ORIGINAL ARTICLE

Multi-ethnic distribution of clinically relevant CYP2C genotypes and haplotypes

S Martis¹, I Peter¹, J-S Hulot², R Kornreich³, RJ Desnick¹ and SA Scott¹

To determine CYP2C19 and CYP2C8 allele frequencies, 28 coding and/or functional variants were genotyped in 1250 African-American, Asian, Caucasian, Hispanic and Ashkenazi Jewish (AJ) individuals. The combined CYP2C19 variant allele frequencies ranged from ~0.30 to 0.41; however, the CYP2C8 frequencies were much lower (~0.04–0.13). After incorporating previously reported CYP2C9 genotyping results from these populations (36 total CYP2C variants), 16 multi-ethnic CYP2C haplotypes were inferred with frequencies >0.5%. Notably, the 2C19*17-2C9*1-2C8*2 haplotype was identified among African-Americans (8%) and Hispanics (2%), indicating that CYP2C19*17 does not always tag a CYP2C haplotype that encodes efficient CYP2C-substrate metabolism. The 2C19*1-2C9*2-2C8*3 haplotype was identified in all populations except African-Americans and additional novel haplotypes were identified in selected populations (for example, 2C19*2-2C9*1-2C8*4 and 2C19*4B-2C9*1-2C8*1), together indicating that both CYP2C19*17 and *2 can be linked with other CYP2C loss-of-function alleles. These results have important implications for pharmacogenomic association studies involving the CYP2C locus and are clinically relevant when administering CYP2C-substrate medications.

The Pharmacogenomics Journal (2013) 13, 369 – 377; doi:10.1038/tpj.2012.10; published online 10 April 2012

Keywords: CYP2C19; CYP2C8; CYP2C9; clinical pharmacogenetics; linkage disequilibrium; haplotype

INTRODUCTION

The hepatic cytochrome P450 (CYP450) superfamily of hemoproteins are the principal enzymes involved in human drug metabolism and bioactivation. Over 50 human CYP450 isozymes have been identified; however, members of the CYP2 and CYP3 families have significant importance as they contribute to the metabolism and bioactivation. Over 50 human CYP450 isozymes have been identified; however, members of the CYP2 and CYP3 families have significant importance as they contribute to the metabolism of the majority of drugs.¹ The most relevant CYP2C subfamily enzymes are encoded by a cluster of polymorphic genes on chromosome 10q23.33, organized as Cen-CYP2C18-CYP2C19-CYP2C9-CYP2C8-Tel.²–⁴ Although the sequences of these four isoforms are >80% identical, they can have distinct substrate specificities, and together are involved in the metabolism of ~20–30% of all medications.⁵ CYP2C19 contributes to the metabolism of a large number of clinically relevant drugs and drug classes such as antidepressants, benzodiazepines, mephentoyin, proton pump inhibitors and the antplatelet prodrug clopidogrel.⁵–⁷ CYP2C9 is involved in the metabolism of tolbutamide, phenytoin, S-warfarin, losartan and numerous anti-inflammatory drugs such as ibuprofen.⁶ CYP2C9 substrates overlap with CYP2C8, including arachidonic acid, several non-steroidal anti-inflammatory drugs and retinoic acid. CYP2C8 also has a direct role in the metabolism of some important therapeutic drugs, including paclitaxel, amiodarone, troglitazone, amiodarone, verapamil, cefazolin and fluvalastatin.¹⁰ Although variant CYP2C18 alleles have been reported,¹¹,¹² CYP2C18 expression is not consistent with a major role in hepatic drug metabolism and specific CYP2C18-substrates have yet to be clearly identified.³

Both common and rare CYP2C19, CYP2C9 and CYP2C8 variant alleles have been identified in different populations, which are cataloged by the Human Cytochrome P450 Allele Nomenclature Committee.¹³ Many of these variant alleles encode reduced or complete loss-of-function, and their frequencies can significantly differ between racial and ethnic populations.¹⁴–¹⁷ Importantly, the ~390 kb sequence that encompasses the CYP2C cluster is in strong linkage disequilibrium (LD)¹⁸,¹⁹ indicating that there is a tendency to jointly inherit alleles that confer specific CYP2C19, CYP2C9 and CYP2C8 metabolic phenotypes. Previous studies interrogating selected CYP2C variants have identified LD between some CYP2C19, CYP2C9 and CYP2C8 alleles in specific ethnic subpopulations,²⁰–²⁴ however, the frequencies of many variant CYP2C alleles and relevant haplotypes remain unknown in most populations.

We previously reported the frequencies of important CYP2C9 alleles (*2, *3, *4, *5, *6, *8, *11 and *13) in the African-American, Asian, Caucasian, Hispanic and Ashkenazi Jewish (AJ) populations.¹⁶,²⁵ and recently identified the novel CYP2C19*4B allele in the AJ population that is defined by both gain-of-function [c.–806C>T (*17)] and loss-of-function [c.1A>G (*4)] alleles on the same haplotype.²⁶ To determine the frequencies of additional CYP2C alleles in these populations, 28 variant CYP2C19 (*2–*10, *12–*17, *22) and CYP2C8 (*2–*10, *12–*14) alleles were genotyped in 250 DNA samples each from healthy African-American, Asian, Caucasian, Hispanic and AJ individuals. These results were then combined with the previously reported CYP2C9 data to identify CYP2C haplotypes and their multi-ethnic frequencies. These results have important implications for pharmacogenomic association studies involving the CYP2C locus and are clinically relevant when administering CYP2C-substrate medications. In addition, given the recent interest in clinical CYP2C19 genetic testing for clopidogrel response,²⁷–³¹ we determined the ABCB1 c.3435C>T³²–³⁵ allele and genotype frequencies for all tested populations.

¹Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY, USA and ²Cardiovascular Research Center, Mount Sinai School of Medicine, New York, NY, USA. Correspondence: Dr SA Scott, Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, Box 1497, 1428 Madison Avenue, New York, NY 10029, USA.

E-mail: stuart.scott@mssm.edu

Received 10 January 2012; revised 16 February 2012; accepted 5 March 2012; published online 10 April 2012
MATERIALS AND METHODS

Study population
Peripheral blood samples from healthy donors who indicated their racial background and gave informed consent for the use of their DNA for research were obtained from the New York Blood Center with Institutional Review Board approval as previously defined.16,25 In addition, blood samples were obtained with informed consent from unrelated healthy 100% AJ individuals from the greater New York metropolitan area.26,36-38 All personal identifiers were removed, and isolated DNA samples were tested anonymously. Genomic DNA was isolated using the Puregene DNA Purification kit (Qiagen, Valencia, CA, USA) according to manufacturer’s instructions. Two hundred fifty samples were genotyped for each of the five tested populations (African-American, Asian, Caucasian, Hispanic and AJ).

Genotyping
The designations of all CYP450 alleles refer to those defined by the Cytochrome P450 Allele Nomenclature Committee (http://www.cypalleles.ki.se/).13 Eleven variant CYP2C19 alleles (*2 – *10, *13, *17) were genotyped using the eSensor 2C19 Test (GenMark Diagnostics, Carlsbad, CA, USA) as per manufacturer’s instructions, and five additional variant CYP2C19 alleles (*12, *14 – *16, *22) and ABCB1 c.3435C>T were genotyped using a custom multiplexed SNAPSHOT single base extension assay (Applied Biosystems, Carlsbad, CA, USA) as previously described.26 Eight variant CYP2C9 alleles (*2 – *6, *8, *11, *13) were genotyped using the Tag-It Mutation Detection Kit (Luminex Molecular Diagnostics, Toronto, ON, USA) and PCR-restriction fragment length polymorphism assays as previously reported.16 All 12 variant CYP2C8 alleles currently defined by the Cytochrome P450 Allele Nomenclature Committee (*2 – *10, *12 – *14) were genotyped using an additional custom multiplexed SNAPSHOT single base extension assay (Applied Biosystems). Multiplexed PCRs were performed in 10 μL containing ~50 ng of DNA, 2 × PCR buffer (Invitrogen, Carlsbad, CA, USA), 1.5 mm MgCl₂, 0.2 mM of each dNTP, forward and reverse primers (CYP2C8 exon 3: 0.8 μM; exons 4, 5, 7 and 9: 0.6 μM; exon 8: 0.4 μM; Supplementary Table S1), and 2.0 units of Platinum Taq DNA Polymerase (Invitrogen). Amplification consisted of an initial denaturation step at 94°C for 5 min followed by 35 amplification cycles (94°C for 30 s, 57°C for 30 s and 72°C for 1 min) and a final incubation at 72°C for 10 min. Amplicons were digested with 3.0 units of shrimp alkaline phosphatase and 2.0 units of Exonuclease I (both from US BioCorporation, Cleveland, OH, USA). SNAPSHOT primer extension reactions were performed in 10 μL containing 1 × SNAPSHOT Reaction Mix (Applied Biosystems), 0.2 μM of each allele-specific primer (Supplementary Table S1) and 3.0 μL of PCR product. Following the recommended thermal cycling, samples were treated with 1.0 unit of shrimp alkaline phosphatase, electropherograms on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems), and analyzed using GeneMarker software v1.95 (SoftGenetics, State College, PA, USA). Representative positive control samples for all identified CYP2C alleles were confirmed by bidirectional sequencing (Supplementary Figure S1), and wild-type (*1) CYP2C19, CYP2C9 and CYP2C8 alleles were assigned in the absence of other detectable variant alleles.

CYP2C19*4B confirmation
Confirmation of potential CYP2C19*4B carriers was performed by cloning and allele-specific sequencing of a 1.2-kb fragment encompassing CYP2C19*17 (c.-806C>T) and *4 (c.1A>G) as previously described.26 For each sample, 6–10 colonies were propagated and bidirectionally sequenced using M13 and T7 vector-specific primers. All plasmid DNA sequence data were analyzed using Mutation Surveyor software v3.30 (SoftGenetics).

Statistical analyses and haplotyping
Observed genotype frequencies were compared with those expected under Hardy–Weinberg equilibrium using the χ²-test for each racial and ethnic group. The χ²-test was also used to detect overall and pairwise differences in allele frequencies between all tested populations. Pairwise LD between tested variants was assessed using Lewontin’s D’ and the squared correlation coefficient between allele frequencies (r²) expressed as a function of D’. The expectation-maximization algorithm was implemented to calculate maximum likelihood estimates of haplotype frequencies assuming Hardy–Weinberg equilibrium. All analyses were conducted using SAS/Genetics software (SAS Institute, Cary, NC, USA).

RESULTS
CYP2C19 allele and genotype frequencies
The CYP2C19 allele and genotype frequencies are summarized in Tables 1 and 2. All alleles were in Hardy–Weinberg equilibrium (P > 0.05) and no studied population carried the *4A, *5, *7, *10, *16 or *22 allele. The overall across-population difference in CYP2C19 allele frequencies was significant for five polymorphic variants (rs12248560 [*17], rs4244285 [*2], rs4986893 [*3], rs17882687 [*15], rs28399504 [*4]; P < 0.02). The CYP2C19*4B allele was detected in both the Caucasian and Hispanic populations and confirmed by cloning and allele-specific sequencing as previously described.16 The combined frequencies of detected variant CYP2C19 alleles were 0.406 (African-American), 0.386 (Asian), 0.304 (Caucasian), 0.296 (Hispanic) and 0.368 (AJ).

Based on their observed genotypes, the African-American, Asian, Caucasian, Hispanic and AJ predicted CYP2C19 metabolic phenotypes3,5,7 were distributed as ultrarapid (3, 2, 2, 3 and 4%), extensive (59, 42, 72, 71 and 67%), intermediate (19, 44, 18, 18 and 1%) and poor (5, 8, 4, 2 and 4%) metabolizers, respectively (Table 2). Some of the variant CYP2C19 alleles (*9, *10, *12 – *16, *22) in the expanded panel currently do not have clear phenotypic consequences, as do compound heterozygous genotypes that include both loss- and gain-of-function alleles (for example, *2/ *17). As such, the frequencies of individuals with unknown predicted metabolic phenotypes using this expanded CYP2C19 genotyping panel in the African-American, Asian, Caucasian, Hispanic and AJ were 14, 4, 4, 7 and 8%, respectively.

ABC1 allele and genotype frequencies
Some studies have found that clopidogrel-treated patients with cardiovascular disease who are homozygous carriers of the synonymous ABCB1 c.3435C>T (p.L11435V) variant have higher rates of adverse cardiovascular events than c.3435C carriers during therapy, which was independent from and compounded by CYP2C19 loss-of-function alleles.22,23,34 However, conflicting data have been reported regarding which allele (c.3435C or c.3435T) was associated with the increased risk.22,23,32,34 Supplementary Tables S2 and S3 summarize the identified ABCB1 c.3435C>T allele and genotype frequencies, which were statistically different between all tested populations (P < 0.0001). Of note, the c.3435T/T genotype frequencies in the African-American, Asian, Caucasian, Hispanic and AJ were 6, 20, 29, 24 and 8%, respectively. In addition, categorizing the tested subjects based on CYP2C19 loss-of-function allele carrier status and ABCB1 c.3435T/T genotype34 indicated that 34–66% of all tested multi-ethnic individuals carried CYP2C19 and ABCB1 genotypes that conferred an increased risk for clopidogrel non-responsiveness and/or adverse effects (Supplementary Table S4; Supplementary Figure S2).

CYP2C8 allele and genotype frequencies
The CYP2C8 allele and genotype frequencies are summarized in Tables 3 and 4. All alleles were in Hardy–Weinberg equilibrium (P > 0.05) and no studied population carried the *5 – *10, *12 or *13 allele. The overall across-population difference in CYP2C8 allele frequencies was highly significant for three polymorphic variants (rs11572080 [*3], rs11572103 [*2], rs10509681 [*3]; P < 0.0001). The combined frequencies of detected variant CYP2C8 alleles were 0.122 (African-American), 0.038 (Asian), 0.130 (Caucasian), 0.116 (Hispanic) and 0.100 (AJ). The African-American, Asian, Caucasian, Hispanic and AJ CYP2C8 genotype frequencies were distributed as homozygous wild-type (77, 92, 77, 79 and 80%), heterozygous...
LD and CYP2C haplotypes

After combining the CYP2C19 and CYP2C8 genotyping results with the previously reported CYP2C9 data, pairwise LD was calculated and visualized using Haploview version 4.1 for each racial and ethnic group (Figure 1). Using 13 polymorphic alleles [CYP2C19*2, *3, *4, *17, CYP2C9*2, *3, *5, *8, *11; CYP2C8*2, *3 (p.R139K), *2 (p.K399R), *4], 33, 18, 19, 22 and 23 non-redundant CYP2C haplotypes were inferred in the African-American, Asian, Caucasian, Hispanic and AJ populations, respectively. However, only 16 of all identified haplotypes had frequencies >0.5% in at least one population and together accounted for ~96--99% of the overall CYP2C cluster haplotypic diversity in these populations (Table 5).

Estimated haplotype frequencies showed considerable variation across the five populations and some of the commonly studied CYP2C19, CYP2C9 and CYP2C8 functional variants were found to exist in more than one haplotype. The two most common variant allele-containing haplotypes were 2C19*2-2C9*1-2C8*1 (12--27%) and 2C19*17-2C9*1-2C8*1 (6--19%). Importantly, a 2C19*17-2C9*1-2C8*2 haplotype was also identified among African-Americans (7.5%) and Hispanics (1.7%), indicating that CYP2C19*17 does not always tag a CYP2C haplotype that encodes efficient CYP2C-substrate metabolism as previously reported in Nordic populations.22 The estimated D' and r² between 2C19*17 and 2C8*2 were 0.813 and 0.325 among African-Americans and 0.626 and 0.057 among Hispanics, respectively (Table 6). In addition, a haplotype containing two CYP2C loss-of-function alleles (2C19*1-2C9*2-2C8*3) was identified in all populations (1.2--8.9%) except African-Americans. Unique ethnic-specific and/or rare haplotypes were also detected at frequencies of 0.5--4.8%, including 2C19*3-2C9*1-2C8*1 (Asians), 2C19*4B-2C9*1-2C8*4 (African-Americans and As) and 2C19*17-2C9*3-2C8*3 (Asians and As).

DISCUSSION

The paucity of frequency data for variant CYP2C19 and CYP2C8 alleles beyond those commonly tested (for example, *2 and *3) prompted our genotyping of 28 functional and/or coding region variants (CYP2C19*2--*10, *12--*17, *22; CYP2C8*2--*10, *12--*14) in the African-American, Asian, Caucasian, Hispanic and AJ populations. Although not all alleles were detected, the combined variant CYP2C19 allele frequencies ranged from ~0.30 to 0.41 in the tested populations; however, the combined CYP2C8 frequencies were much lower (~0.04--0.13). After combining these results with our previously reported CYP2C9 data (36 total variants),16 16 unique CYP2C haplotypes were inferred in the tested populations with frequencies >0.5%. Our haplotype data indicate that CYP2C19*17 does not always tag a CYP2C haplotype encoding efficient CYP2C-substrate metabolism as previously reported in Nordic populations22 and highlight that, despite largely acting as independent loci, CYP2C19*17 and *2 can also be found in LD with other variant CYP2C alleles that influence the metabolizer phenotypes.

The first CYP2C19 loss-of-function allele discovered based on its role in impaired mephenytoin metabolism was *2 (c.681G → A),39 and since then a number of additional variants have been identified in different populations. Some have known effects on CYP2C19 enzyme activity, whereas others do not have clear phenotypic effects.5,12 Consequently, our study using an expanded panel of 16 CYP2C19 variant alleles identified individuals with certain genotypes (for example, *1/*15, *2/*17) that have unknown consequences on CYP2C19-mediated drug metabolism. The identified frequencies of individuals with unknown predicted metabolizer phenotypes ranged from 4 to 14% in the tested...
populations (highest in African-Americans), suggesting that further in-vivo and/or in-vitro phenotyping studies with these specific variant alleles are warranted before their inclusion in clinical genotyping panels. CYP2C19 poor metabolizers typically carry two loss-of-function alleles and the frequencies of these genotypes ranged from ~2 to 8% in the tested populations, which was highest in Asians due to their higher frequencies of both *2 and *3.

CYP2C19 has recently received considerable attention due to its principal role in the bioactivation of the antiplatelet agent clopidogrel. Importantly, CYP2C19 loss-of-function alleles have been associated with lower active metabolite exposure, and increased adverse cardiovascular event rates among clopidogrel-treated subjects, and increased adverse cardiovascular event rates among clopidogrel-treated patients with acute coronary syndromes and/or those undergoing percutaneous coronary intervention. The increased risk among CYP2C19 loss-of-function allele carriers, particularly for poor metabolizers, prompted product insert label revision by the US Food and Drug Administration and additional interest in implementing CYP2C19 clinical testing to guide antiplatelet therapy for some cardiovascular patient populations. Recently, the CYP2C19*4B allele was discovered in the AJ population which has important implications for clinical CYP2C19 testing as the allele harbors both gain-of-function [c.---806C>T (*17)] and loss-of-function [c.1A>G (*4)] variants on the same haplotype. In the current study, CYP2C19*4B was also identified in both the Caucasian and Hispanic populations at lower frequencies (1%-1%); however, no carriers of the *4A allele (c.1A>G without c.---806C>T) were detected in any of the tested populations. Importantly, we previously identified CYP2C19*4A in the Sephardic Jewish population, which confirms the independent existence of these two suballeles.

Although more controversial than CYP2C19, some studies have found that carriers of the ABCB1 (P-glycoprotein) c.3435C>T synonymous variant have higher rates of adverse cardiovascular events during clopidogrel therapy, suggesting that ABCB1 might influence clopidogrel efflux and drug bioavailability. However, conflicting data have been reported regarding both the relationship between c.3435C>T and P-glycoprotein expression and which allele (c.3435C or c.3435T) is associated with the increased cardiovascular risk. Despite this discrepancy, large clinical studies found that c.3435T/T patients had a higher rate of

### Table 2. CYP2C19 genotype frequencies

| Predicted CYP2C19 metabolizer phenotype/ genotype | Observed (expecteda) frequency (%) |
|-------------------------------------------------|-----------------------------------|
| African-American (n = 250)                      | Asian (n = 250)                   | Caucasian (n = 250) | Hispanic (n = 250) | Ashkenazi Jewishb |
| Ultrarapid metabolizer (UM) *17/*17             | 2.8 (3.3)                         | 1.6 (0.4)           | 2.8 (2.5)          | 2.4 (2.3)         | 3.6 (3.9)         |
| Extensive metabolizer (EM)                      |                                   |                     |                   |                   |                   |
| *1/*1                                           | 38.4 (33.5)                       | 36.4 (37.7)         | 49.2 (48.4)       | 50.4 (49.6)       | 41.6 (39.9)       |
| *1/*17                                          | 20.4 (21.6)                       | 5.6 (7.6)           | 22.8 (22.0)       | 20.4 (21.4)       | 25.2 (25.0)       |
| Total                                           | 58.8 (56.9)                       | 42.0 (45.3)         | 72.0 (70.4)       | 70.8 (71.0)       | 66.8 (65.0)       |
| Intermediate metabolizer (IM)                   |                                   |                     |                   |                   |                   |
| *1/*2                                           | 18.4 (23.0)                       | 37.2 (33.9)         | 16.4 (18.4)       | 17.2 (18.0)       | 16.0 (18.5)       |
| *1/*3                                           | 0.4 (0.5)                         | 7.2 (5.9)           | 0.4 (0.6)         | 0.0 (0.0)         | 0.0 (0.0)         |
| *1/*4B                                          | 0.0 (0.0)                         | 0.0 (0.0)           | 0.0 (0.6)         | 0.0 (0.3)         | 2.0 (2.5)         |
| *1/*6                                           | 0.0 (0.0)                         | 0.0 (0.0)           | 0.4 (0.3)         | 0.0 (0.0)         | 0.0 (0.0)         |
| *1/*8                                           | 0.0 (0.0)                         | 0.0 (0.0)           | 0.4 (0.6)         | 0.0 (0.0)         | 0.0 (0.0)         |
| Total                                           | 18.8 (23.5)                       | 44.4 (39.8)         | 17.6 (20.0)       | 17.7 (18.9)       | 18.0 (21.0)       |
| Poor metabolizer (PM)                           |                                   |                     |                   |                   |                   |
| *2/*2                                           | 4.8 (3.8)                         | 6.4 (7.6)           | 2.8 (1.7)         | 2.0 (1.6)         | 2.8 (2.1)         |
| *2/*3                                           | 0.4 (0.2)                         | 1.6 (2.6)           | 0.4 (0.1)         | 0.0 (0.0)         | 0.0 (0.0)         |
| *2/*4B                                          | 0.0 (0.0)                         | 0.0 (0.0)           | 0.8 (0.1)         | 0.0 (0.1)         | 0.8 (0.6)         |
| *3/*3                                           | 0.0 (0.0)                         | 0.4 (0.2)           | 0.0 (0.0)         | 0.0 (0.0)         | 0.0 (0.0)         |
| Total                                           | 5.2 (3.9)                         | 8.4 (10.5)          | 4.0 (2.0)         | 2.0 (1.8)         | 3.6 (2.8)         |
| Unknown                                         |                                   |                     |                   |                   |                   |
| *1/*9                                           | 0.0 (0.0)                         | 0.0 (0.0)           | 0.0 (0.0)         | 0.4 (0.3)         | 0.0 (0.0)         |
| *1/*13                                          | 0.8 (1.4)                         | 0.0 (0.0)           | 0.0 (0.0)         | 0.8 (0.6)         | 0.0 (0.0)         |
| *1/*15                                          | 2.0 (1.7)                         | 0.0 (0.0)           | 0.4 (0.3)         | 0.8 (0.6)         | 0.0 (0.0)         |
| *2/*13                                          | 0.8 (0.5)                         | 0.0 (0.0)           | 0.0 (0.0)         | 0.0 (0.1)         | 0.0 (0.1)         |
| *2/*15                                          | 0.4 (0.5)                         | 0.0 (0.0)           | 0.0 (0.1)         | 0.0 (0.1)         | 0.4 (0.1)         |
| *2/*17                                          | 9.2 (7.1)                         | 3.6 (3.4)           | 3.2 (4.2)         | 4.4 (3.9)         | 6.4 (5.8)         |
| *3/*17                                          | 0.0 (0.1)                         | 0.0 (0.6)           | 0.0 (0.1)         | 0.0 (0.0)         | 0.0 (0.0)         |
| *4B/*15                                         | 0.0 (0.0)                         | 0.0 (0.0)           | 0.0 (0.0)         | 0.0 (0.0)         | 0.0 (0.0)         |
| *4B/*17                                         | 0.0 (0.0)                         | 0.0 (0.0)           | 0.0 (0.1)         | 0.4 (0.1)         | 0.8 (0.8)         |
| *8/*17                                          | 0.4 (0.1)                         | 0.0 (0.0)           | 0.4 (0.1)         | 0.0 (0.0)         | 0.0 (0.0)         |
| *13/*17                                         | 0.8 (0.4)                         | 0.0 (0.0)           | 0.0 (0.0)         | 0.0 (0.1)         | 0.0 (0.0)         |
| *15/*17                                         | 0.4 (0.5)                         | 0.0 (0.0)           | 0.0 (0.1)         | 0.0 (0.1)         | 0.0 (0.2)         |
| Total                                           | 14.4 (12.3)                       | 3.6 (4.0)           | 3.6 (4.9)         | 7.2 (5.9)         | 8.0 (7.4)         |

Abbreviation: n, number of subjects.
*Predicted Hardy–Weinberg frequencies.
Data from Scott et al.
Although some studies classify *1/*17 individuals as ultrarapid metabolizers, the extensive metabolizer classification is consistent with Li-Wan-Po et al.

The Pharmacogenomics Journal (2013), 369 – 377 © 2013 Macmillan Publishers Limited
adverse cardiovascular events than c.3435C homozygotes during clopidogrel therapy, which was independent from and compounded by CYP2C19 loss-of-function alleles.\textsuperscript{33,34} Our study identified a high frequency of c.3435T/T homozygotes in the tested populations (6–30%), and when combined with the CYP2C19 variant frequencies, 34–66% of tested individuals harbored a CYP2C19 loss-of-function allele and/or ABCB1 c.3435T/T, which could influence their response to clopidogrel.

CYP2C8 is involved in the metabolism of a number of drugs and xenobiotics including arachidonic acid, repaglinide and the anticancer agent paclitaxel.\textsuperscript{57–59} Although early in-vitro data suggested that CYP2C8*2 and *3 resulted in impaired activity and decreased metabolism of CYP2C8 substrates, some in-vivo data on the phenotypic consequences of these alleles have yielded contradictory results.\textsuperscript{58–60} Moreover, the effects of the known variant CYP2C8 alleles on activity may be substrate specific.\textsuperscript{60} We genotyped all 12 currently defined variant CYP2C8 alleles (*2–*10, *12–*14) and only detected *2, *3, *4 and *14 in the tested populations. All other alleles were originally discovered at low frequencies in Japanese individuals,\textsuperscript{61–64} which may have been an underrepresented ethnicity in our heterogeneous Asian population. Together, these results suggest that future CYP2C8 pharmacogenetic studies could benefit from additional genotype-phenotype correlation data, and further CYP2C8 sequencing of phenotype outliers in different racial and ethnic populations.

The CYP2C9*2 (p.R144C) reduced function allele previously was found associated with CYP2C8*3 in the Swedish population\textsuperscript{22} and subsequently was reported in all racial and ethnic groups except African-Americans. In contrast, the aforementioned CYP2C8*2-CYP2C9*17 haplotype was identified in all racial and ethnic groups except African-Americans. In contrast, the aforementioned CYP2C8*2-CYP2C9*17-2C9*4B-2C8*1 haplotype was found almost exclusively with wild-type CYP2C9*1 and CYP2C8*1.\textsuperscript{22} However, CYP2C19*17 subsequently was reported in LD with CYP2C8*2 among Brazilian individuals of African descent, prompting these authors to conclude that further multi-ethnic CYP2C haplotype studies including CYP2C19*17 were warranted.\textsuperscript{24}

Interrogating 36 variant CYP2C alleles in five major racial and ethnic populations resulted in 16 inferred CYP2C haplotypes with frequencies >0.5% in our study. Of note, the 2C19*1-2C9*2-2C8*3 haplotype was identified in all racial and ethnic groups except African-Americans. In contrast, the aforementioned 2C19*17-2C9*1-2C8*2 haplotype reported among Brazilians of African descent\textsuperscript{24} was identified in both our African-American (8%) and Hispanic (2%) populations. As Hispanics can be three-way admixtures of Native American, European and West African populations,\textsuperscript{65} our data underscore that CYP2C19*17 should not be used as a sole determinant for extensive CYP2C-substrate metabolism in populations with African descent.\textsuperscript{24} However, 2C19*17-2C9*1-2C8*1 was the more common CYP2C19*17-containing haplotype among all carriers of this variant allele (African-American: 9%; Asian: 6%; Caucasian: 15%; Hispanic: 13%; AJ: 19%). Notably, despite the identification of the 2C19*17-2C9*1-2C8*2 haplotype, CYP2C19*17 still appears to be a marker of extensive CYP2C9 metabolism, which may be more clinically relevant than CYP2C8-mediated drug metabolism.

Other novel haplotypes with multiple variants included 2C19*2-2C9*1-2C8*4 in the African-American and AJ populations, and 2C19*1-2C9*3-2C8*3 in the Asian and AJ populations. In addition, the 2C19*3-2C9*1-2C8*1 and 2C19*4B-2C9*1-2C8*1 haplotypes were found exclusively in the Asian and AJ populations, respectively. Together, these haplotype results are consistent with
### Table 4. CYP2C8 genotype frequencies

| CYP2C8 genotype | Observed (expected) frequency (%) |
|-----------------|------------------------------------|
|                 | African-American (n = 248) | Asian (n = 249) | Caucasian (n = 248) | Hispanic (n = 248) | Ashkenazi Jewish (n = 249) |
| Wild type       | 76.6 (76.9) | 92.0 (92.5) | 77.2 (75.7) | 78.6 (78.3) | 80.0 (81.0) |
| *1/*1           |           |           |           |           |           |
| Heterozygous    |           |           |           |           |           |
| *1/*2           | 18.1 (17.7) | 0.8 (0.8) | 0.4 (0.3) | 4.0 (3.9) | 0.8 (0.7) |
| *1/*3           | 2.0 (1.8) | 4.4 (4.3) | 14.8 (16.4) | 12.9 (13.6) | 15.2 (13.7) |
| *1/*4           | 2.0 (2.1) | 2.4 (2.3) | 4.4 (5.6) | 2.8 (2.9) | 4.0 (3.6) |
| *1/*14          | 0.0 (0.0) | 0.0 (0.0) | 0.4 (0.3) | 0.0 (0.3) | 0.0 (0.0) |
| Total           | 22.2 (21.6) | 7.6 (7.4) | 20.0 (22.6) | 19.8 (20.3) | 20.0 (18.0) |
| Homozygous variant/compound heterozygous |           |           |           |           |           |
| *2/*2           | 0.8 (1.0) | 0.0 (0.0) | 0.0 (0.0) | 0.0 (0.0) | 0.0 (0.0) |
| *2/*3           | 0.4 (0.2) | 0.0 (0.0) | 0.0 (0.0) | 0.0 (0.0) | 0.4 (0.3) |
| *2/*4           | 0.4 (0.2) | 0.0 (0.0) | 0.0 (0.0) | 0.0 (0.0) | 0.4 (0.3) |
| *3/*3           | 0.0 (0.0) | 0.0 (0.0) | 0.8 (0.9) | 0.8 (0.6) | 0.0 (0.6) |
| *3/*4           | 0.0 (0.0) | 0.0 (0.1) | 2.0 (0.6) | 0.4 (0.3) | 0.0 (0.3) |
| Total           | 0.4 (0.3) | 0.0 (0.1) | 2.8 (1.5) | 1.2 (0.9) | 0.0 (0.9) |

Abbreviation: n, number of subjects.
*Predicted Hardy-Weinberg frequencies.

---

**Figure 1.** Linkage disequilibrium (LD) across the CYP2C locus (10q23.33) in each tested population using 13 polymorphic single-nucleotide polymorphisms (SNPs). Pairwise LD between polymorphisms is expressed as $D'$. Significant linkage (logarithm of the odds, LOD $\geq 2$) is illustrated by red shading depending on the magnitude of $D'$ (from pink to bright red), and insignificant linkage (LOD $< 2$) is illustrated by blue (if $D' = 1$) or white (if $D' < 1$) shading. Haplotype blocks were inferred using the ‘Four gamete of LD’ method (Haploview).
Table 5. Haplotype frequencies

| rs12248560 | rs28399504 | rs4986893 | rs4244285 | rs11572103 | rs7900194 | rs28371686 | rs10509681 | rs11572103 | rs1058930 |
|------------|------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| 4          | T; 2C19*17 | (A; 2C19*3) | (G; 4 A; 2C19*2) | (C; T; 2C9*2) | (G; 4 A; 2C9*3) | (4 T; 2C9*11) | (A; 4 C; 2C9*3) | (4 G; 2C8*3) | (G; A; 2C8*3) |
| 0.513      | 0.545      | 0.474     | 0.406     | 0.125     | 0.006     | 0.016     | 0.091     | 0.070     | 0.012     |

Haplotypes

|          | Ashkenazi | American | Asian | Caucasian | Hispanic | Jewish |
|----------|-----------|----------|-------|-----------|----------|--------|
| C A G G C G C A C A A C G | 0.513   | 0.545   | 0.474 | 0.406     | 0.125    | 0.006  |
| C A G A C G C A C A A C G | 0.173   | 0.272   | 0.120 | 0.125     | 0.005    | -      |
| C A G A C G C A C A A G G | 0.005   | -       | -     | -         | -        | 0.006  |
| *3-*1-*1 | -         | -       | -     | -         | -        | ND     |
| *4B-*1-*1| -         | -       | -     | -         | -        | ND     |
| *17-*1-*1| -         | -       | -     | -         | -        | ND     |
| *1-*2-*1 | -         | -       | -     | -         | -        | ND     |
| *1-*2-*3 | -         | -       | -     | -         | -        | ND     |
| *1-*3-*1 | -         | -       | -     | -         | -        | ND     |
| *1-*5-*1 | -         | -       | -     | -         | -        | ND     |
| *1-*8-*1 | -         | -       | -     | -         | -        | ND     |
| *1-*1-*2 | -         | -       | -     | -         | -        | ND     |
| *1-*1-*4 | -         | -       | -     | -         | -        | ND     |

Abbreviation: CI, confidence interval.
aShaded boxes represent variant nucleotides.

The results have important implications for pharmacogenomic association studies involving the CYP2C locus and are clinically relevant when administering CYP2C-substrate medications.

Table 6. Linkage disequilibrium between CYP2C19*17 and CYP2C8*2

|          | Population | CYP2C19*17 frequency | CYP2C8*2 frequency | D’ | r² |
|----------|------------|----------------------|--------------------|----|----|
|          | African-American | 0.182                 | 0.100              | 0.813 | 0.325 | 0.075 |
|          | Asian       | 0.062                 | 0.004              | 0.471 | 0.018 | ND   |
|          | Caucasian   | 0.158                 | 0.002              | 1.000 | 0.028 | ND   |
|          | Hispanic    | 0.152                 | 0.022              | 0.626 | 0.057 | 0.017 |
|          | Ashkenazi   | 0.198                 | 0.004              | 1.000 | 0.017 | ND   |

Abbreviation: ND, not detected.

those previously reported in selected ethnic populations using fewer alleles and extend their findings by identifying both known and novel rare CYP2C haplotypes in other major racial and ethnic groups. Given the vast ethnic diversity prevalent among the Asian racial group, future CYP2C haplotype studies that include additional and more clearly defined ethnic Asian sub-populations are warranted.

In addition, future haplotype studies are warranted as novel CYP2C variants with clinical relevance are identified. For example, an intronic CYP2C9 polymorphism (rs7089580) was recently associated with warfarin dose variability in the African-American population; however, it is currently unclear if it is a functional non-coding variant with a role in gene transcription or if it is in LD with another functional CYP2C9 variant.66 As future studies establish which sequence variant of this potentially novel CYP2C9 allele is functionally relevant, it will be important to include it in CYP2C haplotype studies of the African-American and other populations. These studies could be instructive for the warfarin pharmacogenetics field as CYP2C haplotypes with loss-of-function variants in both CYP2C9 and CYP2C19 could influence dosing variability by affecting S- and R-warfarin pharmacokinetics, respectively. Although the relationship between CYP2C9 loss-of-function alleles and impaired S-warfarin metabolism is well established, a very recent study has reported an association between a CYP2C19 promoter variant (rs3814637) and R-warfarin clearance.67

In conclusion, our study determined the frequencies of 28 variant CYP2C19 and CYP2C8 alleles in the African-American, Asian, Caucasian, Hispanic and AJ populations, which highlight the polymorphic nature of CYP2C19 compared with CYP2C8 in all tested populations. Additionally, the recently described CYP2C19*4B allele, originally discovered in the AJ population, was identified in the Caucasian and Hispanic populations. Combining all genotyping results with our previous CYP2C9 data allowed for CYP2C haplotype structure analyses on all populations, which identified both previously reported and novel haplotypes. Taken together, these results have important implications for pharmacogenomic association studies involving the CYP2C locus and are clinically relevant when administering CYP2C-substrate medications.

CONFLICT OF INTEREST
J-SH has received research grant support from Fondation de France, INSERM, Federation Francaise de Cardiologie, Biotronik and Medco Research Institute; consulting fees from Biotronik and Medco Health Solutions; and lecture fees from Daiichi Sankyo, Eli Lilly and Bristol-Myers Squibb. SAS has been a consultant to USDS, Inc.

ACKNOWLEDGEMENTS
This research was supported in part by the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of
Health, through Grant KL2 RR029885 (SAS). The eSensor 2C19 Test reagents used in this study were generously provided by GenMark Diagnostics (Carlsbad, CA).

REFERENCES

1. Daly AK. Pharmacogenetics of the cytochrome P450. Curr Top Med Chem 2004; 4: 1733 – 1744.
2. Gray IC, Nobile C, Muresu R, Ford S, Spurr NK. A 2.4-megabase physical map spanning the CYP2C gene cluster on chromosome 10q24. Genomics 1995; 28: 328 – 332.
3. Goldstein JA. Clinical relevance of genetic polymorphisms in the human CYP2C subfamily. Br J Clin Pharmacol 2001; 52: 349 – 355.
4. Chen Y, Goldstein JA. The transcriptional regulation of the human CYP2C genes. Curr Drug Metab 2009; 10: 567 – 578.
5. Desta Z, Zhao X, Shu PX, Flockhart DA. Clinical significance of the cytochrome P450 2C19 genetic polymorphism. Clin Pharmacokinet 2002; 41: 913 – 958.
6. Price MJ, Tantry US, Gurbel PA. The influence of CYP2C19 polymorphisms on the pharmacokinetics, pharmacodynamics, and clinical effectiveness of P2Y12 inhibitors. Rev Cardiovasc Med 2011; 12: 1 – 12.
7. Scott SA, Sangkuhl K, Shuldiner AR, Hulot JS, Thorn CF, Altman RB. Genetic determinants of response to clopidogrel and cardiovascular events. N Engl J Med 2009; 360: 363 – 375.
8. Mega JL, Close SL, Wiviott SD, Shen L, Walker JR, Simon T et al. Genetic variants in ABCB1 and CYP2C19 and cardiovascular outcomes after treatment with clopidogrel and prasugrel in the TRITON-TIMI 38 trial: a pharmacogenetic analysis. Lancet 2010; 376: 1312 – 1319.
9. Wallentin L, James S, Storey RF, Armstrong M, Barratt BJ, Horro J et al. Effect of CYP2C19 and ABCB1 single nucleotide polymorphisms on outcomes of treatment with ticagrelor versus clopidogrel for acute coronary syndromes: a genetic substudy of the PLATO trial. Lancet 2010; 376: 1320 – 1328.
10. Scott SA, Edelmann L, Kornreich R, Erazo M, Desnick RJ. CYP2C19 and CYP2D6 allele frequencies in the Ashkenazi Jewish population. Pharmacogenomics 2007; 8: 721 – 730.
11. Scott SA, Edelmann L, Kornreich R, Desnick RJ. Warfarin pharmacogenetics: CYP2C9 and VKORC1 genotypes predict different sensitivity and resistance frequencies in the Ashkenazi and Sephardi Jewish populations. Am J Hum Genet 2008; 82: 495 – 500.
12. Scott SA, Edelmann L, Liu L, Luo M, Desnick RJ, Kornreich R. Experience with carrier screening and prenatal diagnosis for 16 Ashkenazi Jewish genetic diseases. Hum Mutat 2010; 31: 1240 – 1250.
13. de Morais SM, Wilkinson GR, Blasdiell J, Nakamura K, Meyer UA, Goldstein JA. The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans. J Biol Chem 1996; 271: 15419 – 15422.
14. Brandt JT, Close SL, Iturria SJ, Payne CD, Farid NA, Ernest 2nd CS et al. Common polymorphisms of CYP2C19 and CYP2C9 affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel. J Thromb Haemost 2007; 5: 2429 – 2436.
15. Collet JP, Hulot JS, Anzaha G, Pena A, Chastre T, Caron C et al. High doses of clopidogrel to overcome genetic resistance: the randomized crossover CLOLDIS-2 (Clopidogrel and response variability investigation study 2). JACC Cardiovasc Interv 2011; 4: 392 – 402.
16. Hulot JS, Bura A, Villard E, Azzii M, Removes V, Goyenvalle C et al. Cytochrome P450 CYP2C19*1Q loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. Blood 2006; 108: 2244 – 2247.
17. Giusti B, Gori AM, Marucci R, Saracini C, Sestini I, Panizza R et al. Cytochrome P450 CYP2C19*1Q loss-of-function polymorphism, but not CYP3A4 IVS10 + 12G/A and P2Y12 T744C polymorphisms, is associated with response variability to dual antiplatelet treatment in high-risk vascular patients. Pharmacogenet Genomics 2007; 17: 1057 – 1064.
18. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT et al. Cytochrome p-450 polymorphisms and response to clopidogrel. N Engl J Med 2009; 360: 354 – 362.
19. Shuldiner AR, O’Connell JR, Bilden KP, Gandhi A, Ryan K, Horenstein RB et al. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. JAMA 2009; 302: 849 – 857.
20. Collet JP, Hulot JS, Pena A, Villard E, Esteve JB, Silvain J et al. Cytochrome P450 CYP2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. Lancet 2009; 373: 309 – 317.
21. Hulot JS, Collet JP, Silvain J, Pena A, Bellennan-Appaix A, Barthelemy O et al. Cardiovascular risk in clopidogrel-treated patients according to cytochrome P450 2C19
2C19*2 loss-of-function allele or proton pump inhibitor coadministration: a systematic meta-analysis. *J Am Coll Cardiol* 2010; 56: 134 -- 143.

48 Megg JL, Simon T, Collet JP, Anderson JL, Antrim EM, Bilden K et al. Reduced-function CYP2C19 genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for PCI: a meta-analysis. *JAMA* 2010; 304: 1821 -- 1830.

49 Gladding P, Panattoni L, Webster M, Cho L, Ellis S. Clopidogrel pharmacogenomics: next steps: a clinical algorithm, gene-gene interactions, and an elusive outcomes trial. *JACC Cardiovasc Interv* 2010; 3: 995 -- 1000.

50 Roden DM, Shuldiner AR. Responding to the clopidogrel warning by the US food and drug administration: real life is complicated. *Drug Metab Dispos* 2010; 38: 445 -- 448.

51 Neder M, Msc PhD RM. Genetic testing for CYP450 polymorphisms to predict response to clopidogrel: current evidence and test availability. Application: pharmacogenomics. *PLoS Curr* 2010; 2: RRN1180.

52 Lee CC, McMinn GA, Babic N, Melis R, Yeo KT. Evaluation of a CYP2C19 genotype panel on the GenMark eSensor(R) platform and the comparison to the AutoGenomics Infiniti and Luminex CYP2C19 panels. *Clin Chim Acta* 2011; 412: 1133 -- 1137.

53 Cayla G, Hurot JS, O’Connor SA, Pathak A, Scott SA, Gruel Y et al. Clinical, angiographic, and genetic factors associated with early coronary stent thrombosis. *JAMA* 2011; 306: 1765 -- 1774.

54 Hoffmeyer S, Burk O, von Richter Q, Arnold HP, Brockmoller J, Johne A et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci USA* 2000; 97: 3473 -- 3478.

55 Nakamura T, Sakaeda T, Horinouchi M, Tamura T, Aoyama N, Shirakawa T et al. Functional characterization of five novel CYP2C8 variants, G171S, R186X, R186G, K247R, and K383N, found in a Japanese population. *Drug Metab Dispos* 2005; 33: 630 -- 636.

56 Dai D, Zeldin DC, Blaisdell JA, Chanas B, Coulter SJ, Ghanayem BL et al. Polymorphisms in human CYP2C19 decrease metabolism of the anticancer drug paclitaxel and arachidonic acid. *Pharmacogenomics* 2001; 11: 597 -- 607.

57 Totah RA, Rettie AE. Cytochrome P450 2C8: substrates, inhibitors, pharmacogenetics, and clinical relevance. *Clin Pharmacol Ther* 2005; 77: 341 -- 352.

58 Daily EB, Aquilante CL. Cytochrome P450 2C8 pharmacogenomics: a review of clinical studies. *Pharmacogenomics* 2009; 10: 1489 -- 1510.

59 Toyama A, Saito Y, Komamura K, Ueno K, Kamakura S, Otawa S et al. Five novel single nucleotide polymorphisms in the CYP2C8 gene, one of which induces a frame-shift. *Drug Metab Pharmacokinet* 2002; 17: 374 -- 377.

60 Hichia H, Tanaka-Kagawa T, Toyama A, Jinno H, Koyano S, Katori N et al. Impact of the haplotype CYP3A4*1B harboring the Thr185Ser substitution on paclitaxel metabolism in Japanese patients with cancer. *Clin Pharmacol Ther* 2006; 80: 179 -- 191.

61 Saito Y, Katori N, Toyama A, Nakajima Y, Yoshitani T, Kim SR et al. CYP2C8 haplotype structures and their influence on pharmacokinetics of paclitaxel in a Japanese population. *Pharmacogenet Genomics* 2007; 17: 461 -- 471.

62 Mao X, Bigham AW, Mei R, Gutierrez G, Weiss KM, Brutsaert TD et al. A genomewide admixture mapping panel for Hispanic/Latino populations. *Hum Genet* 2007; 120: 1133 -- 1137.

63 Perera MA, Gamaszon E, Cavallari LH, Patel SR, Poiindext S, Kittles RA et al. The missing association: sequencing-based discovery of novel SNPs in VKORC1 and CYP2C9 that affect warfarin dose in African Americans. *Clin Pharmacol Ther* 2011; 89: 406 -- 415.

64 Lane S, Al-Zubiedi S, Hatch E, Matthews I, Jorgensen AL, Deloukas P et al. Population pharmacokinetics of R- and S-warfarin: effect of genetic and clinical factors. *Br J Clin Pharmacol* 2012; 73: 66 -- 76.

65 Rahman A, Korzekwa KR, Grogan J, Gonzalez FJ, Harris JW. Selective biotransformation of taxol to 6 alpha-hydroxytaxol by human cytochrome P450 2C8. *Cancer Res* 1994; 54: 5543 -- 5546.

66 Li-Wan-Po A, Girard T, Farndon P, Cooley C, Lithgow J. Pharmacogenetics of CYP2C19: functional and clinical implications of a new variant CYP2C19*17. *Br J Clin Pharmacol* 2010; 69: 222 -- 230.

---

**Supplementary Information** accompaniments the paper on the *The Pharmacogenomics Journal* website (http://www.nature.com/tpj)