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Association of *CYP2B6* (*G15631T*) polymorphism with Clopidogrel resistance and genetic predisposition to Acute Coronary Syndromes (ACSs) in Morocco

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ABSTRACT

Despite its well-known efficacy as an antiplatelet agent, inter-patient variability of response to Clopidogrel has been documented among patients from different ethnic origins. As polymorphisms in drug metabolizing enzymes contribute to wide heterogeneity in drug pharmacokinetics, response and toxicity, we have tried to investigate the potential association of *CYP2B6* (*G15631T*) polymorphism as one of the less well studied human cytochrome P450 genes, with inter-patient variability of response to Clopidogrel, and to examine its effect on genetic susceptibility to the disease, among a sample of ACS Moroccan patients compared to healthy controls.

We found a higher frequency of the mutant allele among resistant patients compared to non-resistant ones (70% and 56% respectively) ($P = 0.04$). The allelic and genotypic distribution of the polymorphism showed no big difference between ACS cases and healthy controls ($P > 0.05$).

Genetic profiling before adoption of a therapeutic strategy appears to be very useful to identify patients at increased risk for developing cardiovascular events, and may help to choose the most reliable antiplatelet strategy for each patient. The current findings might be preliminary but promising in such field not yet well developed, especially in Moroccan population.

1. Introduction

Clopidogrel is a second-generation thienopyridine platelet inhibitor, used in the management of patients with Coronary Artery Disease (CAD), peripheral vascular disease and stroke. The prodrug requires hepatic bio-transformation steps involving Cytochrome P450 system enzymes, in order to produce the active metabolite that will selectively and irreversibly bind the P2Y12 platelet receptor and generate the desired inhibitory platelet aggregation effect (Idrissi Hassani et al., 2017). Despite the general efficacy of Clopidogrel as antiplatelet agent, interpatient variability in metabolite levels, platelet inhibition, and clinical response has been documented among populations according to ethnicity and genotypic backgrounds (Seripa et al., 2010; Ingelman-Sundberg et al., 1999). About 16–50% of patients under Clopidogrel show High on-Treatment Platelet Reactivity (HTPR), indicating that a portion of P2Y12 receptors are not blocked, despite Clopidogrel treatment (Mallouk et al., 2012). Besides environmental conditions, polymorphisms in genes encoding the hepatic CYP P450 metabolizing enzymes were reported as Clopidogrel resistance biomarkers. Thus, the expression and function of these CYP enzymes definitely have a pivotal role and are associated with the efficiency of Clopidogrel in clinical settings (Yi et al., 2016). In the present study, we tried to access the part of Cyp2B6 enzyme in modulating response to Clopidogrel in Moroccan ACS patients. It is one of the Cytochrome P450 superfamily of enzymes that is mainly expressed in liver, kidney, intestine and lung (Gonzalez et al., 1992; Gervot et al., 1999; Ekins and Wrighton, 1999); it is primarily involved in drugs and xenobiotic biotransformation. In contrast to other mammalian species, CYP2B6 is the only functional enzyme of its subfamily in humans (Nelson et al., 2004). Expression of CYP2B6 is highly variable between and within individuals, owing to many factors including genetics and non-genetic elements, inducibility and inhibition by many compounds (Zanger and Klein, 2013).

The enzyme contributes to 19.4% of the transformation of Clopidogrel to the 2-oxo-Clopidogrel reaction, and 32.9% of the transformation of this intermediate metabolite to the pharmacologically active form, responsible for the inhibitory effect of platelet

Abbreviations: ACSs, Acute Coronary Syndromes; SNPs, Single Nucleotide Polymorphisms; PCR, Polymerase Chain Reaction; RFLP, Restriction Fragment Length Polymorphism

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aggregation (Fig. 5). Polymorphisms in CYP2B6 gene have been reported as interesting predictors of pharmacokinetics and drugs heterogeneity of response (Zanger et al., 2007; Telenti and Zanger, 2008; Rakhmanina and van den Anker, 2010); they were shown to act on several levels of gene expression (mRNA transcription and expression, splice variants generation) and production of proteins; they have also been reported as responsible for complex substrate-dependent and substrate-independent effects (Zanger and Klein, 2013; Zanger et al., 2007; Ekins and Wrighton, 1999; Turpeinen et al., 2006; Hodgson and Rose, 2007; Wang and Tompkins, 2008; Mo et al., 2009; Turpeinen and Zanger, 2012).

Mapped on the long arm of chromosome 19 (19q13.2) (Hoffman et al., 2001), CYP2B6 is one of the most polymorphic CYP genes in humans. Some variants appear to act in haplotypes and affect several functional levels simultaneously. The most common functionally deficient allele is the G15631T being part of the haplotype CYP2B6*6 [Q172H, K262R]. It affects splicing process and lead to lower enzyme expression in liver (Zanger and Klein, 2013).

CYP2B6 enzyme participates in the metabolism of numerous drugs (Ekins and Wrighton, 1999; Lang et al., 2001), however, results about its association with the inter-individual variability of response to Clopidogrel remains non conclusive and divergent. The purpose of our study was therefore to investigate, for the first time in Morocco, the potential impact of G15631T polymorphism of CYP2B6 gene on inter-patient heterogeneity of response to Clopidogrel, and to examine its effect on genetic susceptibility to the disease, among a sample of ACS patients compared to healthy controls.

2. Materiel and methods

2.1. Study population

77 ACS patients from the Department of Cardiology, University Hospital Center Hassan II, Fes, Morocco Vs 93 healthy controls with no history or actual symptoms of ACS or any other pathology were recruited. Clinical data concerning risk factors, biological and demographic parameters, and the platelet test results were collected, and an informed consent was signed by all participants before entering the study. Details about the Verify Now platelet test principle and protocol, and also inclusion/exclusion patients' criteria were previously published by our team (Hassani Idrissi et al., 2017).

2.2. DNA extraction and G15631T CYP2B6 genotyping

Collection of 4 ml whole blood samples from all participants was performed and genomic DNA was extracted from blood leucocytes using the salting-out method as previously described by Miller et al. (Miller et al., 1988). We evaluated DNA quality and quantity using the Nanovue-plus spectrophotometer. Polymerase Chain Reaction (PCR) followed by Restriction Fragment Length Polymorphism method (RFLP) was performed to determine every participant's *G15631T CYP2B6* genotype.

2.3. Statistical analysis

Statistical analysis was performed on SPSS 21.0 software. Both groups of patients and healthy controls were tested for the Hardy-Weinberg-Equilibrium (HWE) using Chi square test (X2) and a significance at P value > 0.05. Odds ratio (OR) were calculated to evaluate the association between genotypes distribution and Clopidogrel resistance, with a confidence interval (CI) of 95% and a significance at P value < 0.05.

Table 1
HWE for the distribution of CYP2B6 (G15631T) among cases and controls.

Cases		Controls	
P value	X2	P value	X2
0.1[a]	4.5	0.0535*	5.86

HWE: Hardy-Weinberg Equilibrium; X2: Chi square test.
[a] Statistically significant.

3. Results

3.1. Study population

Both cases and controls groups were in HWE (Hardy-Weinberg Equilibrium) for the distribution of *G15631T CYP2B6* genotypes (Table 1). Demographic data of all participants are represented in Fig. 1. ACS patients were aged 62 ± 9.22 and 56 ± 9.7 years respectively for resistant and non-resistant ones; for healthy controls, they were 41.38 ± 13.4 years of age.

3.2. Clinical risk factors versus CYP2B6 (G15631T) allelic and genotypic distribution

Fig. 2 shows the correlation of the pathology traditional risk factors with the *CYP2B6* (*G15631T*) allelic distribution: patients having personal and/or familial ACD, diabetes, high blood pressure, smoking and/or dyslipidemia presented a higher frequency of the mutant allele compared to those with no one of these risk factors. However, only association with 'Diabetes' was statistically significant (P = 0.04).

3.3. ACS sub-groups versus CYP2B6 (G15631T) allelic and genotypic distribution

The allelic distribution of *CYP2B6* (*G15631T*) was correlated to the ACS sub-groups, as shown in Fig. 3. The mutant allele was slightly more frequent among ACS ST(−) patients compared to ST(+) ones: 55% of ST(−) patients carried out the mutant allele (55.8% of them under the heterozygous form and 37.2% homozygous mutant) Vs 44% of ST(+) patients that carried out the mutant allele, most of them (70.8%) under the heterozygous form (P = 0.02) (Table 2 and Fig. 3).

3.4. VerifyNow test results versus CYP2B6 (G15631T) allelic and genotypic distribution

Allelic and genotypic distribution of the polymorphism was also correlated to the VerifyNow test results (resistance profile of the patients) (Table 3 and Fig. 4). The mutant allele was more frequent among resistant patients (70%) compared to non-resistant ones (56%) (P = 0.04).

3.5. CYP2B6 (G15631T) allelic and genotypic distribution among cases versus controls

To access the association of the *CYP2B6* (*G15631T*) with ACSs risk of occurrence, we have compared the allelic and genotypic distribution of the polymorphism among ACS cases versus healthy controls. Our results show that the mutant genotype TT was slightly more frequent among cases than controls (25.97% Vs 21.5% respectively). The mutant allele 15631T was also more represented in ACS cases compared to healthy subjects (56.49% Vs 52.69% respectively). No statistical association was found under the three genetic transmission models (P > 0.05) (Table 4).