HHS Public Access

Author manuscript

Pharmacogenomics J. Author manuscript; available in PMC 2014 February 01.

Published in final edited form as:

Pharmacogenomics J. 2013 August ; 13(4): 369–377. doi:10.1038/tpj.2012.10.

Multi-ethnic Distribution of Clinically Relevant *CYP2C* Genotypes and Haplotypes

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Abstract

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 ~0.30–0.41; 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 (e.g., *2C19*2-2C9*1-2C8*4*, *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.

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

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CONFLICT OF INTEREST All other authors declare no conflict of interest.

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 greater than 80% identical, they can have distinct substrate specificities, and together are involved in the metabolism of ~20–30% of all medications.[3]

CYP2C19 contributes to the metabolism of a large number of clinically relevant drugs and drug classes such as antidepressants, benzodiazepines, mephenytoin, proton pump inhibitors, and the antiplatelet prodrug clopidogrel.[5–7] CYP2C9 is involved in the metabolism of tolbutamide, phenytoin, S-warfarin, losartan, and numerous anti-inflammatory drugs such as ibuprofen.[8–9] Some CYP2C9 substrates overlap with CYP2C8, including arachidonic acid, several non-steroidal anti-inflammatory drugs, and retinoic acid. CYP2C8 also plays a direct role in the metabolism of some important therapeutic drugs, including paclitaxel, amodiaquine, troglitazone, amiodarone, verapamil, cerivastatin, and fluvastatin.[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 catalogued by the Human Cytochrome P450 (CYP) 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 of 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*, **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 pharmacogenetic 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,[6, 27–31] we determined the *ABCB1* c. 3435C>T[32–35] allele and genotype frequencies for all tested populations.

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 IRB 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) according to the manufacturer's instructions. Two hundred and 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) as per the 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) 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) and PCR-restriction fragment length polymorphism (PCR-RFLP) 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 PCR reactions were performed in 10 μl containing ~50 ng of DNA, 2× PCR buffer (Invitrogen, Carlsbad, CA), 1.5 mM $MgCl_2$, 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; Supplemental 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 sec, 57°C for 30 sec, 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 (SAP) and 2.0 units of Exonuclease I (both from USB Corporation, Cleveland, OH). SNaPshot® primer extension reactions were performed in 10 μl containing 1× SNaPshot® Reaction Mix (Applied Biosystems), 0.2 μM of each allele-specific primer (Supplemental Table S1) and 3.0 μl of PCR product. Following the recommended thermal cycling, samples were treated with 1.0 unit of SAP, electrophoresed on an ABI Prism 3130 Genetic Analyzer, and analyzed using GeneMarker software v1.95 (SoftGenetics, State College, PA). Representative positive control samples for all identified *CYP2C* alleles were confirmed by bidirectional sequencing (Supplemental 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, six to ten colonies were propagated and bidirectionally sequenced using M13 and T7 vector-specific primers. All plasmid 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 χ^2 test for each racial and ethnic group. The χ^2 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 (r^2) expressed as a function of D'. The expectation-maximization (EM) 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, Inc., Cary, NC).

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.[26] 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 phenotypes[3, 57] were distributed as ultrarapid (3%, 2%, 3%, 2%, 4%), extensive (59%, 42%, 72%, 71%, 67%), intermediate (19%, 44%, 18%, 18%, 18%), and poor (5%, 8%, 4%, 2%, 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 gain- and loss-of-function alleles (e.g., **2/*17*). As such, the frequencies of individuals with unknown predicted metabolic phenotypes using this *CYP2C19* genotyping panel in the African-American, Asian, Caucasian, Hispanic, and AJ were 14%, 4%, 4%, 7%, and 8%, respectively.

ABCB1 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.I1145I) 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.[32–34] However, conflicting data have been reported regarding which allele (c.3435C or c.3435T) was associated with the increased risk.[35]Supplemental 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 genotype[34] indicated that 34 – 66% of all tested multi-ethnic individuals carried *CYP2C19* and *ABCB1* genotypes that conferred an increased risk for clopidogrel nonresponsiveness and/or adverse effects (Supplemental Table S4 and Supplemental 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%, 80%), heterozygous (22%, 8%, 20%, 20%, 20%), and homozygous variant/compound heterozygous (0.4%, 0%, 3%, 1%, 0%), respectively.

Linkage Disequilibrium 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), **3* (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 greater than 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 r^2 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*1* (AJs), *2C19*2-2C9*1-2C8*4* (African-Americans and AJs), and *2C19*1-2C9*3-2C8*3* (Asians and AJs).

DISCUSSION

The paucity of frequency data for variant *CYP2C19* and *CYP2C8* alleles beyond those commonly tested (e.g., **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 – 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 greater than 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 populations[22] 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.[7, 13] Consequently, our study using an expanded panel of 16 *CYP2C19* variant alleles identified individuals with certain genotypes (e.g., **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 – 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 prior to 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 – 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,[40–41] decreased platelet responsiveness *ex vivo* among clopidogrel-treated subjects,[42–45] and increased adverse cardiovascular event rates among clopidogrel-treated patients with acute coronary syndromes and/or those undergoing percutaneous coronary intervention.[33–34, 44–48] The increased risk among *CYP2C19* loss-of-function allele carriers, particularly for poor metabolizers, prompted product insert label revision by the U.S. Food and Drug Administration (FDA) and additional interest in implementing *CYP2C19* clinical testing to guide antiplatelet therapy for some cardiovascular patient populations.[28, 30, 49–52] 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.[26] In the current study, *CYP2C19*4B* was also identified in both the Caucasian and Hispanic populations at lower frequencies (≤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,[26] which confirms the independent existence of these two sub-alleles.

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,[32–35, 53] 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[54–56] and which allele (c.3435C or c.3435T) is associated with the increased cardiovascular risk.[35] Despite this discrepancy, large clinical studies found that c.3435T/T patients had a higher rate of adverse cardiovascular events than c.3435C homozygotes during clopidogrel therapy, which was independent from and compounded by *CYP2C19* loss-of-function alleles.[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.[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.[58–60] Moreover, the effects of the known variant *CYP2C8* alleles on activity may be substrate specific.[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,[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 linked with *CYP2C8*3* in the Swedish population,[20] underscoring the strong LD across the *CYP2C* region.[18–19] This finding highlighted the possibility of jointly inheriting multiple *CYP2C* reduced function alleles on individual haplotypes, which has important implications for the metabolism of common CYP2C9 and CYP2C8 substrates (e.g., arachidonic acid, nonsteroidal anti-inflammatory drugs, etc.). Global variation in *CYP2C9-CYP2C8* haplotype frequencies, including the *2C9*2-2C8*3* haplotype, has been reported in other worldwide populations,[21] and very recent reports have extended these haplotype studies to include *CYP2C19* in selected populations.[22–24] For example, in Nordic populations, the

*CYP2C19*17* gain-of-function allele was found almost exclusively with wild-type *CYP2C9*1* and *CYP2C8*1*.[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.[24]

Interrogating 36 variant *CYP2C* alleles in five major racial and ethnic populations resulted in 16 inferred *CYP2C* haplotypes with frequencies greater than 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[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,[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.[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 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 subpopulations 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 to *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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

This research was supported in part by grant KL2 RR029885 (S.A.S.) from the National Institutes of Health. The eSensor® 2C19 Test reagents used in this study were generously provided by GenMark Diagnostics (Carlsbad, CA).

J.-S.H. has received research grant support from Fondation de France, INSERM, Fédération 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. S.A.S. has been a consultant to USDS, Inc.

SOURCES OF SUPPORT: This research was supported in part by grant KL2 RR029885 (S.A.S.) from the National Institutes of Health. eSensor® 2C19 Test reagents for this study were supplied by GenMark Diagnostics (Carlsbad, CA).

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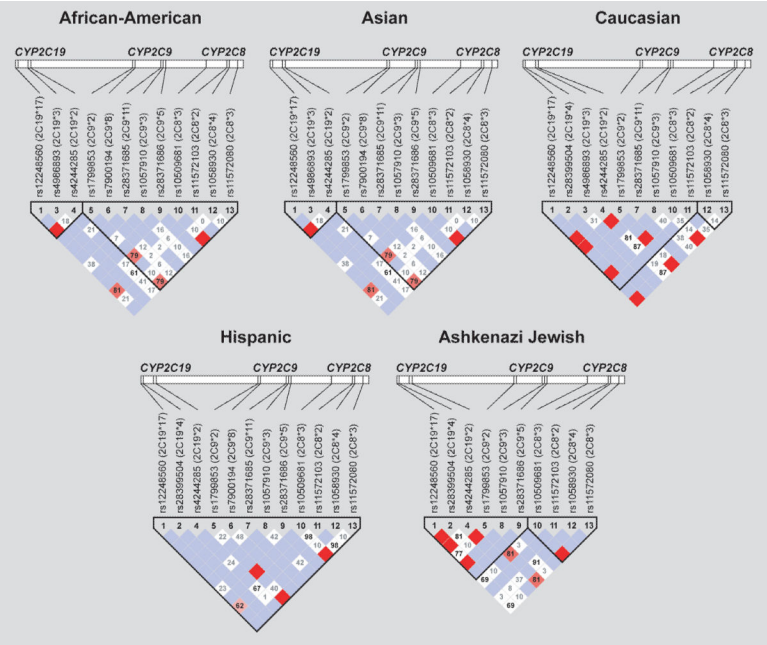

FIGURE 1.

Linkage disequilibrium (LD) across the *CYP2C* locus (10q23.33) in each tested population using 13 polymorphic SNPs. Pairwise LD between polymorphisms is expressed as D'. Significant linkage (logarithm of the odds, LOD≥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).

Pharmacogenomics J. Author manuscript; available in PMC 2014 February 01.

TABLE 1

CYP2C19 Allele Frequencies

	African-American (*n* = 500)		Asian (*n* = 500)		Caucasian (*n* = 500)		Hispanic (*n* = 500)		Ashkenazi Jewish[a] (*n* = 500)	
***CYP2C19* Allele**	**Freq.**	**95% CI**	**Freq.**	**95% CI**	**Freq.**	**95% CI**	**Freq.**	**95% CI**	**Freq.**	**95% CI**
**1*	0.594	0.551–0.637	0.614	0.571–0.657	0.696	0.656–0.736	0.704	0.664–0.744	0.632	0.590–0.674
**2*	0.194	0.159–0.229	0.276	0.237–0.315	0.132	0.102–0.161	0.128	0.099–0.157	0.146	0.115–0.177
**3*	0.004	0.000–0.010	0.048	0.029–0.067	0.004	0.000-0.010	0.000	0.000–0.000	0.000	0.000–0.000
**4A*	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000
**4B*	0.000	0.000–0.000	0.000	0.000–0.000	0.004	0.000–0.010	0.002	0.000–0.006	0.020	0.008–0.032
**5*	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000
**6*	0.000	0.000–0.000	0.000	0.000–0.000	0.002	0.000–0.006	0.000	0.000–0.000	0.000	0.000–0.000
**7*	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000
**8*	0.000	0.000–0.000	0.000	0.000–0.000	0.002	0.000–0.006	0.004	0.000–0.010	0.000	0.000–0.000
**9*	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.002	0.000–0.006	0.000	0.000–0.000
**10*	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000
**12*	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000
**13*	0.012	0.000–0.022	0.000	0.000–0.000	0.000	0.000–0.000	0.004	0.000–0.010	0.000	0.000–0.000
**14*	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000
**15*	0.014	0.004–0.024	0.000	0.000–0.000	0.002	0.000–0.006	0.004	0.000–0.010	0.004	0.000–0.010
**16*	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000
**17*	0.182	0.148–0.216	0.062	0.041–0.083	0.158	0.126–0.190	0.152	0.121–0.183	0.198	0.163–0.233
**22*	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000

n: number of alleles; CI: confidence interval.

[a]Data from Scott et al., 2011.[26]

TABLE 2

CYP2C19 Genotype Frequencies

Predicted CYP2C19 metabolizer phenotype/genotype	Observed (expected[a]) frequency (%)				
	African-American (*n* = 250)	Asian (*n* = 250)	Caucasian (*n* = 250)	Hispanic(*n* = 250)	Ashkenazi Jewish[b] (*n* = 250)
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 (35.3)	36.4 (37.7)	49.2 (48.4)	50.4 (49.6)	41.6 (39.9)
**1/*17*[c]	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 (0.3)	0.4 (0.6)	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 (0.0)
**2/*15*	0.4 (0.5)	0.0 (0.0)	0.0 (0.1)	0.0 (0.1)	0.4 (0.1)

Predicted CYP2C19 metabolizer phenotype/genotype	Observed (expected[a]) frequency (%)				
	African-American (*n* = 250)	Asian (*n* = 250)	Caucasian (*n* = 250)	Hispanic(*n* = 250)	Ashkenazi Jewish[b] (*n* = 250)
**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)	0.4 (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)	0.0 (0.1)	0.4 (0.1)	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)

n: number of subjects.

[a] Predicted Hardy-Weinberg frequencies.

[b] Data from Scott et al., 2011.[26]

[c] Although some studies classify **1/*17* individuals as ultrarapid metabolizers, the extensive metabolizer classification is consistent with Li-Wan-Po, et al., 2010.[68]

TABLE 3

CYP2C8 Allele Frequencies

	African-American (*n* = 500)		Asian (*n* = 500)		Caucasian (*n* = 500)		Hispanic (*n* = 500)		Ashkenazi Jewish (*n* = 500)	
***CYP2C8* Allele**	**Freq.**	**95% CI**	**Freq.**	**95% CI**	**Freq.**	**95% CI**	**Freq.**	**95% CI**	**Freq.**	**95% CI**
**1*	0.878	0.849–0.907	0.962	0.945–0.979	0.870	0.841–0.899	0.884	0.856–0.912	0.900	0.874–0.926
**2*	0.100	0.074–0.126	0.004	0.000–0.010	0.002	0.000–0.006	0.022	0.009–0.035	0.004	0.000–0.010
**3*	0.010	0.001–0.019	0.022	0.009–0.035	0.094	0.068–0.120	0.076	0.053–0.099	0.076	0.053–0.099
**4*	0.012	0.002–0.022	0.012	0.002–0.022	0.032	0.017–0.047	0.018	0.006–0.030	0.020	0.008–0.032
**5*	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000
**6*	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000
**7*	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000
**8*	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000
**9*	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000
**10*	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000
**12*	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000
**13*	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000	0.000	0.000–0.000
**14*	0.000	0.000–0.000	0.000	0.000–0.000	0.002	0.000–0.006	0.000	0.000–0.000	0.000	0.000–0.000

n: number of alleles; CI: confidence interval.

TABLE 4

CYP2C8 Genotype Frequencies

CYP2C8 Genotype	Observed (expected[a]) frequency (%)				
	African-American (*n* = 248)	Asian (*n* = 249)	Caucasian (*n* = 248)	Hispanic (*n* = 248)	Ashkenazi Jewish (*n* = 249)
Wild-type					
**1/*1*	76.6 (76.9)	92.0 (92.5)	77.2 (75.7)	78.6 (78.3)	80.0 (81.0)
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*	0.0 (0.2)	0.0 (0.0)	0.0 (0.0)	0.4 (0.3)	0.0 (0.1)
**2/*4*	0.4 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0(0.1)	0.0 (0.0)
**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)

n: number of subjects.

[a]Predicted Hardy-Weinberg frequencies.

TABLE 5

CYP2C Haplotype Frequencies

Haplotypes[a] 2C19-2C9-2C8	rs12248560 (C>T; *2C19*17*)	rs28399504 (A>G; *2C19*4*)	rs4986893 (G>A; *2C19*3*)	rs4244285 (G>A; *2C19*2*)	rs1799853 (C>%T; *2C9*2*)	rs7900194 (G>%A; *2C9*8*)	rs28371685 (C>%T; *2C9*11*)	rs1057910 (A>%C; *2C9*3*)	rs28371686 (C>%G; *2C9*5*)	rs10509681 (A>;G; *2C8*3*)	rs11572103 (A>%T; *2C8*2*)	rs1058930 (C>%G; *2C8*4*)	rs11572080 (G>%A; *2C8*3*)	Frequency (95% CI)				
														African-American	Asian	Caucasian	Hispanic	Ashkenazi Jewish
**1-*1-*1*	C	A	G	G	C	G	C	A	C	A	A	C	G	0.513 (0.473–0.554)	0.545 (0.505–0.585)	0.474 (0.435–0.513)	0.534 (0. 494–0.573)	0.406 (0.367–0.446)
**2-*1-*1*	C	A	G	A	C	G	C	A	C	A	A	C	G	0.173 (0.142–0.204)	0.272 (0.236–0.308)	0.120 (0.094–0.145)	0.125 (0.099–0.151)	0.132 (0.105–0.160)
**2-*1-*4*	C	A	G	A	C	G	C	A	C	A	A	G	G	0.005 (0.000–0.011)	-	-	-	0.006 (0.000–0.012)
**3-*1-*1*	C	A	A	G	C	G	C	A	C	A	A	C	G	-	0.048 (0.031–0.065)	-	-	-
**4B-*1-*1*	T	G	G	G	C	G	C	A	C	A	A	C	G	-	-	-	-	0.016 (0.006–0.026)
**17-*1-*1*	T	A	G	G	C	G	C	A	C	A	A	C	G	0.091 (0.068–0.115)	0.056 (0.037–0.074)	0.152 (0.124–0.180)	0.134 (0.107–0.161)	0.188 (0.156–0.219)
**17-*1-*2*	T	A	G	G	C	G	C	A	C	A	T	C	G	0.075 (0.054–0.097)	-	-	0.017 (0.007–0.028)	-
**1-*2-*1*	C	A	G	G	T	G	C	A	C	A	A	C	G	0.012 (0.003–0.021)	0.014 (0.005–0.024)	0.059 (0.041–0.078)	0.006 (0.000–0.013)	0.067 (0.047–0.087)
**1-*2-*3*	C	A	G	G	T	G	C	A	C	G	A	C	A	-	0.012 (0.003–0.021)	0.089 (0.067–0.112)	0.070 (0.050–0.090)	0.067 (0.047–0.088)
**1-*3-*1*	C	A	G	G	C	G	C	C	C	A	A	C	G	0.018 (0.007–0.028)	0.021 (0.009–0.032)	0.054 (0.036–0.072)	0.049 (0.032–0.066)	0.080 (0.058–0.102)
**1-*3-*3*	C	A	G	G	C	G	C	C	C	G	A	G	A	-	0.007 (0.000–0.014)	-	-	0.006 (0.000–0.013)
**1-*5-*1*	C	A	G	G	C	G	C	A	G	A	A	C	G	0.012 (0.003–0.021)	-	-	0.011 (0.003–0.019)	-
**1-*8-*1*	C	A	G	G	C	A	C	A	C	A	A	C	G	0.039 (0.023–0.055)	-	-	0.010 (0.002–0.018)	-
**1-*11-*1*	C	A	G	G	C	G	T	A	C	A	A	C	G	0.015 (0.005–0.025)	-	-	0.005 (0.000–0.011)	-
**1-*1-*2*	C	A	G	G	C	G	C	A	C	A	T	C	G	0.008 (0.001–0.015)	-	-	0.008 (0.001–0.015)	-
**1-*1-*4*	C	A	G	G	C	G	C	A	C	A	A	G	G	-	0.010 (0.002–0.019)	0.022 (0.010–0.033)	0.009 (0.001–0.016)	0.008 (0.001–0.015)

n: number of subjects; CI: confidence interval.

[a]Shaded boxes represent variant nucleotides.

TABLE 6

Linkage disequilibrium between *CYP2C19*17* and *CYP2C8*2*

Population	*CYP2C19*17* (rs12248560) Frequency	*CYP2C8*2* (rs11572103) Frequency	D'	r^2	*2C19*17-2C9*1-2C8*2* Frequency
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 Jewish	0.198	0.004	1.000	0.017	ND

ND: not detected.