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Association of CYP2C19*2 polymorphism with clopidogrel resistance among patients with high cardiovascular risk in Northeastern Mexico

*Asociación del polimorfismo CYP2C19*2 con resistencia a clopidogrel en pacientes con alto riesgo cardiovascular en el noreste de México*

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Abstract

Objective: *Oral antiplatelet drugs are a key to modern pharmacotherapy in cardiovascular atherothrombotic diseases. Clopidogrel (CLO) constitutes the main preventive treatment of atherothrombosis. However, a considerable inter-individual variation in CLO response has been documented, resulting in suboptimal therapy and an increased risk of recurrent adverse effects in some patients. The enzyme CYP2C19 has been reported to be the CYP isoform that activates CLO to its active metabolite. Several single nucleotide polymorphisms in the CYP2C19 gene have been identified as strong predictors of CLO-impaired pharmacological response. At least 16 variants have been associated with changes in CYP2C19 activity.* **Materials and Methods:** *The following research was composed of a total of 102 subjects with high cardiovascular risk in the northeast of Mexico, with a maintenance dose of 75 mg of CLO per day. The platelet reactivity was measured with VerifyNow P2Y12 assay, while the presence of CYP2C19*2 was identified by real-time polymerase chain reaction.* **Results:** *Patients were categorized by CYP2C19 metabolizer status based on *2 genotypes using the common consensus star allele nomenclature as normal metabolizer (G/G), intermediate metabolizer (G/A), and poor metabolizer (A/A), respectively. The phenotype frequency for CYP2C19*2 was 74.5% (G/G), 21.6% (G/A), and 3.9% (A/A). The subjects with the A allele presented*

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*≥235 P2Y12 reaction unit levels, classifying them how poor metabolizer. The prevalence of reduced CLO effectiveness was associated with the presence of CYP2C19*2 polymorphism among Mexican patients.* **Conclusion:** *The presence of the CYP2C19*2 allele is related to resistance to the antiplatelet effect of CLO (p = 0.003).*

Key words: *CYP2C19*2. Clopidogrel. High cardiovascular risk. Polymorphism. Resistance. Allele.*

Resumen

Objetivo: *Los antiplaquetarios orales son clave en la farmacoterapia moderna de las enfermedades aterotrombóticas cardiovasculares. Clopidogrel (CLO) constituye el principal tratamiento preventivo de aterotrombosis (AT). Sin embargo, se ha documentado una considerable variación interindividual en la respuesta a CLO, lo que da como resultado una terapia subóptima y mayor riesgo de efectos adversos en algunos pacientes. La enzima CYP2C19 es la isoforma CYP que activa CLO a su metabolito activo. Se han identificado varios polimorfismos de un solo nucleótido en el gen CYP2C19 como fuertes predictores de respuesta farmacológica alterada a CLO. Al menos 16 variantes se han asociado con cambios en la actividad de CYP2C19.* **Método:** *Se reclutaron un total de 102 sujetos con alto riesgo cardiovascular del noreste de México, con dosis de mantenimiento de 75 mg de CLO/día. La reactividad plaquetaria se midió con el ensayo* Verify Now P2Y12*, la presencia de CYP2C19*2 se identificó mediante polymerase chain reaction en tiempo real.* **Resultado:** *Los pacientes fueron clasificados por el estado metabolizador CYP2C19*2 utilizando nomenclatura consenso, como metabolizador normal (G/G), metabolizador intermedio (G/A) y metabolizador pobre (A/A), respectivamente. La frecuencia del fenotipo para CYP2C19*2 fue 74.5% (G/G), 21.6% (G/A) y 3.9% (A/A). Los sujetos con alelo A presentaron ≥235 niveles P2Y12* reaction unit*, clasificándolos como metabolizadores deficientes. La prevalencia de eficacia reducida a CLO se asoció con la presencia del polimorfismo CYP2C19*2 en pacientes mexicanos.* **Conclusiones:** *La presencia del alelo CYP2C19*2 se relaciona con resistencia al efecto antiagregante plaquetario del CLO (p = 0.003).*

Palabras clave: *CYP2C19*2. Clopidogrel. Alto riesgo cardiovascular. Polimorfismo. Resistencia. Alelo.*

Introduction

Atherothrombosis (AT), the leading cause of mortality in the Western world, is defined as an atherosclerotic plaque disruption with superimposed thrombosis[1]. AT is the main cause of ischemic heart disease, ischemic stroke, and peripheral arterial disease[2]. AT may also occur in patients with arrhythmias, such as atrial fibrillation, and in those who have undergone percutaneous or surgical revascularization procedures.

Human platelets are involved in normal and pathological hemostasis. During the AT process, platelets act as essential mediators of several thrombotic and inflammatory events. Clopidogrel (CLO), an antiplatelet drug, inhibits the P2Y12 receptor, which is responsible for mediating the platelet aggregation by adenosine diphosphate[3].

Inhibition of platelet aggregation with drugs such as CLO and acetylsalicylic acid constitutes the main preventive treatment of AT. CLO is one of the most commonly used therapeutic drugs for the secondary prevention of cardiovascular events in patients with acute coronary syndromes[4]. However, considerable inter-individual variation in CLO response has been documented, resulting in suboptimal therapy and an increased risk of recurrent adverse effects in some patients[5].

CLO is a prodrug that requires conversion to an active metabolite by hepatic cytochromes p450 (CYP) to accomplish an antiplatelet effect. The enzyme CYP2C19 has been reported to be the CYP isoform that activates CLO to its active metabolite[6]. Several single nucleotide polymorphisms (SNPs) in the CYP2C19 gene (OMIM124020) have been identified as strong predictors of CLO-diminished pharmacological response[4]. Furthermore, a non-satisfactory effect has been observed in 20% of patients with CLO[7]. CYP2C19 is a liver enzyme that metabolizes a broad range of xenobiotics with clinical importance, such as benzodiazepines, antidepressants, mephenytoin, some proton pump inhibitors, and CLO[8].

It has been demonstrated that genetic variants in the CYP2C19 gene affect the pharmacological and clinical response to the standard 75 mg daily maintenance dose of CLO. Furthermore, at least 16 variants have been associated with changes in CYP2C19 enzymatic activity and seven specific variants result in an inactive enzyme. CYP2C19*1 is the wild-type allele, while CYP2C19*2 (rs4244285) is the null allele (rs4244285; 681G>A) and CYP2C19*3 (rs4986893; 636G>A) results in an inactive enzyme[9]. Recently, the loss-of-function CYP2C19*2 allele has been associated

with decreased activation of CLO, poor antiaggregant effect, and increased cardiovascular events[10]. In high-risk vascular patients, the CYP2C19*2 polymorphism is a strong predictor of adverse cardiovascular events and particularly of stent thrombosis[10].

Aim

The aim is to determine the prevalence of resistance to the antiplatelet effect of CLO and its relationship with the presence of the genetic polymorphism CYP2C19*2 (rs4244285), in a population of Mexican patients with high cardiovascular risk in the northeast of Mexico.

Materials and Methods

Study population

A total of 102 patients from Northeastern Mexico diagnosed with high risk of cardiovascular disease who attended the Cardiology Service of the *Hospital Universitario "Dr. José Eleuterio González"* (UANL) and the *"Institute for Cardiac Arrhythmias and Cardiovascular Diagnosis, S. C."* of Monterrey, Mexico, were included. The individuals were recruited from January to August 2015, with a mean age of 68 (35-91) years. The inclusion criteria were as follows: males and females over 18 years, diagnosed with high risk of cardiovascular disease, and who have received treatment with CLO at a maintenance dose of at least 75 mg daily during the last nine consecutive days before the inclusion to the study. Exclusion criteria were the following: pregnant women, people who are taking prasugrel or ticlopidine, people who are taking proton pump inhibitors, or people who have stopped treatment with proton pump inhibitors for 1-7 days before starting treatment with CLO and history of CLO allergy (Fig. 1). This study was approved by the Ethics Committee of UANL" and registered under the resolution number C414-004. Informed consent was obtained from all patients.

Genotyping

Blood samples were collected in tubes containing EDTA, and DNA was extracted using Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA). DNA quality was assessed according to standard spectrophotometrically procedures using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Genomic DNA was genotyped for the polymorphism CYP2C19*2 (681G>A, dbSNP rs4244285) using real-time polymerase chain reaction (PCR) (quantitative polymerase chain reaction [qPCR]) and Taqman® probes (Applied Biosystems; Thermo Fisher Scientific, Inc., HS) according to the manufacturer's protocol. StepOne System (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used to perform the real-time PCR, and data were analyzed in the SPSS v20 software (IBM Corp., Armonk, NY, USA). The call rate was >99% in the qPCR experiment.

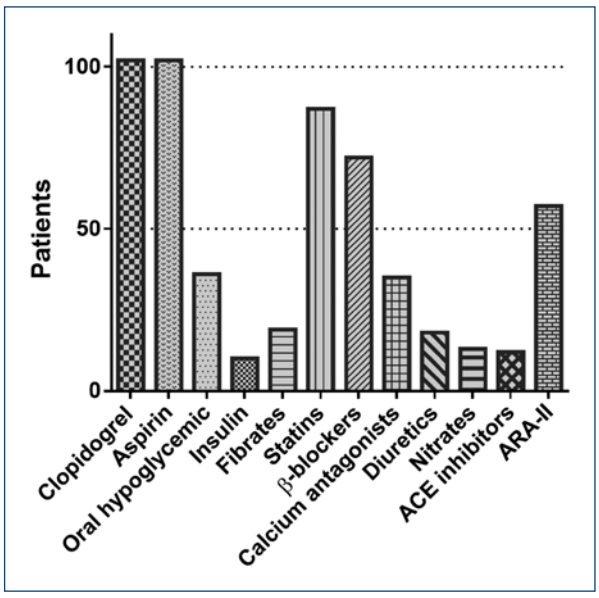

Figure 1. Medications used by the study population. ACE: angiotensin-converting enzyme; ARA-II: angiotensin II receptor antagonists.

Platelet aggregometry

P2Y12 reaction unit (PRU) levels were measured utilizing the VerifyNow P2Y12 assay (Accumetrics, San Diego, CA, USA). This method was used to assay patient's platelet reactivity to antiplatelet medication with CLO. Platelet antiaggregation was determined after a maintenance dose of at least 75 mg/day for a period of not < 9 days.

Statistics

Kolmogorov–Smirnov test was used to determine the distribution of the numerical variables (normal or not normal), and a parametric test was performed with Student's *t*-test for comparison of means. The level of significance was established at a value of $p < 0.05$.

Table 1. Population characteristics

Characteristics (n = 102)	n (%)
Male	64 (62.7)
Female	38 (37.3)
Age mean (range)	68 (35-91)
Obesity	30 (29.4)
Smokers	29 (28.4)
Dyslipidemia	55 (53.9)
Hypertension	59 (57.8)
Diabetes mellitus	36 (35.3)
Acute myocardial infarction	28 (27.4)
Unstable angina	10 (9.8)
Stable angina	42 (41.1)
Transient ischemic attack	5 (4.9)
Supraventricular tachycardia	11 (10.7)
Neurocardiogenic syncope	8 (7.8)
Heart block	4 (3.9)
X syndrome	3 (2.9)
Hypothyroidism	5 (4.9)
Prostatic hyperplasia	5 (4.9)
Dyspepsia	11 (10.7)
Cancer	4 (3.9)

n: sample size; %: percentage.

Table 2. CYP2C19*2 frequency

Allele	Phenotype	Frequency n (%)
GG	Normal	76 (74.5)
GA	Intermediate	22 (21.6)
AA	Poor	4 (3.9)

n: sample size; %: percentage.

Analysis was conducted using the Statistical Package SPSS v20 Software (IBM Corp., Armonk, NY, USA) and GraphPad Prism v6.0 (La Jolla, CA, USA).

Results

One hundred and two patients were included in the study and their demographic characteristics are summarized in Table 1. The mean age was 68 years, and 62.7 % were male; the risk factors among our population were: 57.8 % showed hypertension, 35.3 % showed diabetes mellitus, and 53.9% showed dyslipidemias at the sample collection moment.

Genotype

A total of 102 patients from Northeast Mexico diagnosed with high risk of cardiovascular disease were genotyped for CYP2C19*2 (681G>A, dbSNP rs4244285). The genotype frequencies obtained were 74.5% (G/G), 21.6% (G/A), and 3.9% (A/A). Patients were categorized by CYP2C19 metabolizer status based on *2 genotypes using the common consensus star allele nomenclature as normal metabolizer (G/G), intermediate metabolizer (G/A), and poor metabolizer (A/A), respectively (Table 2)[8].

CLO response

According to the aggregometry results, the patients were classified into three groups. Group 1: 47 patients (46%) were classified as good responders, with a PRU level ≤ 194 evidencing a > 40 % block of the P2Y12 receptor; Group 2: 28 patients (27.5%) with a PRU level of 194-235 (20 to 40% of P2Y12 receptor blocking), classified as intermedium responders; and Group 3: 27 patients (26.5%) with ≥ 235 PRU < 20% of P2Y12 receptor blocking) as poor responders.

Genotype/phenotype

Most of the patients with PRU levels < 235 presented the G/G homozygous genotype for the CYP2C19*1 wild-type isoform. In contrast, patients who presented ≥ 235 PRU levels presented the SNP CYP2C19*2 in at least one allele (G/A or A/A). This finding denotes that CYP2C19-reduced enzymatic function shows a significant difference (p = 0.003) when compared to the enzymatic activity of CYP2C19 wild-type isoform in CLO activation (Fig. 2).

Discussion

CLO has very special and complex pharmacodynamic characteristics, including first pass hepatic metabolism, variation in absorption, drug–drug metabolism, and SNPs in the enzymes responsible for CLO metabolism. As a result, a very variable and unpredictable inter-individual response to this therapy is observed

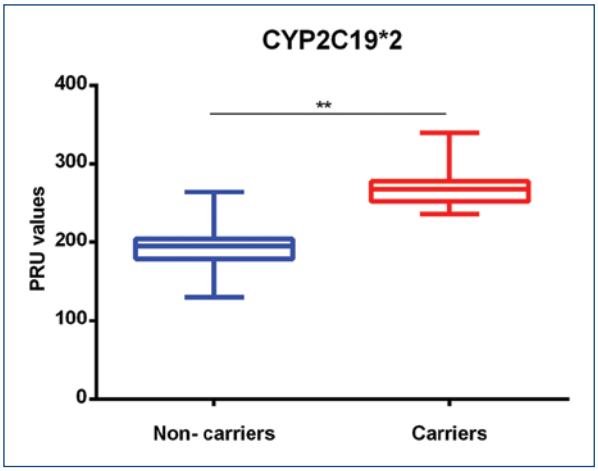

Figure 2. Relationship between the presence of the reduced function allele and platelet antiaggregation (**p = 0.003). PRU (P2Y12 reaction units), non-carriers (G/G/), carriers (G/A, A/A). Error bars: 95% CI (confidence interval).

favoring the treatment failure. The estimated resistance to CLO fluctuates between 4 and 30%[11]. In our specific population, the biomarkers necessary for the measurement of resistance to this therapy have not been explored.

This study describes the CYP2C19*2 (681G > A) presence in a group of patients with high cardiovascular risk from the northeast area of Mexico with genotype frequency of 74.5% for G/G, 21.6% for G/A, and 3.9% for A/A. The allele CYP2C19*2 was present in 25.5% of the patients, with a 3.9% of homozygous genotype among patients. This finding is in agreement with a previous study performed in a sample of 51 Mexican mestizo patients from the central region of Mexico with a 17% genetic frequency and 3.9% of the patients having A/A in a homozygous fashion[12].

CYP2C19*2 accounts for 75-85% of the alleles responsible for the poor metabolizer phenotype in Caucasians and East Asians[13]. This allele is significantly more frequent in East Asian populations (14-39%) than among Caucasians (8-16%) and Africans (18-25%)[13]. However, in Koreans, the frequency of CYP2C19*2 is reported to be 28%, similar to 27% in the Japanese population, but showing a large difference from the Chinese population[13]. CYP2C19*2 transmission pattern has been observed how an autosomal recessive and autosomal codominant traits[14,15].

According to the results of the aggregometry, 46% of the patients were classified as good responders, 27.5% showed of 20-40% of the P2Y12 blocking effect, and 26.5% were classified as poor responders. This observation agrees with the reported by Viveros et al., 2016, where 40% of the patients were good responders while 60% of the patients were non-responders[12]. Most of the patients (98.6%) with the G/G genotype presented PRU levels < 234 were classified as responders. In contrast, carriers of at least one A allele (G/A and A/A) showed a PRU ≥ 235 indicating < 20% blocking effect of the P2Y12 and were classified as resistant or non-responder patients. Discrepancies in response to drugs are partially due to polymorphisms in genes involved in drug metabolism and transport. Furthermore, it has been shown that the frequency, pattern, and impact of these polymorphisms vary among populations[16,17].

The principal problem that physicians face when prescribing antiplatelet agents is the lack of a standardized method for the antiplatelet function. In addition, a cut-point for this function to provide a clear patient classification as responder or non-responder to CLO treatment has not been established[18].

The resistance to CLO can be classified as clinical or laboratory-based resistance. Clinical resistance could be defined when a cardiovascular event happens in an individual who is currently receiving the antiplatelet treatment. Meanwhile, the laboratory-based resistance is defined as the *in vitro* failure of the platelet block activity of an individual who is currently under CLO treatment[17].

Several laboratory methods have been proposed for the diagnosis of CLO antiplatelet resistance, yet all of them present advantages and disadvantages[18]. The big variability reported in the levels of resistance to CLO is due to the absence of a unified definition of the current laboratory tests and to the heterogeneity in the different study groups and protocols.

The results observed in this cohort reflect the importance of both the genotype game of CYP2C19 and the platelet aggregation test as an important predictor to the response and resistance to CLO in patients with high cardiovascular risk.

The prevalence of reduced CLO effectiveness is associated with the presence of CYP2C19*2 polymorphism among patients. This finding denotes that CYP2C19-reduced enzymatic function show a significant difference (p = 0.003) when compared to the enzymatic activity of CYP2C19 wild-type isoform in CLO activation.

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Conflicts of interests

None.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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