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The Effect of Renin Angiotensin System Genetic Variants in Acute Pancreatitis

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Abstract

Objectives—We sought association of genetic variants in the renin angiotensin system (RAS) and Vitamin D system with acute pancreatitis (AP) development and severity.

Summary Background Data—The endocrine RAS is involved in circulatory homeostasis through the pressor action of angiotensin II (ang II) at its AT_1 receptor (AT_1R). However, local RAS regulate growth and inflammation in diverse cells and tissues, and their activity may be suppressed by Vitamin D. Intra-pancreatic ang II generation has been implicated in the development of AP.

Methods—Five hundred and forty-four Caucasian AP patients from three countries (UK 22; Germany 136; Netherlands 386) and 8487 control subjects (UK 7833, Netherlands 717) were genotyped for eight polymorphisms of the RAS/vitamin D systems, chosen based on likely functionality.

Results—The ACE I (rather than D) allele was significantly associated with alcohol-related AP when all cohorts were combined (p=0.03). The *Renin* rs5707 G (rather than A) allele was associated with AP (p=0.002), infected necrosis (p=0.025) and mortality (p=0.046).

Conclusions—The association of two RAS polymorphisms with AP suggests the need for further detailed analysis of the role of RAS/Vitamin D in the genesis or severity of AP, particularly given the ready potential for pharmacological manipulation of this system using existing marketed agents. However, further replication studies will be required before any such association is considered robust, particularly given the significant heterogeneity of AP causation and clinical course.

Keywords

Pancreatitis; RAS; ACE; Genetics

Introduction

Acute pancreatitis (AP) is a common inflammatory disorder that is mild and self-limiting in 80% of cases but which, when severe, may necessitate intensive care admission and lead to organ failure and death. The majority of cases are secondary to either gallstones or alcohol, although a multitude of causes exist. It is a common disease (current UK incidence: 150–420 cases per million per annum) and increasingly prevalent.[1] However, despite recent improvements in intensive care unit management and techniques of organ support, the severity and mortality associated with AP has not decreased since the 1970s[1]: up to 25% of patients will be diagnosed with severe disease[2–5] and approximately 4% of all patients will die.[6,7] This can be partly explained by the fact that no specific prophylactic or therapeutic agent is available, and current management is largely supportive. Better understanding of the molecular drivers of AP is essential for the identification of new therapeutic strategies.

Single-nucleotide polymorphisms (SNPs) are single base-pair variants in the DNA sequence which occur with a population frequency of >1%. Associated biological impacts may result- for instance, if the SNP alters an amino acid in the protein transcribed (thus affecting protein structure and function), or if the SNP lies in a region which affects gene transcription or mRNA stability. Associating such functional variants with specific disease phenotypes is thus one means by which to infer a causal role for the gene product in disease pathogenesis. Gene-association studies, performed to assess the molecular drivers of pancreatitis (Table 1), have generally focussed on the activation of pancreatic enzymes and pro-enzymes (one of the key steps in the initiation and propagation of pancreatic inflammation) or the process of systemic inflammation secondary to acute pancreatitis. However, small cohorts have weakened the ability to detect associations in mixed patient groups.

The renin-angiotensin system (RAS), originally described as a key regulator of intravascular homeostasis, controlling extracellular fluid volume and blood pressure,[18] represents a potential target of such gene-association studies. In response to decreased afferent arteriolar pressure, decreased filtered sodium load or sympathetic nervous stimulation, the renal juxtaglomerular apparatus releases renin,[19] which cleaves hepatically-derived angiotensinogen[20] to yield angiotensin I (ang I). Angiotensin-converting-enzyme (ACE) subsequently hydrolyses ang I to yield the effector peptide angiotensin II (ang II)[21], whose effects are mediated through 2 specific human receptors: the angiotensin II type 1 and 2 receptor (AT_1R and AT_2R).[22] However, local renin-angiotensin systems are now known to exist in diverse cells and tissues, including the pancreas,[23,24] where they have paracrine and autocrine roles in the regulation of metabolism, blood flow, inflammation and healing.[25–27]

Within the pancreas, local generation of ang II influences exocrine and endocrine function through activation of the AT_1R, stimulating increases in pancreatic enzyme secretion[28,29], while reducing islet blood flow and delaying insulin release.[30–32] It is also implicated in the initiation and propagation of AP. Increased expression of RAS components is identified in experimental models of AP,[33,34] where they drive activation of monocytes and macrophages,[35,36] and expression of pro-inflammatory molecules such as interleukin-6 (IL-6), nuclear factor-κβ (NF-κβ) and monocyte chemoattractant protein-1 (MCP-1).[37] Such pro-inflammatory effects may occur through a number of possible AT_1R-mediated mechanisms including generation of reactive oxygen species, matrix metallopeptidase-9 (MMP-9), nicotinamide adenine dinucleotide phosphate (NADPH), NF-κβ, tumour growth factors (TGFs) and Smad, as well as activation of human pancreatic stellate cells.[37–42] RAS inhibition has also been shown to attenuate the expression of pro-inflammatory molecules and mitigate pancreatic cellular injury.[43,44] Further, vitamin D may influence RAS activity by suppressing renin synthesis at the transcriptional level, thus acting as a negative regulator of the RAS.[45–49]

The association of AP (and its severity) with specific variants in key RAS/Vitamin D pathway genes would infer a causal role for such systems in AP pathogenesis. Given the postulated role for RAS/Vit D in AP pathogenesis, we hypothesized that such gene associations would be identified. We thus sought association of common functional variants in the RAS and Vitamin D systems with AP, and its severity, in order to clarify the possible

role for RAS in AP pathogenesis; and in particular to elucidate whether genotypes associated with higher RAS activity were associated with the development or severity of acute pancreatitis.

Methods

Subjects

Blood samples were taken from AP patients from 3 Northern European countries, following local ethical approval (The Joint UCL/UCLH Committees on the Ethics of Human Research (Committee A); Reference No. 08/H0714/90) and written informed consent: (i) University College London Hospitals (UCLH, London, UK), between 2006 and 2009; (ii) Magdeburg University Hospital (Magdeburg, Germany) between 1996 and 2003; (iii) Eight university medical centres and seven major teaching hospitals in the Netherlands between 2004 and 2007, with blood stored at University Medical Centre Utrecht (Utrecht, Netherlands), as part of the PROPATRIA trial, a multicenter, randomized controlled trial (trial registry number ISRCTN38327949).[50]

AP was defined as upper abdominal pain in combination with serum amylase or lipase concentrations raised to at least three times the upper limit of normal. Prospective data, including demographics, predicted severity score (e.g. acute physiology and chronic health evaluation II (APACHE II)), necrosis and in-hospital mortality, were collected. *Patients were classified as having actual (rather than predicted) severe AP in the presence of organ failure of over 48 hours duration and/or local pancreatic or peri-pancreatic complications such as necrosis, fluid collections and pseudocysts (as defined by the revised Atlanta classification*[51]*).*

Controls were 2766 (2711 following quality control) healthy UK Caucasian males who had participated in the second Northwick Park Heart Study (NPHSII) (aged 51 to 60 years, recruited from 9 general medical practices within the UK) for assessment of ACE (rs4646996) and CYP2R1 (rs10741657) genotypes, and 5059 (1334 women and 3725 men) UK civil servants from the Whitehall II study (WHII; aged 35–55 years and working in the London offices of 20 Whitehall departments)[52] for analysis of other genetic variants. Full details of the genotyping and quality control have been published previously.[53] In summary, DNA from WHII was extracted from 6156 individuals from whole blood samples using magnetic bead technology (Medical Solutions, Nottingham, UK) and normalised to a concentration of 50ng/μl. Custom SNP arrays were designed by the Institute of Translational Medicine and Therapeutics, the Broad Institute and the National Heart Lung and Blood Institute supported Candidate-gene Association Resource Consortium (HumanCVD BeadChip)[54] on 5592 of these samples. After restriction to White/European groups and quality control, 5059 samples were utilised for analysis. Seven hundred and seventeen samples were also obtained from blood-bank donors within the Netherlands and utilised as controls for all genotypes (kindly provided by Prof. C. Wijmenga, Department of Genetics, University Medical Center Groningen, Groningen, the Netherlands.

Genetic Analysis

DNA was extracted from UK subjects utilising the 'salting-out' technique[55]; from German subjects using Qiagen® DNA extraction kits (Qiagen, Hilden, Germany); and using the DNA isolation kit I from the Magna Pure LC (Roche Diagnostics, Indianapolis, USA) for the Dutch cohort. Common functional polymorphisms in genes of the human RAS and Vitamin D systems, or those previously associated with pancreatic disease, were selected by review of the published literature (Table 2). *Subjects were genotyped for all pre-selected variants, with genetic analysis performed at University College London (London, UK).*

DNA was measured and standardised using a Nanodrop® 8000 (Thermo Scientific; Waltham, Ma, USA) spectrophotometer and Beckman Coulter Biomek® 2000 (Biodirect; Taunton, Ma, USA) respectively, to a concentration of 15ng/μl stock, prior to further dilution to 5ng/μl working stocks. The ACE insertion/deletion polymorphism was assessed via initial PCR amplification of DNA and subsequent identification of differences in DNA size utilising 7.5% microplate array diagonal gel electrophoresis (MADGE). The remaining genotypes were determined by polymerase chain reaction amplification (PCR), utilising custom-prepared TaqMan® SNP genotyping assay kits (Applied Biosystems; Carlsbad, CA, USA).

Statistical Analysis

Patient data were anonymised. Allele frequencies were tested for deviation from Hardy-Weinberg equilibrium using a χ^2 goodness-of-fit test *and all allele frequencies were in keeping with existing published population data.* Odds ratios and p values were calculated for an additive genetic effect using logistic regression models with adjustment for age, *sex* and region. For ACE and rs5707 genotypes we also tested the recessive model. Analysis was performed using Stata Version 11 (StataCorp, Texas, USA) and a p value of < 0.05 was considered statistically significant. No adjustment was made for multiple comparisons. Power calculations were performed using Quanto (http://hydra.usc.edu/gxe/). Detectable odds ratios were calculated for the sample size of 544 cases assuming an additive effect. In addition we calculated the number of AP cases that would be required to detect an effect of the size observed in this study. The ratio of controls to cases was 5.4 for (rs4646996) and CYP2R1 (rs10741657), 1.4 for AT^2R (rs1403543) and AGT (rs699) and 10.6 for the remaining SNPs.

Results

Combined study groups (Table 3)

Five hundred and forty-four Caucasian patients (304 [55.9%] male) with acute pancreatitis (UK n=22, Germany n=136, Netherlands n=386), and 8487 control subjects (UK NPHS-II n=2711, UK WHII n=5059, Netherlands n=717) were genotyped. Median patient age was 56 years (17–91years). Two hundred and sixty nine (49.5%) cases were secondary to biliary disease, 118 (21.7%) to alcohol and 157 (28.9%) to other causes. One hundred and seventy three (31.8%) patients overall had severe acute pancreatitis (based upon *revised Atlanta criteria*[51]; UK n=4, Germany n=65, Netherlands n=104) and 38 (7.0%) died (Germany n=18 and Netherlands n=20). Infected necrosis occurred in 58 (14.2%) patients in UK and Dutch

cohorts (UK n=2 and Netherlands n=56). Data on infected necrosis were not available in the German cohort. Genotype distributions for cases and controls are shown in Table 4 for the overall cohort. The G allele of the renin rs5707 SNP was associated with AP (p=0.003), infected necrosis (p=0.02) and mortality (p=0.003) in the Dutch cohort, where 60% of deaths occurred secondary to infected necrosis (Table 5). These findings were replicated when the cohorts were combined (AP (OR (95% CI) = 2.19 (1.34–3.60) p=0.002), infected necrosis (2.75 (1.13–6.68) p=0.025) and mortality (2.66 (1.02–6.95) p=0.046) for the recessive model).

There were no other significant genotype associations with the development of AP, severe AP or mortality from AP.

Alcohol-related acute pancreatitis

One hundred and eighteen patients (21.7% of total) had alcohol-related AP (9 UK, 37 Germany, 72 Netherlands). Median age was 47 years (22–88 years) and 98 (83.1%) were male. Of Dutch and UK patients, 11 (14.1%) had infected necrosis (10 Dutch). Fifty (42.4%) patients had actual severe pancreatitis (1 UK, 28 Germany, 21 Dutch), and five (4.2%) died (3 Germany, 2 Netherlands). *The ACE I (rather than D) allele (rs4646994) was associated with alcohol-related AP when the cohorts were combined (OR (95% CI) 0.57 (0.34–0.95) p=0.03 recessive model for DD vs. II/ID).* There were no other significant associations (Table 6).

Biliary acute pancreatitis

Two hundred and seventy patients had biliary AP (49.4% of total: 8 UK, 53 Germany, 209 Netherlands). Median age was 62 years (18–91 years) and 115 (42.6%) patients were male. Of Dutch and UK subjects, 26 (12.1%) patients developed infected necrosis (25 Dutch, 1UK). Seventy (25.9%) had actual severe AP (2 UK, 19 Germany, 49 Netherlands) and 17 (6.3%) died (8 Germany, 9 Netherlands). No significant associations with genotype were identified (Table 7).

Discussion

To our knowledge, this is the largest study thus far investigating the association of RAS genotype with acute pancreatitis. The human ACE gene has a genetic variant in which the absence (Deletion, *D* allele) rather than the presence (Insertion, *I* allele) of a 287 base pair fragment is associated with higher circulating[65] and tissue ACE activity such as that in myocardium,[66] and inflammatory cells.[67] Our analysis of 544 AP patients and 8487 controls demonstrated an association of the ACE I allele (*lower* ACE activity) with alcohol-related acute pancreatitis. To date, no other studies have identified an association of ACE genotype with the development or severity of acute,[68] chronic,[69–72] familial[70] or tropical calcific pancreatitis.[73] However, these studies were small, incorporated mixed aetiologies and disease-types (acute, chronic, familial and tropical calcific pancreatitis), and did not address other RAS variants (see Table 8). *We sought to resolve these issues by using larger sample sizes; the comparison of single aetiologies; the investigation of the effects of multiple RAS genotypes; investigation of alternate systems that may affect the RAS pathway (e.g. the*

vitamin D pathway); and use of multiple cohorts. However, still larger sample sizes may be required to detect effect sizes of the magnitude found (see Table 4) as the sample size utilised here only had sufficient power to detect an odds ratio in the range of 1.20–1.26.

Alcohol has also been shown to directly activate RAS in animal models of alcoholic cardiomyopathy.[74] Despite this, our study demonstrated an association of the *ACE* I allele (*lower* ACE activity) rather than the postulated D-allele (i.e. high activity) with alcohol-related acute pancreatitis. This finding seems contrary to the hypothesis that increasing activity of the RAS may lead to increasing likelihood of developing acute pancreatitis or severe disease. However, ACE inhibitor use has been associated with pancreatitis,[75] and multiple angiotensin I processing enzymes are now known to exist (e.g. chymase, chymotrypsin, tonin, aminopeptidase A, B and N, prolylendopeptidase, and neutral endopeptidase). Further, ACE 2, a homologue of ACE with 42% sequence homology[76] has recently been discovered and its primary product, Ang (1–7), acts through the Mas receptor[77] to negatively regulate the RAS; thereby counter-balancing ACE action. Thus, ACE levels, as assessed by RAS genotypes, may not truly represent overall RAS or ACE activity at a local or systemic level, and further studies may require serum assays or pancreatic biopsy samples for direct assessment of tissue ACE levels.

When the cohorts were combined, the renin rs5707 G allele was associated with AP (p=0.002), infected necrosis (p=0.025) and mortality (p=0.046). The renin rs5707 G allele has previously been associated with hypertension and diabetes, and has been hypothesised to increase the activity of the RAS.[60] *However, although this finding was also present in the Dutch cohort, we could not replicate this finding in cohorts from other countries. Nor was there biological consistency through disease association with other RAS genotypes, or those of (the putatively RAS-regulatory) Vitamin D system investigated here. Such lack of consistency and replication is likely to be secondary to heterogeneity in AP causation and allele frequencies between populations, as well as small individual cohort sample sizes, particularly when single aetiologies were examined. Similarly, the patient cohort contained a high proportion of Dutch and German individuals, whilst the control group was UK-dominated. However, all cohorts were derived from North-European, Caucasian populations with similar ancestry and any minor allelic variations are therefore unlikely to influence the significance of results. Meanwhile, the medical systems and diagnostic criteria utilised in each country were similar, although any national differences in diagnosis, management and outcome would require analysis via long-term, prospective, national registries.*

Although multiple causes exist, a common pathophysiological pathway in AP involves premature activation of various proteolytic pancreatic enzymes such as trypsin, chymotrypsin, carboxypeptidase and kallikrein. Chymotrypsin is capable of converting angiotensinogen to ang I, and trypsin to catalyse ang II to ang III and IV.[78] Therefore, pancreatic enzyme activity may be another crucial factor in the activation of RAS during AP- an effect which may swamp that of RAS genotype. Further, the activation of this common AP-initiating pathway occurs via different mechanisms in biliary- and alcohol-related pancreatitis, and the heterogeneous proportion of each in the three cohorts may further explain the inconsistency of genetic association between the cohorts from various

geographical regions (UK: 40.9% ETOH, 36.4% biliary, 22.7% other; Netherlands: 18.7% ETOH, 54.1% biliary, 27.2% other; Germany: 27.2% ETOH, 39.0% biliary, 33.8% other).

However, such data must be interpreted with caution, and cannot be taken as proof of a role for RAS or Vitamin D in the genesis or severity of AP, perhaps in part due to the study limitations, including the problem of multiple comparison and a relatively small sample size, but also possibly in part due to heterogeneity of AP causation and variations in clinical course. Adjustment for multiple comparison, whilst necessary, may also make the discarding of a 'true positive' finding more likely than that of a 'false positive'. In addition, uniformity in assessment of disease severity is difficult to achieve,[79] and heterogeneity, by its definition, will also weaken power in genetic studies. Further studies should concentrate on large, well-structured study cohorts with clear phenotypes and substantial numbers of individuals in any aetiological group, as well as with tight coconstraints on defined severity, in order to circumvent these issues. In addition, association of any phenotype with one allelic variant may, of course, occur by chance. In addition, the gene variant under study may (through strong linkage disequilibrium) mark activity in an adjacent gene (through which any observed associations are in fact mediated). For reasons such as this, findings of candidate gene association studies require replication if to be considered robust, with subsequent fine-mapping of the genes required.

Further studies should concentrate on large, well-structured study cohorts with clear phenotypes, to attempt to circumvent these issues. However, the association of two RAS polymorphisms *ACE* I and *Renin* rs5707 G ACE I with AP in this study does suggest that this issue warrants further detailed analysis, given the ready potential for pharmacological manipulation of this system using existing marketed agents. Such roles are also worthy of active investigation in diverse pancreatic disease states.

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Abbreviations

ACE	Angiotensin Converting Enzyme
AGT	Angiotensinogen
ANG	Angiotensin
AP	Acute Pancreatitis
DM	Diabetes Mellitus
RAS	Renin Angiotensin System
VDR	Vitamin D Receptor

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Table 1

Selected recent genetic associations with various clinical forms of pancreatitis

Gene	Protein	No. Pts	Association	Authors
PRSS1	Cationic Trypsinogen	108 HCP; 415 ICP; 82 Controls	CP	Teich *et al*, 2002[8]
SPINK1	Pancreatic Secretory Trypsin Inhibitor	**1** 32 CP; 117 Controls **2** 198 Mixed CP; 290 Controls	**1** CP **2** Hereditary CP	Kaneko *et al*, 2001[9] Chandak *et al*, 2004[10]
CFTR	Cystic Fibrosis Transmembrane Regulator	**1** 67 ICP; 60 Controls **2** 25 CP; 236 Controls	**1** Idiopathic CP **2** CP	Weiss *et al*, 2005[11] Tzetis *et al*, 2007[12]
CTLA-4	Cytotoxic T Lymphocyte-Associated Antigen 4	46 AIP; 78 CCP; 200 Controls	Autoimmune Pancreatitis	Chang *et al*, 2007[13]
IL-8	Interleukin-8	92 AP; 200 Controls	Severity of AP	Hofner *et al*, 2006[14]
TGF-β1	Transforming Growth Factor β1	28 CP; 94 Controls	Fibrosis in CP	Bendicho *et al*, 2005[15]
TLR-4	Toll-Like Receptor-4	521 AP; 120 Controls	No association with AP	Guenther *et al*, 2010[16]
TNF-α	Tumour Necrosis Factor α	77AP; 71 Controls	AP Severity	Balog *et al*, 2005[17]

AP- Acute Pancreatitis; **CP**- Chronic Pancreatitis; **AIP**- Autoimmune Pancreatitis; **HCP**- Hereditary Chronic Pancreatitis; **ICP**- Idiopathic Chronic Pancreatitis; **CCP**- Chronic Calcific Pancreatitis

Table 2

A table demonstrating further information on the genetic variants investigated in this study. All variants listed here have previously been associated directly with RAS, indirectly with RAS (vitamin D-metabolising system), or with forms of pancreatic disease. Allele frequencies are based on Caucasian data from HapMap and ABI AoD.

Gene	Gene Symbol	Chrom Location	SNP Name	ID Number	DNA Change	Allele Frequencies	Known Effect
Angiotensin Converting Enzyme 1	*ACE*	17q23.3	I/D	rs4646994	----------	0.54D:0.46I	D Allele: Higher Levels of Tissue & Circulating ACE[56]
Angiotensin II Receptor Type 1	*ATR1*	3q24	A1166C	rs5186	A>C	0.75A/0.25C	C Allele: Increased Receptor Activity[57,58]
Angiotensin II Receptor Type 2	*ATR2*	Xq23	1675	rs1403543	G>A	0.61A:0.39G	G Allele: Increased Receptor Expression[57]
Angiotensinogen	*AGT*	1q42.2	M235T	rs699	C>T	0.62T:0.38C	T Allele: Higher Levels of AGT, Low Prorenin, Susceptibility to DM[59]
Renin	*REN*	1q32	----------	rs5707	G>T	T0.87:G0.13	G Allele: Increased Levels of Renin[60]
Vitamin D Receptor	*VDR*	12q13.11	FokI	rs2228570	C>T	0.56C:0.44T	T Allele: Increased Suceptibility to DM[61]
Vitamin D Hydoroxylase	*CYP2R1*	11p15.2	CYP2R1	rs10741657	A>G	0.63G/0.37A	G Allele: Increased Susceptibility to DM[62]
Vitamin D Binding Protein	*GC*	4q12	----------	rs7041	G>T	0.58G:0.43T	1S & 1S-2: Increased Levels of Circulating Vitamin D Levels[63,64]

SNP- Single Nucleotide Polymorphism; **Chrom**- Chromosome; **DM**- Diabetes Mellitus

Table 3

Baseline characteristics of the 3 study cohorts under investigation.

Study Cohort	Study Group (n=)	Controls (n=)	Aetiology (n=)	Severe AP (n=)	Infected Necrosis (n=)	Mortality (n=)
UK	22	5067 (WHII) 2766 (NPHSII)	9 (40.9%) ETOH 8 (36.4%) Biliary 5 (22.7%) Other	4 (18.2%)	2 (9.1%)	0 (0%)
Netherlands	386	717	72 (18.7%) ETOH 209 (54.1%) Biliary 105 (27.2%) Other	104 (26.9%)	56 (14.5%)	20 (5.2%)
Germany	136	N/A	37 (27.2%) ETOH 53 (39.0%) Biliary 46 (33.8%) Other	65 (47.8%)	N/A	18 (13.2%)

Table 4

Comparison of genotype frequency between combined AP cohorts and controls.

Gene				P value*	*No. AP Patients Required for Statistical Power*
ACE (rs4646994)	**II**	**ID**	**DD**		
Controls[1,3]	839 (24.5)	1656 (48.4)	928 (27.1)	0.63	11167
AP	132 (25.1)	273 (52.0)	120 (22.9)		
OR (95%	1.00	1.22 (0.92–1.60)	0.92 (0.67–1.26)		
AT[1] **R (rs5186)**	**AA**	**AC**	**CC**		
Controls[2,3]	2778 (48.4)	2433 (42.4)	530 (9.2)	0.08	803
AP	266 (52.8)	187 (37.1)	51 (10.1)		
OR (95% CI)	1.00	0.74 (0.58–0.94)	0.87 (0.58–1.30)		
AT[2] **R (rs1403543)**	**AA**	**AG**	**GG**		
Controls [3]	280 (39.8)	146 (20.8)	277 (39.4)	0.31	5114
AP	199 (40.5)	119 (24.2)	174 (35.4)		
OR (95% CI)	1.00	0.88 (0.60–1.29)	0.87 (0.67–1.14)		
CYP2R1 (rs10741657)	**GG**	**GA**	**AA**		
Controls[1,3]	1235 (37.1)	1576 (47.4)	517 (15.5)	0.35	3264
AP	181 (37.7)	227 (47.3)	72 (15.0)		
OR (95% CI)	1.00	1.10 (0.85–1.41)	1.17 (0.82–1.67)		
AGT (rs699)	**AA**	**AG**	**GG**		
Controls[2,3]	2104 (36.6)	2701 (47.0)	945 (16.4)	0.15	1182
AP	190 (37.6)	238 (47.1)	77 (15.3)		
OR (95% CI)	1.00	1.15 (0.90–1.47)	1.27 (0.89–1.81)		
Renin (rs5707)	**AA**	**AC**	**CC**		

Gene				P value*	*No. AP Patients Required for Statistical Power*
Controls[2,3]	3476 (60.6)	2004 (35.0)	253 (4.4)	0.06	647
AP	314 (61.2)	159 (31.0)	40 (7.8)		
OR (95% CI)	1.00	0.99 (0.77–1.27)	2.18 (1.32–3.61)		
VDR (rs2228570)	**GG**	**GA**	**AA**		
Controls[3]	293 (41.6)	304 (43.2)	107 (15.2)	0.18	2220
AP	176 (36.2)	326 (48.6)	74 (15.2)		
OR (95% CI)	1.00	1.28 (0.99–1.66)	1.17 (0.82–1.67)		
GC (rs7041)	**CC**	**CA**	**AA**		
Controls[2,3]	1752 (30.5)	2912 (50.7)	1075 (18.7)	0.29	1975
AP	165 (33.1)	244 (48.9)	90 (18.0)		
OR (95% CI)	1.00	0.77 (0.59–1.00)	0.88 (0.63–1.25)		

[1] NPHSII;

[2] WHII;

[3] Netherlands Blood Bank

* Additive genetic model adjusted for age, *sex*, region

Table 5a

Effect of Renin G allele upon outcome from acute pancreatitis in Dutch AP samples and controls.

	Rs5707	AA	AC	CC	P value*
	Controls	443 (63.9)	224 (32.3)	26 (3.8)	
	AP	214 (59.9)	115 (32.2)	28 (7.8)	
	OR (95% CI)	1.00	1.06 (0.79–1.42)	2.34 (1.30–4.23)	0.003
Infected Necrosis	No	183 (59.6)	104 (33.9)	20 (6.5)	
	Yes	31 (62.0)	11 (22.0)	8 (16.0)	
	OR (95% CI)	1.00	0.64 (0.31–1.33)	2.42 (0.97–6.02)	0.02
Mortality	No	204	112	23	
	Yes	10	3	5	
	OR (95% CI)	1.00	0.53 (0.09–2.12)	4.67 (1.14–16.97)	0.003

*Recessive model

Table 5b

Effect of Renin G allele upon outcome from acute pancreatitis in combined cohort samples and controls.

	Rs5707	AA	AC	CC	P value*
	Controls	3476 (60.6)	2004 (35.0)	253 (4.4)	
	AP	314 (61.2)	159 (31.0)	40 (7.8)	
	OR (95% CI)	1.00	0.99 (0.77–1.27)	2.18 (1.32–3.61)	0.002
Infected Necrosis	No	191 (60.3)	106 (33.4)	20 (6.3)	
	Yes	33 (63.5)	11 (21.2)	8 (15.4)	
	OR (95% CI)	1.00	0.61 (0.30–1.27)	2.37 (0.96–5.88)	0.025
Mortality	No	290 (60.8)	153 (32.1)	34 (7.1)	
	Yes	24 (66.7)	6 (16.7)	6 (16.7)	
	OR (95% CI)	1.00	0.47 (0.19–1.19)	2.18 (0.82–5.80)	0.046

*Recessive model

Table 6

Comparison of genotype frequency between patients with AP secondary to alcohol and combined controls.

Gene				**P value***
ACE (rs4646994)	**II**	**ID**	**DD**	
Alcohol	32 (27.6)	65 (56.0)	19 (16.4)	***0.18 (Additive Model)***
Control[1,3]	839 (24.5)	1656 (48.4)	928 (27.1)	
OR (95% CI)	1.00	1.17 (0.74–1.86)	0.63 (0.34–1.15)	***0.03 (Recessive Model)***
AT[1] **R (rs5186)**	**AA**	**AC**	**CC**	
Alcohol	57 (50.9)	41 (36.6)	14 (12.5)	0.85
Control[2,3]	2778 (48.4)	2433 (42.4)	530 (9.2)	
	1.00	0.75 (0.49–1.16)	1.23 (0.65–2.33)	
AT[2] **R (rs1403543)**	**AA**	**AG**	**GG**	
Alcohol	46 (14.1)	10 (6.4)	45 (14.0)	0.79
Control[3]	280 (39.8)	146 (20.8)	277 (39.4)	
	1.00	1.17 (0.44–3.08)	0.94 (0.60–1.47)	
CYP2R1	**GG**	**GA**	**AA**	
(rs10741657)				
Alcohol	34 (33.3)	47 (46.1)	21 (20.6)	0.18
Control[1,3]	1235 (37.1)	1576 (47.4)	517 (15.5)	
	1.00	1.16 (0.73–1.86)	1.52 (0.84–2.76)	
AGT (rs699)	**AA**	**AG**	**GG**	
Alcohol	45 (40.2)	57 (50.9)	10 (8.9)	0.92
Control[2,3]	2104 (36.6)	2701 (47.0)	945 (16.4)	
	1.00	1.18 (0.78–1.81)	0.79 (0.38–1.64)	
Renin (rs5707)	**AA**	**AC**	**CC**	
Alcohol	72 (64.9)	34 (30.6)	5 (4.5)	0.95
Control[2,3]	3476 (60.6)	2004 (35.0)	253 (4.4)	
	1.00	0.93 (0.60–1.45)	1.15 (0.43–3.10)	
VDR (rs2228570)	**GG**	**GA**	**AA**	
Alcohol	34 (30.6)	60 (54.1)	17 (15.3)	0.17
Control[3]	293 (41.6)	304 (43.2)	107 (15.2)	
	1.00	1.76 (1.09–2.85)	1.30 (0.68–2.51)	
GC (rs7041)	**CC**	**CA**	**AA**	

Gene				**P value***
Alcohol	38 (34.2)	57 (51.4)	16 (14.4)	0.30
Control[2,3]	1752 (30.5)	2912 (50.7)	1075 (18.7)	
	1.00	0.87 (0.56–1.36)	0.72 (0.39–1.35)	

[1] NPHSII;

[2] WHII;

[3] Netherlands Blood Bank

* Additive genetic model adjusted for age, *sex*, region.

Table 7

Comparison of genotype frequency between patients with AP secondary to biliary pathology and combined controls.

Gene				P value*
ACE (rs4646994)	**II**	**ID**	**DD**	
Biliary	72 (27.6)	125 (47.9)	64 (24.5)	0.48
Control[1,3]	839 (24.5)	1656 (48.4)	928 (27.1)	
	1.00	0.95 (0.66–1.37)	0.86 (0.56–1.31)	
AT[1] **R (rs5186)**	**AA**	**AC**	**CC**	
Biliary	123 (50.4)	95 (38.9)	26 (10.7)	0.16
Control[2,3]	2778 (48.4)	2433 (42.4)	530 (9.2)	
	1.00	0.76 (0.54–1.06)	0.80 (0.47–1.36)	
AT[2] **R (rs1403543)**	**AA**	**AG**	**GG**	
Biliary	97 (39.6)	74 (30.2)	74 (30.2)	0.14
Control[3]	280 (39.8)	146 (20.8)	277 (39.4)	
	1.00	0.76 (0.47–1.23)	0.76 (0.53–1.10)	
CYP2R1	**GG**	**GA**	**AA**	
(rs10741657)				
Biliary	96 (41.4)	108 (46.6)	28 (12.1)	0.62
Control[1,3]	1235 (37.1)	1576 (47.4)	517 (15.5)	
	1.00	1.01 (0.72–1.42)	0.84 (0.50–1.41)	
AGT (rs699)	**AA**	**AG**	**GG**	
Biliary	97 (39.4)	110 (44.7)	39 (15.9)	0.58
Control[2,3]	2104 (36.6)	2701 (47.0)	945 (16.4)	
	1.00	1.02 (0.73–1.43)	1.17 (0.73–1.88)	
Renin (rs5707)	**AA**	**AC**	**CC**	
Biliary	161 (63.9)	71 (28.2)	20 (7.9)	0.59
Control[2,3]	3476 (60.6)	2004 (35.0)	253 (4.4)	
	1.00	0.79 (0.56–1.12)	2.11 (1.09–4.08)	
VDR (rs2228570)	**GG**	**GA**	**AA**	
Biliary	96 (39.7)	109 (45.0)	37 (15.3)	0.60
Control[3]	293 (41.6)	304 (43.2)	107 (15.2)	
	1.00	1.02 (0.72–1.44)	1.16 (0.72–1.44)	

Gene				**P value***
GC (rs7041)	**CC**	**CA**	**AA**	
Biliary	78 (32.0)	121 (49.6)	45 (18.4)	0.36
Control[2,3]	1752 (30.5)	2912 (50.7)	1075 (18.7)	
	1.00	0.73 (0.51–1.04)	0.86 (0.54–1.38)	

[1] NPHSII;

[2] WHII;

[3] Netherlands Blood Bank

* Additive genetic model adjusted for age, *sex*, region

Table 8

Previous studies investigating the effect of RAS polymorphisms on outcome from pancreatitis

Gene; SNP	Study Cohorts	Significant Associations	Authors
ACE; I/D	79 AP/95 Controls	None	Oruc *et al*, 2009[68]
ACE; I/D	98 AP & 789 CP/1294 Controls	None	Hucl *et al*, 2009[69]
ACE; I/D	104 CP + 51 FP/163 Controls	None	Oruc *et al*, 2004[70]
ACE; I/D	55 CP/128 Controls	None	Lukic *et al*, 2011[72]
ACE; I/D	91 TCP/99 Controls	None	Bhaskar *et al*, 2006[73]

AP- Acute Pancreatitis; **CP**- Chronic Pancreatitis; **AIP**- Autoimmune Pancreatitis; **HCP**- Hereditary Chronic Pancreatitis; **ICP**- Idiopathic Chronic Pancreatitis; **CCP**- Chronic Calcific Pancreatitis