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Prevalence of *CYP2C19*2* carriers in Saudi ischemic stroke patients and the suitability of using genotyping to guide antiplatelet therapy in a university hospital setup

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Abstract

Objectives: To mitigate the incidence of recurrent stroke in patients, dual antiplatelet therapy comprising aspirin and clopidogrel is usually administered. Clopidogrel is a prodrug and its bioactivation is catalyzed by cytochrome P450 (CYP)2C19. The main objective of this work was to determine the prevalence of *CYP2C19*2* carriers in Saudi ischemic stroke patients and assess the suitability of using genotyping to guide antiplatelet therapy in a university hospital setup.

Methods: This prospective (2018–2019) study was conducted on 256 patients (age 61 ± 12.5) clinically diagnosed with ischemic stroke who were genotyped using Spartan RX *CYP2C19* assay.

Results: From the total patient group (256), upon admission, 210 patients were prescribed either aspirin, clopidogrel or dual antiplatelet therapy. Of the 27 patients with the *CYP2C19*2* allele who were prescribed clopidogrel (18) or dual antiplatelet therapy (9), only 21 patients could be followed up for a period of six months post stroke event, in addition to 21 age- and sex-matched patients with the normal allele. The *CYP2C19*2* allele carriers had a statistically significant increased risk of recurrent stroke compared to patients carrying the normal allele.

Conclusions: This study shows the suitability of using genotyping to guide antiplatelet therapy in ischemic stroke patients in a clinical setting.

Keywords: aspirin; clopidogrel; *CYP2C19*2*; genotyping; stroke.

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Introduction

The active metabolite of the prodrug clopidogrel selectively inhibits purinergic P2RY12 receptor and consequently, inhibits platelet aggregation [1, 2]. This hepatic biotransformation is carried out by the liver enzyme Cytochrome P450 2C19 (*CYP2C19*). However, there is substantial interindividual variability which suggest that variants of the *CYP2C19* gene are associated with poor clopidogrel responsiveness. Although this association between *CYP2C19* loss-of-function alleles and clopidogrel efficacy in a clinical setup has been investigated, the results are controversial [3–6]. Limited data are available concerning the effect of *CYP2C19* polymorphisms on the use of clopidogrel by stroke patients [7]. Moreover, studies have shown that there is an ethnic variation in the incidence of ischemic stroke [8, 9]. People of African and Asian descent, including the population of Saudi Arabia, are more predisposed to ischemic stroke than

their European counterparts [10–12]. Globally, stroke is the leading cause of morbidity and mortality, with a reported 15 million deaths due to stroke [13].

The prevalence of stroke in Saudi Arabia is estimated to be between 40 and 44/100,000, with reports showing that the incidence of ischemic stroke is increasing in the country due to an increase in the aging population [13–15]. In order to improve therapy for stroke patients, it is important to gain a deeper understanding of the relationship between *CYP2C19* polymorphism and clopidogrel's clinical effects. Recent clinical trials have reported that dual antiplatelet therapy, comprising aspirin and clopidogrel, may be effective in preventing recurrent stroke after a minor ischemic stroke or transient ischemic attack (TIA) [16–18]. These trials have shown that while dual antiplatelet therapy reduces the risk of a composite of stroke, MI or death, it increases the risk of a major hemorrhage in these patients. However, clopidogrel has to be metabolized to its active metabolite by the liver Cytochrome P450 to be effective as an antiplatelet therapy. Approximately 28% of the Caucasian population and 26% of the Saudi population carry a non-functional *CYP2C19*2* or **3* allele and therefore the effectiveness of clopidogrel is significantly decreased in these populations [19, 20]. *CYP2C19*2* is due to a splice site mutation in exon 5 (G>A) which alters the reading frame at amino acid 215, while *CYP2C19*3* is due to a nonsense mutation in exon 4 (636G>A) which leads to a truncated protein both of which leads to loss of function variants [21, 22]. Consequently, the use of clopidogrel as part of dual antiplatelet therapy in stroke patients must be reevaluated.

The present study was conducted on a group of ischemic stroke patients in the Eastern Province of Saudi Arabia to determine the percentage of patients who carry the non-functional allele of *CYP2C19* and to assess the suitability of using point-of-care genotyping.

Materials and methods

Over a 2-year period (2018–2019), consecutive patients with primary stroke (n=256) admitted to the Neurology Department, King Fahd Hospital of the University, Al-Khobar, Saudi Arabia, were included in this study after obtaining signed informed consent. Ethical approval of the study was obtained from local Institutional Review Board (IRB) committees and the study was conducted according to the ethical principles of the Declaration of Helsinki and Good Clinical Practice guidelines. All patients had been clinically diagnosed with ischemic stroke through physical and neurological examinations and the use of cranial computed tomography.

After admittance to the hospital following the primary stroke, patients were prescribed the standard treatment described in the Ministry of Health protocol, namely aspirin alone, clopidogrel alone or a combination of aspirin and clopidogrel, known as dual antiplatelet therapy according to the treating physician's discretion. However, some patients in the total cohort had been prescribed other antiplatelet medications or no medication at all. Patients who were prescribed clopidogrel alone or dual antiplatelet therapy was given a 600 mg loading dose of clopidogrel, followed by 75 mg per day from day 2 to day 90. Patients who were given aspirin alone or dual antiplatelet therapy were prescribed a daily dose of aspirin ranging from 50 to 325 mg according to the treating physician, which is consistent with guideline recommendations of the Ministry of Health protocol. The patients in these three groups were also prescribed a proton pump inhibitor.

None of the patients had a history of carotid endarterectomy or carotid stent therapy. From the total study cohort, only 27 patients, heterozygous for the *CYP2C19*2* allele, had been prescribed either clopidogrel or dual antiplatelet therapy. However, due to the COVID-19 pandemic, only 21 of these patients, together with 21 age- and sex-matched controls with the normal allele, could be followed up for a period of six months post the primary stroke event. The primary outcome of this study was determined to be the risk of a composite of ischemic stroke, myocardial infarction, or death from ischemic vascular causes [18]. The treating physician was blinded to the genotyping result, and therefore, no treatment plan was altered as this study was conducted to determine the percentage of patients who carry the non-functional allele of *CYP2C19* and to assess the suitability of using point-of-care genotyping.

Blood samples (5 ml) were collected in EDTA vacutainers from all patients. The Spartan RX CYP2C19 assay using the point-of care Spartan RX device was used to determine the *CYP2C19*2* genotype of patients. The genotyping was further confirmed by TaqMan genotype discrimination method. In this method, DNA is extracted from peripheral leukocytes using QIAamp DNA isolation kit (Qiagen, UK) as per the manufacturer's instructions. DNA concentration and purity were measured using Nanodrop 2000 (Nanodrop, USA). The samples were genotyped using an allele specific Taqman® genotyping assay by real-time PCR (ABI 7500, USA). The ABI TaqMan assay reagents (Cat no. 4351379, Thermo Scientific, USA) were used for the detection of a mutation which used dyes FAM/VIC with excitation at 470 nm, 530 nm and emission at 510 nm, 557 nm corresponding to green and yellow channels, respectively. Binary and other categorical variables were compared using $\chi 2$ test or Fisher's exact test, as appropriate. For continuous data, two-sided unpaired Student's *t*-tests were used. Data were analyzed with SPSS version 24.

Results

In total, 256 patients were included, 166 were male (64.9%) and the mean age of the study cohort was 61 years (range 18–89 years). The patients' basic characteristics are shown in Table 1. Vascular imagining indicated that 63 patients (24.6%) had intercranial disease, 37 (14.5%) had extracranial disease, 92 (35.9%) had no abnormality and for 64 (25.0%) patients, imaging data were not available. Of the total study cohort, 93 (36.3%) had large artery atherosclerosis, 37 (14.5%) had cardio-aortic embolism, 91 (35.5%) had small artery occlusion and in the remaining 35 (13.7%) patients, the stroke was due to undetermined causes.

Furthermore, 53% of the patients had hypertension, 41% had diabetes, 43% had dyslipidemia and 3.9% had chronic kidney disease. Only 5.5% of the patients were smokers. There were no statistically significant differences in the prevalence of these diseases between patients with the normal allele and patients with the mutated allele ($p<0.05$). From the total patient group (256), upon admission, 113 (44.1%) patients had been prescribed aspirin, 56 (21.9%) patients had been prescribed clopidogrel, 41 (16.0%) patients had been prescribed dual antiplatelet therapy, 21 (8.2%) patients had been prescribed other antiplatelet medications and 25 (9.8%) patients had not been prescribed any medication.

The genotyping procedure was timed in the first 50 patients included in the study. It took approximately 90–120 min to complete the results using Spartan RX device. After genotyping, it was determined that 54 (21.1%) patients carried the *CYP2C19*2* allele (Table 2). Two of the patients with the *CYP2C19*2* allele were homozygous for the mutation, while the remaining 52 patients were heterozygous for the mutation. There was no statistically significant difference in the prevalence rates of various comorbidities tested between patients with the normal allele and patients with the mutated allele ($p<0.05$), except for Modified Rankin Scale (mRS) and large artery atherosclerosis ($p>0.05$).

Of the 54 patients with the *CYP2C19*2* allele, 24 patients had been prescribed aspirin, 18 patients had been prescribed clopidogrel, nine patients had been prescribed dual antiplatelet therapy, while the remaining three patients had been prescribed other antiplatelet therapy, namely prasugrel or ticagrelor. The prevalence of large artery atherosclerosis was higher in the *CYP2C19*2* allele group (51.9%) than in the group with the normal allele (32.2%). However, there was no significant difference between the two groups with respect to cardio aortic embolism or small artery occlusion.

Of the 27 patients with the *CYP2C19*2* allele who had been prescribed clopidogrel alone (18 patients) or dual antiplatelet therapy (9 patients), only 21 patients could be followed up for a period of six months post the stroke event. In addition, 21 age- and sex-matched ischemic stroke patients with the normal allele were also followed up. As shown in Table 3, the *CYP2C19*2* allele carriers had a statistically significant increased risk of recurrent stroke compared to patients carrying the normal allele (OR (95% CI) = 47.1 (2.5–878.5), p=0.001). A non-significant trend of increased risk of MI was also observed among *CYP2C19*2* allele carriers compared to normal allele carriers (OR (95% CI) = 5.5 (0.25–122.1), p=0.28, Table 3). The *CYP2C19*2* allele had no impact on mortality due to ischemic vascular causes (OR (95%CI) = 1.0 (0.06–17.1), p=1.00), (Table 3).

Discussion

To mitigate a recurrent stroke, antiplatelet therapy in the form of aspirin alone, clopidogrel alone or dual antiplatelet

Table 1.: Basic characteristic of patients included in the study.

Characteristics	Number of patients, %
Number of patients	256
Gender (male %)	166 (64.9)
Age (mean ± SD), year	61.0 ± 12.5
Age at diagnosis (mean ± SD)	59.7 ± 12.8
Vascular imaging#, n, %	
Intracranial disease	63 (24.6)
Extracranial disease	37 (14.5)
No abnormality	92 (35.9)
Not available	65 (25.0)
Ischemic stroke, n, %	
Large artery atherosclerosis	93 (36.3)
Cardio-aortic embolism	37 (14.5)
Small artery occlusion	91 (35.5)
Undetermined	35 (13.7)
Drug administration, n, %	
Aspirin	113 (44.1)
Clopidogrel	56 (21.9)
Aspirin + clopidogrel	41 (16.0)
Other	21 (8.2)
None	25 (9.8)

Table 2: Distribution of *CYP2C19* mutation carriers (carriers defined as 1*/2* and 2*/2*).

	Fast metabolizers (1*/*1)	Slow metabolizers (1*/2* or 2*/2*)	p-Value
Number of patients, n, %	202 (78.9)	54 (21.1)	
Medical history, n, %			
Hypertension	106 (52.5)	30 (55.6)	0.81
Coronary artery disease	34 (16.8)	7 (13.0)	0.45
Dyslipidemia	90 (44.6)	20 (37.0)	0.26
Diabetes	81 (40.1)	25 (46.3)	0.49
Chronic kidney disease	7 (3.5)	3 (5.6)	0.45
Smoking	11 (5.4)	3 (5.6)	0.99
Drug administered, n, %			
Aspirin	89 (44.1)	24 (44.4)	0.93
Clopidogrel	63 (31.2)	18 (33.3)	
Aspirin + clopidogrel	32 (15.8)	9 (16.7)	
Other	18 (8.9)	3 (5.5)	
mRS >0, n, %	115 (56.9)	43 (79.6)	0.02
Large artery atherosclerosis, n, %	65 (32.2)	28 (51.9)	0.01
Cardio aortic embolism, n, %	31 (15.8)	6 (11.1)	0.40
Small artery occlusion, n, %	76 (37.6)	15 (27.8)	0.15

mRS, Modified Rankin Scale.

Table 3: Comparisons of recurrent stroke, MI, and death between 21 patients with *CYP2C19*2* allele and 21 patients with the normal allele who were on clopidogrel or a combination of clopidogrel and aspirin.

	Recurrent stroke			MI			Death due to ischemic vascular causes, n %		
Allele	**n, %**	**OR (95% CI)**	**p-Value**	**n, %**	**OR (95% CI)**	**p-Value**	**n, %**	**OR (95% CI)**	**p-Value**
*CYP2C19*2*	11 (52.4)	47.1	0.001	2 (9.5)	5.5 (0.25–122.1)	0.28	1 (4.8)	1.0 (0.06–17.1)	1.00
*CYP2C19*1*	0 (0.0)	(2.5–878.5)		0 (0.0)			1 (4.8)		

therapy is commonly prescribed, especially to high-risk patients [16–18]. The simultaneous use of clopidogrel augments the action of aspirin through the P2Y12-receptor pathway. Clinical trials have shown that the use of dual antiplatelet therapy is more effective in preventing a recurrent stroke than those patients who received aspirin alone [17]. Due to clopidogrel's antiplatelet effect and its low cost, it is widely used as the first line therapy to prevent recurrent stroke. However, the pharmacodynamic response to clopidogrel varies between patients and populations as clopidogrel, an inactive prodrug, is activated through CYP450 isoenzymes to form the active thiol metabolite [19, 20].

Previous studies have shown that patients with a reduced function of the mutated allele, *CYP2C19*2*, who had undergone ST-Elevation Myocardial Infarction (STEMI), and who had been treated with Percutaneous Coronary Intervention (PCI), had an increased risk of developing stent thrombosis by approximately 1.5–2 times [23]. In the current study, 21.1% (n=54) of the total cohort carried the *CYP2C19*2* allele. Although the number of patients in our study who have the *CYP2C19*2* allele and were prescribed clopidogrel alone or dual antiplatelet therapy is small, there was a significant difference in the outcome during the six-month follow-up period. Patients with the *CYP2C19*2* allele who were on clopidogrel alone or dual antiplatelet therapy had a higher percentage (9.5%) of MI and a significantly higher percentage of recurrent stroke (52.4%) in comparison to patients who carried the normal allele ($p<0.05$). In the current study, all patients with the *CYP2C19*2* allele and selected controls had been prescribed a proton pump inhibitor, omeprazole, which may interact with clopidogrel. However, these reports provide conflicting conclusions as to this interaction.

Approximately 28% of the Caucasian population and 26% of the Saudi population carry a non-functional *CYP2C19*2* or **3* allele and therefore the effectiveness of clopidogrel is significantly decreased in these patients [20, 24]. Genotyping of *CYP2C19* has become standard procedure in many coronary heart disease centers throughout the world. However, whether the use of genotyping *CYP2C19* and prescribing prasugrel and ticagrelor to stroke patients with the *CYP2C19*2* allele will have a better clinical outcome is still unknown. Although our study sample is limited, it sheds some light on the necessity of genotyping ischemic stroke patients to provide them with the optimum therapy.

It has been shown that there is a net clinical benefit in the use of genotyping to guide antiplatelet therapy in PCI, and therefore, the preliminary data from this study suggests that there may also be a net clinical benefit outcome if ischemic stroke patients with the *CYP2C19*2* allele are treated with alternative antiplatelet therapy that does not require bioactivation by the cytochrome P450 enzyme [25]. In addition, the present study demonstrates the suitability of rapid genotyping in a clinical setting to allow for informed antiplatelet prescribing. It has to be noted that currently in King Fahd Hospital of the University all patients treated by percutaneous coronary intervention (PCI) who are administered dual antiplatelet therapy are genotyped for the *CYP2C19*2* allele to mitigate the risk of stent thrombosis. This form of precision medicine is becoming the norm in many clinical centers as it enhances the clinical outcome. The *3 and *17 alleles are present in the Saudi population, but due to their low prevalence only the *CYP2C19*2* allele was genotyped, which is a limitation to the study [26]. It has been reported that the prevalence of coronary heart disease patients who have an inadequate response to aspirin in our population is low, however we cannot exclude the fact that some of the patients with the *CYP2C19*2* allele included in this study may also have had inadequate response to aspirin [24]. However, due to the high prevalence of *CYP2C19*2* allele in the Saudi population, a prospective clinical trial is required to confirm the above data.

Conclusions

This study has shown the suitability of using genotyping to guide antiplatelet therapy in patients with ischemic stroke in a clinical setting. In addition, the study has shown that

genotyping for the *CYP2C19*2* allele in ischemic stroke patients may lead to an improvement in net clinical benefit outcome if patients with the *CYP2C19*2* allele are treated with alternative antiplatelet therapy that does not require bioactivation by cytochrome P450.

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