (60)	0.831	1.4 (0.1, 2.7)	2.3 (19)	0.036
Visit 2-Visit 4	0.0 (-1.0, 1.0)	0.0 (54)	1.000	0.9 (-0.4, 2.2)	1.4 (18)	0.167
Visit 2-Visit 5	0.3 (-0.6, 1.2)	0.6 (45)	0.529	0.8 (-0.8, 2.4)	1.1 (18)	0.281
Visit 3-Visit 4	-0.4 (-1.2, 0.4)	-1.0 (54)	0.337	-0.4 (-1.3, 0.5)	-1.0 (18)	0.338
Visit 3-Visit 5	-0.4 (-1.2, 0.4)	-1.1 (45)	0.298	-0.5 (-2.0, 1.1)	-0.6 (18)	0.532
Visit 4-Visit 5	-0.5 (-1.4, 0.4)	-1.2 (45)	0.235	-0.1 (-1.6, 1.5)	-0.1 (18)	0.943

*p<0.005 based on multiple paired t-tests with Bonferroni correction is considered statistically significant

Visit 1: baseline IOP, Visit 2: IOP at 1 month, Visit 3: IOP at 3 months, Visit 4: IOP at 6 months, Visit 5: IOP at 12 months post-treatment with topical latanoprost 0.005%

2.3.2.8 Responsiveness and side effects to topical latanoprost 0.005%

Based on the definition of a good responder (25% of IOP reduction from baseline), 64.0% of the subjects were good responders, and 36.0% were poor responders (Table 2.22).

Hypertrichosis was detected at 3 months of treatment (7, 8.6%) and later increased to 85.1% at 6 months. At the end of 12 months, all the remaining patients (65, 100%) developed hypertrichosis. Conjunctival hyperaemia developed in 3 patients at 1 month post treatment with 1 developed grade 1 (mild hyperaemia) and 2 patients developed grade 2 (moderate hyperaemia). Latanoprost induced iris pigmentation was detected in 5 patients at 12 months (Table 2.23).

Table 2.22: Percentage of good and poor responder according to different definition of responsiveness to topical latanoprost 0.005%

Definition of responds	Good responders n (%)	Poor responders n (%)
≥10%	73 (84.9)	13 (15.1)
≥15%	67 (77.9)	19 (22.1)
≥20%	62 (72.1)	24 (27.9)
≥25%	55 (64.0)	31 (36.0)
≥30%	40 (46.5)	46 (53.5)
≥35%	31 (36.0)	55 (64.0)
≥40%	19 (22.1)	67 (77.9)
≥45%	14 (16.3)	72 (83.7)

Table 2.23: Percentage of ocular side effects according to follow-up visits

		Ocular side effects		
Visit	n	**Hypertrichosis N (%)**	**Latanoprost induced iris pigmentation N (%)**	**Conjunctival hyperaemia N (%)**
1 month	86	0 (0)	0 (0)	3 (3.4)
3 months	81	7 (8.6)	0 (0)	0 (0)
6 months	74	63 (85.1)	0 (0)	0 (0)
12 months	65	65 (100.0)	5 (7.7)	0 (0)

2.4 Discussion

2.4.1 Pressure lowering effect of topical Timolol XE 0.5%

Topical Timolol XE 0.5% is a gel forming solution of topical timolol, which is believed to promote compliance and efficacy with a single dosing and minimizing the unwanted systemic side effects. Timolol XE 0.5% has been shown to be as effective as twice dosing of topical timolol aqueous solution (Uusitalo et al, 2006). A total of 97 open angle glaucoma (OAG) patients were recruited and were followed up for 12months. However, only 50 patients (51.5%) completed 12 months monotherapy treatment with topical Timolol XE. Almost half of the recruited patients required additional medication in order to achieve target IOP. The target IOP was individually set up according to the severity of glaucoma and type of glaucoma rather than fixing at certain definitive target. This study was designed to replicate the actual clinical situation rather than the artificial regimental clinical trial. Adherence and persistence to the drug was presumed to be presence in all recruited subjects.

A long term follow up study of Caucasian glaucoma patients treated with topical timolol aqueous solution found that only 17.7% of total 96 patients were still treated with topical timolol monotherapy after 7 years (Watson et al, 2001). At 12 months of follow up, there was 76 glaucoma patients (82.3%) were still treated with topical timolol. A 12 month prospective cohort study was conducted in mainly Caucasians and African Americans (only one Asian glaucoma patient) comparing the effectiveness of topical timolol GFS 0.5% and Timolol XE 0.5%, found that more than half of the patients (69%) achieved target IOP (Schenker and Silver, 2000).

Based on the pressure lowering effect in 50 patients, there was significant pressure lowering effect at 1 month post treatment from the baseline IOP. However, pressure lowering effect of

timolol failed to exert significant effect on subsequent follow-up. Seven years follow up in United Kingdom on glaucoma patients treated with 3 different types of topical beta-blockers: timolol, betaxolol and carteolol showed a similar pattern. In fact, there was slight increase in mean IOP at 3, 6 and 12 months follow up as compared to mean IOP at 1 month in all three types of topical beta blockers (Watson et al, 2001). However, this long term study was based on topical timolol aqueous solution 0.25% twice daily.

In spite of the differences between Malay and Chinese of the Malaysian populations, both do have highly pigmented iris. Perhaps, the high affinity of timolol for reversible binding to melanin is responsible in poor responder rate to topical Timolol XE 0.5% in our population. Timolol has high affinity for and easily bind to melanocyte (Menon et al, 1989). The relationship between timolol and melanin is quite controversial.

Slightly more than half of participants were poor responder to timolol, achieving less than 25% IOP reduction from baseline at their last visit. However, if the meaningful IOP reduction is adjusted to 20%, the number of good responder increased to slightly more than half of the total recruited Malaysian subjects.

2.4.2 Pressure lowering effect of topical Latanoprost 0.005%

Topical Latanoprost 0.005% has been shown to be more effective in Asian population as compared to topical timolol (Hedmann and Larsson, 2002). The current study was initially started at the early era of increasing popularity of prostaglandin analogs in Malaysia and is slowly replacing timolol as the first line antiglaucoma drug. Thus, in the early part of recruitment period there were many patients on adjunctive therapy to timolol and in later part there were more patients on latanoprost monotherapy. At the end of 12 months follow up, 65

subjects were still treated with topical latanoprost. In general, there was significant pressure lowering effect of topical latanoprost at every follow up visit from the baseline. However, there was no significant additional pressure lowering effect in between subsequent visits. For example between 1 month (visit 1) and 3 months (visit 2) post treatment.

Topical latanoprost provides better pressure lowering effect when compared indirectly with topical timolol XE monotherapy in project B in this study. The percentage of IOP reduction reaches around 32% from baseline IOP. Our finding provides additional evidence of better pressure lowering effect of topical latanoprost in Asians population (Hedman and Larsson, 2002; Aquino and Manalo, 2007). However, variations from the general response were observed in certain individual subjects in our study: some failed to achieve any meaningful IOP reduction and there were subjects that demonstrated a 'see-saw' effect on IOP. This inter-individual variation was observed based on individual pattern of 12 months of IOP measurement (not included in the result). In spite of the variation in response to latanoprost, 64.0% of the patients were categorized as good responder (based on ≥25% reduction) and 72.1% based on ≥20% reduction. A retrospective study on newly diagnosed Malay glaucoma patients treated with monotherapy latanoprost demonstrated almost similar percentage of good responders compared to Caucasians (≥20% reduction) (Cheong et al, 2008; Scherer, 2002).

Pressure lowering effect of topical latanoprost as adjunctive therapy has been reported to be lower than monotherapy treatment (Rulo et al, 1994). Surprisingly at 1 month post-treatment, there was almost similar pressure lowering effect between monotherapy and adjunctive therapy even though mean baseline IOP was slightly higher in adjunctive therapy group. However, on the subsequent visits, monotherapy treatment demonstrated clinically better

pressure lowering effect but without any statistically significant difference in comparison between the two treatment modalities. The pressure lowering effect of topical latanoprost is equally effective as monotherapy and adjunctive therapy to topical timolol in Malaysian population. Although, there was statistically significant difference in term of severity of glaucoma (based on HFA) and thinner CCT in adjunctive therapy compared to monotherapy subjects. It is of no surprise; lower target pressure is aimed for more advanced disease and more than a single topical drug is required to achieve the set target pressure.

There was significant difference in pressure lowering effect of latanoprost according to type of glaucoma. Since baseline IOP was found to moderately correlated with the pressure lowering effect of latanoprost, there was significant pressure lowering effect seen in POAG subjects compared to NTG (Bayer et al, 2005). In general, topical latanoprost 0.005% provides good pressure lowering effect in different type of glaucoma in Malaysian population.

CHAPTER 3

The role of ADRB2 and PTGFR gene in susceptibility to glaucoma

Chapter 3

3.1 Objectives

To determine the role of *ADRB2* and *PTGFR* genes as candidates in aetiology of open angle glaucoma.

3.2 Materials and methods

3.2.1 Blood collection for DNA extraction

Venesection was conducted on 183 glaucoma patients recruited based on criteria described in chapter 2. Blood was drawn from an equal number of age, sex and ethnicity-matched control subjects. Five millilitres of venous blood was obtained and kept in an ethylenediaminetetraacetic acid (EDTA) collection tube after written, informed consent was given by both people with glaucoma and control subjects.

Anterior segment examination, gonioscopy to assess the possibility of angle-closure or abnormalities of the angle structure, funduscopy examination to measure the vertical cup to disc ratio, visual field assessment and intraocular pressure measurement using Goldmann applanation tonometry were conducted in all selected control subjects to rule out possibility of glaucoma suspect or undiagnosed glaucoma. Similar examination was conducted in glaucoma patient as detailed in chapter 2. Pedigree charts were drawn to rule out any possibility of family history of glaucoma as well as to rule out the possibility of intermarriages in the family. As genetic polymorphism tends to varies according to population the selected control subjects must be of Malay and Chinese linage for 3 generations.

3.2.2 DNA extraction

DNA extraction was performed using QIAGEN QIAmp DNA Blood Mini Kit (Qiagen Inc., USA) according to the manufacturer's instructions. DNA extraction was conducted at the Human Genome Centre, University Sains Malaysia. Blood in the EDTA collection tubes, tubes and columns were arranged according to the sequence of procedure prior to the DNA extraction. Twenty microlitres of proteinase-K was added directly to 200µl of blood to digest proteins attached to the DNA. This was followed by additional 200µl of buffer AL (lysis buffer) to induce lysis of red blood cells. The sample was then incubated at 56°C for 10 minutes to ensure complete lysis of the red blood cells. After incubation, reaction mixture was centrifuged to collect the lysate at the bottom of the tube. Then, 200 µl of 96% ethanol (BDH Laboratory Supplies, England) was added to the reaction mixture and mixed by pulse-vortexing for 15 seconds, to ensure the precipitation of DNA. The whole volume of reaction mixture was then transferred carefully to QIAmp spin column (with 2 ml collection tube provided by manufacturer) and spun at 8000rpm for 1 minute before discarding the collecting tube containing filtrate.

The filter column was then transferred to another collecting tube before adding 500µl of buffer AW 1 (wash buffer 1) and spun again at 8000rpm for another 1 minute. The spin column was then placed into a new collection tube and the previous collection tube was discarded. Later 500µl of buffer AW 2 (wash buffer 2) was added and spin at 1400rpm for 3 minutes. The filtrate was then discarded and centrifuged again at 14000 rpm for 1 minute to ensure the removal of all the filtrate. The QIAamp spin column was then placed in a new 1.5 ml microcentrifuge tube and 200 µl of buffer AE (elution buffer) was added into the spin column to elute the DNA. Incubation for 5 minutes with elution buffer helped increase the

DNA yield. Buffer AE contained 10 mM Tris-Cl and 0.5 mM EDTA. Finally, the column was spin at 8000rpm for 1 minute before discarding the filter column.

The concentration and purity was obtained using spectrophotometer (BioPhotometer, Eppendorf, Hamburg, Germany) or nanodrop spectrophotometer (Ultroscope 2000, USA). The concentration of DNA and its purity ranged from 30 to 60 ng/µl (260/280 nm) and 1.6 to 1.9 (A_{260}/A_{280}) respectively. Three microlitres of genomic DNA was also selectively analyzed on 1% of agarose gel to ensure it is not degraded. DNA was then kept in -20°C freezer for long term storage.

3.2.3 *ADRB2* gene screening

ADRB2 gene screening was done on 100 glaucoma subjects and 100 control subjects. The reference sequence used for *ADRB2* gene was retrieved from the Gen Bank (accession no. M15169). A single tube multiplex PCR protocol was employed in this study. The entire procedure was conducted at the Human Genome Centre, Universiti Sains Malaysia. For each of the polymorphic loci, two parallel allele-specific reactions were carried out; one with a wild-type specific primer and the other with a mutation specific primer. The primers were initially designed to detect allelic variants at positions 16 (rs56964295), 27 (rs60374884), 164 (rs1800888) and at 5' Untranslated Region (UTR) for nucleotides at position -20 (rs1081704) and -47 (rs1042711) of the β2AR gene. The primers were specifically designed (between 20 to 30 nucleotides in length) to allow reasonable annealing temperature and with specific 3' ends to allow differentiation of single nucleotide changes at specific locus. The G and C contents in the designed primers were almost similar to ensure similar annealing temperatures for multiplex PCR reactions.

The primer sequences are given in Table 3.1 and the schematic diagram showing the amplification scheme is given in figure 3.1. A duplex PCR was carried out for primer Beta16A/G and Beta UTR-20C/T, which has been specifically designed to have almost similar annealing temperature while a triplex PCR was carried out for primer Beta27C/G, Beta164C/T and Beta UTR-47C/T. The primer Beta2-Fw was used as a common forward primer in both duplex and triplex PCR reactions while the Beta 16A/G, Beta UTR-20C/T, Beta 27C/G, Beta UTR-47C/T and Beta 164C/T primers were the reverse primers.

Table 3.1: Primers sequence for multiplex PCR

Primer	Sequence	Size (bp)	Primer concentrations (μM)
Beta2-Fw	5′CAC CAC AGC CGC TGA ATG AGG 3′		1.25
Set A			
Beta2-Fw	5′CAC CAC AGC CGC TGA ATG AGG 3′		1.25
Beta 16A	5′ GTC CGG CGC ATG GCT TCT 3′	179	0.25
Beta utr 20C	5′ CGC GCA GTC TGG CAG GTG 3′	114	0.25
Set B			
Beta2-Fw	5′ CAC CAC AGC CGC TGA ATG AGG 3′		1.25
Beta 16G	5′ GTC CGG CGC ATG GCT TCC 3′	179	0.25
Beta utr 20T	5′ CGC GCA GTC TGG CAG GTA 3′	114	0.25
Set C			
Beta2-Fw	5′ CAC CAC AGC CGC TGA ATG AGG 3′		1.25
Beta 27C	5′ CCA CAC CTC GTC CCT TTG 3′	212	2.5
Beta2 utr 47C	5′ CTG GGG GCG CCT CAG CG 3′	86	2.5
Beta 164C	5′ CTG AAT GGG CAA GAA GGA GG 3′	624	2.5
Set D			
Beta2-Fw	5′ CAC CAC AGC CGC TGA ATG AGG 3′		1.25
Beta 27G	5′ CCA CAC CTC GTC CCT TTC 3′	212	2.5
Beta2 utr 47T	5′ CTG GGG GCG CCT CAG CA 3′	86	2.5
Beta 164T	5′ CTG AAT GGG CAA GAA GGA GA 3′	624	2.5

Reproduction with permission from Zilfalil et al (2006)

The PCR protocol was adapted from the protocol by Zilfalil et al (2006) and was optimized for the PCR compositions and reaction conditions to produce specific bands for both duplex and triplex PCR. For duplex PCR, the final optimized protocol includes a reaction mixture comprised of 1X PCR Buffer (Promega, Madison,WI, USA), 1.5 mM $MgCl_2$, 2 U *Taq*

polymerase (Promega, Madison,WI, USA), 0.25 mM dNTPs, approximately 60 ng genomic DNA, 1.25 µM Beta2-Fw and 0.25µM of each primer in 20 µl total reaction volume.

PCR amplification was performed using PTC200 MJ Research (USA) with pre-denaturation at 95^0C for 3 min, followed by 35 cycles of denaturation at 95^0C for 1 min, annealing at 55^0C for 1 min, extension at 72^0C for 1 min 30sec and final extension at 72^0C for 5 min. The expected fragment sizes for each allele in the duplex PCR reaction was 179bp for allele 16 and 114bp for allele -20 (Figure 3.1).

For triplex PCR, the final optimized conditions comprised of 1X PCR Buffer (Promega, Madison, WI, USA), 1.5 mM $MgCl_2$, 2 U *Taq* polymerase (Promega, Madison, WI, USA), 0.25 mM dNTPs, approximately 60 ng genomic DNA and appropriate primer concentrations in a total volume of 25 µl.

PCR amplification was performed using thermal cycler PTC200 MJ Research, (USA) with pre-denaturation at 95^0C for 3 min, followed by 25 cycles of denaturation at 94^0C for 1 min 30 s, annealing at 62^0C for 40 s, extension at 72^0C for 40 s and final extension for 72^0C for 7 min. The expected fragment sizes for each allele in the triplex PCR reaction was 624bp for allele 164, 212bp for allele 27 and 86bp for allele -47. Triplex PCR reaction was the most challenging part as several of the samples required multiple repeated procedures due to poor quality of gel electrophoresis picture.

Two percent agarose gel was prepared using a mixture of 1g agarose powder and 50ml of electrophoresis buffer, Tris-Base, Borate and EDTA (TBE). After 3 minutes of heating in the microwave, SYBR® Green dye (Qiagen, USA) was added to facilitate visualization of the

band. After solidification of the agarose gel, 10µl of PCR product and loading buffer 1X TBE was then loaded into the well.

Direct sequencing was done on selected samples to reconfirm the findings. Big Dye 3.1 was used according to manufacturer's instruction**.** The purified PCR product was used as template for cycle sequencing. Twenty microlitres reaction contained of 7µl of ddH_2O, 4µl of 5X sequencing buffer, 2µl Big Dye terminator v3.1, 4µl of 5µM of either forward or reverse primer and 3µl of 50ng/µl purified PCR product was subjected to cycle sequencing reaction, which began with initial denaturation at 96^0C for 1 min. Then, it was followed by rapid thermal ramp (1^0C/sec) at 96^0C for 10 sec, 50^0C for 5 sec and 60^0C for 4 min and this step was repeated for a total of 25 cycles. Samples were held at 4^0C before proceeding to ethanol/EDTA precipitation method for purification of the DNA from aqueous solution.

Ethanol precipitation, also known as EDTA precipitation was conducted to facilitate production of consistent signal and to remove the unincorporated dyes. The entire volume of cycle sequencing product was then transferred to 1.5ml microcentrifuge tube and 5 µl of 125 mM EDTA was then added. Extra care was taken to ensure that EDTA reached the bottom of the tube. First volume (60µl) of 100% ethanol was then added to the tube, mixed well by inverting the tube 4 times and left to incubate at room temperature for 15minutes. Later, the reaction mixture was centrifuged at 1300rpm for 15minutes at 4°C (Eppendorf 5415R, Asia Pacific; maximum relative centrifuge force 16,110 x g). The supernatant was carefully removed from the tube with extra care not to reach the bottom of the tube, avoiding accidental removal of DNA.

The second volume (70µl) of 100% ethanol was then added and mixed thoroughly. The reaction mixture was then centrifuged again at 13000rpm for 15minutes at 4°C and the supernatant was carefully removed again. The final step was the addition of 60µl of 70% ethanol to the pellet and mixed thoroughly to break the pellet loose and wash it to remove some of the remaining salts. The reaction mixture was centrifuged again at 1300rpm for 15minutes at 4°C and supernatant was removed again. The pellet was then air-dried using vacuum regulator (Bio-Rad, CA, USA) for 30 minutes and resuspended in 20µl of Hi-Di formamide (Applied Biosystem, Warrington, UK). The pellet was stored at 4°C.

Prior to the sequencing, 10µl of the pellet was then denature for 5 minutes and placed in the ice (-70°C) for 3 minutes. The samples were then placed in the ABI Prism 3100 Genetic Analyzer sequencer (Applied Biosystem, California, USA).

3.2.4 *PTGFR* screening

The examination of PTGFR gene was conducted on 180 samples; 90 glaucoma subjects and 90 unrelated age, sex and race matched controls. A total of 95 pairs of primers were designed to cover the entire PTGFR gene including 3000bp upstream of 5'UTR and 1000bp downstream of 3'UTR (Table 3.2 - 3.5). The reference sequence was based on Ensembl ENSG 00006122420 and NCBI accession number AL136324.6. Sixteen primers were designed for all 4 exons including 1000bp 3'UTR. Four primers were designed for Intron 1, 12 primers for Intron 2 and 59 primers for Intron 3. Detection of microsatellite instability (MSI) or short tandem repeat (STR) was done using special tagged forward primers. Promoter region was the most challenging part with more than 10 primers was designed.

Critical criteria such as the primer length, melting temperature (T_m), specificity, complementary primer sequences, G/C content and 3'-end sequence was strictly followed when designing the primer. The primers were designed between 20 and 28 nucleotides in length; there was no increase in specificity with primers longer than 30 nucleotides. The melting temperature for the pairs of primers was designed to be from 50^0C to 60^0C. The GC content of each of the primer was designed to have a 40 to 60% GC content. In addition, primers were designed to be exactly complementary to the template DNA and there was no complementarity of their 3' ends with the other primers to minimise the chances of primer-dimer formation.

The screening of the PTGFR gene was done in stages at the Human Genome Centre, Universiti Sains Malaysia and Department of Genetics, Institute of Ophthalmology, University College London. Direct sequencing technique was used in screening exons and introns. Genotyping was conducted to detect the presence of MSI or STR. The primers sequence used to screen the PTGFR gene are given in table 3.2-3.4.

Table 3.2: Primers sequence for exons screening of *PTGFR*

Exon	Primer	Sequence	Size (bp)	*T_a (^{0}C)
1	PGFe1-1F	5'cgagagagaagaggaagagg 3'	347	50
	PGFe1-1R	5'ctgggtctagataagcgaag 3'		
2	PGFe2-1F	5'gaaccgcaggcagatatgag 3'	428	55
	PGFe2-1R	5'aacgatgccttggacttctg 3'		
	PGFe2-2F	5'agaagtccaaggcatcgttt 3'	194	55
	PGFe2-2R	5'aagaagtgggcacagaccag 3'		
	PGF8246F	5'ttggtatctgcatggtgt 3'	260	55
	PGF8246R	5'ttgatgtcttctgtgttgtag 3'		
	PGFe2-3F	5'acatcaaagactgggaagatag 3'	213	50
	PGFe2-3R	5'tccaacaaatacaggagacac 3'		
3	PGFe4-1F	5'actggaaggccatatgtttgtt 3'	171	54
	PGFe4-1R	5'ccttaggaaaatcaagctccaa 3'		
4	PGFe3-1F	5'tcattgatttctttctgtcagtat 3'	454	50
	PGFe3-1R	5'ccacacagatttactgtcctatta 3'		
	PGFe3-2F	5'ataggacagtaaatctgtgtgg 3'	480	50
	PGFe3-2R	5'gcatgtgttaattgaggctac 3'		
	PGFe3-3F	5'ggctcagtaaaataaagcactc 3'	453	50
	PGFe3-3R	5'acctatcattggcatgtagc 3'		
	PGF899F	5'aagctacatgccaatgataggtg 3'	331	55
	PGF899R	5'ttggaaaatttggggagaga 3'		
	PGFe3-4F	5'caagcacttggggattatta 3'	503	50
	PGFe3-4R	5'ccctgaatgagagtttcttct 3'		
	PGFe3-5F	5'tggagaagaaactctcattcag 3'	423	50
	PGFe3-5R	5'acagtaaatcgccaagctac 3'		
	PGFe4-6F	5'gtgcacatctgacttaagagtt 3'	376	50
	PGFe4-6R	5'acacctgtaaaaatcctgac 3'		
	PGFe4-7F	5'gtaaggcattatccaagcaac 3'	445	55
	PGFe4-7R	5'aaactcagagtaggcacaaaac 3'		
	PGFe4-8F	5'tccctagaggcagaaagttag 3'	362	50
	PGFe4-8R	5'gtccaacattatttaccaggtg 3'		
	PGFe4-9F	5'tagcttcacctgtatacgatca 3'	453	50
	PGFe4-9R	5'gaaattcttctcatccagtagc 3'		

Table 3.3: Primers sequence for Intron 1 and 2 screening of *PTGFR*

Intron	Primers	Sequence	Size (bp)	T_a (^{0}C)
1	IVS1-1F	5'cgagagagaagaggaagagg 3'	471	50
	IVS1-1R	5'gttagctcgagtacccttcttt 3'		
	IVS1-2F	5'catagagaaagaagggtactcg 3'	465	50
	IVS1-2R	5'ccactcttctctgacctaattc 3'		
	IVS1-3F	5'gaattagctcagagaagagtgg 3'	386	50
	IVS1-3R	5'ttgtcaggcagacataactatc 3'		
	IVS1-4F	5'ggagatagttatgtctgcctga 3'	483	50
	IVS1-4R	5'catctgtaggstaaagggagag 3'		

2	IVS2-1F	5’cgataatgtgtgtctcctgtat 3’	414	50
	IVS2-1R	5’gagaacccagtagctaatatgc 3’		
	IVS2-2F	5’gaacagattgcagtaagtcttg 3’	471	50
	IVS2-2R	5’ggcatgttgtcacttcttaca 3’		
	IVS2-3F	5’gacaacatgccatgaaagaa 3’	222	50
	IVS2-3R	5’gcctaggaaaactgcattagta 3’		
	IVS2-4F	5’agacaaaggtagtggttgtacc 3’	487	50
	IVS2-4R	5’cagtagcagctatcacaaactt 3’		
	IVS2-5F	5’gtgcagttctaattaacgatcc 3’	405	50
	IVS2-5R	5’agtggctccagtgttgttag 3’		
	IVS2-6F	5’ccagtaagagtctgatctgtga 3’	465	50
	IVS2-6R	5’cctcaaagtagaaacccagtaa 3’		
	IVS2-7F	5’agagacataatccggacaact 3’	498	50
	IVS2-7R	5’ctcaactctccataaaagaagc 3’		
	IVS2-8F	5’gatccaccacaggtattacttt 3’	418	50
	IVS2-8R	5’gctgtcactattacagcacaaac 3’		
	IVS2-9F	5’tagattcccatagttgtgctgt 3’	451	50
	IVS2-9R	5’gacccaaaactactcagaaaag 3’		
	IVS2-10F	5’tttgtgcttgacaggaactact 3’	470	50
	IVS2-10R	5’attctgcatgatcctgtaatgg 3’		
	IVS2-11F	5’cattagggctatctctttgtgt 3’	469	50
	IVS2-11R	5’agagacatcaggagaatgtgtt 3’		
	IVS2-12F	5’aacacattctcctgatgtctct 3’	516	50
	IVS2-12R	5’cttgggtaaactcacctatgta 3’		

Table 3.4: Primers sequence for Intron 3 screening of *PTGFR*

Intron	Primer	Sequence	Size (bp)	T_a (^{0}C)
3	IVS3-1F	5’taccttctcttcagggatacag 3’	309	50
	IVS3-1R	5’gtcattagccctttaagacttg 3’		
	IVS3-2F	5’ggagtaacatgacatcatagca 3’	408	50
	IVS3-2R	5’acccactagaatccctcttatt 3’		
	IVS3-3F	5’cttggaatataggtctttcagg 3’	477	50
	IVS3-3R	5’gggatatttgagagaacagaga 3’		
	IVS3-4F	5’tcctctatctgggctatcttta 3’	473	50
	IVS3-4R	5’cttcagaaagtccaagattctc 3’		
	IVS3-5F	5’cttggtggctttactctacatt 3’	493	58
	IVS3-5R	5’gtctgtcggatttttttgag 3’		
	IVS3-6F	5’actatctccctccataaaagt 3’	425	50
	IVS3-6R	5’taaagctaaaactggcaacc 3’		
	IVS3-7F	5’gtactcagagaggacttcatcc 3’	336	50
	IVS3-7R	5’gaagacttgacttgggactatg 3’		
	IVS3-8F	5’ccacttcttagctatgacctta 3’	636	58
	IVS3-8R	5’aggaaacagaggcacagagt 3’		
	IVS3-9F	5’gtatccacttcatagggtggt 3’	420	50
	IVS3-9R	5’cagtttcctgcatatctcttct 3’		
	IVS3-10F	5’ggaagtgtgaaaatgagaagag 3’	532	50
	IVS3-10R	5’gaaggagatagaaagcatgagt 3’		

IVS3-11F	5’acagacgtcttctcctttattg 3’	358	50
IVS3-11R	5’gctatccaaacagttcttgc 3’		
IVS3-12F	5gttagccagttgtcctcaaat 3’	321	50
IVS3-12R	5’atgcaactaatttgcctcac 3’		
IVS3-13F	5’cctaacagcaacgtgcgagtgtccc 3’	667	60
IVS3-13R	5’gaaccaacagagactgactccagg 3’		
IVS3-14F	5’ctaagaatatagagttctttttggc 3’	648	50
IVS3-14R	5’ggccatactggaatgaaataccac 3’		
IVS3-15F	5’ggcacagagtttatagctgttgagtg 3’	695	50
IVS3-15R	5’cccacactgaaacaaattccaaag 3’		
IVS3-16F	5’ccttcctgtgtagttacttatggg 3’	758	50
IVS3-16R	5’caatgaccctctgggttaagaagg 3’		
IVS3-17F	5’gagttgttccaatgggaatttgggcag 3’	489	50
IVS3-17R	5’cctgtattctggtagctgtttcagttc 3’		
IVS3-18F	5’actccggaattctgacatta 3’	331	50
IVS3-18R	5’cagacaaaaaggatatgctagg 3’		
IVS3-19F	5’gtggtaaaggtgactgaatga 3’	598	56
IVS3-19R	5’cccttcctctttcttactctct 3’		
IVS3-20F	5’caggagtggtggcaggag 3’	623	60.2
IVS3-20R	5’gactgattaaatggttgtgacg 3’		
IVS3-21F	5’ctcagagggataactcactcat 3’	611	50
IVS3-21R	5’ccattttacagttccaaagc 3’		
IVS3-22F	5’cggacaaagaaggacaagctgatgagg 3’	645	59.6
IVS3-22R	5’ctacaggcttaacaccatacg 3’		
IVS3-23F	5’ctgtggctcaaagggcctcaggcagat 3’	482	60.2
IVS3-23R	5’agagtacactgatgcaaggagt 3’		
IVS3-24F	5’gtaaacccaatgtctgtataccc 3’	760	50
IVS3-24R	5’cctcctgactgtttcccacacattcc 3’		
IVS3-25F	5’acgtccttatagctgtggttat 3’	341	50
IVS3-25R	5’ggtaaaccctctgaacatacat 3’		
IVS3-26F	5’aatgccctcagtctgataact 3’	500	50
IVS3-26R	5’ggacatctatctttggctctt 3’		
IVS3-27F	5’accataccaaggcacttaaa 3’	647	50
IVS3-27R	5’tagatcacacctttcccttg 3’		
IVS3-28F	5’gctttcaacctagatgacagat 3’	268	50
IVS3-28R	5’ctttgctaaaccaacagagaag 3’		
IVS3-29F	5’gttggtttagcaaaggacat 3’	528	50
IVS3-29R	5’tctctgctcatgttttacac 3’		
IVS3-30F	5’atatcacagctcccatcatt 3’	450	50
IVS3-30R	5’gagataagctttccactctcag 3’		
IVS3-31F	5’tagcctattgtagtgtgcagaa 3’	265	50
IVS3-31R	5’gaatcatggccataacagtaac 3’		
IVS3-32F	5’ccaaccagacactattaccttt 3’	462	50
IVS3-32R	5’cttctgctcttaggagattgtc 3’		
IVS3-33F	5’gggatcaatcatggttaaag 3’	410	50
IVS3-33R	5’atgggaaattaagagagaggag 3’		
IVS3-34F	5’ctgtctgctcaggtaggatagt 3’	350	50
IVS3-34R	5’cgtatgtaccagatgaacaaga 3’		
IVS3-35F	5’gtatgatgctcttgttcatctg 3’	419	50
IVS3-35R	5’gtgcccactattactactaccg 3’		

IVS3-36F	5’gtaagtgtaatggctgttgtagg 3’	501	50
IVS3-36R	5’ggcagataactttctctagctg 3’		
IVS3-37F	5’gcttgtccaaccagacttatt 3’	485	50
IVS3-37R	5’ctaggaatgcttgcatataagg 3’		
IVS3-38F	5’gcttgttggagattgcttac 3’	306	50
IVS3-38R	5’ccaacatactgactacctgtga 3’		
IVS3-39F	5’gtggagtcgctactattgagtat 3’	492	50
IVS3-39R	5’gtattgccagtgagaaagaaag 3’		
IVS3-40F	5’ctttctttctcactggcaatac 3’	342	50
IVS3-40R	5’catttgtccaaagtgtagagc 3’		
IVS3-41F	5’gtatttgacccaagataaccac 3’	504	50
IVS3-41R	5’ctcaccataggagtctgttctt 3’		
IVS3-42F	5’aaaagaacagactcctatggtg 3’	551	50
IVS3-42R	5’gctaattccagaagactacttcc 3’		
IVS3-43F	5’caattggaagtagtcttctgga 3’	315	50
IVS3-43R	5’atactccacagcctctcataaa 3’		
IVS3-44F	5’tgtgaggtgtaaattgacagag 3’	570	50
IVS3-44R	5’ccactagagagaaaatttcagc 3’		
IVS3-45F	5’atacattaccacaagacatggt 3’	324	50
IVS3-45R	5’aggccatcaatctaacagcctat 3’		
IVS3-46F	5’caggaactagatgaaagttggt 3’	549	50
IVS3-46R	5’attctccaggtaacagcctat 3’		
IVS3-47F	5’ggtaatcatatctggaatgagc 3’	436	50
IVS3-47R	5’tgcctggacatctaactatgta 3’		
IVS3-48F	5’cacagaagaccaacacatacat 3’	428	50
IVS3-48R	5’ggaggtacaatgattcagga 3’		
IVS3-49F	5’ccttttattcctgaatcattgtacctcc 3’	708	50
IVS3-49R	5’atgaaactgttccacctcag 3’		
IVS3-50F	5’actagacataggcagatggaac 3’	579	50
IVS3-50R	5’gtgtgacaccactcctttaata 3’		
IVS3-51F	5’gaggtttgagatactgacaggt 3’	370	50
IVS3-51R	5’atagaccttacatctccccata 3’		
IVS3-52F	5’cagaccagaagttaaaggtcat 3’	402	50
IVS3-52R	5’gtgtgacaccactcctttaata 3’		
IVS3-53F	5’cacacaatcaatacagtttggc 3’	628	50
IVS3-53R	5’cagctagctccttgatgatgcatctg 3’		
IVS3-54F	5’catgcctggctagtaaattc 3’	476	50
IVS3-54R	5’ctaaatgtcagcctttcaca 3’		
IVS3-55F	5’agtgctgagactttcttctcac 3’	440	50
IVS3-55R	5’gatgttatcgcatcatctctc 3’		
IVS3-56F	5’ggtagctcttggaaactgtatg 3’	574	50
IVS3-56R	5’gccaaactgtattgattgtg 3’		
IVS3-57F	5’cacacaatcaatacagtttggc 3’	628	50
IVS3-57R	5’cagctagctccttgatgatgcatctg 3’		
IVS3-58F	5’gtccaagcttcacttttcag 3’	600	50
IVS3-58R	5’aactgaccttctcatacttctg 3’		
IVS3-59F	5’tctcagaagtatgagaaggtca 3’	317	50
IVS3-59R	5’ccattgtaacctaga aacgaag 3’		

3.2.4.1 Screening of *PTGFR* exons

Screening of the exons was conducted at the Human Genome Centre, Universiti Sains Malaysia. The designed primers were prepared by 1stBASE, Singapore and sent to Human Genome Centre in wet format. The primers were then diluted to 100µM by adding purified water according to the instruction by the company. Genomic DNA was used as template for PCR amplification by using specific oligonucleotide primers in 20µl reaction, which contained 1.875 mM $MgCl_2$ (Applied Biosystems, California, USA), 1X GeneAmp PCR Buffer II (Applied Biosystems, California, USA), 0.375 mM dNTPs (Applied Biosystems, California, USA), 0.2 µM of each forward and reverse specific primers, 4 ng/µl of template DNA and 0.05 unit of Ampli*Taq* Gold DNA Polymerase (Applied Biosystems, California, USA). PCR was conducted using Eppendorf Mastercycler Gradient (Eppendorf, Hamburg, Germany). Predenaturation process was set at 96°C at 5minutes then followed by 30 cycles of denaturation at 95°C for 1 minutes, specific annealing temperature according to primers (Table 3.2) for 1 minutes and 90 seconds (1 min 30sec) of primary extension at 72°C. The chain reactions ended with final extension at 72°C for 7 minutes. The presence of amplicons was detected using 2% agarose gel electrophoresis.

The amplicons were then purified using GENE ALL PCR purification Kit (General Biosystem, Seoul, Korea). Five volume of buffer PB (PCR purification buffer) was added to a volume of the PCR product (e.g. 75µl of buffer PB was added to 15µl of PCR product) in 1.5ml tube and thoroughly mixed. The mixture was then pipette into GENE ALL spin column and placed in collection tube. DNA was bound selectively to a glassfiber membrane in a GENE ALL Spin Column due to the high concentration of salt in the buffer PB. The mixture was then centrifuged at 13,000 rpm for 30 seconds to accelerate the process where DNA binds to the filter of the column. The flow through was discarded and the spin column was

reinserted into the same collection tube to reduce the plastic waste. Later, 700 µl Buffer NW (column wash buffer N) was added into the spin column, followed by centrifugation at 13,000 rpm for 30 seconds. The flow through was then discarded and the spin column was reinserted back into the collection tube. The spin column was centrifuged for an additional 2 minutes to remove residual wash buffer and eliminate the residual ethanol from buffer NW that might inhibit subsequent enzymatic reaction. A series of rapid "wash and spin" steps removed the contaminating small molecule such as primers, nucleotide and salts while DNA remained bound to the glassfiber membrane. After wash and spin process, the spin column was then transferred into a new 1.5 ml micro centrifuge tube.

Finally, the purified DNA was eluted by a low salt buffer from a glassfiber membrane by adding 50 µl of buffer EB (elution buffer) into the spin column. The elution volume was also reduced to 30 µl for certain samples for higher concentration of elute. It is important to dispense buffer EB directly onto the centre of spin column membrane to ensure the optimal DNA elution. Then, the spin column was allowed to stand for about 1 min and centrifuged at 13,000 rpm for 1 min. The purified DNA was stored in buffer EB at -20^{0}C for long-term storage.

The purified PCR product was used as template for cycle sequencing. Twenty microlitres reaction contained of 7µl of ddH_2O, 4µl of 5X sequencing buffer, 2µl Big Dye terminator v3.1, 4µl of 5µM of either forward or reverse primer and 3µl of 50ng/µl purified PCR product was subjected to cycle sequencing reaction, which began with initial denaturation at 96^{0}C for 1 min. Then, it was followed by rapid thermal ramp (1^{0}C/sec) at 96^{0}C for 10 sec, 50^{0}C for 5 sec and 60^{0}C for 4 min and this step was repeated for a total of 25 cycles. Samples

were held at 4^0C before proceeding to ethanol/EDTA precipitation method for purification of the DNA from aqueous solution. Ethanol precipitation technique was explained in detail in 3.2.3. The purified product was then placed in the ABI Prism 3100 Genetic Analyzer sequencer (Applied Biosystem, California, USA).

3.2.4.2 Screening of *PTGFR* introns

Majority of the screening work on *PTGFR* introns was conducted at the Department of Genetics, UCL Institute of Ophthalmology, UK with some additional work conducted in Human Genome Centre, Malaysia. The primers designed for introns 1 and 2 were prepared by 1stBASE, Singapore. The rest of the primers were prepared by Sigma Aldrich, UK. The primers prepared by Sigma Aldrich, UK were in dry format. The primers were centrifuged before adding purified water according to instruction, and centrifuged again. The stock primers were kept in -20°C. For PCR amplification 1:20 primer concentration was used. In order to facilitate the screening of the large introns, premix extensor hi-fidelity PCR master mix (Thermo Scientific, ABgene, United Kingdom) with or without the dye (ReddyMix) was used. Ready to use master mix eliminated the need to thaw individual components, indirectly reduced the risk of contamination and pipetting errors. The premix contained buffer 2, which is recommended for longer than 12kb or problematic amplifications, 500µM of dNTPS, 2.25mM magnesium chloride and 1.25U ThermoPrime plus *Taq* DNA polymerase. ThermoPrime has the ability to cause 5' to 3' polymerisation and exonuclease activity but lack of 3' to 5' exonuclease activity. A total reaction volume of 17µl, which contained 12µl of premix extensor hi-fidelity PCR, 4µl of 0.2µM forward and 0.2 µM reverse primers and 1µl of genomic DNA was used for PCR amplicification. PCR amplification was done using Techne TC-500 thermal cycler (Techne, Burlington, USA) with predenaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30seconds, specific annealing

temperatures according to primers (table 3.3 and 3.4) and primary extension at 72°C at 1 minute, and final extension at 72°C for 7 minutes. The presence of amplicons was determined by 2% gel electrophoresis.

The amplicons were then purified using ExoSAP-IT (USB, Affymetrix, USA), which contains two hydrolytic enzymes; Exonuclease I and Shrimp Alkaline Phosphatase in a special formulated buffer. Exonuclease I removed residual single-stranded primers and any extraneous single-stranded DNA produced during PCR process, while Shrimp Alkaline Phosphatase removed the remaining dNTPs from the PCR product. One microliter of ExoSAP-IT reagent was added into 13.5µl of purified water and later added directly into 1µl of PCR product. The reaction mixture of 15µl in volume was incubated at 37°C for 15 minutes and inactivated by heating at 80°C at 15 minutes in thermal cycler. The purified PCR product was then ready for cycle sequencing.

The purified PCR product was used as template in cycle sequencing step. The forward and reverse primers were diluted into 10ng/µl from 100ng/µl in concentration by adding 9µl of purified water into 1µl of 100ng/µl primer dilution. One microliter of diluted 10ng/µl forward or reverse primer was then added into the whole volume of purified PCR product together with 0.5µl of Big Dye terminator v3.1 and 3.5µl of 5X sequencing buffer. Twenty microlitres of the reaction mixture was then subjected for 30 cycles of cycle sequencing reaction, which began with denaturation at 96°C for 30 seconds, annealing at 50°C for 30 seconds and primary extension at 60°C for 4 minutes.

The cycle sequencing product was then subjected for multiwell unit purification procedure using gel filtration with Sephadex G-50 superfine beads (SigmaAldrich, Sweden). The

Sephadex plate was prepared prior to the procedure. A multiscreen 96 wells HV plate with durapore PVDF MSHVN 4550 (Millipore, USA), multiscreen column black loader plate MACL 09645 (Millipore, USA), sliding scraper and distilled water were used in this cleaning up procedure. The dry Sephadex beads were poured into the multiscreen column black loader plate and spreaded evenly using the sliding scraper. The sliding scraper was then slided on top of the column loader plate and tapped hard to ensure that the beads filled up each well evenly. The excess of resin was then removed. The multiscreen 96 wells HV plate with durapore MSHVN 4550 was then placed on top of the column black loader plate and inverted upside down. Adequate tapped was given to all side of the plate to ensure that all the beads have fallen into the wells of multiscreen plate. Once all the wells have already filled up with Sephadex beads and 350µl of distilled water was then pipette using multichannel pipetor into each well to expand the resin and referred as Sephadex plate. The Sephadex plate was then allowed to set for 30 minutes (can be left in the room temperature up to 3 hours) and ready to be used for sequencing reaction clean up. The plate can be wrapped with damp tissue and cling film to be used later. It is best to store in 4°C fridge for long term usage.

The 96 wells collection plate was placed under the Durapore membrane of the pre-prepared Sephadex plate. For the plate that has been kept in the 4°C, 100µl of distilled water was added to each well. Each wells of the collection plate must be placed directly below the well of the pre-prepared Sephadex plate containing expanded resin. Both plates were then centrifuged at 910gm for 3 minutes. The excess liquid from the collecting plate was then discarded, the empty collecting plate was again placed under the Sephadex plate and 100µl of distilled water was then added into each well of the Sephadex plate. Both plates were than centrifuged again at 910gm for 5 minutes. The excess liquid in collecting plate must be of similar volume in all 96 wells before discarding them again. Finally, 10µl of sequencing

reaction volume was placed using multichannel pipetor at the centre of each well in the Sephadex plate and 12µl of distilled water was then added. A new 96 wells of PCR microplate with elevated skirt (Axygen, USA) was then placed under the Sephadex plate. A cellophane tap was placed to temporarily bind and aligned both plates and to ensure that each well was properly placed. Both plates were centrifuged again at 910gm for 5 minutes; the purified sequencing product collected in the PCR microplate was then ready to be loaded into the sequencer. ABI Prism 3730xl sequencer Genetic Analyzer (Applied Biosystem, California, USA) was used for sequencing.

3.2.4.3 Screening for microsatellite instability (MSI) in Intron 3 of *PTGFR*

Two areas of MSI or short tandem repeats (STRs) were identified within primer sequence of IVS 3-29 and IVS 3-53 while screening for polymorphisms in introns 3. MSI is a repeat in the number of due to defects in DNA repair. 'CA' repeats are the commonest MSI, which was found in IVS 3-29 and 'TA' repeats were found within the IVS3-53 sequence. Two forward primers were designed and chemically labelled with fluorescent dyes (6 FAM™) to amplify this region (Table 3.5). The reverse primers were not labelled. Generally, the G and C content of the primers were designed between 50 to 55%, closely matched to melting temperature and not exceeding 18 bases. However, in both of the primers in our MSI, the G and C contents were less than 50%. Thus, the designed primers were extended to ensure the melting temperature was less than 45°C and minimized the chances of secondary hybridization. The G or C bases were designed not to exceed more than 2 in the last 5 bases at the 3' end of the primer to avoid mispriming. The primers used for amplification are given in Table 3.5. All primers were prepared by SigmaAldrich, UK.

Table 3.5: Primers sequence for MSI screening of IVS 3-29 and IVS 3-53

Primer	Sequence	Size (bp)	Ta°C
IVS 3-29 MSI	5' (6FAM)ccactgcagcatttgtcattacag 3' 5' ctaacatgcaatacatgctctg 3'	120	57
IVS 3-53 MSI	5' (6FAM) ctgaggctccagaactggaagtttatgcc 3' 5' gggtaacagagttgagactctgtctc 3'	289	60

The primers were used at 100ng/µl. Earlier attempt to use qPCR master mix (Finnzymes, Finland) for amplification of the genomic DNA failed, instead Reddy extensor hi-fidelity PCR master mix (ThermoScientific, Abgene, UK) was used and proven successful. The total reaction volume of 17µl that consisted of 12µl of premix extensor hi-fidelity PCR master mix, 2µl of 0.2µM FAM labelled forward primer, 2µl of 0.2µM reverse primer and 1µl of genomic DNA was used for PCR amplification. The amplification was done using the Techne TC-500 thermal cycler (Techne, Burlington, USA) with predenaturation at 96°C for 15 minutes, followed by 30 cycles of denaturation at 96°C for 30seconds, annealing temperatures according to primers (table 3.5) for 30seconds and primary extension at 72°C at 30seconds, and final extension at 72°C for 5 minutes.

Passive reference dye Genesan™ROX™ 500(Applied Biosystem, USA) was used to label the reaction for quantitative analysis of MSI. The reference dye ROX™ 500 was diluted with high deionised Hi-Di™ formamide 1 in 50; 1000µl of HiDi formamide was added in every 20µl of ROX™. The diluted reference dye was then added to the PCR product and subjected to heat denaturation at 95°C for 3 minutes before placing it on the ice for another 2 minutes.

The reaction mixture was then loaded in the sequencer machine ABI 3730 for genotyping procedure. Analysis of quantification of MSI was done using Genemapper® 4.0 software.

Genemapper®4.0 is designed to identify peaks in predefined 'bins' and ranges, where each bins represent a possible allele within a marker.

3.2.4.4 Screening for polymorphisms in the *PTGFR* promoter region

The promoter region of PTGFR gene was the most challenging part in screening of the entire PTGFR gene. After several fruitless attempts of DNA amplification, the amplification of the genomic DNA was eventually successful using KOD hot start DNA polymerase (Novagen®, Darmstadt, Germany). KOD hot start DNA polymerase is a premixed complex of high fidelity KOD DNA polymerase and two monoclonal antibodies that inhibit the DNA polymerase and 3'-5' exonuclease activities at ambient temperature. KOD DNA polymerase contains enzyme produced by *Thermoccus kodakaraensis* that has the elongation capability of 106 to 138 bases per second and ability to extend more than 300 nucleotide bases in one catalytic reaction by one DNA polymerase molecule. It is ideal for amplification of difficult region and GC-rich target region.

Primers were specifically designed to exceed 21 bases of 3' end complementary to the target sequence due to the strong activity 3'→5' exonuclease activity in KOD hot start DNA polymerase after thermoactivation. The G/C content of the designed primers was between 40 to 60% to ensure better amplification result. Primers PRMLF3 and PRMR2 covered 3219 base pairs upstream from exon 1 of *PTGFR* gene.

Table 3.6: Primers sequence for promoter region screening of PTGFR

Primer	Sequence	Size (bp)	Ta°C	Notes
PRMLF3	5'ccctctctcatcactcgtattccacatag 3'	3219	60	PCR amplification
PRMR2	5'cagcctctggagggatggtaccttg 3'			
PRMF4	5'cacataggtagtggcaacattttac 3'			Sequencing
PRMLF1	5'ctggaccccattccttataccttatac 3'			Sequencing
PRMSEQF1	5'catagaaccaacccaaatgcccatc 3'			Sequencing
PRMLR4	5'gcatagtttgaagtcaggtagcatgatg 3'			Sequencing
PRMLR3	5'gctgaggatgatagcatccagg 3'			Sequencing

The final optimised concentration contained 0.1mM of dNTPs, 1mM of magnesium sulphate, 0.12ml of 10X PCR buffer, 4U of KOD hot start DNA polymerase, 1.5µl of 0.15µM forward and reverse primers respectively and 100ng of genomic DNA in 25µl of total volume reaction. The PCR amplification was conducted with predenaturation at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 30seconds and primary extension at 72°C for 65seconds. Lastly, final extension at 72°C for 3 minutes 20seconds.The duration of primary extension was based on 20seconds for every 1000bases and final extension of 1 minute for every 1000bases. The amplicons were detected in 1% agarose gel. The PCR products were then sequenced as described in section 3.2.4.2.

3.2.4.5 Statistical analysis

The genotype findings for both genes were documented in SPSS PAWS 18.0. Analysis was conducted using SPSS PAWS 18.0 and STATA version 11.0. Allele frequency was calculated based on Hardy Weinberg equilibrium (HWE). HWE is the situation where, in large randomly mating population with a closed gene pool, the allele frequencies remain constant from generation to generation. Allele frequency is defined as the proportion of a particular type of allele to the total of all alleles at this genetic locus in a population. For example, the allele frequency of A in a population of AA (homozygous dominant), Aa (heterozygous) and aa (homozygous recessive), frequency of allele A is calculated as the

summation of frequency of homozygous AA times two and frequecncy of Aa, and divided by total allele (A and a) in the population. The inclusion of two subpopulation; Malays and Chinese in this present study may give rise population stratification. Genomic control analysis was used to detect the possibility of spurious associations or type-1 and type-2 errors due to confounding variables such as sex and race with polymorphisms and disease (Devlin and Roeder, 1999). Genomic control analysis is based on Bayesian outlier test as means of determining which markers exhibit significant linkage disequilibrium with the disorder without the need for Bonferroni correction for multiple tests (Devlin and Roeder, 1999). This analysis is recommended for analysing the possibility of a dense set of SNPs in susceptibility to complex disease such as glaucoma. The inflation factor λ was determined by selecting unrelated SNPs. The selected SNPs must not have vast different mutation rates, not strongly influenced by sub-population specific selection and vary greatly across loci (Chakraborty and Jin, 1992).

Trend test was adopted to look into the trend of susceptibility of certain SNPs to glaucoma. Stratified meta-analysis was conducted to determine the possible association of allele frequency of each of the polymorphism found in ADRB2 and PTGFR between glaucoma and control subjects. The analysis was conducted separately between Malays and Chinese. Breslow-Day test for heterogeneity was used to identify the heterogeneity between the two ethnic groups (Ioannidis et al, 2001). Stratified Cochran Mantel-Haenszel analysis was then conducted to determine the possible increase or decrease susceptibility to glaucoma (Cochran, 1954; Armitage, 1955). Univariate logistic regression was conducted to determine the relationship of predictor variable including age, sex, race and polymorphisms found in each gene to the criterion variables; glaucoma versus control. The ‘goodness of fit’ of the final regression model was checked using the Hosmer- Lemeshow test. Stepwise logistic

regression was then conducted to identify the SNPs that may confer protective or predisposition effect on susceptibility to glaucoma in Malaysian population. The final model was obtained using the likelihood ratio based on maximum likelihood estimate.

Haploview programme (http://www.broad.mit.edu/mpg/haploview/) was used to determine the presence of haplotypes association between the identified polymorphisms as well as the HWE. The eligibility of SNPs to be considered for haplotypes analysis was based on HW p-value >0.05, minimum minor allele frequency (MAF) of 0.1, maximum number of Mendel error of 1 and minimum included genotype of 75%. Quantification of MSI was further analysed using FAMHAP programme (http://www.meb.uni-bonn.de/famhap/) for single marker and possible haplotypes pairing. Global p-value was used in this programme, which combined multiple markers, sexes, 1000 mutations stimulation (Monte Carlo mutation) and Bonferroni correction of 2 (Becker et al, 2005).

3.3 Results

3.3.1: *ADRB2* (Project B)

3.3.1.1 Demographic data

A total of 97 glaucoma (60 Primary Open Angle Glaucoma and 37 Normal Tension Glaucoma patients) and 100 controls were recruited. There was no significant difference in age, sex and race characteristics between glaucoma patients and controls. The breakdown of the samples is given in table 3.7.

Table 3.7 Demographic data of glaucoma patients and controls

	Glaucoma N=97	Control N=100	p-value
Age			
Mean (SD)	64.1 (9.2)	62.2 (9.2)	0.134*
Range			
Sex			
Male	62	66	0.759#
Female	35	34	
Race			
Malay	66	57	0.110#
Chinese	31	43	

p <0.05 is considered statistically significant based on *student t-test and #Pearson chi-square test.

3.3.1.2 *ADRB2* screening

ADRB2 gene screening was successfully conducted in all samples. Figure 3.1 illustrated the gel electrophoresis picture of both duplex and triplex PCR outcome for a single glaucoma (B20) and control (C33) sample. The position of selected polymorphism is illustrated in Figure 3.2.

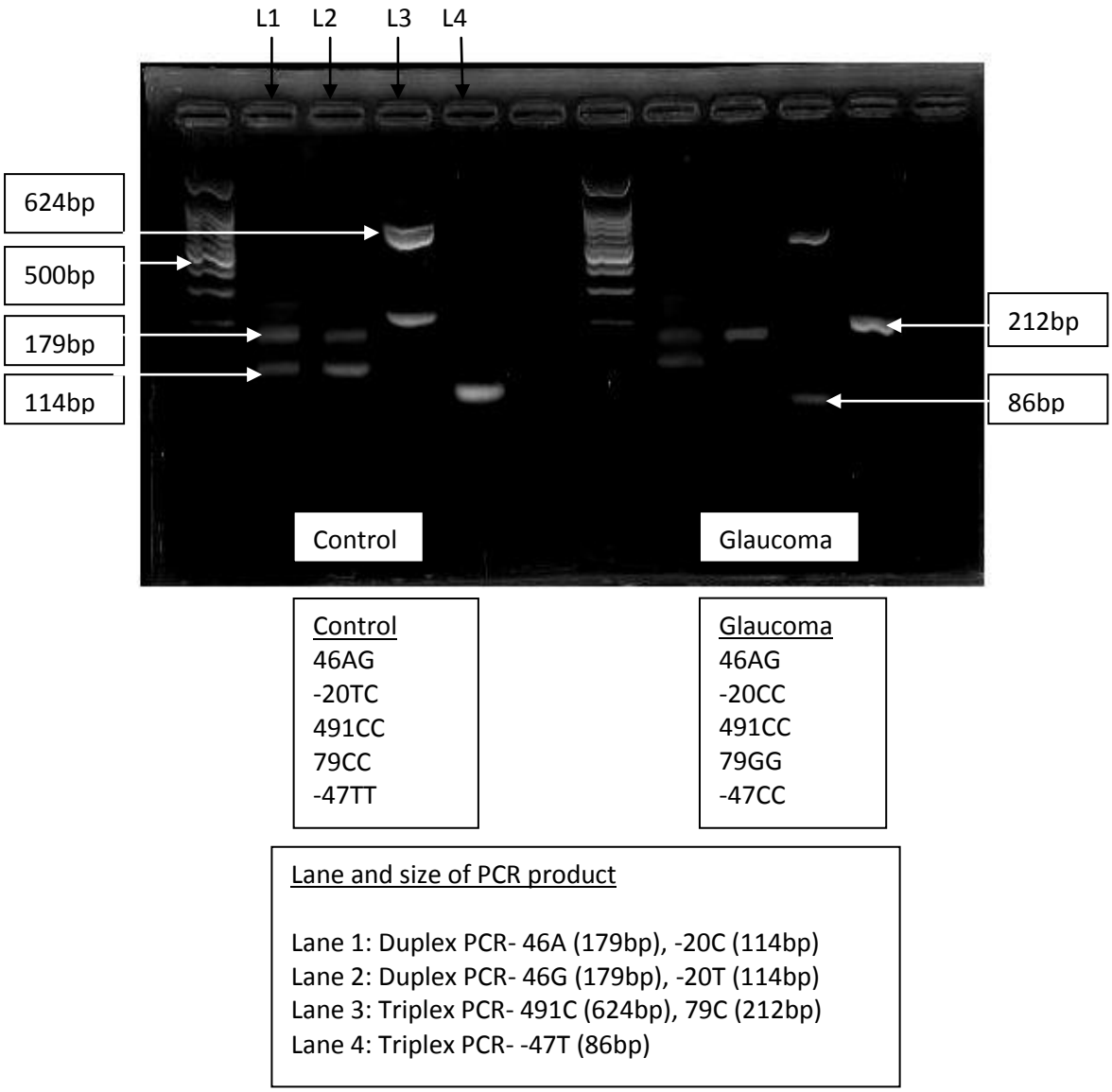


Figure 3.1: Gel electrophoresis of multiplex PCR for *ADRB2* in control and glaucoma patient

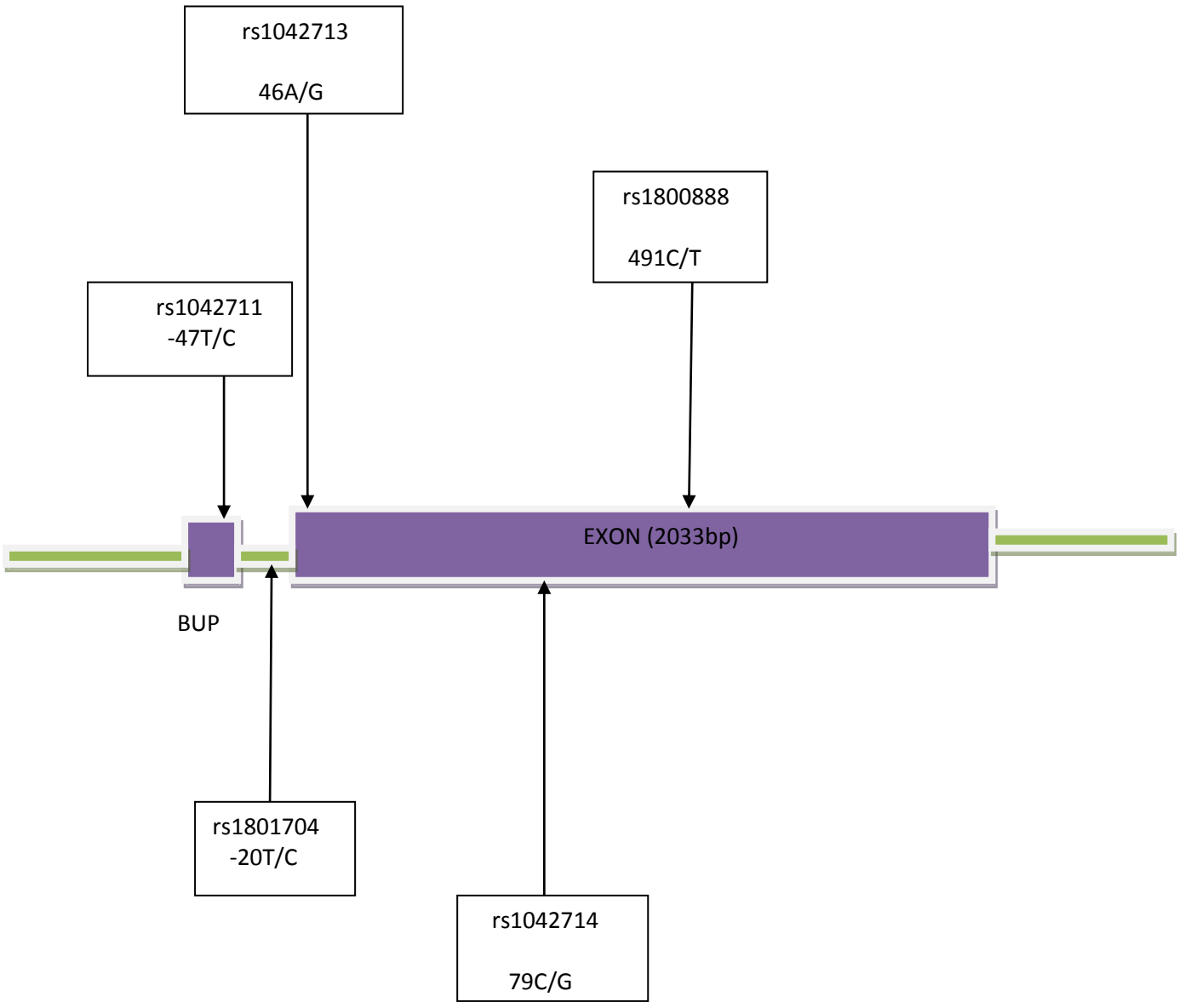


Figure 3.2: Schematic diagram of the position of SNPs found in *ADRB2*

3.3.1.3 Genotype and allele frequency for *ADRB2* between glaucoma and control subjects

There was no significant difference of *ADRB2* between glaucoma and control subjects (table 3.8). There was no polymorphism present at in codon 491C/T in our population (all 491C). There was also no significant difference of allele frequency of polymorphisms in *ADRB2* between glaucoma and control subjects (table 3.9).

Table 3.8 Genotype frequency of *ADRB2* gene polymorphisms between glaucoma and control subjects

Codon	SNP	Glaucoma N=97	Control N=100	LLA, p-value
46	rs1042713			
	AA	0.39	0.28	1.20,
	AG	0.57	0.71	0.273
	GG	0.04	0.01	
79	rs1042714			
	CC	0.84	0.71	2.99,
	CG	0.13	0.26	0.084
	GG	0.03	0.03	
491	rs1800888			
	CC	1.00	1.00	-
	CT			
	TT			
-20	rs1801704			
	TT	0.11	0.09	3.39,
	TC	0.34	0.21	0.066
	CC	0.55	0.70	
-47	rs1042711			
	TT	0.01	0.03	1.61,
	TC	0.10	0.14	0.204
	CC	0.89	0.83	

$p<0.05$ is considered statistically significant difference based on linear-by-linear association (df=1). LLA: linear-by-linear association

Table 3.9: Allele frequency of *ADRB2* polymorphisms between glaucoma and control subjects

Codon	SNPs	Glaucoma N=97	Control N=100	χ^2, p-value
46	rs1042713			
	A	0.675	0.635	0.36,
	G	0.325	0.365	0.550
79	rs1042714			
	C	0.902	0.840	1.59,
	G	0.098	0.160	0.207
491	rs1800888			
	C	1.00	1.00	---
	T			
-20	rs1801704			
	T	0.284	0.195	1.75,
	C	0.716	0.805	0.185
-47	rs1042711			
	T	0.062	0.100	1.09,
	C	0.938	0.900	0.297

$p < 0.05$ is considered statistically significant different based on Pearson chi-square test.

3.3.1.4 Allele frequency of *ADRB2*polymorphisms according to sex and ethnicity in the Malaysian population

There was no significant effect of sex and ethnicity on allele frequency of *ADRB2* polymorphism at codon 46, 79, 491, -47 and -20 (table 3.10). However, genomic control analysis was not conducted due to small number of SNPs screened in this study.

Table 3.10: Allele frequency of *ADRB2* polymorphisms according to sex and ethnicity

Codon	SNP	Sex			Ethnicity		
		Male N=128	Female N=69	χ^2, p-value	Malay N=123	Chinese N=74	χ^2, p-value
46	rs1042713						
	A	0.648	0.667	0.13,	0.646	0.669	0.21,
	G	0.352	0.354	0.717	0.354	0.331	0.648
79	rs1042714						
	C	0.875	0.862	0.12,	0.841	0.919	3.03,
	G	0.125	0.138	0.721	0.159	0.081	0.082
491	rs1800888						
	C	1.000	1.000	1.000	1.000	1.000	1.000
	T						
-20	rs1801704						
	T	0.246	0.225	0.23,	0.776	0.736	0.81,
	C	0.754	0.775	0.634	0.224	0.264	0.368
-47	rs1042711						
	T	0.086	0.072	0.22,	0.098	0.054	2.34,
	C	0.914	0.928	0.640	0.902	0.946	0.126

$P < 0.05$ is considered statistically significant based on Pearson chi square test

3.3.1.5 The role of *ADRB2* polymorphisms in susceptibility of glaucoma in Malays and Chinese

Due to the possibility of population stratification, stratified Cochran Mantel-Haenszel meta-analysis was conducted according race; Malays and Chinese. -47T/C and 491C/T were excluded from the univariate logistic regression. The major allele of 79C/G (p=0.037) and -20T/C is associated with increased susceptibility to glaucoma (p=0.030) (table 3.11). The major allele of -20T/C and 79C/G increased the risk of glaucoma 1.7-fold and 1.9-fold respective as compared to controls. Further analysis using univariate logistic regression (age and sex was added), there was no significant association of *ADRB2* and glaucoma (table 3.12).

Table 3.11: Stratified Mantel-Haenszel meta-analysis on *ADRB2* polymorphisms and susceptibility to glaucoma

	Malays		**Chinese**		**Stratified Meta-analysis**			
	Allele frequency		Allele frequency		OR (95%CI)	SE	P-meta	P-het
SNPs	Glaucoma N=68	Control N=57	Glaucoma N=32	Control N=43				
46 A/G								
A	0.654	0.632	0.710	0.640	1.21	0.21	0.369	0.654
G	0.346	0.368	0.290	0.360	(0.79, 1.84)			
79C/G								
C	0.879	0.798	0.952	0.895	1.93	0.31	**0.037**	0.769
G	0.121	0.202	0.048	0.105	(1.04, 3.57)			
491C/T								
C	1.000	1.000	1.000	1.000	--	--	--	--
T	0	0	0	0				
-20T/C								
T	0.265	0.175	0.323	0.221	1.69	0.24	**0.030**	0.984
C	0.735	0.825	0.677	0.779	(1.05, 2.71)			
-47T/C								
T	0.076	0.123	0.032	0.070	0.55	0.39	0.119	0.769
C	0.924	0.877	0.968	0.930	(0.26, 1.17)			

OR: odd ratio, CI: confidence interval, SE: standard error
Phet: P-value for heterogeneity between both studies (P<0.05 is considered significant heterogeneity based on the Breslow-Day test)
P-meta: P-value for the meta-analysis between Malays and Chinese where the association between alleles and glaucoma status was measured.

Table 3.12: Univariate logistic regression on predictors for glaucoma susceptibility in the Malaysian population

Predictors	OR	SE	95% CI for OR	p-value
46A/G				
AA	--	--	--	--
AG	0.52	0.34	0.27, 1.03	*0.059*
GG	1.44	1.20	0.14, 15.26	0.761
79C/G				
CC	--	--	--	--
CG	0.43	0.53	0.15, 1.20	0.107
GG	1.41	1.22	0.13, 15.32	0.779
-20T/C				
TT	--	--	--	--
TC	1.02	0.57	0.34, 3.10	0.973
CC	0.39	0.55	0.13, 1.15	*0.088*
Sex				
Male	0.93	0.32	0.50, 1.73	0.811
Female	--	--	--	--
Age	1.02	0.02	0.99, 1.05	0.243

OR: odd ratio, CI: confidence interval
The goodness of fit of this model was checked using the Hosmer-Lemeshow test; p=0.994. This result gives no evidence of lack of fit of the model.

3.3.1.6 The role of *ADRB2* in susceptibility to POAG and NTG

There was no evidence of heterogeneity based on Mantel-Haenszel analysis between Malays and Chinese (table 3.13). The minor allele of 79C/G (79G) reduced the risk of POAG 0.3 fold (95% CI 0.1, 0.7) compared to normal healthy volunteers (table 3.13). -20T increased the risk for NTG (OR 2.0[95%CI 1.1, 3.7]) (table 3.15). Age and sex were also included as the predictors for susceptibility to different type of glaucoma.

Table 3.13: Stratified Mantel-Haenszel meta-analysis on *ADRB2* and susceptibility to POAG

	Malays		**Chinese**		**Stratified Meta-analysis**			
SNPs	Allele frequency		Allele frequency		OR (95%CI)	SE	p-meta	p-het
	POAG N=43	Control N=57	POAG N=17	Control N=43				
46A/G								
A	0.686	0.632	0.706	0.640	0.77	0.25	0.293	0.911
G	0.314	0.368	0.294	0.360	(0.47, 1.25)			
79C/G								
C	0.930	0.798	0.971	0.895	0.29	0.44	**0.005**	0.908
G	0.070	0.202	0.029	0.105	(0.12, 0.69)			
491C/T								
C	1.000	1.000	1.000	1.000	--	--	--	--
T	0	0	0	0				
-20T/C								
T	0.256	0.175	0.265	0.221	1.48	0.28	0.157	0.679
C	0.744	0.825	0.735	0.779	(0.86, 2.56)			
-47T/C								
T	0.047	0.123	0.000	0.070	3.72	0.58	**0.022**	0.348
C	0.953	0.877	1.000	0.930	(1.21, 11.48)			

OR: odd ratio, CI: confidence interval, SE: standard error
Phet: P-value for heterogeneity between both studies (P<0.05 is considered significant heterogeneity based on the Breslow-Day test)
P-meta: P-value for the meta-analysis between Malays and Chinese where the association between alleles and glaucoma status was measured.

Table 3.14: Univariate logistic regression exploring the role of *ADRB2* genotypes and other predictors in influencing risk of POAG prevalence relative to unaffected "normal" individuals

Genotype	OR	SE	95% CI for OR LCI, UCI	p-value
79C/G				
CC	--	--	--	--
CG	0.24	0.70	0.06, 0.96	**0.044**
GG	1.11	1.44	0.07, 18.43	0.945
-47T/C				
TT	--	--	--	--
TC	1.256e9	21918.72	0.00	0.999
CC	9.626e8	21918.72	0.00	0.999
Sex				
Male	1.01	0.36	0.50, 2.03	0.987
Female	--	--	--	--
Age	1.02	0.02	0.99, 1.06	0.208

OR: odds ratio, CI: confidence interval, LCI: lower confidence interval, UCI: upper confidence interval.
The goodness of fit of this model was checked using the Hosmer-Lemenshow test; p=0.531. This result gives no evidence of lack of fit of the model.

Table 3.15: Stratified Mantel-Haenszel meta-analysis on *ADRB2* and susceptibility to NTG

	Malays		**Chinese**		**Stratified Meta-analysis**			
SNPs	Allele frequency		Allele frequency		OR (95%CI)	SE	p-meta	p-het
	NTG N=23	Control N=57	NTG N=14	Control N=43				
46A/G								
A	0.609	0.632	0.714	0.640	0.94	0.29	0.814	0.459
G	0.391	0.368	0.286	0.360	(0.54, 1.64)			
79C/G								
C	0.783	0.798	0.929	0.895	0.97	0.38	0.944	0.575
G	0.217	0.202	0.071	0.105	(0.47, 2.03)			
491C/T								
C	1.000 0	1.000 0	1.000 0	1.000 0	--	--	--	--
T								
-20T/C								
T	0.283	0.175	0.393	0.221	2.03	0.31	**0.022**	0.736
C	0.717	0.825	0.607	0.779	(1.11, 3.70)			
-47T/C								
T	0.130	0.123	0.071	0.070	0.94	0.45	0.898	0.965
C	0.870	0.877	0.929	0.930	(0.40, 2.26)			

OR: odd ratio, CI: confidence interval, SE: standard error
Phet: P-value for heterogeneity between both studies (P<0.05 is considered significant heterogeneity based on the Breslow-Day test)
P-meta: P-value for the meta-analysis between Malays and Chinese where the association between alleles and glaucoma status was measured.

Table 3.16: Univariate logistic regression exploring the role of *ADRB2* genotypes and other predictors in influencing risk of NTG prevalence relative to unaffected "normal" individuals

	OR	SE	95% CI for OR LCI, UCI	p-value
79C/G				
CC	--	--	--	--
CG	0.76	0.48	0.30, 1.96	0.565
GG	0.90	0.99	0.13, 6.27	0.914
-20T/C				
TT	--	--	--	--
TC	2.70	0.45	1.12, 6.50	**0.027**
CC	1.93	0.65	0.69, 8.80	0.165
Sex				
Male	0.72	0.41	0.32, 1.60	0.418
Female	--	--	--	--
Age	1.02	0.02	0.97, 1.06	0.479

OR: odd ratio, CI: confidence interval, LCI: lower confidence interval, UCI: upper confidence interval.
The goodness of fit of this model was checked using the Hosmer-Lemenshow test; p=0.500. This result gives no evidence of lack of fit of the model.

3.3.1.7 Haplotypes analysis of *ADRB2*

The possible haplotypes of *ADRB2* gene was further analysed using haplotypes software; Haploview. 46A/G and -47T/C was excluded from the analysis due to statistically significant Hardy Weinberg equilibrium (HWE) for combination of Malay and Chinese (table 3.19). 491C/T was also excluded due to minor allele frequency. Haplotypes analysis was only assessed on 79C/G and -20T/C. There was 84% of association between 79C/G and -20T/C.

Table 3.17: Single marker check for *ADRB2* polymorphisms based on Haploview analysis

Codon	Allele	Observed	Predicted	HW p-value	Minor allele Frequency
Malays (N=123)					
-47	T/C	0.146	0.176	0.080	0.098
-20	T/C	0.285	0.347	0.171	0.226
46	A/G	0.642	0.457	6.791E-6	0.354
79	C/G	0.236	0.267	0.312	0.159
491	C	0	0	1.000	0
Chinese (N=74)					
-47	T/C	0.081	0.102	0.009	0.054
-20	T/C	0.257	0.388	0.362	0.264
46	A/G	0.635	0.443	2.0E-4	0.331
79	C/G	0.135	0.149	0.768	0.081
491	C	0	0	1.000	0
Both (N=197)					
-47	T/C	0.122	0.149	0.001	0.081
-20	T/C	0.274	0.363	0.053	0.239
46	A/G	0.640	0.452	1.531E-9	0.345
79	C/G	0.198	0.225	0.163	0.129
491	C	0	0	1.000	0

3.3.2: *PTGFR* (Project P)

3.3.2.1 Demographic data

A total of 86 glaucoma (64 POAG and 22 NTG) and 90 unrelated control subjects were included in this study. There was no significant different between the glaucoma and control subjects in mean age at recruitment, sex and ethnicity (table 3.18).

Table 3.18: Demographic data of glaucoma and control subjects

	Glaucoma N=86	Control N=90	p-value
Age			
Mean (SD)	67.1 (9.2)	64.5 (11.1)	0.100*
Sex			
Male	60	62	0.899#
Female	26	28	
Race			
Malay	58	62	0.837#
Chinese	28	28	

P<0.05 is considered significant based on *student t-test and # Pearson chi-square test

3.3.2.2 *PTGFR* screening in the Malaysian population

A total 63 SNPs were identified in the *PTGFR* gene including one novel polymorphism rs3766332 at the flanking region of exon 4 (figure 3.3). Only one SNP was found within the exon 4, rs3766331 (figure 3.3). The rest were intronic SNPs with a large number being detected at Intron 3 (47 SNPs). The example of the outcome of *PTGFR* gene screening of Introns 3 is illustrated in figure 3.4 and 3.6. The accidental finding of 'CA' repeats, one of the areas of microsatellite instability (MSI), was noted on the electropherogram of IVS 3-29 (figure 3.5) and further quantification was done using special tagged primer. The finding was illustrated in figure 3.8 and 3.9. A schematic diagram showing all the SNPs investigated is shown in figure 3.10 to 3.12.

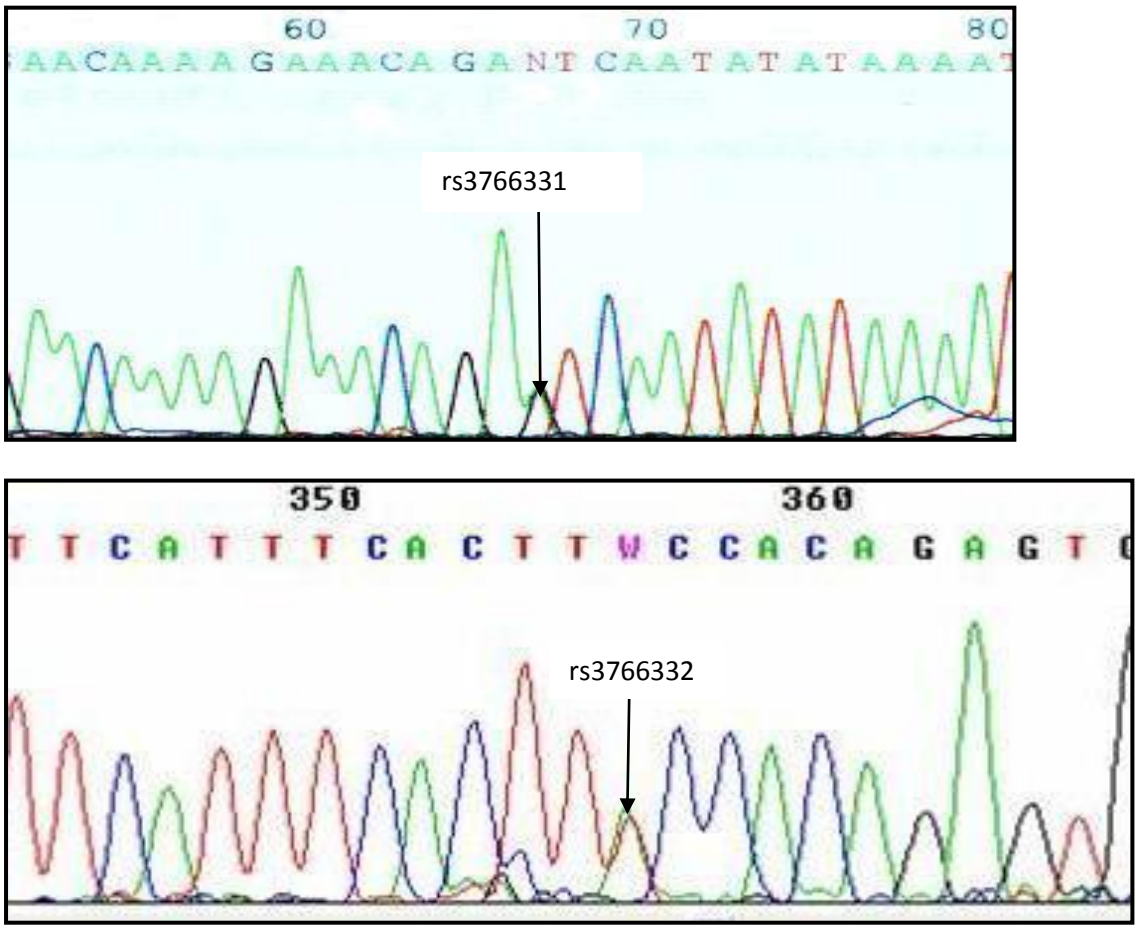


Figure 3.3: Electropherogram of rs3766331 A/G found in the exon 4 of *PTGFR* gene and novel polymorphism rs3766332 A/T (rs3766332AT) found in Malaysian population

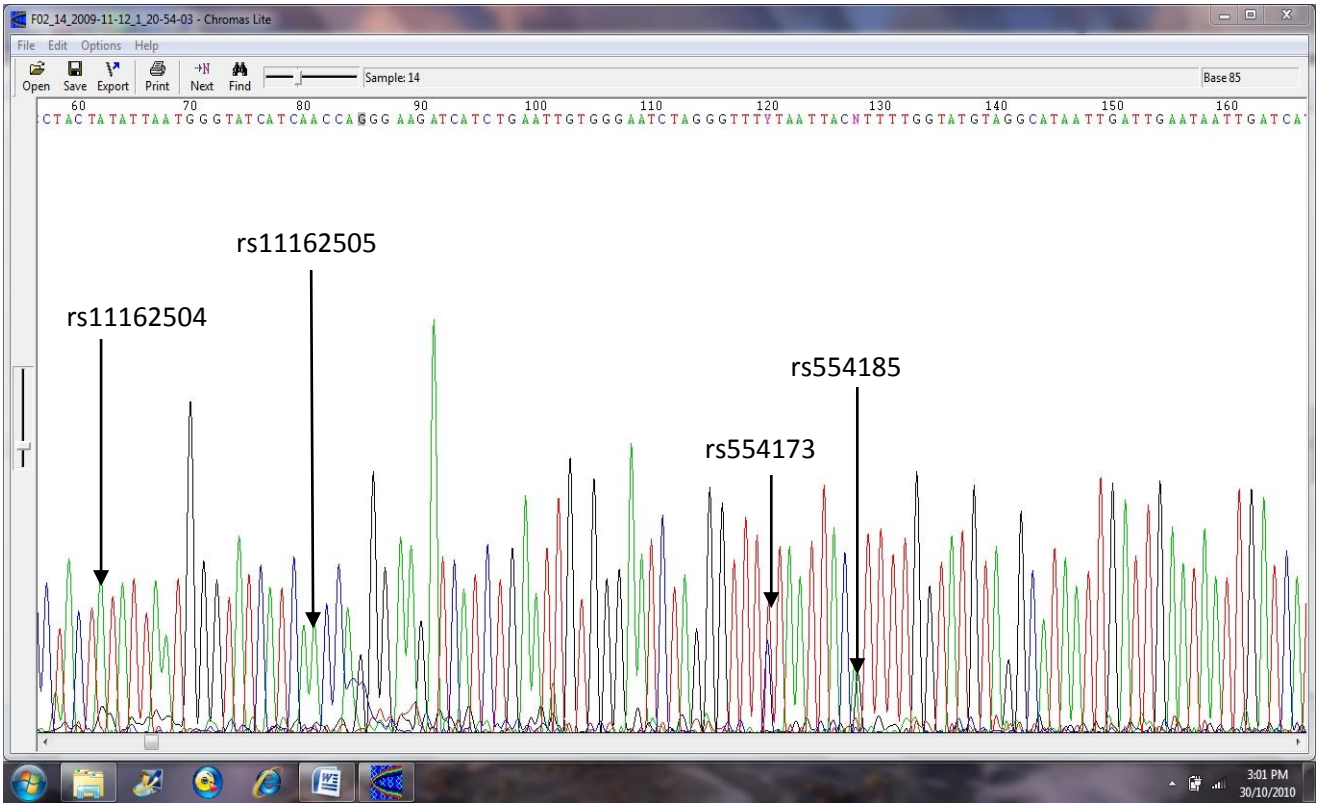


Figure 3.4: Electropherogram of primer IVS 3-45 of glaucoma patient (P18) showing rs11162504AA and rs11162505AA, and rs554173TC and rs554185AG. The position of rs11162504 and rs11162505 is just 18bp apart and 7bp apart between rs554173 and rs554185

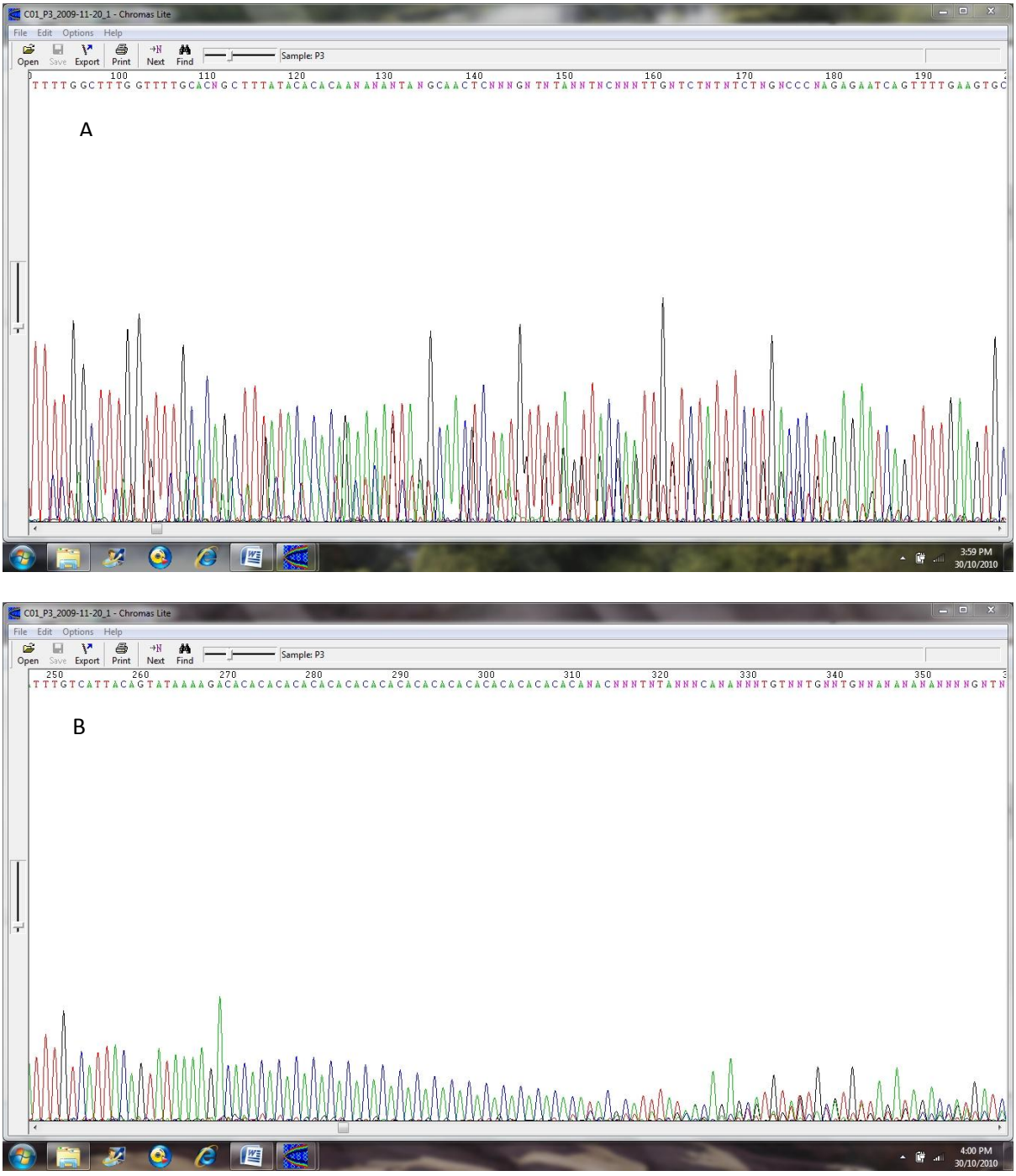


Figure 3.5: Electropherogram of IVS 3-29 showing the presence of an area of MSI, formed by a CA repeat

3.5A: overlapping spike at the beginning of CA repeat

3.5B: a segment of readable CA repeats followed by unreadable sequence towards the end

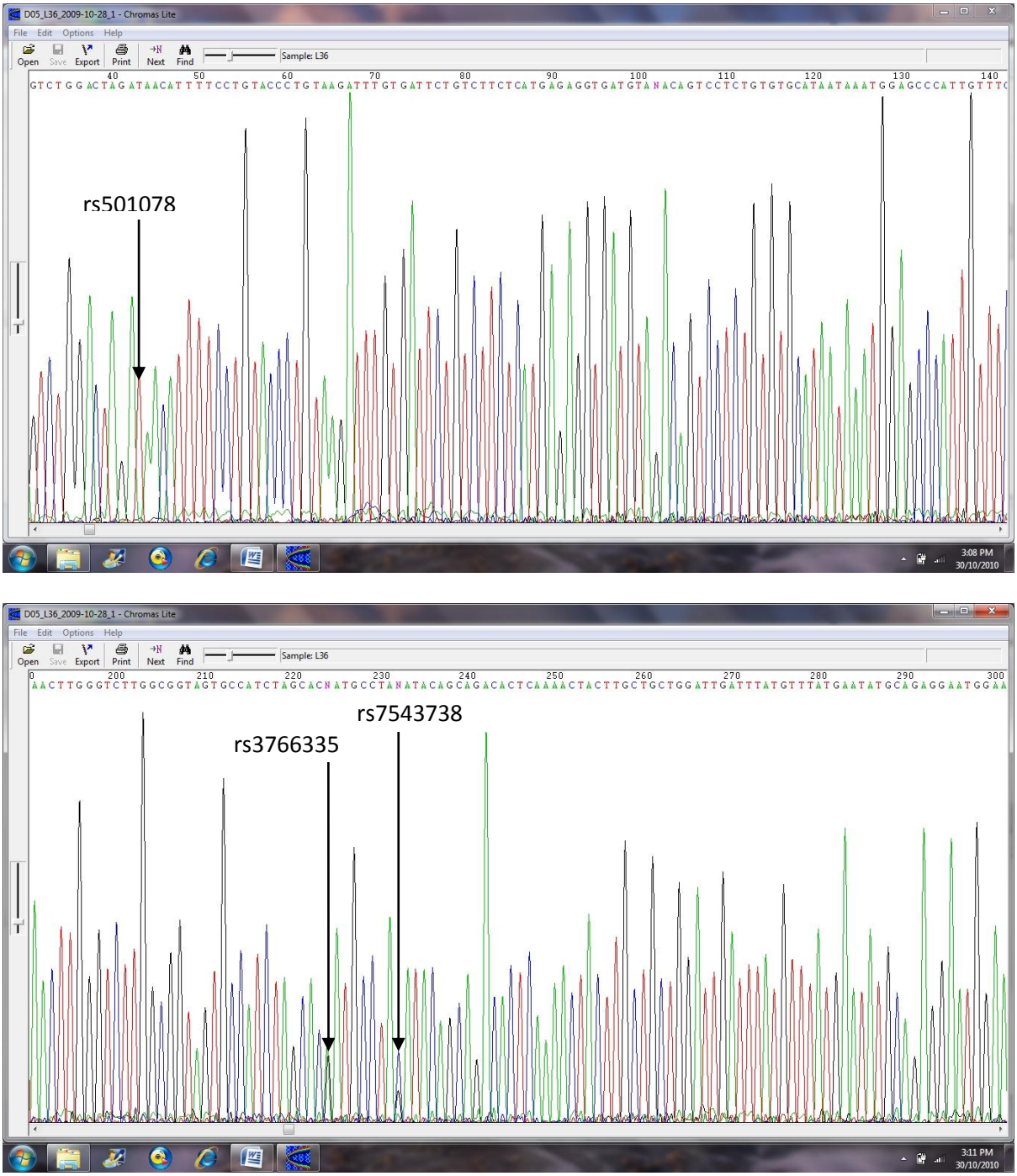


Figure 3.6: Electropherogram of IVS 3-55 of glaucoma patient (P52) showing rs501078TT, rs3766335GA and rs7543738CG.

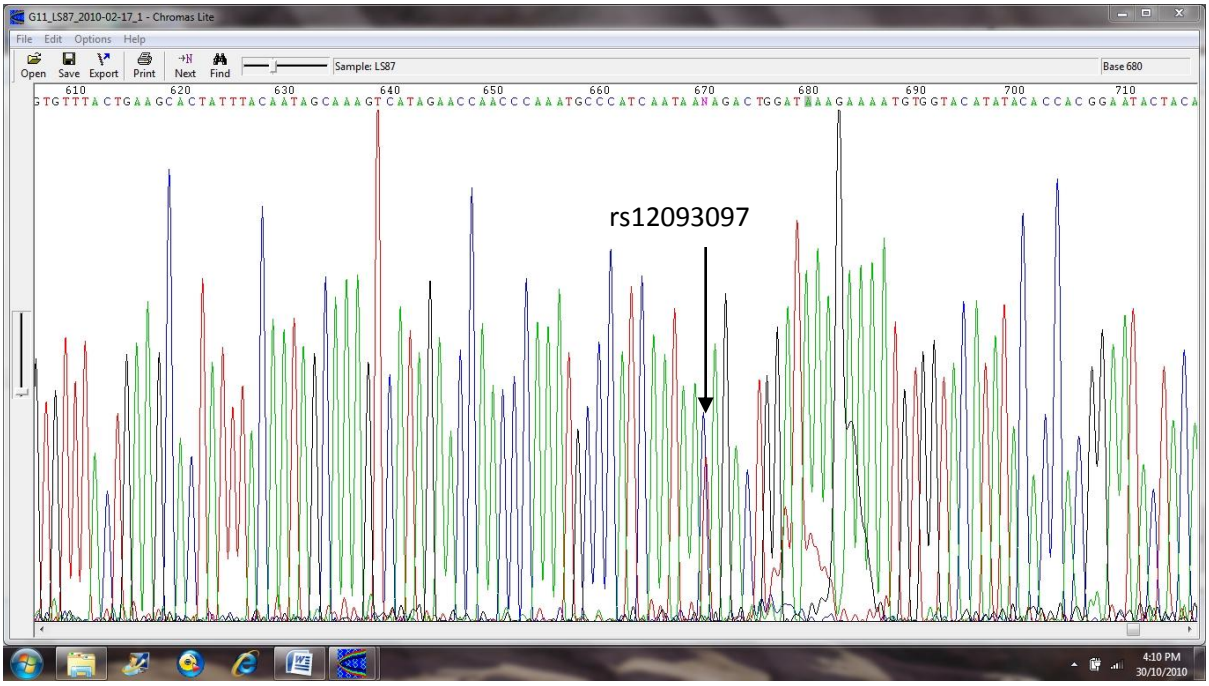


Figure 3.7: Electropherogram of promoter region PRM LR3 primer showing rs12093097CT in glaucoma patient (P50)

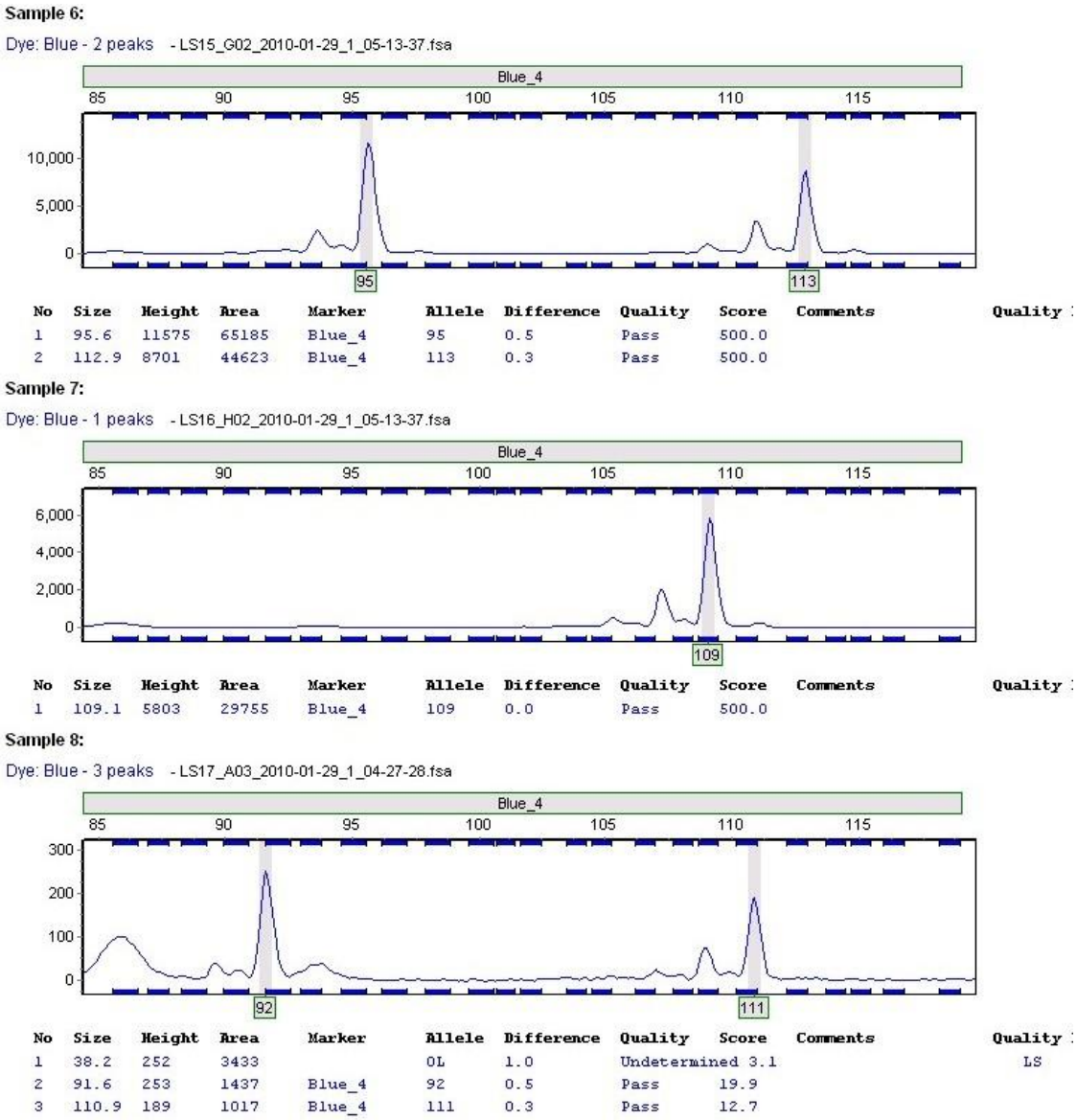
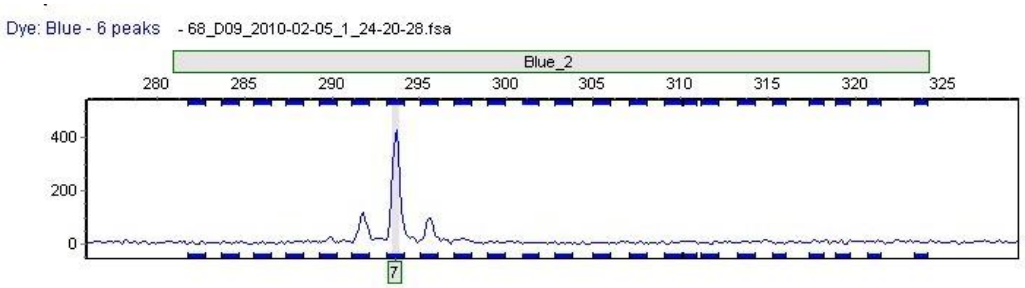
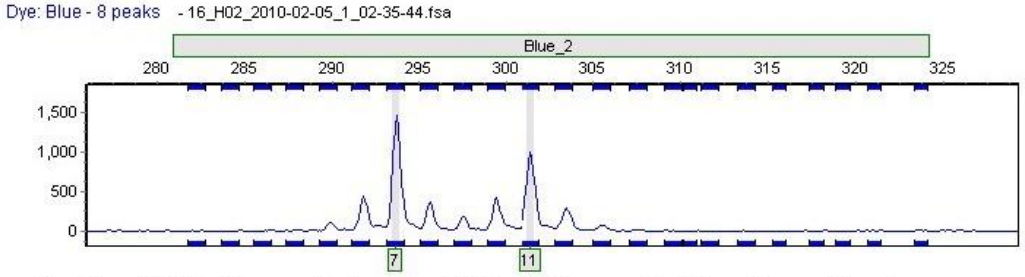


Figure 3.8: Genotyping result of MSI at IVS 3-29 showing of deletion of certain part of MSI



No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments	Quality
1	37.9	326	3266		OL	1.0	Undetermined	1.8		LS
2	42.0	403	7348		OL	1.0	Undetermined	1.2		LS
3	49.6	409	7941		OL	1.0	Undetermined	1.3		LS
4	85.2	515	9081		OL	1.0	Undetermined	3.7		LS
5	232.0	138	3030	Blue_1	232	0.3	Undetermined	0.3		LS
6	293.7	425	2052	Blue_2	7	0.0	Pass	59.4		

Sample 7:



No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments	Quality
1	38.2	401	3709		OL	1.0	Undetermined	1.4		LS
2	41.8	522	9288		OL	1.0	Undetermined	2.5		LS
3	49.9	535	10293		OL	1.0	Undetermined	3.7		LS
4	55.7	548	10062		OL	1.0	Undetermined	1.2		LS
5	85.0	635	10903		OL	1.0	Undetermined	3.7		LS
6	231.4	194	4263	Blue_1	232	0.3	Undetermined	0.4		LS
7	293.7	1450	7430	Blue_2	7	0.0	Pass	305.4		
8	301.4	989	5610	Blue_2	11	0.0	Pass	149.7		

Figure 3.9: Genotyping of IVS 3-56 MSI using capillary electrophoresis showing insertion of MSI

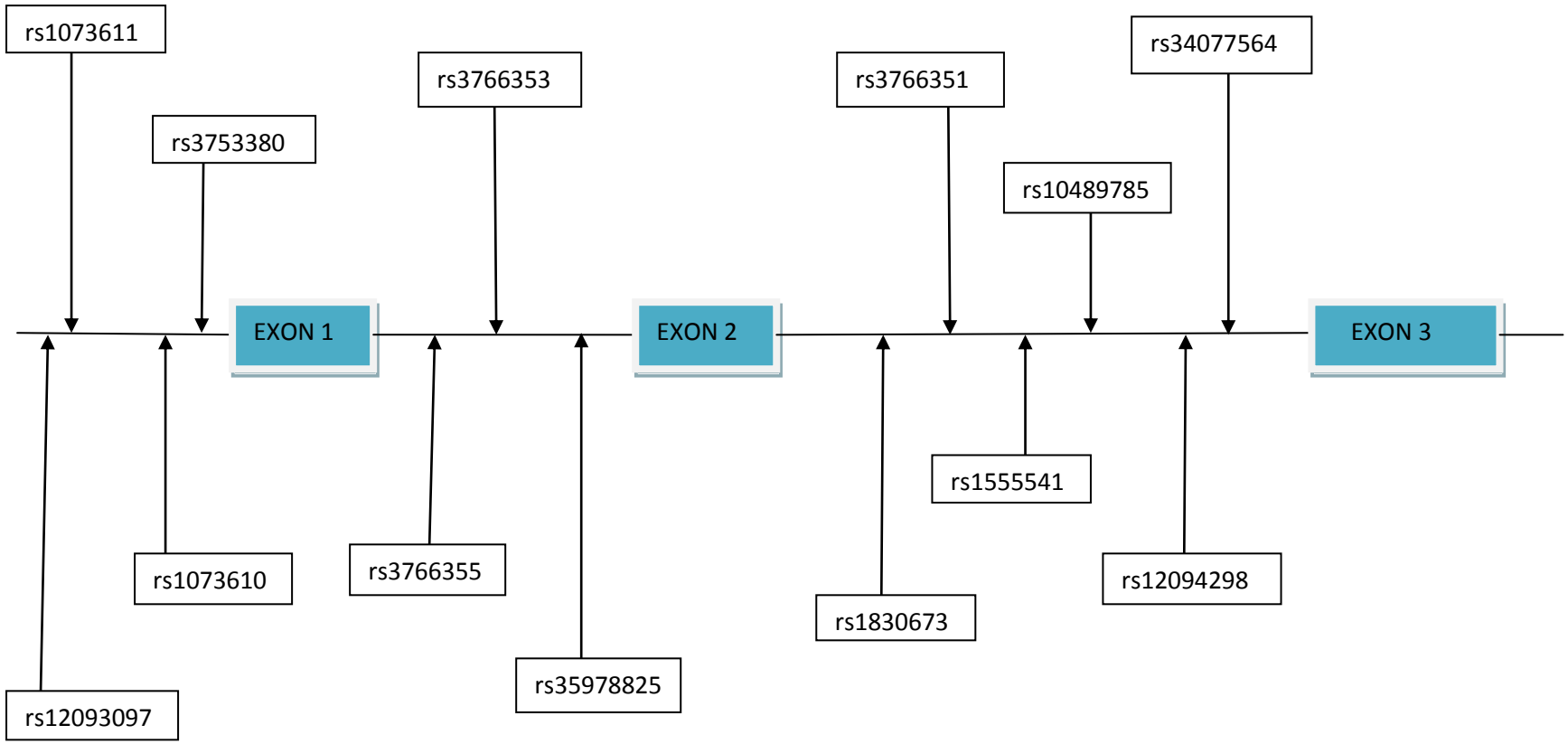


Figure 3.10: Distribution of SNPs found in the promoter, Intron 1 and Intron 2 of *PTGFR* in the Malaysian population

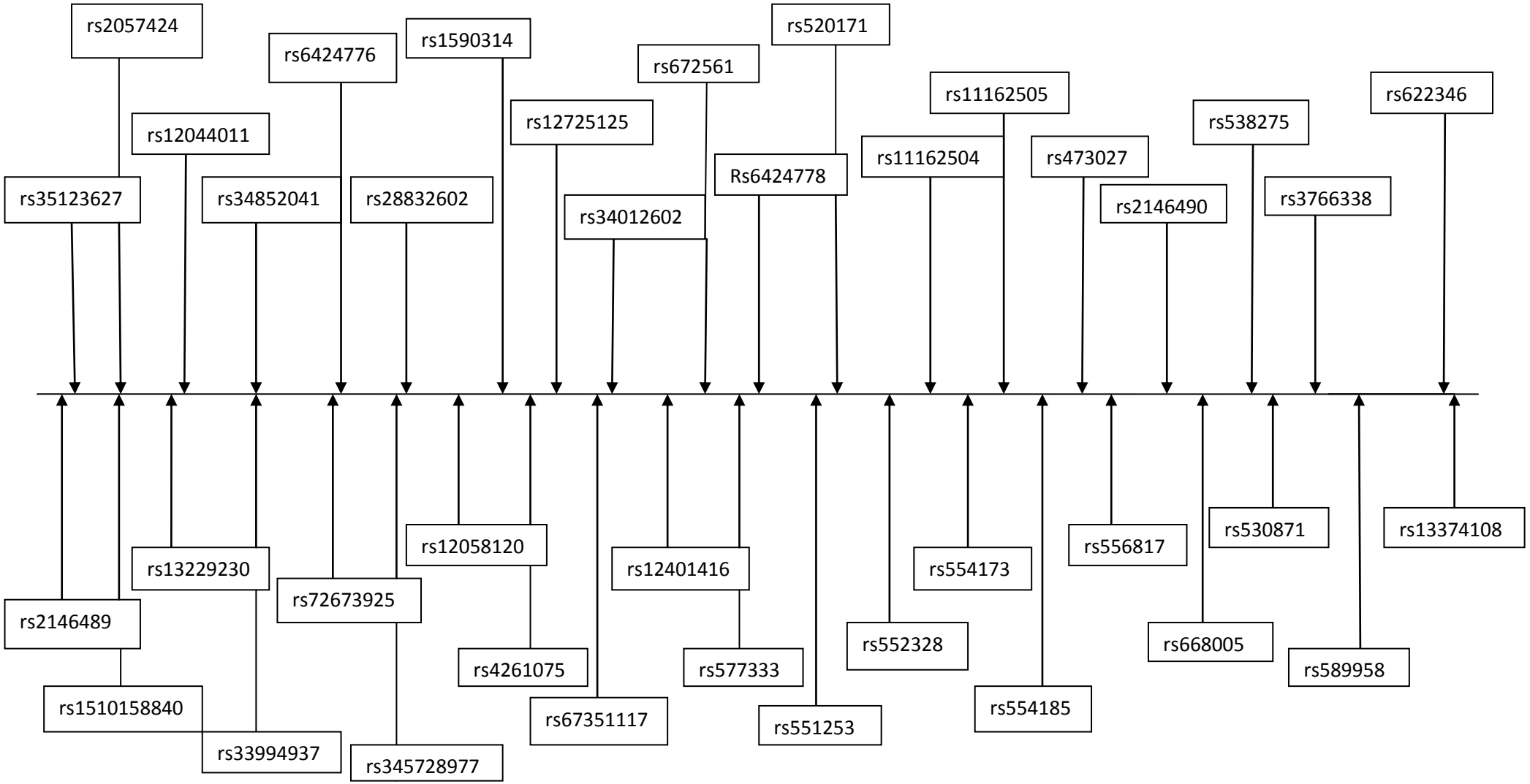


Figure 3.11: Distribution of SNPs found in part of Intron 3 of *PTGFR* in the Malaysian population

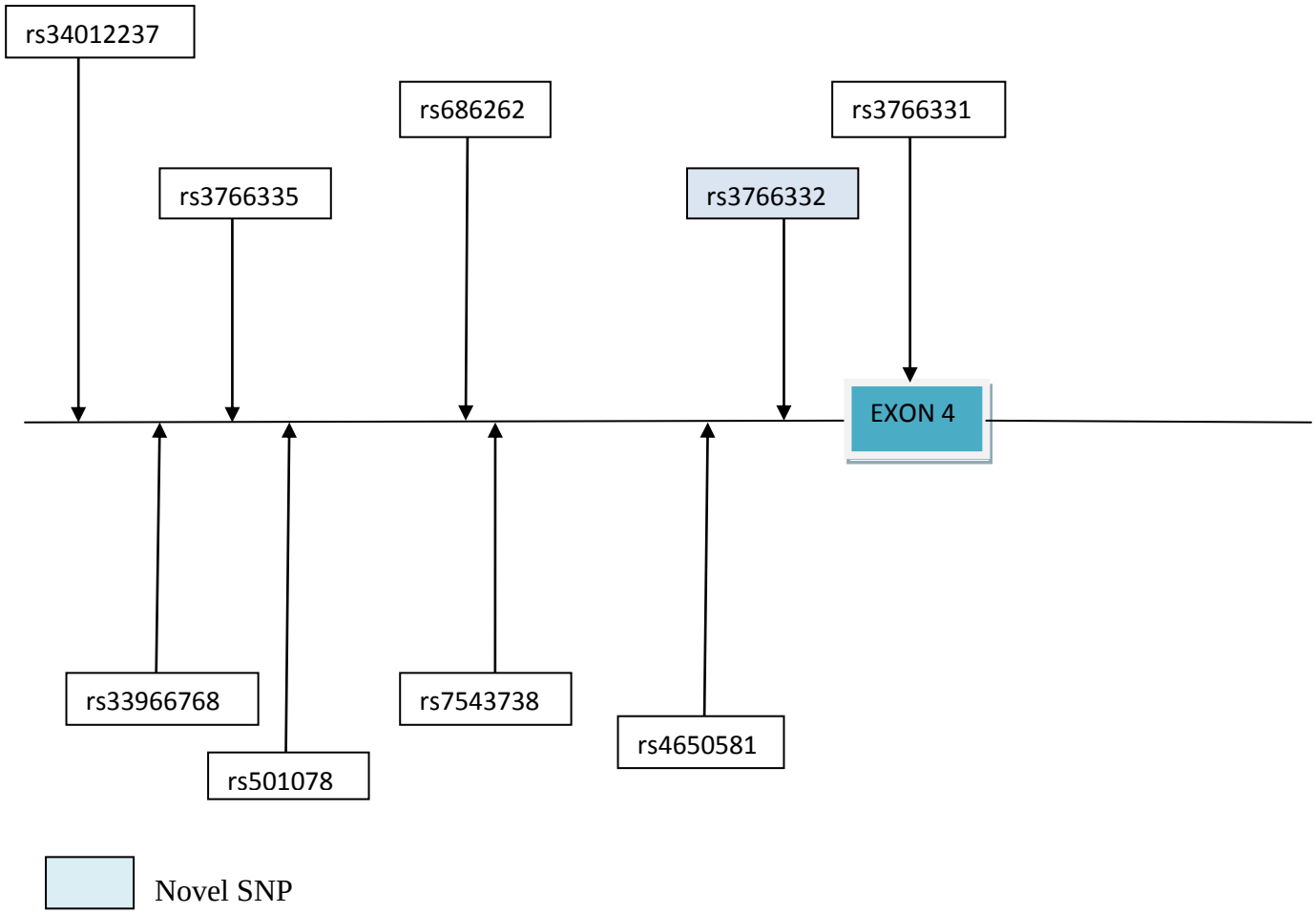


Figure 3.12: Distribution of SNPs in the last part of Intron 3 and Exon 4 of *PTGFR*

3.3.2.3: Genotype and allele frequency of SNPs found in *PTGFR* gene in the Malaysian population

There was significant difference in genotype and allele frequency of rs11162505 and rs554185 between glaucoma cases and control subjects (table 3.19). The homozygous wild state in all significant SNPs increased the apparent susceptibility to glaucoma (table 3.19).

Table 3.19: Genotyping and allele frequency of SNPs found in *PTGFR* gene in the Malaysian population

SNPs		Genotype frequency				Allele frequency		
		Glaucoma N=86	Control N=90	LLA, p-value*		Glaucoma N=86	Control N=90	χ^2, p-value#
rs3766331	AA	60 (69.8)	59 (65.6)	0.15,	A	0.833	0.794	0.52,
	AG	23 (26.7)	25 (27.8)	0.696	G	0.167	0.206	0.471
	GG	3 (3.5)	6 (6.7)					
rs3766355	CC	25 (29.1)	28 (31.1)	0.24,	C	0.535	0.561	0.08,
	CA	42 (48.8)	45 (50.0)	0.622	A	0.465	0.439	0.776
	AA	19 (22.1)	17 (18.9)					
rs3766353	GG	51 (59.3)	45 (50.0)	0.59	G	0.744	0.706	0.23,
	GT	26 (30.2)	37 (41.1)	0.441	T	0.256	0.294	0.635
	TT	9 (10.5)	8 (8.9)					
rs35978825	CC	71 (82.6)	72 (80.0)	0.15,	C	0.907	0.894	0.22,
	CT	14 (16.3)	17 (18.9)	0.698	T	0.093	0.106	0.637
	TT	1 (1.1)	1 (1.1)					
rs1830673	AA	14 (16.3)	14 (15.6)	0.32,	A	0.413	0.383	0.19,
	AG	43 (50.0)	41 (45.6)	0.574	G	0.587	0.617	0.664
	GG	29 (36.4)	35(38.9)					
rs3766351	TT	70 (81.4)	74 (82.2)	0.02,	T	0.866	0.872	0.00,
	TC	9 (10.5)	9 (10.0)	0.895	C	0.134	0.128	1.000
	CC	7 (8.1)	7 (7.8)					
rs1555541	TT	15 (17.4)	14 (15.6)	0.12,	T	0.320	0.339	0.09,
	TC	25 (29.1)	33 (36.7)	0.734	C	0.680	0.661	0.764
	CC	46 (53.5)	43(47.8)					
rs10489785	AA	73 (84.9)	74 (82.2)	0.28,	A	0.913	0.894	0.22,

	AT	11 (12.8)	13 (14.4)	0.599	T	0.087	0.106	0.637
	TT	2 (2.23)	3 (3.3)					
rs12094298	CC	78 (90.7)	79 (87.8)	0.22,	C	0.942	0.928	0.08,
	CA	6 (7.0)	9 (10.0)	0.641	A	0.058	0.072	0.774
	AA	2 (2.3)	2 (2.2)					
rs34077564	AA	73 (84.9)	75 (83.3)	0.06,	A	0.872	0.883	0.05,
	AG	9 (10.5)	9 (10.0)	0.803	G	0.128	0.117	0.831
	GG	4 (4.7)	6 (6.7)					
rs35123627	CC	73 (84.9)	76 (84.4)	0.05,	C	0.913	0.906	0.00,
	CT	11 (12.8)	11 (12.2)	0.833	T	0.087	0.094	1.000
	TT	2 (2.3)	3 (3.3)					
rs2146489	AA	10 (11.6)	19 (21.1)	2.26,	A	0.378	0.456	1.31,
	AG	45 (52.3)	44 (48.9)	0.133	G	0.622	0.544	0.252
	GG	31 (36.1)	27 (30.0)					
rs2057424	AA	48 (55.8)	43 (47.8)	1.46,	A	0.680	0.606	1.07,
	AG	21 (24.4)	23 (25.6)	0.227	G	0.320	0.394	0.301
	GG	17 (19.8)	24 (26.7)					
rs15101588	GG	23 (26.7)	24 (26.7)	0.19,	G	0.523	0.500	0.18,
	GA	44 (51.2)	42 (46.7)	0.667	A	0.477	0.500	0.671
	AA	19 (22.1)	24 (26.7)					
rs34528585	TT	72 (83.7)	77 (85.6)	0.09,	T	0.907	0.917	0.06,
	TA	12 (14.0)	11 (12.2)	0.769	A	0.093	0.083	0.800
	AA	2 (2.3)	2 (2.2)					
rs12044011	TT	23 (26.7)	29 (32.2)	0.08,	T	0.529	0.544	0.02,
	TA	45 (52.3)	40 (44.4)	0.776	A	0.471	0.456	0.887
	AA	18 (21.0)	21 (23.3)					
rs1322930	GG	81 (94.2)	82 (91.1)	0.40,	G	0.965	0.95	0.52,
	GA	4 (4.7)	7 (7.8)	0.529	A	0.036	0.05	0.470
	AA	1 (1.1)	1 (1.1)					
rs34852041	CC	74 (86.0)	72 (80.0)	1.18,	C	0.924	0.889	0.52,
	CT	11 (13.8)	16 (17.8)	0.276	T	0.076	0.111	0.469
	TT	1 (1.2)	2 (2.2)					
rs33994937	TT	74 (86.0)	72 (80.0)	1.18,	T	0.924	0.889	0.52,
	TC	11 (13.8)	16 (17.8)	0.276	C	0.076	0.111	0.469
	CC	1 (1.2)	2 (2.2)					
rs6424776	TT	24 (27.9)	23 (25.6)	0.78,	T	0.547	0.500	0.50,

	TC	46 (53.5)	44 (48.9)	0.378	C	0.453	0.500	0.479
	CC	16 (18.6)	23 (25.6)					
rs72673925	TT	71 (82.6)	75 (83.3)	0.09,	T	0.901	0.911	0.22,
	TG	13 (15.1)	14 (15.6)	0.761	G	0.099	0.089	0.637
	GG	2 (2.3)	1 (1.1)					
rs28832602	CC	69 (80.3)	75 (83.3)	0.22,	C	0.890	0.906	0.22,
	CT	15 (17.4)	13 (14.4)	0.642	T	0.110	0.094	0.637
	TT	2 (2.3)	2 (2.2)					
rs34572897	AA	72 (83.7)	75 (83.3)	0.02,	A	0.907	0.911	0.06,
	AG	12 (14.0)	14 (15.6)	0.898	G	0.093	0.089	0.809
	GG	2 (2.3)	1 (1.1)					
rs1590314	TT	12 (14.0)	12 (13.3)	0.01,	T	0.384	0.389	0.02,
	TC	42 (48.8)	46 (51.1)	0.919	C	0.616	0.611	0.884
	CC	32 (37.2)	32 (35.6)					
rs12058120	CC	71 (82.6)	71 (78.9)	0.08,	C	0.872	0.883	0.05,
	CG	8 (9.3)	17 (18.9)	0.781	G	0.128	0.117	0.831
	GG	7 (8.1)	2 (2.2)					
rs12725125	GG	71 (82.6)	73 (81.1)	0.31,	G	0.872	0.894	0.19,
	GA	8 (9.3)	15 (16.7)	0.577	A	0.128	0.106	0.663
	AA	7 (8.1)	2 (2.2)					
rs4261075	AA	16 (18.6)	13 (14.4)	0.45,	A	0.419	0.383	0.33,
	AG	40 (46.5)	43 (47.8	0.505	G	0.581	0.617	0.564
	GG	30 (34.9)	34 (37.8)					
rs34012602	GG	73 (84.9)	75 (83.3)	0.02,	G	0.911	0.917	0.22,
	GT	11 (12.8)	15 (16.7)	0.899	T	0.089	0.083	0.637
	TT	2 (2.3)	0 (0)					
rs67351117	CC	73 (84.9)	75 (83.3)	0.02,	C	0.907	0.911	0.00,
	CT	10 (11.6)	14 (15.6)	0.901	T	0.093	0.089	1.000
	TT	3 (3.5)	1 (1.1)					
rs672561	TT	74 (86.0)	73 (81.1)	0.52,	T	0.919	0.911	0.00,
	TC	10 (11.6)	15 (16.7)	0.472	C	0.081	0.089	1.000
	CC	2 (2.3)	2 (2.2)					
rs12401416	GG	24 (27.9)	24 (26.7)	0.05,	G	0.529	0.517	0.02,
	GA	43 (50.0)	45 (50.0)	0.816	A	0.471	0.483	0.887
	AA	19 (22.1)	21 (32.2)					
rs6424778	CC	77 (89.5)	84 (93.3)	0.81,	C	0.948	0.967	0.52,
	CT	9 (10.5)	6 (6.7)	0.368	T	0.052	0.033	0.470
	TT	0 (0)	0 (0)					

rs577333	TT TC CC	9 (10.5) 42 (48.0) 35 (40.7)	7 (7.8) 44 (48.9) 39 (43.3)	0.31, 0.579	T C	0.349 0.651	0.322 0.678	0.20, 0.653
rs520171	AA AC CC	43 (50.0) 40 (46.5) 3 (3.5)	47 (52.2) 32 (35.6) 11 (12.2)	0.46, 0.498	A C	0.733 0.267	0.700 0.300	0.22, 0.638
rs551253	GG GC CC	57 (66.3) 25 (29.1) 4 (4.7)	55 (61.1) 17 (18.9) 18 (20.0)	3.68, 0.055	G C	0.808 0.192	0.706 0.294	2.74, 0.098
rs552328	AA AG GG	33 (38.4) 40 (46.5) 13 (15.1)	31 (34.5) 37 (41.1) 22 (24.4)	1.44, 0.231	A G	0.616 0.384	0.550 0.450	1.01, 0.315
rs11162504	AA AG GG	57 (66.3) 25 (29.1) 4 (4.7)	56 (62.2) 28 (31.1) 6 (6.7)	0.45, 0.501	A G	0.808 0.192	0.778 0.222	0.28, 0.599
rs11162505	AA AG GG	76 (87.4) 9 (10.5) 1 (1.1)	59 (65.5) 25 (27.8) 6 (6.7)	**12.62,** **<0.00** **1***	A G	0.936 0.064	0.794 0.206	**9.64,** **0.002**
rs554173	TT TC CC	58 (67.4) 24 (27.9) 4 (4.7)	54 (60.0) 31 (34.4) 5 (5.6)	0.88, 0.347	T C	0.814 0.186	0.772 0.228	0.71, 0.401
rs554185	AA AG GG	37 (43.0) 40 (46.5) 9 (10.5)	31 (34.4) 39 (43.3) 20 (22.2)	*3.60,* *0.058**	A G	0.663 0.337	0.561 0.439	**4.42,** **0.035**
rs556817	AA AG GG	67 (77.9) 15 (17.4) 4 (4.7)	57 (63.3) 28 (31.1) 5 (5.5)	3.19, 0.074	A G	0.866 0.134	0.789 0.211	2.27, 0.132
rs473027	AA AG GG	28 (32.6) 43 (50.0) 15 (17.4)	29 (32.2) 36 (40.0) 25 (27.8)	0.92, 0.338	A G	0.576 0.424	0.522 0.478	0.73, 0.394
rs668005	CC CT TT	36 (41.9) 36 (41.9) 14 (16.2)	30 (33.3) 36 (40.0) 24 (26.7)	2.77, 0.096	C T	0.628 0.372	0.533 0.467	2.05, 0.152
rs2146490	GG GA AA	74 (86.0) 10 (11.7) 2 (2.3)	76 (84.4) 12 (13.3) 2 (2.2)	0.05, 0.819	G A	0.919 0.081	0.856 0.144	1.84, 0.175
rs530871	GG	33 (38.4)	31 (34.4)	1.34,	G	0.605	0.539	1.00,

	GA	38 (44.2)	35 (38.8)	0.247	A	0.395	0.461	0.317
	AA	15 (17.4)	24 (26.7)					
rs538275	GG	32 (37.2)	31 (34.4)	1.12,	G	0.599	0.539	0.73,
	GA	39 (45.3)	35 (38.7)	0.290	A	0.401	0.461	0.391
	AA	15 (17.4)	24 (26.7)					
rs589958	GG	32 (37.2)	30 (33.3)	1.57,	G	0.599	0.528	1.00,
	GA	39 (45.3)	35 (38.7)	0.211	A	0.401	0.472	0.318
	AA	15 (17.4)	25 (27.8)					
rs3766338	TT	50 (58.1)	52 (57.8)	0.02,	T	0.756	0.733	0.24,
	TC	30 (34.9)	28 (31.1)	0.651	C	0.244	0.267	0.626
	CC	6 (7.0)	10 (11.1)					
rs590309	TT	32 (37.2)	29 (32.2)	1.55,	T	0.593	0.522	0.99,
	TC	38 (44.2)	36 (40.0)	0.214	C	0.407	0.478	0.319
	CC	16 (18.6)	25 (27.8)					
rs622346	GG	43 (50.0)	43 (47.8)	0.39,	G	0.709	0.678	0.21,
	GC	36 (41.9)	36 (40.0)	1.530	C	0.291	0.322	0.645
	CC	7 (8.1)	11 (12.2)					
rs13374108	TT	70 (81.4)	67 (74.4)	1.03,	T	0.901	0.867	0.44,
	TA	15 (17.4)	22 (24.4)	0.309	A	0.099	0.133	0.506
	AA	1 (1.2)	1 (1.1)					
rs34012237	TT	75 (87.2)	76 (84.4)	0.17,	T	0.924	0.911	0.06,
	TC	9 (10.5)	12 (13.3)	0.681	C	0.076	0.089	0.800
	CC	2 (2.3)	2 (2.2)					
rs33966768	TT	74 (86.0)	76 (84.4)	0.05,	T	0.919	0.911	0.06,
	TC	10 (11.6)	12 (13.3)	0.819	C	0.081	0.089	0.800
	CC	2 (2.4)	2 (2.2)					
rs501078	CC	14 (16.3)	12 (13.3)	1.34,	C	0.390	0.328	1.07,
	CT	39 (45.3)	35 (38.9)	0.248	T	0.610	0.672	0.301
	TT	33 (38.4)	43 (47.8)					
rs3766335	GG	76 (88.4)	75 (83.3)	0.58,	G	0.930	0.906	0.27,
	GA	8 (9.3)	13 (14.4)	0.446	A	0.070	0.094	0.602
	AA	2 (2.3)	2 (2.2)					
rs7543738	CC	78 (90.7)	84 (93.3)	0.30,	C	0.948	0.961	0.12,
	CG	7 (8.1)	5 (5.6)	0.584	G	0.052	0.039	0.733
	GG	1 (1.2)	1 (1.1)					
rs686262	AA	20 (23.3)	14 (15.6)	2.72,	A	0.448	0.356	1.68,
	AG	37 (43.0)	36 (40.0)	0.099	G	0.552	0.644	0.195
	GG	29 (33.7)	40 (44.4)					

rs4650581	TT	73 (84.9)	75 (83.3)	0.26,	T	0.913	0.894	0.22,
	TA	11 (12.8)	11 (12.2)	0.608	A	0.087	0.106	0.637
	AA	2 (2.3)	4 (4.4)					
rs3766332	AA	47 (54.7)	55 (61.1)	0.13,	A	0.750	0.767	0.11,
	AT	35 (38.9)	28 (31.1)	0.719	T	0.250	0.233	0.741
	TT	4 (4.4)	7 (7.8)					
rs3753380	TT	9 (10.5)	7 (7.8)	0.59,	T	0.337	0.300	0.01,
	TC	40 (46.5)	40 (44.4)	0.444	C	0.663	0.700	0.923
	CC	37 (43.0)	43 (47.8)					
rs12093097	CC	52 (60.5)	65 (72.2)	2.71,	C	0.773	0.844	1.56,
	CT	29 (33.7)	22 (24.4)	0.100	T	0.227	0.156	0.212
	TT	5 (5.8)	3 (3.3)					
rs1073610	GG	53 (61.6)	66 (73.3)	2.55,	G	0.756	0.833	1.50,
	GA	24 (27.9)	18 (20.0)	0.110	A	0.244	0.167	0.220
	AA	9 (10.5)	6 (6.7)					
rs1073611	AA	54 (62.8)	66 (73.3)	2.19,	A	0.762	0.833	1.20,
	AG	23 (26.7)	18 (20.0)	0.139	G	0.238	0.167	0.273
	GG	9 (10.5)	6 (6.7)					

LLA; linear by linear association *p<0.05 based on LLA, #p<0.05 based on Pearson chi-square test

3.3.2.4: The effect of population stratification on *PTGFR* in the Malaysian population

To identify the effect of population stratification in this study, linear by linear association analysis was conducted on the genotype frequency of SNPs between Malays and Chinese recruited in this study. There was significant difference in genotype frequency between Malays and Chinese in 25SNPs identified in this study (table 3.20).

However, after examining 16 unlinked SNPs in the vicinity of the gene, we observed no inflation of their test statistics (λ gc = 0.92), thus suggesting that the significant P-values observed within the gene are unlikely to be due to gross population stratification. Future, more detailed genotyping will be needed to exclude the possibility of cryptic population stratification.

Table 3.20: Allele frequency of SNPs found in *PTGFR* between Malays and Chinese

SNPs	Allele	Malay N=120	Chinese N=56	χ^2	p-value
rs3766331	A	0.817	0.866	1.59	0.207
	G	0.183	0.134		
rs3766353	G	0.758	0.652	2.91	**0.088**^
	T	0.242	0.348		
rs3766355	C	0.567	0.509	0.72	0.395
	A	0.433	0.491		
rs35978825	C	0.888	0.929	0.98	0.323
	T	0.112	0.071		
rs1830673	A	0.421	0.348	1.03	0.309
	G	0.579	0.652		
rs3766351	T	0.842	0.929	3.98	**0.046***
	C	0.158	0.071		
rs1555541	T	0.321	0.348	0.20	0.653
	C	0.679	0.652		
rs10489785	A	0.879	0.955	4.35	**0.037***
	T	0.121	0.045		
rs12094298	C	0.917	0.973	0.41	0.121
	A	0.083	0.027		
rs34077564	A	0.854	0.929	3.27	**0.071**^
	G	0.146	0.071		
rs35123627	C	0.904	0.920	0.24	0.621
	T	0.096	0.080		
rs2146489	A	0.454	0.339	2.53	0.112
	G	0.546	0.661		
rs2057424	A	0.638	0.652	0.02	0.883
	G	0.362	0.348		
rs15101588	G	0.521	0.491	0.18	0.617
	A	0.479	0.509		
rs34528585	T	0.904	0.929	0.58	0.447
	A	0.096	0.071		
rs12044011	T	0.550	0.509	0.32	0.571

	A	0.450	0.491		
rs1322930	G	0.954	0.964	0.12	0.733
	A	0.045	0.036		
rs34852041	C	0.875	0.973	5.84	**0.016***
	T	0.125	0.027		
rs33994937	T	0.879	0.964	4.35	**0.037***
	C	0.121	0.036		
rs6424776	T	0.525	0.518	0.02	0.887
	C	0.475	0.482		
rs72673925	T	0.888	0.946	2.45	0.118
	G	0.112	0.054		
rs28832602	C	0.875	0.946	3.15	**0.076^**
	T	0.125	0.054		
rs34572897	A	0.888	0.955	3.53	**0.060***
	G	0.112	0.045		
rs1590314	T	0.380	0.402	0.08	0.772
	C	0.620	0.598		
rs12058120	C	0.854	0.929	3.27	**0.071^**
	G	0.146	0.071		
rs12725125	G	0.863	0.929	2.61	0.106
	A	0.137	0.071		
rs4261075	A	0.392	0.420	0.19	0.666
	G	0.608	0.580		
rs34012602	G	0.892	0.964	3.53	**0.060^**
	T	0.108	0.036		
rs67351117	C	0.892	0.946	2.45	0.118
	T	0.108	0.054		
rs672561	T	0.879	0.964	4.35	**0.037***
	C	0.121	0.036		
rs12401416	G	0.533	0.500	0.18	0.671
	A	0.467	0.500		
rs6424778	C	0.963	0.946	0.42	0.516
	T	0.037	0.054		

rs577333	T	0.338	0.330	0.02	0.881
	C	0.662	0.670		
rs520171	A	0.683	0.786	3.11	**0.078^**
	C	0.317	0.214		
rs551253	G	0.746	0.777	0.25	0.617
	C	0.254	0.223		
rs552328	A	0.608	0.527	1.31	0.253
	G	0.392	0.473		
rs11162504	A	0.792	0.795	0.03	0.361
	G	0.208	0.205		
rs11162505	A	0.867	0.857	0.04	0.836
	G	0.133	0.143		
rs554173	T	0.817	0.741	1.86	0.172
	C	0.183	0.259		
rs554185	A	0.633	0.563	1.02	0.313
	G	0.367	0.437		
rs556817	A	0.838	0.804	0.54	0.462
	G	0.162	0.196		
rs473027	A	0.583	0.473	2.47	0.119
	G	0.417	0.527		
rs668005	C	0.625	0.482	4.56	**0.033***
	T	0.375	0.518		
rs2146490	G	0.900	0.946	1.80	0.179
	A	0.100	0.064		
rs530871	G	0.613	0.482	3.41	**0.065^**
	A	0.387	0.518		
rs538275	G	0.625	0.446	6.52	**0.011***
	A	0.375	0.554		
rs589958	G	0.613	0.455	4.52	**0.033***
	A	0.387	0.545		
rs3766338	T	0.808	0.607	8.68	**0.003#**
	C	0.192	0.393		
rs590309	T	0.613	0.438	5.79	**0.016***
	C	0.387	0.562		

rs622346	G	0.717	0.643	1.47	0.225
	C	0.293	0.357		
rs13374108	T	0.900	0.848	1.29	0.257
	A	0.100	0.152		
rs34012237	T	0.896	0.964	2.77	**0.096**^
	C	0.104	0.036		
rs33966768	T	0.892	0.964	2.77	**0.096**^
	C	0.108	0.036		
rs501078	C	0.371	0.330	0.35	0.553
	T	0.629	0.670		
rs3766335	G	0.892	0.973	4.92	**0.027***
	A	0.108	0.027		
rs7543738	C	0.946	0.973	0.52	0.470
	G	0.054	0.027		
rs686262	A	0.421	0.357	0.76	0.384
	G	0.579	0.643		
rs4650581	T	0.883	0.946	2.45	0.118
	A	0.117	0.054		
rs3766332	A	0.771	0.732	0.43	0.514
	T	0.229	0.268		
rs3753380	T	0.341	0.268	1.16	0.282
	C	0.659	0.732		
rs12093097	C	0.804	0.821	0.13	0.778
	T	0.196	0.179		
rs1073610	G	0.788	0.813	0.13	0.724
	A	0.112	0.187		
rs1073611	G	0.792	0.813	0.13	0.724
	A	0.108	0.187		

p<0.1, *p<0.05, #p<0.01 based on Pearson chi-square test

3.3.2.5: The role of *PTGFR* on susceptibility to glaucoma in Malays and Chinese

Gender and racial group were identified as factors that may influence the genotype frequency of SNPs in *PTGFR*. Based on linear by linear association and Mantel-Haenszel test, rs11162505, rs554185, rs551253, rs556817 and rs668005 were identified to significantly associate with glaucoma susceptibility in Malays (table 3.21 and 3.22). There was significant difference in genotype of rs11162505 between glaucoma and controls of Malay ethnicity (p=1.72E-4).

Table 3.21: Linear by linear association analysis between genotype frequency of *PTGFR* and susceptibility to glaucoma in Malays and Chinese

SNPs		Malays			Chinese		
		Glaucoma N=58	Control N=62	LLA, p-value	Glaucoma N=28	Control N=28	LLA, p-value
rs3766331	AA	38(65.5)	39(62.9)	0.00, 0.952	22(78.6)	20(71.4)	0.08, 0.783
	AG	18(31.0)	17(27.4)		5(17.9)	8(28.6)	
	GG	2(3.5)	6(9.7)		1(3.6)	0	
rs3766353	GG	38(67.7)	33(53.2)	1.35, 0.246	13(46.4)	12(42.9)	0.04, 0.851
	GT	16(25.8)	24(38.7)		10(35.7)	13(46.4)	
	TT	4(6.5)	5(8.1)		5(17.9)	3(10.7)	
rs3766355	CC	18(31.0)	19(30.6)	0.01, 0.943	7(25.0)	9(32.1)	0.79, 0.373
	CA	30(51.7)	32(53.2)		12(42.9)	13(46.4)	
	AA	10(17.2)	11(16.1)		9(32.1)	6(21.4)	
rs35978825	CC	46(79.3)	49(7.9)	0.00, 0.984	25(89.3)	23(82.1)	0.57, 0.449
	CT	11(19.0)	12(19.4)		3(10.7)	3(10.7)	
	TT	1(1.7)	1(1.6)		0	0	
rs1830673	AA	10(17.2)	10(16.1)	1.91, 0.167	4(14.3)	4(14.3)	0.88, 0.348
	AG	34(58.6)	27(43.5)		9(32.1)	14(50.0)	
	GG	14(24.1)	25(40.3)		15(53.6)	10(35.7)	
rs3766351	TT	45(77.6)	48(77.4)	0.01, 0.916	25(89.3)	26(92.9)	0.31, 0.580
	TC	8(13.8)	8(12.9)		1(3.6)	1(3.6)	
	CC	5 (8.6)	6(9.7)		2(7.1)	1(3.6)	
rs1555541	TT	10(17.2)	7(11.3)	0.04, 0.842	5(17.9)	7(25.0)	0.69, 0.408
	TC	18(31.0)	25(40.3)		7(25.0)	8(28.6)	
	CC	30(51.7)	30(48.4)		16(57.1)	13(46.4)	
rs10489785	AA	48(82.8)	48(77.4)	0.51, 0.477	25(89.3)	26(92.9)	0.22, 0.642
	AT	8(13.8)	11(17.7)		3(10.7)	2(7.1)	
	TT	2(3.4)	3(4.8)		0	0	
rs12094298	CC	51 (89.7)	52(83.9)	0.45, 0.504	26(92.9)	27(96.4)	0.35, 0.556
	CA	4 (6.9)	8(12.9)		2(7.1)	1(3.6)	
	AA	2 (3.4)	2(3.2)		0	0	
rs34077564	AA	48 (82.8)	49(79.0)	0.00, 0.981	25(89.3)	26(92.9)	0.31, 0.580
	AG	3 (5.2)	8(12.9)		1(3.6)	1(3.6)	
	GG	7 (12.1)	5(8.1)		2(7.1)	1(3.6)	
rs35123627	CC	49(84.5)	52(83.9)	0.00, 0.964	24(85.7)	24(85.7)	0.10, 0.748

	CT	7(12.1)	8(12.9)		4(14.3)	3(10.7)	
	CC	2(3.4)	2(3.2)		0	1(3.6)	
rs2146489	AA	8(13.8)	16(25.8)	0.49, 0.483	2(7.1)	3(10.7)	2.83, **0.093^**
	AG	34(58.6)	27(43.5)		11(39.3)	17(60.7)	
	GG	16(27.6)	19(30.6)		15(53.6)	8(28.6)	
rs2057424	AA	33(56.9)	26(41.9)	4.28, **0.038**	15(53.6)	17(60.7)	0.59, 0.444
	AG	17(29.3)	18(29.0)		4(14.3)	5(17.9)	
	GG	8(13.8)	18(29.0)		9(32.1)	6(21.4)	
rs1510158840	GG	16(27.6)	16(25.8)	0.17, 0.681	7(25.0)	8(28.6)	0.03, 0.859
	GA	30(51.7)	31(50.0)		14(50.0)	11(39.3)	
	AA	12(20.7)	15(24.2)		7(25.0)	9(32.1)	
rs34528585	TT	48(82.8)	52(83.9)	0.00, 0.963	24(85.7)	25(89.3)	0.44, 0.505
	TA	9(15.5)	8(12.9)		3(10.7)	3(10.7)	
	AA	1(1.7)	2(3.2)		1(3.6)	0	
rs12044011	TT	15(25.9)	20(32.3)	0.05, 0.832	8(28.6)	9(32.1)	0.03, 0.863
	TA	33(8.9)	29(46.8)		12(42.9)	11(39.3)	
	AA	10(17.2)	13(21.0)		8(28.6)	8(28.6)	
rs1322930	GG	55(94.8)	55(88.7)	1.78, 0.182	26(92.9)	27(96.4)	0.69, 0.407
	GA	3(5.2)	6(9.7)		1(3.6)	1(3.6)	
	AA	0	1(1.6)		1(3.6)	0	
rs34852041	CC	47(81.0)	46(74.2)	0.87, 0.351	27(96.4)	26(92.9)	0.35, 0.556
	CT	10(17.2)	14(22.6)		1(3.6)	2(7.1)	
	TT	1(1.7)	2(3.2)		0	0	
rs33994937	TT	48(82.8)	46(74.2)	1.29, 0.256	26(92.9)	26(92.9)	0.00, 1.000
	TC	9(16.5)	14(22.6)		2(7.1)	2(7.1)	
	CC	1(1.7)	2(3.2)		0	0	
rs6424776	TT	16(27.6)	15(24.2)	1.20, 0.274	8(28.6)	8(28.6)	0.00, 1.000
	TC	33(56.9)	31(50.0)		13(46.4)	13(46.4)	
	CC	9(15.5)	16(25.8)		7(25.0)	7(25.0)	
rs72673925	TT	47(81.0)	49(79.0)	0.00, 0.985	24(85.7)	26(92.9)	0.73, 0.392
	TG	9(15.5)	12(19.4)		4(1.3)	2(7.1)	
	GG	2(3.4)	1(1.6)		0	0	
rs28832602	CC	45(77.6)	49(79.0)	0.03, 0.857	24(85.7)	26 (92.9)	0.73, 0.392
	CT	11(19.0)	11(17.7)		4(14.3)	2(7.1)	
	TT	2(3.4)	2(3.2)		0	0	
rs34572897	AA	47(81.0)	49(79.0)	0.00, 0.985	25(89.3)	26(92.9)	0.22, 0.642
	AG	9(15.5)	12(19.4)		3(10.7)	2(7.1)	
	GG	2(3.4)	1(1.6)		0	0	
rs1590314	TT	7(12.1)	7(11.3)	0.08, 0.774	5(17.90	5(17.9)	0.31, 0.580
	TC	31(53.4)	32(51.6)		11(39.3)	14(50.0)	
	CC	20(34.5)	23(51.1)		12(42.9)	9(32.1)	
rs12058120	CC	47(81.0)	46(74.2)	0.00, 0.979	24(85.7)	25(89.3)	0.44, 0.505
	CG	5(8.6)	14(22.6)		3(10.7)	3(10.7)	
	GG	6(10.3)	2(3.2)		1(3.6)	3(10.7)	
rs12725125	GG	47(81.0)	48(77.4)	0.11, 0.741	24(85.7)	25(89.3)	0.44,0.505
	GA	5(8.6)	12(19.4)		3910.7)	3(10.7)	
	AA	6(10.3)	2(3.2)		1(3.6)	0	
rs4261075	AA	10(17.2)	7(11.3)	0.93, 0.335	6(21.4)	6(21.4)	0.03, 0.860
	AG	29(50.0)	31(50.0)		11(39.3)	12(42.9)	
	GG	19(32.8)	24(38.7)		11(39.3)	10(35.7)	
rs34012602	GG	46(79.3)	50(80.6)	0.34, 0.563	27(96.4)	25(89.3)	1.06, 0.304
	GT	10(17.2)	12(19.4)		1(3.6)	3(10.7)	
	TT	2(3.4)	0		2(7.1)	0	
rs67351117	CC	48(82.8)	49(79.0)	0.05, 0.826	25(89.3)	26(92.9)	0.53, 0.465
	CT	8(13.8)	12(19.4)		2(7.1)	2(7.1)	
	TT	2(3.4)	1(1.6)		1(3.6)	0	
rs672561	TT	48(82.8)	47(75.8)	0.54, 0.463	26(92.9)	26(92.9)	0.00, 1.000
	TC	8(13.8)	13(21.0)		2(7.1)	2(7.1)	
	CC	2(3.4)	2(3.2)		0	0	
rs12401416	GG	16(27.6)	16(25.8)	0.09, 0.762	8(28.6)	8(28.6)	0.00, 1.000

	GA	31(53.4)	30(53.2)		12(42.9)	12(42.9)	
	AA	11(19.0)	13(21.0)		8(28.6)	8(28.6)	
rs6424778	CC	54(93.1)	57(91.9)	0.06, 0.809	23(82.1)	27(96.4)	2.93, **0.087**^
	CT	4(6.9)	5(8.1)		5(17.9)	1(3.6)	
	TT	0	0		0	0	
rs577333	TT	5(8.6)	4(6.5)	2.11, 0.147	4(14.3)	3(10.7)	0.93, 0.336
	TC	34(58.6)	29(46.8)		8(28.6)	15(53.6)	
	CC	19(32.8)	29(46.8)		16(57.1)	10(35.7)	
rs520171	AA	26(44.8)	29(46.8)	0.24, 0.625	17(60.7)	18(64.3)	0.20, 0.655
	AC	29(50.0)	25(40.3)		11(39.3)	7(25.0)	
	CC	3(5.2)	8(12.9)		0	3(10.7)	
rs551253	GG	39(67.2)	35(56.5)	4.76, **0.029**	18(64.3)	20(71.4)	0.04, 0.851
	GC	17(29.3)	14(22.6)		8(28.6)	3(10.7)	
	CC	2(3.4)	13(21.0)		2(7.1)	5(17.9)	
rs552328	AA	26(44.8)	23(37.1)	2.48, 0.116	7(25.0)	8(28.6)	0.04, 0.848
	AG	25(43.1)	23(37.1)		15(53.6)	14(50.0)	
	GG	7(12.1)	16(25.8)		6(21.4)	6(21.4)	
rs11162504	AA	40(69.0)	37(59.7)	0.92, 0.337	17(60.7)	19(67.9)	0.05, 0.823
	AG	15(25.9)	21(33.9)		10(35.7)	7(25.0)	
	GG	3(5.2)	4(6.5)		1(3.6)	2(7.1)	
rs11162505	AA	54(93.1)	38(61.3)	16.59,**1.72E-4**	22(78.6)	21(75.0)	0.23, 0.635
	AG	4(6.9)	20(32.3)		5(17.9)	5(17.9)	
	GG	0(0)	4(6.5)		1(3.6)	2(7.1)	
rs554173	TT	42(72.4)	39(62.9)	0.54, 0.463	16(57.1)	15(53.6)	0.400, 0.526
	TC	13(22.4)	21(33.9)		11(39.3)	10(35.7)	
	CC	3(5.2)	2(3.2)		1(3.6)	3(10.7)	
rs554185	AA	29(50.0)	23(37.1)	4.56, **0.033**	8(28.6)	8(28.6)	0.04, 0.840
	AG	24(41.4)	24(38.7)		16(57.1)	15(53.6)	
	GG	5(8.6)	15(24.2)		4(14.3)	5(17.9)	
rs556817	AA	47(81.0)	39(62.9)	2.57, 0.109	20(71.4)	18(64.3)	0.74, 0.391
	AG	8(13.8)	21(33.9)		7(25.0)	7(25.0)	
	GG	3(5.2)	2(3.2)		1(3.6)	3(10.7)	
rs473027	AA	22(37.9)	22(35.5)	1.75, 0.186	6(21.4)	7(25.0)	0.03, 0.854
	AG	29(50.0)	23(37.1)		14(50.0)	13(46.4)	
	GG	7(12.1)	17(27.4)		8(28.6)	8(28.6)	
rs668005	CC	30(51.7)	23(37.1)	4.19, **0.041**	6(21.4)	7(25.0)	0.00, 1.000
	CT	21(36.2)	23(37.1)		15(53.6)	13(46.4)	
	TT	7(12.1)	16(25.8)		7(25.0)	8(28.6)	
rs2146490	GG	50(86.2)	50(80.6)	0.37, 0.541	24(85.7)	26(92.9)	0.73, 0.392
	GA	6(10.3)	10(16.1)		4(14.3)	2(7.1)	
	AA	2(3.4)	2(3.2)		0	0	
rs530871	GG	27(46.6)	24(38.7)	2.78, 0.095	6(21.4)	7(25.0)	0.00, 1.000
	GA	24(41.4)	21(33.9)		14(50.0)	14(50.0)	
	AA	7(12.1)	17(27.4)		8(28.6)	7(25.0)	
rs538275	GG	26(44.8)	25(40.3)	1.86, 0.172	6(21.4)	6(21.4)	0.00, 1.000
	GA	26(44.8)	22(33.5)		13(46.4)	13(46.4)	
	AA	6(10.4)	15(24.2)		9(32.1)	9(32.1)	
rs589958	GG	26(44.8)	24(38.7)	2.10, 0.147	6(21.4)	6(21.4)	0.03, 0.853
	GA	25(43.1)	22(35.5)		14(50.0)	13(46.4)	
	AA	7(12.1)	16(25.8)		8(28.6)	9(32.1)	
rs3766338	TT	42(72.4)	38(61.3)	1.76, 0.184	8(28.6)	14(50.0)	0.53, 0.465
	TC	14(24.1)	20(32.3)		16(57.1)	8(28.6)	
	CC	2(3.4)	4(6.5)		4(14.3)	6(21.4)	
rs590309	TT	27(46.6)	23(37.1)	3.75, **0.053**^	5(17.9)	6(21.4)	0.31, 0.575
	TC	25(43.1)	22(35.5)		13(46.4)	14(50.0)	
	CC	6(10.3)	17(27.4)		10(35.7)	8(28.6)	
rs622346	GG	33(56.9)	31(50.0)	0.61, 0.435	10(35.7)	12(42.9)	0.00, 1.000
	GC	20(34.5)	24(38.7)		16(57.1)	12(42.9)	
	CC	5(8.6)	7(11.3)		23(7.1)	4(14.3)	

rs13374108	TT	50(86.2)	47(75.8)	2.43, 0.119	20(71.4)	20(71.4)	0.07, 0.790
	TA	8(13.8)	14(22.6)		7(25.0)	8(28.6)	
	AA	0	1(1.6)		1(3.6)	0	
rs34012237	TT	49(84.5)	40(80.6)	0.168,0.682	26(92.9)	26(92.9)	0.00, 1.000
	TC	7(12.1)	10(16.1)		2(7.1)	2(7.1)	
	CC	2(3.4)	2(3.2)		0	0	
rs33966768	TT	48(82.8)	50(80.6)	0.05, 0.832	26(92.9)	26(92.9)	0.00, 1.000
	TC	8(13.8)	10(16.1)		2(7.1)	2(7.1)	
	CC	2(3.4)	2(3.2)		0	0	
rs501078	CC	11(19.0)	8(12.9)	1.03, 0.309	3(10.7)	4(14.3)	0.33, 0.564
	CT	25(43.1)	26(41.9)		14(50.0)	9(32.1)	
	TT	22(37.9)	28(45.2)		11(39.3)	15(53.6)	
rs3766335	GG	49(84.5)	49(79.0)	0.34, 0.558	27(96.4)	26(92.9)	0.35, 0.556
	GA	7(12.1)	11(17.7)		1(3.6)	2(7.1)	
	AA	2(3.4)	2(3.2)		0	0	
rs7543738	CC	51(87.9)	58(93.5)	0.75, 0.386	27(96.4)	26(92.9)	0.35, 0.556
	CG	6(10.3)	3(4.8)		1(3.6)	2(7.1)	
	GG	1(1.7)	1(1.6)		0	0	
rs686262	AA	15(25.9)	10(16.1)	1.62, 0.204	5(17.9)	4(14.3)	1.20, 0.273
	AG	24(41.4)	27(43.5)		13(46.4)	9(32.1)	
	GG	19(32.8)	25(40.3)		10(35.7)	15(53.6)	
rs4650581	TT	48(82.8)	50(80.6)	0.28, 0.597	25(89.3)	25(89.3)	0.00, 1.000
	TA	8(13.8)	8(12.9)		3(10.7)	3(10.7)	
	AA	2(3.4)	4(6.2)		0	0	
rs3766332	AA	35(60.3)	38(61.3)	0.579, 0.447	12(42.9)	17(60.7)	03.15, 0.076
	AT	22(37.9)	17(27.4)		13(46.4)	11(39.3)	
	TT	1(1.7)	7(11.3)		3(10.7)	0	
rs3753380	TT	6(10.3)	5(8.1)	2.38, 0.123	3(10.7)	2(7.1)	0.66, 0.418
	TC	33(56.9)	27(43.5)		7(25.0)	13(46.4)	
	CC	21(39.9)	30(48.4)		18(64.3)	13(46.4)	
rs12093097	CC	32(55.2)	47(75.8)	3.86, **0.049**	20(71.4)	18(64.3)	0.001, 1.000
	CT	23(39.7)	12(19.4)		6(21.4)	10(35.7)	
	TT	3(5.2)	3(4.8)		2(7.1)	0	
rs1073610	GG	32(55.2)	48(77.4)	4.18, **0.041**	21(75.0)	18(64.3)	0.05, 0.829
	GA	20(34.5)	9(14.5)		4(14.3)	9(32.1)	
	AA	6(10.3)	5(8.1)		3(10.7)	1(3.6)	
rs1073611	GG	33(56.9)	48(77.4)	3.62, **0.057**^	21(75.0)	18(64.3)	0.05, 0.827
	GA	19(32.8)	9(14.5)		4(14.3)	9(32.1)	
	AA	6(10.3)	5(8.1)		3(10.7)	1(3.6)	

P<0.05 based on linear-by-linear association analysis (LLA), df=1

There was statistically significant difference of allele frequency of rs11162505 and rs2057424 between the Malays and Chinese based on Breslow-Day test for homogeneity (table 3.22), suggesting significant population stratification. The minor allele for rs11162505 (OR 0.3 [95% CI 0.1, 0.5]), rs554185 (OR 0.7 [95% CI 0.4, 1.0) and rs551253 (OR 0.6 [95% CI 0.4, 0.9]) is associated with decrease susceptibility to glaucoma in Malays (table 3.22).

Table 3.22: Stratified Mantel-Haenszel meta-analysis on *PTGFR* and susceptibility to glaucoma in Malays and Chinese

	Malays		Chinese		Stratified meta-analysis			
SNPs	Allele frequency		Allele frequency		OR	SE	P-meta	P-Het
	Glaucoma N=58	Control N=62	Glaucoma N=28	Control N=28				
rs3766331								
A	0.810	0.766	0.875	0.857	0.79	0.28	0.388	0.862
G	0.190	0.234	0.125	0.143	(0.46, 1.35)			
rs3766353								
G	0.793	0.726	0.643	0.661	0.82	0.24	0.397	0.369
T	0.207	0.274	0.357	0.339	(0.51, 1.31)			
rs3766355								
C	0.569	0.565	0.464	0.554	1.11	0.21	0.632	0.414
A	0.431	0.435	0.536	0.446	(0.73, 1.69)			
rs35978825								
C	0.888	0.887	0.946	0.875	0.98	0.37	0.953	0.940
T	0.112	0.113	0.054	0.054	(0.48, 2.01)			
rs1830673								
A	0.466	0.379	0.304	0.393	0.88	0.22	0.559	0.115
G	0.534	0.621	0.696	0.607	(0.58, 1.35)			
rs3766351								
T	0.845	0.839	0.911	0.946	1.07	0.32	0.840	0.473
C	0.155	0.161	0.089	0.054	(0.57, 1.99)			
rs1555541								
T	0.328	0.315	0.304	0.393	1.09	0.23	0.697	0.348
C	0.672	0.685	0.696	0.607	(0.70, 1.70)			
rs10489785								
A	0.897	0.863	0.946	0.964	0.82	0.37	0.582	0.458
T	0.103	0.137	0.054	0.036	(0.40, 1.67)			
rs12094298								
C	0.931	0.903	0.964	0.982	0.80	0.44	0.613	0.402
A	0.069	0.097	0.036	0.018	(0.34, 1.89)			
rs34077564								
A	0.853	0.855	0.911	0.946	1.12	0.33	0.728	0.520
G	0.147	0.145	0.089	0.054	(0.59, 2.14)			
rs35123627								
C	0.905	0.903	0.929	0.911	0.92	0.37	0.819	0.790
T	0.095	0.097	0.071	0.089	(0.44, 1.90)			
rs2146489								

A	0.431	0.476	0.268	0.411	1.37	0.22	0.147	0.335
G	0.569	0.524	0.732	0.589	(0.90, 2.10)			
rs2057424								
A	0.716	0.565	0.607	0.696	0.73	0.22	0.148	**0.028**
G	0.284	0.435	0.393	0.304	(0.47, 1.12)			
rs15101588								
G	0.534	0.508	0.500	0.482	0.91	0.21	0.657	0.940
A	0.466	0.492	0.500	0.518	(0.60, 1.38)			
rs34528585								
T	0.905	0.903	0.911	0.946	1.13	0.38	0.739	0.511
A	0.095	0.097	0.089	0.054	(0.54, 2.37)			
rs12044011								
T	0.543	0.556	0.500	0.518	1.06	0.21	0.781	0.970
A	0.457	0.444	0.500	0.482	(0.70, 1.62)			
rs1322930								
G	0.974	0.935	0.946	0.982	0.69	0.54	0.489	0.101
A	0.026	0.065	0.054	0.018	(0.24, 1.98)			
rs34852041								
C	0.897	0.855	0.982	0.964	0.62	0.38	0.269	0.802
T	0.103	0.145	0.018	0.036	(0.30, 1.30)			
rs33994937								
T	0.905	0.855	0.964	0.964	0.66	0.38	0.268	0.658
C	0.095	0.145	0.036	0.036	(0.32, 1.38)			
rs6424776								
T	0.560	0.492	0.518	0.518	0.83	0.21	0.382	0.549
C	0.440	0.508	0.482	0.482	(0.55, 1.26)			
rs72673925								
T	0.888	0.887	0.929	0.964	1.14	0.37	0.727	0.444
G	0.112	0.113	0.071	0.036	(0.55, 2.34)			
rs28832602								
C	0.871	0.879	0.929	0.964	1.21	0.36	0.596	0.496
T	0.129	0.121	0.071	0.036	(0.60, 2.42)			
rs34572897								
A	0.888	0.887	0.946	0.964	1.07	0.37	0.867	0.670
G	0.112	0.113	0.054	0.036	(0.51, 1.92)			
rs1590314								
T	0.388	0.371	0.375	0.429	1.02	0.22	0.916	0.529
C	0.612	0.629	0.625	0.571	(0.67, 1.57)			
rs12058120								
C	0.853	0.855	0.911	0.946	1.12	0.33	0.723	0.520
G	0.147	0.145	0.089	0.054	(0.59, 2.14)			

rs12725125								
G	0.888	0.871	0.911	0.946	1.00	0.33	0.987	0.405
A	0.112	0.129	0.089	0.054	(0.50, 1.98)			
rs4261075								
A	0.422	0.363	0.411	0.429	0.87	0.22	0.505	0.487
G	0.578	0.637	0.589	0.571	(0.57, 1.33)			
rs34012602								
G	0.879	0.903	0.982	0.946	1.07	0.36	0.408	0.793
T	0.121	0.097	0.089	0.054	(0.50, 2.26)			
rs67351117								
C	0.897	0.887	0.929	0.964	1.06	0.37	0.872	0.392
T	0.103	0.113	0.071	0.036	(0.51, 2.20)			
rs672561								
T	0.897	0.863	0.964	0.964	0.76	0.37	0.457	0.770
C	0.103	0.137	0.036	0.036	(0.37, 1.57)			
rs12401416								
G	0.543	0.500	0.500	0.500	0.95	0.21	0.809	0.868
A	0.457	0.452	0.500	0.500	(0.63, 1.44)			
rs6424778								
C	0.966	0.960	0.911	0.982	1.59	0.54	0.387	0.137
T	0.034	0.040	0.089	0.018	(0.56, 4.57)			
rs577333								
T	0.379	0.298	0.286	0.375	0.89	0.23	0.597	0.115
C	0.621	0.702	0.714	0.625	(0.57, 1.38)			
rs520171								
A	0.698	0.669	0.804	0.768	0.86	0.24	0.516	0.884
C	0.302	0.331	0.196	0.232	(0.54, 1.37)			
rs551253								
G	0.819	0.677	0.786	0.768	0.57	0.25	**0.027**	0.225
C	0.181	0.323	0.214	0.232	(0.35, 0.94)			
rs552328								
A	0.664	0.556	0.518	0.536	0.76	0.22	0.199	0.257
G	0.336	0.444	0.482	0.464	(0.49, 1.16)			
rs11162504								
A	0.819	0.766	0.786	0.804	0.83	0.26	0.484	0.446
G	0.181	0.234	0.214	0.196	(0.50, 1.39)			
rs11162505								
A	0.966	0.774	0.875	0.839	0.27	0.36	**<0.001**	**0.015**
G	0.057	0.226	0.125	0.161	(0.13, 0.54)			
rs554173								
T	0.836	0.798	0.768	0.714	0.77	0.27	0.321	0.962
C	0.164	0.202	0.232	0.286	(0.46,			

					1.29)			
rs554185								
A	0.706	0.565	0.571	0.554	0.65	0.22	**0.049**	0.241
G	0.293	0.435	0.429	0.446	(0.42, 1.00)			
rs556817								
A	0.879	0.798	0.839	0.768	0.57	0.29	*0.055*^	0.800
G	0.121	0.202	0.161	0.232	(0.33, 1.01)			
rs473027								
A	0.629	0.540	0.464	0.482	0.80	0.22	0.299	0.341
G	0.371	0.460	0.536	0.518	(0.52, 1.22)			
rs668005								
C	0.698	0.556	0.482	0.482	0.67	0.22	*0.065*^	0.188
T	0.302	0.444	0.518	0.518	(0.43, 1.03)			
rs2146490								
G	0.914	0.887	0.929	0.964	0.92	0.38	0.818	0.288
A	0.086	0.113	0.071	0.036	(0.43, 1.94)			
rs530871								
G	0.672	0.556	0.464	0.500	0.76	0.22	0.198	0.170
A	0.328	0.444	0.536	0.500	(0.49, 1.16)			
rs538275								
G	0.672	0.581	0.446	0.446	0.77	0.22	0.230	0.398
A	0.328	0.419	0.554	0.554	(0.50, 1.18)			
rs589958								
G	0.664	0.565	0.464	0.446	0.74	0.22	0.162	0.452
A	0.336	0.435	0.536	0.554	(0.48, 1.13)			
rs3766338								
T	0.845	0.774	0.571	0.643	0.87	0.25	0.577	0.136
C	0.155	0.226	0.429	0.357	(0.53, 1.42)			
rs590309								
T	0.681	0.548	0.411	0.464	0.74	0.22	0.162	*0.093*^
C	0.319	0.452	0.589	0.536	(0.48, 1.13)			
rs622346								
G	0.741	0.694	0.643	0.643	0.86	0.23	0.506	0.628
C	0.259	0.306	0.357	0.357	(0.54, 1.35)			
rs13374108								
T	0.931	0.871	0.839	0.857	0.71	0.34	0.304	0.229
A	0.069	0.129	0.161	0.143	(0.36, 1.37)			
rs34012237								
T	0.905	0.726	0.964	0.964	1.28	0.39	0.394	0.719
C	0.095	0.113	0.036	0.036	(0.59, 2.76)			
rs33966768								
T	0.897	0.887	0.964	0.964	0.92	0.39	0.827	0.929

C	0.103	0.113	0.036	0.036	(0.43, 1.96)			
rs501078								
C	0.405	0.339	0.357	0.304	0.76	0.22	0.223	0.930
T	0.595	0.661	0.643	0.696	(0.49, 1.18)			
rs3766335								
G	0.905	0.879	0.982	0.964	0.73	0.40	0.420	0.736
A	0.095	0.121	0.018	0.036	(0.33, 1.58)			
rs7543738								
C	0.931	0.960	0.982	0.964	1.38	0.52	0.535	0.335
G	0.069	0.040	0.018	0.036	(0.50, 3.78)			
rs686262								
A	0.466	0.379	0.411	0.304	0.68	0.22	*0.075*^	0.811
G	0.534	0.621	0.589	0.696	(0.44, 1.04)			
rs4650581								
T	0.897	0.871	0.946	0.946	0.82	0.37	0.578	0.788
A	0.103	0.129	0.054	0.054	(0.40, 1.67)			
rs3766332								
A	0.793	0.750	0.661	0.804	1.09	0.25	0.725	*0.064*^
T	0.207	0.250	0.339	0.196	(0.67, 1.78)			
rs3753380								
T	0.388	0.298	0.232	0.304	0.84	0.23	0.441	0.132
C	0.612	0.702	0.768	0.696	(0.54, 1.31)			
rs12093097								
C	0.750	0.855	0.821	0.821	1.59	0.27	*0.090*^	0.255
T	0.250	0.145	0.179	0.179	(0.93, 2.72)			
rs1073610								
G	0.724	0.847	0.821	0.804	1.61	0.27	*0.073*^	0.137
A	0.276	0.153	0.179	0.196	(0.96, 2.72)			
rs1073611								
G	0.733	0.847	0.821	0.804	1.56	0.27	*0.095*^	0.159
A	0.267	0.153	0.179	0.196	(0.93, 2.64)			

OR: odd ratio, CI: confidence interval, SE: standard error
Phet: P-value for heterogeneity between both studies (P<0.05 is considered significant heterogeneity based on the Breslow-Day test)
P-meta: P-value for the meta-analysis between Malays and Chinese where the association between alleles and glaucoma status was measured.

Univariate logistic regression was then conducted on the selected SNPs, sex, and age at presentation on Malays and Chinese separately (table 3.23 and table 3.24). rs551253GC (OR 7.1 [95% CI 1.0, 50.0]) and rs554185AG (OR 17.8 [95% CI 1.5, 213.7]) are found to increase

the susceptibility to glaucoma in Malays (table 3.23). Whereas, rs551253GC, rs554185AG and rs3766338TC showed association with glaucoma in Chinese (table 3.24). Chinese men were less susceptible to glaucoma compared to Chinese women (table 3.24).

Table 3.23: Univariate logistic regression analysis on predictors for glaucoma susceptibility in Malays

Predictors	OR	SE	p-value	95% CI for OR (LCI, UCI)
rs2146489				
AA	--	--	--	--
AG	4.19	0.75	*0.057*	0.96, 18.32
GG	2.54	0.75	0.212	0.59, 11.00
rs551253				
GG	--	--	--	--
GC	7.08	0.99	*0.050*	1.00, 50.02
CC	7.60	1.59	0.203	0.33, 172.39
rs11162505				
AA	--	--	--	--
AG	0.01	1.62	**0.002**	0.00, 0.13
GG	0.00	760.92	0.999	0.00
rs554185				
AA	--	--	--	--
AG	17.77	1.27	**0.023**	1.47, 213.73
GG	157.27	2.58	*0.050*	1.01, 24519.85
rs556817				
AA	--	--	--	--
AG	0.05	1.02	**0.003**	0.01, 0.36
GG	0.07	2.27	0.238	0.00, 5.91
rs668005				
CC	--	--	--	--
CT	0.27	1.03	0.198	0.04, 2.00
TT	0.15	1.65	0.244	0.01, 3.70
rs686262				
AA	--	--	--	--
AG	0.76	0.64	0.663	0.22, 2.65
GG	1.56	0.80	0.577	0.33, 7.50
rs12093097				
CC	--	--	--	--
CT	0.65	1.04	0.684	0.09, 5.05
TT	1.19	1.10	0.877	0.14, 10.20
Sex				
Male	2.32	0.54	0.119	0.81, 6.70
Female	--	--	--	--
Age at presentation	1.01	0.03	0.642	0.96, 1.06

The goodness of fit of this model was checked using the Hosmer-Lemenshow test; p=0.673. This result gives no evidence of lack of fit of the model.

Table 3.24: Univariate logistic regression analysis on predictors for glaucoma susceptibility in Chinese

Predictors	OR	SE	p-value	95% CI for OR (LCI, UCI)
rs551253				
GG	--	--	--	--
GC	15.32	1.31	**0.037**	1.18, 199.29
CC	2.48	2.46	0.712	0.02, 305.25
rs3766338				
TT	--	--	--	--
TC	36.43	1.67	**0.031**	1.39, 954.22
CC	14.92	1.60	0.092^	0.65, 345.37
rs11162505				
AA	--	--	--	--
AG	0.29	1.59	0.435	0.01, 6.51
GG	6.18	2.80	0.516	0.03, 1505.84
rs554185				
AA	--	--	--	--
AG	0.03	1.72	**0.041**	0.00, 0.87
GG	0.09	1.77	0.168	0.00, 2.79
Sex				
Male	0.01	1.73	**0.009**	0.00, 0.32
Female	--	--	--	--
Age at presentation	1.00	0.04	0.913	0.94, 1.08

OR: odd ratio, LCI: low confidence interval, UCI: upper confidence interval
The goodness of fit of this model was checked using the Hosmer-Lemenshow test; p=0.953. This result gives no evidence of lack of fit of the model.

3.3.2.6 *PTGFR* and susceptibility to glaucoma in the Malaysian population

Predictors that were found to be statistically significant in univariate logistic regression in Malays and Chinese were included in stepwise logistic regression. rs11162505, rs556817, rs551253, rs554185, rs3766338 and sex were included. Backward stepwise logistic regression was used as the final model.

rs11162505AG and rs556817AG conferred strong protective against glaucoma with reduction of risk of 0.2 fold (95%CI 0.0, 0.6) and 0.1 fold (95%CI 0.0, 0.5) respectively (table 3.25). rs551253GC increases the susceptibility to glaucoma 3.3fold (95% CI 1.2, 9.4). Similarly, rs3766338TC increases the susceptibility to develop glaucoma in both Malay and Chinese up to 4.2fold (95% CI 1.2, 14.0).

Table 3.25: Stepwise logistic regression exploring the *PTGFR* in influencing the risk of glaucoma relative to unaffected normal individuals

Predictors	OR_c	OR_r	SE	p-value	95% CI (UCI, LCI
rs551253					
HW-GG	--	--	--	--	--
HT-GC	2.57	3.33	0.53	**0.023**	1.18, 9.40
HM-CC	1.30	1.40	0.97	0.731	0.21, 9.32
rs11162505					
HW-AA	--	--	--	--	--
HT-AG	0.09	0.16	0.68	**0.007**	0.04, 0.61
HM-GG	0.02	0.10	1.44	0.114	0.01, 1.73
rs556817					
HW-AA	--	--	--	--	--
HT-AG	0.08	0.13	0.66	**0.002**	0.04, 0.46
HM-GG	0.22	1.11	0.98	0.916	0.16, 7.52
rs3766338					
HW-TT	--	--	--	--	--
HT-TC	3.40	4.15	0.62	**0.022**	1.23, 13.95
HM-CC	0.43	0.59	0.81	0.507	0.12, 2.85

OR_c: crude odd ratio, OR_r: logistic odd ratio, CI: confident interval, OR: odd ratio
The goodness of fit of the model was checked using the Hosmer-Lemeshow test (p= 0.092). This result gives no evidence of lack of fit of the model.

3.3.2.7 *PTGFR* and susceptibility to POAG in the Malaysian population

Patients diagnosed as POAG, NTG and OHT were included in this study. Due to small number of patients with OHT, patients with OHT were excluded from the analysis. There were 2 SNPs that demonstrated significant difference in 2 subpopulation; Malays and Chinese (rs11162505 and rs2057424). Minor allele frequency of rs12094298, rs551253, rs11162505, rs12093097, rs686262 and rs1073610 were found to demonstrate statistically significant association with POAG (table 3.26). However, Breslow-Day test for heterogeneity was statistically significant in rs11162505 suggesting the possible effect of population stratification.

Table 3.26: Stratified Mantel-Haenszel meta-analysis on *PTGFR* and susceptibility to POAG relative to unaffected normal individuals in Malays and Chinese

	Malays		**Chinese**		**Stratified meta-analysis**			
SNPs	Allele frequency		Allele frequency		OR	SE	P-meta	P-Het
	Glaucoma N=40	Control N=62	Glaucoma N=24	Control N=28				
rs3766331								
A	0.800	0.766	0.875	0.857	0.83	0.30	0.532	0.946
G	0.200	0.234	0.125	0.143	(0.46, 1.49)			
rs3766353								
G	0.763	0.726	0.625	0.661	0.95	0.26	0.825	0.508
T	0.238	0.274	0.375	0.339	(0.57, 1.56)			
rs3766355								
C	0.550	0.565	0.458	0.554	1.19	0.23	0.461	0.509
A	0.450	0.435	0.542	0.446	(0.75, 1.87)			
rs35978825								
C	0.900	0.887	0.979	0.875	0.59	0.41	0.202	0.111
T	0.100	0.113	0.021	0.054	(0.26, 1.33)			
rs1830673								
A	0.425	0.379	0.313	0.393	0.96	0.24	0.863	0.372
G	0.575	0.621	0.438	0.607	(0.60, 1.53)			
rs3766351								
T	0.863	0.839	0.958	0.946	0.82	0.37	0.592	0.940
C	0.138	0.161	0.042	0.054	(0.40, 1.70)			
rs1555541								

T	0.338	0.315	0.292	0.393	1.10	0.25	0.713	0.283
C	0.663	0.685	0.708	0.607	(0.68, 1.77)			
rs10489785								
A	0.938	0.863	0.979	0.964	0.44	0.49	0.091	0.815
T	0.063	0.137	0.021	0.036	(0.17, 1.14)			
rs12094298								
C	0.988	0.903	0.979	0.982	0.21	0.77	**0.044**	0.145
A	0.013	0.097	0.021	0.018	(0.05, 0.96)			
rs34077564								
A	0.863	0.855	0.979	0.946	0.84	0.39	0.643	0.451
G	0.138	0.145	0.021	0.054	(0.39, 1.78)			
rs35123627								
C	0.938	0.903	0.958	0.911	0.56	0.47	0.216	0.740
T	0.063	0.097	0.042	0.089	(0.23, 1.40)			
rs2146489								
A	0.425	0.476	0.271	0.411	1.41	0.24	0.151	0.408
G	0.575	0.524	0.729	0.589	(0.88, 2.24)			
rs2057424								
A	0.725	0.565	0.625	0.696	0.71	0.24	0.153	**0.046**
G	0.275	0.435	0.375	0.304	(0.44, 1.14)			
rs15101588								
G	0.488	0.508	0.458	0.482	1.09	0.23	0.708	0.978
A	0.513	0.492	0.542	0.518	(0.69, 1.72)			
rs34528585								
T	0.913	0.903	0.938	0.946	0.96	0.43	0.925	0.779
A	0.088	0.097	0.063	0.054	(0.42, 2.22)			
rs12044011								
T	0.538	0.556	0.458	0.518	1.14	0.23	0.567	0.740
A	0.463	0.444	0.542	0.482	(0.72, 1.80)			
rs1322930								
G	0.975	0.935	0.938	0.982	1.13	0.52	0.814	0.219
A	0.025	0.065	0.063	0.018	(0.41, 3.15)			
rs34852041								
C	0.913	0.855	1.000	0.964	0.49	0.46	0.126	0.329
T	0.088	0.145	0.000	0.036	(0.20, 1.22)			
rs33994937								
T	0.925	0.855	0.979	0.964	0.49	0.46	0.120	0.890
C	0.075	0.145	0.021	0.036	(0.20, 1.20)			
rs6424776								
T	0.513	0.492	0.479	0.518	1.00	0.23	1.000	0.141
C	0.975	0.508	0.521	0.482	(0.64, 1.56)			

rs72673925								
T	0.900	0.887	0.958	0.964	0.92	0.43	0.842	0.792
G	0.100	0.113	0.042	0.036	(0.40, 2.11)			
rs28832602								
C	0.900	0.879	0.958	0.964	0.86	0.42	0.720	0.738
T	0.100	0.121	0.042	0.036	(0.38, 1.96)			
rs34572897								
A	0.900	0.887	0.979	0.964	0.83	0.44	0.664	0.751
G	0.100	0.113	0.021	0.036	(0.35, 1.95)			
rs1590314								
T	0.363	0.371	0.354	0.429	1.14	0.24	0.577	0.582
C	0.638	0.629	0.646	0.571	(0.71, 1.83)			
rs12058120								
C	0.888	0.855	0.938	0.946	0.82	0.39	0.608	0.629
G	0.113	0.145	0.062	0.054	(0.39, 1.75)			
rs12725125								
G	0.888	0.871	0.938	0.946	0.92	0.39	0.824	0.736
A	0.113	0.129	0.062	0.054	(0.43, 1.97)			
rs4261075								
A	0.413	0.363	0.396	0.429	0.92	0.24	0.711	0.488
G	0.588	0.637	0.604	0.571	(0.58, 1.46)			
rs34012602								
G	0.913	0.903	1.000	0.946	0.68	0.47	0.410	0.145
T	0.088	0.097	0.00	0.054	(0.27, 1.71)			
rs67351117								
C	0.913	0.887	0.958	0.964	0.82	0.44	0.643	0.694
T	0.088	0.113	0.042	0.036	(0.35, 1.92)			
rs672561								
T	0.925	0.863	1.000	0.964	0.44	0.49	0.096	0.353
C	0.075	0.137	0.000	0.036	(0.17, 1.16)			
rs12401416								
G	0.513	0.500	0.458	0.500	1.10	0.23	0.693	0.814
A	0.488	0.452	0.542	0.500	(0.69, 1.73)			
rs6424778								
C	0.963	0.960	0.896	0.982	1.91	0.56	0.247	0.130
T	0.038	0.040	0.104	0.018	(0.64, 5.71)			
rs577333								
T	0.363	0.298	0.271	0.375	0.98	0.25	0.917	0.140
C	0.638	0.702	0.729	0.625	(0.60, 1.58)			
rs520171								
A	0.700	0.669	0.813	0.768	0.84	0.26	0.493	0.824
C	0.300	0.331	0.188	0.232	(0.50,			

					1.40)			
rs551253								
G	0.863	0.677	0.813	0.768	0.45	0.29	**0.006**	0.178
C	0.138	0.323	0.188	0.232	(0.25, 0.80)			
rs552328								
A	0.638	0.556	0.542	0.536	0.80	0.24	0.337	0.524
G	0.363	0.444	0.458	0.464	(0.50, 1.27)			
rs11162504								
A	0.850	0.766	0.813	0.804	0.69	0.30	0.211	0.433
G	0.150	0.234	0.188	0.196	(0.38, 1.24)			
rs11162505								
A	1.000	0.774	0.896	0.839	0.15	0.50	**<0.001**	**0.002**
G	0.000	0.226	0.104	0.161	(0.06, 0.41)			
rs554173								
T	0.813	0.798	0.771	0.714	0.84	0.28	0.546	0.722
C	0.188	0.202	0.229	0.286	(0.48, 1.47)			
rs554185								
A	0.688	0.565	0.583	0.554	0.68	0.24	0.111	0.414
G	0.313	0.435	0.417	0.446	(0.43, 1.09)			
rs556817								
A	0.838	0.798	0.833	0.768	0.73	0.30	0.292	0.811
G	0.163	0.202	0.167	0.232	(0.40, 1.31)			
rs473027								
A	0.613	0.540	0.500	0.482	0.81	0.23	0.355	0.646
G	0.388	0.460	0.500	0.518	(0.51, 1.27)			
rs668005								
C	0.700	0.556	0.500	0.482	0.66	0.24	*0.080*	0.269
T	0.300	0.444	0.500	0.518	(0.41, 1.05)			
rs2146490								
G	0.930	0.887	0.958	0.964	0.62	0.47	0.310	0.479
A	0.063	0.113	0.042	0.036	(0.25, 1.56)			
rs530871								
G	0.675	0.556	0.479	0.500	0.75	0.24	0.220	0.234
A	0.325	0.444	0.521	0.500	(0.47, 1.19)			
rs538275								
G	0.663	0.581	0.458	0.446	0.79	0.24	0.312	0.543
A	0.338	0.419	0.542	0.554	(0.49, 1.25)			
rs589958								
G	0.650	0.565	0.479	0.446	0.76	0.24	0.240	0.644
A	0.350	0.435	0.521	0.554	(0.48, 1.20)			
rs3766338								
T	0.825	0.774	0.542	0.643	1.01	0.27	0.969	0.172

C	0.175	0.226	0.458	0.357	(0.60, 1.70)			
rs590309								
T	0.675	0.548	0.417	0.464	0.76	0.24	0.248	0.141
C	0.325	0.452	0.583	0.536	(0.48, 1.21)			
rs622346								
G	0.738	0.694	0.646	0.643	0.87	0.25	0.579	0.696
C	0.263	0.306	0.354	0.357	(0.53, 1.43)			
rs13374108								
T	0.925	0.871	0.854	0.857	0.72	0.37	0.363	0.403
A	0.075	0.129	0.146	0.143	(0.35, 1.47)			
rs34012237								
T	0.925	0.726	0.854	0.964	0.53	0.47	0.178	0.942
C	0.075	0.113	0.146	0.036	(0.21, 1.34)			
rs33966768								
T	0.925	0.887	0.854	0.964	0.63	0.47	0.324	0.939
C	0.075	0.113	0.146	0.036	(0.25, 1.58)			
rs501078								
C	0.425	0.339	0.375	0.304	0.70	0.24	0.145	0.926
T	0.575	0.661	0.625	0.696	(0.44, 1.13)			
rs3766335								
G	0.925	0.879	0.854	0.964	0.59	0.47	0.256	0.985
A	0.075	0.121	0.146	0.036	(0.23, 1.47)			
rs7543738								
C	0.950	0.960	0.854	0.964	1.03	0.60	0.959	0.579
G	0.050	0.040	0.146	0.036	(0.32, 3.32)			
rs686262								
A	0.500	0.379	0.417	0.304	0.61	0.24	**0.038**	1.000
G	0.500	0.621	0.583	0.696	(0.38, 0.97)			
rs4650581								
T	0.913	0.871	0.958	0.946	0.67	0.43	0.346	0.870
A	0.088	0.129	0.042	0.054	(0.29, 1.54)			
rs3766332								
A	0.763	0.750	0.688	0.804	0.84	0.26	0.488	0.592
T	0.238	0.250	0.313	0.196	(0.50, 1.39)			
rs3753380								
T	0.375	0.298	0.208	0.304	0.92	0.25	0.751	0.121
C	0.625	0.702	0.792	0.696	(0.57, 1.51)			
rs12093097								
C	0.725	0.855	0.792	0.821	1.97	0.29	**0.018**	0.227
T	0.275	0.145	0.208	0.179	(1.12, 3.44)			
rs1073610								

G	0.700	0.847	0.792	0.804	2.09	0.28	**0.008**	0.556
A	0.300	0.153	0.208	0.196	(1.21, 3.61)			
rs1073611								
G	0.713	0.847	0.792	0.821	1.73	0.28	*0.052*	0.225
A	0.288	0.153	0.208	0.179	(0.99, 3.02)			

OR: odd ratio, CI: confidence interval, SE: standard error
Phet: P-value for heterogeneity between both studies (P<0.05 is considered significant heterogeneity based on the Breslow-Day test)
P-meta: P-value for the meta-analysis between Malays and Chinese where the association between alleles and glaucoma status was measured.

Age at presentation, sex, rs12094298, rs551253, rs11162505, rs12093097, rs686262 and rs1073610 were included in stepwise logistic regression analysis. Age remains a significant predictor for susceptibility to POAG on stepwise logistic regression analysis (table 3.27). The presence of the minor allele rs11162505G in the heterozygous state (rs11162505AG) reduces the susceptibility to POAG 0.2 fold (95% CI 0.0, 0.6) in the Malaysian population (table 3.27).

Table 3.27: Stepwise logistic regression analysis on *PTGFR* and predictors for susceptibility to POAG in the Malaysian population

Predictors	**OR_c**	**OR_r**	**SE**	**p-value**	**95%CI for OR (LCI, UCI)**
rs11162505					
AA	--	--	--	--	--
AG	0.17	0.15	0.73	**0.010**	0.04, 0.63
GG	0.00	0.00	15884.90	0.999	0.00,---
rs551253					
GG	--	--	--	--	--
GC	3.22	2.78	0.53	*0.051*	0.10,7.79
CC	0.72	0.60	1.29	0.690	0.05, 7.53
Age (at presentation)	1.06	1.04	0.02	**0.022**	1.01, 1.08

OR_c: crude odd ratio, OR_r: logistic odd ratio, CI: confidence interval, OR: odd ratio, LCI: lower confidence interval, UCI: upper confidence interval
The goodness of fit of the model was checked using the Hosmer-Lemeshow test (p= 0.261). This result gives no evidence of lack of fit of the model.

3.3.2.8 *PTGFR* and susceptibility to NTG in the Malaysian population

Breslow-Day test on heterogeneity between the sub-populations was found to be significant in several SNPs found in *PTGFR* (table 3.28). The minor allele of four SNPs (rs7543738G, rs556817G, rs577333C and rs6424776C) was found to be significantly associated with NTG based on stratified meta-analysis between the two sub-populations (table 3.28).

Table 3.28: Stratified Mantel-Haenszel meta-analysis on *PTGFR* and susceptibility to NTG relative to unaffected normal individuals in Malays and Chinese

	Malays		**Chinese**		**Stratified meta-analysis**			
SNPs	Allele frequency		Allele frequency		OR	SE	P-meta	P-Het
	Glaucoma N=18	Control N=62	Glaucoma N=4	Control N=28				
rs3766331								
A	0.833	0.766	0.875	0.857	0.97	0.54	0.956	0.900
G	0.167	0.234	0.125	0.143	(0.34, 2.78)			
rs3766353								
G	0.861	0.726	0.750	0.661	0.62	0.45	0.287	0.945
T	0.139	0.274	0.250	0.339	(0.25, 1.50)			
rs3766355								
C	0.611	0.565	0.500	0.554	0.90	0.34	0.752	0.631
A	0.389	0.435	0.500	0.446	(0.46, 1.76)			
rs35978825								
C	0.861	0.887	0.750	0.875	1.68	0.48	0.279	0.190
T	0.139	0.113	0.250	0.054	(0.66, 4.32)			
rs1830673								
A	0.556	0.379	0.500	0.393	0.52	0.34	0.053^	0.740
G	0.444	0.621	0.500	0.607	(0.27, 1.01)			
rs3766351								
T	0.806	0.839	0.625	0.946	1.82	0.42	0.150	**0.031**
C	0.194	0.161	0.375	0.054	(0.80, 4.12)			
rs1555541								
T	0.306	0.315	0.375	0.393	1.05	0.36	0.892	0.970
C	0.694	0.685	0.625	0.607	(052, 2.14)			
rs10489785								
A	0.806	0.863	0.750	0.964	1.95	0.44	0.132	0.113
T	0.194	0.137	0.250	0.036	(0.82, 4.64)			
rs12094298								

C	0.806	0.903	0.875	0.982	2.52	0.49	0.058^	0.403
A	0.194	0.097	0.125	0.018	(0.97, 6.56)			
rs34077564								
A	0.833	0.855	0.625	0.946	1.79	0.43	0.178	**0.028**
G	0.167	0.145	0.375	0.054	(0.77, 4.17)			
rs35123627								
C	0.833	0.903	0.875	0.911	1.79	0.47	0.236	0.847
T	0.167	0.097	0.125	0.089	(0.69, 4.66)			
rs2146489								
A	0.044	0.476	0.500	0.411	1.63	0.35	0.163	0.199
G	0.694	0.524	0.500	0.589	(0.82, 3.25)			
rs2057424								
A	0.694	0.565	0.500	0.696	0.76	0.35	0.441	0.098
G	0.306	0.435	0.500	0.304	(0.38, 1.52)			
rs1510158840								
G	0.778	0.508	0.750	0.482	0.52	0.35	0.064^	0.500
A	0.361	0.492	0.250	0.518	(0.26, 1.04)			
rs34528585								
T	0.889	0.903	0.750	0.946	1.66	0.51	0.320	0.151
A	0.111	0.097	0.250	0.054	(0.61, 4.52)			
rs12044011								
T	0.556	0.556	0.750	0.518	0.77	0.34	0.444	0.114
A	0.444	0.444	0.250	0.482	(0.39, 1.50)			
rs1322930								
G	0.972	0.935	1.000	0.982	0.39	1.08	0.377	0.806
A	0.028	0.064	0.000	0.018	(0.05, 3.18)			
rs34852041								
C	0.861	0.855	0.875	0.964	1.12	0.50	0.818	0.291
T	0.139	0.145	0.125	0.036	(0.42, 2.98)			
rs33994937								
T	0.861	0.855	0.875	0.964	1.12	0.50	0.818	0.291
C	0.139	0.145	0.125	0.036	(0.42, 2.98)			
rs6424776								
T	0.667	0.492	0.750	0.518	0.46	0.36	**0.030**	0.749
C	0.333	0.508	0.250	0.482	(0.23, 0.93)			
rs72673925								
T	0.861	0.887	0.750	0.964	1.77	0.48	0.238	0.085
G	0.139	0.113	0.250	0.036	(0.69, 4.55)			
rs28832602								
C	0.806	0.879	0.750	0.964	2.22	0.45	0.076^	0.150
T	0.194	0.121	0.250	0.036	(0.92, 5.36)			

rs34572897								
A	0.861	0.887	0.750	0.964	1.77	0.48	0.238	0.085
G	0.139	0.113	0.250	0.036	(0.69, 4.55)			
rs1590314								
T	0.444	0.371	0.500	0.429	0.74	0.34	0.378	0.984
C	0.556	0.629	0.500	0.571	(0.38, 1.45)			
rs12058120								
C	0.778	0.855	0.750	0.946	2.03	0.43	0.098	0.246
G	0.222	0.145	0.250	0.054	(0.88, 4.69)			
rs12725125								
G	0.778	0.871	0.750	0.946	2.29	0.43	0.056^	0.306
A	0.222	0.129	0.250	0.054	(0.98, 5.36)			
rs4261075								
A	0.444	0.363	0.500	0.429	0.72	0.34	0.337	0.951
G	0.556	0.637	0.500	0.571	(0.37, 1.41)			
rs34012602								
G	0.806	0.903	0.875	0.946	2.29	0.48	0.084	0.932
T	0.194	0.097	0.125	0.054	(0.90, 5.85)			
rs67351117								
C	0.861	0.887	0.750	0.964	1.77	0.48	0.238	0.850
T	0.139	0.113	0.250	0.036	(0.69, 4.55)			
rs672561								
T	0.833	0.863	0.750	0.964	1.69	0.46	0.250	0.078
C	0.167	0.137	0.250	0.036	(0.69, 4.12)			
rs12401416								
G	0.611	0.500	0.750	0.500	0.61	0.35	0.163	0.423
A	0.389	0.452	0.250	0.500	(0.31, 1.22)			
rs6424778								
C	0.972	0.960	1.000	0.982	0.61	1.10	0.654	0.754
T	0.028	0.040	0.000	0.018	(0.07, 5.30)			
rs577333								
T	0.417	0.298	0.375	0.375	0.66	0.35	0.238	0.551
C	0.583	0.702	0.625	0.625	(0.34, 1.31)			
rs520171								
A	0.694	0.669	0.750	0.768	0.93	0.37	0.833	0.825
C	0.306	0.331	0.250	0.232	(0.45, 1.91)			
rs551253								
G	0.722	0.677	0.625	0.768	0.97	0.37	0.927	0.311
C	0.278	0.323	0.375	0.232	(0.47, 1.99)			
rs552328								
A	0.722	0.556	0.375	0.536	0.66	0.35	0.231	0.107
G	0.278	0.444	0.625	0.464	(0.33,			

					1.31)			
rs11162504								
A	0.750	0.766	0.625	0.804	1.30	0.38	0.500	0.372
G	0.250	0.234	0.375	0.196	(0.61, 2.75)			
rs11162505								
A	0.889	0.774	0.750	0.839	0.60	0.47	0.280	0.170
G	0.111	0.226	0.250	0.161	(0.25, 1.52)			
rs554173								
T	0.889	0.798	0.750	0.714	0.57	0.48	0.244	0.615
C	0.111	0.202	0.250	0.286	(0.23, 1.46)			
rs554185								
A	0.750	0.565	0.500	0.554	0.55	0.36	0.101	0.217
G	0.250	0.435	0.500	0.446	(0.27, 1.12)			
rs556817								
A	0.972	0.798	0.875	0.768	0.19	0.74	**0.024**	0.323
G	0.028	0.202	0.125	0.232	(0.04, 0.80)			
rs473027								
A	0.667	0.540	0.250	0.482	0.80	0.35	0.516	0.087
G	0.333	0.460	0.750	0.518	(0.41, 1.57)			
rs668005								
C	0.694	0.556	0.375	0.482	0.69	0.35	0.288	0.231
T	0.306	0.444	0.625	0.518	(0.35, 1.37)			
rs2146490								
G	0.861	0.887	0.750	0.964	1.77	0.48	0.238	0.085
A	0.139	0.113	0.250	0.036	(0.69, 4.55)			
rs530871								
G	0.667	0.556	0.375	0.500	0.77	0.35	0.451	0.257
A	0.333	0.444	0.625	0.500	(0.39, 1.52)			
rs538275								
G	0.694	0.581	0.375	0.446	0.72	0.35	0.354	0.363
A	0.306	0.419	0.625	0.554	(0.36, 1.44)			
rs589958								
G	0.694	0.565	0.375	0.446	0.68	0.35	0.282	0.323
A	0.306	0.435	0.625	0.054	(0.34, 1.37)			
rs3766338								
T	0.889	0.774	0.750	0.643	0.47	0.48	0.114	0.745
C	0.111	0.226	0.250	0.357	(0.19, 1.20)			
rs590309								
T	0.694	0.548	0.375	0.464	0.66	0.35	0.237	0.250
C	0.306	0.452	0.625	0.536	(0.33, 1.31)			
rs622346								
G	0.750	0.694	0.625	0.643	0.82	0.38	0.594	0.687

C	0.250	0.306	0.375	0.357	(0.39, 1.71)			
rs13374108								
T	0.944	0.871	0.750	0.857	0.69	0.57	0.507	0.155
A	0.056	0.129	0.250	0.143	(0.23, 2.08)			
rs34012237								
T	0.861	0.726	0.875	0.964	1.22	0.51	0.695	0.328
C	0.139	0.113	0.125	0.036	(0.70, 3.34)			
rs33966768								
T	0.833	0.887	0.875	0.964	1.75	0.49	0.255	0.510
C	0.167	0.113	0.125	0.036	(0.67, 4.57)			
rs501078								
C	0.444	0.339	0.250	0.304	0.97	0.36	0.928	0.700
T	0.639	0.661	0.750	0.696	(0.48, 1.96)			
rs3766335								
G	0.861	0.879	1.000	0.964	1.08	0.54	0.889	0.560
A	0.139	0.121	0.000	0.036	(0.37, 3.16)			
rs7543738								
C	0.833	0.960	1.000	0.964	3.76	0.60	**0.026**	0.270
G	0.167	0.040	0.000	0.036	(1.17, 12.09)			
rs686262								
A	0.389	0.379	0.375	0.304	0.91	0.35	0.786	0.751
G	0.611	0.621	0.625	0.696	(0.46, 1.80)			
rs4650581								
T	0.861	0.871	0.875	0.946	1.23	0.50	0.685	0.523
A	0.139	0.129	0.125	0.054	(0.46, 3.28)			
rs3766332								
A	0.861	0.750	0.000	0.804	1.19	0.39	0.651	**0.001**
T	0.139	0.250	1.000	0.196	(0.56, 2.54)			
rs3753380								
T	0.417	0.298	0.375	0.304	0.62	0.35	0.171	0.821
C	0.583	0.702	0.625	0.696	(0.31, 1.23)			
rs12093097								
C	0.806	0.855	1.000	0.821	1.03	0.47	0.953	0.140
T	0.194	0.145	0.000	0.179	(0.41, 2.56)			
rs1073610								
G	0.778	0.847	1.000	0.804	1.12	0.44	0.803	0.104
A	0.222	0.153	0.000	0.196	(0.47, 2.67)			
rs1073611								
G	0.778	0.847	1.000	0.804	1.12	0.44	0.803	0.104
A	0.222	0.153	0.000	0.196	(0.47, 2.67)			

OR: odd ratio, CI: confidence interval, SE: standard error
Phet: P-value for heterogeneity between both studies (P<0.05 is considered significant heterogeneity based on the Breslow-Day test)
P-meta: P-value for the meta-analysis between Malays and Chinese where the association between alleles and glaucoma status was measured.

Age at presentation, sex, rs6424776, rs12725125, rs556817 and rs7543738 were included in the stepwise logistic regression analysis. The final model of stepwise logistic regression, demonstrated that the minor allele rs556817G in the heterozygous state (rs556817AG) increases the risk of NTG 5.4 fold (95%CI 1.1, 26.1), whereas the homozygousity for the most common allele at rs12725125 (rs12725125AA) reduces the risk of NTG (OR 0.1 [95%CI 0.0, 0.8]) in the Malaysian population (table 3.29).

Table 3.29: Stepwise logistic regression on *PTGFR* and susceptibility to NTG in the Malaysian population

Predictors	OR_c	OR_r	SE	p-value	95%CI for OR (LCI, UCI)
rs556817					
AA	--	--	--	--	--
AG	4.44	5.43	0.80	**0.035**	1.13, 26.11
GG	3.06E8	0.00	856.88	0.999	0.00,--
rs12725125					
GG	--	--	--	--	--
GA	2.77	2.22	0.82	0.327	0.45, 10.99
AA	0.13	0.12	0.96	**0.028**	0.02, 0.80

OR_c: crude odd ratio, OR_r: logistic odd ratio, CI: confidence interval, OR: odd ratio, LCI: lower confidence interval, UCI: upper confidence interval
The goodness of fit of the model was checked using the Hosmer-Lemeshow test (p= 0.989). This result gives no evidence of lack of fit of the model.

3.3.2.9: Haplotypes analysis on *PTGFR*

The possible linkage disequilibrium of polymorphisms found in *PTGFR* gene was analysed using available software Haploview (http://www.broad.mit.edu/mpg/haploview/). Based on single marker check, rs3766351, rs34077564, rs2057424, rs12058120, rs12725125, rs551253 and rs1073611 were found to violate HWE and were excluded from further analysis. There was significant difference in haplotypes frequency of rs11162504G and rs556817G between glaucoma and control subjects. There were six possible haplotypes blocks (Figure 3.13). Haplotypes of GG and GA of rs11162505 and rs554185 in block 3 demonstrated statistically significant association with glaucoma (table 3.30). Haplotypes of rs556817C, rs473027G and rs668005G also shown significant association with glaucoma (p=0.026).

Table 3.30: Association of haplotypes of *PTGFR* between glaucoma and control subjects

Block	Haplotypes	Frequency	Allele frequency		χ^2	p-value
			Glaucoma	Control		
1	**CT**	0.898	0.907	0.889	0.31	0.576
	TC	0.097	0.081	0.111	0.89	0.346
2	**AA**	0.790	0.808	0.772	0.69	0.406
	GG	0.136	0.070	0.200	12.65	**4.0E-4**
	GA	0.071	0.122	0.022	13.22	**3.0E-4**
3	**TAA**	0.610	0.661	0.561	3.73	*0.054*
	TGA	0.180	0.147	0.211	2.46	0.116
	CGG	0.167	0.122	0.211	4.97	**0.026**
	CGA	0.033	0.051	0.016	3.21	*0.073*
4	**GG**	0.562	0.599	0.528	1.80	0.179
	AA	0.423	0.384	0.461	2.16	0.142
5	**TT**	0.553	0.592	0.516	2.06	0.151
	CC	0.243	0.225	0.260	0.57	0.450
	TC	0.197	0.176	0.218	0.98	0.322
6	**GTTTCG**	0.345	0.380	0.312	1.79	0.181
	GTTTTG	0.243	0.254	0.232	0.22	0.639
	CTTTTG	0.202	0.189	0.214	0.33	0.567
	GATTTG	0.101	0.069	0.132	3.86	*0.050*

CTCCTA	0.082	0.076	0.089	0.21	0.650
CTTTCG	0.011	0.008	0.014	0.25	0.620

Block 1: rs6424776 and rs72673925
Block 2: rs11162505 and rs554185
Block 3: rs556817, rs473027 and rs668005
Block 4: rs3766338 and rs590309
Block 5: rs622346 and rs13374108
Block 6: rs34012237, rs33966768, rs501078, rs3766335, rs7543738 and rs686262

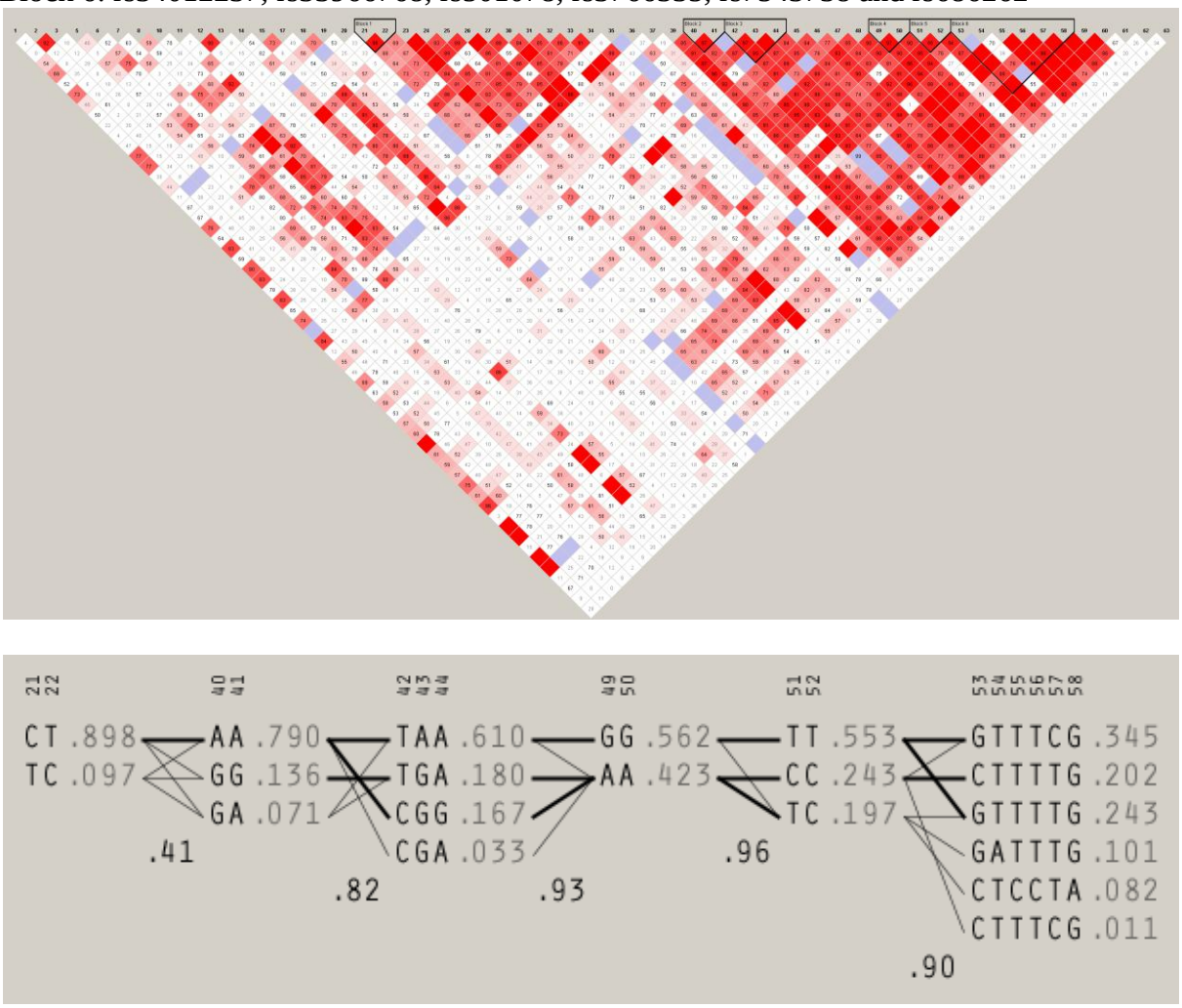


Figure 3.13: LD plot of *PTGFR*

3.3.2.10: Microsatellite instability (MSI) of *PTGFR* in the Malaysian population

There were 2 (areas of) MSI found in Intron 3; 'CA' deletion was found within primer IVS 3-29 and 'TA' insertion within primer IVS 3-56. There were multiple combinations of quantitative analysis of MSI based on FAMHAP programme (http://www.meb.uni-bonn.de/famhap/) in PTGFR gene. Based on global p-value of FAMHAP programme, both MSIs showed no statistically significant difference between glaucoma patients and normal individuals (table 3.31 and 3.32).

Table 3.31: Association of MSI size found within primer IVS 3-29 between glaucoma and control subjects

MSI size	Frequency		OR	95% CI OR LCI, UCI	p-value*
	Glaucoma N=86	Control N=90			
96	0.167	0.156	1.09	0.62, 1.91	0.603
111	0.067	0.078	0.85	0.38, 1.89	
92	0.256	0.328	0.70	0.45, 1.11	
109	0.122	0.089	1.43	0.72, 2.82	
105	0.083	0.100	0.82	0.40, 1.68	
107	0.050	0.078	0.62	0.26, 1.48	
113	0.133	0.089	1.58	0.81, 3.08	
90	0.011	0.006			
94	0.011	0.011	1.00	0.14, 7.18	
80	0.006	0.000			
89	0.006	0.000			
95	0.011	0.000			
98	0.011	0.011	1.00	0.14, 7.18	
93	0.044	0.022	**2.05**	0.61, 6.92	
102	0.006	0.000			
103	0.006	0.017	0.33	0.03, 3.20	
97	0.000	0.011	0.00	-9.00, -9.00	
115	0.000	0.011	0.00		
100	0.000	0.006			

*p-value is based on global p-value for single marker using FAMHAP software
OR: odd ratio, LCI: lower confident interval, UCI: upper confident interval

Table 3.32: Association of MSI size within primer of IVS3-56 between glaucoma and control subjects

MSI size	Frequency		OR	95% CI of OR LCI, UCI	p-value*
	Glaucoma N=86	Control N=90			
294	0.206	0.283	0.65	0.40, 1.06	0.077
318	0.050	0.028	**1.84**	0.61,5.61	
299	0.061	0.072	0.84	0.36, 1.92	
316	0.033	0.028	**1.21**	0.36, 4.03	
298	0.133	0.100	**1.38**	0.72, 2.65	
314	0.044	0.106	0.39	0.17, 0.93	
296	0.156	0.072	**2.37**	1.18, 4.73	
311	0.028	0.022	**1.26**	0.33, 4.76	
292	0.017	0.000			
307	0.011	0.000			
315	0.011	0.011	1.00	0.14, 7.18	
301	0.050	0.078	0.62	0.26, 1.48	
286	0.039	0.011	**3.60**	0.74, 17.58	
290	0.006	0.000			
312	0.050	0.028	**1.84**	0.61, 5.61	
310	0.028	0.017	**1.69**	0.40, 7.16	
287	0.011	0.017	0.66	0.11, 4.02	
317	0.011	0.000			
320	0.006	0.017	0.33	0.03, 3.20	
303	0.033	0.011	**3.07**	0.61, 15.41	
319	0.006	0.011	0.50	0.04, 5.53	
313	0.006	0.000			
308	0.006	0.050	0.11	0.07, 0.85	
306	0.000	0.017	0.00	-9.00, -9.00	
309	0.000	0.011	0.00	-9.00, -9.00	
300	0.000	0.011	0.00	-9.00, -9.00	

*p-value is based on global p-value for single marker using FAMHAP software
OR: odd ratio, LCI: lower confident interval, UCI: upper confident interval

There were 130 haplotypes pairings identified between MSI found in introns 3 PTGFR gene, but only certain pairing was illustrated in table 3.33. The selected haplotypes were those with frequency found in both glaucoma and control subjects.

Table 3.33: Association of the haplotypes pairing of the identified MSI in *PTGFR* between glaucoma patients and controls

Haplotypes		Frequency		OR	95% CI of OR LCI, UCI	p-value*
		Glaucoma N=86	Control N=90			
105	**294**	0.006	0.026	0.21	0.02, 1.81	0.103
105	**312**	0.022	0.011	**2.02**	0.37, 11.19	
107	**294**	0.006	0.021	0.27	0.03, 2.45	
107	**314**	0.011	0.038	0.28	0.06, 1.38	
109	**294**	0.028	0.030	0.92	0.27, 3.16	
111	**294**	0.025	0.028	0.89	0.25, 3.21	
111	**299**	0.006	0.012	0.47	0.04, 5.09	
111	**301**	0.006	0.011	0.50	0.04, 5.53	
113	**294**	0.039	0.022	**1.83**	0.52, 6.42	
113	**298**	0.034	0.034	0.99	0.32, 3.09	
113	**299**	0.010	0.018	0.54	0.09, 3.36	
92	**287**	0.002	0.011	0.21	0.01, 5.84	
92	**294**	0.025	0.046	0.52	0.16, 1.69	
92	**296**	0.040	0.017	**2.46**	0.63, 9.61	
92	**298**	0.052	0.036	**1.45**	0.52, 4.04	
92	**299**	0.023	0.030	0.76	0.21, 2.77	
92	**301**	0.017	0.046	0.35	0.09, 1.35	
92	**311**	0.006	0.011	0.50	0.04, 5.53	
92	**314**	0.007	0.044	0.16	0.03, 1.02	
92	**316**	0.006	0.011	0.50	0.04, 5.53	
92	**318**	0.028	0.018	**1.53**	0.37, 6.23	
96	**294**	0.061	0.072	0.85	0.37, 1.94	
96	**296**	0.052	0.014	**3.88**	0.95, 15.38	
96	**312**	0.011	0.011	1.00	0.14, 7.18	
98	**294**	0.006	0.011	0.50	0.04, 5.53	

*p-value is based on global p-value for haplotypes markers using FAMHAP software
OR: odd ratio, LCI: lower confident interval, UCI: upper confident interval

3.4 Discussion

3.4.1 The role of *ADRB2* as glaucoma susceptibility gene in the Malaysian population

ADRB2 has been intensively studied in various diseases in many populations (Weir et al, 1998; Xie et al, 2000; Hahntow et al, 2006). Most studies emphasized Arg16Gly and Gln27Glu polymorphisms, which were believed to be important in determining risk of asthma and hypertension (Brodde, 2008; Xie et al, 2000; Scott et al, 1999). However, after more than 2 decades the functional role of 46A/G and 79C/G are still uncertain due to inconsistent findings (Kotanko et al, 1997; Lee et al, 2004; Kato et al, 2001). Recently, the SNPs found within 1.5kb 5 untranslated region (UTR) upstream from the ATG start codon or the promoter region; -20 T/C, -47 T/C, -367 T/C, -468 C/G, -654 G/A, -1343 A/G and -1429 T/A has created new interest, especially as these SNPs are believed to play an important role in transcriptional regulatory activity of *ADRB2* (Scott et al, 1999).

Great emphasis was given to codon 16 and 27 of *ADRB2*, which was reflected in various studies involving many diseases (Brodde, 2008; Xie et al, 2000; Scott et al, 1999). Previous studies have suggested that mutations/polymorphisms within *ADRB2* were not associated with an increased risk of glaucoma in Turkish, Caucasians and African Americans populations (Güngör K et al, 2003; McLaren N et al, 2006). There was no significant association between 46A/G and 79C/G with susceptibility to glaucoma in Japanese but there was significant difference in other phenotypes: age at diagnosis and IOP at diagnosis (Inagaki et al, 2007). Japanese POAG patients with 46AG and 46GG of *ADRB2* were found to diagnose with glaucoma at younger age compared to 46AA (Inagaki et al, 2007). While those with 79CG and 79GG was found to have significantly higher IOP at presentation compared to Gln/Gln (homozygous wild).

In this study, five codons that were believed to be functionally important were selected 46A/G, 79C/G, 491C/G, -20T/C and -47T/C. The minor allele of 79C/G (79G) and -20T/C (-20C) was found to associate with the susceptibility to glaucoma. Genomic control analysis was not conducted due to the small number of markers included in this current study. In addition, the minor allele of 79C/G (79G) was found to confer protective effect against POAG (OR 0.3[95%CI 0.1, 0.7]) and -20C increases the susceptibility to NTG up to 2.0 folds (95%CI 1.1, 3.7). Thus, 79C/G and -20T/C of *ADRB2* may play a role in the susceptibility to glaucoma in the Malaysian population.

3.4.2 *PTGFR* as glaucoma susceptibility gene in Malaysian population

The role of *PTGFR* as a risk modifying gene for glaucoma has not been studied before. A study on knock-out mice found that *PTGFR* was crucial in functionality of latanoprost (Crowston et al, 2004). In addition, *PTGFR* was found to play an important role in pharmacogenetics of topical latanoprost has been observed in normal volunteers in Japan (Sakurai et al, 2008). SNPs at the promoter rs3753380 and at introns 1 rs3766355 of *PTGFR* were significantly associated with short term response to topical latanoprost in normal healthy volunteers (Sakurai et al, 2008). In our preliminary screening of the exons of *PTGFR* in a small sample of Malaysia population, we have identified 2 SNPs including 1 novel SNP in Malaysian population (Hoh et al, 2007). Novel SNP was identified at -97 upstream of exon 3 (IVS-97A>T) (Hoh et al, 2007). The possibility of functional importance of this novel SNP was not explored in this early report (Hoh et al, 2007).

The majority of the SNPs identified are intronic (non-coding) polymorphisms. In general, *PTGFR* confers protective against glaucoma in the Malaysian population. Minor allele of rs11162505 and rs554185 reduce the susceptibility of glaucoma. These two intronic SNPs are

in linkage disequilibrium. There was significant association of GG ($p=4.0 \times 10^{-4}$) and GA ($p=3.0 \times 10^{-4}$) haplotypes of rs11162505 and rs554185 respectively with susceptibility to glaucoma. There was also significant heterogeneity between Malay and Chinese in allele frequency of rs11162505.

Based on these findings, *PTGFR* appear not only to influence the response to topical prostaglandin analogues as previously reported, but a potential candidate gene for development of glaucomatous optic neuropathy (both POAG and NTG) in Malaysian people. This finding was based on a mixed cohort of Malay and Chinese people, and as such, population stratification is a concern. Genomic control analysis had shown no inflation suggesting lack of the possibility of gross population stratification. However, there were several SNPs found to associate with glaucoma in Malays but not among our Chinese people and vice versa.

Microsatellite instability (MSI) was also identified in *PTGFR* in Malaysian population. There was 'CA' deletion and 'TA' insertion identified within Intron 3. However, based on the global p-value adopted in FAMHAP software, there was no significant association between the identified MSI and the susceptibility to develop glaucoma. The importance of MSI in glaucoma susceptibility remains to be explored. MSI was proven important in susceptibility of hereditary non-polyposis colorectal cancer, responsible in causing frameshift mutation that alter tumour suppressor gene (Peltomaki et al, 1993).

CHAPTER 4

Pharmacogenetics of topical timolol and latanoprost in glaucoma patients

Chapter 4

4.1 Objective

To determine the association of *ADRB2* and *PTGFR* gene polymorphisms with the responsiveness to topical timolol XE 0.5% and topical latanoprost 0.005% respectively

4.2 Material and method

4.2.1 Recruitment of subjects

A total of 183 glaucoma patients were recruited; 97 of them were newly diagnosed glaucoma (58 primary open angle glaucoma and 37 normal tension glaucoma) and treated with monotherapy of topical Timolol 0.5% XE. Another 86 patients were treated with topical latanoprost 0.005% either as monotherapy (41 patients) or adjunctive therapy to topical timolol (49 patients). The detail of selection criteria is available in chapter 2. Systemic co-morbidity such as hypertension, diabetes mellitus, hyperlipidemia, cardiovascular disease and cerebrovascular accident were also documented based on medical record review. The detail of definition of systemic co-morbidity is available in chapter 2.

After confirmation of the diagnosis as described in chapter 2, intraocular pressure (IOP) was taken using Goldmann applanation tonometry (GAT) between 8am to 12pm during the first visit before the commencement of medication, and was termed as baseline IOP. Topical Timolol 0.5% XE and latanoprost 0.005% was then prescribed to the subjects accordingly after demonstration of proper instillation. The importance of adherence and persistence was also explained to the recruited patients and their family members. Target pressure was set up for each individual patient. Target pressure was determined based on type and severity of glaucoma. IOP was measured after a month of medication. A subsequent visit was then scheduled at 3 months, 6 months and 12 months post commencement of treatment. IOP

measurement was taken and documentation of side effects was conducted during each visit. Humphrey visual field 30-2 test was conducted at 6 months and 12 months post commencement of treatment. Whenever the patients failed to achieve target IOP or demonstrated visual field progression, they were discontinued from the treatment protocol and scheduled for routine glaucoma clinic follow up. However, the available clinical data (up to the date of discontinuation) was included for phenotype and genotype association analysis. The detail of treatment schedule is available in chapter 2.

At the end of the follow up period, the subjects were categorised as either responders or non responders based on the percentage different between IOP at the final follow up and baseline IOP. The cut off point for classification as responders was based on the mean IOP of respective topical antiglaucoma drugs. All responders (good responder) to topical Timolol XE 0.5% was defined as more or equal to 20% reduction from the baseline IOP. All responders (good responder) to topical latanoprost 0.005% were identified as having at least a 25% reduction from the baseline IOP. Both cut off points were based on the mean percentage IOP reduction of Timolol XE 0.5% (22.9 SD 18.1%) and latanoprost 0.005% (26.7 SD 19.3%) respectively.

4.2.2 Genetics screening of *ADRB2* and *PTGFR*

Venesection was performed and 3ml of venous blood was obtained for genetic screening of *ADRB2* and *PTGFR*. DNA extraction was done using commercially available QIAGEN QIAmp DNA Blood Mini Kit (Qiagen Inc., USA). The detail procedure for genomic DNA extraction was explained in chapter 3.

Genomic DNA was then used for further screening for *ADRB2* and *PTGFR*. A single tube multiplex PCR was conducted to screen 5 codons of *ADRB2* gene; allelic variants at positions 16, 27, 164 and at 5' Untranslated Region (UTR) for nucleotides at positions -20 and -47 on the *ADRB2* gene. For each of the polymorphic loci, two parallel allele-specific reactions were carried out; one with a wild-type specific primer and the other with a mutation specific primer. *ADRB2* screening was conducted on 97 glaucoma subjects and 100 age and race matched unrelated control.

A duplex PCR was carried out for primer Beta16A/G and Beta UTR-20C/T and triplex PCR was carried out for primer Beta27C/G, Beta164C/T and Beta UTR-47C/T. The primer Beta2-Fw was used as a common forward primer in both duplex and triplex PCR reactions while the Beta 16A/G, Beta UTR-20C/T, Beta 27C/G, Beta UTR-47C/T and Beta 164C/T primers were the reverse primers. For detail explanations of the procedures and thermocycler settings referred to chapter 3. The presence of amplicons was determined by 2% agarose gel electrophoresis. Direct sequencing was then conducted for selected sample to confirm the findings.

Screening of *PTGFR* gene was conducted on 176 samples; 86 glaucoma subjects and 90 unrelated controls, matched for age and ethnicity. The screening of *PTGFR* was more complicated due to its size and the presence of microsatellite instability (MSI) or short tandem repeats (STR). In total 95 primers were designed to cover the entire *PTGFR* including 3000bp's upstream of 5'UTR and 1000bp's downstream of 3'UTR. Detection of MSI was done using special tagged forward primers. The detail of primer design is available in chapter 3.

The amplification of genomic DNA using specific primers was described in chapter 3. PCR product was then purified using two hydrolytic enzymes; Exonuclease I and Shrimp Alkaline Phosphatase (ExoSAP-IT) (USB, Affymetrix, USA) or using GENE ALL PCR purification Kit (General Biosystem, Seoul, Korea). The purified PCR products were then used as template for cycle sequencing as described in chapter 3. The cycle sequencing products were later purified using ethanol/alcohol precipitation technique or using gel filtration with Sephadex G-50 superfine beads (SigmaAldrich, Sweden) as explained in chapter 3. The purified product was loaded into ABI Prism 3730xl Genetic Analyzer (Applied Biosystem, California, USA) or ABI Prism 3100 Genetic Analyzer sequencer (Applied Biosystem, California, USA).

Two MSIs or STRs were identified within primer sequence of IVS 3-29 and IVS 3-53 while screening for polymorphisms in introns 3. The quantification of MSI was done using capillary electrophoresis using forward primers that were designed and chemically labelled with fluorescent dyes (6 FAM™). The reverse primers were not labelled. The primer sequence was illustrated in table 3.5 of chapter 3. PCR amplification was conducted using premix extensor hi-fidelity PCR master mix (Thermo Scientific, ABgene, United Kingdom). The details of the volumes of the reagents needed were explained in detail in chapter 3.
Passive reference dye Genesan™ROX™ 500(Applied Biosystem, USA) was used during labelling reaction on fluorescent dye for quantitative analysis of MSI. The reaction mixture then was uploaded in the sequencer machine ABI 3730xl for genotyping procedure. Analysis of quantification of MSI was done using Genemapper® 4.0 software. The detail explanation of MSI screening is available in chapter 3.

4.2.3 Statistical analysis

The descriptive analysis has been outlined in chapters 2 and 3. In this chapter, the analysis mainly aimed to explore the associations between *ADRB2* and *PTGFR* genetic structure and pressure lowering effects of topical timolol and latanoprost respectively. Wright-Fisher module (Fisher RA, 1930; Wright S, 1931) was used to determine the contribution of a single biallelic quantitative trait locus (QTL) of the gene towards the mean and variance in the population, as well as the covariance between relatives. Genotype value in this study was analysed based on the mean IOP reduction for each SNP. Additive genotype value is often chosen for complex disease such as glaucoma. Due to limited number of SNPs that expressed additive genotype effect, SNPs with significant dominant value were also selected.

One way ANOVA test was conducted to determine the effect of SNPs at 5 codons of *ADRB2* and selected SNPs of *PTGFR* on mean IOP reduction at each follow-up visits. Association between responsiveness to topical Timolol XE 0.5% and topical latanoprost 0.005% to *ADRB2* and *PTGFR* respectively was assessed by stratified Cochran Mantel-Haenszel meta-analysis. Univariate logistic regression was then conducted on Malays and Chinese glaucoma patients. The effect of *ADRB2* and *PTGFR* on the responsiveness to topical Timolol XE 0.5% and topical latanoprost 0.005% respectively was then determined by stepwise logistic regression. The final model was based backward model on combination of Malay and Chinese glaucoma patients. Age at initial presentation, sex, type of glaucoma, baseline IOP, central cornea thickness and mean deviation of Humphrey visual field analysis were also included in the final model of stepwise logistic regression.

4.3 Results

4.3.1 Pressure lowering effect of topical timolol and *ADRB2*

4.3.1.1 Demographic data

A total of 97 glaucoma subjects were recruited for monotherapy treatment with topical timolol XE 0.5% (60 POAG and 37 NTG); 66 of them were Malays and 31 of them were Chinese. Almost two thirds of them were male (63%). Average "mean defect" on visual field analysis was -11.51dB (SD 8.82) and mean vertical cup: disc ratio (VCDR) of the optic disc was 0.80 (SD 0.60). Detailed demographic data is available in chapter 2.

4.3.1.2 Long term pressure lowering effect of topical Timolol XE 0.5% monotherapy

The mean IOP reduction of topical timolol 0.5% monotherapy was 5.4 (SD 5.1) mmHg, based on the difference between baseline IOP and mean IOP during follow-up. The mean percentage IOP reduction was 22.9% (SD 18.1). Table 2.6 in chapter 2 illustrated the details of IOP reduction from baseline, 1 month, 3 months, 6 months and 12 months post treatment with topical timolol.

After 12 months of follow up, only 50 patients were still on topical timolol XE 0.5% monotherapy. More than 2/3 of POAG patients failed to achieve target pressure within 12 months follow up (Table 4.1). In addition, glaucoma patients with more advanced glaucoma based on mean VCDR, MD and PSD and thinner CCT were associated with failure to achieve target pressure within 12 months follow up (Table 4.1).

Table 4.1: Univariate analysis on the predictors affecting the long term efficacy of topical Timolol XE 0.5%

Characteristics	Completed 12 months treatment N=50	Incomplete 12 months treatment** N=47	p-value
Age			
Mean (SD)	64.9 (8.7)	63.4 (9.6)	0.430*
Range	41-81	42-82	
Sex (n (%))			
Male	32 (64.0)	30 (63.8)	0.00,
Female	18 (36.0)	17 (36.2)	0.986#
Race (n (%))			
Malay	30 (60.0)	36 (76.6)	3.07,
Chinese	20 (40.0)	11 (23.4)	0.080#
Hypertension^ (n (%))			
Yes	28 (56.0)	27 (57.4)	0.02,
No	22 (44.0)	20 (42.6)	0.886#
Diabetes mellitus^ (n (%))			
Yes	19 (38.0)	19 (40.4)	0.06,
No	31 (62.0)	28 (59.6)	0.807#
Hyperlipidemia^ (n (%))			
Yes	25 (50.0)	18 (38.3)	1.34,
No	25 (50.0)	29 (61.7)	0.246#
Cardiovascular disorder^ (n (%))	7 (14.0)	4 (8.5)	0.73,
Yes	43 (86.0)	43 (91.5)	0.394#
No			
Cerebrovascular accident^ (n (%))			
Yes	3 (6.0)	0 (0.0)	0.243@
No	47 (94.0)	47 (100.0)	
Type of glaucoma (n (%))			
POAG	24 (48.0)	36 (76.6)	8.40,
NTG	26 (52.0)	11 (23.4)	**0.006@**
Responder (n (%))			
Good	33 (66.0)	23 (48.9)	2.89,
Poor	17 (34.0)	24 (51.1)	0.089#
Baseline IOP (mmHg)			

Mean	21.7 SD 6.8	23.7 SD 4.1	0.092*
Range	12-46	14-32	
Central Corneal Thickness (µm)			
Mean	517 SD 33	501 SD 35	**0.027***
Range	431-565	438-563	
Vertical CDR			
Mean	0.78 SD 0.05	0.82 SD 0.06	**0.007***
Range	0.7-0.9	0.7-0.9	
Mean defect on HFA testing (dB)			
Mean	-9.14 SD 7.21	-14.03 SD 9.73	**0.006***
Range	-34.28 - -2.08	-33.33 - -0.44	
Pattern Standard Deviation (PSD) in HFA testing			
Mean	5.37 SD 2.81	6.74 SD 3.02	**0.024***
Range	1.13 -13.73	1.96- 12.78	

P<0.05 is considered statistically significant based on Pearson chi-square test#, Fischer-exact test@ and student t-test*

**Incomplete 12 months treatment referred to patients who failed to complete 12 months treatment with topical Timolol XE 0.5%.

^ Systemic disease such as hypertension, diabetes mellitus, cardiovascular diseases, cerebrovascular disease and hyperlipidemia were diagnosed based on medical record and information from general practitioner as stated in chapter 2. Cardiovascular disease such as angina pectoris, myocardial infarction was included.

POAG: primary open angle glaucoma, NTG: normal tension glaucoma, IOP: intraocular pressure, VCDR: vertical cup disc ratio, HFA: Humphrey visual field analysis.

4.3.1.3 Predictors affecting the long term efficacy of monotherapy treatment with topical timolol XE 0.5%

Sex, race, type of glaucoma, systemic co-morbidities and responsiveness to topical timolol were included as the categorical predictors. Age, baseline IOP, CCT, VCDR and parameters for visual field were included as the numerical predictors. There was no significant association of factors affecting long term efficacy of topical Timolol XE 0.5% in univariate logistic regression analysis. In spite of the absence of significant factor, baseline IOP, MD, PSD, CCT, type of glaucoma and responsiveness to topical Timolol were selected for stepwise logistic regression model. POAG patients have 3.7 fold (95% CI 1.4, 9.5) risk of poor long term efficacy of topical Timolol XE 0.5% (table 4.2).

Table 4.2: Factors affecting long term efficacy of topical Timolol XE 0.5% treatment by stepwise logistic regression model

Variables	$OR_{(c)}$	$OR_{(r)}$	SE	95% CI (LCI, UCI)	p-value
Type of glaucoma					
POAG	3.34	3.68	0.48	1.43, 9.46	**0.007**
NTG	--	--	--	--	
Responder					
Good	0.43	0.45	0.47	0.18, 1.12	0.087
Poor	--	--	--	--	-

POAG: primary open angle glaucoma, NTG: normal tension glaucoma.
OR: odd ratio, $OR_{(c)}$: crude odd ratio, $OR_{(r)}$: logistic odd ratio, CI: confidence interval, LCI: lower confidence interval, UCI: upper confidence interval
Multicollinearity and interaction terms were checked and not found. The goodness of fit of the backward model was checked using the Hosmer-Lemenshow test; p=0.808. This result gives no evidence of lack of fit of the model.

4.3.1.4 *ADRB2* and genotype value based on Fisher model

Based on Fisher model, -20T/C was found to express additive genotype value in term of respond to topical Timolol XE 0.5% (table 4.3).

Table 4.3: Fisher model on genotype value of *ADRB2*

SNPs	Additive effect N=97		Dominant effect N=97		Both N=97	
	f	p-value	f	p-value	f	p-value
46A/G	0.09	0.767	0.52	0.473	0.36	0.695
79C/G	0.22	0.642	0.02	0.887	0.31	0.732
-47T/C	1.34	0.250	0.62	0.433	1.12	0.331
-20T/C	3.70	*0.057*	0.01	0.904	1.90	0.156

4.3.1.5: Association between *ADRB2* and pressure lowering effect of topical Timolol XE 0.5% monotherapy

One-way ANOVA was conducted to examine the association between *ADRB2* and pressure lowering effect of topical Timolol XE 0.5%. In general, *ADRB2* exerted more effect on Malay glaucoma patients compared to Chinese on pressure lowering effect of Timolol XE 0.5% (table 4.4). Mean baseline IOP was significantly higher in Malays with 79CC and -47CC (table 4.4). Similarly, -47CC demonstrated significantly higher mean baseline IOP in Chinese glaucoma patients. There was also significant difference in mean IOP of 46A/G at 12 months post-treatment in Malays patients (table 4.4). 79C/G and -47T/C shown significant association with mean IOP on analysis combining Malays and Chinese (table 4.5). Mean baseline IOP was significantly higher in patients with 79CC and -47CC (table 4.5).

Table 4.4: Association between *ADRB2* and pressure lowering effect of topical Timolol XE 0.5% in Malays and Chinese

Visits	Malays Mean IOP (SD)				Chinese Mean IOP (95% CI)			
	46A/G				46A/G			
	AA (n)	AG (n)	GG (n)	p-value	AA (n)	AG (n)	GG (n)	p-value
Baseline	25.2(5.8) (25)	21.8(5.6) (37)	22.8(5.9) (4)	*0.080*^	21.2(5.7) (13)	22.2(5.5) (18)	--	0.601
Visit 1	18.0(4.7) (25)	16.9(5.2) (37)	18.5(2.4) (4)	0.618	17.3(5.0) (13)	16.6(3.6) (18)	--	0.658
Visit 2	17.0(3.1) (21)	15.6(3.6) (32)	18.0(8.0) (4)	0.307	16.6(4.6) (12)	16.6(3.4) (16)	--	0.989
Visit 3	16.9(3.0) (15)	15.5(2.4) (24)	12.0 (--) (1)	0.113	15.3(4.4) (9)	15.8(4.8) (14)	--	0.821
Visit 4	14.9(2.9) (11)	14.5(3.2) (19)	22.0 (--) (1)	**0.010**	14.3(3.7) (7)	14.8(3.7) (12)	--	0.795
	79C/G				79C/G			
	CC (n)	CG (n)	GG (n)	p-value	CC (n)	CG (n)	GG (n)	p-value
Baseline	24.0(5.7) (53)	19.7(4.2) (10)	18.7(9.1) (3)	**0.037**	22.3(5.3) (28)	17.3(6.1) (3)	--	0.143
Visit 1	18.0(5.0) (53)	15.5(3.5) (10)	13.0(1.7) (3)	*0.088*^	17.2(4.2) (28)	14.0(3.5) (3)	--	0.216
Visit 2	16.9(3.9) (45)	13.8(2.2) (9)	15.0(4.6) (3)	*0.070*^	16.8(4.0) (25)	15.0(1.7) (3)	--	0.464
Visit 3	16.4(2.7) (30)	14.4(2.2) (7)	15.3(3.1) (3)	0.224	15.8(4.8) (20)	14.3(2.1) (3)	--	0.613
Visit 4	15.1(3.3) (21)	15.3(4.8) (7)	13.3(3.1) (3)	0.713	14.7(3.9) (16)	14.0(1.0) (3)	--	0.771
	-20T/C				-20T/C			
	TT (n)	CT (n)	CC (n)	p-value	TT (n)	CT (n)	CC (n)	p-value
Baseline	22.4(4.8) (7)	21.9(5.0) (21)	23.9(6.4) (38)	0.429	23.3(9.2) (4)	20.5(4.9) (12)	22.4(5.0) (15)	0.583
Visit 1	19.6(8.0) (7)	17.1(4.2) (21)	17.2(4.6) (38)	0.470	15.5(4.1) (4)	16.9(4.2) (13)	17.3(3.8) (15)	0.771
Visit 2	16.7(3.7) (6)	16.2(4.0) (18)	16.3(3.9) (33)	0.963	15.3(3.8) (4)	16.6(4.6) (9)	16.9(3.6) (15)	0.753
Visit 3	15.0(3.5) (4)	15.6(2.2) (11)	16.3(2.9) (25)	0.591	14.3(4.3) (4)	15.6(3.8) (7)	16.1(5.2) (12)	0.798
Visit 4	15.0(6.1) (3)	15.4(4.7) (8)	14.8(2.8) (20)	0.932	13.0(4.8) (4)	14.4(3.6) (7)	15.5(3.2) (8)	0.547
	-47T/C				-47T/C			
	TT (n)	CT (n)	CC (n)	p-value	TT (n)	CT (n)	CC (n)	p-value
Baseline	12.0(--) (1)	20.3(4.6) (8)	23.7(5.8) (57)	**0.043**	--	14.0(2.8) (2)	22.3(5.2) (29)	**0.036**
Visit 1	12.0(--) (1)	15.9(3.7) (8)	17.7(5.0) (57)	0.329	--	12.0(0.0) (2)	17.2(4.2) (29)	*0.090*^
Visit 2	11.0(--) (1)	13.9(2.4) (7)	16.7(3.8) (49)	*0.065*^	--	14.5(2.1) (2)	16.7(3.9) (26)	0.439
Visit 3	12.0(--) (1)	15.0(2.0) (5)	16.2(2.8) (34)	0.227	--	14.0(2.8) (2)	15.8(4.7) (21)	0.611
Visit 4	10.0(--) (1)	16.4(5.3) (5)	14.9(3.1) (25)	0.256	--	13.5(0.7) (2)	14.7(3.8) (17)	0.667

n: number of patients, Visit 1: IOP at 1 month, Visit 2: IOP at 3 months, Visit 3: IOP at 6 months, Visit 4: IOP at 12 months
P<0.05 and ^p<0.10 based on One-way ANOVA

Table 4.5: Association between *ADRB2* and pressure lowering effect of topical Timolol XE 0.5% monotherapy in glaucoma patients

Visits	**Mean IOP (95%CI)**			**p-value**
	46A/G			
	AA (n)	AG (n)	GG (n)	
Baseline	23.8 (21.8, 25.8) (38)	21.9 (20.4, 23.4) (55)	22.8(13.4, 32.1) (4)	0.309
1 month	17.8 (16.2, 19.3) (38)	16.8 (15.5, 18.1) (55)	18.5 (14.7, 22.3) (4)	0.537
3 months	16.8 (15.5, 18.1) (33)	15.9 (14.9, 17.0) (48)	18.0 (15.2, 30.8) (4)	0.410
6months	16.3 (14.8, 17.8) (24)	15.6 (14.5, 16.8) (38)	12.0 (--) (1)	0.418
12 months	14.7 (13.1, 16.2) (18)	14.6 (13.4, 15.8) (31)	22.0 (--) (1)	**0.012***
	79C/G			
	CC (n)	CG (n)	GG (n)	
Baseline	23.4 (22.2, 24.6) (81)	19.2 (16.4, 21.9) (13)	18.7(13.9, 21.2) (3)	**0.020***
1 month	17.7 (16.7, 18.8) (81)	15.2 (13.1, 17.2) (13)	13.0 (8.7, 17.3) (3)	**0.048***
3 months	16.8 (15.9, 17.8) (70)	14.1 (12.8, 15.4) (12)	15.0 (13.6, 26.4) (3)	*0.055*
6months	16.1 (15.1, 17.2) (50)	14.4 (12.9, 15.9) (10)	15.3 (7.7, 22.9) (3)	0.344
12 months	14.9 (13.8, 16.1) (37)	14.9 (12.1, 17.8) (10)	13.3 (9.7, 20.9) (3)	0.762
	-20T/C			
	TT (n)	TC (n)	CC (n)	
Baseline	22.7 (18.5, 27.0) (11)	21.4 (19.6, 23.1) (33)	23.5(21.8, 25.2) (53)	0.259
1 month	18.1 (13.5, 22.7) (11)	17.1 (15.5, 18.6) (33)	17.2 (16.0, 18.4) (53)	0.813
3 months	16.1 (13.5, 18.7) (10)	16.3 (14.7, 17.9) (27)	16.5 (15.4, 17.6) (48)	0.953
6months	14.6 (11.6, 17.7)	15.6 (14.2, 17.0)	16.2 (14.8, 22.9)	0.470

	(8)	(18)	(37)	
12 months	13.9 (9.3, 18.5) (7)	14.9 (12.7, 17.2) (15)	15.0 (13.9, 16.1) (28)	0.746
		-47T/C		
	TT (n)	TC (n)	CC (n)	
Baseline	12.0 (1)	19.0 (15.5, 22.5) (10)	23.3 (22.1, 24.5) (89)	**0.013***
1 month	12.0 (1)	15.1 (12.5, 17.7) (10)	17.6 (16.6, 18.6) (86)	0.152
3 months	11.0 (1)	14.0 (12.3, 15.7) (9)	16.8 (15.9, 17.6) (78)	**0.044***
6months	12.0 (1)	14.7 (12.8, 16.6) (7)	16.0 (15.1, 17.0) (55)	0.348
12 months	10.0 (1)	15.6 (11.3, 19.8) (7)	14.8 (13.8, 15.9) (42)	0.345

*P<0.05 is considered statistically significant based on One-way ANOVA

4.3.1.5: Responsiveness to topical Timolol XE 0.5% monotherapy and *ADRB2*

The cut off point for definition of good responder to timolol was predetermined as more than a 20% reduction at the last IOP measurement from the baseline. Mean IOP reduction of topical Timolol XE 0.5% is 5.5 SD 5.0 mmHg, based on the different between the baseline IOP and summation of IOP divided by the number of visits. The mean percentage of reduction was 23 SD 18%. Based on stratified meta-analysis, there was no significant association of *ADRB2* and response to monotherapy treatment of topical Timolol XE 0.5% (table 4.6).

Age at presentation, sex, type of glaucoma, CCT and baseline IOP were included as possible predictors for respond to topical timolol in univariate logistic regression analysis. Baseline IOP was found to be significant predictors for responds to topical timolol (table 4.7).

Table 4.6: Stratified Mantel-Haenszel meta-analysis on *ADRB2* and responsiveness to topical Timolol XE 0.5% monotherapy

	Malays		**Chinese**		**Stratified Meta-analysis**			
SNPs	Allele frequency		Allele frequency		OR (95%CI)	SE	P-meta	P-het
	Good responder N=39	Poor responder N=27	Good responder N=17	Poor responder N=14				
46A/G								
A	0.705	0.593	0.676	0.780	0.79	0.31	0.446	0.205
G	0.295	0.407	0.324	0.250	(0.43, 1.45)			
79C/G								
C	0.897	0.852	0.971	0.929	0.60	0.49	0.303	0.705
G	0.103	0.148	0.029	0.071	(0.23, 1.58)			
491C/T								
C	1.000	1.000	1.000	1.000	--	--	--	--
G	0	0	0	0				
-20T/C								
T	0.243	0.296	0.324	0.321	0.84	0.32	0.595	0.681
C	0.757	0.704	0.676	0.679	(0.45, 1.58)			
-47T/C								
T	0.051	0.111	0.000	0.071	3.07	0.64	*0.079*	0.312
C	0.949	0.889	1.000	0.929	(0.88, 10.73)			

OR: odd ratio, CI: confidence interval, SE: standard error
Phet: P-value for heterogeneity between both studies (P<0.05 is considered significant heterogeneity based on the Breslow-Day test)
P-meta: P-value for the meta-analysis between Malays and Chinese where the association between alleles and glaucoma status was measured.

Table 4.7: Univariate logistic regression on the predictors for responsiveness to topical Timolol XE 0.5% monotherapy

Predictors	OR	SE	95% CI for OR	p-value
46A/G				
AA	--	--	--	--
AG	0.19	1.45	0.01, 3.34	0.258
GG	0.18	1.39	0.01, 2.70	0.213
79C/G				
CC	--	--	--	--
CG	0.60	1.67	0.02, 15.83	0.260
GG	0.11	2.11	0.00, 6.69	0.289
-20T/C				
CC	--	--	--	--
CT	0.69	0.89	0.12, 3.98	0.678
TT	1.08	0.53	0.38, 3.04	0.885
Sex				
Male	0.71	0.51	0.27, 1.92	0.502
Female	--	--	--	--
Age	1.00	0.03	0.94, 1.05	0.856
Type of glaucoma				
POAG	3.66	0.83	0.72, 18.68	0.118
NTG	--	--	---	--
Baseline IOP	0.81	0.09	0.68, 0.96	**0.014**
CCT	0.98	0.01	0.96, 1.01	0.248

OR: odd ratio, CI: confidence interval, POAG: primary open angle glaucoma, NTG: normal tension glaucoma, IOP: intraocular pressure, CCT: central corneal thickness
The goodness of fit of this model was checked using the Hosmer-Lemenshow test; p=0.576. This result gives no evidence of lack of fit of the model.

4.3.2: Pressure lowering effect of topical latanoprost 0.005% and *PTGFR*

4.3.2.1: Demographic data

Details of demographic characteristics are available in table 2.11 of chapter 2. A total of 87 glaucoma (64 POAG and 22 NTG) patients were recruited; 39 of them were treated with topical latanoprost 0.005% monotherapy and 47 of them required adjunctive therapy. At the end of 12 months follow up only 65 (74.7%) patients were still part of study treatment protocol. As was the case with the entire cohort of subjects, more than half received adjunctive treatment of latanoprost, with the majority being POAG sufferers.

Glaucoma patients who completed a full 12 months treatment were younger with less severe glaucoma based on vertical cup disc ratio (VCDR) and visual field parameter. Thicker CCT and lower baseline IOP are also found to associate with failure to complete 12 months treatment (table 4.8).

Table 4.8: Univariate analysis on the potential clinical predictors that affect the ability to complete 12 months treatment of topical latanoprost 0.005%

Characteristic	Completed 12 months treatment N=65	Incomplete 12months treatment** N=21	p-value
Age at presentation (Mean (SD))	66.7 SD 8.8	68.1 SD 10.5	0.532#
Ethnicity (n (%))			
Malay	44(67.7)	14(66.7)	0.01,
Chinese	21(32.3)	7(33.3)	0.931*
Gender (n (%))			
Male	44 (67.7)	16 (76.2)	0.54,
Female	21 (32.3)	5 (23.8)	0.467*
Mean baseline IOP (mmHg)	22.0 SD 3.6	23.7 SD 5.5	0.103#
VCDR (Mean (SD))	0.79 SD 0.08	0.80 SD 0.08	0.583#
Humphrey visual field (Mean SD)			
MD	-10.91 SD 8.22	-11.99 SD 9.21	0.624#

PSD	6.42 SD 3.14	6.80 SD 3.37	0.648#
CCT (Mean SD in µm)	512 SD 37	502 SD 46	0.309#
Type of glaucoma (n (%))			
POAG	46 (67.7)	18 (81.8)	0.393@
NTG	19 (27.9)	3 (13.7)	
Treatment modalities (n (%))			
Monotherapy	32 (49.2)	7 (33.3)	1.62,
Adjunctive	33 (50.8)	14 (66.7)	0.203*
Hypertension^			
Yes	44 (67.7)	11 (52.4)	1.61,
No	21 (32.3)	10 (47.6)	0.204*
Diabetes mellitus^			
Yes	29 (44.6)	5 (23.8)	2.87,
No	36 (55.4)	16 (76.2)	0.090*
Cardiovascular diseases^			
Yes	10 (15.4)	1 (4.8)	0.281@
No	55 (84.6)	20 (95.2)	
Hyperlipidemia^			
Yes	29 (44.6)	9 (42.9)	0.20,
No	36 (55.4)	12 (57.1)	0.888*

P<0.05 is considered statistical significant based on Pearson chi-square test*, Fischer exact test@ and student t-test#.

**Incomplete treatment referred to patients who failed to complete 12 months treatment with topical Latanoprost 0.005%.

^ Systemic disease such as hypertension, diabetes mellitus, cardiovascular diseases and hyperlipidemia were diagnosed based on medical record and information from general practitioner as stated in chapter 2. Cardiovascular disease such as angina pectoris, myocardial infarction was included.

POAG: primary open angle glaucoma, NTG: normal tension glaucoma, IOP: intraocular pressure, CCT: central corneal thickness, VCDR: vertical cup disc ratio, HFA: Humphrey visual field analysis.

4.3.2.2 Predictors affecting the long term efficacy of topical Latanoprost 0.005%

Sex, race, systemic co-morbidities and responder were included as the categorical predictors. Age, baseline IOP, CCT, VCDR and parameters for visual field were included as the numerical predictors. There was no significant association of factors affecting long term efficacy of topical latanoprost 0.005% in simple logistic regression model. In spite of the absence of significant factor based on simple logistic regression, baseline IOP, MD, PSD, CCT and responder were selected for stepwise logistic regression model. It is no surprise that good responsiveness to topical latanoprost and higher baseline IOP were identified as significant predictor for long term efficacy of topical latanoprost 0.005% (table 4.9).

Table 4.9: Stepwise logistic regression on factors affecting the ability of glaucoma patients to complete treatment protocol

Variables	$OR_{(c)}$	$OR_{(r)}$	95% CI (LCI, UCI)$_{(a)}$	SE	p-value
HFA MD	0.95	0.96	0.87, 1.06	0.05	0.447
HFA PSD	0.89	0.92	0.77, 1.12	0.09	0.376
Baseline IOP	0.82	0.83	0.71, 0.98	0.08	**0.024**
Responder*					
Good	6.91	4.55	1.22, 16.98	0.67	**0.024**
Poor	-	-	-	-	-

*Good responder is based on ≥25% IOP reduction from baseline; Poor responder is based on <25% IOP reduction from baseline

$_{(a)}$: Backward LR multiple logistic regression model was applied; OR: odd ratio, $OR_{(c)}$: crude odd ratio, $OR_{(r)}$: logistic odd ratio, CI: confidence interval, LCI: lower confidence interval, UCI: upper confidence interval

Multicollinearity and interaction terms were checked and not found.

The goodness of fit of the backward model was checked using the Hosmer-Lemenshow test; p=0.835. This result gives no evidence of lack of fit of the model.

4.3.2.3 SNPs of *PTGFR* and genotype value based on Fisher model

The overall mean IOP reduction for each SNP was selected as phenotype value. Based on Fisher's model, rs4650581 was found to exert a significant dominant effect (p=0.007) and combination of additive and dominant effect (p=0.014). On the other hand, rs2146490, rs34012237, rs33966768, rs3766335 and rs7543738 were significant as dominant effect but borderline significant on combination of both effects. The SNP rs35978825 was borderline significant as dominant effect (p=0.054) and also on combination of effect (p=0.075). There was no significant quantitative trait locus (QTL) as addictive effect (table 4.10). Additive genotype value is commonly used in analysis of complex disease such as glaucoma. Thus, rs4650581, rs2146490, rs33966768, rs3766335, rs7543738 and rs35978825 were selected for subsequent analysis.

Table 4.10: Quantitative trait locus on IOP reduction in *PTGFR* based on Fisher model

SNPs N=90	**Addictive effect**		**Dominant effect**		**Both**	
	f	p-value	f	p-value	F	p-value
rs3766331	1.37	0.245	0.02	0.898	1.32	0.273
rs3766355	0.15	0.703	0.72	0.398	0.40	0.673
rs3766353	1.25	0.267	2.08	0.153	1.15	0.320
rs35978825	1.25	0.267	3.81	*0.054*	2.66	*0.075*
rs1830673	0.04	0.840	0.06	0.803	0.07	0.933
rs3766351	0.27	0.602	0.05	0.817	0.25	0.776
rs1555541	0.10	0.748	0.01	0.938	0.05	0.949
rs10489785	1.75	0.189	1.43	0.235	0.92	0.401
rs12094298	1.67	0.200	3.46	*0.066*	1.73	0.183
rs34077564	0.01	0.903	0.90	0.346	0.46	0.633
rs35123627	1.62	0.206	3.25	*0.075*	1.63	0.201
rs2146489	0.08	0.779	0.04	0.834	0.04	0.956
rs2057424	0.08	0.779	0.12	0.734	0.13	0.875
rs15101588	0.99	0.322	0.01	0.913	0.52	0.599
rs34528585	1.74	0.190	3.22	*0.076*	1.61	0.205
rs12044011	0.71	0.401	0.08	0.772	0.38	0.687
rs1322930	0.19	0.664	0.08	0.779	0.10	0.909
rs34852041	0.42	0.517	1.02	0.316	0.59	0.557
rs33994937	0.40	0.531	1.74	0.191	1.32	0.273
rs6424776	0.44	0.511	0.01	0.933	0.22	0.805
rs72673925	1.60	0.209	3.33	*0.072*	1.69	0.191

rs28832602	1.57	0.214	3.79	*0.055*	1.98	0.145
rs34572897	1.73	0.192	1.66	0.201	0.97	0.383
rs1590314	0.40	0.528	0.01	0.908	0.20	0.816
rs12058120	0.24	0.622	1.14	0.288	0.57	0.567
rs12725125	0.24	0.622	1.14	0.288	0.57	0.567
rs4261075	0.00	0.987	0.03	0.870	0.01	0.986
rs34012602	1.68	0.198	2.37	0.127	1.21	0.302
rs67351117	3.13	*0.080*	1.49	0.226	1.58	0.213
rs672561	1.73	0.192	1.67	0.199	0.99	0.377
rs12401416	0.00	0.996	0.69	0.410	0.35	0.709
rs6424778	0.00	0.992	0.68	0.398	0.14	0.712
rs577333	1.04	0.311	0.19	0.667	0.52	0.597
rs520171	2.19	0.142	1.43	0.235	1.14	0.326
rs551253	0.03	0.858	0.04	0.836	0.11	0.899
rs552328	2.64	0.108	1.19	0.279	1.50	0.230
rs11162504	0.21	0.651	1.25	0.266	0.73	0.484
rs11162505	0.06	0.814	0.18	0.674	0.11	0.897
rs554173	0.95	0.333	0.88	0.352	0.54	0.585
rs554185	0.66	0.417	0.22	0.637	0.82	0.445
rs556817	1.14	0.289	0.10	0.747	0.68	0.510
rs473027	0.49	0.488	0.13	0.720	0.26	0.770
rs668005	0.76	0.387	0.35	0.553	0.43	0.650
rs2146490	1.59	0.211	4.92	**0.029**	2.59	*0.081*
rs530871	0.27	0.606	0.31	0.580	0.23	0.798
rs538275	0.00	0.970	0.01	0.911	0.01	0.994
rs589958	0.10	0.749	0.11	0.742	0.15	0.864
rs3766338	0.07	0.792	0.35	0.558	0.18	0.836
rs590309	0.00	0.982	0.28	0.601	0.15	0.863
rs622346	0.07	0.796	0.00	0.961	0.06	0.939
rs13374108	0.34	0.560	0.09	0.767	0.26	0.773
rs34012237	1.60	0.210	5.38	**0.023**	2.84	*0.064*
rs33966768	1.61	0.208	4.20	**0.043**	2.15	0.122
rs501078	0.23	0.634	0.60	0.442	0.34	0.714
rs3766335	1.61	0.208	5.48	**0.022**	2.86	*0.062*
rs7543738	1.33	0.252	4.56	**0.036**	2.70	*0.073*
rs686262	0.22	0.639	0.14	0.705	0.20	0.816
rs4650581	1.52	0.220	7.58	**0.007**	4.49	**0.014**
rs3766332	2.88	0.093	0.09	0.765	2.30	0.106
rs3753380	1.07	0.303	0.10	0.753	0.54	0.582
rs12093097	0.04	0.840	0.00	0.949	0.02	0.976
rs1073610	0.63	0.429	3.72	0.057	1.87	0.160
rs1073611	0.69	0.409	3.30	*0.073*	1.65	0.198

P< 0.05 is considered statistically significant based on Fisher model

4.3.2.4 *PTGFR* and long term pressure lowering effect of topical latanoprost 0.005%

At the end of 12 months follow up, 65 patients were still on topical latanoprost. One way ANOVA was conducted on the mean IOP (SD) on each follow up visit according to genotype of selected SNPs. The selection of SNPs of *PTGFR* was based on genotype value derived from Fisher model (table 4.10). There was no significant difference in mean IOP at each visit among Malay patients with open angle glaucoma (table 4.11). In general, those with homozygous wild presented with higher mean baseline IOP but with relatively good IOP reduction on subsequent visits.

Due to relatively small number of glaucoma patients from Chinese descent were recruited in this study, majority of the SNPs were devoid of homozygous minor alleles. Chinese glaucoma patients with rs2146490GG were found to have significantly higher mean IOP compared to those with rs2146490GC at 12 months (table 4.11). There was no significant difference between mean IOP at each follow up visit and genotype frequency of selected SNPs found in *PTGFR* (table 4.12).

Table 4.11: Association between mean IOP at each follow up visits and genotype of selected SNPs of *PTGFR* in Malays and Chinese

Visits	Malays N=58 Mean IOP (SD) (mmHg)				Chinese N=28 Mean IOP (SD) (mmHg)			
	rs4650581				**rs4650581**			
	TT (n)	TA (n)	AA (n)	p-value	TT (n)	TA (n)	AA (n)	p-value
Baseline	22.1(4.6) (48)	23.1(3.4) (8)	18.5(0.7) (2)	0.422	22.7(3.5) (25)	24.3(4.7) (3)	--	0.458
Visit 1	15.4(2.8) (48)	15.6(1.3) (8)	15.5(0.7) (2)	0.974	16.1(4.0) (25)	16.3(2.1) (3)	--	0.916
Visit 2	15.5(4.8) (44)	14.0(2.7) (8)	16.0(0.0) (2)	0.669	15.4(4.1) (24)	13.0(3.0) (3)	--	0.339
Visit 3	14.9(3.1) (40)	13.9(1.8) (8)	16.0(0.0) (2)	0.555	16.0(3.1) (21)	13.0(3.0) (3)	--	0.137
Visit 4	14.5(2.5) (34)	15.3(3.5) (8)	13.5(3.5) (2)	0.667	15.4(2.7) (18)	13.3(3.5) (3)	--	0.247
	rs34012237				**rs34012237**			
	TT (n)	TC (n)	CC (n)	p-value	TT (n)	TC (n)	CC (n)	p-value
Baseline	22.1(4.5) (49)	23.3(3.7) (7)	18.5(0.7) (2)	0.406	22.8(3.5) (26)	23.5(6.4) (2)	--	0.797
Visit 1	15.3(2.8) (49)	15.5(0.7) (7)	15.5(0.7) (2)	0.749	16.1(3.9) (26)	16.0(2.8) (2)	--	0.968
Visit 2	15.2(4.8) (45)	15.6(2.6) (7)	16.0(0.0) (2)	0.962	15.4(4.0) (25)	11.5(2.1) (2)	--	0.190
Visit 3	14.9(3.1) (42)	13.8(1.7) (6)	16.0(0.0) (2)	0.606	16.0(3.0) (22)	11.5(2.1) (2)	--	*0.056*
Visit 4	14.4(2.5) (36)	16.5(3.0) (6)	13.5(3.5) (2)	0.159	15.5(2.6) (19)	11.5(2.1) (2)	--	*0.053*
	rs3766335				**rs3766335**			
	GG (n)	GA (n)	AA (n)	p-value	GG (n)	GA (n)	AA (n)	p-value
Baseline	22.1(4.5) (49)	23.3(3.7) (7)	18.5(0.7) (2)	0.406	22.7(3.5) (27)	28.0(0.0) (1)	--	0.145
Visit 1	15.3(2.8) (49)	16.1(1.5) (7)	15.5(0.7) (2)	0.749	16.0(3.9) (27)	18.0(0.0) (1)	--	0.622
Visit 2	15.2(4.8) (45)	15.6(2.6) (7)	16.0(0.7) (2)	0.962	15.2(4.1) (26)	13.0(0.0) (1)	--	0.598
Visit 3	14.9(3.1) (44)	13.9(1.7) (7)	16.0(0.0) (2)	0.606	15.7(3.2) (23)	13.0(0.0) (1)	--	0.420
Visit 4	14.4(2.5) (36)	16.5(3.0) (6)	13.5(3.5) (2)	0.159	15.2(2.8) (20)	13.0(0.0) (1)	--	0.456
	rs2146490				**rs2146490**			
	GG (n)	GA (n)	AA (n)	p-value	GG (n)	GA (n)	AA (n)	p-value
Baseline	22.3(4.5) (50)	22.3(3.8) (6)	18.5(0.7) (2)	0.498	22.9(3.5) (24)	22.5(4.4) (4)	--	0.833
Visit 1	15.5(2.8) (55)	14.8(1.6) (6)	15.5(0.7) (2)	0.845	16.3(4.0) (24)	15.3(2.2) (4)	--	0.636
Visit 2	15.0(4.8) (46)	14.8(2.3) (6)	16.0(0.0) (2)	0.945	15.7(4.1) (23)	12.0(1.8) (4)	--	*0.092*
Visit 3	14.8(3.1)	14.0(1.9)	16.0(0.0)	0.676	15.9(3.1)	14.0(3.4)	--	0.286

	(42)	(6)	(2)		(20)	(4)		
Visit 4	14.5(2.4) (36)	15.6(3.8) (6)	13.5(3.5) (2)	0.435	15.7(2.4) (17)	12.5(3.3) (4)	--	**0.035**
		rs33966768				**rs33966768**		
	TT (n)	TC (n)	CC (n)	p-value	TT (n)	TC (n)	CC (n)	p-value
Baseline	22.3(4.5) (48)	22.4(4.3) (8)	18.5(0.7) (2)	0.498	22.8(3.5) (26)	23.5(6.4) (2)	--	0.797
Visit 1	15.4(2.8) (48)	15.6(2.0) (8)	15.5(0.7) (2)	0.974	16.1(3.9) (26)	16.0(2.8) (2)	--	0.968
Visit 2	15.4(4.9) (44)	15.8(2.7) (8)	16.0(0.0) (2)	0.971	15.4(4.0) (25)	11.5(2.1) (2)	--	0.190
Visit 3	14.9(3.1) (41)	13.6(1.7) (7)	16.0(0.0) (2)	0.438	16.0(3.0) (22)	11.5(2.1) (2)	--	*0.056*
Visit 4	14.4(2.5) (35)	16.1(2.9) (7)	13.5(3.5) (2)	0.233	15.5(2.6) (19)	11.5(2.1) (2)	--	*0.053*
		rs7543738				**rs7543738**		
	CC (n)	CG (n)	GG (n)	p-value	CC (n)	CC (n)	GG (n)	p-value
Baseline	22.1(4.5) (51)	23.0(3.5) (6)	18.0(--) (1)	0.582	22.7(3.5) (27)	28.0(0.0) (1)	--	0.145
Visit 1	15.4(2.8) (51)	15.8(1.3) (6)	15.0(--) (1)	0.917	16.0(3.9) (27)	18.0(0.0) (1)	--	0.622
Visit 2	15.3(4.7) (47)	15.6(2.8) (6)	16.0(--) (1)	0.968	15.2(4.1) (26)	13.0(0.0) (1)	--	0.598
Visit 3	15.0(3.0) (44)	14.2(2.0) (5)	16.0(--) (1)	0.828	15.7(3.2) (23)	13.0(0.0) (1)	--	0.420
Visit 4	14.6(2.6) (38)	15.4(4.0) (5)	16.0(--) (1)	0.676	15.2(2.8) (20)	13.0(0.0) (1)	--	0.456
		rs67351117				**rs67351117**		
	CC (n)	CT (n)	TT (n)	p-value	CC (n)	CT (n)	TT (n)	p-value
Baseline	22.2(4.6) (48)	22.9(3.7) (8)	18.5(0.7) (2)	0.459	23.3(3.5) (25)	19.5(0.7) (2)	18.0(0.0) (1)	0.131
Visit 1	15.3(2.8) (48)	16.5(1.1) (8)	15.5(0.7) (2)	0.467	16.3(3.9) (25)	13.0(1.4) (2)	17.0(0.0) (1)	0.499
Visit 2	15.2(4.9) (44)	15.8(2.6) (8)	16.0(0.0) (2)	0.931	15.6(4.0) (24)	10.5(0.7) (2)	13.0(0.0) (1)	0.201
Visit 3	14.8(3.1) (41)	14.6(2.1) (7)	16.0(0.0) (2)	0.826	16.0(3.1) (21)	12.0(2.8) (2)	15.0(0.0) (1)	0.251
Visit 4	14.6(2.8) (36)	15.2(1.5) (6)	13.5(3.5) (2)	0.745	15.3(2.7) (18)	12.5(3.5) (2)	17.0(0.0) (1)	0.338

Visit 1: 1 month, Visit 2: 3 months, Visit 3: 6 months and Visit 4: 12 months post treatment with topical latanoprost 0.005%

P<0.05 is considered statistically significant based on One –way ANOVA

Table 4.12: Association between mean IOP at each follow up visit and genotype frequency of selected SNPs of *PTGFR*

Visits	Mean IOP (95%CI)			p-value
	rs4650581			
	TT (n)	TA (n)	AA (n)	
Baseline	22.3(21.3, 23.3) (73)	24.5(21.0, 25.9) (11)	18.5(12.2, 24.9) (2)	0.289
1 month	15.6(14.9, 16.4) (76)	15.8(14.9, 16.7) (12)	15.5(9.2, 21.9) (2)	0.989
3 months	15.7(14.6, 16.8) (71)	14.0(12.3, 15.7) (12)	16.0(16.0, 16.0) (2)	0.457
6months	15.4(14.6, 16.1) (63)	13.8(12.5, 15.0) (12)	16.0(16.0,16.0) (2)	0.218
12 months	14.9(14.2, 15.6) (54)	14.5(12.3, 16.7) (12)	13.5 (-18.3, 45.3) (2)	0.708
	rs34012237			
	TT (n)	TC (n)	CC (n)	
Baseline	22.6(21.6, 23.6) (78)	24.4(20.8, 28.0) (10)	18.5(12.2, 24.9) (2)	0.197
1 month	15.7(15.0, 16.4) (78)	16.1(15.0, 17.2) (10)	15.5(9.2, 21.9) (2)	0.924
3 months	15.6(14.5, 16.6) (73)	14.9(12.8, 17.0) (10)	16.(16.0, 16.0) (2)	0.899
6months	15.3(14.6, 16.1) (66)	13.4(12.0, 15.0) (9)	16.0(16.0, 16.0) (2)	0.194
12 months	14.8(14.2, 15.5) (57)	14.9(12.2, 17.6) (9)	13.5(-18.3, 25.3) (2)	0.793
	rs3766335			
	GG (n)	GA (n)	AA (n)	
Baseline	22.5(21.6, 23.5) (79)	25.0(21.2, 28.8) (9)	18.5(12.2, 24.9) (2)	0.119
1 month	15.7(15.0, 16.4) (79)	16.3(15.3, 17.4) (9)	15.5(9.2, 21.9) (2)	0.830
3 months	15.5(14.4, 16.6) (74)	15.4(13.6, 17.3) (9)	16.0(16.0, 16.0) (2)	0.986
6months	15.2(14.5, 16.0) (67)	13.9(12.6, 15.2) (8)	16.0(16.0, 16.0) (2)	0.440
12 months	14.8(14.1, 15.5) (58)	15.5(12.9, 18.1) (8)	13.5(-18.3, 25.3) (2)	0.615

		rs2146490		
	GG (n)	GA (n)	AA (n)	
Baseline	22.7(21.7, 23.7) (77)	23.5(20.1, 26.8) (11)	18.5(12.2, 24.9) (2)	0.357
1 month	15.8(15.1, 16.6) (77)	15.1(14.0, 16.2) (11)	15.5(9.2, 21.9) (2)	0.745
3 months	15.7(14.6, 16.8) (72)	14.0(12.3, 15.7) (11)	16.0(16.0, 16.0) (2)	0.493
6months	15.3(14.5, 16.0) (64)	14.1(12.6, 15.6) (11)	16.0(16.0, 16.0) (2)	0.448
12 months	15.0(11.8, 16.8) (55)	14.3(11.8, 16.8) (11)	13.59-18.3, 25.3) (2)	0.595
		rs33966768		
	TT (n)	TC (n)	CC (n)	
Baseline	22.7(21.7, 23.7) (77)	23.6(20.0, 27.3) (11)	18.5(12.2, 24.9) (2)	0.328
1 month	15.8(15.0, 16.5) (77)	15.7(14.5, 17.0) (11)	15.5(9.2, 21.9) (2)	0.993
3 months	15.6(14.5, 16.7) (72)	14.6(12.7, 16.6) (11)	16.0(16.0, 16.0) (2)	0.790
6months	15.4(14.6, 16.1) (65)	13.3(12.0, 14.7) (10)	16.0(16.0, 16.0) (2)	0.114
12 months	14.9(14.2,15.6) (56)	14.8(12.4, 17.2) (10)	13.5(-18.3, 25.3) (2)	0.792
		rs7543738		
	CC (n)	CG (n)	GG (n)	
Baseline	22.5(21.6, 23.5) (81)	25.0(20.8, 29.2) (8)	18.0(--) (1)	0.189
1 month	15.7(15.0, 16.4) (81)	16.1(15.0, 17.3) (8)	15.0(--) (1)	0.911
3 months	15.5(14.4, 16.5) (76)	15.5(13.3, 17.7) (8)	16.0(--) (1)	0.993
6months	15.2(14.5, 16.0) (69)	14.1(12.5, 15.8) (7)	16.0(--) (1)	0.647
12 months	14.8(14.1, 15.5) (60)	14.6(11.2, 17.9) (7)	16.0(--) (1)	0.888
		rs67351117		
	CC (n)	CT (n)	TT (n)	
Baseline	22.9(21.9, 24.0) (77)	22.2(19.6, 24.8) (10)	18.3(16.9, 19.8) (3)	0.203
1 month	15.7(15.0, 16.5)	15.8(14.5, 17.1)	16.0(13.5, 18.5)	0.987

	(77)	(10)	(3)	
3 months	15.6(14.5, 16.7)	14.7(12.4, 17.0)	15.0(10.7, 19.3)	0.817
	(72)	(10)	(3)	
6months	15.3(14.5, 16.0)	14.0(12.2, 15.8)	15.7(14.2, 17.1)	0.483
	(65)	(9)	(3)	
12 months	14.9(14.1, 15.6)	14.5(12.7, 16.3)	14.7(6.7, 22.7)	0.939
	(57)	(8)	(3)	

P<0.05 based on one-way ANOVA

4.3.2.5 *PTGFR* polymorphisms and responsiveness to topical latanoprost 0.005%

A good respond to latanoprost is defined as 25 % or more difference between baseline IOP and the final measurement. The minor allele frequency of rs686262 (rs686262G) demonstrated significant association to good respond to topical latanoprost (table 4.13). Test of heterogeneity was significant in a number of SNPs (rs3766353, rs12093097, rs1073610 and rs1073611) suggesting responds to topical timolol is relatively affected by racial difference (table 4.13). Baseline IOP remains the strongest predictor that determines the respond to topical latanoprost in glaucoma patients in this present study (table 4.14 and 4.16). rs686262, rs37666332, rs501078, rs33994937 and rs15101588 were included in analysis of Malays. rs686262 and rs3766332 were included in analysis of Chinese glaucoma patients (table 4.15). For stepwise linear regression, only rs686262 was included (table 4.16). Age at the initial presentation, sex, type of glaucoma, central cornea thickness, baseline IOP and mean deviation of HFA were also included as predictors. rs686262GG predisposes to poor responds to topical latanoprost (OR 6.3[95%CI 1.3, 31.0]) (table 4.16). Baseline IOP remains as a strong predictor for responsiveness to topical latanoprost.

Table 4.13: Stratified Mantel-Haenszel analysis on *PTGFR* and responsiveness to topical latanoprost 0.005%.

	Malays N=58		**Chinese N=28**		**Stratified meta-analysis**			
	Allele frequency		Allele frequency		OR (95%CI)	SE	P-meta	P-Het
SNPs	Good N=35	Poor N=23	Good N=20	Poor N=8				
rs3766331								
A	0.829	0.783	0.850	0.938	0.86	0.44	0.707	0.485
G	0.171	0.217	0.150	0.063	(0.37, 2.02)			
rs3766353								
G	0.757	0.848	0.725	0.438	0.90	0.37	0.778	**0.021**
T	0.243	0.152	0.275	0.563	(0.44, 1.84)			
rs3766355								
C	0.529	0.630	0.475	0.438	1.28	0.32	0.440	0.421
A	0.471	0.370	0.525	0.563	(0.68, 2.42)			
rs35978825								
C	0.886	0.891	0.925	1.000	1.38	0.58	0.577	0.290
T	0.114	0.109	0.075	0	(0.45, 4.26)			
rs1830673								
A	0.471	0.457	0.300	0.313	0.97	0.33	0.929	0.873
G	0.529	0.543	0.700	0.688	(0.51, 1.85)			
rs3766351								
T	0.842	0.847	0.875	1.000	1.44	0.50	0.461	0.164
C	0.158	0.152	0.125	0	(0.55, 3.80)			
rs1555541								
T	0.316	0.333	0.275	0.375	1.27	0.34	0.479	0.680
C	0.684	0.667	0.725	0.625	(0.65, 2.47)			
rs10489785								
A	0.900	0.891	0.925	1.000	1.23	0.58	0.726	0.259
T	0.100	0.109	0.075	0	(0.39, 3.85)			
rs12094298								
C	0.929	0.935	0.950	1.000	1.44	0.72	0.612	0.401
A	0.071	0.065	0.050	0	(0.35, 5.94)			
rs34077564								
A	0.855	0.813	0.875	1.000	1.32	0.50	0.574	0.145
G	0.145	0.188	0.125	0	(0.50, 3.51)			
rs35123627								
C	0.900	0.913	0.925	0.938	1.18	0.58	0.776	0.976
T	0.100	0.089	0.075	0.063	(0.38, 3.64)			
rs2146489								
A	0.414	0.457	0.250	0.313	1.23	0.33	0.531	0.855
G	0.586	0.543	0.750	0.688	(0.64, 2.35)			
rs2057424								

A	0.743	0.674	0.650	0.500	0.65	0.34	0.212	0.697
G	0.257	0.326	0.350	0.500	(0.33, 1.28)			
rs15101588								
G	0.500	0.587	0.300	0.563	1.75	0.32	0.084	0.298
A	0.500	0.413	0.700	0.438	(0.93, 3.30)			
rs34528585								
T	0.914	0.891	0.925	0.875	0.70	0.53	0.509	0.879
A	0.086	0.109	0.075	0.125	(0.25, 2.00)			
rs12044011								
T	0.529	0.565	0.550	0.375	0.91	0.32	0.754	0.228
A	0.471	0.436	0.450	0.625	(0.48, 1.69)			
rs1322930								
G	0.974	0.979	0.925	1.000	2.79	1.13	0.366	0.359
A	0.026	0.021	0.075	0	(0.30, 25.62)			
rs34852041								
C	0.871	0.935	0.975	1.000	2.30	0.69	0.228	0.661
T	0.129	0.065	0.025	0	(0.59, 8.87)			
rs33994937								
T	0.871	0.957	0.950	1.000	3.79	0.80	0.095	0.611
C	0.129	0.043	0.050	0	(0.79, 18.13)			
rs6424776								
T	0.543	0.587	0.550	0.438	0.99	0.32	0.984	0.372
C	0.457	0.413	0.450	0.563	(0.53, 1.86)			
rs72673925								
T	0.871	0.913	0.900	1.000	2.09	0.61	0.224	0.302
G	0.129	0.087	0.100	0	(0.64, 6.83)			
rs28832602								
C	0.857	0.891	0.900	1.000	1.81	0.56	0.289	0.273
T	0.143	0.109	0.100	0	(0.61, 5.41)			
rs34572897								
A	0.908	0.854	0.925	1.000	1.00	0.55	0.995	0.216
G	0.092	0.146	0.075	0	(0.34, 2.97)			
rs1590314					1.02			
T	0.371	0.413	0.400	0.313	(0.53,	0.33	0.959	0.449
C	0.629	0.587	0.600	0.688	1.94)			
rs12058120								
C	0.857	0.847	0.925	0.875	0.83	0.47	0.694	0.654
G	0.143	0.153	0.075	0.125	(0.33, 2.08)			
rs12725125								
G	0.857	0.847	0.925	0.875	0.83	0.47	0.694	0.654
A	0.143	0.156	0.075	0.125	(0.33, 2.08)			
rs4261075								
A	0.429	0.413	0.400	0.438	1.00	0.32	0.999	0.759
G	0.571	0.587	0.600	0.563	(0.53, 1.89)			
rs34012602								
G	0.886	0.870	0.975	1.000	0.95	0.56	0.927	0.499

T	0.114	0.130	0.025	0	(0.31 2.87)			
rs67351117								
C	0.900	0.891	0.950	0.875	0.73	0.53	0.553	0.451
T	0.100	0.109	0.050	0.125	(0.26 2.06)			
rs672561								
T	0.900	0.891	0.950	1.000	1.12	0.59	0.847	0.355
C	0.100	0.109	0.050	0	(0.35, 3.59)			
rs12401416								
G	0.557	0.522	0.525	0.438	0.82	0.32	0.526	0.768
A	0.443	0.478	0.475	0.563	(0.44, 1.53)			
rs6424778								
C	0.971	0.957	0.900	0.875	0.72	0.68	0.623	0.893
T	0.029	0.043	0.100	0.125	(0.19, 2.72)			
rs577333								
T	0.357	0.413	0.250	0.375	1.39	0.33	0.319	0.636
C	0.643	0.587	0.750	0.625	(0.73, 2.67)			
rs520171								
A	0.671	0.739	0.825	0.750	1.14	0.36	0.714	0.343
C	0.329	0.261	0.175	0.250	(0.56, 2.32)			
rs551253								
G	0.857	0.761	0.725	0.875	0.86	0.40	0.700	*0.086*
C	0.143	0.239	0.275	0.125	(0.39, 1.87)			
rs552328								
A	0.657	0.674	0.475	0.625	1.27	0.33	0.470	0.461
G	0.343	0.326	0.525	0.375	(0.66, 2.45)			
rs11162504								
A	0.800	0.848	0.775	0.813	1.35	0.42	0.476	0.910
G	0.200	0.152	0.225	0.188	(0.59, 3.07)			
rs11162505								
A	0.971	0.957	0.875	0.875	0.83	0.67	0.779	0.748
G	0.029	0.043	0.125	0.125	(0.23, 3.06)			
rs554173								
T	0.843	0.826	0.800	0.688	1.28	0.40	0.471	0.570
C	0.157	0.174	0.200	0.313	(0.59, 2.81)			
rs554185								
A	0.700	0.717	0.550	0.625	1.17	0.34	0.647	0.759
G	0.300	0.283	0.450	0.375	(0.50, 2.30)			
rs556817								
A	0.857	0.913	0.875	0.750	1.03	0.47	0.954	0.142
G	0.143	0.087	0.125	0.250	(0.41, 2.56)			
rs473027								
A	0.600	0.674	0.450	0.500	1.33	0.33	0.391	0.867
G	0.400	0.326	0.550	0.500	(0.70, 2.54)			
rs668005								
C	0.714	0.673	0.475	0.750	0.87	0.32	0.670	0.834

T	0.286	0.326	0.525	0.250	(0.47, 1.63)			
rs2146490								
G	0.914	0.913	0.900	1.000	1.50	0.63	0.516	0.212
A	0.086	0.087	0.100	0	(0.44, 5.12)			
rs530871								
G	0.671	0.673	0.500	0.375	0.86	0.33	0.650	0.473
A	0.329	0.327	0.500	0.625	(0.45, 1.65)			
rs538275								
G	0.686	0.652	0.450	0.438	0.89	0.33	0.720	0.888
A	0.314	0.348	0.550	0.563	(0.46, 1.71)			
rs589958								
G	0.671	0.652	0.500	0.375	0.81	0.33	0.514	0.558
A	0.329	0.348	0.500	0.625	(0.42, 1.54)			
rs3766338								
T	0.857	0.826	0.450	0.313	0.68	0.40	0.336	0.663
C	0.143	0.174	0.550	0.688	(0.31, 1.49)			
rs590309								
T	0.700	0.652	0.450	0.313	0.72	0.34	0.328	0.620
C	0.300	0.348	0.550	0.688	(0.37, 1.39)			
rs622346								
G	0.757	0.717	0.675	0.500	0.68	0.34	0.256	0.466
C	0.243	0.283	0.325	0.500	(0.35, 1.33)			
rs13374108								
T	0.929	0.935	0.800	0.938	1.77	0.60	0.342	0.349
A	0.071	0.065	0.200	0.063	(0.54, 5.79)			
rs34012237								
T	0.914	0.891	0.950	1.000	0.98	0.61	0.968	0.320
C	0.086	0.109	0.050	0	(0.30, 3.19)			
rs33966768								
T	0.914	0.870	0.950	1.000	0.80	0.58	0.695	0.274
C	0.086	0.130	0.050	0	(0.26, 2.47)			
rs501078								
C	0.457	0.326	0.400	0.250	0.55	0.34	0.081	0.857
T	0.543	0.674	0.600	0.750	(0.29, 1.08)			
rs3766335								
G	0.914	0.891	0.975	1.000	0.87	0.62	0.825	0.477
A	0.086	0.109	0.025	0	(0.26, 2.94)			
rs7543738								
C	0.943	0.913	0.975	1.000	0.76	0.70	0.698	0.441
G	0.057	0.087	0.025	0	(0.19, 3.02)			
rs686262								
A	0.543	0.348	0.475	0.250	0.43	0.34	**0.011**	0.796
G	0.457	0.652	0.525	0.750	(0.22, 0.82)			
rs4650581								
T	0.886	0.913	0.925	1.000	1.76	0.62	0.363	0.344

A	0.114	0.087	0.075	0	(0.52, 5.91)			
rs3766332								
A	0.757	0.848	0.600	0.813	2.10	0.41	*0.067*	0.581
T	0.243	0.152	0.400	0.187	(0.95, 4.66)			
rs3753380								
T	0.371	0.413	0.275	0.125	0.94	0.34	0.848	0.202
C	0.629	0.587	0.725	0.875	(0.48, 1.84)			
rs12093097								
C	0.700	0.826	0.900	0.625	1.05	0.37	0.892	**0.004**
T	0.300	0.174	0.100	0.375	(0.51, 2.16)			
rs1073610								
G	0.686	0.783	0.900	0.625	0.94	0.36	0.886	**0.008**
A	0.214	0.217	0.100	0.375	(0.47, 1.90)			
rs1073611								
G	0.700	0.783	0.900	0.625	0.90	0.36	0.758	**0.010**
A	0.300	0.217	0.100	0.375	(0.44, 1.81)			

OR: odd ratio, CI: confidence interval, SE: standard error
Phet: P-value for heterogeneity between both studies (P<0.05 is considered significant heterogeneity based on the Breslow-Day test)
P-meta: P-value for the meta-analysis between Malays and Chinese where the association between alleles and glaucoma status was measured.

Table 4.14: Predictors affecting response to topical latanoprost 0.005% in Malay glaucoma patients

Predictors	**OR**	**SE**	**95% CI for OR**	**p-value**
rs686262				
AA	--	--	--	--
AG	0.76	1.56	0.04, 16.06	0.858
GG	4.03E9	25730.22	0.00	*0.099*
rs3766332				
AA	--	--	--	--
AT	2.03	0.97	0.30, 13.66	0.468
TT	3.56	47723.36	0.00	1.000
rs3399437				
TT	--	--	--	--
TC	0.21	1.29	0.02, 2.60	0.222
CC	0.00	40192.97	0.00	0.999
rs501078				
CC	--	--	--	--
CT	0.48	1.66	0.02, 12.48	0.662
TT	0.00	25730.22	0.00	0.999
rs15101588				
GG	--	--	--	--
GA	0.37	1.17	0.04, 3.71	0.399

AA	0.24	1.48	0.01, 4.41	0.3440
Sex				
Male	0.97	1.40	0.11, 8.25	0.974
Female	--	--	--	--
Age (at presentation)	0.99	0.05	0.91, 1.00	0.989
Glaucoma type				
POAG	0.95	1.27	0.08, 11.45	0.968
NTG	--	--	--	--
Baseline IOP	0.72	0.15	0.54, 0.96	**0.024**
Central corneal thickness	1.02	0.01	0.99, 1.04	0.280
Mean deviation				
HFA	1.01	0.07	0.88, 1.15	0.923

P<0.05 based on univariate logistic regression. OR: odd ratio, CI: confidence interval, POAG: primary open angle glaucoma, NTG: normal tension glaucoma, OHT: ocular hypertension, IOP: intraocular pressure. The goodness of fit of this model was checked using the Hosmer-Lemenshow test; p=0.738. This result gives no evidence of lack of fit of the model.

Table 4.15: Factor affecting response to topical latanoprost 0.005% in Chinese glaucoma patients

Predictors	**OR**	**SE**	**95% CI for OR**	**p-value**
rs686262				
AA	--	--	--	--
AG	9.16E6	24854.65	0.00	0.999
GG	3.03E4	50218.26	0.00	1.000
rs3766332				
AA	--	--	--	--
AT	0.00	11.18	0.00, 1008.68	0.180
TT	0.00	24.97	0.00,2.45E18	0.792
Sex				
Male	1.31	2.19	0.02, 96.20	0.903
Female	--	--	--	--
Age (at presentation)	0.84	0.19	0.58, 1.20	0.334
Type of glaucoma				
POAG	5.10E10	16989.57	0.00, 0.00	0.999
NTG	--	--	--	--
Baseline IOP	0.10	1.75	0.00, 3.16	0.102

Central corneal thickness	0.91	0.09	0.76, 1.09	0.304
Mean deviation HFA	2.59	0.82	0.52, 12.93	0.246

P<0.05 based on univariate logistic regression. OR: odd ratio, CI: confidence interval, POAG: primary open angle glaucoma, NTG: normal tension glaucoma, OHT: ocular hypertension, IOP: intraocular pressure. The goodness of fit of this model was checked using the Hosmer-Lemenshow test; p=0.990. This result gives no evidence of lack of fit of the model.

Table 4.16: Stepwise logistic regression on factor affecting response to topical latanoprost 0.005% in both Malay and Chinese glaucoma patients

Predictors	**OR_c**	**OR_r**	**SE**	**p-value**	**95%CI**
rs686262					
AA	--	--	--	--	--
AG	1.49	1.34	0.79	0.712	0.28, 6.36
GG	6.19	6.31	0.81	**0.023**	1.28, 31.02
Baseline					
IOP	0.76	0.78	0.77	**0.001**	0.67, 0.90
CCT	1.01	1.01	0.01	0.102	1.00, 1.03

P<0.05 based on stepwise logistic regression. OR: odd ratio, CI: confidence interval, IOP: intraocular pressure.

The goodness of fit of this model was checked using the Hosmer-Lemenshow test; p=0.193. This result gives no evidence of lack of fit of the model.

4.4 Discussion

4.4.1 Pressure lowering effect of timolol and *ADRB2*

ADRB2 has been extensively studied in many diseases. The potential effect of *ADRB2* and drugs response has also been studied particularly bronchodilators (Munakata et al, 2006; Weir et al, 1998; Tsai et al, 2006). 46A/G and 76C/G have been implicated to be responsible for agonist induced receptor desensitization or downregulation, with 46A enhanced down-regulation and 76C reduced down-regulation in beta agonist drugs (Green et al, 1994; Moore et al, 2000). However, the association between *ADRB2* and drug response is inconclusive. It is believed to be due to ethnic variation in allele frequency of *ADRB2* (Xie et al, 2000; Lee et al, 2004; Brodde, 2008).

Topical Timolol XE 0.5% provides reasonable pressure lowering effect in this study. However, only 50 glaucoma patients (51.5%) are still treated with topical timolol monotherapy at the end of the study protocol. 76CC and -47TT are significantly associated with higher mean baseline IOP. Glaucoma patients with 76CC demonstrated higher mean IOP at 1 and 3 months post-treatment. 76C/G was found to associate with higher IOP and younger age at presentation in Japanese glaucoma patients (Inagaki et al, 2006).

Genetic variation of 76C/G was found to be reasonably convincing in reduction of bronchodilator response in the asthmatic patients (Munakata et al, 2006; Weir et al, 1998; Tsai et al, 2006). The impact of 76C/G in hypertension and cardiovascular diseases is not as convincing as in agonist response in asthmatic patients. Similarly 76C/G has not been identified as potential pharmacodynamic candidate gene in glaucoma management. Instead 76C/G was found to be associated with higher likelihood to achieve ≥20% reduction from baseline with topical beta blockers (McCarty et al, 2008). There was significant association

between glaucoma patients carrying the 76C and achieving clinical significant IOP reduction. In fact, those with 76C have 2-fold (95%CI 1.00, 4.02) more likely to achieve clinical significant IOP reduction in this population based study involving Caucasians (McCarty et al, 2008).

McCarty et al (2008) conducted a population based study but the phenotype data were obtained retrospectively that may lead to various biases especially on the accuracy of obtaining IOP measurement. In addition the specific topical beta blockers were not mentioned. It is perhaps possible, although unlikely, that those who were 76GG or 76CG were treated with topical betoptic, a cardioselective beta blocker that is known to be less potent compared to timolol in reducing the pressure. Moreover, the definition of clinical significant IOP reduction at fixed or absolute figure (20% reduction) may not clinically represent the effectiveness of the drug. Thus, we used the IOP reduction over the time period in this study as a more clinically relevant endpoint. Prospective study on the pressure lowering effect of timolol as monotherapy allows observation of long term effect of timolol.

Ethnic variation of allele frequency may also play an important role in explaining the difference between our study and McCarty et al (2008). For example, 76C is considered as wild type or common variation in Asian population (Xie et al, 2000; Lee et al, 2004) but an alternative variation in Caucasian population (Tomaszewski et al, 2002; McCarty et al, 2008). On contrary, there was no association between pressure lowering effect of timolol on healthy volunteers at 8 hours post instillation and *ADRB2* in Caucasians residing in Europe (Fuchsjager-Mayrl et al, 2005). Pressure lowering effect of timolol in healthy volunteers may not be the same as that seen in glaucoma patients with higher baseline IOP (Katz et al, 1976; Boger et al, 1978). IOP reduction at 8 hours post instillation may not be ideal or reflective of

the actual effect of timolol due to up and down regulation of the ADRB2 receptors over a longer period of time. The role of *ADRB2* as pharmacodynamic gene in glaucoma management remains elusive. There are various other factors that may affect the pressure lowering effect of timolol including pharmacokinetic gene such as *CYP2D6.*

4.4.2 Pressure lowering effect and *PTGFR*

Topical latanoprost is one of the first prostaglandin analogs introduced to the glaucoma pharmaceutical market. It has been proven effective in various populations and almost all type of glaucoma (Zhang et al, 2001). The variation of response has also been reported (Scherer, 2002; Cheong et al, 2008). Latanoprost provided better pressure lowering effect in Asians and Mexican as compared to other population (Hedmann and Larsson, 2002).

To date, latanoprost and bimatoprost have been found to only activate in cell and tissue that express functional FP receptors (Woodwards et al, 2007; Liang et al, 2003). It is postulated that genetic variation of *PTGFR* may influence the pressure lowering effect of latanoprost. It was found that in homozygous mutant FP knock-out mice, latanoprost do not lower the pressure (Crowston et al, 2004). However, minimal pressure lowering effect was observed in heterozygous knock-out mice (Crowston et al, 2004). The minimal IOP reduction in heterozygous knock-out mice is probably due to reduction of transcription of FP mRNA. Larger pressure lowering effect was observed in homozygous wild mice (Crowston et al, 2004).

Sakurai et al (2007) found that *PTGFR* variation was associated with short term response to latanoprost in normal healthy Japanese volunteers. SNP at the promoter region, rs373380 and rs3766355 at introns 1 of *PTGFR* was found to be associated with lower percentage of

pressure reduction (Sakurai et al, 2007). It was then postulated that these SNPs may be responsible in downregulation of *PTGFR* expression. In the present study, these two SNPs were also identified in Malaysian population. However, there was no significant association of these SNPs with pressure lowering effect of latanoprost. Based on our pilot study screening the *PTGFR* in Malaysian population, rs3766332 was identified as novel SNP in Malaysian population (Hoh et al, 2007). Upon completion of this study, we found that the minor allele frequency rs376632 may predisposes to good respond to topical latanoprost but without statistically significant association (p=0.067). However, the presence of the minor allele of rs686262G in the homozygous state of rs686262GG was found to predispose to poor respond to latanoprost up to 6.3fold (95% CI 1.3, 31.0) in the present study.

Based on the Fisher's model for single QTL, seven SNPs in the introns 3 were identified to have potential dominant effect; rs4650581, rs34012237, rs3766335, rs2146490, rs33966768, rs6543738 and rs675351117. Interestingly, these SNPs were positioned quite closed to each other. The different between the present study and Sakurai et al (2007) could be due to the ethnic difference that resulted in different allele frequency. McCarty et al (2011) attempted to replicate the study by Sakurai et al (2007) as part of the Marshfield Clinic Personalized Medicine Research Project but failed to find significant association of rs373380 and rs3766355 to responsiveness to topical prostaglandin analogs.

In addition, the difference in phenotypic characteristics may potentially affect the findings. The present study was conducted on glaucoma patients that may result in higher reduction of IOP. Greater reduction of IOP has been observed in POAG patients treated with latanoprost as compared to NTG patients, which is probably explicable on the basis of higher baseline IOP. The pressure reduction in normal healthy volunteers may differ from diseased patients

(Camras et al, 1992, Alm and Villumsen, 1991). Moreover, the present study conducted a longer duration of observation as compared to the earlier study. The possibility of short term escape or long term drift may be addressed. As latanoprost acts on receptor, similar up and down regulation of the receptor may occur as it has been reported with long term treatment with topical beta blocker (Boger, 1983). However, adherence is an important issue associated with long term treatment.

Earlier study by Sakurai et al (2007) defined the phenotype based on specific cut off point of IOP reduction without considering the time effect. In this present study, the data was also analysed using cut off point. Higher cut off point was adopted in this present study (25% reduction) compared to previous studies (Sakurai et al., 2007; McCarty et al., 2011). Perhaps, lower cut off point will give different outcome.

PTGFR is potentially an important determinant of variability of response to latanoprost in Malaysian population. However, how the identified SNPs caused change in the *PTGFR* product structure and function is not known, especially most of the SNPs were found in the intronic region. The variant in *PTGFR* has been reported in human ciliary body with at least six different isoforms (Liang et al, 2008). There is also the possibility of interaction of multiple genes involved in pathway of mechanism of action of topical latanoprost.

CHAPTER 5

Discussion

Chapter 5

Similar to essential hypertension, glaucoma is a chronic disease. According to the mechanical theory, glaucoma results in glaucomatous optic neuropathy due to direct injury caused by increased intraocular pressure. The vascular theory explains the possibility of reduced blood supply to the optic nerve head due to impaired autoregulation, microvascular insult, and atherosclerosis; similar mechanisms have been postulated for systemic hypertension (Flammer et al., 1999; Flammer et al., 2007). Both essential hypertension and glaucoma are complex diseases, and both genetic and environmental factors (such as corticosteroid consumption and trauma) are responsible for the disease onset (Langman et al., 2005). Complex disease is often associated with multiple other factors and display genetic predisposition but without specific pattern of inheritance. However, the relationship between essential hypertension and glaucoma is still inconclusive. The current mainstay of treatment for essential hypertension is similar to that of glaucoma: pressure reduction to minimise target organ damage. Retardation of further nerve fibre layer damage is the main aim of glaucoma therapy. Although it is well established that intraocular pressure (IOP) is the only modifiable risk factor, pressure reduction does not confer a protective effect against further nerve fibre damage in certain patients.

Topical pressure-lowering drugs are an effective, non-invasive treatment for glaucoma. Similar to other systemic drugs, variations have been reported in the response to topical pressure-lowering drugs between different populations and within the same population (Otaleju and Ajayi, 1999; Higginbotham et al., 2002; Netland et al., 2001; Piltz et al., 2000; Scherer, 2000). Variations in the response to topical ophthalmic drugs were first observed in 1971 with the topical mydriatic drugs topical epinephrine 4% and homatropine 4% (Emiru,

1971). At similar drug concentrations, a longer duration of treatment was required to achieve mydriasis in Africans than in Caucasians (Emiru, 1971; Salminen et al., 1985). Ethnic influence is an intriguing issue in drug response variation. Higher amount of pigment, particularly in the iris, has also been implicated in drug response variation in certain populations (Otaleju and Ajayi, 1999; Higginbotham et al., 2002; Netland et al., 2001; Piltz et al., 2000). Genetic variation is believed to be responsible for both individual and population variations.

5.1 Topical timolol and *ADRB2*

5.1.1 Pressure lowering effect of topical Timolol XE 0.5%

5.1.1.1 Mean IOP reduction

Gellan gum, a special ingredient in timolol gel-forming solution (GFS), is a purified anionic heteropolysaccharide that turns into gel once it comes in contact with divalent cations in the precorneal tear film (Shedden, 1994). Ocular bioavailability of timolol GFS was nearly 4-fold higher than that of the aqueous solution in albino and pigmented rabbits (Rozier et al., 1989). Prolongation of ocular bioavailability reduces the instillation frequency to once daily without reducing efficacy. Theoretically, it also reduces systemic absorption and minimises the systemic side effects of non-selective topical beta-blockers (Dickstein and Aarsland, 2001; Uusitalo et al., 2001).

Timolol GFS was found to provide almost similar pressure reduction as the aqueous solution in healthy volunteers and glaucoma patients, with slightly better pressure-lowering effect in glaucoma patients (Roselund, 1996; Shedden et al., 2001). Timolol GFS was found to produce 1–2 mmHg more hypotensive effect than the aqueous solution (Laurence et al., 1993). The greater ocular hypotensive effect of the GFS was best observed between 4–8

hours post-instillation compared to aqueous solution (Laurence et al., 1993). However, timolol in aqueous solution showed better pressure-lowering effect than the GFS at 24 hours post-instillation (Laurence et al., 1993). This study reported short-term observations on the effectiveness of timolol GFS.

A 3-month multicentre control trial conducted on 223 Caucasian patients showed no significant difference between the pressure-lowering effects of the GFS and aqueous solution (Roselund, 1996). Superiority of the timolol GFS was also not observed in a longer study (Shedden et al., 2001). However, most studies were conducted on Caucasians. Patients with pigmented irises required a higher concentration of timolol in the aqueous solution to achieve the same effect as patients with less iris pigmentation (Otaleju and Ajayi, 1999; Ong et al., 2005; Katz and Berger, 1979). The amount of melanin is believed to be responsible for the variation in topical timolol responses. Reversible binding of the active ingredient in timolol to melanin is believed to reduce the effectiveness of timolol (Menon et al., 1989; Katz and Berger, 1979). This interaction is also believed to act as a slow release depot to provide longer pressure-lowering effects. Because GFS prolongs ocular bioavailability, perhaps it also improves the effectiveness of timolol in pigmented iris individuals, particularly Asian patients.

However, limited literature is available on the effectiveness of timolol GFS in Asian patients. In a retrospective review involving 76 Asian glaucoma patients treated with timolol GFS monotherapy, the mean IOP reduction was 5.7 (standard deviation [SD] 5.3) mmHg with a mean percentage of reduction was 23.1% over 12 months of treatment (Ong et al., 2005). This study revealed that the morning dosing of timolol GFS produced a significantly better pressure-lowering effect than the night dosing; the morning dosing provided twice the

percentage of IOP reduction compared to the night dosing (Ong et al., 2005). Based on this finding, morning dosing was used in the current prospective study.

The mean IOP reduction of timolol GFS (5.4 [SD 5.1] mmHg) in our study was almost similar to that of the retrospective study conducted by Ong et al. (2005). However, our calculation was based on the difference between baseline IOP and summation of IOP measurements over the follow-up period, whereas the study conducted by Ong et al. (2005) was based on the difference between IOP at 12 months of treatment and baseline IOP. Schenker et al. (2000) conducted a similar long-term study over a 12-month period to compare Timolol XE and timolol maleate GFS. The mean IOP reduction from baseline in their study ranged from 4.1 mmHg to 5.3 mmHg in glaucoma patients treated with Timolol XE 0.5% (Schenker et al., 2000).

The mean IOP reduction from baseline in our study ranged from 6.1 mmHg to 6.8 mmHg, which is higher than the studies conducted by Schenker et al. (2000) and Shedden et al. (2001a) on mixed populations comprising a majority of Caucasians. The mean IOP reduction in a multicentre, double-masked, 6-month trial on Timoptic XE® 0.5% in a mixed population comprising a majority of Caucasians ranged between 6.0 and 6.4 mmHg at peak (11 am) and 5.6 and 5.9mmHg at trough (9am)(Shedden et al., 2001a). An equal distribution of glaucoma patients with pigmented iris and lightly pigmented iris were recruited. In our study, IOP was taken between 9am to 12noon, majority were taken between 10 and 11am. IOP was obtained only once without considering the fluctuation of pressure. If the baseline IOP is taken at trough and subsequent IOP is taken at peak or vice versa, the mean IOP reduction from baseline will differ. There is a possibility of skewness towards pressure taken at peak rather

than trough, giving better pressure reduction compared to previous studies (Schenker et al, 2000; Shedden et al, 2001a).

The lack of timolol efficacy in individuals with pigmented iris, as in the Malaysian population, was thought to be due to the affinity of timolol for melanin (Salazar-Bookaman et al., 1994; Menon et al., 1989). Based on the observations in pigmented rabbits, the duration of action of timolol GFS was longer than that of the aqueous solution at similar concentration (Ohno et al., 2001). There was no evidence of increased beta-receptor occupancy rate in the timolol GFS group compared to the aqueous solution group (Ohno et al., 2005). Thus, it is unlikely that the binding affinity of timolol GFS for the beta receptor is responsible for the better pressure-lowering effect in our study. Longer retention of timolol GFS in the ocular cul-de-sac may counter the timolol-melanin binding effect and thereby improve the efficacy of timolol as a net effect in our study. Reversible binding of the drug to melanin in individuals with highly pigmented irises is believed to be responsible for the greater pressure-lowering effect of timolol. Topical timolol in aqueous solution was reported to elicit up to a 30% pressure reduction from baseline in Caucasians. However, 31 patients in our study demonstrated a good response to topical timolol GFS with up to 30% and more pressure reduction from baseline. Perhaps, melanin is not entirely responsible for the variation in timolol responses.

Oral-ophthalmic drugs interaction has been reported in patients treated with oral quinidine and cimetidine with topical beta blocker (Edeki et al, 1995; Ishii et al, 2000). Glaucoma is more prevalent among elderly. Thus systemic co-morbidities such as hypertension are not uncommon among glaucoma patients. Salim and Shields (2010) found that 73% of glaucoma patients were also hypertensive in a retrospective record review study. In our present study,

more than half of our glaucoma patients were also hypertensive. Inadvertently, systemic medications may affect the effectiveness of topical Timolol XE 0.5%. In our setting, the main first line systemic antihypertensive medication is calcium channel blocker such as Amlodipine. In most developing countries, due to cost effectiveness, systemic beta blockers are the main first line antihypertensive medication. Our study was conducted in a teaching hospital, where many clinical trials have been conducted and more expensive drugs were available.

There was no evidence of the effect oral calcium channel blockers on pressure lowering effect of timolol when given concomitantly in healthy Japanese volunteers (Yatsuka et al., 1998). However, topical calcium channel blockers such as diltiazem and verapamil have potential effect as pressure lowering drug in animals and human (Shayegan et al., 2009; Siegner at al., 2000). Systemic beta blockers such as metoprolol are believed to reduce the efficacy of topical timolol and increase the risk of bradycardia (Schuman JS., 2000). Based on the available data of General Practice Research Network database in Australia, 20% of glaucoma patients were co-prescribed with systemic beta-blockers (Goldberg and Adena, 2007). Systemic beta blockers were co-prescribed in 18.2% (10 patients) of our recruited glaucoma patients. However, there was no significant effect of systemic beta blockers on pressure lowering effect of topical Timolol XE 0.5%. Higher mean IOP reduction was observed in those who were on oral beta blockers (6.5 [3.7] mmHg) compared to oral calcium channel blockers (4.3 [3.8] mmHg).

5.1.1.2 Long term efficacy of topical Timolol XE 0.5%

Some patients had demonstrated a lack of IOP reduction after good IOP control during the treatment period. This phenomenon is known as ‘long-term drift’, and was first coined by

Boger et al. (1978), who observed that timolol lost its effectiveness in some patients who had previously demonstrated good reduction in pressure over a long period. Long-term drift is believed to be due to the down-regulation of beta-adrenergic receptors in the eye (Boger et al., 1978). The pattern of mean IOP in our study did not reveal any evidence of long-term drift. In fact, the mean IOP reduction from baseline was greatest at the final follow-up measurement. However, based on the individual mean IOP reduction patterns, there was evidence of long-term drift and short-term escape in some patients. The disappearance of these effects in the final 12-month IOP patterns could be due to the fact that patients were dropped from the study when they failed to reach target pressure. Due to this selectivity, only those who demonstrated a good response to timolol GFS were analysed at the end of the study.

Target pressure was individualised according to the severity and type of glaucoma in our study. Individualisation of target pressure represented the actual clinical setting. At the same time, it was a source of bias due to lack of standardisation in this study. In general, based on the mean deviation (MD) of the Humphrey visual field analysis (HFA), a majority of the recruited patients were in the severe stage (mean MD of -11.51dB [8.82]). Thus, a more stringent reduction in target pressure (30–50% reduction from baseline IOP) was adopted resulting in additional drug being added to the topical Timolol XE monotherapy treatment. A majority of our ‘drop outs’ from follow-up were not due to the lack of effectiveness of Timolol XE but more due to not reaching the target pressure.

At the end of the 12-month treatment, only 51.5% completed the entire monotherapy treatment. Schenker et al. (2000) found that only 71% achieved a clinically relevant response, predetermined as >5 mmHg IOP reduction from baseline or at least 21 mmHg IOP reduction.

Based on our findings, Asians patients treated with timolol GFS exhibited a better pressure-lowering effect but failed to sustain its effectiveness through the long duration of treatment. A 7-year prospective study on glaucoma patients treated with topical timolol aqueous solution, betaxolol, and carteolol in the United Kingdom found that less than half the patients were able to sustain the monotherapy treatment for 5 years (Watson et al., 2001). At 12 months, 82.3% patients were still on monotherapy treatment. The main reason for withdrawal was inadequate pressure-lowering effect regardless of target pressure (Watson et al., 2001). Similarly, in our current study, failure to achieve target pressure was the main reason. The more stringent individualised target pressure adopted in our study was perhaps responsible for the lower proportion of our patients completing the 12-month monotherapy treatment. Due to selectivity based on target pressure, the lowest mean IOP (14.8 [SD 3.5] mmHg) with the highest percentage of reduction from baseline (27.8 [SD 18.7] %) was recorded at the 12-month follow-up.

Lower target pressure was advocated for patients with more advanced glaucoma. Thus, those with more advanced vertical cup to disc ratio (CDR) and visual field defects were found to be less likely to complete the 12-month monotherapy treatment with Timolol XE in our study. Aggressive treatment and lower target pressure were advocated for advanced glaucoma to halt further nerve fibre damage. A higher mean baseline IOP was also found to be a predictor for failure to complete the 12-month monotherapy treatment. As expected, based on the mean IOP reduction in our study, a higher IOP may need more than a single therapy to achieve target pressure. Adherence and persistence were also related to the pressure-lowering effect of timolol in our population. However, we did not calculate the adherence or persistence in this study. Weighing the medication bottles, teaching proper instillation, and the counselling

conducted in our clinic were believed to be able to ascertain the adherence of our recruited patients.

A higher baseline IOP is theoretically associated with a higher IOP reduction once treatment is advocated (Rulo et al., 1996). Factors that affect the baseline IOP are theoretically useful in predicting subsequent IOP control during the follow-up period. Collaborative Initial Glaucoma Treatment Study (CIGTS), a prospective, multicentre cohort study conducted on 607 newly diagnosed patients with open angle glaucoma found that age, pseudoexfoliative glaucoma (type of open angle glaucoma), women, and the presence of positive relative afferent pupillary defects were significantly associated with baseline IOP (Musch et al., 2008). Multivariate analysis was also conducted to identify predictors that affect the baseline IOP of the recruited patients in our study. Age, sex, race, type of glaucoma, central corneal thickness (CCT), and systemic co-morbidities, including hyperlipidemia, were included as possible predictors in our model.

Normal tension glaucoma (NTG) was identified to be associated with significantly lower baseline IOP than primary open angle glaucoma (POAG). The baseline IOP for NTG was 8 - fold lower than that of POAG (95% confidence interval [CI]: -10.1, -6.7). The pressure-lowering effect of Timolol XE on NTG was found to be significantly lower than that on POAG based on repeated measure (RM) analysis of variance (ANOVA) analysis of the 12-month follow-up measurements. Patients with NTG experienced a mean IOP reduction of 2.0 mmHg from baseline (95% CI: 2.3, 3.7 mmHg) at 1 month compared to 7.0 mmHg (95% CI: 5.4, 8.5 mmHg) in POAG patients. The highest mean IOP reduction of 3.5 mmHg (95% CI: 2.6, 4.4 mmHg) or 21% reduction from baseline in NTG patients was seen at the 12-month follow-up. Our IOP reduction percentage was much lower than that advocated by the

Collaborative Normal Tension Glaucoma Study Group (1998). The group recommended a 30% IOP reduction to prevent further visual field loss. A meta-analysis conducted on 15 publications on common topical pressure-lowering drugs prescribed for NTG patients found that topical timolol aqueous solution provided mean peak and trough IOP reductions of 2.4 mmHg (95% CI: 2.0, 2.8 mmHg) and 3.0 mmHg (95% CI: 1.7, 4.3 mmHg), respectively, from the baseline (Cheng et al., 2009). Even though we did not include the peak or trough IOP, the mean IOP reduction by timolol GFS in our study was almost similar to the mean peak in a previous study conducted by Cheng et al (2009). Thus, baseline IOP is a good predictor for determining the subsequent IOP reduction during the follow-up period.

Based on univariate analysis, glaucoma patients without hyperlipidemia showed significant higher mean baseline IOP than those with hyperlipidemia in our study. However, RM ANOVA revealed no significant differences between glaucoma patients with and without hyperlipidemia. Glaucoma patients with hyperlipidemia demonstrated more stable pressure reduction than those without hyperlipidemia. All our hyperlipidemia patients were treated with statins, the mainstay of treatment for hypercholesterolemia. Statins are known to increase cerebral circulation in patients with cerebrovascular disease (Vaughan and Delanty, 1999). Statins also inhibit rho kinase activity, which increases the aqueous outflow and lowers IOP (Rao et al., 2001). NTG patients treated with simvastatin for hyperlipidemia have a 60% reduction of risk for further visual field progression (Leung et al., 2010). Leung et al. (2010) also reported that patients on simvastatin exhibited slightly lower IOP that was not statistically significant. Confocal scanning laser polarimetry in glaucoma suspects has shown that statins retard the progression of structural damage to the optic nerve (de Castro et al., 2007).

5.1.1.3 Side effects of topical Timolol XE 0.5%

Two patients without any known risks of respiratory disease developed symptoms suggestive of respiratory impairment, and Timolol XE was discontinued. Prolonged ocular bioavailability reduces systemic absorption and partially protects against systemic side effects (Dickstein and Aarsland, 1996). The plasma concentration of timolol GFS was found to be lower than that of the aqueous solution; however, neither form of medication exceeded 1 ng/mL (Shedden et al., 2001b). The 1 ng/mL plasma concentration of timolol was found to be more likely to induce systemic side effects. In addition, our strict selection criteria excluding those with respiratory and cardiovascular co-morbidities further reduced the potential systemic side effects.

Diggory et al. (1994) found that timolol caused respiratory function impairment even in patients without any history of reversible airways disease. In fact, the affected patients were asymptomatic. His findings were later challenged for the absence of a control group, and the much lower values of the respiratory functions FEV_1 and FVC suggest the possibility that patients with undiagnosed respiratory disease were recruited in his study. Betaxolol and timolol in aqueous solution were included in this early report on respiratory impairment in elderly glaucoma patients. Timolol GFS was not available at that time. Stewart et al. (2001) found no significant difference in $FEV_{1,}$ FVC, and FEV_1/FVC in elderly glaucoma patients treated with timolol GFS and aqueous solution. The effects of timolol GFS on heart rate and blood pressure did not differ significantly from those of the aqueous solution (Stewart et al., 1999).

5.1.2 *ADRB2* and susceptibility to glaucoma

ADRB2 gene (*ADRB2)* has been implicated in various diseases and responsiveness to various beta-blocker agonists (Kotanko et al., 1997). The main purpose of the present study was to evaluate the effect of *ADRB2* polymorphism on the responsiveness to timolol GFS. The potential role of *ADRB2* as the susceptibility gene for glaucoma needs to be assessed. Beta 2 adrenoreceptors (ADRB2s) have been implicated in the regulation of aqueous humour formation and outflow (Trope and Clark, 1982; Nathanson, 1981; Erickson-Lamy and Nathanson, 1992). ADRB2s are also present in the blood vasculature of the ciliary process (Wax and Molinoff, 1987). ADRB2s were expressed at moderate-to-high levels in transected optic nerves in humans and were not expressed in areas with dead astrocytes (Mantyh et al., 1995). There is a strong possibility that the *ADRB2*, which governs the functionality of ADRB2, is responsible for the pathogenesis and susceptibility to glaucoma via IOP regulation, disruption of perfusion to the optic nerve and ciliary process, or direct effect on nerve fibre damage.

Five important single nucleotide polymorphisms (SNPs) of *ADRB2*; 46A/G, 79C/G, 491C/T, -20T/C and -47T/C, were examined in the present study because of their functional importance in the alteration of receptor function and receptor expression at the translational level (Scott et al., 1999; Green et al., 1994; Liggett, 1997). Reports on the potential role of *ADRB2* as a susceptibility gene in glaucoma are limited. Three SNPs, 46A/G, 79C/G, and 491C/T, were studied in POAG and primary congenital glaucoma in a Turkish population (Güngör et al., 2003). However, no association was found between *ADRB2* and susceptibility to glaucoma (Güngör et al, 2003). Similarly, *ADRB2* is not a susceptibility gene for POAG in Caucasians and African Americans, as revealed by individual SNP (46A/G and 79C/G) and haplotypes analyses (McLaren et al., 2007). Another study by Inagaki et al. (2006) found no

association between *ADRB2* polymorphisms (46G/A and 79C/G) and POAG in a Japanese population. Despite this negative association, Inagaki et al. (2006) found that POAG diagnosis was made at a younger age in patients with 46AG and 46GG. 79CG and 79GG were associated with higher IOP compared to 79CC. *ADRB2* exerts an influence on POAG in Japanese patients through the endophenotype of glaucoma.

In our study, we found no significant difference in the allele and genotype frequencies of *ADRB2* between glaucoma patients and control subjects. However, based on more robust statistical analysis; stratified meta-analysis shown that 79C/G and -20T/C is a potential susceptibility locus for POAG in the Malaysian population. Our finding contradicts the previous studies conducted in various other populations (Güngör et al., 2003; McLaren et al., 2007; Inagaki et al., 2006). The frequency of the 79C/G genotype in the Malaysian population was almost similar to the reported frequency in Chinese and Japanese populations (Xie et al., 1999; Inagaki et al., 2006). The frequencies of the common *ADRB2* SNPs markedly vary with the population (Xie et al., 1999). Xie et al. (1999) found that the frequency of 79GG was lower in healthy Chinese volunteers than in Caucasians and African Americans.

The Malaysian population involved in the present case-control study is an admixture of individuals of Malay and Chinese descent. The difference in *ADRB2* SNP frequencies between different populations is the main cause of the inconsistent findings in case-control association studies on systemic diseases, such as essential hypertension (Kotanko et al., 1997; Kato et al., 2001; Xie et al., 2000). There is a possibility that the outcome of our case-control association study was influenced by population genetic variation rather than direct association with the disease.

Genomic control analysis addressed the cryptic relatedness and population heterogeneity between affected individuals and selected normal control subjects by calculating the inflation factor, λ (Devlin and Roeder, 1999). Inflation factor is best derived from at least 30 unrelated markers. Thus, it is not feasible to obtain the inflation factor for *ADRB2* in the present study due to limited SNPs and several SNPs were reported to be in linkage disequilibrium (Dewar et al, 1998; Drysdale et al, 2000). Stratified meta-analysis on 5 codons of *ADRB2* in the present study found no significant heterogeneity between the Malays and Chinese.

SNPs at the UTR of *ADRB2* were also examined in our study. A significant difference was observed in the -20T/C and -47T/C genotype frequencies between glaucoma patients and control subjects. Unfortunately, -47T/C and 46A/G violated the Hardy-Weinberg Equilibrium (HWE) and were excluded from haplotypes analysis. The violation of HWE persists even after the analysis was conducted according to sub-population; Malays and Chinese. There was also no significant difference between Malays and Chinese based on Breslow-Day test of heterogeneity in stratified Mantel-Haenszel meta-analysis. It therefore appears unlikely that population stratification is responsible for the departure from HWE.

The most common cause of departure from HWE is genotyping error (Hosking et al, 2004). The single tube multiplex PCR method adopted in our study is inexpensive, fast, and a relatively reliable technique. However, contamination may affect its accuracy (Zilfalil et al., 2006). We repeated the test more than once for samples with non-satisfactory, unreliable, or suspected contaminated gel electrophoresis results. To further ascertain the results, sequencing was also performed for a majority of samples. The relatively small sample size may also have contributed to the departure from HWE. *ADRB2* screening was conducted on

197 samples in the present study. Although we reached the targeted sample size with 80% power, it was still considered relatively small. There could be other biological effects, which are not known yet. For example the SNPs could be associated with other conditions that are not tested in this present study.

Other possible reason is the presence of copy number variations (CNVs). CNVs are structural variations resulted from errors during mitosis and meiosis, causing duplications and deletions of large genomic segment that differ from reference sequence. CNVs are found in 12% to 15% of human genome and 56% of CNVs are found within known gene (Redon et al., 2006; Iafrate et al., 2004). There is the possibility that SNPs may fall in the CNV region. Lee et al. (2008) conducted Bayesian analysis to study the potential effect of CNV on the behaviour of SNPs. They concluded that violation of HWE could be due to the SNP falls in the CNV region (Lee et al., 2008). Xu et al. (2011) reported common and rare CNVs in 3 main ethnic groups in Singapore; Chinese, Malays and Indians. There is ethnic difference in distribution of CNVs in these 3 main ethnic groups.

Vine and Curtis (2009) contributed marked departure from HWE in genome wide association involving 463842 markers from 1504 British subjects to gene harbouring embryonic survival. However, our study was unable to rule out these possibilities. In addition, meta-analysis study conducted on 72 primary gene –disease studies found that only 46 studies reported HWE violation (Minelli et al., 2007). It is suggested no benefit to exclude the findings from these studies unless there is valid ground for rejection (Minelli et al., 2007; Trikalinos et al., 2006). Moreover, it was found that excluding the SNP that violate HWE does not alter the common odd ratios of other SNPs in case-control association studies (Trikalinos et al., 2006). Any

finding including statistically significant association derived from 46A/G and -47T/C in the present study were excluded or ignored.

The frequency of non-synonymous SNP at position 491 (491C/T) is rather low in the Asian population. There was no variation from the homozygous for the common allele of 491C/T in our population. Heterozygous form of 491C/T was considered a minor allele in our study population. The homozygous 491TT (Ile164) exhibited extensive signalling defects in an in vitro study (Green et al., 1993). Güngör et al. (2003) reported only the presence of homozygous for the common allele and heterozygous form of 491C/T in both glaucoma patients and control subjects. The 491CT (Thr164Ile) variant is very rare, occurring in 2–4% of all populations (Small et al., 2003). Ligget et al (1998) found that heterozygous form 491C/T was associated with significant poor survival and increased the needs of heart transplant in patients with congestive heart failure. Perhaps due to rather small sample size and selection bias, the heterozygous form of 491C/T was not found in the current study.

It is also known that SNPs in the 5′-leader cistron and the coding region are linked and form specific haplotypes. Haplotypes allow genotype combinations to produce cumulative effects on the phenotype. Strong linkage disequilibrium in *ADRB2* was observed between 79G and -47T resulted in subjects homozygous for the most common allele for -47T, almost all are also homozygous for 46G/A (Small et al, 2003). The most common haplotypes was -47T46G79G491C (Arg19Gly16Glu27Thr164). Haplotypes frequency varies with the population. Linkage disequilibrium reduces the need to study all the functional SNPs of *ADRB2*, because the SNP variations can be predicted with some certainty from the linkage disequilibrium. In the present study, haplotypes analysis was only conducted on -20T/C and

79C/G. 46G/A and -47T/C were excluded due to violation of HWE. Non-polymorphic Thr164Ile was also excluded.

ADBR2 screening of peripheral blood leukocytes from the Malaysian glaucoma patients and control subjects suggested that -20T was associated with a 1.7-fold difference (95% CI: 1.1, 2.7) in the susceptibility to glaucoma. 79G confers potential protective effects against susceptibility to glaucoma. These two SNPs were in 84% linkage disequilibrium. 79C/G is resistant to agonist-promoted down-regulation (Liggett, 1997). A higher concentration of isoprenaline was needed to down-regulate the 79GG to achieve similar results as the 79CC in human airway smooth muscle (HASM) cells (Green et al., 1995). Locally applied isoprenaline produced a larger increase in forearm blood flow and vein dilatation in subjects with 79GG (Dishy et al., 2001). Aqueous humour formation is believed to be regulated by the adrenergic system, particularly ADRB2. The production of aqueous humour is related to activation of adenyl cyclase and synthesis of cAMP from ATP. Ciliary process ADRB2 is stimulated by circulating agonists, such as catecholamine, epinephrine, and norepinephrine, during aqueous humour production, and the presence of antagonist drugs, such as timolol, reduces aqueous humour production (Nathanson, 1980).

Assuming that the functional alteration induced by genetic variants of *ADRB2* in blood leukocytes are similar to those in ciliary processes, the lower resistance of 79CC to agonist-promoted down-regulation presumably results in the maintenance of the aqueous humour production rate. Subsequently, if the outflow remains constant, production and outflow are in equilibrium and IOP is maintained within normal range. Presumably, there is also no change in blood flow in the ciliary processes, as ADRB2 also governs vessel activity. The end result is an absence of IOP elevation and a reduction in the risk of glaucoma. Perhaps, this

assumption helps to explain the protective effect of 79G in our POAG patients. 79G reduced the risk of POAG by 0.3-fold (95% CI: 0.1, 0.7; p = 0.005) but not the risk of NTG.

To the best of our knowledge, no studies have been conducted on the promoter region of *ADRB2* in glaucoma patients to date. The promoter region, including the beta upstream peptide (BUP), is believed to act as a translational inhibition system. SNPs in the promoter region have shown the potential to alter *ADRB2* expression (Scott et al., 1999).The functional role of -20T/C has not been well studied compared to -47T/C. The functionality of the SNPs in the promoter region is studied using luciferase reaction. Luciferase is used to report the transcriptional activity in certain cells that are transfected with a genetic construct containing luciferase gene under the control of the promoter of certain gene. Luciferase activity was significantly reduced in COS-7 cells transfected with -20C and -47C (Scott et al., 1999). -47 is located within a small open reading frame (spanning -102 to -42) that encodes a 19-amino acid polypeptide, which is thought to modulate the translation of *ADRB2* mRNA (Parola and Kobilka, 1994). -47C changes the amino acid from cysteine to arginine and causes down-regulation of receptor expression in ^{125}Iodine radioligand-binding experiments and luciferase assays (McGraw et al., 1998). In contrast, -47T/C and -367T/C were found to have no effects on *ADRB2* expression either as single polymorphisms or as haplotypes in human peripheral blood mononuclear cells derived from asthmatic patients (Lipworth et al., 2002). The effect of isoprenaline on cAMP regulation was also unaffected. The allele and genotype frequencies of -47T/C were significantly different between glaucoma patients and control subjects in our study. Allele frequency of -47C was higher in glaucoma patients, suggesting that alteration of ADRB2 expression may play a role in susceptibility to glaucoma. However, the role of -47T/C in susceptibility to glaucoma needs to be interpreted with caution in the presence of HWE violation.

Despite the evidence indicating a role for -20T/C in receptor expression in vitro, -20T/C does not alter known transcription factor-binding sites and is deemed functionless as an individual variant (Lipworth et al., 2002; Panebra et al., 2007). Moreover, there is no evidence for the involvement of -20T/C in the susceptibility to hypertension and risk of myocardial infarction in 2 large studies involving European-derived populations (Herrmann et al., 2002). Despite these negative associations, -20T/C demonstrated a strong association with glaucoma susceptibility in our study. -20T also increased the risk of NTG 2.0fold (95%CI 1.1, 3.7) in this present study. Variation in genotype frequency between races may contribute to this observation. Due to little emphasis on the promoter region of *ADRB2*, comparison with other populations was not possible.

Strong linkage disequilibrium was not only observed between SNPs in the promoter and coding regions but also within the promoter region. Johnatty and co-workers (2002) studied -468C/G, -367T/C, -47T/C and -20T/C, 4 important SNPs in the promoter region, and identified 2 haplotypes GCCT and CTCT that cause a 3-fold reduction in luciferase activity compared to the reference haplotypes (CTTT). They concluded that polymorphisms in the promoter region interact to alter *ADRB2* expression. The degree and direction of alteration was haplotypes-dependent with significant impact attributable to the -47C variant (Johnatty et al., 2002; Panebra et al., 2010). Haplotypes analysis with -47T/C was not possible in our study due to the departure from HWE. Nevertheless, we found that *ADRB2* is a potential susceptibility gene for glaucoma in our population. The next question was does *ADRB2* alter the response to timolol in our glaucoma patients?

5.1.3 *ADRB2* and pressure lowering effect of topical Timolol XE 0.5%

The role of *ADRB2* in the pharmacological response to agonist drugs for various conditions, particularly asthma, has been extensively studied (Tan et al., 1997; Martinez et al., 1997; Israel et al., 2000). Despite several replication studies in various populations on beta-agonists in asthmatic patients, there is no conclusive evidence on the influence of *ADRB2*. Similarly, studies on systemic beta-antagonists used for essential hypertension treatment failed to produce concrete evidence on the influence of *ADRB2* (Kotanko et al., 1997; Kato et al., 2001; Tomaszewski et al., 2002).

The phenotypic end points in our study are presented in mean IOP over 12 months of treatment and also using predetermined cut-off point of responsiveness to topical Timolol XE. A predetermined cut-off point for drug responsiveness has been adopted in many genotype-phenotype association studies. A good response to topical Timolol XE was defined at a predetermined level of 20% from baseline. This level was selected based on the mean percentage of IOP reduction in our study (23 [SD 18] %). Adjusting to different predetermined levels will result in different outcomes. For example, if the reduction is lowered to 15%, more patients would have been categorised as good responders. Moreover, this value is rather artificial and is particularly related to IOP. IOP fluctuation occurs over 24 hours and seasonally (David et al., 1992). The significance of IOP fluctuation in the progression of glaucoma remains inconclusive.

Glaucoma patients with 79CC demonstrated significantly higher mean baseline IOP compared to those with 79GG and 79CG. Our finding contradicts the previous finding in Japanese patients with POAG (Inagaki et al, 2006). Japanese patients with POAG demonstrated higher baseline IOP in 79GG and 79CG. Population variation could be

responsible for this observation. In fact, 79CC exerted strong effect on mean baseline IOP in Malays but not in Chinese in the present study. In the present study, more robust stratified Mantel-Haenszel meta-analysis on the allele frequency was conducted compared to Pearson chi square test on genotype frequency conducted in previous study. The previous study, may creates biased by combining the genotypic variant (79GG and 79CG) as one group and assumed 79CC as a reference (Inagaki et al, 2006).

Glaucoma patients with 79CC showed significant higher mean IOP at 1 month and 3 months post-treatment compared to 79GG and 79CG. The genotypic effect diminished after 3 months of treatment. This could be due to the diminishing number of patients on monotherapy treatment with topical timolol GFS and diluting the genotypic effect. Quite substantial number of patients failed to achieve target pressure and 'dropped out' from the study protocol. 79C/G may play a role in determining the pressure lowering effect of topical timolol in Malaysian glaucoma patients.

Glaucoma patients with -47TT also demonstrated significant higher mean baseline IOP compared to -47CC and -47TC. However, there was no significant difference of mean IOP on subsequent follow-up. There was also significant association between 46A/G and mean IOP at 12 months post-treatment in our glaucoma patients when 46GG was included. However, only 1 patient with 46GG completed the 12-month monotherapy treatment, and excluding this patient resulted in no significant association. Depleted sample size due to incomplete monotherapy treatment reduced the power of the study. In addition, the role 46G/A and -47T/C in pressure lowering effect of topical timolol in this present study needs to be interpreted with caution in the presence of HWE violation.

Fuchsjager-Mayrl et al. (2005) conducted a study on 89 healthy volunteers with genotypes that expressed the 3 main haplotypes of *ADRB2* coding region non-synonymous SNPs: 46A76C, 46G76G, and 46G76C. Topical timolol was prescribed and IOP was measured at 8.00 AM, 12.00 noon, and 6.00 PM. IOP reduction patterns and RM ANOVA was used for genotype-phenotype analysis (Fuchsjager-Maryl et al., 2005). There was no significant association between these haplotypes and the short term IOP reduction patterns. In spite of recruiting healthy non-smoker Caucasian volunteers, the IOP at 4 hours post-instillation was found to exhibit a 40% reduction from baseline, which is almost twice the reduction in our population. This provides additional evidence that timolol may produce a better pressure-lowering effect in less pigmented individuals. *ADRB2* does not play a role in determining short term pressure lowering effect of topical timolol in Caucasians.

Contradictory finding was observed in The Marshfield Clinic Personalized Medicine Research Project (PMRP) on 210 patients treated with topical beta-blockers for 3 months found that 79C/G was associated with the responder rate in topical beta-blocker treatment (McCarty et al., 2008). McCarty et al. (2008) also studied SNPs in *MYOC*, *OPTN*, *ADRB1*, and *CYP2D6*. Only two SNPs of *ADRB2*, 46A/G and 79C/G, were studied. The promoter region of *ADRB2* was not included, and haplotypes analysis was not conducted. A predetermined cut-off point of 20% and higher from baseline was adopted in this study. Patients with 79C were 2.0 times (95% CI: 1.0, 4.0) more likely to achieve 20% or more IOP reduction from baseline (McCarty et al., 2008). The selected cut-off point for clinically meaningful IOP reduction was similar to that in our study. We believe that the ethnic variation in our population is may be partly responsible for the contradictory results. However, the topical beta-blockers prescribed to the patients treated in Marshfield Clinic were not specific (McCarty et al., 2008). Most likely both non-selective and selective beta-

blockers were included. The possibility of topical timolol GFS use was not mentioned. Thus, if betaxolol had been included the overall percentage of IOP reduction would have definitely been different. The pressure-lowering effect of betaxolol is significantly lower than that of timolol or carteolol (Watson et al., 2001). Furthermore, a 3-month follow-up period was too short for short-term escape or long-term drift to have taken occurred. Inevitably, a higher proportion of patients achieved 20% or more IOP reduction from baseline. Despite certain drawbacks of this study, *ADRB2* is a candidate pharmacodynamic gene in determining the response to topical beta-blockers in Caucasian populations (McCarty et al., 2008).

The difference in phenotypic observation may be responsible to the contradictory finding between Fushjager-Maryl et al (2005) and McCarty et al (2008) in Caucasian population. Similarly, our study showed contradictory observation in difference phenotypic clinical observation. There was no significant association between *ADRB2* and responsiveness to topical timolol GFS based on predetermined cut off point of 20% reduction of pressure from baseline.

ADRB2 is a potential pharmacodynamic gene in Malaysian glaucoma patients. Timolol is a non-selective beta-blocker, and the drug target receptor also involves ADRB1 and interacts with other receptors, particularly the serotonin receptor (5-HT_{1A}). The molecular structure of the serotonin receptor (5-HT_{1A}) is almost similar to that of ADRB; both are GPCRs. Due to structural similarity, timolol has some affinity for the 5-HT_{1A} receptor in ciliary processes (Inoue-Matshuhisa et al., 2003). To further understand the variation in responsiveness to timolol, other target receptor genes, such as *ADRB1* and *5-HT_{1A}*, should be screened. Timolol is partially metabolised by cytochrome P450 (CYP2D6). Yuan et al (2010) conducted a study 123 glaucoma patients treated with topical timolol aqueous solution to look into association

of responsiveness to timolol and heart rate. CYP2D6 rs16947 was found to increase the susceptibility to timolol-induced bradycardia. In addition, oral cimetidine given together with topical timolol causes further reduction of heart rate and improved pressure lowering effect of timolol (Ishii et al, 2000). Drug-metabolising enzymes, such as cytochrome P450 (*CYP2D6*), and other downstream pathways, such as adenylyl cyclase, and ion channels may also interact and result in individual and population variations in response to topical timolol.

5.2 Topical latanoprost and *PTGFR*

5.2.1 Pressure lowering effect of topical latanoprost 0.005%

Latanoprost was reported to be a more effective glaucoma treatment than timolol in Asian and Mexican populations, based on 8 clinical trials conducted in the USA, UK, Scandinavia, Mexico, China, Philippines, Korea and Japan (Hedmann and Larsson, 2002). In those studies, diurnal intraocular pressure (IOP) was measured in the morning, at noon, and in the afternoon of the baseline visit and again at 3 and 6 months after initiation of treatment. The mean diurnal IOP reduction was reported to be 7.9 (SEM 0.3) mm Hg (32%) in patients treated once daily with latanoprost and 6.4 (0.3) mm Hg (26%) in patients treated with timolol twice daily. This difference was statistically significant and demonstrated superior IOP reduction by topical latanoprost once daily. This study also noted that the largest difference in mean diurnal IOP between the 2 treatments was observed in Asians and Mexicans. It was postulated that differences in iris pigmentation in these subjects might underlie this difference. In contrast, another study revealed no significant differences between topical beta-blockers and prostaglandin analogues in IOP reduction in African American and Caucasian patients with ocular hypertension (OHT) (Mansberger et al., 2007).

The mean IOP reduction from baseline and mean percentage IOP reduction by latanoprost in our study were 7.1 (4.2) mm Hg and 27% (19%), respectively. This was a slightly smaller reduction than the previous reports on Asian populations (Hedmann and Larsson, 2002; Thomas et al., 2005; Aquino et al., 1999). However, our patients were treated for up to 12 months whereas the previous studies treated for 3–6 months (Hedmann and Larsson, 2002; Thomas et al., 2005; Aquino et al., 1999). It is believed that the greatest effect of latanoprost is seen within 6 weeks of treatment initiation, during which time changes in the ciliary muscle extracellular matrix has almost completed and leads to improvement in uveoscleral outflow (Lindsey et al., 1997; Weinreb et al., 1997). A study conducted in India reported mean IOP reductions from baseline of 9.4 (1.9) mm Hg at 6 weeks post-treatment with latanoprost and 8.9 (1.7) mm Hg at 12 weeks post-treatment (Thomas et al., 2005). Moreover, in the earlier studies, long-term follow-up at 2 years showed that IOP reduction was stable without drift or tachyphylaxis in the European populations of the earlier studies (Hedmann et al., 2002; Alm et al., 1997).

Our calculation of mean IOP reduction was based on the difference between the sum of the IOPs at each visit during the follow-up period and the IOPs at baseline. Thus, a slight increase in IOP from one visit to another would result in lower estimates of IOP reduction. In a previous study, the reduction in IOP was calculated as the difference between IOP at baseline and at the final follow-up visit (Cheong et al, 2008). Unlike the studies conducted by Hedmann and Larsson (2002) and Aquino et al. (1999), our study did not assess diurnal fluctuations in IOP. IOP was measured once between 8:00 AM and 12 noon, and patients were asked to instil the medication at 8:00 PM. Evening dosing of latanoprost is known to be more effective than morning dosing (Alm et al., 1995). In a previous study (Larsson et al., 2002), IOP was assessed over 24 hours in OHT patients treated with topical latanoprost in the

evening and topical timolol GFS (gel-forming solution) in the morning. Latanoprost was shown to provide better IOP reduction; however, there was a slight spike in IOP between 8:00 PM and midnight in patients treated with topical latanoprost (Larsson et al., 2002). Thus, we speculate that the time of IOP measurement in our study (8:00 AM) may be indirectly responsible for the smaller pressure-lowering effect of latanoprost compared with earlier studies.

In the present study, latanoprost was prescribed as either monotherapy or adjunctive therapy to topical Timolol XE 0.5%, with slightly more patients treated with adjunctive therapy. While we were recruiting for this study, latanoprost was gaining in popularity as first line medication but the cost of treatment was a major drawback. However, latanoprost is currently available as a standard therapy in our practice and most of the patients receive it at no cost, in particular those in lower socioeconomic groups. Because they have different mechanisms of action, it has been reported that the pressure-lowering effects of latanoprost plus timolol are additive (Bron et al., 2001; Alm et al., 1995; Rulo et al., 1994; Toris et al., 1993). In contrast, we found that latanoprost was more effective as a monotherapy (mean IOP reduction 7.2 (3.2) mm Hg) than as adjunctive treatment to Timolol XE 0.5% (7.0 (4.8) mm Hg), but this difference was not statistically significant. In a study conducted in Italian glaucoma patients, IOP reduction achieved from switching timolol monotherapy to latanoprost monotherapy was similar to when switching to adjunctive therapy of latanoprost and timolol (Bucci et al., 1999). In another study, adjunctive therapy of latanoprost and timolol was compared with monotherapy of either latanoprost or timolol in aqueous solution (Higginbotham et al., 2002). The mean IOP reduction of the adjunctive therapy was comparable to latanoprost monotherapy but significantly superior to timolol monotherapy (Higginbotham et al., 2002).

Timolol gel forming solution (GFS) provides slightly better IOP reduction than aqueous solution due to the increased ocular bioavailability of the GFS formulation (Rozier et al., 1989; Roselund, 1996). Theoretically, the additive pressure-lowering effect of latanoprost to timolol GFS should be more pronounced than with timolol in aqueous solution. However, there have been no studies to date comparing efficacy between these formulations as additive therapy to latanoprost. Clinically, timolol GFS has a slightly better pressure-lowering effect compared with timolol in aqueous solution, but the difference is not significant (Roselund, 1996; Shedden et al., 2001a). Thus, it can be postulated that the additive effect of timolol GFS will not be as pronounced as expected. Reversible binding of timolol to ocular melanin might reduce its efficacy and render the additive effect of timolol plus latanoprost less pronounced (Menon et al., 1989; Ong et al., 2005).

A retrospective review of topical latanoprost as monotherapy in Malay patients showed that the mean IOP reduction from baseline was 7.8 (5.3) mm Hg (Cheong et al., 2008). This reduction is higher than the mean IOP reduction for both latanoprost monotherapy and adjunctive therapy in the present study. Comparatively, the baseline IOP in our study was much lower than previous studies involving Asian patients (Cheong et al., 2008; Aquino et al., 1999; Mishima et al., 1996; Hedmann and Larsson, 2002). A higher baseline IOP has been observed to cause higher IOP reduction once treatment is advocated (Rulo et al., 1996). This may explain the reason of lower IOP reduction in the present study.

We found that topical latanoprost treatment resulted in a mean reduction of IOP from baseline of 26.7% (19.3%), ranging from 28.9% (13.4%) at 1 month to 31.9% (13.0%) at 6 months post-treatment. Although this percentage reduction was lower than in other latanoprost studies, it was superior to the effects of topical timolol XE based on indirect

comparison to our patients treated with topical timolol GFS (Project B). Furthermore, 65 (75.6%) of our recruited patients completed 12 months treatment. Although our study had a lower mean IOP reduction than the study conducted by Cheong et al. (2008) in Malay patients treated with topical latanoprost monotherapy, our percentage of good responders (≥25% IOP reduction) was virtually the same. Cheong et al. (2008) set a predetermined cut-off point for response of ≥20% IOP reduction from baseline. The retrospective nature of that study is a drawback and the conclusions may be affected by other confounding factors (Cheong et al., 2008).

Statistical analyses of the present study showed that the baseline IOP had a significant effect on the patient responsiveness to latanoprost. In other studies with topical latanoprost, patients with higher baseline IOP experienced greater IOP reductions post-treatment (Hedmann and Larsson, 2002; Alm et al., 1995; Denis et al., 2010). Baseline IOP was found to moderately correlate with the pressure-lowering effect of latanoprost in a retrospective study involving 186 cases (Bayer et al., 2005). OHT patients with high baseline IOP were found to have better pressure reduction than those with lower baseline IOP (Mansberger et al., 2007). Paradoxically, lower baseline IOP was found to reduce the risk of failure in completing 12 months of treatment in our study (0.8-fold; 95% CI [0.7–1.0]). It is perhaps not surprising that the most significant IOP reductions are observed in patients with higher baseline IOP, but the individualised target pressure reductions set for these patients may not be achieved. These patients are also more likely to require additional treatment or changes in medication.

In the present study, adjunctive therapy was advocated to patients diagnosed with more advanced disease based on Humphrey visual field analysis. The target IOP was set at lower levels for patients with advanced disease in an attempt to retard further optic nerve damage.

Inevitably, most of these patients are transitioned from monotherapy to adjunctive therapy during the follow up period. We also found that patients with advanced glaucomatous damage had thinner central corneal thicknesses (CCT). CCT correlated with the severity of primary open-angle glaucoma (POAG), based on the Advanced Glaucoma Intervention Study score, the mean deviation of visual field, and the vertical and horizontal cup-to-disc ratios at the initial examination by glaucoma specialists (Herndon et al., 2004). The Ocular Hypertension Treatment Study found that thinner CCT was also a significant predictor for progression of OHT to POAG when race was included in the analysis (Gordon et al., 2002). Another study evaluated the impact of race on the response to beta-blockers and PG analogues and found that baseline IOP and CCT affected the responsiveness to treatment in African American and Caucasians (Mansberger et al., 2007).

Furthermore, thicker CCT is associated with greater ocular rigidity, which reduces the sensitivity of detection of IOP differences following treatment (Brandt et al., 2004). However, thin CCT was also associated with underestimation of IOP by Goldmann applanation tonometry (Ehlers and Hansen, 1975). Ethnic differences also influence CCT; Asians tend to have thinner CCT compared to Caucasians (Aghaian et al., 2004; Shimmyo et al., 2003). In the present study, CCT was evaluated by non-contact specular microscopy. Non-contact specular microscopy provides acceptable CCT measurement and comparable with ultrasonic pachymetry but tends to give thinner measurement (Kawana et al., 2004; Módis et al., 2001). Thus, there is possibility of overestimation of CCT measurement that may leads to underestimation of IOP. However, there was no significant association between CCT and baseline IOP in the present study. It is not the aim of our study to evaluate the accuracy of non-contact specular microscopy. Nevertheless, caution must be taken in interpretation of CCT in this present study.

In the present study, patients diagnosed with normal-tension glaucoma (NTG) recorded less IOP fluctuation than patients with POAG. Topical latanoprost as monotherapy or adjunctive therapy provides good pressure-lowering effects with stable pressure reduction for 12 months. In our population, topical latanoprost is a better choice of first line treatment than timolol. However, compared with other Asians population the pressure-lowering effect of latanoprost was slightly lower in our study (Aquino et al., 2007; Cheong et al., 2008; Thomas et al., 2005). These findings suggest there may be ethnic variation in the response to latanoprost treatment.

5.2.2 Side effects of topical latanoprost 0.005%

The side effects of latanoprost have also been postulated to be affected by ethnicity. This affect may lie in the unusual relationship between various topical pressure-lowering drugs and melanin. Latanoprost increases pigmentation of the iris, lashes, and periocular area, but this is not observed with timolol, which has a higher affinity for melanin (Watson and Stjernschantz, 1996). In our patients, hypertrichosis was the most common side effect and was detected as early as 3 months post-treatment. At the end of 12 months, all patients remaining on latanoprost had developed hypertrichosis. The incidence of hypertrichosis has previously been found to increase with the duration of treatment (Chiba et al., 2004). Hypertrichosis is regarded as a cosmetic side effect that was well accepted in this study, especially among the women. Thus, none of the patients raised concerns or expressed the desire to discontinue treatment.

Latanoprost-induced iridial pigmentation (LIID) is a major concern among Caucasians, especially LIID causing brown patches on blue, gray, or green eyes (Watson and

Stjernschantz, 1996; Alm et al., 1995). The mechanism for this intriguing side effect has been widely studied. It is thought that the majority of the pathology occurs at the anterior stroma of the iris and results from increased melanin production by melanocyte, rather than melanocyte proliferation (Cracknell et al., 2003; Arranz-Marquez et al., 2004). In our study, we observed LIID in 5 patients (5.6%) at 12 months post-treatment. When latanoprost first became the treatment of choice for glaucoma, individuals with homogenous dark brown irises, especially Africans and Asians, were thought not to be affected by LIID. However, as the popularity of latanoprost escalated, LIID was also detected in Asians (Chou et al., 2005; Chiba et al., 2004). Detection of LIID in dark brown irises is more difficult than in lighter coloured irises. Chou et al. (2005) used the Boys-Smith pigment gradation lens, which gives a more objective assessment, for semi-quantitative measurement of iris pigment in a study in the Japanese population. In our study, detection of LIID was based on the anterior segment photographs and slit lamp evaluations. The serial photographs gave a reasonably objective assessment of LIID but were insufficiently accurate. Thus, there is strong possibility that LIID was underdetected in our study, which may be partly responsible for the lower incidence of LIID compared to studies conducted in other Asians population (Chou et al., 2005; Chiba et al., 2004).

Although conjunctival hyperaemia is not a permanent side effect of latanoprost, it is the most concerning ocular side effect. We reported only a 3.4% incidence of conjunctival hyperaemia and none of the patients wished to terminate their involvement in this study. Based on the classification system suggested by Stewart et al. (2002), the patients' conjunctival hyperaemia was mild and had disappeared by 3 months post-treatment. Latanoprost is believed to cause less conjunctival hyperaemia than other prostanoid analogues due to its selectivity for the PTGFR (Honrubia et al., 2009; Feldman., 2003). Naturally occurring PGs

have the highest affinity for their respective receptors but they are also relatively non-selective (Sharif et al., 2003). Latanoprost is more selective for PTGFR than are circulating $PGF_{2\alpha}$ or other PG analogue drugs such as bimatoprost and travoprost (Sharif et al., 2003). For example, bimatoprost acid exhibits relatively high affinity for FP, EP_1 and EP_3 (Cantor et al., 2007). Bimatoprost acid is the product of hydrolytic conversion of bimatoprost and can be detected in the aqueous humour (Cantor et al., 2007). However, it has been suggested that the cause of conjunctival hyperaemia resulting from latanoprost treatment may be due to the high concentration of the preservative benzalkonium chloride (200 mg/ml) in those preparations, which is almost double the concentration in preparations of timolol (Alm et al., 1995). Although the presence of conjunctival hyperaemia is not thought to influence the efficacy of topical latanoprost (Stewart et al., 2003), a recent study has found a significant correlation between IOP and the severity of conjunctival hyperaemia (Kobayashi and Kobayashi, 2011).

Despite the effectiveness of latanoprost for glaucoma therapy in a number of populations, there are reports of unresponsiveness to treatment and tachyphylaxis. In addition, some but not all patients develop conjunctival hyperaemia. The possible causes of such variation in the clinical pharmacology of latanoprost should be identified and addressed, not only to ensure patients receive maximally effective treatment to halt further insult to the damaged optic nerve, but also to minimise side effects and promote compliance to medication in the long-term management of glaucoma. This subject raises the key question: are there interactions between the genetic make-up of glaucoma patients and their responsiveness to treatment with PG analogues?

5.2.3 *PTGFR* and susceptibility to glaucoma

A phase 1 genome-wide association study (GWAS) of European and Asians patients with bipolar disorder identified *PTGFR* as a candidate gene (Chen et al., 2011). The PTGFR is abundant in the brain and optic nerve and PGF2α has been shown to exacerbate hypoxic neuronal injury in neuron-enriched primary cultures (Li et al., 2008), suggesting a possible role for *PTGFR* as a susceptibility gene for glaucoma. *PTGFR* has been shown to influence the contractility of myometrium during labour (Hay et al., 2010, Sugimoto et al., 1997). Ciliary muscle is a smooth muscle with similar structural properties to myometrium; thus, the regulation of PTGFR in ciliary muscle relaxation may be analogous to that in myometrium. Relaxation of ciliary muscle has been proposed to facilitate aqueous outflow through an unconventional pathway. Preliminary data from studies of gene therapy for glaucoma have found that the prostaglandin pathway is a potential therapeutic target for gene therapy for sustained lowering of IOP (Barraza et al., 2010).

In the present study, the entire *PTGFR* gene, including the promoter region, was directly sequenced to identify polymorphisms. A total of 63 SNPs were identified, including a novel SNP rs3766332 that was reported by Hoh et al (2007) and 2 regions of microsatellite instability (MSI). The majority of the SNPs were found in the introns and 1 SNP, rs3766331, was found in exon 4. Four SNPs were found at the 5'UTR. There is accumulating evidence of heterogeneity in various genes among Asian population (Cornes et al., 2012; Tan et al., 2010). Our study included 2 major racial groups and the analysis was stratified according to Malays and Chinese. We found a significant difference between Malays and Chinese in the allele frequencies of 12 SNPs and an additional 9 SNPs had a near-significant difference ($p < 0.1$), suggesting a potential population stratification. However, genomic control analysis showed no inflation of λ_{gc}, suggesting that the finding in the present study is unlikely to be

due to population stratification. Ideally, the genomic control analysis should be conducted on 30 unlinked markers, whereas we examined only 16 markers because of the close proximity of the identified SNPs and the presence of linkage disequilibrium between the SNPs.

Based on the Cochran-Mantel-Haenszel test for stratified samples, we identified 4 intronic SNPs (rs11162505, rs554185, rs551253 and rs556817) as potentially associated with glaucoma in Malays. Interestingly, none of the 63 identified SNPs showed significantly different frequencies between glaucoma patients and control subjects in Chinese residents of Malaysia. The inability to detect significant associations between *PTGFR* SNPs and susceptibility to glaucoma in this population may be due to the small patient sample size. Tests for heterogeneity were significant in rs11162505. Genotype frequency rs11162505 shown significant difference between glaucoma and controls (p=1.72E-4) in Malays but not among the Chinese. The minor allele of rs11162505 (rs11162505G) exerts a strong and significant effect as a protective SNP against glaucoma (OR 0.3; 95% CI 0.1, 0.5). The minor allele of rs554185 also confers protection against glaucoma in Malaysian population (OR 0.7; 95%CI 0.4, 1.0). The position of rs11162505 and rs554185 is just 49bp apart and in strong linkage disequilibrium (LD). Allele frequency of haplotypes GG of rs11162505 and rs554185 shown significant difference between glaucoma patients and controls ($p=4.0x10^{-4}$). Another haplotypes GA of these two SNPs also showed significant association with glaucoma ($p=3.0x10^{-4}$). In addition, the minor allele of another intronic polymorphism rs551253 was also found to confer protective effect against glaucoma. However, rs551253 violated HWE and excluded from further analysis.

On univariate analysis, the minor allele of rs11162505G in the heterozygous state (rs11162505GC) confers strong protective effect against glaucoma in Malays but not in

Chinese, which provide further support the significant difference in heterogeneity test. Interestingly, rs554185AG was found to increase the susceptibility to glaucoma in Malays but reduces the risk of glaucoma in Chinese. Stepwise logistic regression combining Malays and Chinese demonstrated rs11162505GC provides strong protective effect against glaucoma but the effect of rs554185AG was no longer significant. On the other hand, rs556817AG was also found to reduce the risk of glaucoma and rs3766338TC increases the risk of glaucoma 4.2 folds (95% CI 1.2, 14.0). Based on the findings of our study, *PTGFR* is a potential protective gene against glaucoma.

Further analysis was conducted according to the type of glaucoma: POAG and NTG. The minor allele of rs11162505, rs686262 and rs551253 was found to confer significant protective effect against POAG. The minor allele of two SNPs; rs12093097 and rs1073610 found in the promoter region, increase the risk of POAG. However, on the stepwise logistic regression, only rs11162505AG was found to confer significant protective effect against POAG. The minor allele of rs556817 and rs6424776 showed significant effect in reducing the risk of NTG. On contrary, the minor allele of rs7543738 increases the risk of NTG 3.8folds (95% CI 1.2, 12.1). In general, rs11162505 and rs556817 seems to confer protective effect against POAG and NTG respectively.

In the present study, the majority of SNPs were found in *PTGFR* introns. In general, intronic SNPs are noncoding polymorphisms that do not affect protein expression. However, the rapid progress of genetic research, especially following the Human Genome Project, has resulted in the identification of numerous susceptibility alleles for varying diseases. With respect to ocular diseases, an intronic SNP and common variant in the complement factor H gene (*CFH*) were found to increase susceptibility to age-related macular degeneration by 7.4-fold

(95% CI 2.9–19) (Klein et al., 2005). The patients in that study were recruited from the Age-Related Eye Disease Study (AREDS Research Group, 2001). The association of SNP rs11162505 in *PTGFR* in the present study does not reach the significance (set at 10^{-7}) of a genome wide association study (GWAS); nonetheless, this allele was demonstrated to have a significant protective effect against glaucoma. Replication study in other populations is necessary to ascertain the role of *PTGFR* in glaucoma.

SNP markers of CDKN2B-AS1 gene (located at chromosome 9p21) were found to associate with the risk of glaucoma in European derived population (Ramdas et al., 2011). The minor allele of a locus of CDKN2B-AS1 gene was found to confer protective effect against this large sample of glaucoma patients of European derived population. Replication study conducted in Japanese population found similar GWAS significant of CDKN2B-AS1 gene (Nakano et al., 2012). However, marker of CDKN2B-AS1 gene was found to significantly increase susceptibility to glaucoma. Nakano et al (2012) contributed this different was due to smaller sample size and the inclusion of NTG.

Dinucleotide microsatellite instability (MSI) identified in this study was shown to not associate with susceptibility to glaucoma. However, it is possible than an interaction between the regions of MSI and SNPs may be responsible for susceptibility to glaucoma or responsiveness to medication. A large-scale multi-centre study conducted on colorectal cancer patients found 3 SNPs that were associated with the methylation status of the *MLH1*. These SNPs caused loss of MLH1 protein and induced MSI leading to microsatellite unstable (MSI-H) colorectal cancer (Mrkonjc et al., 2010). The role of MSI in pathogenesis of colorectal cancer is well established but the role in complex disease such as glaucoma is not known. Intronic MSI was found to associate with susceptibility with a number of

neurodegenerative diseases such as Friedreich ataxia, asparagines synthetase gene causing acute lymphoblastic leukaemia and NOS3 gene in hypertension (Bidichandani et al., 1998; Akagi et al., 2006; Jemaa et al., 2009). Thus, the possibility remains that the MSI and SNPs found in the present study could cause amino acid changes or other alterations of PTGFR function.

There are reports of alternative splice variants of *PTGFR* in various tissues, including ocular tissue (Hay et al., 2010; Vielhauer et al., 2004; Liang et al., 2008). Two alternatively spliced isoforms of the FP receptor, designated FPA and FPB, have been cloned from human corpus luteum, placenta, uterus, heart, and ocular tissue (Pierce et al., 1997; Vielhauer et al., 2004). The isoforms have almost identical structures except at the carboxyl terminus and have indistinguishable radioligand binding activity. However, they differ in their signalling capacity, specifically in their functional coupling to phosphatidylinositol hydrolysis. Liang et al. (2008) reported the presence of 6 alternatively spliced mRNAs in human ciliary body, resulting from the insertion of 5 additional exons between exons 2 and 3 of wild-type *PTGFR*. Of note, almost two-thirds of the SNPs identified in the present study are located in Intron 3 (between exons 3 and 4, exon 3 is also part of the identified spliced variant), including rs11162505, rs554185 and rs556817. Thus, it is possible that these SNPs are located within the additional exons in the *PTGFR* splice variants isolated from human ciliary body.

The role of *PTGFR* in the pathogenesis of glaucoma has not previously been studied. Loss of Heterozygosity of *PTGFR* has been shown to increase susceptibility to breast cancer (Soosey-Alaoui et al., 2001). Because PTGFR is abundant in the brain (Yanai et al., 2005), changes in PTGFR function could be associated with chemical or structural changes that may be

responsible for glaucoma. PTGFR is also expressed at the retina and optic nerve where it is postulated to provide a neuroprotective effect (Ocklind et al., 1996; Nakanishi et al., 2006). It was found that PTGFR is not only activated and desensitised by $PGF_{2\alpha}$ but also by F_2 isoprostanes (Kunapuli et al., 1997). F_2 isoprostanes, especially 12-*iso*-$PGF_{2\alpha,}$ activate PTGFR in a specific and saturable manner (Kunapuli et al., 1997). 12-*iso*-$PGF_{2\alpha}$ is produced in large amounts as a result of free radical cyclization (O'Connor et al., 1984), which usually occurs under conditions of oxidative stress, as has been postulated to contribute to glaucoma and heart failure. It is thus possible that genetic variation in the *PTGFR* gene may influence the affinity of 12-*iso*-$PGF_{2\alpha}$ for PTGFR.

COX is the rate-limiting enzyme in the biosynthesis of prostaglandins. Two isoforms, COX-1 and COX-2, have been identified and characterised (Vane et al., 1998). COX-1 is expressed in most tissues and is known as a housekeeping enzyme, while COX-2 is expressed in specific tissues under normal physiological conditions but its transcription is upregulated by factors such as pro-inflammatory cytokines (Beiche et al., 1996; Yamagata et al., 1993). Lack of COX-2 expression in non-pigmented epithelium of the ciliary body at various stages of POAG suggested the potential role of COX-2 in POAG (Maihöfner et al., 2001). Glucocorticoid inhibit expression of COX-2, suggesting a role for COX-2 in juvenile glaucoma and the possibility of an interaction with the trabecular meshwork inducible glucocorticoid response gene (*TIGR)* also known as Myocilin (*MYOC)* (Maihöfner at al., 2001). PTGFR is localized close to COX-2 and TIGR in ciliary epithelium and ciliary body. *PTGFR, COX-2* and *MYOC* are located in close proximity on chromosome 1q at 13.1, 25 and 24.3 respectively (Michels-Rautenstrauss et al., 1998; Tay et al., 1994; Betz et al., 1999). Since glaucoma is a complex disease, perhaps the genetic variations of these genes may actually increase the susceptibility to glaucoma.

5.2.4 *PTGFR* and pressure lowering effect of topical latanoprost 0.005%

Latanoprost failed to reduce IOP in homozygous PTGFR knockout mice (FPKO) over a short duration (Crowston et al., 2002). This indicates that PTGFR is important in determining the functionality of prostaglandin analogues. Surprisingly, bimatoprost and unoprostone have no IOP-lowering effect in FPKO mice (Ota et al., 2005; Crowston et al., 2005), suggesting that these analogues stimulate the PTGFR either directly or indirectly through unknown mechanisms and that most PG analogues act in a PTGFR-dependent manner. Recent work on prostanoid gene therapy found that injection of cats with lentiviral vectors encoding codon-optimised COX-2 and codon-optimised FP receptor (FPR) produced sustained reductions in IOP of up to 35% (Barraza et al., 2010). The *PTGFR* gene is thus important in determining the pressure-lowering effect of latanoprost and is a potential pharmacodynamic gene.

We selected certain SNPs in *PTGFR* based on the Fisher model of mapping quantitative trait loci. As glaucoma is a complex disease, ideally SNPs with significant additive genotype values should be selected. However, no SNP showed significant additive genotype value, and instead, SNPs with significant dominant and combination genotype values were selected. These were rs4650581, rs34012237, rs3766335, rs2146490, rs3966768, rs7543738 and rs6735117. One-way ANOVA was used to analyse the mean IOP at each visit of the patients with the selected SNP genotype. Due to the possibility of an effect of race on IOP, the analysis was further divided for Malays and Chinese. There was no significant difference in the mean IOP at each visit for patients with any of the selected SNPs, except for rs2146490 in Chinese glaucoma patients. Patients with rs2146490GG had significantly higher mean IOP measurements at 12 months post-treatment compared to patients with rs2146490GA.

Sakurai et al. (2007) conducted a short-term study on the pressure-lowering effect of latanoprost on healthy Japanese volunteers. There was significant association between the presence of rs3753380 and rs3766355 and percentage latanoprost-induced IOP reduction from baseline at 7 days post-treatment. Patients were defined as low responders to latanoprost (less than 10% reduction in IOP), medium responders (between 10% and 25% reduction) and good responders (more than 25% reduction). However, studying the responsiveness to latanoprost in healthy volunteers is not a fair representation of the phenotype because healthy volunteers have normal baseline IOP. Moreover, the percentage latanoprost-induced IOP reduction is dependent on the baseline IOP (Herndon et al, 2004; Bayer et al., 2005; Mansberger et al., 2007), with patients with higher baseline IOP achieving higher percentage of IOP reduction (Alm et al., 1997).

The SNPs identified by Sakurai et al. (2007) at the promoter region (rs3753380) and intronic region (rs376355) were also identified in our study. However, our study showed that these 2 SNPs are not susceptibility genes for glaucoma, nor are they potential candidates as pharmacodynamic genes. Differences in the study populations may explain the difference in genotype-phenotype association of the *PTGFR*. McCarty et al. (2011) suggested that a discrepancy in the minor allele of rs3766335 in Europeans and Japanese is responsible for the lack of replication between the studies. These authors genotyped a population based at the Marshfield Clinic Personalized Medicine Research Project for the *PTGFR* polymorphisms rs3753380 and rs376355 in an attempt to replicate the study conducted in the Japanese population. However, the population included glaucoma patients seen at the Marshfield clinic whereas the Japanese study was conducted on healthy volunteers. McCarty et al. (2011) observed no association between the presence of SNPs rs375580 and rs376355 in *PTGFR* and the patient response to PG analogues at 90 days post–treatment.

In the present study, the responsiveness to latanoprost was defined as good or poor based on a predetermined cut-off point of 25% reduction in IOP from baseline. This cut-off point was selected based on the mean IOP reduction of latanoprost in our study and was higher than in previous studies conducted in Japanese (Sakurai et al., 2007) and European (McCarty et al., 2011) populations. A stratified Cochran Mantel-Haenszel analysis of 63 SNPs was conducted on the good (≥25% reduction from baseline) and poor (<25% reduction from baseline) responders. There was a significant association between SNP rs686262 and the responsiveness to latanoprost. The major allele of rs686262 (rs686262A) provides a protective effect against poor responder or reduces risk of poor responder to topical latanoprost 0.005% (0.4 folds (95% CI 0.2, 0.8). The final model of stepwise logistic regression showed that rs686262GG was associated with increased risk of poor responsiveness to latanoprost 6.3-fold (95% CI 1.3–31.0). We identified a novel SNP rs3766332 earlier during screening of *PTGFR* exons, the minor allele of rs3766332 shown borderline significant as predictor for responsiveness to treatment (Hoh et al., 2007). This is perhaps due to rather small sample size in the present study.

Based on the findings from the present study, we consider *PTGFR* to be a potential pharmacodynamic gene. However, future studies aimed at replicating our findings should focus on topical latanoprost as monotherapy rather than combining the adjunctive and monotherapy. The postulated mechanism of action of latanoprost is complex and not well established. *MMP* has also been identified as a potential pharmacodynamic gene. To improve our understanding of the pharmacogenetics of latanoprost, genes that may be involved in the postulated mechanism of action of latanoprost, such as *COX-2* and *MMP*, should be screened.

More robust genetic screening techniques, such as microarray techniques, might provide better detection of potential pharmacodynamic and pharmacokinetic genes.

5.3 Limitation and recommendation

The major limitation in this present study was relatively small number of total recruits. This is mainly due to high number of drop-out. Most of the 'drop-out' was those who even failed to turn up during the first visit, 1 month post treatment. The number of actual recruits was more than the total recruits reported in this study.

Lack of randomisation, is also a potential source of bias. For example, newly diagnosed glaucoma patients with suspicious of respiratory impairment or history of chronic smoking were automatically assigned to Project P. Moreover, those with more advanced disease or higher IOP were more likely to be assigned to Project P. This is based on the reported effectiveness of topical latanoprost 0.005% in Asians (Hedmann and Larsson., 2002). This may be responsible for quite a large number of patients were treated with topical latanoprost as adjunctive therapy in Project P.

Setting individualised target pressure emulates the actual clinical setting. At the same time, individualised target pressure was the main reason for patients been 'dropped-out' from the study protocol. Even if topical timolol or latanoprost provide good pressure lowering but may not reach the targeted pressure. Pressure lowering effect of the drugs may not be accurately reflective in this study. In the future, perhaps a randomised control trial study should be adopted with a definitive target pressure.

5.4 Conclusion: Pharmacogenetics in glaucoma management

The present study provides additional evidence of the potential role of pharmacogenetics in glaucoma management. Timolol XE 0.5% monotherapy provided good pressure reduction and higher levels of reduction than reported in previous studies. The mean baseline IOP of genotype -47CC of *ADRB2* was significantly higher than of genotypes -47GG and -47CG. However, there was no association of *ADRB2* with responsiveness to topical Timolol XE. In addition, 79G and -20C of *ADRB2* were found to be potential susceptibility markers for glaucoma.

The pressure-lowering effects of topical latanoprost 0.005% was better than that of topical Timolol XE based on indirect observation, but was lower than the mean IOP reduction observed in other studies conducted in Asian glaucoma patients. We identified one SNP in the flanking region of exon 4, rs686262, was found to associate with responsiveness to topical latanoprost. There was significant association between the genotype frequency of rs11162505 and glaucoma in Malays. The minor allele of rs11162505, rs554185 and rs551253 appeared to confer a strong protective effect against glaucoma.

Perhaps, in the future, a simple fast genetic screening is useful in helping ophthalmologist to choose appropriate treatment for glaucoma patients. *ADRB2* and *PTGFR* are the potential markers to be included in the future genetic screening tool. The pharmacokinetic genes such as *CYP2D6* and other potential genes that play a role in the mechanism of action of the drugs such as *MMP* should also be included as the potential markers.

REFERENCES

References

Abdalla MI and Hamdi M, "Applanation ocular tension in myopia and emmetropia", *Br J Ophthalmol*, 1970; vol. 54, pp. 122-125.

Abel MH and Baird DT, "The effect of 17 beta-estradiol and progesterone on prostaglandin production by human endometrium maintained in organ culture", *Endocrinology*, 1980; vol. 106, pp. 1599-1606.

Abrahamsson T, Böstrom S, Bräutigam J, Lagerstrom PO, Regardh CG, Vauqelin G, "Binding of β-blocker timolol and H216/44 to ocular melanin", *Exp Eye Res*, 1988; vol. 47, pp. 565-77

Abramovitz M, Boie Y, Nquyen T, Rushmore TH, Bayne MA, Metters KM, Slipetz DM, Grygorczyk R, "Cloning and expression of a cDNA for the human prostanoid FP receptor", *J Biol Chem*, 1994; vol. 269, pp. 2632-2636.

Aghaian E, Choe JE, Lin S, Stamper RL, "Central cornea thickness of Caucasians, Chinese, Hispanics, Filippinos, African Americans and Japanese in a glaucoma clinic", *Ophthalmology*, 2004; vol. 111, pp. 2211-2219.

Ahlquist RP, "A study of the adrenotropic receptors", *Am J Physiol*, 1948; vol. 153, pp. 586-599.

Aihara M, Lindsey JD, Weinreb RN, "Episcleral venous pressure of mouse eye and effect of body position", *Curr Eye Res*, 2003; vol. 27, no. 6, pp. 355-362.

Akagi T, Yin D, Kawamata N, Bartram CR, Hoffmann WK, Wolf I, Miller CW, Koeffler HP, " *Leukemia*, 2006, vol 20, pp 1303-1306.

Albert DM, Gangnon RE, Grossniklaus HE, Green WR, Darkatmoko S, Kulkarni AD, "A study of histopathological features of latanoprost-treated irides with or without darkening compared with non-latanoprost-treated irides", *Arch Ophthalmol*, 2008; vol. 126, pp. 626-631.

Albert DM, Gangnon RE, Grossniklaus HE, Green WR, Darjatmoko S, Kulkarni AD, "A Study of Histopathological Features of Latanoprost-Treated Irides With or Without Darkening Compared With Non-Latanoprost-Treated Irides", *Archives of Ophthalmology*, 2008; vol. 126, no. 5, pp. 626-631.

Algovik M, Lagercarntz J, Westgren M, Nordenkjold A, "No mutations found in candidate genes for dystocia", *Hum Reprod*, 1999; vol. 14, pp. 2451-2454.

Allingham RR, Liu Y, Rhee DJ, "The genetics of primary open-angle glaucoma: a review", *Exp Eye Res*, 2009; vol. 88, pp. 837-844.

Allingham RR, Loftsdottir M, Gottfredsdottir MS, Thorgeirsson E, Jonasson S, Sverisson T, Hodge WG, Damji KF, Stefansson E, "Pseudoexfoliation syndrome in Icelandic families", *Br J Ophthalmol*, 2001; vol. 85, pp. 702-707.

Allingham RR, Liu Y, Rhee DJ, “The genetics of primary open-angle glaucoma: a review”, *Exp Eye Res*, 2009, vol 88, pp 837-844

Alm A and Stjernschantz J, Scandinavian Latanoprost Study Group, "Effects on intraocular pressure and side effects of 0.005% latanoprost applied once daily, evening or morning. A comparison to timolol", *Ophthalmology*, 1995; vol. 102, pp. 1743-1752.

Alm A, "Prostaglandin derivates as ocular hypotensive agents", *Progress in Retinal and Eye Research*, 1998; vol. 17, no. 3, pp. 291-312.

Alm A, Grierson I, Shields MB, "Side Effects Associated with Prostaglandin Analog Therapy", *Survey of Ophthalmology*, 2008; vol. 53, no. 6, Supplement 1, p. S93-S105.

Alm and Villumsen J, “PhXA34- a potent ocular hypotensive drug. A study on effect-side effect and aqueous humour dynamics in healthy volunteers”, Arch Ophthalmol 1991; vol. 109, pp. 1564-8

Alm A, Camras CB, Watson PG, “Phase III latanoprost studies in Scandinavia, the United Kingdom and the United States”, *Surv Ophthalmol*, 1997, vol 41 (Suppl 2), pp S105-S110

Alvarado J, Murphy C, Polansky J, Juster R, "Age-related changes in trabecular meshwork cellularity", *Invest Ophthalmol Vis Sci*, 1981; vol. 21, no. 5, pp. 714-727.

Alward WL, Kwon YH, Kawase K, Craig JE, Hayreh SS, Johnson AT, Khanna CL, Yamamoto T, Mackey DA, Roos BR, Afatigato LM, Sheffield VC, Stone EM, "Evaluation of Optineurin sequence variations in 1,048 patients with open-angle glaucoma", *Am J Ophthalmol*, 2003; vol. 136, pp. 904-910.

Alward WL, Fingert JH, Coote MA, Johnson AT, Lerner SF, Jungua D, Durcan FJ, McCartney PJ, Mackey DA, Sheffield VC, Stone EM, "Clinical features associated with mutations in the chromosome 1 open angle glaucoma gene (GLC1A)", *N Engl J Med*, 1998; vol. 338, pp. 1022-1027.

Ambache N, "Properties of irin, a physiological constituent of the rabbit's iris", *J Physiol*, 1957; vol. 135, pp. 114-132.

Anderson DR, Grant WM, "The influence of position on intraocular pressure", *Invest Ophthalmol Vis Sci*, 1973; vol. 12, pp. 201-212.

Anderson DR, Drance SM, Schulzer M, Collaborative Normal-Tension Glaucoma Study Group, "Factors that predict the benefit of lowering intraocular pressure in normal tension glaucoma", 2003, *Am J Ophthalmol*, vol. 136, pp. 820-829.

Anderson LE, Wu YL, Tsai SJ, Wittbank MC, “Prostaglandin $F_{2\alpha}$ receptor in the corpus luteum: recent information on the gene messenger ribonucleic and protein”, *Biol Reprod* 2001, vol 64, pp 1041-1047

Anthony LD, Lindsey JD, Weinreb RN, "Latanoprost effects on TIMP-1 and TIMP-2 expression in human ciliary muscle cells", *Invest Ophthalmol Vis Sci*, 2002; vol. 43, pp. 3705-3711.

Aragon-Martin JA, Ritch R, Liebmann J, O'Brien C, Blaaow K, Mercieca F, Spiteri A, Cobb CJ, Damiji KF, Tarkkanen A, Rezaie T, Child AH, Sarfarazi M, "Evaluation of *LOXL1* gene polymorphisms in exfoliation syndrome and exfoliation glaucoma", *Mol Vis*, 2008; vol. 14, pp. 533-541.

Armaly MF, "The genetic determinant of ocular pressure in the normal eye", *Arch Ophthalmol*, 1967; vol. 78, pp. 187-192.

Armaly MF, "Effect of corticosteroids on intraocular pressure and fluid dynamics. II. The effect of dexamethasone in the glaucomatous eye", *Arch Ophthalmol*, 1963, vol 70, pp 492-499.

Armitage P, "Test for linear trends in proportions and frequencies", *Biometrics*, 1955, vol 11, pp 375-386.

Arranz-Marquez E, Teus MA, Saomil MA, Mendez MC, Gil R, "Analysis of irises with latanoprost-induced change in iris color", *Am J Ophthalmol*, 2004, vol 138, pp 625-630

Asrani S, Zeimer R, Wilensky J, Gieser D, Vitale S, Lindenmuth K, "Large diurnal fluctuations in intraocular pressure are an independent risk factor in patients with glaucoma", 2000, *J Glaucoma*, vol. 9, pp. 134-142.

Astin M and Stjernschantz J, "Mechanism of prostaglandin E2-, F2alpha- and latanoprost acid-induced relaxation of submental veins", *Eur J Pharmacol*, 1997; vol. 340, pp. 195-201.

Aquino MV, Lat-Luna M, "The effect of latanoprost and timolol on intraocular pressure in patients with glaucoma and ocular hypertension", *Asian J Ophthalmol*, 1999, vol 1, pp3-7.

Aquino NA and Manalo JCP, "Post-marketing surveillance of the safety, tolerability, and efficacy of latanoprost (Xalatan^{TM}) for primary open angle glaucoma and ocular hypertension" Asian J Ophthalmol 2007; vol. 9: pp. 8-12

Aynacioglu AS, Cascobi I, Güngör K Ozkur M, Bekir N, Roots I, Brockmoller J, Population frequency, mutation linkage and analytical methodology for the Arg16Gly, Glu 27Gln and Thr164Ile polymorphisms in β_2 adrenergic receptor in Turks. J Clin Pharmacol 1999; 48: 761-764

Bagga H, Liu. JHK, Weinreb RN, "Intraocular pressure measurement throughout the 24hours", 2009, *Curr Opin Ophthalmol*, vol. 20, pp. 79-83.

Bahna SL and Bjerkedahl T, "Smoking and intraocular pressure", *Am J Ophthalmol*, 1948; vol. 31, pp. 1597.

Barraza RA, McLaren JW, Poeschla EM, "Prostaglandin pathway gene therapy for sustained reduction of intraocular pressure. Mol Ther", 2010; vol. 18, pp. 491-501

Baskaran M, Raman K, Ramani KK, Roy J, Vijaya L, Badrinath SS, "Intraocular pressure changes and ocular biometry during Sirsana (headstand posture) in yoga practitioners", *Ophthalmology*, 2006; vol. 113, no. 8, pp. 1327-1332.

Bateman DN, Clark R, Azuara-Blanco A, Bain M, Forrest J, "The effects of new topical treatments on management of glaucoma in Scotland: an examination of ophthalmological health care", *Br J Ophthalmol*, 2002; vol. 86, pp. 551-554.

Baun O, Heegaard S, Kessing SV, Prause JU, "The morphology of conjunctiva after long term topical anti-glaucoma treatment. A quantitative analysis", *Acta Ophthalmol Scand*, 1995; vol. 73, pp. 242-245.

Baudouin O, Hamard P, Liang H, Creuzot-Gharcer C, Bensoussan L, Brignole F, "Conjunctival epithelial cell expression of interleukins and inflammatory markers in glaucoma patients over the long term treatment", *Ophthalmology*, 2004; vol. 111, pp. 2186-2192.

Bayer A, Henderer JD, Kwak T, Myers J, Fontanarosa J, Spaeth GL, Clinical predictors of latanoprost treatment effect", J Glaucoma 2005; 14: 260-263

Bean GW, Camras CB,"Commercially available prostaglandin analogs for the reduction of intraocular pressure: similarities and differences", *Surv Ophthalmol*, 2008, vol 53 (suppl 1), pp S69-S84

Becker B, Kolker AE, Roth FD, "Glaucoma family study", *Am J Ophthalmol*, 1960; vol. 50, pp. 557-567.

Becquet F, Goldschild M, Moldovan MS, Ettaiche M, Gastaud P, Baudoin C, "Histopathological effects of topical ophthalmic preservative on rat conjunctival surface", *Curr Eye Res*, 1998; vol. 17, pp. 419-425.

Bengtsson B, Leske MC, Hyman L, Heijl A, Early Manifesto Glaucoma Trial Group, "Fluctuation of intraocular pressure and glaucoma progression in the Early Manifest Glaucoma Trial", 2007, *Ophthalmology*, vol. 114, pp. 205-209.

Bengttson B and Heijl A, "Diurnal fluctuation: not an independent risk factor for glaucomatous visual field loss in high-risk ocular hypertension", 2005, *Graefe Arch Clin Exp Ophthalmol*, vol. 243, pp. 513-518.

Betz R, Lagercrantz J, Kedra D, Dumanski JP, Nordenskjold A, "Genomic structure, 5' flanking sequences, and precise localization in 1P31.1 of the human prostaglandin F receptor gene", *Biochem Biophys Res Comm*, 1999; vol. 254, pp. 413-416.

Beiche F, Schuerer S, Brune K, Geisslinger G, Goppelt-Struebe M, "Up-regulation of cyclooxygenase-2 mRNA in the rat spinal cord following peripheral inflammation. FEBS Lett 1996; vol. 390, pp. 165-169

Bhopal R, "Spectre of racism in health and healthcare: Lessons from history and United States", *BMJ*, 1998, vol 316, pp 1970-1973

Bhopal R, "Is research into ethnicity and health racist, unsound, or important science?", *BMJ*, vol 314, pp 1751-1756

Bhopal R, Rankin J, "Concepts and terminology in ethnicity, race and health: be aware of the ongoing debate", *Br Dental J*, 1999, vol 186, pp 483-484

Bidichandani SI, Ashizawa T, Patel PI, “The GAA triplet repeat expansion in Friedreich ataxia interferes with transcription and may be associated with an unusual DNA structure”, *Am J Hum Genet*, 1998, vol 62, pp 111-121

Bigger JF, "Glaucoma with elevated episcleral venous pressure", *South Med J*, 1975; vol. 68, pp. 1444-1448.

Bito LZ, Camras CB, Gum GG, Resul B, "The ocular hypotensive effects and side effect of prostaglandins on the eyes of experimental animals", *Prog Clin Biol Res*, 1989; vol. 312, pp. 343-368.

Bito LZ, Stjernschantz J, “The Ocular Effects of prostaglandins and Other Eicosanoids. Bito LZ, Camras CB Gum CG Resul B. The ocular hypotensive effects and side effects of prostaglandins on the eyes of experimental animals. 349-368. 1989. New York, Alan R Liss. Serial (Book, Monograph)

Boger WP, "Short-term "escape" and long-term "drift'. The dissipation effects of the beta adrenergic blocking agents", *Surv Ophthalmol*, 1983; vol. 28, pp. 235-242.

Boger WP III, Steinert RF, Puliafito CA, Pavan-Langston D, "A double-masked clinical trial comparing timolol ophthalmic solution and pilocarpine in the therapy of open-angle glaucoma", *Am J Ophthalmol*, 1978; vol. 86, pp. 8-18.

Boger WP, Puliafito C, Steinert RF, Langston DP, "Long-term experience with timolol ophthalmic solution in patients with open-angle glaucoma", *Ophthalmology*, 1978; vol. 85, pp. 259-267.

Brandt JD, Beiser JA, Gordon MO, Kass MA, “Central corneal thickness and measured IOP response to topical ocular hypotensive medication in the ocular hypertension treatment study”, *Am J Ophthalmol*, 2004; vol. 138, pp. 717-22.

Broadway DC, Grierson I, O’Brien C, Hitching RA, "Adverse effects of topical antiglaucoma medication. I. The conjunctival cell profile", *Arch Ophthalmol*, 1994; vol. 112, pp. 1437-1445.

Broadway DC, Grierson I, O’Brien C, Hitching RA, "Adverse effects of topical antiglaucoma. II. The outcome of filtration surgery", *Arch Ophthalmol*, 1994; vol. 112, pp. 1446-1454.

Brodde OE, "Beta-adrenoceptor blocker treatment and cardiac beta-adrenoceptor-G-protein (s)-adenyl cyclase system in chronic heart failure", *Naunyn Schmiedebergs Arch Pharmacol*, 2007; vol. 374, pp. 361-372.

Brodde OE, "ß-1 and ß-2 adrenoreceptor polymorphisms: Functional importance, impact on cardiovascular diseases and drug responses", *Pharmacol Ther*, 2008; vol. 117, pp. 1-29.

Brody S, Erb C, Veit R, Rau H, "Intraocular pressure changes: the influence of psychological stress and the valsalva manoeuvre", *Biol Psychol*, 1999; vol. 51, pp. 43-57.

Bromberg BB, Gregory DS, Sears ML, “Beta-adrenergic receptors in ciliary processes of the rabbits”, *Invest Ophthalmol Vis Sci*, 1980; vol 19: pp203-207

Bron AM, Dennis P, Nordmann JP, Rouland JF, Sellem E, Johansson M, “Additive IOP-reducing effect of latanoprost in patients insufficiently controlled on timolol”, *Acta Ophthalmol Scand*, 2001, vol 79, pp 289-293

Bron AM, Creuzot-Garcher C, Goudeau-Boutillon S, D’Athis P, "Falsely elevated intraocular pressure due to increased central corneal thickness", *Graefes Arch Clin Exp Ophthalmol*, 1999; vol. 237, pp. 220-224.

Brubaker RF, "Flow of aqueous humour in humans", *Invest Ophthalmol Vis Sci*, 1991; vol. 32, no. 13, pp. 3145-3166.

Brubaker RF, "Delayed functional loss in glaucoma", *Am J Ophthalmol*, 1996; vol. 121, pp. 473-482.

Brubaker RF, Nagataki S, Townsend DJ, Burns RR, Higgins RG, Wentworth W, "The effect of age on aqueous humor formation in man", *Ophthalmology*, 1981; vol. 88, no. 3, pp. 283-288.

Bruck H, Leineweber K, Beifulb A, Weber M, Heusch G, Phillipp T, Brodde O-E, "Genotype-dependent time course of lymphocyte beta2-adrenergic receptor down-regulation", *Clin Pharmacol Ther*, 2003; vol. 74, no. 3, pp. 255-263.

Bruck H, Leineweber K, Buscher R, Ulrich A, Radke J, Insel PA, Brodde OE, "The Gln27Glu $ß_2$-adrenoceptor polymorphism slows the onset of desensitization of cardiac functional responses in vivo", *Pharmacogenetics* , 2003; vol. 13, pp. 59-66.

Bucci MG, Italian Latanoprost Study Group, “Intraocular pressure-lowering effects of latanoprost monotherapy versus latanoprost or pilocarpine in combination with timolol: a randomized, observer-masked multicenter study in patients with open-angle glaucoma. Italian Latanoprost Study Group”, *J Glaucoma*, 1999, vol 8, pp 24-30.

Buckingham T and Young R, "The rise and fall of intra-ocular pressure: the influence of physiological factors", *Ophthalmic Physiol Opt*, 1986; vol. 6, pp. 95-99.

Bulpitt CJ, Hodes C, Everitt MG, "Intraocular pressure and systemic blood pressure in the elderly", *Br J Ophthalmol*, 1975; vol. 59, pp. 717-720.

Büscher R, Eilmes KJ, Grasemann H, "$ß_2$ adrenoreceptor gene polymorphisms in cystic fibrosis lung disease", *Pharmacogenetics*, 2000; vol. 12, pp. 347-354.

Camras CB, Schumer RA, Marsk A, Lustgarten JS, Serle JB, Stjernschantz J, Bito LZ, Podos SM, ”Intraocular pressure with PhXA34, a new prostaglandin analogue, in patients with ocular hypertension” Arch Ophthalmol 1992; 110: 1733-8

Camras CB, Hedman K, US Latanoprost Study Group, "Rate of response to latanoprost or timolol in patients with ocular hypertension or glaucoma", *J Glaucoma*, 2003; vol. 12, pp. 466-469.

Caprioli J, “Intraocular pressure fluctuation: an independent risk factor for glaucoma?”, *Arch Ophthalmol*, 2007; vol.125, pp. 1124-1125

Caprioli J and Coleman AL, "Intraocular pressure fluctuation. A risk factor for visual field progression at low intraocular pressures in the Advanced Glaucoma Intervention Study", 2008, *Ophthalmology*, vol. 115, pp. 1123-1129.

Caprioli J, Garway-Heath DF & International Glaucoma Think Tank, "A critical revaluation of current glaucoma management: International Glaucoma Think Tank, July 27-29, 2006, Taormina, Sicily", *Ophthalmology*, 2007; vol. 114 (Suppl 11), pp. S1-41.

Caprioli JC, Sears M, Bausher L, Gregory D, Mead A, "Forskolin lowers intraocular pressure by reducing aqueous outflow", *Invest Ophthalmol Vis Sci*, 1984; vol. 25, pp. 268-277.

Carel RS, Korczyn A, Rock M, Goya I, "Association between ocular pressure and certain health parameters", *Ophthalmology*, 1984; vol. 91, pp. 311-314.

Chakrabarti S, Rao KN, Kaur I, Parikh RS, Mandal AK, Chandrasekhar G, Thomas R, "The *LOXL1* gene variations are not associated with primary open-angle and primary angle-closure glaucomas", *Invest Ophthalmol Vis Sci*, 2008; vol. 49, pp. 2343-2347.

Chakraborty R, Jin L,"Heterozygote deficiency, population substructure and their implications in DNA fingerprinting", *Hum Genet*, 1992, vol 88, pp 267-72

Chandrasekaran S, Rochtchina E, Mitchell P, "Effects of caffeine on intraocular pressure: the Blue Mountains Eye Study", *J Glaucoma*, 2005; vol. 14, pp. 504-507.

Chen D-B, Westfall S, Fong HW, Roberson MS, Davis JS, "Prostaglandin $F_{2\alpha}$ stimulates the Raf/MEK1/mitogen-activated protein kinase signalling cascade in bovine luteal cells", *Endocrinology*, 1998; vol. 139, pp. 3876-3885.

Chen D, Fong HW, Davis JS, "Induction of c-*fos* and c-*jun* messenger ribonucleic acid expression by prostaglandin $F_{2\alpha}$ is mediated by a protein kinase C-dependent extracellular signal-regulated kinase mitogen-activated protein kinase pathway in bovine luteal cells", *Endocrinology*, 2001; vol. 142, pp. 887-895.

Chen DT, Jiang X, Akula N, Shugart YY, Wendand JR, Steele CJ, Kassem L, Park JH, Chatterjee N, Jamain S, Cheng A, Leboyar M, Muglia P, Schulze TG, Cichon S, Nothem MM, Rietschel M, Bigs, McMahon FJ, "Genome-wide association study meta-analysis of European and Asian-ancestry samples identifies three novel loci associated with bipolar disorder", 2011; vol ; pp1-11

Cheong MT, Kodisvary M, Selva Raja V, Azhany Y, Zilfalil BA, Liza-Sharmini AT, "Response to latanoprost monotherapy among Malay patients with glaucoma", Asian J Ophthalmol, 2008; 10: 118-22

Chew PT, Aung T, Aguino MV, Rojanapongpun P, Exact Study Group, "Intraocular pressure-reducing effects and safety of latanoprost versus timolol in patients with chronic angle-closure glaucoma", *Ophthalmology*, 2004; vol 111, pp. 427-434.

Chiba T, Kashiwagi K, Ishijima K, Furuichi M, Kogure S, Abe K, Chiba N, Tsukahara S, "A prospective study of iridial pigmentation and eyelash changes due to ophthalmic treatment with latanoprost", *Jpn J Ophthalmol*, 2004; vol. 48, pp. 141-147.

Chieng LL, Ong LB, Cheong MT, Vengadasalam SR, Ng GL, Tajudin LSA, " Visual impairment and disc cupping among newly diagnosed patients with glaucoma", *Asian J Ophthalmol* 2005, vol 7, pp 140-145.

Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jones DW, Materson BJ, Oparil S, Wright JT, Roccella EJ, The National High Blood pressure Education Program Coordinating Committee, "Seventh report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure", *Hypertension*, 2003; vol. 42, pp. 1206-1252.

Chong LK, Chowdry J, Ghahramani P, Peachell PT, "Influence of genetic polymorphisms in the beta2-adrenoceptor on desensitization in human lung mast cells", *Pharmacogenetics*, 2000; vol. 10, pp. 153-162.

Chou SY, Chou C-K, Kuang TM, Hsu WM, "Incidence and severity of iris pigmentation on latanoprost-treated glaucoma eyes", *Eye*, 2005; vol. 19, pp. 784-787.

Coakes RL and Brubaker RF, "The mechanism of timolol in lowering intraocular pressure", *Arch Ophthalmol*, 1978; vol. 96, pp. 2045-2048.

Coffey M, Reidy A, Wormald R, Xian WX, Wright L, Coutrney P, "Prevalence of glaucoma in the west Ireland", *Br J Ophthalmol*, 1993; vol. 77, pp. 17-21.

Coleman AL, Diehl DL, Jampel HD, Bachorik PS, Quigley HA, "Topical timolol decreases plana-high-density lipoprotein cholesterol level", *Arch Ophthalmol*, 1990; vol. 108, pp. 1260-1263.

Collaborative normal-tension glaucoma study group, "The effectiveness of intraocular pressure reduction in the treatment of normal-tension glaucoma", 1998, *Am J Ophthalmol*, vol. 126, pp. 498-505.

Collaborative normal-tension glaucoma study group, "The Collaborative Normal-Tension Glaucoma Study. Comparison of glaucomatous progression between untreated patients with normal tension glaucoma and patients with therapeutically reduced intraocular pressure", *Am J Ophthalmol* , 1998; vol. 126, pp. 487-497.

Congdon NG, Broman AT, Bandeen-Roche K, Grover D, Quigley HA, "Central corneal thickness and corneal hysteresis associated with glaucoma damage", 2006, *Am J Ophthalmol*, vol. 141, pp. 868-875.

Copin B, Brezin AP, Valtot F, Dascotte JC, Bechetoille A, Garchon H-J, "Apolipoprotein E-promoter single-nucleotide polymorphisms affect the phenotype of primary open-angle glaucoma and demonstrate interaction with the Myocilin gene", *Am J Hum Genet*, 2002; vol. 70, pp. 1575-1581.

Cornes BK, Khor CC, Nongpiur ME,Xu L, Tay W-T, Zheng Y, Lavanya R, Li Y, Wu R, Sim X, Wang YX, Chen P, Teo YY, Chia K-S, Seielstad M, Liu J, Hibberd ML, Cheng C-Y, Saw S-M, Tai E-S, Jonas JB, Vithana EN, Wong TY, Aung T, 'Identification of four novel variants that influence central corneal thickness in multi-ethnic Asian populations", Hum Mol Genet, 2012; vol 21: pp437-445

Cracknell K and Grierson I, "Prostaglandin analogues in the anterior eye: Their pressure lowering action and side effects", *Exp Eye Res*, 2009; vol. 88, pp. 786-791.

Cracknell KP, Grierson I, Hogg P, Appleton P, Pfeiffer N, "Latanoprost-induced iris darkening: a morphometric study of human peripheral iridectomies", *Exp Eye Res*, 2003; vol. 77, pp. 721-730.

Craig JE, Baird PN, Healey DL, McNaught AL, McCartney PJ, Rait JL, Dickinson JL, Reo L, Fingert JH, Stone EM, Mackey DA, "Evidence for genetic heterogeneity within eight glaucoma families, with the GLC1A Gln368STOP mutation being an important phenotype modifier", *Ophthalmology*, 2001; vol. 108, pp. 1607-1620.

Cristiansson J, "Intraocular pressure in diabetes mellitus', Acta Ophthalmol (Copenh), vol 39, pp. 155-167

Crowston JG, Lindsey J. Aihara M, Weinreb RN, "Effect of latanoprost on intraocular pressure in mice lacking the prostaglandin FP receptor", *Invest Ophthalmol Vis Sci*, 2004; vol. 45, pp. 3555-3559.

Damji KF, Behki R, Wang L, "Target IOP workshop participants. Canadian perspectives in glaucoma management: setting target intraocular pressure range", *Can J Ophthalmol*, 2003; vol. 38, pp. 189-197.

Dandona L, Dandona R, Srinivas M, Mandal P, John RK, McCarty CA, Rao GN, "Open-angle glaucoma in an urban population in Southern India. The Andhra Pradesh Eye Disease Study", *Ophthalmology*, 2000; vol. 107, pp. 1702-1709.

Dasgupta S, Oates V, Bookhart BK, Vaziri B, Schwartz GF, Mozaffari E, "Population-based persistency rates for topical glaucoma medications measured with pharmacy claims data", *Am J Manag Care*, 2002; vol. 8 (Suppl), p. S255-S256

David R, Zangwill L, Briscoe D, Dagan M, Yagev R, Yassur Y, "Diurnal intraocular pressure variations: an analysis of 690 diurnal curves", 1992, *Br J Ophthalmol*, vol. 76, pp. 280-283.

David R, Zangwill LM, Tessler Z, Yassur Y, "The correlation between intraocular pressure and refractive status", *Arch Ophthalmol*, 1985; vol. 103, pp. 1812-1815.

Davis JS, Weakland LL, Weiland DA, Farese RV, West LA, "Prostaglandin $F_{2\alpha}$ stimulates phosphatidylinositol 4,5-biphosphate hydrolysis and mobilizes intracellular calcium in bovine luteal cells", *Proc Natl Acad Sci USA*, 1987; vol. 84, pp. 3728-3732.

De Saint Jean M, Brignole F, Bringuier AF, Bauchet A, Feldmann G, Baudoin C, "Effects of benzalkonium chloride on growth and survival of Chang conjunctival cells", *Invest Ophthalmol Vis Sci*, 1999; vol. 40, pp. 619-630.

Debbasch C, Pisella PJ, De Saint Jean M, rat P, Warnet JM, Baundoin C, "Mitochondrial activity and glutathione injury in apoptosis induced by unpreserved and preserved beta-blockers on Chang conjunctival cells", *Invest Ophthalmol Vis Sci*, 2001; vol. 42, pp. 2525-2533.

de Castro DK, Punjabi OS, Bostrom AG, Stamper RL, Lietman TM, Ray K, Lin SC, “Effect of Statin drugs and aspirin on progression in open-angle glaucoma suspects using confocal scanning laser ophthalmology”. *Clin Exp Ophthalmol*, 2007; vol 35: 506-513

Deokule S and Weinreb RN, "Relationships among systemic blood pressure, intraocular pressure, and open-angle glaucoma", 2008, *Can J Ophthalmol*, vol. 43, pp. 302-307.

Department of Statistics, Malaysia, 2000. http://www.statistics.gov.my

Dewar JC, Wheatley AP, Venn A, Morrison JF, Britton J, Hall IP, “Beta2-adrenoreceptor polymorphisms are in linkage disequilibrium, but are not associated with asthma in adult population”, *Clin Exp Allergy*, 1998, vol 28, pp 442-448

Devlin B, Roeder K, “Genomic control for association studies”, *Biometric*, 1999, vol 55, pp 997-1004.

Dickstein K, Hapnes R, Aarsland T, "Comparison of aqueous and gellan ophthalmic timolol with placebo on the 24-hour heart rate response in patients on treatment for glaucoma", 2001, *Am J Ophthalmol*, vol. 132, pp. 626-631.

Dielemans I, Vingerlin JR, Wolfs RC, Hofman A, Grobbee DE, de Jong PT, "The prevalence of primary open-angle glaucoma in a population-based study in The Netherlands. The Rotterdam study", *Ophthalmology*, 1994; vol. 101, pp. 1851-1855.

Dielemans I, Vingerlin JR, Algra D, Hofman A, Grobbee DE, de Jong PT, "Primary open-angle glaucoma, intraocular pressure, and systemic blood pressure in the general elderly population. The Rotterdam Study", *Ophthalmology*, 1995; vol. 102, pp. 54-60.

Diggory P and Franks WA, "Glaucoma therapy may take your breath away", 1997, *Age and Ageing*, vol. 26, pp. 63-67.

Diggory P, Cassel-Brown A, Vail A, Hillman JS, "Randomised, controlled trial of spirometric changes in elderly people receiving timolol or betaxolol as initial treatment for glaucoma", *Br J Ophthalmol*, 1998; vol. 82, pp. 146-149.

Diggory P, Heyworth P, Chau Geordon Mckenzie S, Sharma A, "Unsuspected bronchospasm in association with topical timolol- a common problem in elderly people: can we easily identify those affected and do cardioselective agents lead to improvement", *Age and Ageing*, 1994; vol. 23, pp. 17-21.

Dishy V, Sofowora GG, Xie HG, Kim RB, Byrne DW, Stein CM, Wood AJ, “The effect of common polymorphisms of the beta2-adrenergic receptor on agonist-mediated vascular desensitization”. *N Engl J Med*, 2001; vol 345, pp1030-5

Doshi V, Ying-Lai M, Azen SP, Varma R, Los Angeles Latino Eye Study Group, "Sociodemographic, family history, and lifestyle risk factors for open-angle glaucoma and ocular hypertension. The Los Angles Latino Eye Study", 2008; *Ophthalmology*, vol. 115, pp. 639-647.

Doughty MJ and Zaman ML, "Human corneal thickness and its impact on intraocular pressure measures: a review and meta-analysis approach", *Surv Ophthalmol*, 2003; vol. 44, pp. 367-408.

Drago F, Marino A, La Manna C, "Alpha-methyl-p-tyrosine inhibits latanoprost-induced melanogenesis in vitro", *Exp Eye Res*, 1978; vol. 17, pp. 511-514.

Drance SM, "The significance of the diurnal tension variations in normal and glaucomatous eyes", *Arch Ophthalmol*, 1960; vol. 64, pp. 494-501.

Drance SM, "Diurnal variation of intraocular pressure in treated glaucoma", *Arch Ophthalmol*, 1963; vol. 70, pp. 302-311.

Drance SM, "Some factor in the production of low tension glaucoma", Br J Ophthalmol, 1972; vol.56, pp. 229-242.

Drysdale CM, McGraw DW, Stack CB, Stephens JC, Judson RS, Nandabalan K, Arnold K, Ruano G, Liggett SB, "Complex promoter and coding region ß$_2$-adrenergic receptor haplotypes alter receptor expression and predict in vivo responsiveness", *Proc Natl Acad Sci USA*, 2000; vol. 97, pp. 10483-10488.

Duke-Elder WS 1941, *Textbook of Ophthalmology* Mosby-Year Book Inc, St Louis, Mo.

Duncan AM, Anderson LL, Funk CD, Abramovitz M, Adam M, "Chromosomal localization of the human prostanoids receptor gene family", *Genomics*, 1995; vol. 25, pp. 740-742.

Dunham CN, Spaide RF, Dunham G, "The contralateral reduction of intraocular pressure by timolol", *Br J Ophthalmol*, 1994; vol. 78, pp. 38-40.

Edeki TI, He H, Wood AJ,"Pharmacogenetic explanation for excessive beta-blockade following timolol eye drops. Potential for oral-ophthalmic drug interaction", *JAMA*, 1995, vol 274, pp 1611-1613

Ehlers N, Bransem T, Sperling S, "Applanation tonometry and central corneal thickness", *Acta Ophthalmol (Copenh)*, 1975; vol. 53, pp. 34-43.

Ehlers N, Hansen FK, "Central corneal thickness in low-tension glaucoma", *Acta Ophthalmol*, 1974; vol 52, pp740-6.

Eichelbaum M, Ingelman-Sundberg M, Evans WE, "Pharmacogenomics and individualized drug therapy", *Annu Rev Med*, 2006; vol. 57, no. 119, p. 137.

Emiru VP, "Response to mydriatics in the African", *Br J Ophthalmol*, 1971; vol. 55, pp. 538-543.

Era P, Parssinen O, Kallinen M, Suominen H, "Effect of bicycle ergometer test on intraocular pressure in elderly athletes and controls", *Acta Ophthalmol (Copenh)*, 1993; vol. 71, no. 3, pp. 301-307.

Erickson-Lamy KA, Nathanson JA,"Epinephrine increases facility of outflow and cyclic AMP content in the human eye in vitro'. *Invest Ophthalmol Vis Sci*, 1992; vol 33: pp2672-2678

European Glaucoma Prevention Study (EGPS) group, "Results of European Glaucoma Prevention Group", *Ophthalmology*, 2005; vol. 112, pp. 366-375.

European Glaucoma Preventive Study (EGPS) Group, "Predictive factors for open-angle glaucoma among patients with ocular hypertension in the European Glaucoma Preventive Study", *Ophthalmology*, 2007; vol. 114, pp. 3-9.

European Glaucoma Society 2008, "Treatment Principles and Options," in *Terminology and guidelines for glaucoma*, 3rd edition, European Glaucoma Society, ed., European Glaucoma Society, Savona, Italy, pp. 117-169.

Evans DAP, "N-Acetyltransferase", *Pharmacol Ther*, 1989; vol. 42, pp. 157-234.

Evans WE, McLeod HI, "Pharmacogenomics- drug disposition, drug targets, and side effects." *N Engl J Med*, 2003; vol. 348, pp. 538-549.

Evans WE and Relling MV, "Moving towards individualized medicine with pharmacogenomics", *Nature*, 2004; vol. 429, pp. 464-468.

Feinstein AR, "On white-coat effects and the electronic monitoring of compliance", *Arch Intern-Med*, 1990; vol. 150, pp. 1377-1378.

Feldman RM, "Conjunctival hyperaemia and the use of topical prostaglandins in glaucoma and ocular hypertension", *J Ocul Pharm Ther*, 2003, vol 19, pp 23-35.

Feltgen N, Leifert D, Funk J, "Correlation between central corneal thickness, applanation tonometry, and direct intracameral IOP readings", *Br J Ophthalmol*, 2001; vol. 85, pp. 85-87.

Fisher RA. The genetical theory of natural selection. Clarendon Press, Oxford, UK. 1930

Fingert JH, Heon E, Liebmann JM, Yamamoto T, Craig JE, Rait J, Kawase K, Hoh ST, Buys YM, Dickinson J, Hockey RR, Williams-Lyn D, Trope-G, Kitazawa Y, Ritch R, Mackey DA, Alward WL, Sheffield VC, Stone EM, "Analysis of Myocilin mutations in 1703 glaucoma patients from five different populations", *Hum Mol Genet*, 1999; vol. 8, pp. 899-905.

Flammer J, Haefliger IO, Orgul S, Resink T, "Vascular dysregulation: a principal risk factor for glaucomatous damage?" *J Glaucoma* , 1999; vol. 8, pp. 212-219.

Flammer J, Orgul S, Costa VP, Orzalesi N, Krieglstein GK, Serra LM, Renard JP, Steffanson E, "The impact of ocular blood flow in glaucoma", *Prog Retina Eye Res*, 2002; vol. 21, pp. 359-393.

Fogagnolo P, Rossetti L, Mazzolani F, Orzalesi N, "Circadian variations in central corneal thickness and intraocular pressure in patients with glaucoma", *Br J Ophthalmol*, 2006; vol. 90, pp. 24-28.

Foord SM, Bonner TI, Neubig RR, Rosser EM, Pin JP, Davenport AP, Spedding M, Harmar AJ, "International Union of Pharmacology. XLVI. G protein-coupled receptor list", *Pharmacol Ther*, 2005; vol. 57, no. 2, pp. 278-288.

Forbes M, Pico G, Grolman B, "A noncontact applanation tonometer. Description and clinical evaluation", *Arch Ophthalmol*, 1974; vol. 91, pp. 134-140.

Ford EB, "Polymorphism and taxonomy," in *The New Systematic*, Huxley J, ed., Clarendon Press, Oxford, 1940; pp. 493-573.

Foster PJ, Johnson GJ, "Glaucoma in China; how big is the problem?" *Br J Ophthalmol*, 2001; vol. 85, pp. 1277-1282.

Foster PJ, Baasanhu J, Alsbirk PH, Munkhbayar D, Uranchimeg D, Johnson GJ, "Glaucoma in Mongolia. A population-based survey in Hövsgöl province, Northern Mongolia", *Arch Ophthalmol*, 1996; vol. 114, pp. 1235-1241.

Foster PJ, Baasanhu J, Alsbirk PH, Munkhbayar D, Uranchimeg D, Johnson GJ, "Central corneal thickness and intraocular pressure in a Mongolian population", *Ophthalmology*, 1998; vol. 105, pp. 969-973.

Foster PJ, Buhrmann R, Quigley HA, Johnson GJ, "The definition and classification of glaucoma in prevalence survey", *Br J Ophthalmol*, 2002; vol. 86, pp. 238-242.

Foster PJ, Machin D, Wong TY, Ng TP, Kirwan JF, Johnson GJ, Khaw PT, Seah SKL "Determinants of intraocular pressure and its association with glaucomatous optic neuropathy in Chinese Singaporeans: the Tanjong Pagar Study", 2003, *Invest Ophthalmol Vis Sci*, vol. 44, pp. 3885-3891.

Foster PJ, Oen FT, Machin D, Ng TP, Devereux JG, Johnson GJ, Khaw PT, Seah SK, "The prevalence of glaucoma in Chinese residents of Singapore. A cross-sectional population survey of the Tanjong Pagar District", *Arch Ophthalmol*, 2000; vol. 118, pp. 1105-1111.

Foster PJ, Alsbirk PH, Baasanhu J, Munkhbayar D, Uranchimeg D, Johnson JG, "Anterior chamber depth in Mongolians: variation with age, sex, and method of measurement", *Am J Ophthal*, 1997; vol.124(1), pp. 53-60.

Francis BA, Hsieh A, Lai MY, Chopra V, Pena F, Azen S, Varma R, Los Angeles Latino Eye Study Group, "Effects of corneal thickness, corneal curvature, and intraocular pressure level on Goldmann applanation tonometry and dynamic contour tonometry", *Ophthalmology*, 2007; vol. 114, pp. 20-26.

Francois J, "Genetics and primary open-angle glaucoma", *Am J Ophthalmol*, 1966; vol. 61, pp. 652-665.

Fredholm BB, Hohfelt T, Milligan G, "G-protein-coupled receptors: an update", *Acta Physiol*, 2007; vol. 190, no. 1, pp. 3-7.

Freedman ML, Reich D, Penny KL, McDonald GJ, Mignault AA, Patterson N, Gabriel SB, Topol EJ, Smoller JW, Pato CN, Pato MT, Petryshen TL, Kolonel LN, Lander ES, Sklar P, Henderson B, Hirschhorn JN, Altshuler D, "Assessing the impact of population stratification on genetic association studies", *Nature Genetics*, 2004; vol. 36, no. 4, pp. 388-393.

Friberg TR, Sanborn G, Weinreb RN, "Intraocular and episcleral venous pressure during inverted posture", *Am J Ophthalmol*, 1987; vol. 103, pp. 523-526.

Friedenwald JS, "Contribution to the theory and practice of tonometry", *Am J Ophthalmol*, 1937; vol. 20, pp. 985-1024.

Friedenwald JS, Becker B, "Aqueous humor dynamics", *Arch Ophthalmol*, 1955; vol. 54, p. 799.

Friedman DS, Hahn SR, Gelb L, Tan J, Shah SN, Kim EE, Zimmerman TJ, Quigley HA, "Doctor-patient communication and health-related beliefs: Results from the Glaucoma Adherence and Persistency Study (GAPS)", *Ophthalmology*, 2008; vol. 115, pp. 1320-1327.

Friedman DS, Quigley HA, Gelb L, Tan J, Margolis J, Shah SN, Kim EE, Zimmerman T, Hahn SR, "Using pharmacy claims data to study adherence to glaucoma medications methodology of the Glaucoma Adherence and Persistency Study (GAPS)", *Invest Ophthalmol Vis Sci*, 2007; vol. 48, pp. 5052-5057.

Friedman DS, Wolfs RC, O'Colmain BJ, "Eye Diseases Prevalence Research Group. Prevalence of open-angle glaucoma among adults in the United States", *Arch Ophthalmol*, 2004; vol. 122, pp. 532-538.

Fuchsjager-Maryl G, Markovic O, Losert D, Lucas T, Wachek V, Muller M, Schmetterer, "Polymorphism of the β-2 adrenoreceptor and IOP lowering potency of topical timolol in healthy subjects", *Mol Vis*, 2005; 11: 811-5.

Fuchsjager-Maryl G, Wally B, Georgopoulos M, Rainer G, Kircher K, Buehl W, Amoako-Mensah T, Eichler HG, Vass C, Schmetterer L, "Ocular blood flow and systemic blood pressure in patients with primary open-angle glaucoma and ocular hypertension", *Invest Ophthalmol Vis Sci*, 2004; vol. 45, pp. 834-9.

Fujita S, "Diurnal variation in human corneal thickness", *Jpn J Ophthalmol*, 1980; vol. 24, pp. 444-456.

Funayama T, Ishikawa K, Ohtake Y, Tanino T, Kurosaka D, Kimura I, Suzuki K, Ideta H, Nakamoto K, Yasuda N, Fujimaki T, Murukami A, Asaoka R, Hotta Y, Tanihara H, Kanamoto T, Mishima H, Fukuchi T, Abe H, Iwata T, Shimada N, Kudoh J, Shimizu N, Mashima Y, "Variants in Optineurin gene and their association with tumor necrosis factor-alpha polymorphisms in Japanese patients with glaucoma", *Invest Ophthalmol Vis Sci*, 2004; vol. 45, pp. 4359-4367.

Funayama T, Masima Y, Ohtake Y, Ishikawa K, Fuse N, Yasuda N, Fukuchi T, Murakami A, Hotta Y, Shimada N, Glaucoma Gene Research Group, "SNPs and interaction analyses of noelin 2, myocilin and optineurin genes in Japanese patients with open-angle glaucoma", *Invest Ophthalmol Vis Sci*, 2006; vol. 47, pp. 5368-5375.

Fuse N, Takahashi K, Akiyama H, Nakazawa T, Seimiya M, Kuwahara S, Tamai M, "Molecular genetic analysis of optineurin gene for primary open-angle and normal tension glaucoma in the Japanese population", *J Glaucoma*, 2004; vol. 13, pp. 299-303.

Gabelt BT, Gottanka J, Lütjen-Drecoll E, Kaufman PL, "Aqueous humor dynamics and trabecular meshwork and anterior ciliary muscle morphologic changes with age in rhesus monkeys", *Invest Ophthalmol Vis Sci*, 2003; vol. 44, no. 5, pp. 2118-2125.

Galin MA, McIver JW, Magruder GB, "Influence of position on intraocular pressure", *Am J Ophthalmol*, 1963; vol. 55, pp. 720.

Ghanouni P, Gryczynski Z, Steenhuis JJ, Lee TW, Farrens DL, Lakowicz JR, Kobilka BK, "Functionally different agonists induce distinct conformations in the G protein coupling domain of the beta 2 adrenergic receptor", *J Biol Chem*, 2001; vol. 276, pp. 24433-24436.

Giuffre G, "The effects of prostaglandin $F_{2\alpha}$ in the human eye", *Graefe's Arch Clin Exp Ophthalmol*, 1985; vol. 222, pp. 139-141.

Gibson JM, Rosenthal AR, Lavery J, "A study of the prevalence of eye disease in the elderly in an English community", *Trans Ophthalmol Soc UK*, 1985, vol 104 (Pt 2), pp 196-203

Glaucoma Laser Trial Research Group, "The Glaucoma Laser Trial (GLT) and Glaucoma Laser Trial Follow-up Study, 7.results", *Am J Ophthalmol*, 1995; vol. 120, pp. 718-731.

Glaucoma panel, *The Preferred Practice Pattern for Primary Open-Angle Glaucoma* San Francisco , CA, 1989.

Goldberg I,"Relationship between intraocular pressure and preservation of visual field in glaucoma", *Surv Ophthalmol*, 2003, vol. 48, no. Suppl 1, pp. 3-7.

Goldberg I, Adena MA, "Co-prescribing of topical and systemic beta-blockers in patients with glaucoma: a quality use of medicine issue in Australian practice", *Clin Experiment Ophthalmol*, 2007, vol 35, pp 700-705.

Goldmann H, Schmidt TH, "Über applanationstonometrie", *Ophthalmologica*, 1957; vol. 134, pp. 221-242.

Gordon MO, Beiser JA, Brandt JD, Heuer DK, Higginbotham EJ, Johnson CA, Keltner JL, Miller JP, Parrish RK, Wilson MR, Kass MA, The Ocular Hypertension Treatment Study Group, "The ocular hypertension treatment study. Baseline factors that predict the progression of primary open-angle glaucoma", *Arch Ophthalmol*, 2002; vol. 120, pp. 714-720.

Grant DM, "Molecular genetics of the N-acetyltransferase", *Pharmacogenetics*, 1993; vol. 3, pp. 45-50.

Greenfield DS, "Glaucoma associated with elevated episcleral venous pressure", *J Glaucoma*, 2000; vol. 9(2), pp.190-4.

Gasser P, Flammer J, "Blood cell velocity in the nail fold capillaries of patients with normal-tension and high tension glaucoma", *Am J Ophthalmol*, 1991; vol. 111, pp. 585-8.

Green CM, Kaerns LS, Wu J, Barbour JM, Wilkinson RM, Ring MA, Craig JE, Wong TL, Hewitt AW, Mackey DA, "How significant is a family history of glaucoma? Experience from the Glaucoma Inheritance Study in Tasmania (GIST)", *Clin Experiment Ophthalmol*, 2007; vol. 35, pp. 793-799.

Green K, "Marijuana smoking vs. cannabinoids for glaucoma therapy", *Arch Ophthalmol*, 1998; vol. 116, no. 11, pp. 1433-1437.

Green SA, Cole G, Jacinto M, Innis M, Liggett SB, "A polymorphism of the human beta2-adrenergic receptor within the fourth transmembrane domain alters ligand binding and functional properties of the receptor", *J Biol Chem*, 1993; vol. 268, no. 31, pp. 23116-23121.

Green SA, Turki J, Innis M, Liggett SB, "Amino-terminal polymorphisms of the human beta2-adrenergic receptor impart distinct agonist-promoted regulatory properties", *Biochemistry*, 1994; vol. 33, pp. 9414-9.

Green SA, Turki J, Bejarano P, Hall IP, Liggett SB, "Influence of beta2-adrenergic receptor genotypes on signal transduction in human airway smooth muscle cells", *Am J Respir Cell Moll Biol*, 1995; vol. 13, pp. 25-33.

Green SA, Turki J, Innis M, Liggett SB, "Amino-terminal polymorphisms of the human beta2-adrenergic receptor impart distinct agonist-promoted regulatory properties", *Biochemistry*, 1994; vol. 33, pp. 9414-9419.

Güngör K, Ozkur M, Cascorbi I, Brockmoller J, Bekir N, Roots I, Aynacioglu AS, "Beta2-adrenergic receptor polymorphism and susceptibility to primary congenital and primary open angle glaucoma", *Eur J Clin Pharmacol*, 2003; vol. 59, pp. 527-31

Gusovsky F, "Prostaglandin receptor in NIH 3T3 cells: coupling of one receptor to adenyl cyclase and of a second receptor to phospholipase C", *Mol Pharmacol*, 1991; vol. 40, pp. 633-638.

Hahntow IN, Koopmans RP, Micheal MC, "The ß$_2$-adrenoceptor gene and hypertension: is it the promoter or the coding region or neither?", *J Hypertens*, 2006; vol. 24, pp. 1003-1007.

Hansen KF, Ehlers N, "Elevated tonometer readings caused by a thick cornea", *Acta Ophthalmol*, 1971; vol. 49, pp. 775-778.

Hata AN, Breyer RM, "Pharmacology and signaling of prostaglandin receptors: Multiple roles in inflammation and immune modulation", *Pharmacol Ther*, 2004; vol. 103, pp. 147-166.

Hauser MA, Allingham RR, Linkroum K, Wang J, LaRocque-Abramson K, Figueiredo D, Santiago-Turla C, del Bono EA, Haines JL, Pericak-Vance MA, Wiggs JL, "Distribution of WDR36 DNA sequence variants in patients with primary open-angle glaucoma", *Invest Ophthalmol Vis Sci*, 2006; vol. 47, pp. 2542-2546.

Hay A, Wood S, Olson D, Slater DM, "Labour is associated with decreased expression of the $PGF_{2\alpha}$ receptor (PTGFR) and a novel PTGFR splice variant in human myometrium but not deciduas", *Mol Hum Reprod*, 2010; vol. 16, pp 752-760

He M, Foster PJ, Ge J, Huang W, Zheng Y, Friedman DS, Lee PS, Khaw PT, "Prevalence and clinical characteristics of glaucoma in adult Chinese: a population-based study in Liwan District, Guangzhou", *Invest Ophthalmol Vis Sci*, 2006; vol. 47, pp. 2782-2788.

Hedman K and Larsson LI, "The effect of latanoprost compared with timolol in African-American, Asian, Caucasian and Mexican open-angle glaucoma or ocular hypertensive patients", *Surv Ophthalmol*, 2002; vol. 47 (Suppl 1), p. S77-S89.

Hedman K, Watson PG, Alm A, "The effect of latanoprost on intraocular pressure during 2 years of treatment", *Surv Ophthalmol*, 2002; vol. 47 (Suppl 1), p. S65-S76.

Hedner J, Svedmyr N, Lunde H, Mandahl A, "The lack of respiratory effects of the ocular hypertensive drug latanoprost in patients with moderate asthma", *Surv Ophthalmol*, 1997; vol. 41 (Suppl 2), pp. S111-S115.

Heijl A, Leske MC, Bengtsson B, Hyman L, Bengtsson B, Hussein M. 2002, "Reduction of intraocular pressure and glaucoma progression. Results from the Early Manifest Glaucoma Trial", *Arch Ophthalmol*, vol. 120, pp. 1268-1279.

Hennis A, Wu SY, Nemesure B, Leske MC, Barbados Eye Studies Group, "Hypertension, diabetes, and longitudinal changes in intraocular pressure", *Ophthalmology*, 2003; vol. 110, no. 5, pp. 908-914.

Hepler RS, Frank IR, "Marihuana smoking and intraocular pressure", *JAMA*, 1971; vol. 217, no. 10, p. 1392.

Helper RS, Frank IM, Petrus R, "The ocular effects of marihuana smoking", in the Pharmacology of Marihuana (eds: Braude MC, Szara S). New York: Raven Press.

Herndon LW, Weizer JS, Stinnett SS, "Central corneal thickness as risk factor for advanced glaucoma damage", *Arch Ophthalmol*, 2004; vol 122; pp 17-21

Herndon LW, Williams RD, Wand M, Aarani S, "Increased periocular pigmentation with ocular hypotensive lipid use in African Americans", *Am J Ophthalmol*, 2003; vol. 135, pp. 713-715.

Herrmann SM, Nicaud V, Tiret L, Evans A, Kee F, Ruidavats JB, Arveiler D, Luc G, Morrison C, Hoehe MR, Paul M, Cambien F, "Polymorphisms of the β_2 –adrenoreceptor (ADRB2) gene and essential hypertension: the ECTIM and PEGASE studies'. *J Hypertens*, 2002; vol 20: pp229-235

Hewitt A, Samples JR, Allingham RR, Jarvela I, Kitsos G, Krishnadas SR, Richards JE, Lichter PR, Petersen MB, Sundaresan P, Wiggs JL, Mackey DA, Wirtz MK, "Investigation of founder effects for the Thr377Met Myocilin mutation in glaucoma families from differing ethnic backgrounds", *Mol Vis*, 2007; vol. 13, pp. 492.

Hewitt AW, Mackey DA, Craig JE, "Myocilin allele-specific glaucoma phenotype database", *Hum Mutat*, 2008; vol. 29, pp. 207-211.

Higginbotham EJ, Feldman R, Stiles M, Dubiner H, "Latanoprost and timolol combination therapy vs. monotherapy. One year randomized trial", *Arch Ophthalmol*, 2002, vol 120, pp 915-922

Hiller R, Sperduto RD, Krueger DE, "Race, iris pigmentation, and intraocular pressure", *Am J Epidemiol*, 1982; vol. 115, pp. 674-683.

Hirata T, Ushikubi F, Kakizuka A, Okuma M, Narumiya S, "Two thromboxane A 2 receptor isoforms in human platelets: opposite coupling to adenylate cyclase with different sensitivity to Arg to Leu mutation", *J Clin Invest*, 1996; vol. 97, pp. 949-956.

Hirvela H, Tuulonen A, Laatikainen L, "Intraocular pressure and prevalence of glaucoma in elderly people in Finland: a population-based study", *Int Ophthalmol*, 1994; vol. 18, pp. 299-307.

Hitchings R, Tan J, "Target pressure", 2001, *J Glaucoma*, vol. 10 (Suppl 1), p. S68-S70.

Hoh BP, Zahary MN, Tajudin LS, Lin CL, Teck CM, Sidek MR, Zulkifli A, Zilfalil BA, A novel single nucleotide polymorphism, IVS2 -97 A>T, in the prostaglandin F2α receptor gene was identified among the Malaysian patients with glaucoma. *Kobe J Med Sci*; 2007; vol. 53, pp. 49-52

Hoffman C, Zurn A, Bunemann M, Lohse MJ, "conformational changes in G-protein-coupled receptors - the quest for functionally selective conformations is open", *Br J Pharmacol*, 2008; vol. 153, p. S358-S366.

Hoffstedt J, Iliaudo A, Pedersen NL, Schalling M, Arner P, "The effect of the $beta_2$ adrenoceptor gene Thr164Ile polymorphism on human adipose tissue lipolytic function", *Br J Pharmacol*, 2001; vol. 133, pp. 708-712.

Hollows FC, Graham PA, "Intraocular pressure, glaucoma, and glaucoma suspects in a defined population", *Br J Ophthalmol*, 1966; vol. 50, pp. 570-586.

Holmstrom S, Buchholz P, Walt J, Wickstrom J, Aagren M, "The cost-effectiveness of bimatoprost, latanoprost and timolol in treatment of primary open angle glaucoma in five European countries", *Curr Med Res Opin*, 2006; vol. 22, pp. 897-905.

Honrubia F, Garcia-Sanchez J, Polo V, de La Casa JM, Soto J, "Conjunctival hyperemia with the use of latanoprost versus other prostaglandin analogues in patients with ocular hypertension or glaucoma: a meta-analysis of randomised clinical trials", *Br J Ophthalmol*, 2009; vol. 93, pp. 316-321.

Houle RE, Grant WM, "Alcohol, vasopressin, and intraocular pressure", *Invest Ophthalmol*, 1967; vol. 6, no. 2, pp. 145-154.

Hosking L, Lumsden S, Lewis K, Yeo A, McCarthy L, Bansal A, Riley J, Purvis I, Xu CF, "Detection of genotyping errors by Hardy-Weinberg equilibrium testing''. *Eur J Hum Genet*, 2004; vol 12: pp 395-399.

Huber T, Menon S, Sakmar TP, "Structural basis for ligand binding and specificity in adrenergic receptors: implications for GPCR-targeted drug discovery", *Biochemistry*, 2008; vol. 47, pp. 11013-11023.

Hylton C, Robin AL, "Update on prostaglandin analogs", *Curr Opin Ophthalmol*, 2003; vol. 14, pp. 65-69.

Iafrate AJ, Feuk L, Rivera MN, Listerwnik ML, Donahoe PK, Qi Y, Scherer SW, Lee C, "Detection of large-scale variation in the human genome", *Nat Genet*, 2004, vol 36, pp 949-951.

Idle JR, Smith RL, "Polymorphisms of oxidation at carbon centers of drugs and their clinical significance", *Drug Metab Rev*, 1979; vol. 9, pp. 301-317.

Inagaki Y, Mashima Y, Fuse N, Funayama T, Ohtake Y, Yasuda N, Murakami A, Hotta Y, Fukuchi T, Tsubota K, "Polymoprphisms of β-adrenergic receptor and susceptibility to open-angle glaucoma", *Mol Vis*, 2006; vol. 12, pp. 673-80

Inoue-Matsuhisa E, Sogo S, Mizota A, Taniai M, Takenaka H, Mano T, ‘Effect of MCI-9042, a 5-HT_2 receptor antagonist, on retinal ganglion cell death and retina ischemia’’ *Exp Eye Res*, 2003, vol 76, pp 445-452

Ishii Y, Nakamura K, Tsutsumi K, Kotegawa T, Nakano S, Nakatsuka K, “Drug interaction between cimetidine and timolol ophthalmic solution: effect on heart rate and intraocular pressure in healthy Japanese volunteers”, *J Clin Pharmacol*, 2000; vol 40, pp. 193-199

Ismail M and Rohaizat Y, "Health care reform and changes: the Malaysian experience", 2002; *Asia Pac J Public Health*, vol. 14(1), pp. 17-22.

Israel E, Drazen JM, Liggett SB, Boushey HA, Cherniack RM, Chincilli VM, Cooper DM, Fahy JV, Fish JE, Ford JG, Kraft M, Kunselman S, Lazarus SC, Lemanske RF, Martin RJ, McLean DE, Peters SP, Silverman EK, Sorkness CA, Szefler SJ, Weiss ST, Yandava CN, ”The effect of polymorphisms of the beta(2)-adrenergic receptor on the response to regular use of albuterol in asthma”, *Am J Respir Crit Care Med*, 2000; vol 162, pp 75-80

Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidia DG, “Replication validity of genetic association studies*”, Nat Genet,* 2001; vol 29, pp. 306-309

Jampel HD, "Target pressure in glaucoma therapy", 1997, *J Glaucoma*, vol. 6, pp. 132-138.

Jemaa R, Ali SB, Kallel A, Feki M, Elasmi M, Taieb SH, Sanhaji H, Omar S, Kaabachi N,”Association of a 27-bp repeat polymorphism in introns 4 of endothelial constitutive nitric oxide synthase gene with hypertension in a Tunisian population”, *Clin Biochem*, 2009, vol 42, pp 852-856

Johnatty SE, Abdellatif M, Shimmin L, Clark RB, Boerwinkle E, “β_2 adrenergic receptor 5’haplotypes influence promoter activity”. *Br J Pharmacol*, 2002; vol 137, pp. 1213-1216

Johnson M, "Mechanisms of action of ß-adrenoceptor agonists," in *Beta-agonists in the treatment of asthma*, Costello JF and Mann RD, ed., Parthenon, Carnforth,1992, pp. 27-42.

Johnson M, "The ß-adrenoceptor", *Am J Respir Crit Care*, 1998; vol. 158, no. 5, p. S146-S153.

Johnstone MA, "Hypertrichosis and increased pigmentation of eyelashes and adjacent hair in the region of the ipsilateral eyelids of patients treated with unilateral topical latanoprost", *Am J Ophthalmol*, 1997; vol. 124, pp. 544-547.

Jonas JB, Holbach L, "Central corneal thickness and thickness of the lamina cribrosa in human eyes", *Invest Ophthalmol Vis Sci*, 2005; vol. 46, pp. 1275-1279.

Jonasson F, Damji KF, Arnarsson A, Sverrisson T, Wang L, Sasaki H, Sasaki K, the Reykjavik Eye Study Group, "Prevalence of open-angle glaucoma in Iceland: Reykjavik Eye Study", *Eye*, 2003; vol. 17, pp. 747-753.

Jørgensen JS, Guthoff R, "[The role of episcleral venous pressure in the development of secondary glaucoma]", *Klin Monbl Augenhelikd*, 1988; vol. 193, pp. 471-475.

Juzych MS and Zimmerman TJ, "Beta-blockers," 1997, in *Textbook of Ocular Pharmacology*, Zimmerman TJ, ed., Lippincott-Raven, Philadelphia, pp. 261-275.

Kalow W, "Interethnic variation of drug metabolism", *Trends Pharmacol Sci*, 1991; vol. 12, pp. 102-107.

Kalow W, "Pharmacogenetics in biological perspective", *Pharmacol Rev*, 1997; vol. 49, pp. 369-379.

Kunapuli P, Lawson JA, Rokachi J, FitzGerald GA, "Functional characterization of the ocular prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) receptor", *J Biol Chem*, 1997, vol 272, pp27147-27154

Kashiwagi K, Tsukamoto K, Suzuki M, Tsukahara S, "Effects of isopropyl unoprostone and latanoprost on melanogenesis in mouse epidermal melanocytes", *J Glaucoma*, 2002; vol. 11, pp. 57-64.

Kato N, Sugiyama T, Morita H, Kurihara H, Sato T, Yamori Y, Yazaki Y, "Association analysis of β_2-adrenergic receptor polymorphisms with hypertension in Japanese", *Hypertension*, 2001; vol. 37, pp. 286-292

Katz IM and Berger ET, "Effects of iris pigmentation on response of ocular pressure to timolol", *Surv Ophthalmol*, 1979; vol. 23, pp. 395-398.

Kaushik S, Pandav SS, Ram J, "Neuroprotection in glaucoma", *J Postgrad Med*, 2003; vol. 49, pp. 90-95.

Kawana K, Tokunaga T, Miyata K, Okamoto F, Kiuchi T, Oshika T, "Comparison of corneal thickness measurements using Orbscan II, non-contact specular microscopy and ultrasonic pachymetry in eyes after laser in situ keratomileusis", *Br J Ophthalmol*, 2004, vol 88, pp 466-468

Kawase K, Tomidokoro A, Araie M, Iwase A, Yamamoto C, Tajimi Study Group, Japan Glaucoma Society, "Ocular and systemic factors related to intraocular pressure in Japanese adults: the Tajimi study", *Br J Ophthalmol*, 2008; vol. 92, pp. 1175-1179.

Kay LJ, Chong LK, Rostami-Hodjegan A, Peachell PT, "Influence of the Thr164Ile polymorphism in the beta2-adrenoceptor on the effects of beta-adrenoceptor agonists on human lung mast cells", *Int Immunopharmacol*, 2003; vol. 3, pp. 91-95.

Kerstetter JR, Brubaker RF, Wilson SE, Kullerstrand LJ, "Prostaglandin $F_{2\alpha}$-1-isopropylester lowers intraocular pressure without decreasing aqueous humor flow", *Am J Ophthalmol*, 1988; vol. 105, pp. 30-34.

Kida T, Liu JH, Weinreb RN, "Effect of 24-hour corneal biomechanical changes on intraocular pressure measurement", *Invest Ophthalmol Vis Sci*, 2006; vol. 47, pp. 4422-4426.

Kida T, Liu JHK, Weinreb RN, "Effects of aging on corneal biomechanical properties and their impact on 24-hour measurement of intraocular pressure", *Am J Ophthalmol*, 2008; vol. 146, pp. 567-572.

Kirchheiner J, Brockmoller J, "Clinical consequences of cytochrome P450 2C9 polymorphisms", *Clin Pharmacol Ther*, 2005; vol. 77, pp. 1-16.

Kirchheiner J, Heesch C, Bauer S, Miesel C, Seringer A, Goldammer M, Tzvetkov M, Meineke I, Roots I, Brockmoller J, "Impact of the ultrarapid metabolizer genotype of

cytochrome P450 2D6 on metoprolol pharmacokinetics and pharmacodynamics", *Clin Pharmacol Ther*, 2004; vol. 76, pp. 302-312.

Klein BE, Klein R, Sponsel WE, Franke T, cantor LB, Martone J, Menage MJ, "Prevalence of glaucoma. The Beaver Dam Eye study", *Ophthalmology*, 1992; vol. 99, pp. 1499-1504.

Klein BE, Klein R, Knudtson MD, "Intraocular pressure and systemic blood pressure: longitudinal perspective: the Beaver Dam Eye Study", *Br J Ophthalmol*, 2005; vol. 89, pp. 284-287.

Klein BE, Klein R, Moss SE, "Intraocular pressure in an American community. The Beaver Dam Eye Study", *Invest Ophthalmol Vis Sci*, 1992; vol. 33, pp. 2224-2228.

Klein BE, Klein R, Ritter LL, "Relationship of drinking alcohol and smoking to prevalence of open-angle glaucoma. The Beaver Dam Eye Study", *Ophthalmology*, 1993, vol 100, pp 1609-1613.

Klein RJ, Zeiss C, Chew EY, Tsai J-Y, Sackler RS, Haynes C, Henning AK, SanGiovanni JP, Mane SM, Mayne ST, Bracken MB, Ferris FL, Ott J, Barnstable C, Hoh J, "Complement Factor H polymorphism in age related macular degeneration", *Science*, 2005; vol. 308, pp. 385-389.

Kobayashi H, Kobayashi K, "A correlation between latanoprost-induced conjunctival hyperemia and intraocular pressure-lowering effects", *J Glaucoma*, 2011; vol. 20, pp. 3-6.

Kobilka BK, "G protein coupled receptor structure and activation", *Biochim Biophys Acta*, 2007; vol. 1768, pp. 794-807.

Kobilka BK, Dixon RA, Frielle T, Dohlman HG, Bolanowski MA, Sigal IS, Yang-Feng TL, Francke U, Caron MG, Lefkowitz RJ, "cDNA for the human beta 2-adrenergic receptor: a protein with multiple membrane-spanning domains and encoded by a gene whose chromosomal location is shared with that of the receptor for platelet-derived growth factor", *Proc Natl Acad Sci USA*, 1987; vol. 84, pp. 46-50.

Kohlhass M, Boehm AG, Spoerl E, Pursten A, Grein HJ, Pillunat LE, "Effect of central corneal thickness, corneal curvature, and axial length on applanation tonometry", *Arch Ophthalmol*, 2006; vol. 124, pp. 471-476.

Kotanko P, Binder A, Tasker J, DeFreitas P, Kamdar S, Clark AJ, Skrabal F, Caulfield M, "Essential hypertension in African Caribbean associates with a variant of the β_2-adrenoceptor", *Hypertension*, 1997; vol. 30, pp. 773-776

Kunapuli P, Lawson JA, Rokach J, FitzGerald GA, "Functional characterization of the ocular prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) receptor. Activation by the isoprostone, 12-*iso*- $PGF_{2\alpha}$. *J Biol Chem*, 1997; vol 272: 27147-27154

Laiquzzaman M, Bhojwani R, Cunliffe I, Shah S, "Diurnal variation of ocular hysteresis in normal subjects: relevance in clinical context", *Clin Experiment Ophthalmol*, 2006; vol. 34, pp. 114-118.

Lama PJ, "Systemic adverse effects of beta-adrenergic blockers: An evidence-based assessment", *Am J Ophthalmol*, 2002; vol. 134, pp. 749-760.

Lands AM, "Sympathetic receptor action", *Am J Physiol*, 1952; vol. 169, pp. 11-21.

Lands AM, Arnold A, McAuliff JP, Luduena FP, Brown TG Jr, "Differentiation of receptor systems activated by sympathomimetic amine", *Nature*, 1967; vol. 214, pp. 597-598.

Langman MJS, Lancashire RJ, Cheng KK, Stewart PM, "Systemic hypertension and glaucoma: mechanisms in common and co-occurrence", *Br J Ophthalmol*, 2005, vol 89, pp 960-963.

Langley D, MacDonald RK, "Clinical method of observing changes in the rate of flow of aqueous humour in the human eye. II. In glaucoma", *Br J Ophthalmol*, 1952; vol. 36, pp. 499-505.

Lanigan LP, Clark CV, Hill DW, "Intraocular pressure responses to systemic autonomic stimulation", *Eye*, 1989; vol. 3, no. Pt 4, pp. 477-483.

Laquizzaman M, Bhojwani R, Cunliffe I, Shah S, "Diurnal variation of ocular hysteresis in normal subjects: relevance in clinical context", *Clin Experiment Ophthalmol*, 2006; vol. 34, pp. 114-118.

Larson RS, Brubaker RF, "Isoproterenol stimulates aqueous flow in humans with Horner's syndrome", *Invest Ophthalmol Vis Sci*, 1988; vol. 29, p. 621.

Larsson LI, Mishima HK, Takamatsu M, Orzalesi N, Rossetti L, "The effect of latanoprost on circadian intraocular pressure", *Surv Ophthalmol*, 2002; vol. 47(Suppl 1), p. S90-S96.

Lass JH, Pavan-Langston D, "Timolol therapy in secondary angle-closure glaucoma post penetrating keratoplasty", *Ophthalmology*, 1979; vol. 86, pp. 51-59.

Lee YW, Oh VM, Garcia E, Taylor EA, Wu H, Yap EP, Kazeem GR, Gaulfield MJ, Munroe PB, "Haplotypes of the ß$_2$-adrenergic receptor gene are associated with essential hypertension in a Singaporean Chinese population", *J Hypertens*, 2004; vol. 22, pp. 2111-2116.

Lee JS, Lee SH, Oum BS, Chung JS, Cho BM, Hong JW, "Relationship between intraocular pressure and systemic health parameters in a Korean population", *Clin Experiment Ophthalmol*, 2002; vol. 30, pp.237-41.

Lee S, Kasif S, Weng Z, Cantor CR, "Quantitative analysis of single nucleotide polymorphisms within copy number variations", *PLOS ONE*, 2008; vol. 3(12), pp.e3906.

Lefkowitz RJ, "Seven transmembrane receptors: something old, something new", *Acta Physiol*, 2007; vol. 190, pp. 9-19.

Leier CV, Baker D, Weber PA, "Cardiovascular effects of ophthalmic timolol", 1986 *Annals of Internal Medicine*, vol. 104, pp. 197-199.

Leighton DA, Philips CI, "Systemic blood pressure in open angle glaucoma, low tension glaucoma and the normal eye", *Br J Ophthalmol*, 1972; vol.56, pp.447-53.

Leineweber K, Brodde OE, "ß$_2$ -adrenoceptor polymorphisms: relation between in vitro and in vivo phenotypes", *Life Sci*, 2004; vol. 74, pp. 2803-2814.

Leineweber K, Buscher R, Bruck H, Brodde OE, "Beta-adrenoceptor polymorphism", *Naunyn Schmiedebergs Arch Pharmacol*, 2004; vol. 369, pp. 1-22.

Lemmelä S, Forsman E, Onkamo P, Nurmi H, Laivuori H, Kivela T, Puska P, Heger M, Eriksson A, Forsius H, Jarvela I, "Association of *LOXL1* gene with Finnish exfoliation syndrome patients", *J Hum Gen*, 2009; vol. 54, pp. 289-297.

Lempert P, Cooper KH, Culver JF, Traduce TJ, "The effect of exercise on intraocular pressure", *Am J Ophthalmol*, 1967; vol. 63, no. 1673-1676.

Leske MC, Heijl A, Hyman L, Bengtsson B, "Early manifest glaucoma trial. Design and baseline data", *Ophthalmology*, 1999; vol. 106, pp. 2144-2153.

Leske MC, Heijl A, Hyman L, Bengtsson B, Dong L, Yang Z, EMGT Group, "Predictors of long term-progression in the Early Manifest Glaucoma Trial", *Ophthalmology*, 2007; vol. 114, pp. 1965-1972.

Leske MC, Wu SY, Hennis A, Hyman L, Nemesure B, Yang L, Schachat AP, Barbados Eye Study Group, "Hyperglycemia, blood pressure, and the 9 year incidence of diabetic retinopathy: the Barbados Eye Studies", *Ophthalmology*, 2005; vol.112(5), pp.799-805.

Leung DYL, Li FCH, Kwong YYY, Tham CCY, Chi SCC, Lam DSC, " Simvastatin and disease stabilization in normal tension glaucoma: a cohort study", *Ophthalmology*, 2010, vol 117: pp 471-476

Li W, Wu S, Hickey RW, Rose ME, Chen J, Graham SH, "Neuronal cyclooxygenase-2 activity and prostaglandins and $PGF_{2\alpha}$ exacerbate hypoxia neuronal injury in neuron-enriched primary culture", *Neurochem Res*, 2008, vol 33, pp 490-499

Liang Y, Woodward DF, Guzman VM, Li C, Scott DF, Wang JW, Wheeler LA, Garst ME, Landsverk K, Sachs G, Krauss AH, Cornell C, Martos J, Pettit S, Fliri H, "Identification and pharmacological characterization of prostaglandin FP receptor and FP receptor variant complexes", *Br J Pharmacol*, 2008; vol. 154, pp. 1079-1093.

Liggett SB, "Molecular and genetic basis of $ß_2$-adrenergic receptor function", *J Allergy Clin Immunol*, 1999; vol. 103, p. S42-S46.

Liggett SB, "Polymorphisms of the beta2-adrenergic receptor and asthma". *Am J Respir Crit Care Med*, 1997; vol 156: ppS156-62

Ligget SB, Wagoner LE, Craft LL, Hornung RW, Hoit BD, McIntosh TC, Walsh RA,"The Ile164 beta-adrenergic receptor polymorphism adversely affects the outcome of congestive heart failure", *J Clin Invest*, 1998, vol 102, pp 1534-1539.

Lin HY, Hsu WM, Chou P, Liu CJ, Chou JC,Tsai SY, Cheng CY, "Intraocular pressure measured with a noncontact tonometer in an elderly Chinese population: the Shihpai Eye Study", *Arch Ophthalmol*, 2005; vol. 123, pp. 381-386.

Lin LL, Galin MA, Obstabaum SA, Katz I, "Longterm timolol therapy", 1979, *Surv Ophthalmol*, vol. 23, pp. 377-380.

Linder BJ, Trick GL, Wolf ML, "Altering body position affects intraocular pressure and visual function", *Invest Ophthalmol Vis Sci*, 1988; vol. 29, no. 10, pp. 1492-1497.

Lindsey JD, Kashiwagi K, Kashiwagi F, Weinreb RA, "Prostaglandins alter extracellular matrix adjacent to human ciliary muscle cells in vitro", *Invest Ophthalmol Vis Sci*, 1997; vol. 38, pp. 2214-2223.

Lipworth B, Koppelman GH, Wheatley AP, Le Juene I, Coutie W, Meurs H, Kauffman HF, Postma DS, Hall IP, "β_2 adrenoreceptor promoter polymorphisms: extended haplotypes and functional effects in peripheral blood mononuclear cells". *Thorax*, 2002; vol 57; pp 61-66

Litonjua AA, Silverman EK, Tantisira KG, Sparrow D, Sylvia JS, Weiss ST, "Beta2-adrenergic receptor polymorphisms and haplotypes are associated with airways hyperresponsiveness among nonsmoking men", *Chest*, 2004; vol. 126, pp. 66-74.

Liu JH, Bouligny RP, Kripke DF, Weinreb RN, "Nocturnal elevation of intraocular pressure is detectable in the sitting position", *Invest Ophthalmol Vis Sci*, 2003; vol. 44, pp. 4439-4442.

Liu JH, Kripke DF, Twa MD, Hoffman RE, Mansberger SL, Rex KM, Girkin CA, Weinreb RN, "Twenty-four-hour pattern of intraocular pressure in the aging population", *Invest Ophthalmol Vis Sci*, 1999; vol. 40, pp. 2912-2917.

Liu JHK,"Diurnal measurement of intraocular pressure", *J Glaucoma*, 1998; vol. 10 (Suppl 1), p. S39-S41.

Liu Y, Schmidt S, Qin X, Gibson J, Hutchins K, Santiago-Turla C, Wiggs JL, Budenz DL, Akafo F, Challa P, Herndon LW, Hauser MA, Allingham RR, "Lack of association between LOXL1 variants and primary open-angle glaucoma in three different populations", *Invest Ophthalmol Vis Sci*, 2008; vol. 49, pp. 3465-3468.

Liza-Sharmini AT, Abdul MO, Madhavan M, "The effects of topical antiglaucoma drugs on the conjunctival cell profile of Asian patients", *Asian J Ophthalmol*, 2007; vol. 9, pp. 17-22.

Lou Y, Liu J, Huang Y, Liu J, Wang Z, Liu Y, Li Z, Li Y, Xie Y, Wen S, "A46G and C79G polymorphisms in the β2 adrenergic receptor (ADRB2) and essential hypertension risk: a meta-analysis", *Hypertens Res*, 2010; vol. 33, pp. 1114-23

Lou Y, Liu J, Li Y, Wang Z, Liu K, Wu H, Niu Q, Gu W, Guo Y, Li Z, Wen S, "Association study of the β2-adrenergic receptor gene polymorphisms and hypertension in Northern Han Chinese", *PLoS One*, 2011; vol. 6, pp. e18590

Lütjen-Drecoll E, Tamm E, "Morphological study of the anterior segment of cynomolgus monkey eyes following treatment with prostaglandin F2 alpha", *Exp Eye Res*, 1988; vol. 47, pp. 761-769.

Mackey DA, Healy DL, Fingert JH, Coote MA, Wong TL, Wilkinson CH, McCartney PJ, Rait JL, de Graaf AP, Stone EM, Craig JE, "Glaucoma phenotype in pedigrees with the myocilin Thr377Met mutation", *Arch Ophthalmol*, 2003; vol. 121, pp. 1172-1180.

Maepea O, Bill A, "The pressure in the episcleral veins, Schlemn's canal and the trabecular meshwork in monkeys; effects of changes in intraocular pressure", *Exp Eye Res*, 1989; vol.49, pp.645-663.

Maihöfner C, Schlötzer-Schrehardt U, Gübrig H, Zeilhofer HU, Naumann GO, Pahl A, Mardin C, Tamm ER, Brune K, "Expression of cyclooxygenase-1 and -2 in normal and glaucomatous human eyes", *Invest Ophthalmol, Vis*, 2001; vol. 42, pp. 2616-2624

Mansberger SL, Hughes BA, Gordon MO, Spaner SD, Beiser JA, Cioffi GA, Kass MA; Ocular Hypertension Treatment Study Group, "Comparison of initial intraocular pressure response with topical β-adrenergic antagonists and prostaglandin analogues in African American and white individuals in the ocular hypertension treatment study", *Arch Ophthalmol*, 2007, vol 125, pp 454-459.

Mantyh PW, Rogers SD, Allen CJ, Catton MD, Ghilardi JR, Levin LA, Maggio JE, Vigna SR, "β_2 –adrenergic receptors are expressed by glial *in vivo* in the normal and injured central nervous systemic in the rat, rabbit and human", *J Neurosci*, 1995; vol 15, pp152-164

Marez D, Legrad M, Sabbagh N, Lo Guidice JM, Spire C, Laffite JJ, Meyer UA, Broly F, "Polymorphism of the cytochrome P450 CYP2D6 gene in a European population: characterization of 48 mutations and 53 alleles, their frequencies and evolution", *Pharmacogenetics*, 1997; vol. 7, pp. 293-302.

Martin B, Harris A, Hammel T, Malinovsky V, "Mechanism of exercise induced ocular hypotension", *Invest Ophthalmol Vis Sci*, 1999; vol. 40, pp. 1011-1015.

Martin MJ, Sommer A, Gold EB, Diamond EL, "Race and primary open-angle glaucoma", *Am J Ophthalmol*, 1985; vol. 99, no. 4, pp. 383-387.

Martinez FD, Graves PE, Baldini M, Solomon S, Erickson R,"Association between genetic polymorphisms of the β_2-adrenoreceptor and response to albuterol in children with and without a history of wheezing', *J Clin Invest*, 1997, vol 100, pp 3184-88.

Maruyama I, Ohguro H, Ikeda Y, "Retinal ganglion cells recognized by serum auto antibody against gamma-enolase found in glaucoma patients", *Invest Ophthalmol Vis Sci*, 2000; vol. 41, pp. 1657-1665.

Masimirembwa CM, Johansson I, Hasler JA, Ingelman-Sundberg M, "Genetic polymorphism of cytochrome P450 2D6 in a Zimbabwean population", *Pharmacogenetics*, 1993; vol. 3, pp. 275-280.

Matthew HW, "Racial, ethnic and gender differences in response to medicine", *Drug Metabol Drug Interact*, 1995; vol. 12, pp. 77-91.

Mäpea O, Bill A, "The pressures in the episcleral veins, Schlemm's canal and the trabecular meshwork in monkeys: effects of changes in intraocular pressure", *Exp Eye Res*, 1989; vol. 49, pp. 645-663.

McCarty CA, Burmester JK, Mukesh BN, Patchett RB, Wilker RA, "Intraocular pressure response to topical β-blockers associated with an ADRB2 single nucleotide polymorphism", *Arch Ophthalmol*, 2008; vol. 126, pp. 959-63

McCarty CA, Berg R, Patchett R, Wilker RA, Burmester JK, "Lack of association between polymorphisms in the prostaglandin F(2α) receptor and solute carrier organic anion

transporter family 2A1 genes and intraocular pressure to prostaglandin analogs", *Ophthalmic Genet*; 2011; vol. 33, pp. 74-76

McGraw DW, Forbes SL, Kramer LA, Liggett SB, "Polymorphisms of the 5' leader cistron of the human beta2-adrenergic receptor regulate receptor expression", *J Clin Invest*, 1998; vol. 102, pp. 1927-1932.

McLaren NC and Moroi SE, "Clinical implications of pharmacogenetics for glaucoma therapeutics", *Pharmacogenomics J*, 2003; vol. 3, pp. 197-201.

McLaren N, Reed DM, Musch DC, Downs CA, Higashi ME, Santiago C, Radenbaugh PA, Allingham RR, Richards JE, Moroi SE, "Evaluation of the β_2-adrenergic receptor gene as a candidate glaucoma gene in 2 ancestral populations", *Arch Ophthalmol*, 2007; vol. 125, pp. 105-111

McLeod SD, West SK, Quigley HA, Fozard JL, "A longitudinal study of the relationship between intraocular and blood pressure", *Invest Ophthalmol Vis Sci*, 1990; vol. 124, pp. 1631-1636.

McNaught AI, Allen JG, Healy DL, McCartney PJ, Coote MA, Wong TL, Craig JA, Green CM, Rait JL, Mackey DA, "Accuracy and implications of a reported family history of glaucoma: experience from the Glaucoma Inheritance Study in Tasmania", *Arch Ophthalmol*, 2007; vol. 118, pp. 900-904.

Medeiros FA, Weinreb RN, Zangwill LM, Alencar LM, Sample PA, Vasile C, Bowd C, "Long-term intraocular pressure fluctuations and risk of conversion from ocular hypertension to glaucoma", 2008, *Ophthalmology*, vol. 115, pp. 934-940.

Medeiros FA, Alencar LM, Sample PA, Zangwill LM, Susanna J, Weinreb RN, "The Relationship between Intraocular Pressure Reduction and Rates of Progressive Visual Field Loss in Eyes with Optic Disc Hemorrhage", *Ophthalmology*, 2010; vol. 117, no. 11, pp. 2061-2066.

Memarzadeh F, Ying-Lai M, Azen SP, Hooper C, Foong AW, Varma R, "Associations with intraocular pressure in Latinos: the Los Angeles Latino Eye Study", *Am J Ophthalmol*, 2008; vol. 146, pp. 69-76.

Menon IA, Trope GE, Basu PK, Wakeham DC, Persad SD, "Binding of timolol to iris-ciliary body and melanin: an in vitro model for assessing the kinetics and efficacy of long acting antiglaucoma drugs", *J Ocul Pharmacol*, 1989; vol. 5(4), pp. 313-324.

Michels-Rautenstrauss KG, Mardin CY, Budde WM, Liehr T, Polansky J, Nguyen T, Timmerman V, Van Broeckhoven C, Naumann GO, Pfeiffer RA, Rautenstrauss BW, 'Juvenile open angle glaucoma: fine mapping of the TIGR gene to 1q24.3-q25.2 and mutational analysis", *Hum Genet*, 1998; vol. 102, pp. 103-106

Migdal C, Gregory W, Hitchings R, "Long-term functional outcome after early surgery compared with laser and medicine in open-angle glaucoma", *Ophthalmology*, 1994; vol. 101, pp. 1651-1656.

Minelli C, Thompson JR, Abrams KR, Thakkinstian A, Attia J, “How should we use information about HWE in the meta-analyses of genetic association studies?”, *Int J Epidemiol*, 2007, vol 37, pp 136-146

Mishima KH, Masuda K, Kitazawa Y, Azuma I, Araie M, “A comparison of latanoprost and timolol in primary open-angle glaucoma and ocular hypertension. A 12-week study”, *Arch Ophthalmol*, 1996, vol 114, pp929-932

Mitchell P, S. W. A. K. 1996, "Prevalence of open-angle glaucoma in Australia. The Blue Mountains Eye study", *Ophthalmology*, vol. 103, pp. 1661-1669.

Mitchell R, Rochtchina E, Lee A, Wang JJ, Mitchell P, Blue Mountains Eye Study, "Iris color and intraocular pressure: The Blue Mountains Eye Study", *Am J Ophthalmol*, 2003; vol. 135, pp. 384-386.

Miyake K, Ota I, Maekubo K, Ichihashi S, Miyake S, "Latanoprost accelerates disruption of the blood-aqueous barrier and the incidence of angiographic cystoid macular edema in early postoperative pseudophakias", *Arch Ophthalmol*, 1999; vol. 117, pp. 34-40.

Moffet S, Mouilac B, Bonin H, Bouvier M, "Altered phosphorylation and desensitization patterns of human ß$_2$-adrenergic receptor lacking the palmitoylated Cys^{341}", *EMBO J*, 1993; vol. 12, pp. 349-356.

Monemi S, Spaeth G, Da Silva A, Popinchalk S, Ilitcher E, Liebmann J, Ritch R, Heon E, Crick A, Sarfazi M, “Identification of a novel adult-onset primary open-angle glaucoma (POAG) gene on 5q22.1”’ Hum Mol Genet, 2005, vol 14, pp725-733.

Moore PE, Laporte JD, Abraham JH, Schwartzman IN, Yandava CN, Silverman ES, Drazen JM, Wand MP, Panettieri RA Jr, Shore SA, "Polymorphism of the ß$_2$-adrenergic receptor gene and desensitization in human airway smooth muscle", *Am J Respir Crit Care Med*, 2000; vol. 162, pp. 2117-2124.

Morgan RW, Drance SM, "Chronic open-angle glaucoma and ocular hypertension", *Br J Ophthalmol*, 1975; vol. 59, pp. 211-215.

Mori K, Ando F, Nomura H, Sato Y, Shimokata H, "Relationship between intraocular pressure and obesity in Japan", *Int J Epidemiol*, 2000; vol. 29, no. 4, pp. 661-666.

Módis LJ, Lagenbucker A, Seitz B, “Corneal thickness measurements with contact and noncontact specular microscopic and ultrasonic pachymetry”, *Am J Ophthalmol*, 2001, vol 132, pp 517-521

Mrkonjic M, Roslin NM, Greenwood CM, Raptis S, Pollett A, Laird PW, Pethe W, Chiang T, Daftary D, Dicks E, Thibodeau SN, Gallinger S, Parfrey PS, Younghusband HB, Potter JD, Hudson TJ, McLaughlin JR, Green RC, Zanke BW, Newcomb PA, Paterson AD, Bapat B, “Specific variants in the MLH1 gene region may drive DNA methylation, loss of protein expression, and MSI-H colorectal cancer”, *PLoS ONE*, 2010, vol. 5, pp. e13314”,

Mukhopadhyay A, Komatireddy S, Acharya M, Bhattacharjee A, Mandal AK, Thakur SK, Chandrasekhar G, Banerjee A, Thomas R, Chakrabarti S, Ray K, "Evaluation of optineurin as a candidate gene in Indian patients with primary open angle glaucoma", *Mol Vis*, 2005; vol. 11, pp. 792-797.

Mukhopadhyay P, Bian L, Yin H, Bhattacherjee P, Paterson CA, "Localization of EP_1 and FP receptors in human ocular tissues by in situ hybridization", *Invest Ophthalmol Vis Sci*, 2001; vol. 42, pp. 424-428.

Munukata M, Harada Y, Ishida T, Saito J, Nagabukuro A, Matsushita H, Koga N, Ohsaki M, Imagawa K, Shiratsuchi T, "Molecular-based haplotype analysis of ß 2-adrenergic receptor gene (ADRB2) in Japanese asthmatic and non-asthmatic subjects", *Allergology International*, 2006; vol. 55, pp. 191-198.

Murphy E, "One cause? Many causes? The argument from bimodal distribution", *J Chron Dis*, 1964; vol. 17, pp. 301-324.

Murphy G, Docherty AJ, "The matrix metalloproteinases and their inhibitors", *Am J Respir Cell Mol Biol*, 1992, vol 7, pp 120-125

Musch DC, Gillespie BW, Lichter PR, Niziol LM, Janz NK. 2009, "Visual field progression in the Collaborative Initial Glaucoma Treatment Study. The impact of treatment and other baseline factors", *Ophthalmology*, vol. 116, pp. 200-207.

Musch DC, Lichter PR, Guire KE, Standardi CL, "The Collaborative Initial Glaucoma Treatment Study. Study design, methods and baseline characteristics of enrolled patients", *Ophthalmology*, 1999; vol. 106, pp. 653-662.

Myatt L, Lye SJ, "Expression, localization and function of prostaglandin receptors in myometrium", *Prostaglandins Leukot Essent Fatty Acids*, 1984; vol. 70, pp. 137-148.

Nakanishi Y, Nakamura M, Mukuno H, Kanamori A, Seigel GM, Negi A, "Latanoprost rescues neuro-glial cells from apoptosis by inhibiting caspase-3, which is mediated by p44/p42 mitogen activated protein kinase", *Exp Eye Res*, 2006; vol 83: pp 1108-1117

Nakano M, Ikeda Y, Tokuda Y, Fuwa M, Omni N, Ueno M, Imai K, Adachi H, Kageyama M, Mori K, Kinoshita S, Tashiro K, "Common variants in CDKN2B-AS1 associated with optic-nerve vulnerability of glaucoma identified by genome-wide association studies in Japanese", *PLoS One*, 2012, vol 7, pp e33389

Namba T, Sugimoto Y, Negishi M, Irie A, Ushikubi F, Kakizuka A, Ito S, Ichikawa A, Narumiya S, "Alternative splicing of C-terminal tail of prostaglandin E receptor subtype EP_3 determines G-protein specificity", *Nature*, 1993; vol. 365, pp. 166-170.

Nathanson JA, "Adrenergic regulation of intraocular pressure: Identification of $ß_2$-adrenergic-stimulated adenylate cyclase in ciliary process epithelium", *Proc Natl Acad Sci USA*, 1980; vol. 77, pp. 7420-7424.

Nathanson JA, "Human ciliary process adrenergic receptor: pharmacological characterization", *Invest Ophthalmol Vis Sci*, 1981; vol. 21, pp. 798-804

Nelson WL, Fraunfelder FT, Sills JM, Pharm D, Arrowsmith JB, Kuritsky JN, "Adverse respiratory and cardiovascular events attributed to timolol ophthalmic solution, 1978-1985", *Am J Ophthalmol*, 1986; vol. 102, pp. 606-611.

Nemesure B, He Q, Mendell N, Wu SY, Hejtmancik JF, Hennis A, Leske MC, Barbados Family Study Group, "Inheritance of open-angle glaucoma in the Barbados Family Study", *Am J Med Genet*, 2001; vol. 103, pp. 36-43.

Nemesure B, Wu SY, Hennis A, Leske MC, Barbados Eye Study Group, "Corneal thickness and intraocular pressure in the Barbados eye studies", *Arch Ophthalmol*, 2003; vol. 121, pp. 240-244.

Nemesure B, Honkanen R, Hennis A, Wu SY, Leske MC; Barbados Eye Studies Group, "Incident open-angle glaucoma and intraocular pressure", *Ophthalmology*, 2007; vol.114, pp.1810-5.

Netland PA, Landry T, Sullivan EK, Andrew R, Silver L, Weiner A, Mallick S, Dickerson J, Bergamini MV, Robertson SM, Davis AA; Travoprost Study Group, "Travoprost compared with latanoprost and timolol in patients with open-angle glaucoma or ocular hypertension", 2001, *Am J Ophthalmol*, vol 132, pp 472-484.

Neufeld AH, "Experimental studies on the mechanism of action of timolol", *Surv Ophthalmol*, 1979; vol. 23, pp. 363-370.

Neufeld AH, Jampol LM, Sears ML, "Cyclic AMP in the aqueous humor. The effects of adrenergic agents", *Exp Eye Res*, 1972; vol. 14, pp. 242-250.

Neufeld AH, Zawistowski KA, Page ED, Bromberg BB, "Influences on the density of beta adrenergic receptors in the cornea and iris-ciliary body of rabbit", *Invest Ophthalmol Vis Sci*, 1978; vol. 17, p. 1069.

Nieminen T, Uusitalo H, Mäenpää J, Turjanmaa V, Rane A, Lundgren S, Ropo A, Rontu R, Lehtimaki T, Kahonen M, 'Polymorphisms of genes CYP2D6, ADRB1 and GNAS1 in pharmacokinetics and systemic effects of ophthalmic timolol. A pilot study", *Eur J Clin Pharmacol* 2005; vol. 61, pp. 811-9

Nomura H, Shimokata H, Ando F, Miyake Y, Kuzuya F, "Age-related changes in intraocular pressure in a large Japanese population: a cross sectional and longitudinal study", *Ophthalmology*, 1999; vol. 106, pp. 2016-2022.

Nordstrom BL, Friedman DS, Mozaffari E, Quigley HA, Walker AM, "Persistence and adherence with topical glaucoma therapy", *Am J Ophthalmol*, 2005; vol. 140, pp. 598-606.

Nouri-Mahdavi K, Hoffman D, Coleman AL, Liu G, Li G, Gaasterland D, Caprioli J, "Predictive factors for glaucomatous visual field progression in Advanced Glaucoma Intervention Study", 2004, *Ophthalmology*, vol. 111, pp. 1627-1635.

Nüsing RM, Hirata M, Kakizuka A, Eki T, Ozawa K, Narumiya S, "Characterization and chromosomal mapping of the human thromboxane A_2 receptor gene", *J Biol Chem*, 1993; vol. 268, pp. 253-259.

Obstbaum SA , Podos SM, Kolker AE, "Low-dose oral alcohol and intraocular pressure", *Am J Ophthalmol*, 1973; vol. 76, no. 6, pp. 926-928.

Ocklind A, Lake S, Wentzel P, Nister M, Stjernschantz J, "Localization of the prostaglandin $F_{2\alpha}$ receptor messenger RNA and protein in the cynomologus monkey eye", *Invest Ophthalmol Vis Sci*, 1996; vol. 37, pp. 716-726.

O'Connor DE, Mihelich ED, Coleman MC,"Stereochemical course of the auto oxidative cyclization of lipid hydroperoxides to prostaglandin-like bicyclo endoperoxides", *J Am Chem Soc*, 1984, vol 106, pp3577-3584

Ogawa Y, Tanaka I, Inoue M, Yoshitake Y, Isse N, Nakagawa O, Usui T, Itoh H, Yoshimasa T, Narumiya S, "Structural organization and chromosomal assignment of the human prostacyclin receptor gene", *Genomics*, 1995; vol. 27, pp. 142-148.

Oh DJ, Martin JL, Williams AJ, Rusell P, Birk DE, Rhee DJ, "Effect of latanoprost on the expression of matrix metalloproteinases and their tissue inhibitors in human trabecular meshwork cells", *Invest Ophthalmol Vis Sci*, 2006; vol. 47, pp. 3887-3895.

Oh SW, Lee S, Park C, Kim DJ, "Elevated intraocular pressure is associated with insulin resistance and metabolic syndrome", *Diabetes Metab Res Rev*, 2005; vol. 21, pp. 434-440.

Ohno Y, Takoyanagi R, Iga T, Yamada Y, Nagahara M, Araie M, "Pharmacokinetic and pharmacodynamic analysis of systemic effect of topically applied timolol maleate ophthalmic gelling vehicle (Rysmon®TG), *Curr Eye Res*, 2005, vol 30, pp 319-328.

Oien HG, Mandel LR, Humes JL, Taub D, Hoffsommer RD, Kuehl FA Jr, "Structural requirement for the binding of prostaglandins", *Prostaglandins*, 1975; vol. 9, pp. 985-995.

Ong LB, Liza-Sharmini AT, Chieng LL, Cheong MT, Vengadasalam SR, Shin HC, Balaravi P, "The efficacy of timolol in gel-forming solution after morning or evening dosing in Asian glaucomatous patients", *J Ocul Pharmacol Ther*, 2005; vol. 21, pp. 388-394.

Orssengo GJ, Pye DC, "Determination of the True Intraocular Pressure and Modulus of Elasticity of the Human Cornea in vivo", *Bulletin of Mathematical Biology*, 1999; vol. 61, no. 3, pp. 551-572.

Osborne NN, Chidlow G, "Do beta-adrenoreceptors and serotonin 5-HT1A receptors have similar functions in the control of intraocular pressure in the rabbit?" *Ophthalmologica*, 1996; vol. 210, pp. 308-314.

Ota T, Aihara M, Narumiya S, Araie M, "The effects of prostaglandin analogues on IOP in prostanoid FP-receptor-deficient mice", *Invest Ophthalmol Vis Sci*, 2005; vol. 46, pp. 4159-4163.

Olateju SO and Ajayi AA, "The lack of efficacy of topical beta-blockers, timolol and betaxolol on intraocular pressure in Nigerian healthy volunteers", *Eye*, 1999; vol. 13, pp. 758-763.

Ozaki M, Lee KY, Vithana EN, Yong VH, Thalamuthu A, Mizoguchi T, Venkatraman A, Aung T, "Association of *LOXL1* gene polymorphisms with pseudoexfoliation in the Japanese", *Invest Ophthalmol Vis Sci*, 2008; vol. 49, pp. 3976-3980.

Özcura F, Aydin S, Uzgoren N, "Effects of central corneal thickness, central corneal power, and axial length on intraocular pressure measurement assessed with Goldmann applanation tonometry", *Jpn J Ophthalmol*, 2008; vol. 52, pp. 353-356.

Panebra A, Schwarb MR, Glinka CB, Liggett SB, "Allele-specific binding of airway nuclear extracts to polymorphic β_2-adrenergic receptor 5' sequence". *Am J Respir Cell Mol Biol*, 2007; vol 36: pp654-660

Panebra A, Wang WC, Malone MM, Pitter DRG, Weiss ST, Hawkins GA, Liggett SB,"Common *ADRB2* haplotypes derived from 26 polymorphic sites direct β_2- adrenergic receptor expression and regulation phenotypes", PLoS ONE, 2010, vol 5, pp e11819

Palmberg P, "How clinical trial results are changing our thinking about target pressures", 2002, *Curr Opin Ophthalmol*, vol. 13, pp. 85-88.

Park BC, Tibudan M, Samarameera M, Shen X, Yue BX, "Interaction between two glaucoma genes, optineurin and myocillin", Genes cells, 2007, vol 12, pp 969-979.

Parola AL, Kobilka BK, "The peptide product of a 5' leader cistron in the ß$_2$ adrenergic receptor mRNA inhibits receptor synthesis", *J Biol Chem*, 1994; vol. 269, no. 6, pp. 4497-4505.

Passo MS, Palmer EA, Van Buskirk EM, "Plasma timolol in glaucoma patients", *Ophthalmology*, 1984; vol. 91, pp. 1361-1363.

Peczon JD, Grant WM, "Glaucoma, alcohol and intraocular pressure", *Arch Ophthalmol*, 1965; vol. 73, p. 495.

Peeters A, Webers CA, Prins MH, Zeegers MP, Hendrikse F, Schouten JS, "Quantifying the effect of intraocular pressure reduction on the occurence of glaucoma", *Acta Ophthalmol*, 2010; vol. 88, pp. 5-11.

Peltomaki P, Lothe RA, Aaltonen LA, Pylkkanen L, Nystrom-Lahti M, Seruca R, David L, Holm R, Ryberg D, Haugen A, "Microsatellite instability is associated with tumors that characterize the hereditary non-polyposis colorectal carcinoma syndrome", *Cancer Res*, 1993; vol. 53, pp. 5853-5855

Pemp B, Georgopoulos M, Vass C, Fuchsjäger-Maryl G, Luksch A, Rainer G, Schmetterer L, "Diurnal fluctuation of ocular blood flow parameters in patients with primary open-angle glaucoma and healthy subjects", *Br J Ophthalmol*, 2009, vol 93, pp 486-491.

Phillips CI, Bartholomew RS, Levy AM, Grove J, Vogel R, "Penetration of timolol eye drops in human aqueous humor: the first hour", *Br J Ophthalmol*, 1985; vol. 69, pp. 217-218.

Pierce KL, Bailey TJ, Hoyer PB, Gil DW, Woodward DF, Regan JW, "Cloning of a carboxyl-terminal isoform of the prostanoid FP receptor", *J Biol Chem*, 1997; vol. 272, pp. 883-887.

Pierce KL, Fujino H, Srinivasan D, Regan JW, "Activation of FP prostanoid receptor isoforms leads to Rho-mediated changes in cell morphology and in the cell cytoskeleton", *J Biol Chem*, 1999; vol. 274, pp. 35944-35949.

Piltz J, Gross R, Shin DH, Beiser JA, Dorr DA, Kass MA, Gordon MO, "Contralateral effect of topical beta-adrenergic antagonists in initial one-eyed trials in the ocular hypertension treatment study", *Am J Ophthalmol*, 2000; vol. 130, pp. 441-453.

Planning and Development Division, Ministry of Health Malaysia 2009, "Health Facts 2008", *Ninth Malaysia Plan* pp. 1-12.

Posner A, Schlossman A, "Role of inheritance in glaucoma", *Arch Ophthalmol*, 1949; vol. 41, pp. 125-150.

Price EL, Gray LS, Humphries L, Zweig C, Button NF, "Effect of exercise on intraocular pressure and pulsatile ocular blood flow in a young normal population", *Optom Vis Sci*, 2003; vol. 80, pp. 460-466.

Pritchard JK, Rosenberg WA, "Use of linked genetic markers to detect population stratification in association studies", *Am J Hum Genet*, 1999; vol. 65, pp. 220-228.

Probert LA, "A survey of hereditary glaucoma", *Can Med Assoc J*, 1952; vol. 66, pp. 563-568.

Quigley HA, "Number of people with glaucoma worldwide", *Br J Ophthalmol*, 1996; vol. 80, pp. 389-393.

Quigley HA and Broman AT, "The number of people with glaucoma worldwide in 2010 and 2020", *Br J Ophthalmol*, 2006; vol. 90, pp. 262-267.

Quigley HA, West SK, Rodriguez J, Munoz B, Klein R, Snyder R, "The prevalence of glaucoma in a population-based study of Hispanic subjects: Proyecto Ver.", *Arch Ophthalmol*, 2001; vol. 119, pp. 1819-1826.

Qureshi IA, "The effects of mild, moderate and severe exercise on intraocular pressure in glaucoma patients", *Jpn J Physiol*, 1995a; vol. 45, pp. 561-569.

Qureshi IA, "Effects of mild, moderate and severe exercise on intraocular pressure of sedentary subjects", *Ann Hum Biol*, 1995b; vol. 22, pp. 545-553.

Qureshi IA, Xi XR, Huang YB, Wu XD, "Magnitude of decrease in intraocular pressure depends upon intensity of exercise", *Korean J Ophthalmol*, 1996; vol. 10, pp. 109-115.

Ramakrishnan R, Nirmalan PK, Krishnadas R, Thulasirai RD, Tielsch JM, Katz J, Friedman DS, Robil AL, "Glaucoma in a rural population of southern India: The Aravind Comprehensive Eye Survey", *Ophthalmology*, 2003; vol. 110, pp. 1484-1490.

Ramdas WD, van Koolwijk LM, Lemij HG, Pasutto F, Cree AJ, Thorleifsson G, Janssen SF, Jacoline TB, Amin N, Rivadeneira F, Wolfs RC, Walters GB, Jonasson F, Weisschuh N, Mardin CY, Gibson J, Zegers RH, Hofman A, de Jong PT, Uitterlinden AG, Costra BA, Thorsteinsdottir U, Gramer E, Welgen-Lüssen UC, Kirwan JF, Bergen AA, Reis A, Stefansson K, Lotery AJ, Vingerling JR, Jansonius NM, Klaver CC, van Duijn CM, “Common genetic variants associated with open-angle glaucoma”, *Hum Mol Genet*, 2011, vol 15, pp2464-2471

Rao PV, Deng PF, Kumar J, Epstein DL, “Modulation of aqueous humor outflow facility by the Rho kinase specific inhibitor”, *Invest Ophthalmol Vis Sci*, 2001, vol 42, pp 1029-1037.

Rasmussen SG, Choi HJ, Rosenbaum DM, Kobilka TS, Thian FS, Edwards PC, Burghammer M, Ratnala VR, Sanishvili R, Fischetti RF, Schertler GF, Weis WI, Kobilka BK, "Crystal structure of the human beta2 adrenergic G-protein-coupled receptor", *Nature*, 2007; vol. 450, pp. 383-387.

Reardon G, Schwartz GF, Mozaffari E, "Patient persistency with topical ocular hypotensive therapy in a managed care population", *Am J Ophthalmol*, 2004; vol. 137 (Suppl), pp. S3-12.

Reddy SC, Rampal L, Nurulaini O, "Prevalence and causes of visual impairment and blindness in a rural population in Sepang district, Selangor", 2003; *Med J Malaysia*, vol. 59 (2), pp. 212-217.

Redon R, Ishikawa S, Fitch KR, Fecile L, Perry GH, Andrews TD, Fiegler H, Shapero MH, Carson AR, Chen W, Cho EK, Dallaire S, Freeman JL, Gonzàlez JR, Gratacòs M, Huang J, Kalaitzopoulos D, Komura D, MacDonald JR, Marshall CR, Mei R, Montgomery L, Nishimura K, Okamura K, Shen F, Somerville MJ, Tchinda J, Valsesia A, Woodwark C, Yang F, Zhang J, Zerjal T, Zhang J, Armengol L, Conrad DF, Estivill X, Tyler-Smith C, Carter NP, Aburatani H, Lee C, Jones KW, Scherer SW, Hurles ME, “Global variation in copy number in the human genome”, *Nature*, 2006, vol 444, pp 44-454

Rein DB, Wittenborn JS, Lee PP, Wirth KE, Sorensen SW, Hoerger TJ, Saaddine JB, "The cost-effectiveness of routine office-based identification and subsequent medical treatment of primary open-angle glaucoma in the United States", *Ophthalmology*, 2009; vol. 116, pp. 823-832.

Reiss GR, Lee DA, Topper JA, Brubaker RF, "Aqueous humor flow during sleep", *Invest Ophthalmol Vis Sci*, 1984; vol. 25, p. 776.

Resnikoff S, Pascolini D, Etya’ale D, Kocur I, Parajasegaram R, Pokharel GP, Mariotti SP, "Global data on visual impairment in the year 2002", *Bull World Health Org*, 2004; vol. 82, pp. 844-851.

Resul B, Stjernschantz J, "Structure-activity relationships of prostaglandin analogues as ocular hypotensive agent", *Curr Opin Ther Patients*, 1993; pp. 781-795.

Rezaie T, Child A, Hitchings R, Brice G, Miller L, Coca-Prados M, Heon E, Krupin T, Ritch R, Kreutzer D, Crick RP, Sarfarazi M, "Adult-onset primary open-angle glaucoma caused by mutations in optineurin", *Science*, 2002; vol. 295, pp. 1077-1079.

Rezaie T, Child A, Hitchings R, Brice G, Miller L, Coca-Prados M, Heon E, Krupin T, Ritch R, Kreutzer D, Crick RP, Sarfarazi M, “Adult onset primary open angle glaucoma caused by mutations in optineurin”, *Science*, 2002; vol. 295, pp. 1077-9.

Richter M, Krauss AH, Woodward DF, Lütjen-Drecoll E, "Morphological changes in the anterior eye segment after long-term treatment with different receptor selective prostaglandin agonists and a prostamide", *Invest Ophthalmol Vis Sci*, 2003; vol. 44, pp. 4419-4426.

Rochtchina E, Mitchell P, Wang JJ, "Relationship between age and intraocular pressure: the Blue Mountains Eye Study", *Clin Experiment Ophthalmol*, 2002; vol. 30, pp. 173-175.

Roselund EF, "The intraocular pressure lowering effect of timolol in gel-forming solution", Acta *Ophthalmol Scand*, 1996, vol. 74, pp. 160-162.

Rozier A, Manuel C, Groove J, Plazonet B, "Gelrite: Novel, non-activated, in-situ gelling polymer for ophthalmic vehicles. Effect on bioavailability of timolol." *Int J Pharm*, 1989; vol. 57, pp. 163-168.

Rudnicka AR, Mt-Isa S, Owen GG, Cook DG, Ashby D, "Variation in primary open angle glaucoma prevalence by age, gender and race: a Bayesian meta-analysis", *Invest Ophthalmol Vis Sci*, 2006; vol.47, pp.4254-61.

Rulo AH, Greve EL, Hyong PF, "Additive effect of latanoprost, a prostaglandin $F_{2\alpha}$ analogue and timolol in patients with elevated intraocular pressure", *Br J Ophthalmol* 1994, vol 7, pp 899-902

Rulo AH, Greve EL, Geijssen HC, Hyong PF, "Reduction of intraocular pressure with treatment of latanoprost once daily in patients with normal-pressure glaucoma", *Ophthalmology*, 1996, vol 103, pp 1276-1282

Saccà SC, Rolando M, Marletta A, Macri A, Cergueti P, Ciurlo G, "Fluctuation of intraocular pressure during the day in open-angle glaucoma, normal tension glaucoma and normal subjects", *Ophthalmologica*, 1998; vol. 212, pp. 115-119.

Sahin A, Bayer A, Ozge G, Mumcuoglu T, "Corneal biomechanical changes in diabetes mellitus and their influence on intraocular pressure measurements", *Invest Ophthalmol Vis Sci*, 2009; vol. 50, no. 10, pp. 4597-4604.

Sakamoto K, Ezashi T, Miwa K, Okuda-Ashitaka E, Houtani T, Sugimoto T, Ito S, Hayaishi O, "Molecular cloning and expression of a cDNA of the bovine prostaglandin $F_{2\alpha}$ receptor", *J Biol Chem*, 1994; vol. 269, pp. 3881-3886.

Sakamoto K, Kamimura M, Kurozumi S, Ito S, "Prostaglandin $F_{2\alpha}$ receptor", *J Lipid Mediat Cell Signal*, 1995; vol. 12, pp. 405-411.

Sakurai M, Higashide T, Takahashi M, Sugiyama K, "Association between genetic polymorphisms of the prostaglandin $F_{2\alpha}$ receptor gene and response to latanoprost", *Ophthalmology*, 2007; vol. 114, pp. 1039-1045.

Salazar-Bookaman MM, Wainer I, Patil PN, "Relevance of drug-melanin interactions to ocular pharmacology and toxicology". *J Ocul Pharmacol Ther*, 1994; vol 10: pp 217-239

Saleh TA, Adams M, McDermott B, Claridge KG, Ewings P, "Effects of corneal parameters on measurements using the pulsatile ocular blood flow tonograph and Goldmann applanation tonometer", *Clin Exp Ophthalmol*, 2006, vol 34, pp 51-61

Sales KJ, Grant V, Jabbour HN, "Prostaglandin E_2 and $F_{2\alpha}$ activate the FP receptor and up-regulate cyclooxygenase-2 expression via the cyclic AMP response element", *Mol Cell Endocrinology*, 2008; vol. 285, pp. 51-61.

Salim S, Shields MB," Glaucoma and systemic diseases", *Surv Ophthalmol*, 2010, vol 55, pp 64-77.

Salminen L, Imre G, Huupponen R, "The effect of ocular pigmentation on intraocular pressure response to timolol", *Acta Ophthalmol*, 1985; vol. 173 (Suppl), pp. 15-18.

Sarfarazi M, Child A, Stoilova D, Brice G, Desai T, Trifan OC, Poinoosawmy D, Crick RP, "Localization of the fourth locus (GLC1E) for adult-onset primary open-angle glaucoma to the 10p15-p14 region", *Am J Hum Genet*, 1998; vol. 62, pp. 641-652.

Schenker HI, Yablonski ME, Podos SM, Linder L, "Fluorophotometric study of epinephrine and timolol in human subjects", *Arch Ophthalmol*, 1981; vol. 99, pp. 1212-1216.

Schenker HI and Silver LH, "Long-term intraocular pressure-lowering efficacy and safety of timolol maleate gel forming solution 0.5% compared to Timoptic XE 0.5% in a 12 months study", *Am J Ophthalmol*, 2000, vol.130, pp 145-50

Scherer WJ, "A retrospective review of non-responders to latanoprost", *J Ocul Pharmacol Ther*, 2002; vol. 18, pp. 287-291.

Schiøtz H, "Ein neues Tonometer", *Arch Augenh*, 1905; vol. 52, p. part IV.

Schmitt CJ, Lotti VJ, LeDouarec JC, "Penetration of timolol into the rabbit eye. Measurements after ocular instillation and intravenous injection", *Arch Ophthalmol*, 1980; vol. 98, pp. 547-551.

Schötzer-Schrehardt U, Zenkel M, Nusing RM, "Expression and localization of FP and EP prostanoid receptor subtypes in human ocular tissues", *Invest Ophthalmol Vis Sci*, 2002; vol. 43, pp. 1475-1487.

Schuettauf F, Quinto K, Naskar R, Zurakowski D, "Effects of anti-glaucoma medications on ganglion cell survival: the DBA/2J mouse model", *Vision Res*, 2002; vol. 42, pp. 2333-2337.

Schuman JS, "Effects of systemic beta-blocker therapy on the efficacy and safety of topical brominidine and timolol", *Ophthalmology*, 2000, vol.107, pp.1171-7

Schwartz GF, Quigley HA, "Adherence and persistence with glaucoma therapy", *Surv Ophthalmol*, 2008; vol. 53 (Suppl), pp. 57-68.

Schwartz GF, Reardon G, Mozaffari E, "Persistency with latanoprost or timolol in primary open-angle glaucoma suspects", *Am J Ophthalmol*, 2004; vol. 137 (Suppl), pp. S13-16.

Scott MG, Swan C, Wheatley AP, Hall IP, "Identification of novel polymorphisms within the promoter region of the human $ß_2$- adrenergic receptor gene", *Br J Pharmacol*, 1999; vol. 126, pp. 841-844.

Seah SK, Foster PJ, Chew PT, Jap A, Oen F, Fam HB, Lim AS, "Incidence of acute primary angle-closure glaucoma in Singapore. An island-wide survey", *Arch Ophthalmol*, 1997; vol. 115, pp. 1436-1440.

Semes L, Shaikh A, McGwin G, Bartllet JD, "The relationship among race, iris color, central corneal thickness, and intraocular pressure", *Optom Vis Sci*, 2006; vol. 83, pp. 512-515.

Senior PA, Bhopal R, "Ethinicity as a variable in epidemiological research", *BMJ*, 1994, vol 305, pp 327-330

Shaikh MY, Bodla AA, "Hypertrichosis of the eyelashes from prostaglandin analog use: a blessing or a bother to the patient?", *J Ocul Pharmacol Ther*, 2006; vol. 22, pp. 76-77.

Sharif NA, Kelly CR, Crider JY, Williams GW, Xu SX, "Ocular hypotensive FP prostaglandin (PG) analogs: PG receptor subtype binding affinities and selectivities and agonist potencies at FP and other PG receptor cultured cells", *J Ocul Pharmacol Ther*, 2003; vol. 19, pp. 501-515.

Sharif FM, Selvarajah S, "The outcome of trabeculectomy for primary glaucoma in adult patients in UKM", *Med J Malaysia*, vol 52, pp 17-25.

Shayegan MR, Boloorian AA, Kianoush S, "Comparative study of topical application of timolol and verapamil in patients with glaucoma within 6 months", *J Ocul Pharmacol Ther*, 2009; vol. 25(6), pp.551-3.

Shedden AH, "Timolol maleate in gel forming solution: A novel formulation of timolol maleate". *Chibret Int I Ophthalmol*, 1994; vol 10: pp32-36

Shedden A, Laurence J, Tipping R, for the Timoptic-XE 0.5% Study Group, "Efficacy and tolerability of timolol maleate ophthalmic gel-forming solution versus timolol ophthalmic solution in adults with open-angle glaucoma or ocular hypertension: a six-month, double-masked, multicenter study", *Clin Ther*, 2001a; vol. 23, pp. 440-450.

Shedden AH, Laurence J, Barrish A, Olah TV, "Plasma timolol concentrations of timolol maleate: timolol gel forming solution (TIMOPTIC-XE®) once daily versus timolol maleate ophthalmic solution twice daily", *Doc Ophthalmol*, 2001b; vol. 103, pp. 73-79.

Shen SY, Wong TY, Foster PJ, Loo JL, Rosman M, Loon SC, Wong WL, Saw SM, Aung T, "The prevalence and types of glaucoma in Malay people: The Singapore Malay Eye Study", *Invest Ophthalmol Vis Sci*, 2008; vol. 49, pp. 3846-3851.

Shephard RJ, Ponsford E, Basu PK, LaBarre R, "Effects of cigarette smoking on intraocular pressure and vision", *Br J Ophthalmol*, 1978; vol. 62, pp. 682-687.

Sherwood MB, Grierson I, Millar L, Hitchings RA, "Long term morphology effects of antiglaucoma drugs on the conjunctiva and tenon's capsule in glaucomatous patients", *Ophthalmology*, 1989; vol. 96, pp. 327-335.

Shimmyo M, Ross AJ, Moy A, Mostafavi R, "Intraocular pressure, Goldmann applanation tension, corneal thickness, and corneal curvature in Caucasians, Asians, Hispanics and African Americans", *Am J Ophthalmol* , 2003; vol. 136, pp. 603-613.

Shin DH, Becker B, Kolker AE, "Family history in primary open-angle glaucoma", *Arch Ophthalmol*, 1977; vol. 95, pp. 598-600.

Shiose Y, "The aging effect on intraocular pressure in apparently normal population", *Arch Ophthalmol*, 1984; vol. 102, no. 6, pp. 883-887.

Shiose Y, Kawase Y, "A new approach to stratified normal intraocular pressure in a general population", *Am J Ophthalmol*, 1986; vol. 101, pp. 714-721.

Shiose Y, Kitazawa Y, Tsukahara S, Akamatsu T, Mizokami K, Futa R, Katsushima H, Hosaki H, "Epidemiology of glaucoma in Japan- a nationwide glaucoma survey", *Jpn J Ophthalmol*, 1991; vol. 35, pp. 133-155.

Siegner SN, Netland PA, Schroedar A, Erickson Ka, "Effect of calcium channel blockers alone and in combination with antiglaucoma medications an intraocular pressure in the primate eye", *J Glaucoma*, 2000; vol.9(4), pp.334-9.

Silverman EK, Kwiatkowski DJ, Sylvia JS, Lazarus R, Drazen J, Lange C, Laird N, Weiss S, "Family-based association analysis of ß$_2$-adrenergic receptor polymorphisms in the childhood asthma management program", *J Allergy Clin Immunol*, 2003; vol. 112, pp. 870-876.

Singh K, Shrivastava A, "Intraocular pressure fluctuations: how much do they matter?" *Curr Opin Ophthalmol*, 2009; vol. 20, pp. 84-87.

Singh K, Spaeth G, Zimmerman T, Minckler D, "Target pressure-glaucomatologist's holey grail", *Ophthalmology*, 2000; vol. 107, pp. 629-630.

Sjögren H, "A study in pseudoglaucoma (glaucoma without hypertension)", Acta Ophthalmol, 1946; vol. 24, pp. 239-294.

Sjöquist B, Stjernschantz J, "Ocular and systemic pharmacokinetics of latanoprost in humans", *Surv Ophthalmol*, 2002; vol. 47 (Suppl 1), p. S6-S12.

Small KM, Tanguay DA, Nandalan K, Zhan P, Stephens JC, Ligget SB, "Gene and protein domain-specific patterns of genetic variability within the G-protein coupled receptor superfamily", *Am J Pharmacogenomics*, 2003; vol 3, pp. 65-71

Smith RJ, "Medical versus surgical therapy in glaucoma simplex", *Br J Ophthalmol*, 1972; vol. 56, pp. 277-283.

Sommer A, "Intraocular pressure and glaucoma", *Am J Ophthalmol*, 1989; vol.107, pp.186-8.

Sonntag JR, Brindley GO, Shields MB, "Effect of timolol therapy on outflow facility", *Invest Ophthalmol Vis Sci*, 1978; vol. 17, pp. 293-296.

Sossey-Alaoui K, Kitamura E, Cowell JK, "Fine mapping of the PTGFR gene to 1p31 region and mutation analysis in human breast cancer", *Int J Mol Med*, 2001; vol. 7, pp. 543-546.

South East Asia Glaucoma Interest Group 2008, *Asia Pacific Glaucoma Guidelines*, Second edn, Scientific Communications, Hong Kong.

Spina D, Rigby PJ, Paterson JW, Goldie RG, "Autoradiographic localization of ß-adrenoceptor in asthmatic human lung", *Am Rev Respir Dis*, 1989; vol. 140, pp. 1410-1415.

Spooner JJ, Bullano MF, Ikeda LI, Cockerham TR, Waugh WJ, Johnson T, Mozaffari E, "Rates of discontinuation and change of glaucoma therapy in a managed care setting", *Am J Manag Care*, 2002; vol. 8, no. Suppl, pp. S262-270.

Sripriya S, Uthra S, Sangeetha R, George RJ, Hemamalini A, Paul PG, Amali J, Vijaya L, Kumaramanickavel G, "Low frequency of myocillin mutations in Indian primary open-angle glaucoma patients", *Clin Genet*, 2004; vol. 65, pp. 333-337.

Stewart RH, LeBlanc R, Becker B, "Effect of exercise on aqueous dynamics", *Am J Ophthalmol*, 1970; vol. 69, p. 245.

Stewart WC, Day DG, Holmes KT, Stewart JA, "Effect of timolol 0.5% gel and solution in pulmonary function in older glaucoma patients", 2001, *J Glaucoma*, vol. 10, pp. 227-232.

Stewart WC, Kolker AE, Stewart JA, Leech J, Jackson AL, "Conjunctival hyperemia in healthy subjects after short-term dosing with latanoprost, bimatoprost, and travoprost", *Am J Ophthalmol*, 2003; vol. 135, pp. 314-320.

Stewart WC, Day DG, Sharpe ED, Rubiner HB, Holmes KT, Stewart JA, "Efficacy and safety of timolol solution once daily vs. timolol gel added to latanoprost", *Am J Ophthalmol*, 1999, vol 128, pp 692-696.

Stjernschantz J, Alm A, "Latanoprost as a new horizon in the medical management of glaucoma", *Curr Opin Ophthalmol*, 1996; vol. 7, pp. 11-17.

Stjernschantz JW, Albert DM, Hu DN, Drago F, Wistrand PJ, "Mechanism and clinical significance of prostaglandin-induced iris pigmentation", *Surv Ophthalmol*, 2002; vol. 47, no. Suppl 1, pp. 162-175.

Stjernschantz J, Selεn G, Astin M, Resul B, "Microvascular effects of selective prostaglandin analogues in the eye with special reference to latanoprost and glaucoma treatment", *Progress in Retinal and Eye Research*, 2000; vol. 19, no. 4, pp. 459-496.

Stone EM, Fingert JH, Alward WL, Nguyen TD, Polansky JR, Sunden SL, Nishimura D, Clark AF, Nystuen A, Nichols BE, Mackey DA, Ritch R, Kalenak JW, Craven ER, Sheffield VC, "Identification of a gene that causes primary open angle glaucoma", *Science*, 1997; vol. 275, pp. 668-670.

Strosberg AD, "Structure, function and regulation of adrenergic receptors", *Protein Science*, 1993; vol. 2, pp. 1198-1209.

Sugimoto Y, Yamasaki A, Segi E, Tsuboi K, Aze Y, Nishimura T, Oida H, Yoshida N, Tanaka T, Katsuyama M, Hasumoto K, Murata T, Hirata M, Ushikubi F, Negishi M, Ichikawa A, Narumiya S, "Failure of parturition in mice lacking the prostaglandin F receptor", *Science*, 1997, vol 277, 681-683

Suzuki S, Suzuki Y, Iwase A, Araie M, "Corneal thickness in an ophthalmologically normal Japanese population", *Ophthalmology*, 2005; vol. 112, pp.1327-1336.

Tan S, Hall IP, Dewar J, Dow E, Lipworth B, "Association between beta 2-adrenoreceptor polymorphism and susceptibility to bronchodilator desentisation in moderately severe stable asthmatics', *Lancet*, 1997, vol 350, pp 995-999.

Tan JT, Ng PK, Nurbaya S, Ye S, Lim XL, Leong H, Set LT, Siew WF, Kon W, Wong TY, Saw SM, Aung T, Chia KS, Lee J, Chew SK, Seielstad M, Tai ES, "Polymorphisms identified through genome-wide association studies and their associations with type 2 diabetes in Chinese, Malays, and Asian-Indians in Singapore", *J Clin Endocrinol Metab*, 2010, vol 95, pp 390-397

Takeda Y, Azuma I, "Diurnal variations in outflow facility", *Ann Ophthalmol*, 1978; vol. 10, pp. 1575-1580.

Talty P, O'Brien PD, "Does extended wear of a tight necktie cause raised intraocular pressure?", *J Glaucoma*, 2005; vol. 14, pp. 508-510.

Tay A, Squire JA, Goldberg H, Skorecki K, "Assignment of the human prostaglandin-endoperoxide synthase 2 (PTGS2) gene to 1q25 by fluorescence in situ hybridization", *Genomics*, 1994; vol. 23, pp. 718-719

Taylor P, "Cholinergic agonists," in *Goodman and Gilman's The pharmacological basis of therapeutics*, 8th edn, Gilman AG, Rall TW, Nies AS, and Taylor P eds, 1990; pp. 122-130, Pergamon Press, New York.

Teng C, Gurses-Ozden R, Liebmann JM, Tello C, Ritch R, "Effect of tight necktie on intraocular pressure", *Br J Ophthalmol*, 2003; vol. 87, pp. 946-948.

Tezel G, Wax MB, "The mechanisms of hsp27 antibody-mediated apoptosis in retinal neuronal cells", *J Neurosci*, 2000; vol. 20, pp. 3552-3562.

The AGIS investigators, "The Advanced Glaucoma Intervention Study (AGIS): 1. Study design and methods and baseline characteristics of study patients", *Control Clin Trials*, 1994; vol. 15, pp. 299-325.

The AGIS investigators, "The Advanced Glaucoma Intervention Study (AGIS):7. The relationship between control of intraocular pressure and visual field deterioration", *Am J Ophthalmol*, 2000; vol. 130, pp. 429-440.

The European Glaucoma Prevention Study (EGPS) Group, "The European Glaucoma Prevention Study design and baseline description of the participants", *Ophthalmology*, 2002; vol. 109, pp. 1612-1621.

Thomas R, Parikh R, Sood D, Vijaya L, Sekhar GC, Sood NN, Baskaran M, Prasad KK, Latanoprost India Study Group, "Efficacy and safety of latanoprost for glaucoma treatment: a three-month multicentric study in India", *Indian J Ophthalmol*, 2005; vol. 53, pp 23-30

Thorleifsson G, Magnusson KP, Sulem P, Walters GB, Gudbjartsson DF, Stefansson H, Jonsson T, Jonasdottir A, Jonasdottir A, Stefansdottir G, Masson G, Hardarson GA, Petursson H, Arnarsson A, Motallebipour M, Wallerman O, Wadelius C, Gulcher JR, Thorsteinsdottir U, Kong A, Jonasson F, Stefansson K, "Common sequence variants in the LOXL1 gene confer susceptibility to exfoliation glaucoma", *Science*, 2007; vol. 317, pp. 1397-1400.

Thylefors B and Négrel AD, "The global impact of glaucoma", *Bull World Health Org*, 1994; vol. 72, pp. 323-326.

Tielsch JM, Katz J, Quiqley HA, Javitt JC, Sommer A, "Diabetes, intraocular pressure, and primary open-angle glaucoma in Baltimore Eye Survey", *Ophthalmology*, 1995; vol. 102, pp. 48-53.

Tielsch JM, Katz J, Sommer A, Quigley HA, Javitt JC, "Family history and risk of primary open angle glaucoma. The Baltimore Eye Survey", *Archives of Ophthalmology*, 1994; vol. 112, pp. 69-73.

Tielsch JM, Sommer A, Katz J, Royal RM, Quigley HA, Javitt J, "Racial variations in the prevalence of primary open-angle glaucoma. The Baltimore Eye Survey", *JAMA*, 1991, vol, pp. 369-374.

Timothy CO, Nneli RO, "The effects of cigarette smoking on intraocular pressure and arterial blood pressure of normotensive young Nigerian male adult", *Nigerian J Physiol Sci*, 2007; vol. 22, pp. 31-35.

Tomaszewski M, Brain NJ, Charchar FJ, Wang WY, Lacka B, PadmanaBahn S, Clark JS, Anderson NH, Edwards HV, Zukowska-Szczechowska E, Grzeszczak W, Dominiczak AF, ”Essential hypertension and beta2-adrenergic receptor gene: linkage and association analysis”, *Hypertension* 2002; vol. 40, pp. 286-91

Tomlinson A, Phillips CI, "Applanation tension and axial length of the eyeball", *Br J Ophthalmol*, 1970; vol. 54, pp. 548-553.

Topper JE, Brubaker RF, "Effects of timolol, epinephrine, and acetalzolamide on aqueous flow during sleep", *Invest Ophthalmol Vis Sci*, 1985; vol. 26, p. 1315.

Toris CB, Camras CB, Yablonski ME. "Effects of PhXA41, a new prostaglandin $F_{2\alpha}$ analog, on aqueous humor dynamics in human eyes", *Ophthalmology*, 1993; vol. 100, pp. 1297-1304.

Toris CB, Gabelt BT, Kaufman PL, "Update on the mechanism of action of topical prostaglandins for intraocular pressure reduction", *Surv Ophthalmol*, 2008; vol. 53 (Suppl 1), p. S107-S120.

Trevan J, "The error of determination of toxicity", *Proc R Soc Lond Biol Sci*, 1927; vol. 101, pp. 483-514.

Trikalinos TA, Salanti G, Khoury MJ, Ioannidis JP, “Impact of violations and deviations in Hardy-Weinberg equilibirium on postulated gene-disease associations” *Am J Epidemiology*, 2006; vol 163: pp 300-309.

Trope GE, Clark B, “Beta adrenergic receptors in pigmented ciliary process,”*Br J Ophthalmol”,* 1982, vol 66, pp 788-792

Tsai HJ, Shaikh N, Kho JY, Battle N, Nagvi M, Navarro D, Matallana H, Lilly CM, Eng CS, Kumar G, Thyne S, Watson HG, Meade K, LeNoir M, Choudry S, Burchard EG, Study of African Americans, Asthma, Gene Enviroments (SAGE), "$ß_2$-adrenergic receptor polymorphisms: pharmacogenetics responder to bronchodilator among African American asthmatics", *Hum Genet*, 2006; vol. 119, pp. 547-557.

Tsai JC, McClure CA, Ramos SE, Schlundt DG, Pichert JW, "Compliance barriers in glaucoma: a systematic classification", *J Glaucoma*, 2003; vol. 12, pp. 393-398.

Turki J, Lorenz JN, Green SA, Donnelly ET, Jacinto M, Liggett SB, "Myocardial signalling defects and impaired cardiac function of a human beta2-adrenergic receptor polymorphism expressed in transgenic mice", *Proc Natl Acad Sci USA*, 1996; vol. 93, pp. 10483-10488.

Turner ST, Schwartz GL, Chapman AB, Hall WD, Boerwinkle E, "Antihypertensive pharmacogenetics: getting the right drug into the right patient", *J Hypertens*, 2001; vol. 19, pp. 1-11.

Ueki J, Rhodes CG, Hughes JM, De Silva R, Lefroy DC, Ind PW, Qing F, Brady F, Luthra SK, Steel CJ, "In vivo quantification of pulmonary ß-adrenoceptor density in humans with (S)-^{11}C-CGP12177 and PET", *J Appl Physiol*, 1993; vol. 75, pp. 559-565.

Urban JD, Clarke WP, von Zastrow M, Nicholas DE, Kobilka B, Weinstein H, Javith JA, Roth BL, Christopaulus A, Sexton PM, Miller KJ, Spedding M, Mailman RB, "Functional selectivity and classical concepts of quantitative pharmacology", *J Pharmacol Exp Ther*, 2007; vol. 320, pp. 1-13.

Uusitalo H, Kahonen M, Ropo A, Maenpaa J, Bjarnhall G, Hedenstrom H, Turjanmaa V, "Improved systemic safety and risk-benefit ratio of topical 0.1% timolol hydrogel compared with 0.5% timolol aqueous solution in the treatment of glaucoma", 2006, *Graefe's Arch Clin Exp Ophthalmol*, vol. 244, pp. 1491-1496.

Van de Valk R, Weber CA, Schouten JS, Zeeger MP, Hendrikse F, Prins MH, "Intraocular pressure lowering effects of all commonly used glaucoma drugs. A meta-analysis of randomized clinical trials", 2005, *Ophthalmology*, vol. 112, pp. 1177-1185.

Vane JR, Bakhle YS, Botting RM, "Cyclooxygenase 1 and 2", *Annu Rev Pharmacol Toxicol*", 1998; vol 38, pp 97-120.

Vareilles P, Silvestone D, Plazzonet B, Le Douarec JC, Sears ML, Stone CA, "Comparison of the effects of timolol and other adrenergic agents on intraocular pressure in the rabbit", *Invest Ophthalmol Vis Sci*, 1977; vol. 16, pp. 987-996.

Vane JR, Bakhle YS, Botting RM, "Cyclooxygenase 1 and 2", *Annu Rev Pharmacol Toxicol*, 1998, vol 38, pp 97-120

Vaughan CJ, Delanty N, "Neuroprotective properties of statin in cerebral ischemia and stroke", *Stroke*, 1999; vol.30, pp. 1969-73.

Vesell ES, "Pharmacogenetics perspective gained from twin and family studies," in *Pharmacogenetics of drug metabolism* , vol. 137 Kalow W, ed., Pergamon Press, New York, 1992; pp. 843-868.

Vieira GM, Oliveira HB, de Andrade DT, Bottaro M, Ritch R, "Intraocular pressure variation during weight lifting", *Arch Ophthalmol*, 2008; vol. 124, pp. 1251-1254.

Vielhauer GA, Fujino H, Regan JW, "Cloning and localization of hFP_s: a six-transmembrane mRNA splice variant of the human FP prostanoid receptor", *Arch Biochem Biophys*, 2004; vol. 421, pp. 175-185.

Vijaya L, George R, Baskaran M, Arvind H, Raju P, Ramesh SV, Kumaramanickavel G, McCarty C, "Prevalence of primary open-angle glaucoma in an urban south Indian population

and comparison with a rural population. The Chennai Glaucoma Study", *Ophthalmology*, 2008; vol. 115, pp. 648-654.

Vine AE, Curtis D, "Markers typed in genome-wide analysis identify regions showing deviation from Hardy-Weinberg equilibrium", BMC Research Notes, 2009; vol 9: pp.29

von Graefe A, "Beitrage zur Pathologie und Therapie des Glaukoms", *Arch Ophthalmologie*, 1869; vol. 15, pp. 108-252.

Walters TR, DuBiner HB, Carpenter SP, Khan B, VanDenburgh AM, Bimatoprost Circadian IOP Study Group, "24 hour IOP control with once-daily bimatoprost, timolol gel-performing solution, or latanoprost: a 1-month, randomized, comparative clinical trial", *Surv Ophthalmol*, 2004; vol. 49, no. Suppl 1, pp. 26-35.

Wand M, Gilbert CM, Liesegang TJ, "Latanoprost and herpes simplex keratitis", *Am J Ophthalmol*, 1999; vol. 127, pp. 602-604.

Warwar RE, Bullock JD, Ballal D, "Cystoid macular edema and anterior uveitis associated with latanoprost use. Experience and incidence in a retrospective review of 94 patients", *Ophthalmology*, 1998; vol. 105, pp. 263-268.

Watanabe K, Chiou GC, "Action mechanism of timolol to lower the intraocular pressure in rabbits", *Ophthalmic Res*, 1983; vol. 15, pp. 160-167.

Watson PG, Barnett MF, Parker V, Haybittle J, "A 7 year prospective comparative study of three topical ß blockers in the management of primary open angle glaucoma", *Br J Ophthalmol*, 2001; vol. 85, pp. 962-968.

Watson P, Stjernschantz J, "A six-month, randomized, double-masked study comparing latanoprost with timolol in open-angle glaucoma and ocular hypertension. The Latanoprost Study Group", *Ophthalmology*, 1996, vol 103, pp 126-137

Wax MB, Molinoff PB, "Distribution and properties of ß-adrenergic receptors in human iris/ciliary body", *Invest Ophthalmol Vis Sci*, 1987; vol. 28, pp. 420-430.

Wax MB, Molinoff PB, Alvarado J, Polansky J, "Characterization of ß-adrenergic receptors in cultured human trabecular cells and in human trabecular meshwork", *Invest Ophthalmol Vis Sci*, 1989; vol. 30, pp. 51-57.

Webers CA, Beckers HJ, Zeegers MP, Nuijts RM, Hendrikse F, Schouten JS, "The Intraocular Pressure-Lowering Effect of Prostaglandin Analogs Combined with Topical [beta]-Blocker Therapy: A Systematic Review and Meta-analysis", *Ophthalmology*, 2010; vol. 117, no. 11, pp. 2067-2074.

Weih LM, Mukesh BN, McCarty CA, Taylor HR, "Association of demographic, familial, medical, and ocular factors with intraocular pressure", *Arch Ophthalmol*, 2001; vol. 119, pp. 875-880.

Weinreb RN, Cook J, Friberg TR, "Effect of inverted body position on intraocular pressure", *Am J Ophthalmol*, 1984; vol. 98, p. 784.

Weinreb RN, Kashiwagi K, Kashiwagi F, Tsukahara S, Lindsey JD, “Prostaglandin increase matrix metalloproteinase release from human ciliary smooth muscle cells”, *Invest Ophthalmol Vis Sci*, 1997, vol 38, pp2772-2780.

Weinreb RN and Lindsey JD, “Metalloproteinase gene transcription in human ciliary muscle cells with latanoprost”. *Invest Vis Sci*, 2002; vol 43: pp716-722

Weir TD, Mallek N, Sandford AJ, Bai TR, Awadh N, Fitzgerald JM, Cockcroft D, James A, Liggett SB, Pare PD, "ß$_2$-adrenergic receptor haplotypes in mild, moderate and fatal/near fatal asthma", *Am J Respir Crit Care Med*, 1998; vol. 158, pp. 787-791.

Wentworth WO, Brubaker RF, "Aqueous humor dynamics in a series of patients with third neuron Horner's syndrome", *Am J Ophthalmol*, 1981; vol. 92, p. 407.

Whitacre MM, Stein R, "Source of error with use of Goldmann-type tonometers", *Surv Ophthalmol*, 1993; vol. 38, pp. 1-30.

Wilensky JT, "Diurnal variation of intraocular pressure", *Trans Am Ophthalmol Soc*, 1991; vol. 89, pp. 757-790.

Wolfs RC, Borger PH, Ramrattan RS, Klaver CCW, Hulsman CAA, Hofman A, Vingerling JR, Hitchings RA, Jong PTVM, "Changing views on open-angle glaucoma: Definitions and prevalences-The Rotterdam Study", *Invest Ophthalmol Vis Sci*, 2000; vol. 41, pp. 3309-3321.

Wolfs RC, Klaver CC, Vingerlin JR, Grobbee DE, Hofman A, de Jong PT , "Distribution of central corneal thickness and its association with intraocular pressure", *Am J Ophthalmol*, 1997; vol. 123, pp. 767-772.

Wolfs RCW, Klaver CCW, Ramrattan RS, Duijn CMV, Hofman A, Jong PTVM, "Genetic risk of primary open-angle glaucoma. Population-based familial aggregation study", 1998; *Arch Ophthalmol*, vol. 116, pp. 1640-1645.

Wong TY, Foster PJ, Seah SK, Chew PT, "Rates of hospital admissions for primary angle closure glaucoma among Chinese, Malays and Indians in Singapore", *Br J Ophthalmol*, 2000; vol. 84, pp. 990-992.

Wong TY, Klein BE, Klein R, Knudtson M, Lee KE, "Refractive errors, intraocular pressure, and glaucoma in a white population", *Ophthalmology*, 2003; vol. 110, pp. 211-217.

Woodward DF, Regan JW, Lake S, Ocklind A, "The molecular biology and ocular distribution of prostanoid receptors", *Surv Ophthalmol*, 1997; vol. 41, no. Suppl 2, p. S15-S21.

Wright S, “Evolution in Mendelian populations”, *Genetics* 1931, vol; 16: 97-159

Wu SY, Leske MC, "Associations of intraocular pressure in the Barbados Eye Study", *Arch Ophthalmol*, 1997; vol. 115, pp. 1572-1576.

Wu SY, Nemesure B, Hennis A, Leske MC, Barbados Eye Studies Group, "Nine-year changes in intraocular pressure. The Barbados Eye Studies", *Arch Ophthalmol*, 2006; vol. 124, pp. 1631-1636.

Xie HG, Stein CM, Kim RB, Xiao ZS, He N, Zhou HH, Gainer JV, Brown NJ, Haines JL, Wood AJ, "Frequency of functionally important beta-2 adrenoceptor polymorphisms varies markedly among African-American, Caucasian and Chinese individuals", *Pharmacogenetics*, 1999; vol. 9, pp. 511-516.

Xie HG, Stein CM, Kim RB, Gainer JV, Sofowora G, Dishy V, Brown NJ, Goree RE, Haines JL, Wood AJ, "Human ß$_2$-adrenergic receptor polymorphisms: No association with essential hypertension in black and white Americans", *Clin Pharmacol Ther*, 2000; vol. 67, pp. 670-675.

Xu H, Poh WT, Sim X, Ong RTH, Sho C, Tay WT, Khor CC, Seielstad M, Liu J, Aung T, Tai ES, Wong TY, Chia KS, Teo YY, "Sg-D CNV, a database for common and rare copy number variants in three Asian population", *Hum Mutat*, 2011; vol.32, pp. 1341-9.

Xu L, Wang H, Wang Y, Jonas JB, "Intraocular pressure correlated with arterial blood pressure: the Beijing Eye Study", *Am J Ophthalmol*, 2007, vol 144, pp 461-462

Yablonski ME, Zimmerman TJ, Waltman SR, Becker B, "A fluorophotometric study of the effect of topical timolol on aqueous humor dynamics", *Exp Eye Res*, 1978; vol. 27, pp. 135-142.

Yamagata K, Andreasson KI, Kaufmann WE, Barnes CA, Worley PF, "Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids", *Neuron*, 1993; vol 11, pp 371-386

Yanai I, Benjamin H, Shmoish M, Chalifa-Caspi V, Shklar M, Ophir R, Bar-Even A, Horn-Saban S, Safran M, Domany E, Lancet D, Shmueli O, "Genome-wide midrange transcription profiles reveal expression level relationship in human tissue specification", *Bioinformatics*, 2005, vol 21, pp650-659

Yatsuka YI, Tsutsumi K, Nakamura K, Nakano S, Nakatsuka K, "Interaction between timolol eyedrops and oral nicardipine or oral diltiazem in healthy Japanese subjects", Eur *J Clin Pharmacol*, 1998; vol.54(2), pp.149-54.

Yip JL, Foster PJ, "Ethnic differences in primary angle-closure glaucoma", *Curr Opin Ophthalmol*, 2006; vol. 17, pp. 175-180.

Yousufzai SYK, Gao G, Abdel-Latif AA, "Mitogen-activated protein kinase inhibitors suppress prostaglandin $F_{2\alpha}$-induced myosin-light chain phosphorylation and contraction in iris sphincter smooth muscle", *Eur J Pharmacol*, 2000; vol. 407, pp. 17-26.

Yuan H, Yu M, Yang Y, Wu K, Lin X, Li J "Association of Cyp2D6 single-nucleotide polymorphism with response to ophthalmic timolol in primary open-angle glaucoma- a pilot study'. *J Ocul Pharm Ther*, 2010; vol 26, pp497-501

Zainal M, Ismail SM, Ropilah AR, Elias H, Arumugam G, Alias D, Fathilah J, Lim TO, Ding LM, Goh PP, "Prevalence of blindness and low vision in Malaysian population: results from the National Eye Survey 1996", *Br J Ophthalmol*, 2002; vol. 86, pp. 951-956.

Zaragoza DB, Wilson R, Eyster K, Olson DM, "Cloning and characterization of the promoter region of the human prostaglandin $F_{2\alpha}$ receptor gene", *Biochim et Biophysica Acta*, 2004; vol. 1676, pp. 193-202.

Zaragoza DB, Wilson RR, Mitchell BF, Olson DM, “The interleukin 1beta-induced expression of human prostaglandin F_{2alpha} receptor messenger RNA in human myometrial-derived ULTR cells requires the transcription factor, NFkappaB[1”]. *Biol Reprod.* 2006; vol 75, pp 697-704

Zeimer RC, "Circadian variations in intraocular pressure," in Ritch R, Shields MB, Krupin T (eds): *The Glaucomas. St Louis, Mosby*, 1996; vol. 1, pp. 429-445.

Zeimer RC, Wilensky JT, Gieser DK, Viana MA, "Association between intraocular pressure peaks and progression of visual field loss", *Ophthalmology*, 1991; vol. 98, pp. 64-69.

Zilfalil BA, Hoh BP, Nizam MZ, Liza-Sharmini AT, Teh LK, Ismail R,”An improved rapid genotyping method for the study of beta-2 adrenergic receptor gene polymorphisms using single tube allele specific multiplex PCR”, *J Clin Pharm Ther*, 2006, vol 31, pp637-640.

Zhang WY, Po AL, Dua HS, Azuara-Blanco A, "Meta analysis of randomised controlled trials comparing latanoprost and timolol in the treatment of patients with open angle glaucoma and ocular hypertension", *Br J Ophthalmol*, 2001; vol. 85, pp. 983-990.

Zhou YH, Wei YT, Bai YJ, Duan S, Lin MK, Saragovi HU, Ge J, "Pro370Leu MYOC gene mutation in a large Chinese family with juvenile-onset open angle glaucoma: correlation between genotype and phenotype", *Mol Vis*, 2008; vol. 14, pp. 1533-1539.

Zimmerman TJ and Boger WP III, "The beta-adrenergic blocking agents and the treatment of glaucoma", *Surv Ophthalmol*, 1979; vol. 23, pp. 347-362.

Zimmerman TJ, Kaufman HE, "Timolol: dose response and duration of action", *Arch Ophthalmol*, 1977; vol. 95, pp. 605-607.

Zimmerman TJ, Hahn SR, Gelb L, Tan H, Kim EE, "The impact of ocular adverse effects in patients treated with topical prostaglandin analogs: Changes in prescription patterns and patient persistence", *J Ocul Pharmacol Ther*, 2009; vol. 25, pp. 145-152

APPENDICES

PATIENT INFORMATION SHEET AND CONSENT FORM

Pharmacogenetics of glaucoma; a study of the role of beta2-ar and prostanoid (FP) receptor gene polymorphisms in pressure lowering effect of topical timolol and latanoprost

PROJECT B: Topical timolol

Introduction

You are invited to take part voluntarily in a research study involving topical Timolol. Timolol is one of the commonly used anti-glaucoma drugs. Before agreeing to participate in this research study, it is important that you read and understand this form. It describes the purpose, procedures, benefits, risks, discomforts, and precautions of the study. It also describes your right to withdraw from the study at anytime. If you agree to participate, you will receive a copy of this form to keep for your records.

Purpose of the Study

The purpose of this study is to determine whether the variation of the genetic make-up of patients, who are on timolol therapy, affects the intraocular pressure lowering effect of timolol.

All the information obtained in this study will be kept **CONFIDENTIAL**. It is possible that information collected during this study will be analyzed by the sponsor in the future for other possible uses or other medical or scientific purposes other than those currently proposed.

Requirements to participate

The doctor in charge of this study or a member of the study staff has discussed with you the requirements for participation in this study. It is important that you are completely truthful with the doctor and staff about you health history. You should not participate in this study if you do not meet all the requirements.

Some of the requirements to be in this study are –

- Adults aged above 40 years.
- Had been diagnosed to have primary open angle glaucoma, normal tension glaucoma or ocular hypertension.
- Prescription of topical timolol upon confirmation of diagnosis

You cannot participate in this study if you are -

- Unwilling to be started on topical timolol XE 0.5%
- Allergic to topical timolol
- Known to suffer from:-
  Chronic obstructive airway disease, bronchial asthma, pulmonary disease, psychiatric illness, liver and renal disease.

You need to agree to use the drug as instructed by the doctor and staff of the research project and to return any unused drug and containers at the end of the study or as otherwise instructed by the doctor.

Procedure of the study

Visit 1

If you are diagnosed to have primary open angle glaucoma, normal tension glaucoma or ocular hypertension during your visit to the Eye Clinic and agree to participate in this study, you will receive treatment with topical timolol. You will undergo several baseline ocular examinations to check your intraocular pressure, optic nerve head assessment and a visual field test. Three ml of blood will be taken for genetic analysis. Any remaining blood after the genetic analysis will be discarded. You will then be prescribed with topical timolol XE 0.5% on morning. You must follow the instructions given by the doctor involved in this study with regards to the dosage and follow-up plan.

You will then be given an appointment for your second visit.

Visit 2

During your second visit (one month after your first visit), a repeat eye examination on your intraocular pressure, optic nerve head and visual field test will be carried out. Should you choose to withdraw from the study, you will still be followed up for your disease. You'll be asked regarding any side effects of timolol. If all goes well, you would then be continued on topical timolol XE.

You will then be given an appointment for your third visit.

Visit 3

During your third visit (2 months after your second visit), a repeat eye examination on your intraocular pressure, optic nerve head and visual field test will be carried out. You'll be asked again regarding any side effects of timolol.

During this time, if

- your intraocular pressure is uncontrolled
- there is sign of progression of the disease
- the side effects is intolerable

Your treatment maybe changed or you may be given additional medications or an operation maybe planned. If this happens, you will be automatically excluded from the study. On the other hand, if topical timolol XE is effective in controlling your disease, you will be continued on topical timolol XE and given another appointment for your fourth visit.

Visit 4

During your forth visit (3 months after your third visit), a repeat eye examination on your intraocular pressure, optic nerve head and visual field test will be carried out. You'll be asked again regarding any side effects of timolol

During this time, if

- your intraocular pressure is uncontrolled
- there is sign of progression of the disease
- the side effects is intolerable

Your treatment maybe changed or you may be given additional medications or an operation maybe planned. If this happens, you will be automatically excluded from the study. On the other hand, if topical timolol XE is effective in controlling your disease, then you will be continued on topical timolol XE and given another appointment for your fifth visit. (The appointment 3 months from this visit will not be included in the study but for continuation of your treatment. You need to continue your follow-up accordingly.)

Visit 5

During your fifth visit (6 months from your fourth visit), you'll be on topical timolol XE for 12 months. As usual, a repeat eye examination on your intraocular pressure, optic nerve head and visual field test will be carried out.

During this time, if

- your intraocular pressure is uncontrolled
- there is sign of progression of the disease
- the side effects is intolerable

Your treatment maybe changed or you may be given additional medications or an operation maybe planned. If this happens, you will be automatically excluded from the study. This is the last visit for this study. If all goes well, you will be continued on topical timolol XE and you will continue your follow-up and treatment in the Eye Clinic.

Risks

There may be some risks if you participate in this study, which relate to the usage of standard glaucoma medication. You may encounter some ocular or systemic side effects. Examples of side effects include burning sensation or ocular discomfort or pain, breathlessness and palpitations. Some of the side effects are transient and mild. However, if the side effects are hazardous to health, topical timolol will be stopped immediately. In addition to the risk named above, the study procedures may have other unknown risks. There may be unknown risks of possible harmful interaction with other medication you may be taking. These risks would be present if you used the mediation outside of this research project.

You should follow carefully the doctor's directions for taking this study drug to avoid undesirable incidence.

Other Treatments

You do not have to take part in this study to be treated for your illness or condition. Other treatments and therapies for your condition are available, including your current therapy, including the medication used in this research project. The doctor who is involved in this study can discuss these treatments and therapies with you.

Participation in the Study

Your participation in this study is entirely voluntary. You may refuse to take part in the study or you may stop your participation in the study at anytime, without a penalty or loss of benefits to which you are otherwise entitled.

The doctor who is involved in this study may stop your participation even without your consent.

If you stop being part of this study, the doctor or the staff member will talk to you about any medical issues regarding the discontinuation of your participation.

Treatment and Compensation for Injury

If you follow the directions of the study doctor and staff and you are physically injured due to any substance or procedure properly given under the plan for this study, the sponsor will pay the medical expenses for the treatment of that injury which are not covered by your medical insurance, by a government program, or by any other third party.

Possible Benefits

The drug and the study procedures will be provided at no cost to you. You may receive information about your health from any physical examination and laboratory tests to be done in this study.

You will be paid RM 50 to reimburse you for transportation, parking, meal, or other expenses related to your participation in this study. If you withdraw from the study early, you will be paid for these expenses for the portion of the study that you did complete.

Information obtained from this study will benefit the sponsor of the study (Ministry of Science, Technology and Information of Malaysia) and may benefit patients in the future.

Investigator Payment

The sponsor (Ministry of Science, Technology and Information of Malaysia) is paying the study doctor and/or her institution for their work in this study.

Questions

If you have any question about this study or your rights, please contact

- Dr. Liza Sharmini Ahmad Tajudin 0976664563/0199179227

If you have any questions about your rights as a participant in a research study, please contact the Ethical Review Board of the University Hospital.

Confidentialty

Your medical information will be kept confidential by the doctor and staff involved in this study and will not be made publicly available unless disclosure is required by the law.

Data obtained from this study that does not identify you individually will be given to the sponsor and/or its representatives and may be published. Your original medical records may

be reviewed by the sponsor and/or its representatives, the Ethical Review Board (ERB) for this study, and regulatory authorities for the purpose of verifying clinical trial procedures and/or data. Your medical information may be held and processed on a computer.

By signing this consent form, you authorize the record review, information storage and data transfer described above.

Signatures

To be entered into the study, you or a legal representative must sign and date the signature page (see Attachment 1)

Attachment 1: Consent Form

Information and consent form
Attachment 1
Consent form

To take part in this study, you or your legal guardian must sign this page.
Thereby signing this form, I certified that:

- **I have read and understand all the information provided in this information consent form.**
- **I have been given enough time to consider it**
- **All my questions had been answered satisfactorily**
- **I voluntarily agree to take part in this study, and will obey all the procedures and willing to give all the information required to the investigators when needed.**
- **I can terminate my participation at any time without any reason**
- **I have received a copy of the information and consent form for my own safe keeping.**

_____ _____

Patient's signature
Patient's name: **Registration number**
IC number:

_____ _____

Signature of legal guardian **Date: (DD/MM/YY)**
Name :
IC number:

_____

Name of the investigator

_____ _____

Signature of the investigator **Date: (DD/MM/YY)**

_____ _____

Signature of the witness **Date: (DD/MM/YY)**

Name :
IC number:

PATIENT INFORMATION SHEET AND CONSENT FORM

Pharmacogenetics of glaucoma; a study of the role of beta2-ar and prostanoid (FP) receptor gene polymorphisms in pressure lowering effect of topical timolol and latanoprost

PROJECT P: Topical Latanoprost

Introduction

You are invited to take part voluntarily in a research study of the drug topical Latanoprost. Latanoprost is one of the medicines used for treating disease called glaucoma and you will receive this medication as your glaucoma treatment. Before agreeing to participate in this research study, it is important that you read and understand the information written on this form. It describes the purpose, procedures, benefits, risks, discomforts, and precautions of the study. It also explains about your right to withdraw yourself from this study at any stage of the study. If you agree to participate, you will receive a copy of this information sheet for your reference.

Purpose of the Study

The purpose of this study is to determine whether the variation of the genetic make-up (polymorphisms) of prostanoid receptor in glaucoma patient treated with topical latanoprost therapy is associated with the effectiveness pressure lowering effect of the drug.

All the information obtained in this study will be kept **CONFIDENTIAL**. It is possible that information collected during this study will be analyzed by the sponsor in the future for other possible uses or other medical or scientific purposes other than those currently proposed.

Requirements to participate

The doctor involved in this study or a member of the staff must discuss with you the requirements for participation in this study. It is important that you are completely truthful with the doctor and staff about you health history. You should not participate in this study if you do not meet all the requirements.

The requirements to be recruited in this study are:

- Adults aged above 40 years.
- Had been diagnosed to have primary open angle glaucoma, normal tension glaucoma or ocular hypertension.
- Previously treated with topical timolol but the pressure is not well control or evidence of progression of glaucoma

You cannot participate in this study if you are -

- Unwilling to be started on topical latanoprost 0.005%
- Allergic to topical latanoprost
- History of previous trabeculectomy surgery

You need to agree to use the drug as instructed by the doctor and staff of the research project and to return any unused drug and containers at the end of the study or as otherwise instructed by the doctor.

Procedure of the study

Visit 1

If you are diagnosed to have primary open angle glaucoma, normal tension glaucoma or ocular hypertension during your visit to the Eye Clinic and agree to participate in this study, you will receive treatment with topical latanoprost. You will undergo several baseline ocular examinations to check your intraocular pressure, optic nerve head assessment and visual field test. Photograph of your optic nerve and anterior part of the eye will also be taken. Three ml of blood will be taken for genetic analysis. Any remaining blood after the genetic analysis will be discarded. You will then be prescribed with topical latanoprost 0.005% on nocte. You must follow the instructions given by the doctor involved in this study with regards to the dosage and follow-up plan.

You will then be given an appointment for your second visit.

Visit 2

During your second visit (one month after your first visit), a repeat eye examination on your intraocular pressure, optic nerve head and visual field test will be carried out. Should you choose to withdraw from the study, you will still be followed up for your disease. You'll be asked regarding any side effects of timolol. However, if your intraocular pressure fails to reduce to the target level, you will be advised to either to add or switching to another drug. Once this happen, you will be excluded from the study. If all goes well, you would then be continued on topical latanoprost.

You will then be given an appointment for your third visit.

Visit 3

During your third visit (2 months after your second visit), a repeat eye examination on your intraocular pressure, optic nerve head, visual field test and anterior segment photograph will be carried out. You'll be asked again regarding any side effects of topical latanoprost.
During this time, if

- your intraocular pressure is uncontrolled
- there is sign of progression of the disease
- the side effects is intolerable

Your treatment maybe changed or you may be given additional medications or an operation maybe planned. If this happens, you will be automatically excluded from the study. On the

other hand, if topical latanoprost is effective in controlling your disease, you will be continued on the treatment and given another appointment for your fourth visit.

Visit 4

During your forth visit (3 months after your third visit), a repeat eye examination on your intraocular pressure, optic nerve head, visual field test and anterior segment photograph will be carried out. You'll be asked again regarding any side effects of latanoprost
During this time, if

- your intraocular pressure is uncontrolled
- there is sign of progression of the disease
- the side effects is intolerable

Your treatment maybe changed or you may be given additional medications or an operation maybe planned. If this happens, you will be automatically excluded from the study. On the other hand, if topical latanoprost is effective in controlling your disease, then you will be continued on the treatment and given another appointment for your fifth visit. (The appointment 3 months from this visit will not be included in the study but for continuation of your treatment. You need to continue your follow-up accordingly.)

Visit 5

During your fifth visit (6 months from your fourth visit), you'll be on topical latanoprost for 12 months. As usual, a repeat eye examination on your intraocular pressure, optic nerve head, visual field test and anterior segment photography will be carried out.
During this time, if

- your intraocular pressure is uncontrolled
- there is sign of progression of the disease
- the side effects is intolerable

Your treatment maybe changed or you may be given additional medications or an operation maybe planned. If this happens, you will be automatically excluded from the study. This is the last visit for this study. If all goes well, you will be continued on topical latanoprost and you will continue your follow-up and treatment in the Eye Clinic.

Risks

There may be some risks if you participate in this study, which relate to the usage of standard glaucoma medication. You may encounter some ocular or systemic side effects. Examples of side effects include burning sensation or ocular discomfort or pain, breathlessness and palpitations. Some of the side effects are transient and mild. However, if the side effects are hazardous to health, topical latanoprost will be stopped immediately. In addition to the risk named above, the study procedures may have other unknown risks. There may be unknown risks of possible harmful interaction with other medication you may be taking. These risks would be present if you used the mediation outside of this research project.

Other Treatments

You do not have to take part in this study to be treated for your illness or condition. Other treatments and therapies for your condition are available, including your current therapy and/or the treatment being studied in this research project. The doctor who is involved in this study can discuss these treatments and therapies with you.

Participation in the Study

Your participation in this study is entirely voluntary. You may refuse to take part in the study or you may stop your participation in the study at anytime, without a penalty or loss of benefits to which you are otherwise entitled.

The doctor who is involved in this study may stop your participation even without your consent.

If you stop from being part of this study, the doctor or the staff member will talk to you about any medical issues regarding the discontinuation of your participation.

Treatment and Compensation for Injury

If you follow the directions of the study doctor and staff and you are physically injured due to any substance or procedure properly given under the plan for this study, the sponsor will pay the medical expenses for the treatment of that injury which are not covered by your medical insurance, by a government program, or by any other third party.

Possible Benefits

The drug and the study procedures will be provided at no cost to you. You may receive information about your health from any physical examination and laboratory tests to be done in this study.

You will be paid RM 50 to reimburse you for transportation, parking, meal, or others expense related to your participation in this study. If you withdraw from the study early, you will be paid for these expenses for the portion of the study that you did complete.

Information obtained from this study will benefit the sponsor of the study (Ministry of Science, Technology and Information of Malaysia) and may benefit patients in the future.

Questions

If you have any question about this study or your rights, please contact

- Dr. Liza Sharmini Ahmad Tajudin 0976664563/0199179227

If you have any questions about your rights as a participant in a research study, please contact The Ethical Review Board of the University Hospital.

Confidentiality

Your medical information will be kept confidential by the doctor and staff involved in this study and will not be made publicly available unless disclosure is required by the law.

Data obtained from this study that does not identify you individually will be given to the sponsor and/or its representatives and may be published. Your original medical records may be reviewed by the sponsor and/or its representatives, the ERB for this study, and regulatory authorities for the purpose of verifying clinical trial procedures and/or data. Your medical information may be held and processed on a computer.

By signing this consent form, you authorize the record review, information storage and data transfer described above.

Signatures

To be entered into the study, you or a legal representative must sign and date the signature page (see Attachment 1)

Attachment 1: Consent Form

Information and consent form

Attachment 1
Consent form

To take part in this study, you or your legal guardian must sign this page.
Thereby signing this form, I certified that:

- **I have read and understand all the information provided in this information consent form.**
- **I have been given enough time to consider it**
- **All my questions had been answered satisfactorily**
- **I voluntarily agree to take part in this study, and will obey all the procedures and willing to give all the information required to the investigators when needed.**
- **I can terminate my participation at any time without any reason**
- **I have received a copy of the information and consent form for my own safe keeping.**

_____ _____

Patient's signature
Patient's name: **Registration number**
IC number:

_____ _____

Signature of legal guardian **Date: (DD/MM/YY)**
Name :
IC number:

_____

Name of the investigator

_____ _____

Signature of the investigator **Date: (DD/MM/YY)**

_____ _____

Signature of the witness **Date: (DD/MM/YY)**
Name :

IC number:

CLINICAL RECORD FORM: PROJECT B

Index no: ☐☐☐☐

A. Demographic data

Name: Address:

RN: ☐☐☐☐☐☐

Age: ☐☐ year (at diagnosis)

Sex: ☐ Male

☐ Female Contact no:

Race: ☐ Malay

☐ Chinese Date of first presentation: ☐☐☐☐☐☐

☐ Indian

☐ Other specify _____

☐Y ☐N Interracial marriage within 3 generation (If yes, do not enrol)

B Inclusion / Exclusion criteria

1. Laterally ☐ Bilateral

☐ Unilateral ☐ OD

☐ OS

2. Confirmation of diagnosis (Baseline)

* Gonioscopic findings ☐ Open Date: _____

☐ Closed (If closed, do not enrol)

* CDR (cup to disc ratio) OD OS

☐ ☐ Date: _____

(Date of qualifying optic disc

If not seen, give reason _____ _____ examination)

Glaucomatous features Y N Y N

(If no, do not enrol unless for OHT)

* Visual field (VF) OD OS Date: _____

PSD ☐ ☐ (reliable VF within 3 months

MD ☐ ☐ of diagnosis)

FP ☐

FN ☐

FL

Y N Y N

Glaucomatous changes

(If no, do not enrol unless for OHT)

* IOP(mmHg) OD OS

☐ ☐

☐ ☐

* CCT

3. Diagnosis:

☐ POAG

☐ OHT

☐ NTG

4. Systemic diseases

Y | N DM Drugs: _____

Y | N HPT Drugs: _____

Y | N CVA Drugs: _____

Y | N Hyperlipidemia Drugs: _____

Y | N IHD / MI Drugs: _____

Respiratory problem: Asthma Y | N

COPD Y | N

PTB Y | N

Lung Cancer Y | N

Smoker Y | N (If yes, do not enrol)

4. Any history of allergy to beta blocker Y | N

(If yes, do not enrol)

C. OUTCOME I (effectiveness)

Schedule visit	Date	IOP		CDR		VF		Comment
Visit 1 (1 month)		OD	OS	OD	OS	OD	OS	Progression Y N If yes, state reason:_____ Adequate IOP co Y N If no, state additional drug_____ Surgical intervent Y N If yes, state the procedure:_____
						PSD MD		
Visit 2 (3 months)		OD	OS	OD	OS	OD	OS	Progression Y N If yes, state reason:_____ Adequate IOP co Y N If no, state additional drug_____ Surgical intervention Y N If yes, state the procedure:_____
						PSD MD		
Visit 3 (6 months)		OD	OS	OD	OS	OD	OS	Progression Y N If yes, state reason:_____ Adequate IOP co Y N If no, state additional drug_____ Surgical interventi Y N If yes, state the procedure:_____
						PSD MD		
Visit (12 months)		OD	OS	OD	OS	OD	OS	Progression Y N If yes, state reason:_____ Adequate IOP control Y N If no, state additio drug_____ Surgical intervent Y N If yes, state the procedure:_____
						PSD MD		

C. OUTCOME II (genotype)

	Genotype		
Allele	Homozygous Wild type	Homozygous Mutant	Heterozygous
16			
27			
164			
-20			
-47			

CLINICAL RECORD FORM: PROJECT P

Index no: ☐☐☐☐

A. Demographic data

Name: Address:

RN: ☐☐☐☐☐☐☐

Age: ☐☐ year (at diagnosis)

Sex: ☐ Male

☐ Female Contact no:

Race: ☐ Malay

☐ Chinese Date of first presentation: ☐☐☐☐☐☐

☐ Indian

☐ Other specify _____

Y N Interracial marriage within 3 generations (If yes, do not enrol)

B Inclusion / Exclusion criteria

1. Laterally ☐ Bilateral

☐ Unilateral ☐ OD

☐ OS

2. Confirmation of diagnosis (Baseline)

* Gonioscopic findings ☐ Open Date: _____

☐ Closed (If closed, do not enrol)

* CDR (cup to disc ratio) OD OS

☐ ☐ Date: _____

(Date of qualifying optic disc

If not seen, give reason _____ _____ examination)

Glaucomatous features Y N Y N (If no, do not enrol)

* Visual field (VF) OD OS Date: _____

PSD ☐ ☐ (reliable VF within 3 months

MD ☐ ☐ of diagnosis)

FP ☐

FN ☐

FL

Glaucomatous changes Y N Y N

(If no, do not enrol unless OHT)

*CCT ☐ ☐

* IOP OD OS

☐ ☐

☐

* Diagnosis ☐ POAG

☐ OHT

☐ NTG

3. Treatment ☐ Monotherapy

☐ Adjunctive therapy

(Only enrol, if Timolol is the first line drug)

☐ Switch therapy

(Only enrol, if Timolol is the only previous drug)

4. Ocular problem

Dry eye Y N

Inflammatory eye disease Y N

(Uveitis/ sclerotic)

Ocular injury Y N

Filtrating surgery Y N

(If yes, do not enrol)

5. Any history of allergy to latanoprost Y N (If yes, do not enrol)

C. OUTCOME I (effectiveness)

Schedule visit	Date	IOP		CDR		VF		Comment
Visit 1 (1 month)		OD	OS	OD	OS	OD	OS	Progression Y N If yes, state reason:_____ Adequate IOP co Y N If no, state additional drug_____ Surgical intervent Y N If yes, state the procedure:_____
						PSD MD		
Visit 2 (3 months)		OD	OS	OD	OS	OD	OS	Progression Y N If yes, state reason:_____ Adequate IOP co Y N If no, state additional drug_____ Surgical intervention Y N If yes, state the procedure:_____
						PSD MD		
Visit 3 (6 months)		OD	OS	OD	OS	OD	OS	Progression Y N If yes, state reason:_____ Adequate IOP co Y N If no, state additional drug_____ Surgical intervent Y N If yes, state the procedure:_____
						PSD MD		
Visit (12 months)		OD	OS	OD	OS	OD	OS	Progression Y N If yes, state reason:_____ Adequate IOP control If no, state additio Y N drug_____ Surgical intervent Y N If yes, state the procedure:_____
						PSD MD		

D. OUTCOME II (side effect)

Schedule visit	Date	Conjunctival hyperaemia	Hypertrichosis	LIID	Iritis	Comments
Visit 1 (1 month)						
Visit 2 (3 months)						
Visit 3 (6 months)						
Visit 4 (12 months)						

E. POLYMORPHISM (genotype)

UNIVERSITI SAINS MALAYSIA

PEJABAT PELANTAR KESIHATAN
HEALTH PLATFORM OFFICE

Date : 26 March 2008

Dr Liza Sharmini Ahmad Tajudin
School of Medical Sciences
Ophthalmology Department
Health Campus
Universiti Sains Malaysia
16150 Kubang Kerian

Dear Dr,

APPLICATION FOR ETHICAL APPROVAL

Protocol/ Research Title:

Topical Antiglaucoma Drugs: Beta 2 and Prostanoid (FP) Receptors Polymorphisms and their implications

I refer to your application of 28th February 2008.

I am pleased to inform you that the Research Ethics Committee (Human), Universiti Sains Malaysia has reviewed your application and has approved the extension of the study duration starting from October 2006 until October 2009.

Thank you.

"GLOBAL COMPETITIVENESS: OUR COMMITMENT"

Yours sincerely,

(Professor Ab. Rahman Esa)
Chairman of Research Ethics Committee (Human)

c.c Secretary of Ethics Committee, USM

KAMPUS KESIHATAN HEALTH CAMPUS
Universiti Sains Malaysia, 16150, Kubang Kerian, Kelantan, Malaysia
Tel: +609-766 3000 ext. ~~4919~~ 3585 / 3760 / 3117. Fax: 609-~~765 6291~~ 7648064. Website: www.usm.my

LONDON SCHOOL OF HYGIENE
& TROPICAL MEDICINE

ETHICS COMMITTEE

APPROVAL FORM
Application number: **5406**

Name of Principal Investigator	**Paul J Foster**
Department	**Infectious and Tropical Diseases**
Head of Department	**Professor Simon Croft**

Title: **Pharmacognetics of glaucoma; a study of the role of beta 2 andrenoreceptor (ADRB2) and prostanoid (FP) receptor gene polymorphisms in pressure lowering effect of tropical timolol and latanoprost**

This application is approved by the Committee.

Chair of the Ethics Committee ....

Date .....19 November 2008.....

Approval is dependent on local ethical approval having been received.

Any subsequent changes to the application must be submitted to the Committee via an E2 amendment form.