Genetic polymorphism of the CYP2C19 gene in the Foshan area of Guangdong Province

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Abstract

Background: The CYP2C19 gene is highly polymorphic, and CYP2C19 is involved in the broad interindividual variability of the clinical efficacy of certain clinical medications, such as clopidogrel. However, data on the CYP2C19 genotype in the Chinese population of the Foshan area of Guangdong Province are scarce. The purpose of this study was to determine CYP2C19 genetic polymorphisms in patients in the Foshan area and to compare the CYP2C19 genotype frequencies in different populations to determine the allele distribution pattern to identify the most appropriate prescription.

Methods: The CYP2C19 gene was detected in 1231 patients on a gene chip platform, and the genotype frequencies of CYP2C19 in Foshan populations from different populations were compared.

Results: The frequencies of CYP2C19*1, *2 and *3 in the Foshan population were 63.89%, 30.46% and 5.65%, respectively. For the three metabolic types, the frequency associated with the rapid metabolism type (*1/*1) was 41.51 [95% confidence interval (CI) 40.11 to 42.91%]; that for the intermediate metabolism type (*1/*2, *1/*3) was 44.76% (95% CI 43.34 to 46.18and that for the slow metabolism type (*2/*2, *2/*3, *3/*3) was 13.73% (95% CI 12.75 to 14.71%). In the Foshan population, the frequencies of the CYP2C19 *2 and *3 alleles were similar to those previously reported for Chinese and other Asian populations.

Conclusion: Our study the genetic basis of CYP2C19 polymorphism in the Foshan population. Our results will potentially contribute to the improvement of pharmacotherapy effectiveness by providing personalized medicine for the Foshan population. Key words: Polymorphism; Genetic; Pharmacogenetics

Introduction

Coronary heart disease is one of the main causes of disability and death worldwide [1]. Clopidogrel, which blocks the platelet adenosine diphosphate (ADP) receptor pathway to inhibit platelet aggregation, is an antiplatelet drug that is usually used in the treatment of thromboembolic diseases such as coronary artery disease (CAD), ischaemic stroke or peripheral atherosclerotic diseases. [2] However, the pharmacodynamic efficacy of clopidogrel is often reduced because of interindividual variability. According to report, ~ 4–30% of patients appear nonresponsive or show low responsiveness to clopidogrel (clopidogrel resistance), which could increase the risk of major adverse cardiovascular events (MACE) [3].

Cytochrome P450 (CYP450) is an enzyme that is essential for the degradation and biosynthesis of medications, toxins, and endogenous substances [4]. In the last few years, many CYPP450 enzymes that participate in phase I reactions of drug metabolism, including CYP2D6, CYP1A2, CYP2C19, and CYP2C9, have been reported [5, 6]. CYP2C19 participates in the metabolism of approximately 10% of commonly prescribed medications, such as antidepressants, antipsychotics, clopidogrel, and proton pump inhibitors [7]. Genetic variations in CYP2C19, which is highly polymorphic, may affect the ability to metabolize the related drugs. Interindividual differences in enzyme activity divide the population into the extensive

metabolizer (EM), intermediate metabolizer (IM) and poor metabolizer (PM) phenotypes [8–9]. To date, at least 34 alleles of CYP2C19 have been identified. Among these alleles, CYP2C19*3 and CYP2C19*2 are the most frequent loss-of-function (LOF) alleles and are responsible for the reduced efficacy of clopidogrel and increased rate of cardiovascular events [10–12]. In March 2010, the US Food and Drug Administration (FDA) proposed a boxed warning about clopidogrel, which suggests that physicians might consider the use of replacement treatment strategies in individuals with PM genotypes [13]. The polymorphism of CYP2C19 varies greatly among regions and ethnic groups [14]. Multiple studies on the differences in the genotype frequencies of CYP2C19 among different populations have been performed to date.

However, few studies on CYP2C19 genetic polymorphisms and genotype frequencies have been conducted among populations in Guangdong Province. Our study aims to determine the CYP2C19 genetic polymorphisms among patients in Foshan City of Guangdong Province. We also compared the genotype frequencies of CYP2C19 in different populations.

Materials And Methods

Subjects

Our study was retrospective. The study was approved by the ethics committee of Nan Hai Hospital affiliated with Southern Medical University, and written informed consent was obtained from all participants (number: 20160106). We selected 1231 patients (ages 19–92 years) who visited Nan Hai Hospital affiliated with Southern Medical University between January 2016 and May 2018 for inclusion in this study. All subjects were Han Chinese individuals residing in the Foshan area of Guangdong Province with more than three generations of paternal ancestry of their ethnicity. Subjects with liver diseases or severe heart failure or kidney disease was excluded. All of the chosen subjects had undergone clopidogrel therapy and were unrelated.

DNA extraction

Venous blood samples were taken from each subject in 5 ml tubes containing ethylene diaminetetraacetic acid (EDTA). Genomic DNA was isolated using the MagPure Fast Blood DNA KF Kit (Magen, Chuangzhou, China) according to the manufacturer’s instructions.

DNA genotyping

Detection of the CYP2C9*1, CYP2C9*2 and CYP2C19*3 alleles was performed by using a commercially available kit (BaiO Technology Co, Ltd, Shanghai, China). In brief, 5 μl of sample DNA was mixed with 19 μl of CYP2C19 amplification solution 1 or 2 and 1 μl of reaction solution A provided in the kit. Each polymerase chain reaction (PCR) assay included positive and negative controls. Amplification was carried out with an ABI VeritiDx PCR system (Applied Biosystems, USA). The cycling conditions were as follows: 50 °C for 5 min, an initial denaturation step of 5 min at 94 °C, followed by 35 cycles of 94 °C for

25 s for denaturation, 48 °C for 40 s and 72 °C for 30 s, with a final extension step at 72 °C for 5 min. Then, the PCR products were transferred to a hybridization reaction chamber for hybridization reactions. Finally, BaioRBE-2.0 software was utilized to detect the CYP2C19 alleles. Positive and negative controls were included in each experiment.

Statistical analysis

Statistical analyses were performed with Excel and SPSS 19.0.

The gene counting method was used to calculate allele frequencies and genotype frequencies. A chi-square test was used to analyse the differences in the allele frequencies between our population and other populations. P values < 0.05 were considered significant. Hardy-Weinberg equilibrium was calculated for each allele using the chi-square test.

Results

CYP2C19 genotype and allele frequencies of all subjects

A total of 1231 individuals (range, 19–92 years old) were included in the study.

The frequencies of the CYP2C19*1, CYP2C19*2 and CYP2C19*3 alleles were 63.89%, 30.46% and 5.65%, respectively (Table 1). The allele frequency of CYP2C19*3 was lower than that of CYP2C19*2 by nearly one-fifth. Among the 1231 subjects, 511 (41.51%, 95% CI 40.11 to 42.91%) were wild-type homozygous; 478 (38.83%, 95% CI 37.44 to 40.22%) were heterozygous; and 108 (8.77%, 95% CI 7.96 to 9.58%) were mutant homozygous for CYP2C19*2 (Fig. 1). There were 73 (5.93%, 95% CI 5.26 to 6.60%) heterozygotes and 5 (0.41%, 95% CI 0.23 to 0.59%) mutant homozygotes for CYP2C19*3. The total 1231 subjects were divided into three phenotypes on the basis of genetic polymorphism. As shown in Fig. 1, there were 511 (41.51%, 95% CI 40.11 to 42.91%) patients with EM (CYP2C19*1/*1); 551 (44.76%, 95% CI 43.34 to 46.18%) patients with IM (CYP2C19*1/*2 or CYP2C19*1/*3); and 169 patients (13.73%, 95% CI 12.75 to 14.71%) with PM (CYP2C19*2/*2, CYP2C19*3/*3, or CYP2C19*2/*3). The CYP2C19 allele and genotype frequencies in all subjects were in Hardy-Weinberg equilibrium ($\chi 2 = 8.00$, $P > 0.05$).

Table 1
The distribution of genetic polymorphisms

Gene	Allele	Allele frequency [n(%)]
CYP2C19	*1	2467 (63.91)
	*2	1186 (30.73)
	*3	207 (5.36)

CYP2C19 allele frequency in our study compared with previous reports

We further compared the CYP2C19 allele frequencies between our data and previously published studies from different countries and ethnic groups (Table 2). Our results showed that the CYP2C19*1 allele frequency in our population was lower than those in other Caucasian and Asian populations. In addition, the CYP2C19*2 and CYP2C19*3 allele frequencies in the subjects of our study were higher than those in studies of Caucasians.

Table 2
CYP2C19 allele frequencies in our study and in previous studies.

Population	Number	CYP2C19 allele frequency (%)			Reference
		*1	*2	*3	
Asian					
Foshan	1231	63.89	30.46	5.65	Present study
Hakka	6686	64.33	31.06	4.61	18
Chinese Dai	193	66	30	3	19
Chinese Li	100	74	25	1	20
Chinese Uighur	214	65.42	32.48	2.1	21
Chinese Hui	164	45.43	49.39	5.18	21
Chinese Mongolian	158	54.11	41.46	4.43	21
Japanese	140	53.9	35	11.1	22
Korean	103	67	21	12	23
Vietnamese	90	62	24	14	23
Thai	1051	63	27	1	24
Caucasian					
Swedish	83	85	14	0.1	25
Russian	290	88.3	11.4	0.3	26
Italian	360	88.9	11.1	0	27
Bolivian	778	92.1	7.8	0.1	28
Faroese	312	97.1	2.9	0	29
African					
Tanzanian	251	81.5	17.9	0.6	30
Ethiopian	114	84	14	2	31
Zimbabwean	84	86.9	13.1	0	32

Discussion

CYP2C19 participates in the metabolization of approximately 10% of commonly prescribed medications, such as antidepressants, antipsychotics, clopidogrel, and proton pump inhibitors [7]. The genetic polymorphism of CYP2C19 plays an important role in related drug metabolism and may lead to inter-individual and inter-ethnic variation in patient responsiveness and adverse drug reactions [15]. Currently, at least 34 alleles of CYP2C19 have been identified. Among these alleles, the CYP2C19*2 and CYP2C19*3 alleles are responsible for the reduced activation of metabolized drugs and an increased rate of serious adverse effects that undermine clinical therapeutics [16–17]. Several studies have analysed genetic polymorphisms of the CYP2C19 gene in Chinese populations, but few studies have focused on Han populations from Guangdong Province. Therefore, our results provide new data on the CYP2C19 allele frequency that could help to establish a new database for functional research and guide personalized medicine for the Han populations of Guangdong. The CYP2C19 allele and genotype frequencies in all subjects were in Hardy-Weinberg equilibrium ($\chi 2 = 8.00$, $P > 0.05$).

In our current study, a total of three different alleles and six genotypes were detected. The CYP2C19*1 allele (wild-type) frequency in the Foshan population was lower than that in Caucasian populations and Asian populations [25–32]. According to previous studies, the highest CYP2C9*2 allele frequency was found in Asians (21.0-49.39%) [18–24]; however, the lowest frequency was found in Caucasians (2.9–14%) [25–29]. The CYP2C19*2 allele frequency in the subjects of our study was higher than that reported for Caucasians. Interestingly, the CYP2C19*2 allele frequency in our group (30.46%) was closer to those of Hakka (31.06%) and Chinese-Dai (30%) populations [18–19]. As shown in Table 2, the CYP2C19*2 allele frequency in our group was closer to that reported for other Asian populations.

The CYP2C9*3 allele frequency shows an increasing order in different groups of subjects as follows: Caucasians (0.0-0.3%) [25–29], Africans (0.0–2.0%) [30–32], Asians (1.0-11.1%) [18–24]. The CYP2C19*3 allele frequency was reported to be 5.65% in the Foshan population, which is consistent with those in other Chinese populations [19–21]. However, the frequency of the CYP2C19*3 variant in our population was lower than has been reported in the Japanese, Korean, Vietnamese, and Thai populations [22–24]. In addition, the CYP2C19 *2 and CYP2C19 *3 alleles cause diverse responses to medications such as clopidogrel; therefore, clinicians should be cautious when using clopidogrel in populations with a high loss-of-function allele frequency, especially in China.

Recent reports have provided evidence that the polymorphisms of CYP2C19 might affect its activity in the metabolization of related drugs. Interindividual differences in enzyme activity divide the population into EM, IM and PM phenotypes [8–9]. In our current study, the proportion of the IM phenotype (44.76%) was slightly higher than that of the EM phenotype (41.51%). The proportion of the PM phenotype was 13.73%, which is closer to that recorded in other Asian populations. Similar to findings in the Hakka population, the CYP2C19*2 allele was the most important variation in our group and was chiefly responsible for the PM phenotype, accounting for 80.48% of the study population.

Conclusions

In summary, our study provides the CYP2C19 genetic polymorphisms present in the Foshan population. The CYP2C19 allele frequencies and genotype frequencies were compared with those of other populations. This study demonstrates that the CYP2C19 allele frequency in the Foshan population is closest to that of Hakka populations and is distinct from that of African and Caucasian populations. In conclusion, our study provides important information about CYP2C19 polymorphisms in the Han population of Guangdong, which could be beneficial for guiding personalized medicine for the Han population of Guangdong.

Abbreviations

CI: confidence interval; EM: extensive metabolizer; IM: intermediate metabolizer; PM: poor metabolizer; PCR: POLYMERASE chain reaction

Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee of Nan Hai Hospital affiliated with Southern Medical University, and written informed consent was obtained from all participants (number: 20160106).

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due to protecting the participants' anonymity but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

ZYL, SYY and QQ coordinated and performed all sample analyses. XWY and XFZ drafted and revised the manuscript. All the authors read and approved the final manuscript.

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Figures

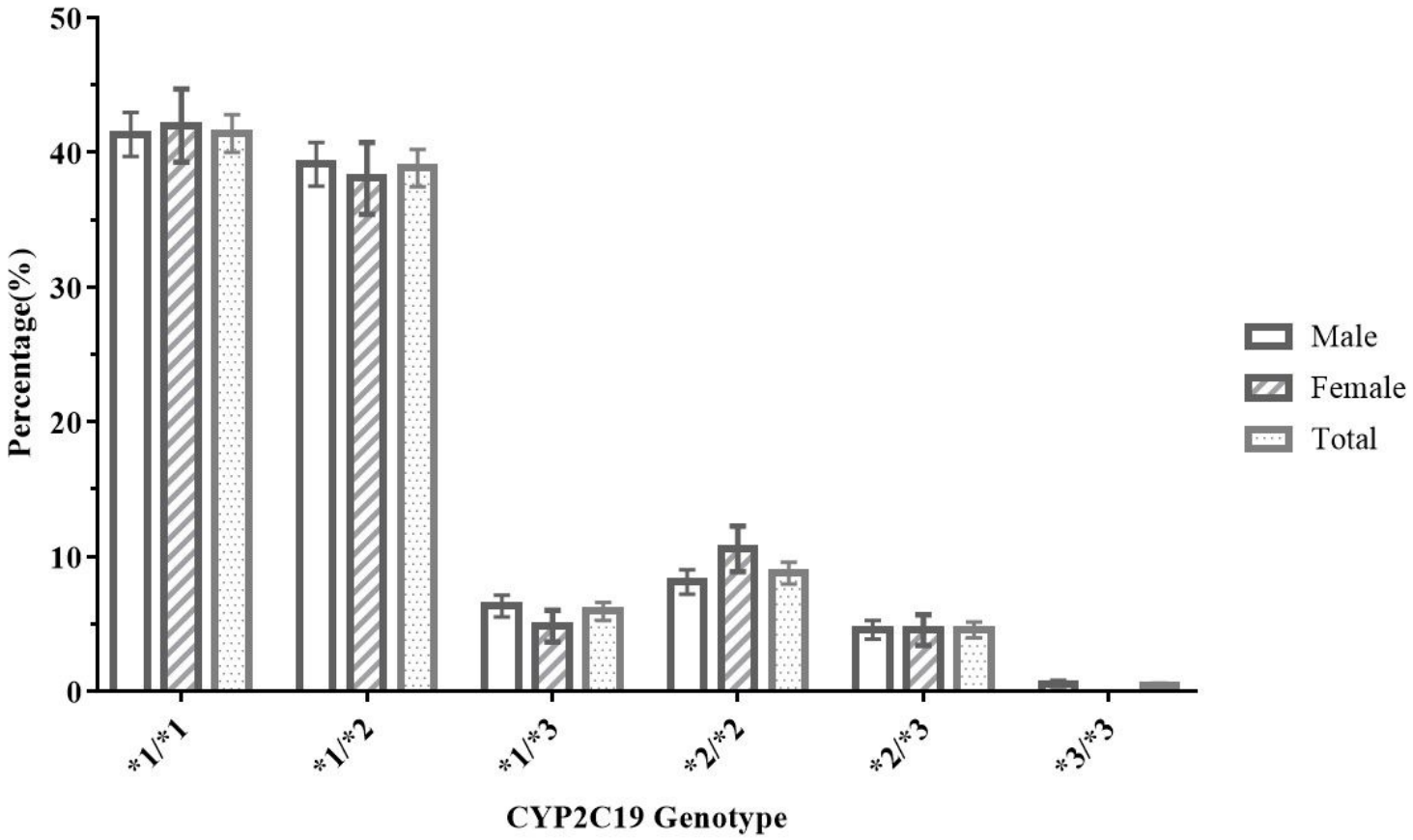

Figure 2

CYP2C19 genotype frequencies in 1231 patients in the Foshan area of Guangdong Province