Original Article

CYP2C19 genetic polymorphisms in Maltese patients on clopidogrel therapy

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Abstract

Introduction and Aims: The CYP2C19 enzyme is involved in the metabolism of various therapeutically-important drugs including clopidogrel.

The aims were to determine *CYP2C19 *2* and **17* variant allele frequencies and *CYP2C19* genotype distribution in a cohort of Maltese patients on clopidogrel and to compare observed frequencies of the *CYP2C19 *2* allele and **2/*2* genotype in this cohort to other populations bordering the Mediterranean Sea.

Methods: *CYP2C19 g*enotyping in a cohort of Maltese patients on clopidogrel was performed using TaqMan™ Drug Metabolism Genotyping Assays. Frequencies of the *CYP2C19 *2* and **17* variant alleles and six genotypes (**1/*1*, **1/*2*, **2/*2*, **1/*17*, **17/*17*, **2/*17*) were determined. Observed frequencies of the **2* allele and **2/*2* genotype were compared to fourteen populations bordering the Mediterranean Sea.

Results: Frequency of the *CYP2C19 *2* and **17* allele in the 244 Maltese patients genotyped was 12.3% and 15.4% respectively. *CYP2C19* genotype distribution was: **1/*1* (52.1%), **1/*17* (22.5%), **1/*2* (18.0%), *2/*17* (6.6%), **17/*17* (0.8%) and *2/*2 (0). There was no statistically significant difference in **2* allele frequency between the Maltese cohort and all fourteen populations bordering the Mediterranean Sea.

Conclusions: This study reports the frequency of *CYP2C19 *2* and **17* variant alleles in a cohort of Maltese patients treated with clopidogrel. The high percentage of patients genotyped as carriers of the **2* (25%) or **17* (23%) variant alleles indicates that CYP2C19 genotyping could be used to guide clinicians in the individualisation of antiplatelet therapy.

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Keywords

clopidogrel; *CYP2C19* polymorphisms; drug metabolism; Maltese; Mediterranean

Introduction

The CYP2C19 enzyme is involved in the metabolism of a number of therapeutically-important drugs, including the thienopyridine inactive prodrug

clopidogrel. Biotransformation in the liver is required to form the pharmacologically active metabolite of clopidogrel, which selectively and irreversibly antagonises the $P2Y_{12}$ component of the adenosine diphosphate receptor on the platelet surface, consequently attenuating platelet aggregation.[1] Two sequential hepatic oxidative steps are involved in clopidogrel bioactivation[1] and CYP2C19 is the principal enzyme involved in both steps.[2] *CYP2C19* single nucleotide polymorphisms have been identified as significantly and consistently being associated with variability in clopidogrel response.[3-5] Identifying patients' genotype and ability to effectively transform clopidogrel to the active metabolite is crucial for individualisation of treatment in cardiology.

The cytochrome P (CYP) 450 isoenzyme 2C19 (CYP2C19) is highly polymorphic and more than 30 variant alleles have been identified.[6] The *CYP2C19* '**1*' 'wild-type' allele is associated with normal 'functional' CYP2C19-mediated metabolism and is assigned when variant alleles are not identified. The '**2*' variant allele is the most prevalent loss-of-function allele which translates into decreased drug metabolism and the '**17'* allele is a gain-of-function allele which may result in increased activity due to enhanced expression.[3]

Frequencies of the *CYP2C19 *2* and **17* alleles in forty-one healthy Maltese volunteers have been reported.[7] The aims of this study were to determine the frequency of the *CYP2C19 *2* and **17* alleles and *CYP2C19* genotype distribution in a cohort of Maltese patients on clopidogrel and to compare the frequencies of the **2* allele and **2/*2* genotype observed in this cohort to other populations bordering the Mediterranean Sea.

Methodology

Ethics approval

The study protocol was approved by the University of Malta Research Ethics Committee.

Study design and setting

This cohort study was undertaken at Mater Dei Hospital. Patients were prospectively identified from the cardiac catheterisation suite at the Department of Cardiology and *CYP2C19* genotyping was performed at the Molecular Diagnostics Unit of the Department of Pathology.

Patient recruitment and sample collection

Maltese patients ≥ 18 years undergoing percutaneous coronary intervention (PCI) with stent placement and prescribed dual antiplatelet therapy with aspirin and clopidogrel were recruited by non-probability sampling over a twelve-month period (January-December 2014). The advantages of this sampling method are that it is cost and time-effective. Although non-probability sampling does not guarantee that each patient has equal probability of being selected, the sample is a good representation of the population since the patients were recruited over a one-year period. After obtaining written informed consent, 5 mL of peripheral blood was collected from each patient in a purple-top ethylenediaminetetraacetic (EDTA) vacutainer at the time of PCI.

Genomic DNA extraction and CYP2C19 genotyping

Genomic DNA was extracted from 200 µL of the EDTA-blood sample using the QIAamp® DNA Mini Kit on the fully automated QIAcube *(Qiagen)*. *CYP2C19* genotyping for the **2* (rs4244285) and **17* (rs12248560) alleles was performed with TaqMan™ Drug Metabolism Genotyping Assays *(Thermo Fisher Scientific)*, which involve DNA amplification and homogeneous solution hybridisation using fluorescence resonance energy transfer, on the 7500 ABI real-time polymerase chain reaction (PCR) system *(Applied Biosystems)*. Each well in the PCR plate had a final reaction volume of 25 µl consisting of gDNA, an allele-specific probe labelled with VIC® dye and another with 6FAM™ dye, forward and reverse primers and TaqMan™ Universal PCR Master Mix *(Thermo Fisher Scientific)*. Thermal cycling conditions consisted of initial denaturation at 95 °C for 10 minutes, followed by 50 denaturation cycles at 92 °C for 15 seconds and annealing/extension at 60 °C for 90 seconds. Patients were genotyped as homozygous (**1/*1, *2/*2, *17/*17*) or heterozygous (**1/*17, *1/*2, *2/*17*) for the *CYP2C19* alleles.

Categorisation of patients into metaboliser phenotypes for clopidogrel

The observed genotypes were classified into four clopidogrel metaboliser phenotypes according to the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for *CYP2C19*

genotype and clopidogrel therapy[4], namely extensive metabolisers - EMs (**1/*1*), ultra-rapid metabolisers - UMs (**1/*17*, **17/*17*), intermediate metabolisers - IMs (**1/*2*, **2/*17*) or poor metabolisers - PMs (**2/*2*). The caring cardiologists were informed of the genotype and phenotype results.

Comparison of CYP2C19 polymorphisms in populations bordering the Mediterranean Sea

The observed frequencies of the *CYP2C19 *2* allele and **2/*2* genotype in the Maltese patient cohort on clopidogrel were compared to fourteen populations bordering the Mediterranean Sea, namely Albanian, Bosnian, Croatian, Egyptian, Greek, Israeli, Lebanese, Moroccan, Slovenian, Southern French, Southern Italian, Southern Spanish, Tunisian and Turkish populations.

Statistical analysis

IBM SPSS Statistics 24 was used for statistical analysis. Observed and expected *CYP2C19* genotype frequencies were compared using the Hardy-Weinberg (H-W) equilibrium calculation. The Fisher's exact test was used to determine whether the observed data supports the null hypothesis that the cohort is in H-W equilibrium by adopting a 0.05 level of significance. Observed proportions of the *CYP2C19 *2* allele and **2/*2* genotype in the study cohort were compared to the fourteen populations bordering the Mediterranean Sea using the difference of two proportions z-test. A p-value less than 0.05 indicates that the proportions differ significantly, while a p-value greater than 0.05 indicates a non-significant (NS) difference, hence comparable proportions.

Results

Two hundred and forty-four (29%) Maltese patients on clopidogrel (75% male, mean age 65.43 ±1.24 years, all Caucasian, 45% undergoing PCI following admission with acute coronary syndrome) out of the total 843 Maltese and non-Maltese patients who underwent PCI from January to December 2014 were genotyped for the *CYP2C19 *2* and **17* alleles.

*CYP2C19 *1*, **2* and **17* allele frequencies were 72.3%, 12.3% and 15.4% respectively. *CYP2C19* genotype distribution of the 244 patients was **1/*1* (52.1%), **1/*17 (*22.5%), **1/*2* (18.0%), **2/*17* (6.6%) and **17/*17* (0.8%). No patients were genotyped as **2/*2*. Since there was a discrepancy between the observed frequencies and the corresponding expected frequencies, particularly for the **2/*17*, **2/*2* and **17/*17* genotypes, the Fisher's exact p-value obtained (0.051) is very close to the 0.05 threshold for H-W equilibrium (Table 1).

Table 1*: Observed and expected CYP2C19 genotypes (N=244)*

CYP2C19 **genotype**	**Observed number (%)**	**Expected number (%)** *H-W*	**X^2; p-value** *(Fisher's exact test)* *H-W*
**1/*1*	127 (52.1)	127.5 (52.3)	$X^2(5) = 11.04$; p=0.051
**1/*17*	55 (22.5)	54.3 (22.3)	
**17/*17*	2 (0.8)	5.8 (2.4)	
**1/*2*	44 (18.0)	43.4 (17.8)	
**2/*17*	16 (6.6)	9.3 (3.8)	
**2/*2*	0 (0)	3.7 (1.5)	

H-W: Hardy-Weinberg

***Table 2**: Distribution of CYP2C19 *2 allele and *2/*2 genotype: Maltese cohort compared to other populations bordering the Mediterranean Sea*

Population	Number of patients (number of alleles)	Frequency % (*p*-value)	Frequency % (*p*-value)
		*CYP2C19 *2*	*CYP2C19 *2/*2*
Maltese *Present study*	**244 (488)**	**12.3**	**0**
Albanian[8]	40 (80)	20.0 (NS)	2.5 **(S)**
Bosnian[9]	77 (154)	16.9 (NS)	2.6 **(S)**
Croatian[10]	200 (400)	15.0 (NS)	3.0 **(S)**
Egyptian[11]	247 (494)	10.9 (NS)	0.8 (NS)
Greek[12]	283 (566)	13.1 (NS)	2.1 **(S)**
Israeli[13]	140 (280)	15.0 (NS)	2.9 **(S)**
Lebanese[14]	161 (322)	13.4 (NS)	3.1 **(S)**
Moroccan[15]	290 (580)	11.4 (NS)	0.3 (NS)
Slovenian[16]	129 (258)	15.9 (NS)	0.8 (NS)
Southern French[17] *(Marseille, Nimes)*	213 (426)	12.0 (NS)	1.0 (NS)
Southern Italian[18] *(Messina)*	360 (720)	11.1 (NS)	1.7 **(S)**
Southern Spanish[19] *(Valencia)*	362 (724)	13.1 (NS)	1.9 **(S)**
Tunisian[20]	100 (200)	11.5 (NS)	0 (NS)
Turkish[21]	404 (808)	12.0 (NS)	1.0 (NS)

S – significant; NS - not significant

When classifying the patients according to metaboliser phenotype relative to clopidogrel, 52.1% of the patients were EMs, 24.6% were IMs, 23.4% were UMs and no patients were PMs.

Frequencies of the **2* allele ranged from 10.9% in Egyptians to 20% in Albanians (Maltese patients 12.3%). Prevalence of the **2* allele in the Maltese cohort is comparable (NS) to all fourteen populations bordering the Mediterranean Sea. Frequencies of the **2/*2* genotype ranged from 0% in Tunisians to 3.1% in Lebanese (Maltese patients 0%). Prevalence of the **2/*2* genotype in the Maltese patient cohort is comparable (NS) to six populations bordering the Mediterranean Sea, namely Egyptian, Moroccan, Southern French, Slovenian, Turkish and Tunisian populations (Table 2).

Discussion

This is the first report on the frequency of *CYP2C19 *2* and **17* genetic polymorphisms in Maltese patients on clopidogrel therapy.

The frequency of the *CYP2C19 *2* allele in this cohort of Maltese patients taking clopidogrel (12.3%) is lower than the reported prevalence in healthy Maltese volunteers (20%)[7] and in Europeans and Africans (18%).[22] The **2* allele frequency in the patient cohort studied is comparable to the fourteen populations bordering the Mediterranean Sea included in the comparison since no statistically significant difference was observed.

The reported prevalence of the *CYP2C19 *17* allele in healthy Maltese volunteers (26%)[7] and in Europeans and Africans (22.4% and 23.5% respectively)[22] is higher than the frequency observed in this Maltese patient cohort (15.4%). The prevalence of the *CYP2C19 *17* allele was studied in three populations bordering the Mediterranean Sea, namely Southern French[17], Southern Spanish[19] and Greek[23], with a higher observed frequency (20%) compared to the Maltese cohort (15.4%). However, the difference was not statistically significant for all three populations.

Prevalence of *CYP2C19* PMs is reported to be between 1 and 7% in Caucasians and Africans.[4,24,25] In Europe, a north-south gradient, with a decreased prevalence of PMs in Southern Europe, has been observed.[21] No patients in this cohort were genotyped as homozygous for the *CYP2C19 *2* allele and the frequency of the **2/*2* genotype was comparable to only six of the fourteen populations bordering the Mediterranean Sea included in the comparison.

Twenty-five percent of this Maltese patient cohort was genotyped as heterozygous for the *CYP2C19 *2* allele and phenotyped as IMs, while 23% of the patients were phenotyped as UMs. These findings have relevant clinical implications vis-à-vis clopidogrel since these patients are at an increased risk of unwanted outcomes due to compromised clopidogrel activity.

The *CYP2C19 *2* allele is clinically important with respect to clopidogrel and has been associated with reduced formation of active metabolites and higher on-clopidogrel platelet reactivity (PR), leading to increased risk of adverse cardiovascular events in IMs and PMs compared to EMs.[26-29] The strongest association is reported in patients with acute coronary syndrome undergoing PCI with stent placement, where carriers of the **2* allele are at higher risk of stent thrombosis compared to non-carriers.[27,30,31] According to the CPIC guidelines, an alternative $P2Y_{12}$-receptor inhibitor, such as ticagrelor or prasugrel, should be considered in carriers of the **2* allele (25% in this patient cohort) provided there is no contra-indication.[5]

There are mixed results on the clinical relevance of the *CYP2C19 *17* allele with respect to clopidogrel (23% in this patient cohort), where some studies reported lower on-clopidogrel PR, enhanced response to clopidogrel and increased risk of bleeding, while other studies reported no effect of this allele on clinical outcomes.[32-35] The CPIC guidelines recommend standard dosage of clopidogrel in UMs.[5]

The UM phenotype is clinically relevant for other drugs where *CYP2C19* genetic polymorphisms are implicated in variability of interpatient response, such as for proton pump inhibitors (PPIs), tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs) and voriconazole. Further study is recommended to assess the prevalence and clinical implications of *CYP2C19* genetic polymorphisms in patients taking these drugs.

For PPIs, UMs have shown less effective gastric acid suppression and decreased *Helicobacter pylori* eradication rates, hence an increase in dose is recommended.[36] For TCAs (amitriptyline, clomipramine, doxepin, imipramine, trimipramine) and SSRIs (citalopram, escitalopram, sertraline), the UM phenotype is associated with increased metabolism and risk of sub-optimal response, hence the CPIC guidelines suggest an alternative drug not metabolised by CYP2C19.[37,38] With respect to voriconazole, UMs are less likely to attain therapeutic concentrations with standard dosing and selection of an alternative agent not dependent on CYP2C19 metabolism as primary therapy is recommended.[39]

Conclusion

This study reports the frequency of *CYP2C19 *2* and **17* variant alleles in a cohort of Maltese patients on clopidogrel therapy. The high percentage of patients phenotyped as IMs (25%) indicates that CYP2C19 pharmacogenetic testing could be used to guide clinicians in the individualisation of antiplatelet therapy. This study serves as an example of pharmacogenetic testing to achieve precision medicine.

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