Study on genetic polymorphisms and phenotypic frequency distribution of CYP2C9, CYP2C19 and CYP2D6 in Han Chinese population

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Abstract
Purpose Genetic mutations and phenotypic changes of CYP2C9, CYP2C19 and CYP2D6 are vital for individual variations in clinical drug responses. Elucidating the mutating frequencies and phenotypic distributions of these genes shall facilitate the implementation of preemptive pharmacogenetic testing. We analyzed the gene polymorphisms and phenotypic frequencies of CYP2C9, CYP2C19 and CYP2D6 in Han Chinese population.

Methods Tests of CYP2C9, CYP2C19 and CYP2D6 were performed in over 3200 (3276-3293) samples in Han Chinese population; furthermore, systematic review was employed for analyzing the mutation frequency and phenotypic distribution of CYP2C9 and CYP2C19 in Han Chinese population.

Results Among 9062 samples, the mutation frequency of CYP2C9 was 11.49% and the frequency of phenotypic changes 7.1%; in 8222 samples, the mutation frequency of CYP2C19 was 66.07% and the frequency of phenotypic changes 63.75%; among 3931 samples, the mutation frequency of CYP2D6 was 88.04% and the frequency of phenotypic changes 3.87%. Among 2690 samples, gene mutations and phenotypic distributions of CYP2C9, CYP2C19 and CYP2D6 were simultaneously examined and it was found that 96.36% samples became mutated and the frequency of phenotypic changes was 66.51%.

Conclusions In Han Chinese population, the gene mutations and phenotypic changes of CYP2C9, CYP2C19 and CYP2D6 are all relatively frequent. Prior to dosing, preemptive pharmacogenetic testing of CYP2C9, CYP2C19 and CYP2D6 is recommended.

Introduction
Genetic polymorphism of metabolic enzymes Cytochrome P450 (abbreviated as CYP450) is one of the important factors causing individual variations in clinical drug responses [1]. As stressed by the U.S. Food and Drug Administration (FDA) and other pharmacogenetic (PGx) guideline-formulating agencies, such as Clinical Pharmacogenetics Implementation Consortium (CPIC), Canadian Pharmacogenomics Network for Drug Safety (CPNDS), Dutch Pharmacogenetics Working Group (DPWG) and French National Network (Réseau) of Pharmacogenetics, most genetic polymorphisms which influences drug responses are related with P450 metabolic enzymes [2]. And the genetic
polymorphisms of CYP2C9, CYP2C19 and CYP2D6 have been most thoroughly examined so far in P450 metabolic enzymes [3]. However, there is still an ongoing debate on guiding drug dosing by detecting these main PGx genes [4]. Even the guideline of CPIC only discussed the issue of transforming PGx genotype into clinical phenotype [5]. There was no recommendation of whether or not conducting preemptive pharmacogenetic testing. Existing confusion of PGx genotype testing was mainly due to a lack of convincing evidences for large-scaled clinical trials for confirming the clinical values of PGx [6]. Recently more and more such trials of PGx are performed. For example, CHANCE study has confirmed that, in patients with mild ischemic stroke or transient ischemic attack (TIA), as compared with aspirin dosing alone, a combined use of aspirin and clopidogrel could only lower the occurring rate of new stroke among patients not carrying CYP2C19 loss-of-function allele [7]. As demonstrated by Genetic Informatics Trial (GIFT), among patients undergoing elective hip/knee joint replacement and receiving warfarin therapy during perioperative period, as compared with routine clinical drug dosing, genotype-guided dosing of warfarin could lower the risks of major hemorrhage, venous thrombus/embolism and death [8]. As confirmed by a randomized clinical trial of Syn NL et al, the dosing scheme based upon a formula of pharmacogenetic might reduce the dose of warfarin and better predict its maintenance dose in Asian populations. With the elapsing of time, a growing body of convincing evidences from clinical trials shall reiterate the needs of conducting preemptive pharmacogenetic testing [9]. And many current controversies of pharmacogenetic testing may be settled.

However, in addition to the strong clinical research evidence of preemptive pharmacogenetic testing, we should not ignore the influence of genetic polymorphisms and phenotype frequency distribution on preemptive pharmacogenetic testing. If the frequency of genetic polymorphism is low and a small variation exists in phenotypic frequency, then the cost effectiveness of pharmacogenetic test shall be minimal. Thus implementing pharmacogenetic test is to restrain clinically. Other than providing study evidences, we should also pay attention to the frequencies of these VIP PGx genes and their phenotypes. Thus it is possible to evaluate objectively the value of preemptive pharmacogenetic testing. For such well-established P450 enzymes as CYP2C9, CYP2C19 and CYP2D6, examining their
polymorphisms and phenotypes may provide more comprehensive rationales of guiding clinical drug
dosing with preemptive pharmacogenetic testing in Han Chinese population.

Methods
Allele frequency Data
Collaborating with Pharmacogenomics database (PGxC, www.chnpgxc.com), Third Xiangya Hospital of
Central South University utilized an Illumina HI SEQ X-10 sequencer for detecting multiple VIP PGx
genes, including CYP2C9, CYP2C19 and CYP2D6 polymorphisms, in Han Chinese population of 3200
(3276-3293) subjects. The study was approved by the ethics committee of the Third Xiangya Hospital
of Central South University. Furthermore, the PubMed database was searched for the literature
reports of CYP2C9 and CYP2C19 polymorphism frequency in healthy Han Chinese population having a
sample size of over 100. Ultimately systematic reviews were conducted for analyzing the distributions
of CYP2C9 and CYP2C19. Wildtype frequency = (1 — overall mutant frequency) ×100%. Due to the
complexity of CYP2D6 polymorphism involving variation in copy number, only samples supplied by
PGxC were detected for CYP2D6 and no systematic review was performed.
Allele nomenclature and definitions
Based upon the Human CYP Allele Nomenclature Database (https://www.pharmvar.org/) and
PharmGKB database (https://www.pharmgkb.org/), star (*) allele was adopted for naming and
functional notes. CYP allelic genotypic functions included the increased function, normal function,
decreased function and no function. Based upon standardized pharmacogenetic terms from the CPIC,
the phenotypes of CYP enzymes were classified as ultrarapid, rapid, normal, intermediate and poor
metabolizers [10].

Results
The genotypes and phenotypes of CYP2C9, CYP2C19 and CYP2D6 were evaluated in Han Chinese
population. And their sample sizes were 9062, 8222 and 3931 respectively (Table 1, supplemental
materials).

The percentage of wildtype CYP2C9 was approximately 88.51% in Han Chinese population and that of
mutated CYP2C9 genotype approximately 11.49% (Table 1). And the predominant genotype of
functionally mutated CYP2C9 was *1/*3 and its mutating frequency stood at approximately 6.50%. For
other mutations, the genotypic frequency was all under 1%. The phenotypic distribution of CYP2C9 was as follows normal metabolizer (88.5%), intermediate metabolizer (6.89%), poor metabolizer (0.21%), probable intermediate metabolizer (1.64%) and probable poor metabolizer (1.43%). And the phenotypes of approximately 1.33% genotype remained indeterminate (Table 2).

Wildtype CYP2C19 accounted for approximately 33.93% in Han Chinese population. And the percentage of mutated CYP2C19 genotype was approximately 66.07% (Table 1). Common functional mutated CYP2C19 genotypes included *1/*2 (37.95%), *2/*2 (9.67%), *1/*3 (5.32%), *2/*3 (3.8%), *2/*15 (1.56%) and *1/*17 (1.78%). The phenotypic distribution of CYP2C19 was as follows: normal metabolizer (34.7%), intermediate metabolizer (47.36%), poor metabolizer (14.61%), rapid metabolizer (1.78%) and probable intermediate metabolizer (1%). And the phenotypes of approximately 0.55% genotype remained indeterminate (Table 2).

Wildtype CYP2D6 accounted for 11.96% in Han Chinese population and mutated CYP2D6 genotype was approximately 88.04% (Table 1). In Han Chinese population, the predominant genotype of CYP2D6 was *1/*10 and *10/*10, mutating frequency 24.57% and 28.8%. However, activity score (AS) of *1/*10 and *10/*10 were 1.5 and 1 respectively [11]. Both were of normal metabolizer of CYP2D6.

Thus the frequency of phenotypic changes for CYP2D6 was lower than those for CYP2C9 and CYP2C19. For CYP2D6, the predominant genotype was *10/*60; intermediate metabolizer (3.35%) (Activity fraction = 0.5); poor metabolizer (0.52%) (AS = 0) (Table 2).

Among 2690 samples, we simultaneously examined the mutating status and phenotypic distribution of genes CYP2C9, CYP2C19 and CYP2D6. The non-mutated samples of CYP2C9, CYP2C19 and CYP2D6 accounted for 3.64%; 96.36% samples became mutated, the number of enzymatic mutation was one (36.58%), two (56.10%) and three (3.68%) (Table 1). The distribution of phenotypes was as follows: normal enzymatic activity of CYP2C9/CYP2C19/CYP2D6 (32.93%); altered enzymatic activity (66.51%). And the number of altered enzymatic activity was one (59.78%), two (6.54%) and three (0.19%) (Table 2).

Discussion
Systemic studies were performed for the genotypic and phenotypic distributions of CYP2C9, CYP2C19...
and CYP2D6 in Han Chinese population. Furthermore, we also examined the co-existing patterns of genotypes and phenotypic changes for CYP2C9, CYP2C19 and CYP2D6.

In this study, we conducted systematic review of the frequencies of common CYP2C9 gene polymorphisms. It was discovered that, among Han Chinese population, the major genotype of causing CYP2C9 functional alterations was *1/*3 with a mean mutating frequency of 6.50% (3.5%-9.81%). The frequency of this genotype was lower in Chinese populations than that in Caucasian (11.33%), Middle Eastern (14.32%) and South/Central Asian (16.0%) counterparts and yet higher than in African (1.79%) and African American (2.02%) counterparts. The figure was somewhat closer to that of Americas (5.79%). As for CYP2C9, the frequencies of intermediate and poor metabolizers were 6.89% and 0.21% respectively. Both were lower than those of Caucasian (31.99%, 4%), Middle Eastern (35.52%, 5.33%), South/Central Asian (33.30%, 4.45%), African American (23.47%, 1.84%), African (23.06%, 1.77%) and Americas (19.70%, 1.23%) (supplemental materials).

In this study, we systematic review the frequencies of common gene polymorphisms of CYP2C19. It was found that, in Han Chinese population, the predominant allelic genotypes of *2 and *3 induced the functional changes of CYP2C19 and lowered enzymatic activity. In Han Chinese population, the frequencies of intermediate and poor metabolizers of CYP2C19 metabolizing enzyme (47.36%, 14.61%) were higher than those of Caucasian (26.8%, 2.5%), Middle Eastern (26.4%, 2.4%), African American (32.3%, 4.3%), African (24.1%, 4.8%) and Americas (25.9%, 2.3%) populations. The figures were closer to those of South/Central Asian (45.6%, 12.4%). However, the frequency of CYP2C19 rapid metabolizer (1.78%) was lower than that of Caucasian (26.9%), Middle Eastern (25.9%), African American (23.6%), African (13.6%), Americas (21.8%) and South/Central Asian (16.4%) populations (supplemental materials).

Based upon the samples of PGxC, the common polymorphisms of CYP2D6 were detected in Han Chinese population. It was found that *10 allelic genes had the highest mutation frequency. The frequencies of both *1/*10 (24.57%) and *10/*10 (28.80%) were higher than those of Caucasian (0.75%, 0.08%), Middle Eastern (1.85%, 0.12%), African American (1.34%, 0.017%), African (0.95%, 0.44%), Americas (1.92%, 0.07%) and South/Central Asian (10.47%, 3.01%) populations. Yet both
*1/*10 and *10/*10 were of normal metabolizer. Thus the frequency of CYP2D6 phenotypic changes was relatively low. And the frequencies of CYP2D6 intermediate and poor metabolizers were 3.35% and 0.52% respectively. Both were lower than those of Caucasian (7.18%, 6.07%), Middle Eastern (5.64%, 1.20%), African American (13.21%, 3.05%), African (12.56%, 1.88%), Americas (4.48%, 3.66%) and South/Central Asian (6.31%, 3.011.28%) populations. In our study, the predominant genotype of inducing altered enzymatic activity of CYP2D6 was *10/*60. The limitation of our study lied in the fact that copy number of CYP2D6 was not analyzed. Thus the data were still lacking for rapid/ultrarapid metabolizer of CYP2D6 in Han Chinese population (supplemental materials).

In the mean time, we also analyzed the genotypes and phenotypes of CYP2C9, CYP2C19 and CYP2D6. The overall mutation rate was as high as 96.36%; and the ultimate mutating frequency of inducing altered enzymatic activity was up to 66.51%. It hinted that adjusting drug doses was essential for at least half of Chinese populations using common substrate drugs of CYP2C9, CYP2C19 and CYP2D6. In Han Chinese population, the genetic mutations and phenotypic changes of CYP2C9, CYP2C19 and CYP2D6 are all relatively frequent. It is worth noting that the mutations and phenotypic changes of CYP2C19 have surpassed 60%; there is a higher prevalence of triple-gene mutations and phenotypic changes. This study shall facilitate the implementation of preemptive pharmacogenetic testing in Chinese population.

Declarations
Authors’ contributions All authors including LH, SC, JL, XX, LH, YK, YZ, HT, QP, GY and CG have made substantial, direct and intellectual contribution to the work and approved it for publication.
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Conflict of interest The authors declare that they have no conflict of interest.
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Tables

| Gene | Sample size | Genotype               | Frequency (%) |
|------|-------------|------------------------|---------------|
| CYP2C9 | 9062       | *1/*1                 | 88.51         |
|       |            | *1/*3                 | 6.50          |
|       |            | Other*                | 4.99          |
|       |            | Overall mutation      | 11.49         |
| CYP2C19 | 8222     | *1/*1                 | 33.93         |
|       |            | *1/*2                 | 37.95         |
|       |            | *1/*3                 | 5.32          |
|       |            | *1/*17                | 1.78          |
|       |            | *2/*2                 | 9.67          |
|       |            | *2/*3                 | 3.80          |
|       |            | *2/*15                | 1.56          |
|       |            | Other*                | 5.99          |
|       |            | Overall mutation      | 66.07         |
| CYP2D6 | 3931       | *1/*1                 | 11.96         |
|       |            | *1/*2                 | 4.76          |
|       |            | *1/*10                | 24.57         |
|       |            | *1/*41                | 2.16          |
|       |            | *1/*60                | 1.3           |
|       |            | *2/*2                 | 2.59          |
|       |            | *2/*10                | 10.99         |
|       |            | *10/*10               | 28.8          |
|       |            | *10/*14B              | 1.32          |
|       |            | *10/*41               | 3.31          |
|       |            | *10/*60               | 2.16          |
|       |            | Other*                | 6.08          |
|       |            | Overall mutation      | 88.04         |
| CYP2C9+CYP2C19+CYP2D6 | 2690   | Wildtype              | 3.64          |
|       |            | One enzyme with altered enzymatic activity | 36.58 |
|       |            | Two enzymes with altered enzymatic activity | 56.10 |
|       |            | Three enzymes with altered enzymatic activity | 3.68 |
|       |            | Overall mutation      | 96.36         |
Other* as clustering of non-common genotypes

Table 2 Distribution frequencies of CYP2C9, CYP2C19 and CYP2D6 phenotypes

| Gene                      | Sample size | Genotype                        | Frequency (%) |
|---------------------------|-------------|---------------------------------|---------------|
| CYP2C9                    | 9062        | Normal metabolizer              | 88.5          |
|                           |             | Intermediate metabolizer        | 6.89          |
|                           |             | Probable intermediate metabolizer | 1.64         |
|                           |             | Poor metabolizer                | 0.21          |
|                           |             | Probable poor metabolizer       | 1.43          |
|                           |             | Indeterminate                   | 1.33          |
| CYP2C19                   | 8222        | Rapid metabolizer               | 1.78          |
|                           |             | Normal metabolizer              | 34.7          |
|                           |             | Intermediate metabolizer        | 47.36         |
|                           |             | Probable intermediate metabolizer | 1            |
|                           |             | Poor metabolizer                | 14.61         |
|                           |             | Indeterminate                   | 0.55          |
| CYP2D6                    | 3931        | Normal metabolizer              | 95.43         |
|                           |             | Intermediate metabolizer        | 3.35          |
|                           |             | Poor metabolizer                | 0.52          |
|                           |             | Indeterminate                   | 0.7           |
| CYP2C9+CYP2C19+CYP2D6     | 2690        | Normal enzymatic activity       | 32.93         |
|                           |             | One enzyme with altered enzymatic activity | 59.78 |
|                           |             | Two enzymes with altered enzymatic activity | 6.54 |
|                           |             | Three enzymes with altered enzymatic activity | 0.19 |
|                           |             | Altered overall enzymatic activity | 66.51 |

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