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Identification of *CYP2C19*4B*: pharmacogenetic implications for drug metabolism including clopidogrel responsiveness

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Abstract

CYP2C19 is a principal enzyme involved in the bioactivation of the antiplatelet prodrug clopidogrel and common *CYP2C19* loss-of-function alleles are associated with adverse cardiovascular events. To assess the impact of the *CYP2C19*17* increased activity allele in the Ashkenazi Jewish (AJ) and Sephardi Jewish (SJ) populations and to determine the frequencies of additional variant alleles, 250 AJ and 135 SJ individuals were genotyped for *CYP2C19*2–*10*, **12–*17*, **22* and P-glycoprotein (*ABCB1*) c.3435C>T. Importantly, *CYP2C19*4*, a loss-of-function allele, was identified in linkage disequilibrium with **17*. This novel haplotype, designated *CYP2C19*4B*, significantly alters the interpretation of *CYP2C19* genotyping when testing **17*. Moreover, genotyping *CYP2C19*17* changed the frequency of extensive metabolizers from ~70 to ~40%, reclassifying ~30% as ultrarapid metabolizers. Combining *CYP2C19* and *ABCB1* identified ~1 in 3 AJ and ~1 in 2 SJ individuals at increased risk for adverse responses to clopidogrel. These data underscore the importance of including **4B* and **17* when clinically genotyping *CYP2C19*.

Keywords

CYP2C19; *CYP2C19*4B*; *CYP2C19*17*; clopidogrel; Ashkenazi Jewish; Sephardi Jewish

Introduction

The human cytochrome P450 2C (CYP2C) subfamily of enzymes is comprised of CYP2C8, CYP2C9, CYP2C18 and CYP2C19, which together are involved in the hepatic metabolism of ~25% of prescription and over-the-counter drugs.[1] Like many other *CYP450* superfamily members, the *CYP2C19* gene is highly polymorphic with over 25 known variant alleles.[2,3] For example, *CYP2C19*2* (c.681G>A; rs4244285) and **3* (p.W212X; rs4986893) are the most commonly studied *CYP2C19* loss-of-function alleles and their frequencies vary among racial groups (average multiethnic allele frequencies of ~18 and ~2%, respectively).[4–6] In contrast to these and other variant *CYP2C19* alleles with decreased enzyme activity (**4–*8*), the **17* promoter variant allele (c.−806C>T; rs12248560) is unique in that it results in increased activity as a consequence of enhanced transcription.[7,8] Based on identified *CYP2C19* genotypes, individuals can be categorized into four CYP2C19 metabolizer phenotypes:[9–11] ultrarapid (**1/*17* or **17/*17*), extensive (**1/*1*), intermediate (deficient allele heterozygote) and poor (deficient allele compound heterozygote or homozygote).

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Conflict of interest
The authors declare no conflict of interest.

CYP2C19 is involved in the metabolism of various clinically important drugs, including some antiulcer medications, antidepressants, β-adrenoceptor blockers and the anticonvulsant drug S-mephenytoin.[4,12] However, recent attention has focused on its role in the bioactivation of the antiplatelet agent clopidogrel,[13,14] which is commonly prescribed in patients with acute coronary syndrome and those undergoing percutaneous coronary intervention. Importantly, carriers of *CYP2C19* loss-of-function alleles have reduced responsiveness to clopidogrel with significantly increased risks of stent thrombosis, stroke, myocardial infarction and death following treatment.[11,15–19] However, these adverse effects may be reduced among patients with certain indications such as non-ST-segment elevation acute coronary syndrome and atrial fibrillation.[20] The growing body of literature implicating *CYP2C19* loss-offunction alleles in adverse clopidogrel responses prompted the United States Food and Drug Administration to implement a recent boxed warning on the clopidogrel label describing the relationship between *CYP2C19* pharmacogenetics and drug response, particularly noting the diminished effectiveness in poor metabolizers.[21] Additionally, these studies offer a compelling rationale for pharmacogenetic-guided anti-platelet drug selection and/or dosing in this patient population.[22–26]

In contrast to the deficient activity of *CYP2C19* alleles, *CYP2C19*17* recently has been associated with an enhanced platelet response to clopidogrel with an accompanying slight increase in bleeding risk during treatment.[27–30] *CYP2C19*17* appears to be common in Caucasians and less so in Asian populations (allele frequencies of~20 and~5%, respectively);[31] however, its frequency has not been adequately established in other racial and ethnic groups. Moreover, recent studies have suggested that previously reported *CYP2C19* allele, genotype and metabolizer phenotype frequencies should be reassessed in light of the recent identification of the **17* allele.[32]

We previously reported the *CYP2C19*2–*8* allele frequencies for the Ashkenazi Jewish (AJ) population and found that they were similar to those in other European Caucasian populations.[33] Notably, the **4* (c.1A>G; rs28399504) null mutation frequency was found to be ~2%, slightly higher than its reported frequencies in Caucasian and Hong Kong Chinese cohorts (~0.6%).[34,35] To assess the impact of including **17* in AJ testing panels and to determine the population structure of additional variant *CYP2C19* alleles in both the AJ and Sephardi Jewish (SJ) populations, we regenotyped the AJ individuals previously tested for *CYP2C19*2–*8*, for *CYP2C19*2–*10*, **12–*17* and **22*, and compared these results with a large SJ cohort. Additionally, the ATP-binding cassette, subfamily B (MDR/TAP), member 1 (*ABCB1*) c.3435C>T (rs1045642) polymorphism recently associated with impaired clopidogrel absorption and adverse clinical response[17,36–38] was interrogated in both populations. By employing an expanded *CYP2C19* genotyping panel coupled with allele-specific cloning and sequencing, our study identified a novel haplotype, designated *CYP2C19*4B*, that has important pharmacogenetic implications for drug metabolism, particularly when clinically assessing *CYP2C19* for clopidogrel responsiveness among patients initiating antiplatelet therapy. Moreover, genotyping *CYP2C19*17* in the AJ and SJ populations significantly changed the frequency of extensive metabolizers by reclassifying ~30% as ultrarapid metabolizers. Importantly, when combining all of the studied *CYP2C19* and *ABCB1* variants, ~1 in 3 AJ and ~1 in 2 SJ individuals may be at increased risk for adverse cardiovascular responses to clopidogrel.

Materials and methods

Specimens

Peripheral blood samples were obtained with informed consent from self-reported unrelated AJ (n=250) and SJ (n=135) individuals from the greater New York metropolitan area undergoing routine carrier screening for Jewish genetic diseases.[33,39,40] All personal

identifiers were removed, and the samples were tested anonymously. Genomic DNA was isolated using the Puregene DNA Purification kit (Qiagen, Valencia, CA, USA) as per the manufacturer's instructions.

Genotyping

The designations of all *CYP2C19* alleles refer to those defined by the Cytochrome P450 Allele Nomenclature Committee (http://www.cypalleles.ki.se/cyp2c19.htm).[3] Eleven variant *CYP2C19* alleles (**2*–**10*, **13* and **17*) were genotyped using the eSensor 2C19 Test (GenMark Diagnostics, Carlsbad, CA, USA) as per the manufacturer's instructions. The wild-type *CYP2C19***1* allele was assigned in the absence of other detectable variant alleles. Representative positive control samples for identified alleles were confirmed by bidirectional sequencing (see below).

Genotyping of *CYP2C19***12*, **14*, **15*, **16*, **22* and *ABCB1* c.3435C>T was performed using a multiplexed SNaPshot single base extension assay (Applied Biosystems, Carlsbad, CA, USA), whereby *CYP2C19* exons 1, 4 and 9 were initially PCR amplified with exon 27 of *ABCB1*. Reactions were performed in 10 μl containing ~50 ng of DNA, 1× PCR buffer (Invitrogen, Carlsbad, CA, USA), 3.0mM $MgCl_2$, 0.2mM of each dNTP, forward and reverse primers (*CYP2C19* exon 1: 0.4 μM; exon 4: 0.5 μM; exon 9: 0.2 μM; *ABCB1* exon 27: 0.4 μM; Supplementary Table S1) and 1.0 unit of Platinum *Taq* DNA Polymerase (Invitrogen). Amplification consisted of an initial denaturation step at 94 °C for 5 min followed by 38 amplification cycles (94 °C for 30 s, 58 °C for 30 s and 72 °C for 1 min) and a final incubation at 72 °C for 5 min. Amplicons were digested with 3.0 units of shrimp alkaline phosphatase and 2.0 units of Exonuclease I (both from USB Corporation, 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 4.0 μl of PCR product. Following the recommended thermal cycling, samples were treated with 1.0 unit of shrimp alkaline phosphatase, electrophoresed on an ABI Prism 310 Genetic Analyzer, and analyzed using GeneMarker software v1.85 (SoftGenetics, State College, PA, USA). Representative positive control samples for identified alleles were confirmed by bidirectional sequencing (see below).

CYP2C19 promoter and exon 1 cloning

Cloning and allele-specific sequencing of a 1.2-kb fragment encompassing *CYP2C19***17* (c.−806C>T) and **4* (c.1A>G) was performed on samples that harbored both alleles as determined by targeted genotyping. An initial PCR was performed in 25 μl containing ~100 ng of DNA, 1× Pfu DNA polymerase reaction buffer (Agilent Technologies, La Jolla, CA, USA), 0.2mM of each dNTP, 0.4 μM of forward and reverse primers (Supplementary Table S1) and 2.5 units of *PfuTurbo* Hotstart DNA polymerase (Agilent Technologies). Amplification consisted of an initial denaturation step at 95 °C for 5 min followed by 35 amplification cycles (95 °C for 30 s, 58 °C for 30 s and 72 °C for 2 min) and a final incubation at 72 °C for 10 min. A 3′-deoxyadenosine over-hang was added to blunt-ended amplicons by incubation with 1.0 unit of Platinum *Taq* DNA Polymerase (Invitrogen) at 72 °C for 10 min. These products were purified and cloned into the pCR2.1-TOPO vector using the TOPO TA Cloning Kit (Invitrogen) as per the manufacturer's instructions. For each sample, 6 to 10 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).

CYP2C19 sequencing

CYP2C19 exon sequencing was performed by an initial PCR in 25 μl containing ~100 ng of DNA, 1× PCR buffer (Invitrogen), 1.5mM $MgCl_2$, 0.2mM of each dNTP, 0.4 μM of

forward and reverse primers and 1.0 unit of Platinum *Taq* DNA Polymerase (Invitrogen). Amplification consisted of an initial denaturation step at 95 °C for 5 min followed by 35 amplification cycles (95 °C for 30 s, T_a (Supplementary Table S1) for 30 s and 72 °C for 1 min) and a final incubation at 72 °C for 10 min. Amplicons were digested with 2.5 units of shrimp alkaline phosphatase and 10.0 units of Exonuclease I (both from USB Corporation), and bidirectionally sequenced using amplification primers and/or nested sequencing primers as noted in Supplementary Table S1. In addition, an upstream *CYP2C19* promoter fragment encompassing the c.−3402C>T (rs11188072) allele common to **17* was sequenced in selected samples as described above. All bidirectional sequence data were analyzed using Mutation Surveyor software v3.30 (SoftGenetics).

Results

Identification of *CYP2C19**4B

To assess the impact of including **17* in AJ testing panels and to determine the population structure of variant *CYP2C19* alleles in the AJ population, 250 AJ individuals previously tested for *CYP2C19**2–**8* were re-genotyped for *CYP2C19**2–**10*, **12*, **13*, **16*, **17* and **22*. The initial *CYP2C19**1, **2*, **4* and **17* allele frequencies detected among the 250 AJ individuals were 0.619, 0.145, 0.020 and 0.216, respectively; *CYP2C19**3, **5–**10*, **12*, **13*, **16* and **22* were not detected among the 500 AJ alleles tested. Of note, complete concordance for **2* and **4* was observed between the previously reported genotyping platform[33] and that used in the current study. However, interestingly, four subjects were identified with three alleles each: two with **2*/**4*/**17* and two with **4*/**17*/**17*, inappropriately resulting in 504 total alleles among the 250 individuals. Moreover, the genotype frequencies deviated from Hardy–Weinberg equilibrium as no AJ individuals were identified with a **1*/**4* genotype, even though the **1* and **4* allele frequencies predicted that 2.5% of the population (approximately six individuals in 250) should have the genotype. In fact, all other **4* heterozygotes (n=6) were also heterozygous for **17*, resulting in a **4*/**17* genotype frequency (2.4%) greater than what was predicted (0.9%). Taken together, these results suggested that *CYP2C19**4*, a loss-of-function poor metabolizer allele,[34] occurred on a **17* background in the AJ population.

To confirm that *CYP2C19**4* and **17* were *in cis* among the **4* carriers in our AJ cohort, a 1.2-kb promoter/exon 1 amplicon encompassing the **4* and **17* variants was PCR amplified from each informative carrier and cloned into the pCR2.1-TOPO vector. Six to 10 colonies from the eight **4* and **17* heterozygotes were propagated and bidirectionally sequenced, and in all individuals, the **4* and **17* variants were on the same *CYP2C19* allele. To further define the coding sequence of this novel allele, all nine *CYP2C19* exons and exon/intron boundaries were bidirectionally sequenced, which identified two additional coding variants (c.99C>T (rs17885098) and c.991A>G (rs3758581)) consistent with the previously defined **4* haplotype.[3,4,34]

Detailed sequencing results of all identified single-nucleotide polymorphisms, including intronic variants, among the **4* carriers are summarized in Table 1. The identified haplotype of the novel allele (NG_008384.1:c.[−3402T; −806T; 1G; 99T; 991G]) was submitted for review by the Cytochrome P450 Allele Nomenclature Committee[2,3] and subsequently named *CYP2C19**4B*. All sequencing and genotyping data were correlated with known *CYP2C19* haplotypes[3] and the identified diplotypes among the **4B* carriers, including their coding region variants, are illustrated in Figure 1.

Interestingly, one **1*/**4B* AJ individual was found by sequencing to be heterozygous for the *CYP2C19**15* (p.I19L; rs17882687) allele, suggesting a **4B*/**15* genotype (Subject: AJ 8; Table 1; Figure 1). The unanticipated identification of **15* prompted its subsequent

inclusion into the multiplexed SNaPshot assay, in addition to the neighboring *CYP2C19*14* (p.L17P; rs55752064) allele (see Materials and methods).

CYP2C19 allele and genotype frequencies

After accounting for the novel *CYP2C19*4B* allele and incorporating **14* and **15* into the genotyping panel, the revised AJ *CYP2C19* allele and genotype frequencies were in Hardy–Weinberg equilibrium and are summarized in Tables 2 and 3. The *CYP2C19*1, *2, *4B, *15* and **17* allele frequencies among the 250 AJ individuals were 0.632, 0.146, 0.020, 0.004 and 0.198, respectively. Based on their genotypes, the reassigned metabolizer phenotypes in the AJ population were distributed as ultrarapid (29%), extensive (42%), intermediate (18%) and poor (4%) metabolizers.

In addition to the AJ cohort, 135 individuals of SJ descent were genotyped for all 16 variant *CYP2C19* alleles. Interestingly, in contrast to the AJ population, both the original *CYP2C19*4A* allele (without c.−806C>T) and the novel **4B* allele (with c.−806C>T), as well as the **8* (p.W120R; rs41291556) loss-of-function allele, were detected in the SJ population. Like the AJ *CYP2C19*4B* carriers, all informative SJ carriers were confirmed by allele-specific cloning and sequencing (Table 1; Figure 1). The *CYP2C19*1, *2, *4A, *4B, *8, *15* and **17* allele frequencies among the SJ individuals were 0.722, 0.096, 0.004, 0.011, 0.004, 0.004 and 0.159, respectively (Table 2). Although additional variant alleles were detected in the SJ compared with the AJ, the *CYP2C19* allele and genotype frequencies did not reach statistical significance between the two populations (P=0.07 and 0.16, respectively). Based on their genotypes, the assigned metabolizer phenotypes in the SJ population were distributed as ultrarapid (27%), extensive (53%), intermediate (16%) and poor (3%) metabolizers (Table 3).

ABCB1 allele and genotype frequencies

Several studies have found that homozygous carriers of the *ABCB1* c.3435C>T (p.I1145I) synonymous variant have higher rates of adverse cardiovascular events than c.3435C homozygotes during clopidogrel therapy, which was independent from and compounded by *CYP2C19* loss-of-function alleles.[17,36–38] However, conflicting data have been reported regarding which allele (c.3435C or c.3435T) is associated with the increased risk.[41] Supplementary Tables S2 and S3 summarize the identified *ABCB1* c.3435C>T allele and genotype frequencies, which were statistically different between the AJ and SJ populations (P=0.0002 and 0.0005, respectively). Of note, ~1 in 13 (8.4%) AJ and ~1 in 5 (19.3%) SJ individuals were c.3435T homozygotes.

Combined *CYP2C19* and *ABCB1* frequencies

Figure 2 and Supplementary Table S4 summarize the combined *CYP2C19* metabolizer phenotype and *ABCB1* genotype frequency profiles for the AJ and SJ, which were statistically different between the two populations (P<0.0001). Of note, when combining the *CYP2C19* and *ABCB1* frequency data, ~60–65% of AJ and SJ individuals carried a *CYP2C19* allele (**2, *4A, *4B, *8, *17*) and/or *ABCB1* genotype (c.3435T/T) that could influence their response to clopidogrel. However, increased risk for clopidogrel non-responsiveness and adverse cardiovascular events has largely been reported among individuals carrying a *CYP2C19* loss-of-function allele and/or those with an *ABCB1* c.3435T/T genotype.[17,38] Categorizing the AJ and SJ subjects using these specific criteria indicated that 34% of AJ (~1 in 3) and 43% of SJ (~1 in 2) individuals carried *CYP2C19* and *ABCB1* genotypes that conferred an increased risk for clopidogrel nonresponsiveness and/or adverse effects (P<0.0001; Figure 3; Supplementary Table S5).

Discussion

The growing body of literature on clopidogrel pharmacogenetics and the paucity of frequency data for variant *CYP2C19* alleles beyond the commonly studied **2* and **3* loss-of-function alleles prompted our population structure investigation of *CYP2C19* and *ABCB1* in the AJ and SJ populations. Importantly, our study identified a novel *CYP2C19* allele, designated *CYP2C19***4B*, in both Jewish ethnicities that has significant pharmacogenetic implications for drug metabolism, particularly when clinically assessing *CYP2C19* for clopidogrel responsiveness. The haplotype of the *CYP2C19***4B* allele (NG_008384.1: c.[−3402T; −806T; 1G; 99T; 991G]) is a combination of two previously identified alleles, **4* (NG_008384.1:c.[1G; 99T; 991G]) and **17* (NG_008384.1:c.[−3402T; −806T; 99T; 991G]), each with opposing phenotypic consequences. *CYP2C19***4* is a loss-of-function 'poor metabolizer' allele and **17* is an increased activity 'ultrarapid metabolizer' allele.[7,8,34]

The c.1A>G mutation of *CYP2C19***4* abolishes the ATG initiation codon and was originally identified by sequencing *CYP2C19* in Caucasians who were poor mephenytoin metabolizers.[34] The defective activity of *CYP2C19***4* was confirmed *in vitro* by its lack of recombinant protein expression in yeast and failure to translate CYP2C19 peptides in a coupled transcription/translation assay. However, *CYP2C19***4* was able to transcribe mRNA *in vitro*, indicating that the failure of CYP2C19 protein expression was at the level of translation.[34] Consequently, *CYP2C19***4B* would also fail to produce CYP2C19 protein, regardless of the increased transcriptional capacity mediated by the upstream c.–806C>T promoter variant.

Although the frequency of *CYP2C19***4B* was only ~2% in the AJ and SJ populations, its inclusion in genotyping panels is important, particularly given the high frequency of *CYP2C19***17* in Caucasian populations and the increasing interest in adding **17* to panels that include the commonly tested **2* and **3* alleles. For example, testing for **2*, **3* and **17* without **4B* would have misclassified ~1 in 25 AJ individuals, including eight intermediate metabolizers who would have been incorrectly classified as ultrarapid metabolizers and two poor metabolizers who would have been incorrectly classified as intermediate metabolizers. Testing for **2*, **3* and **17* without **4B* in the SJ would have misclassified ~1 in 45 individuals, including one intermediate metabolizer who would have been incorrectly classified as an ultrarapid metabolizer and two poor metabolizers who would have been incorrectly classified as intermediate metabolizers.

Recently, *CYP2C19***17* has been reported to be in linkage disequilibrium with the neighboring *CYP2C8***1* and *CYP2C9***1* wild-type alleles among Nordic individuals.[42] The identified linkage disequilibrium between **17* and **4* (comprising the **4B* allele) in the AJ and SJ populations was not observed in the Nordic cohort nor was **4B* identified in a recent *CYP2C19* sequencing study of Han Chinese individuals,[43] together suggesting that **4B* may be specific to Jewish subpopulations. However, preliminary studies in our laboratory have identified the **4B* allele in both Caucasians and Hispanics.

The inclusion of *CYP2C19***17* alone also significantly altered the frequencies of the predicted metabolizer phenotypes. For example, *CYP2C19***17* changed the frequency of extensive metabolizers (**1*/**1*) in both populations from ~70 to ~40%, with ~30% of individuals reclassified as ultrarapid metabolizers (**1*/**17* or **17*/**17*). Very recently, Sibbing *et al.*[29] assessed the impact of *CYP2C19***2* and **17* on clopidogrel responsiveness among patients undergoing clopidogrel maintenance therapy. They determined that individuals with a **1*/**17* or **17*/**17* genotype had lower residual ADP-induced platelet aggregation compared with wild-type individuals, suggesting that the **17* allele resulted in

the enhanced bioactivation of clopidogrel.[29] In addition, they reported that individuals with a *2/*17 genotype had higher residual platelet aggregation compared with wildtype individuals, but less than those carrying *2 without *17, suggesting that the ultrarapid *17 allele could not completely compensate for a heterozygous null allele. Although this implies that individuals with a *2/*17 genotype (or other loss-of-function allele/*17 compound heterozygotes) are intermediate metabolizers, in the absence of independent validation these genotypes were provisionally classified as having an 'unknown' metabolizer phenotype in our current study.

The *CYP2C19*15* variant allele was originally identified in an African population;[44] however, its phenotypic consequence is unknown. Therefore, the detected *1/*15, *2/*15 and *4B/*15 individuals in our study were also classified as having an 'unknown' metabolizer phenotype (Table 3). Although missense alterations at the amino-terminal region of CYP450 enzymes are often considered benign,[44] aberrant splicing, protein misfolding and/or expression alterations mediated by the *CYP2C19*15* allele could not be ruled out. As more ethnic subpopulation-specific *CYP2C19* alleles are identified and commercial genotyping panels are expanded, appropriate clinical assessment of unique genotype combinations will be critical to assign the appropriate metabolizer phenotypes and for the clinical application of expanded *CYP2C19* pharmacogenetic panels, particularly for clopidogrel responsiveness testing.

The *ABCB1* gene encodes the P-glycoprotein membrane efflux transporter, which is involved in the intestinal absorption and bioavailability of clopidogrel. Previously, the c.3435C>T allele was associated with duodenal protein expression and lower bioavailability of established P-glycoprotein substrates,[45,46] suggesting that *ABCB1* c.3435C>T might influence clopidogrel efflux and drug bioavailability. [36] However, given the conflicting data available on c.3435C>T and P-glycoprotein expression,[45–47] expanded studies are warranted to identify *ABCB1* haplotypes and assess their relationship to gene expression. Despite this discrepancy, some 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.[17,38] Our study identified a high frequency of c.3435C>T homozygotes in both Jewish populations (~10–20%), and when combined with the *CYP2C19* data, the majority (~60–65%) of AJ and SJ individuals harbored a *CYP2C19* and/or *ABCB1* genotype that could influence their response to clopidogrel.

In conclusion, our study identified a novel allele in the AJ and SJ populations, designated *CYP2C19*4B*, that is a variant of the *4 loss-of-function allele occurring on a *17 increased transcriptional background. The high frequency of *17 without the *4 variant in our cohorts suggests that the c.−806C>T (*17) promoter variant predates the initial occurrence of the c.1A>G (*4) allele. Three other important conclusions can be made from our population structure study: *CYP2C19*4B* would significantly alter the interpretation of *CYP2C19* genotyping when testing for *17 without the *4 mutation; inclusion of *CYP2C19*17* in our genotyping panel significantly changed the frequency of extensive metabolizers in the AJ and SJ cohorts; and, when combining *CYP2C19* and *ABCB1* genotypes, ~1 in 3 AJ and ~1 in 2 SJ individuals could have an increased risk for an adverse response to the commonly prescribed antiplatelet prodrug clopidogrel. Taken together, these data underscore the importance of including both *4B and *17, in addition to the commonly tested *2 and *3 alleles, when clinically assessing *CYP2C19* for pharmacogenetic-guided dosing, particularly when testing for clopidogrel responsiveness among patients initiating antiplatelet therapy. Moreover, these data suggest that additional prospective clinical studies on clopidogrel response are warranted that include the common *ABCB1* c.3435C>T polymorphism in addition to variant *CYP2C19* alleles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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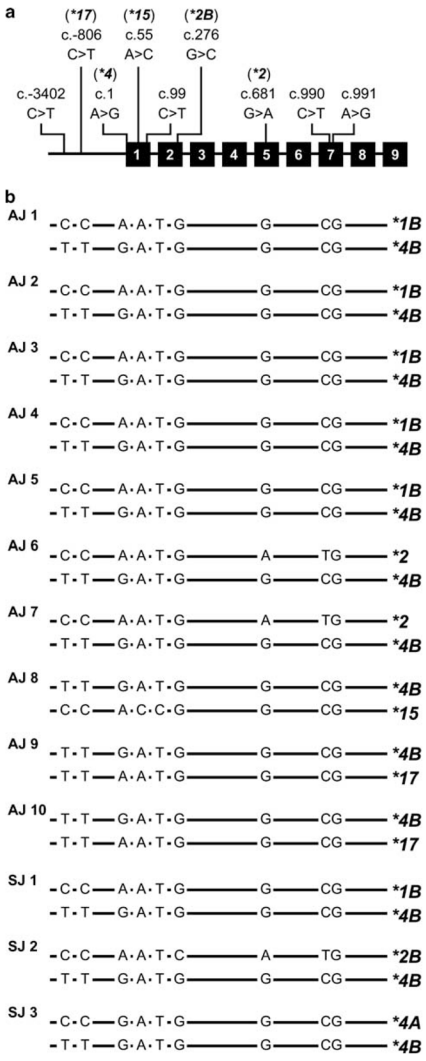

Figure 1.

Sequence and genotyping results for the Ashkenazi Jewish (AJ) and Sephardi Jewish (SJ) *CYP2C19*4B* carriers. (**a**) Illustration of the *CYP2C19* gene and location of relevant polymorphisms (not to scale). Exons are represented by numbered black boxes. (**b**) Identified *CYP2C19* haplotypes of the AJ and SJ *CYP2C19*4B* carriers with related alleles for **1B*/**4B*, **2*/**4B*, **4A*/**4B*, **4B*/**15* and **4B*/**17* genotypes. For detailed sequencing data, see Table 1.

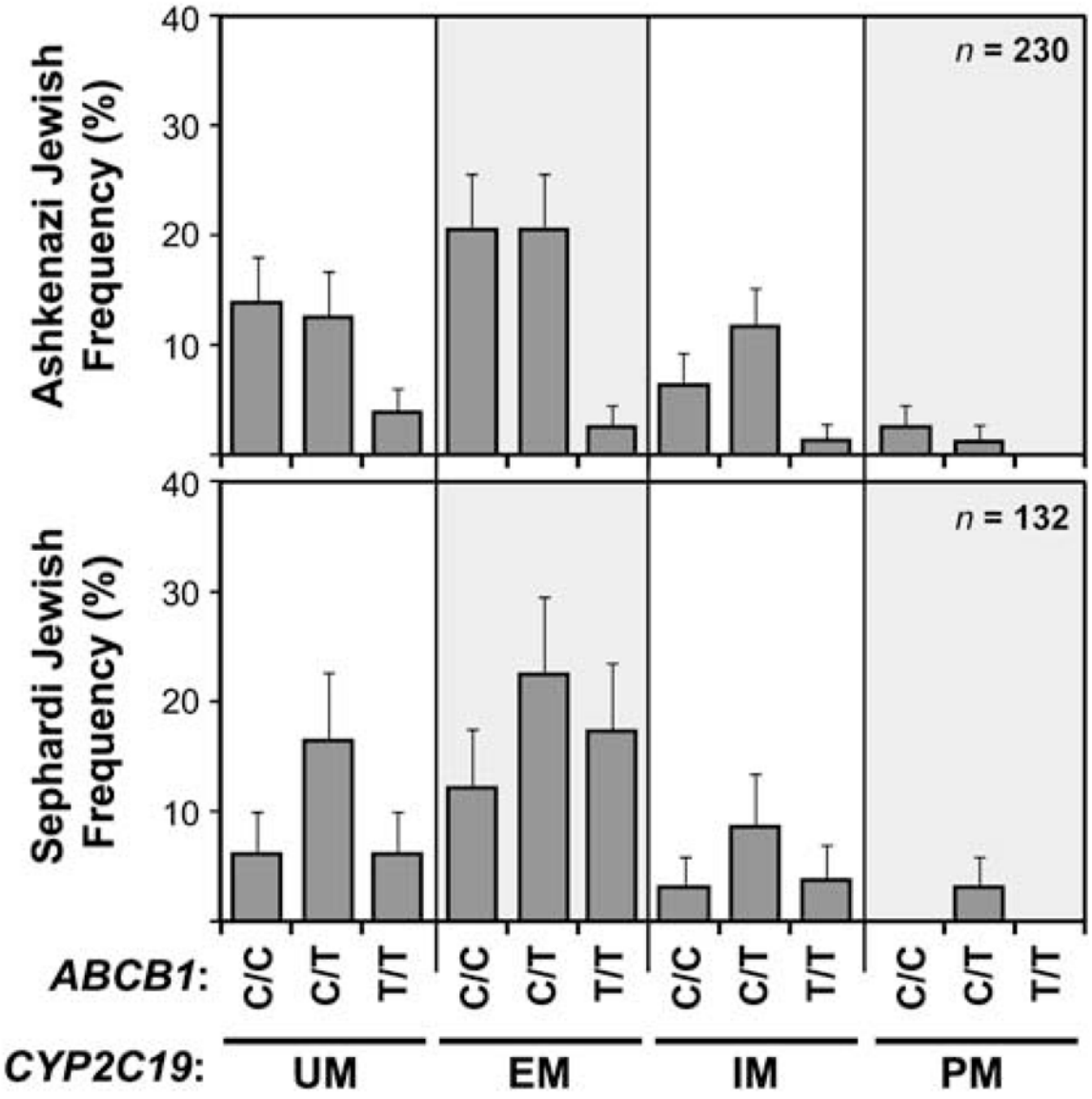

Figure 2.

Combined *CYP2C19* and *ABCB1* genotype frequency profiles in the Ashkenazi Jewish (AJ) and Sephardi Jewish (SJ) populations. Frequencies were subdivided based on *CYP2C19* predicted metabolizer phenotype and *ABCB1* c.3435C>T genotype. Genotypes with 'unknown' predicted metabolizer phenotypes were not included in this analysis (see Table 3 and Supplementary Table S4). Statistically distinct *CYP2C19* metabolizer phenotype and *ABCB1* genotype profiles were detected between the AJ and SJ populations ($P<0.0001$). Error bars represent 95% confidence intervals; EM, extensive metabolizer; IM, intermediate metabolizer; *n*, number of subjects; PM, poor metabolizer; UM, ultrarapid metabolizer.

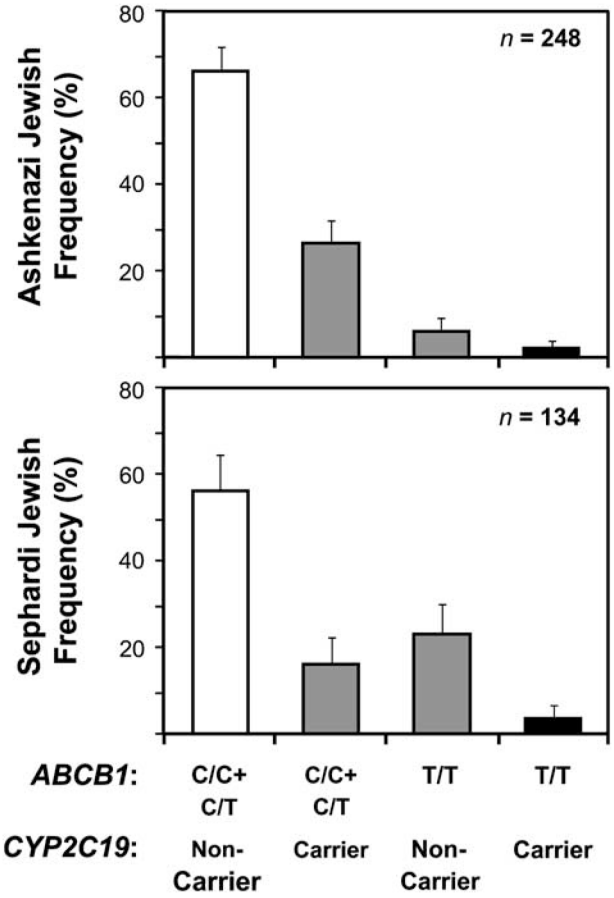

Figure 3.

Combined genotype frequencies subdivided by *CYP2C19* loss-of-function allele carrier status (noncarriers vs carriers) and *ABCB1* c.3435C>T genotype (C/C+C/T vs T/T) in the Ashkenazi Jewish (AJ) and Sephardi Jewish (SJ) populations. Genotype categories conferring an increased risk for clopidogrel nonresponsiveness are highlighted by gray bars and genotype categories conferring the highest risk are highlighted by black bars. Of note, 34% of AJ and 43% of SJ individuals carried *CYP2C19* and *ABCB1* genotypes with an increased or high risk for clopidogrel nonresponsiveness. Genotypes that included the *CYP2C19*15* allele were not included in this analysis (see Table 3 and Supplementary Table S5). Statistically distinct *CYP2C19* and *ABCB1* genotype category frequencies were detected between the AJ and SJ populations ($P<0.0001$). Error bars represent 95% confidence intervals; *n*, number of subjects.

Table 1

CYP2C19 sequencing results for the Ashkenazi Jewish (AJ) and Sephardi Jewish (SJ) *CYP2C19*4* carriers

Subject	Genotype	Exon 1						Exon 2		Exon 3	Exon 5	Exon 6	Exon 7			Exon 8
		c.−3402 C>T (rs11188072)	*c.−98T>C (rs4986894)*	*c.1A>G (*4) (rs28399504)*	*c.55A>C (*15) (rs17882687)*	*c.99C>T (rs17885098)*	*c.168+ 96T>C*	*c.169− 231G>A (rs7916649)*	*c.276 G>C (*2B) (rs17878459)*	*c.332− 23A>G (rs12769205)*	*c.681G>A (*2) (rs4244285)*	*c.820−51 C>G (rs4417205)*	*c.962−196 T>A (rs28399513)*	*c.990 C>T (rs3758580)*	*c.991 A>G (rs3758581)*	*c.1150−106 T>C (rs4917623)*
AJ 1	**1B/*4B*	C/T	T/T	A/G	A/A	T/T	T/C	G/A	G/G	A/A	G/G	C/C	T/T	C/C	G/G	T/C
AJ 2	**1B/*4B*	C/T	T/T	A/G	A/A	T/T	T/T	G/A	G/G	A/A	G/G	C/C	T/T	C/C	G/G	T/C
AJ 3	**1B/*4B*	C/T	T/T	A/G	A/A	T/T	T/T	G/A	G/G	A/A	G/G	C/C	T/T	C/C	G/G	T/C
AJ 4	**1B/*4B*	C/T	T/T	A/G	A/A	T/T	T/T	G/A	G/G	A/A	G/G	C/C	T/T	C/C	G/G	T/T
AJ 5	**1B/*4B*	C/T	T/T	A/G	A/A	T/T	T/T	G/A	G/G	A/A	G/G	C/C	T/T	C/C	G/G	T/C
AJ 6	**2/*4B*	C/T	T/C	A/G	A/A	T/T	T/T	A/A	G/G	A/G	G/A	C/G	T/A	C/T	G/G	T/T
AJ 7	**2/*4B*	C/T	T/C	A/G	A/A	T/T	T/T	A/A	G/G	A/G	G/A	C/G	T/A	C/T	G/G	T/T
AJ 8	**4B/*15*	C/T	T/T	A/G	A/C	C/T	T/T	G/A	G/G	A/A	G/G	C/C	T/T	C/C	G/G	T/T
AJ 9	**4B/*17*	T/T	T/T	A/G	A/A	T/T	T/T	A/A	G/G	A/A	G/G	C/C	T/T	C/C	G/G	T/T
AJ 10	**4B/*17*	T/T	T/T	A/G	A/A	T/T	T/T	A/A	G/G	A/A	G/G	C/C	T/T	C/C	G/G	T/T
SJ 1	**1B/*4B*	C/T	T/T	A/G	A/A	T/T	T/T	G/A	G/G	A/A	G/G	C/C	T/T	C/C	G/G	T/T
SJ 2	**2B/*4B*	C/T	T/C	A/G	A/A	T/T	T/T	A/A	G/C	A/G	G/A	C/G	T/A	C/T	G/G	T/T
SJ 3	**4A/*4B*	C/T	T/T	G/G	A/A	T/T	T/T	G/A	G/G	A/A	G/G	C/C	T/T	C/C	G/G	T/C

Table 2

Ashkenazi Jewish (AJ) and Sephardi Jewish (SJ) *CYP2C19* allele frequencies

Allele	AJ (*n* =500)				SJ (*n* =270)	
	CYP2C19*2–*8 *panel*[a]		CYP2C19*2–*10, *12–*17, *22 *panel*		CYP2C19*2–*10, *12–*17, *22 *panel*	
	Frequency	**95% CI**	**Frequency**	**95% CI**	**Frequency**	**95% CI**
**1*	0.834	0.801–0.867	0.632	0.590–0.674	0.722	0.669–0.776
**2*	0.146	0.115–0.177	0.146	0.115–0.177	0.096	0.061–0.131
**4A*	ND	ND	0.000	0.000–0.000	0.004	0.000–0.011
**4B*	0.020[b]	0.008–0.032[b]	0.020	0.008–0.032	0.011	0.000–0.024
**8*	ND	ND	0.000	0.000–0.000	0.004	0.000–0.011
**15*	ND	ND	0.004	0.000–0.010	0.004	0.000–0.011
**17*[c]	ND	ND	0.198	0.163–0.233	0.159	0.116–0.203

Abbreviations: CI, confidence interval; *n*, number of alleles; ND, not determined.

[a]Partial data from Scott *et al.*, 2007.[33]

[b]Previously reported as *CYP2C19*4*.[33]

[c]*CYP2C19*17* allele frequency does not include the c.−806C>T polymorphism of the **4B* haplotype.

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Table 3

Ashkenazi Jewish (AJ) and Sephardi Jewish (SJ) *CYP2C19* genotype frequencies

Predicted metabolizer phenotype by genotype	AJ (*n* = 250)		SJ (*n* = 135)
	CYP2C19*2–*8 *panel*[a]	CYP2C19*2–*10, *12–*17, *22 *panel*	CYP2C19*2–*10, *12–*17, *22 *panel*
	Observed (expected)[b] frequency (%)	Observed (expected)[b] frequency (%)	Observed (expected)[b] frequency (%)
Ultrarapid metabolizer (UM)			
**1/*17*	ND (ND)	25.2 (25.0)	23.0 (23.0)
**17/*17*	ND (ND)	3.6 (3.9)	3.7 (2.5)
Total	ND (ND)	28.8 (28.9)	26.7 (25.5)
Extensive metabolizer (EM)			
**1/*1*	70.4 (69.6)	41.6 (39.9)	52.6 (52.2)
Intermediate metabolizer (IM)			
**1/*2*	22.8 (24.4)	16.0 (18.5)	14.1 (13.9)
**1/*4B*	3.2 (3.3)[c]	2.0 (2.5)	0.7 (1.6)
**1/*8*	0.0 (0.0)	0.0 (0.0)	0.7 (0.5)
Total	26.0 (27.7)	18.0 (21.0)	15.6 (16.0)
Poor metabolizer (PM)			
**2/*2*	2.8 (2.1)	2.8 (2.1)	1.5 (0.9)
**2/*4B*	0.8 (0.6)	0.8 (0.6)	0.7 (0.2)
**4A/*4B*	0.0 (0.0)	0.0 (0.0)	0.7 (0.0)
Total	3.6 (2.8)	3.6 (2.8)	3.0 (1.2)
Unknown[d,e]			
**1/*15*	ND (ND)	0.0 (0.5)	0.7 (0.5)
**2/*15*	ND (ND)	0.4 (0.1)	0.0 (0.1)
**2/*17*	ND (ND)	6.4 (5.8)	1.5 (3.1)
**4B/*15*	ND (ND)	0.4 (0.0)	0.0 (0.0)
**4B/*17*	ND (ND)	0.8 (0.8)	0.0 (0.4)
Total	ND (ND)	8.0 (7.4)	2.2 (4.2)

Abbreviations: *n*, number of subjects; ND, not determined.

[a] Partial data from Scott *et al.*, 2007.[33]

[b] Predicted Hardy–Weinberg frequencies.

[c] Previously reported as *CYP2C19*1/*4*.[33]

[d] The predicted phenotypes of these genotypes are currently unknown.

[e] See Discussion for comments relating to these genotypes.