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ABCB1 C3435T and CYP2C19*2 polymorphisms in a Palestinian and Turkish population: A pharmacogenetic perspective to clopidogrel

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ABSTRACT

Clopidogrel is an antiplatelet drug used to prevent recurrent ischemic events after acute coronary syndrome and/or coronary stent implantation. Single nucleotide polymorphisms (SNPs) such as CYP2C19*2 and ABCB1 C3435T have been found to play a role in different individual responses to clopidogrel. Since the prevalence of these SNPs is generally known to differ from one population to another, the aim of this study was to examine their prevalence in both a Palestinian and Turkish population. One hundred unrelated Palestinian subjects and 100 unrelated Turkish subjects were analyzed for CYP2C19*2 and ABCB1 C3435T polymorphisms by the amplification refractory mutation system (ARMS).
Results showed an ABCB1 3435 T allele frequency of 0.46 (95% CI 0.391 to 0.529) in the Palestinian sample and 0.535 (95% CI 0.4664 to 0.6036) in the Turkish sample. CYP2C19*2 allele frequency was 0.095 (95% CI 0.0558 to 0.134) in the Palestinian sample and 0.135 (95% CI 0.088 to 0.182) in the Turkish sample.
Our results provide information about the prevalence of the polymorphisms related to clopidogrel response in both the Palestinian and Turkish populations, in order to improve the safety and efficacy of clopidogrel through use of genetically guided, individualized treatment. The prevalence of these clinically significant alleles shed light on the importance of testing them before prescribing clopidogrel.

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Introduction

Genetic polymorphisms are known to have pronounced clinical significance in determining inter-patient variability towards drug response. It is the dominant influencing factor for individual and inter-ethnic variations in drug responses (Evans and Johnson, 2001). Clopidogrel is one such drug whose pharmacokinetic and pharmacodynamic efficiency can be predicted based on an individual's or population's genetic makeup (Angiolillo et al., 2007a; Brandt et al., 2007; Hulot et al., 2006). Clopidogrel administration along with aspirin is the guide line-approved standard of care in acute coronary syndromes and following stent implantation (Antman et al., 2008; Chen et al., 2005; Yusuf et al., 2001). Pharmacodynamic responses to clopidogrel vary greatly among patients (Gurbel et al., 2003); patients with lesser degrees of platelet inhibition are more likely to experience recurrent ischemic events (Hochholzer et al., 2006).

The *ABCB1* (ATP-binding cassette, subfamily B, member1) gene encodes the intestinal efflux transporter P-glycoprotein (P-gp), which modulates the absorption of clopidogrel (Gros et al., 1986; Taubert et al., 2006). Amongst several SNPs within this gene, the ABCB1 C3435T (rs1045642) has been shown to hinder the absorption of clopidogrel (Taubert et al., 2006). Individuals with TT homozygotes for the C3435T variant have lower levels of the active drug metabolite and may have higher rates of adverse clinical outcomes (Taubert et al., 2006; Simon et al., 2009).

Clopidogrel requires biotransformation to its active metabolite through two cytochrome P450-dependent steps (Reese et al., 2012). In particular, the isoenzyme CYP2C19 is involved in both steps contributing to an estimated 45% and 21% of the first and second steps respectively (Kazui et al., 2010). The most common SNP designated CYP2C19*2 (c.G681A) (rs4244285) leads to a splicing defect that functionally affects the enzyme. There are more than 25 known polymorphic groups (Trenk et al., 2008). The CYP2C19*1 allele is the normal (wild-type) copy that has full enzymatic activity. The CYP2C19*2 and CYP2C19*3 alleles are the most common variants resulting in complete loss of enzymatic activity. Carriers of CYP2C19*2 and CYP2C19*3 alleles have reduced metabolism of clopidogrel and demonstrate diminished clopidogrel-induced platelet inhibition (Collet et al., 2009). Previous pharmacogenetic studies have shown the prevalence of *CYP2C19* alleles to vary between different populations (Sameer et al., 2009; Hamdy et al., 2002). In March 2010, the FDA added a boxed warning to the product label of clopidogrel, alerting the clinicians on the risk of reduced clopidogrel efficacy in CYP2C19 poor-metabolizers (annon, 2010).

As several studies indicate, a higher clopidogrel dose could potentially provide a more intense antiplatelet effect in patients with a genetic predisposition to a diminished response to standard dose therapy by providing a greater substrate for biotransformation into the active metabolite (Angiolillo et al., 2004; Angiolillo et al., 2007b; Gurbel et al., 2005; Mega et al., 2011).

The aim of the study is to investigate the frequency of ABCB1 C3435T and CYP2C19*2 polymorphisms involved in clopidogrel pharmacokinetics in Palestinian and Turkish sample populations, in order to contribute to the use of appropriate strategies for clopidogrel therapy in these populations.

Materials and methods

Study population

Two study groups were investigated in this study. Group one included 100 unrelated Palestinian subjects (24–84 years old) geographically originating from the West Bank and Jerusalem. The work on these samples took place in Makassed Islamic Charitable Hospital in Jerusalem. The second group included 100 unrelated Turkish subjects (20–66 years old) and was genotyped in Yeditepe University in Istanbul. Informed consent was obtained from the 200 subjects. The study was approved by the Research and Ethical Committee both at Makassed Hospital and Yeditepe University. Three ml of whole blood in EDTA tubes was collected from each subject.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes following a standard salting-out method (Miller et al., 1988). The CYP2C19*2 c.G681A (rs4244285) and ABCB1 c.C3435T (rs1045642) polymorphisms were screened using the amplification refractory mutation system (ARMS). The primers designed for the study are shown in Table 1.

The PCR reaction was carried out using Go Taq Green Master Mix (Promega, USA) in a final volume of 15 μl, containing 100 ng of purified genomic DNA and 100 ng of each primer. The PCR amplification conditions for ABCB1 C3435T polymorphism included an initial denaturation step at 95 °C for 5 min, followed by 28 cycles with a second denaturation step at 95 °C for 15 s, annealing at 62 °C for 15 s, and extension at 72 °C for 15 s, then a final extension step at 72 °C for 5 min for 28 cycles. PCR amplification conditions for CYP2C19*2 polymorphism included an initial denaturation step at 95 °C for 5 min, followed by 25 cycles with a second denaturation step at 95 °C for 15 s, annealing at 61 °C for 15 s and extension at 72 °C for 15 s, then a final extension step at 72 °C for 5 min. The amplified product was analyzed using 2% agarose gel electrophoresis (OWL Ltd, USA), and stained with ethidium bromide. Bands were visualized on an ultraviolet illuminator and photographed with a Polaroid camera (Fuji Photo Film Co., Japan).

Control samples included normal and heterozygous DNA for CYP2C19*2 and ABCB1 C3435T polymorphisms, that were previously identified by ARMS and confirmed by direct DNA sequencing. DNA sequencing amplification was performed in 100 μl reaction containing 400 ng of genomic DNA, 0.4 μg of each primer (Table 1), and 50 μl of GoTaq Green Master Mix, 2X (Promega, USA). Cycling parameters included an initial denaturation step of 4 min at 94 °C, followed by 35 cycles of 94 °C for 30 s, 61 °C for 30 s, 72 °C for 30 s, with a final extension step of 10 min at 72 °C.

The amplification products (355 bp) were separated on 2% agarose gel and purified using GFX PCR DNA and Gel Band Purification Kit (Amersham Pharmacia Biotech). Following purification, DNA segments were sequenced using a 3130 Genetic Analyzer (Applied Biosystems, USA). The sequence of each sample was then compared to the wild-type gene obtained from the NCBI gene bank accession number GI: 51467897.

Statistical analysis

Data were presented as genotype and allele frequencies. The frequency of each allele is given together with the 95% confidence interval. Genotype frequencies were tested for deviations from Hardy–Weinberg equilibrium (HWE) through chi-square analysis and at $P = 0.05$.

Table 1
Primers used for the amplification of the ABCB1 C3435T and CYP2C19*2 polymorphisms.

Name	Primer sequence (5′ to 3′)	Fragment size (bp)
ARMS primers		
CYP2C19*2 common	TACGCAAGCAGTCACATAACTAAGC	205
CYP2C19*2 mutated	TCCCACTATCATTGATTATTTCCCA	
CYP2C19*2 normal	TCCCACTATCATTGATTATTTCCCG	
ABCB1 C3435T common	CTCACAAGGAGGGTCAGGT	
ABCB1 C3435T mutated	GTGGTGTCACAGGAAGAGATT	278
ABCB1 C3435T normal	GTGGTGTCACAGGAAGAGATC	
Sequencing Primers		
CYP2C19*2	Forward: TCATCTTTGATTCTCTTGTCAGAA	355
	Reverse: TAAAGTCCCGAGGGTTGTTG	
ABCB1 C3435T	Forward: AAGTGTGCTGGTCCTGAAGTT	372
	Reverse: AAGGGTGTGATTTGGTTGC	

Results and discussion

This is the first study to discuss the relationship of these polymorphisms for an individualized approach to clopidogrel treatment in both Turkish and Palestinian populations. Genotype and allele frequencies of ABCB1 C3435T and CYP2C19*2 polymorphisms in both Palestinian and Turkish subjects are shown in Table 2. The results were in good accordance with the expected genotype distributions of the tested genes, calculated by HWE (Table 2). This means that both population samples followed HWE for these two locations.

The importance of this study relates to the fact that an understanding of the worldwide distribution of SNPs is crucial for the future application of pharmacogenomic-based algorithms to different population groups. Such applications have recently been performed in warfarin therapy (Ross et al., 2010).

Clopidogrel, as a prodrug, requires metabolic activation before inhibiting platelet aggregation. The ABCB1C3435T has been previously associated with loss of function of P-glycoprotein which decreased the active metabolite of clopidogrel (Taubert et al., 2006). ABCB1 3435 T allele frequency was 0.46 (95% CI 0.391 to 0.529) in the Palestinian sample and 0.535 (95% CI 0.4664 to 0.6036) in the Turkish sample. Previous studies have shown conflicting results on the effect of C3435T polymorphism on clinical outcome in patients receiving clopidogrel (Jaitner et al., 2012; Mega et al., 2010; Price et al., 2012; Simon et al., 2009). A recently published meta-analysis delineated the association between ABCB1 C3435T and the risk of poor clinical outcome in patients treated with clopidogrel. It was indicated that the 3435 T allele was related to the risk of major adverse cardiovascular events in patients on clopidogrel, and that TT homozygotes decreased the outcome of bleeding compared with CC homozygotes (Su et al., 2012).

The frequency of the clinically significant genotype TT in this study was observed as 18% in the Palestinian sample and 28% in the Turkish sample. The 'T' allele frequency was (46%) among Palestinians and (53.5%) in the Turkish sample. The presence of these clinically important alleles in this percentage of population makes their genotyping worth testing before drug prescription. Similar to other Caucasian subjects, in studies on Spanish (Bernal et al., 2003), German (Hoffmeyer et al., 2000), and Polish (Mrozikiewicsz et al., 2007) subjects, the 'T' allele frequency was 48%, 48%, and 49% respectively.

Being the allele responsible for the most deficient metabolizers, CYP2C19*2 was genotyped in the Palestinian and Turkish populations and the allele frequency of *2 to was found to be 0.095 (95% CI 0.0558 to 0.134) in Palestinians and 0.135 (95% CI 0.088 to 0.182) in Turkish. Concerning the genotype distribution, the *1/*2 genotype was the main risk genotype in our samples since it consisted of 19% in Palestinians and 27% in the Turkish sample.

Even though Palestine and Turkey do not share common borders, the genetic similarities found in this study could be attributed to a common genealogy knowing that Turks had ruled Palestine for about four centuries.

Table 2
Allelic and genotypic frequencies of ABCB1 C3435T and CYP2C19*2 in the Palestinian and Turkish populations.

Nucleotide change and genotype	Palestinian	HWE ΣX^2	Turkish	HWE ΣX^2
	100		100	
	N (%)		*N* (%)	
CYP2C19*2		1.09		2.43
*1/*1	81 (81)		73 (73)	
*1/*2	19 (19)		27 (27)	
*2/*2	0		0	
*2 allele	0.095		0.135	
ABCB1C3435T		1.61		0.062
CC	26 (26)		21 (21)	
CT	56 (56)		51 (51)	
TT	18 (18)		28 (28)	
T allele	0.46		0.535	

HWE Hardy–Weinberg equilibrium test ($P = 0.05$).

Palestinians and Turkish populations showed similarities in CYP2C19*2 allele frequency with some of other populations in the Middle East (Djaffar Jureidini et al., 2011; Hamdy et al., 2002; Yousef et al., 2012). The homozygous mutant genotype of CYP2C19*2 (*2/*2) was not detected in neither the Palestinian population sample nor the Turkish sample while other studies reported the presence of this genotype in their population samples (Akhlaghi et al., 2011; Aynacioglu et al., 1999; Sameer et al., 2009; Zalloum et al., 2012).

The variability in the distribution of both CYP2C19*2 and ABCB1 C3435T among populations reflects a population specific pattern which stresses the need to genotype these alleles for the Palestinian and Turkish populations as well. A possible solution to overcome the decreased response to clopidogrel especially in patients who are CYP2C19 poor metabolizers is to increase the loading dose from 300 mg to 600 mg (Gurbel et al., 2005) or increase the maintenance dose from 75 mg to 150 mg (Angiolillo et al., 2007b).

Interestingly, in a study conducted to evaluate the cost effectiveness of genotype-guided antiplatelet therapy, researchers found that genotyping patients before selecting antiplatelet therapy could offer more value in the clinical setting than assigning drug therapy without regard to pharmacogenomic test results (Reese et al., 2012).

The data presented in this study will facilitate physicians prescribing clopidogrel by providing an approximate percentage of poor metabolizers. It is therefore recommended to test the polymorphisms CYP2C19*2 and ABCB1 C3435T before prescribing clopidogrel. This would allow physicians to give a genotype-guided treatment, which could be more cost-effective and result in a reduction of clinical adverse outcomes.

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