Distribution of CYP2D6 Alleles and Phenotypes in the Brazilian Population

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Abstract

The CYP2D6 enzyme is one of the most important members of the cytochrome P450 superfamily. This enzyme metabolizes approximately 25% of currently prescribed medications. The CYP2D6 gene presents a high allele heterogeneity that determines great inter-individual variation. The aim of this study was to evaluate the variability of CYP2D6 alleles, genotypes, and predicted phenotypes in Brazilians. Eleven single nucleotide polymorphisms and CYP2D6 duplications/multiplications were genotyped by TaqMan assays in 1020 individuals from North, Northeast, South, and Southeast Brazil. Eighteen CYP2D6 alleles were identified in the Brazilian population. The CYP2D6*1 and CYP2D6*2 alleles were the most frequent and widely distributed in different geographical regions of Brazil. The highest number of CYP2D6 alleles observed was six and the frequency of individuals with more than two copies ranged from 6.3% (in Southern Brazil) to 10.2% (Northern Brazil). The analysis of molecular variance showed that CYP2D6 is homogeneously distributed across different Brazilian regions and most of the differences can be attributed to inter-individual differences. The most frequent predicted metabolic status was EM (83.5%). Overall 2.5% and 3.7% of Brazilians were PMs and UMs respectively. Genomic ancestry proportions differ only in the prevalence of intermediate metabolizers. The IM predicted phenotype is associated with a higher proportion of African ancestry and a lower proportion of European ancestry in Brazilians. PM and UM classes did not vary among regions and/or ancestry proportions therefore unique CYP2D6 testing guidelines for Brazilians are possible and could potentially avoid ineffective or adverse events outcomes due to drug prescriptions.

Introduction

Cytochrome P450 (CYP) is a superfamily of proteins responsible for metabolizing a wide spectrum of different substrates, both endogenous and xenobiotics. The CYP system has a central role in pharmacogenetics area since most medication prescribed is a substrate for these enzymes. CYP2D6 enzyme is one of the most important members of this family. Though CYP2D6 comprises only 2% of human hepatic CYP450 enzymes, it metabolizes more than 25% of all currently prescribed medications and it is not induced by environmental factors [1–6].

CYP2D6 spans a 4.3 Kb region on chromosome 22q13.1 and forms a cluster with two pseudo-genes CYP2D8P and CYP2D7P [7]. At present, more than 100 allelic variants and sub variants are described [8]. They are the result of different mutation types mainly single nucleotide polymorphism (SNPs), but insertion/deletion (indel), gene rearrangements, and deletion or duplications of the entire gene have also been observed. This high polymorphic level results in four CYP2D6 phenotypes: poor metabolizers (PM; two inactive alleles), intermediate metabolizers (IM; one reduced activity allele and one inactive allele or two reduced activity alleles), extensive metabolizers (EM; at least one functional allele), and ultrarapid metabolizers (UM; three or more functional copies) [9–11].

CYP2D6 allele and phenotype frequencies have been investigated in all major ethnic and geographical populations and present significant differences in allele frequencies within and between populations [2,10,12–14].

Brazilians form one of the most heterogeneous populations in the world. Amerindian people occupied the Brazilian territory when the Portuguese arrived in 1500 and colonized the country. Then between the 16th and 19th centuries, Africans were brought to Brazil as slaves. In addition to the Portuguese, other migratory waves occurred in the 19th and 20th centuries, mainly from Italy, Germany and Spain [15]. All of these migratory events contributed to the formation of a multi-ethnic and highly admixed
population. This heterogeneity was documented in several genetic studies that used uniparental or autosomal markers to demonstrate a typical, although non-uniform, tri-ethnic (European, African and Amerindian) pattern for the Brazilian population. This admixture process occurred in different ways in the various geographic regions of the country. In Northeastern Brazil, the African contribution is high; in the North, the contribution of Native Americans is pronounced; and in the South, the influences are predominantly European with reduced Amerindian and African contributions when compared to other Brazilian geographic regions [16–20].

Since the knowledge of allele distribution and frequency is required to effectively translate pharmacogenetics to the clinics and given the paucity of \textit{CYP2D6} data in the Brazilian population, the purpose of this study was to evaluate the influence of ethnic classifications, geographical origins and genetic ancestry in \textit{CYP2D6} alleles, genotype and predicted phenotype distributions in Brazilians.

### Results

A total of 18 different alleles and duplications were identified (Table 1). \textit{CYP2D6} *1, *2, *4, *5, *10, *17, *29, *35, *41, *7\times2, *2\times2 alleles were polymorphic in all Brazilian regions. These 11 alleles account for more than 97% of all investigated chromosomes. Some alleles and/or duplications were found exclusively in one region, e.g. \textit{CYP2D6}*34 and \textit{CYP2D6}*35\times2 in the Northeast, \textit{CYP2D6}*17\times2 in the South (Table 1).

| Allele frequency % (Number of chromosomes) |
|-------------------------------------------|
| \textit{CYP2D6} allele | Total sample | North | Northeast | Southeast | South |
| *1 | 39.9 (814) | 40.3 (198) | 36.8 (192) | 40.7 (210) | 41.9 (214) |
| *2 | 21.5 (438) | 24.4 (120) | 24.1 (126) | 18.8 (97) | 18.6 (95) |
| *3 | 0.7 (14) | 0.6 (3) | 0.8 (4) | 0.6 (3) | 0.8 (4) |
| *4 | 9.4 (191) | 10.8 (53) | 9.6 (50) | 7.7 (40) | 9.4 (48) |
| *5 | 4.6 (94) | 2.7 (13) | 5.4 (28) | 5.4 (28) | 4.9 (25) |
| *9 | 1.1 (23) | 1.0 (5) | 0.9 (5) | 1.3 (7) | 1.2 (6) |
| *10 | 2.05 (42) | 1.4 (7) | 1.7 (9) | 3.1 (16) | 1.9 (10) |
| *17 | 5.6 (114) | 5.3 (26) | 4.4 (23) | 7.4 (38) | 5.3 (27) |
| *29 | 3.4 (69) | 2.2 (11) | 2.3 (12) | 4.3 (22) | 4.7 (24) |
| *34 | 0.05 (1) | - | 0.2 (1) | - | - |
| *35 | 2.6 (53) | 2.2 (11) | 3.1 (16) | 2.9 (15) | 2.2 (11) |
| *39 | 0.8 (17) | 1.4 (7) | 0.4 (2) | - | 1.6 (8) |
| *41 | 5.5 (112) | 4.1 (20) | 7.8 (41) | 5.0 (26) | 4.9 (25) |
| *7\times2 | 0.55 (12) | 0.8 (4) | 0.6 (3) | 0.6 (3) | 0.4 (2) |
| *7\times3 | 0.05 (1) | 0.2 (1) | - | - | - |
| *7\times2 | 0.55 (11) | 0.6 (3) | 0.4 (2) | 0.8 (4) | 0.4 (2) |
| *7\times5 | 0.05 (1) | - | 0.2 (1) | - | - |
| *4\times2 | 0.2 (4) | 0.4 (2) | - | 0.4 (2) | - |
| *17\times2 | 0.05 (1) | - | - | - | 0.2 (1) |
| *35\times2 | 0.05 (1) | - | 0.2 (1) | - | - |
| Others* | 1.3 (27) | 1.6 (8) | 1.1 (6) | 1.0 (5) | 1.6 (8) |
| Total number of chromosomes | 2040 | 492 | 522 | 516 | 510 |

*SNP combination that could not be assigned to a known allele, for more information see Table S3.

\(\chi^2 = 70.184\) and \(p\text{-value} = 0.069\) for the comparison of the allele frequencies among the four regions.

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| \textit{CYP2D6} copy number | Total sample | North | Northeast | Southeast | South |
|-----------------------------|--------------|-------|-----------|-----------|-------|
| 3                           | 67           | 21    | 18        | 14        | 14    |
| 4                           | 16           | 3     | 4         | 7         | 2     |
| 5                           | 3            | 1     | 2         | 0         | 0     |
| 6                           | 1            | 0     | 1         | 0         | 0     |
| Number of individuals with more than two copies | 87 (8.5%) | 25 (10.2%) | 25 (9.6%) | 21 (8.1%) | 16 (6.3%) |

Table 2. Copy number variation of the \textit{CYP2D6} in the Brazilian population.

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genotypes observed in Brazilians are listed in Table S1. CYP2D6 copy number varied from three to six (Table 2). The frequency of individuals with more than two copies ranged from 6.3% (in Southern Brazil) to 10.2% (Northern Brazil). According to the genotyping procedure, the duplications could not be unambiguously assigned to an allele in some heterozygous individuals, therefore an underestimation of some duplicated alleles occurred. Overall CYP2D6 allele distribution is homogeneous in Brazil.

AMOVA showed that more than 99% of the variability observed in allele frequencies is due to differences among individuals; the differences between regions and self-reported skin color accounted respectively for 0.2% and 0.5% of the total variance observed (Table 3). Pairwise FST results obtained among regions and among self-reported skin color are shown in Table 4. All FST estimates were low and no differences among regions or self-reported skin color were observed.

Table 5 shows the frequencies of alleles classified by predicted activity. Alleles for reduced function (CYP2D6*9, *10, *17, *29, *41) were observed in 18% of the chromosomes investigated, 15.1% were non-functional alleles (CYP2D6*3, *4, *4×2, *5), 64% were functional alleles (CYP2D6*1, *2A, *35, *39), and 2.6% resulted in increased enzyme activity (CYP2D6*1×2, *1×3, *2×2, *35×2, *2×5). No differences in the prevalence of alleles grouped by function were observed among Brazilian regions ($\chi^2 = 10.7$; degrees of freedom = 9; p-value = 0.29).

Predicted phenotypes (UM, EM, IM, PM) frequencies stratified by Brazilian region and self-reported color are shown in Table 6. As expected, the most frequent predicted metabolic status was EM (83.5%). Overall 2.5% and 3.7% of Brazilians were PMs and UMs respectively. No differences were observed in predicted phenotype frequencies among Brazilian regions (p = 0.467) or self-reported skin color (p = 0.089).

Genomic ancestry based on the individual proportions of European, African and Amerindian ancestry independent of self-reported color was investigated in this cohort as a continuous variable. African ($p<0.001$) and European ($p<0.01$) proportions were significantly different among predicted phenotypes whereas Amerindian proportions did not vary among these categories.
Discussion

This is the most comprehensive study of variation at the CYP2D6 locus in the Brazilian population. A total of 18 different CYP2D6 alleles including duplications were identified in this Brazilian cohort, displaying a unique and complex allele distribution. In a country of continental dimensions like Brazil with distinct immigration patterns and different admixture processes, a homogeneous distribution of CYP2D6 alleles across geographical regions and skin color categories was unexpected. The homogeneity seen is in contrast with the results of previous pharmacogenetic studies in this population that demonstrated a highly heterogeneous distribution of several pharmacogene polymorphisms across geographical regions, as well as within the color/race categories adopted by the Brazilian census [21–27].

There are marked differences in CYP2D6 allele frequencies in populations of different continental origins. Certain alleles were observed in high frequencies in different populations such as CYP2D6*4 in Europeans, CYP2D6*10 in Asians and CYP2D6*17 in Africans [5,28]. In Brazilians the prevalence of CYP2D6*4 (9.2%) and CYP2D6*17 (5.6%) are intermediate between those described for Europeans and Africans as expected for an admixed population.

Few CYP2D6 screening surveys were previously performed in Brazilian subjects. Two studies described CYP2D6*3 and CYP2D6*4 allele frequencies in a southeastern population based on one SNP PCR-RFLP methodology. These allele frequencies were 0.04 and 0.14 for white subjects and 0.03 and 0.10 for black individuals, respectively, which are higher than those described herein [29,30]. Larger studies were performed in the southern Brazilian population [31–33]. Overall the allele frequencies were very similar to those observed herein, except for CYP2D6*4 which was observed in 18% of breast cancer patients [33]. These discrepancies in results among studies possibly reflect methodological issues such as sample sizes, subject characteristics such as different admixture proportions, healthy subjects or disease patients or different study designs.

The frequency of CYP2D6 duplications/multiplications alleles carriers is about 1–2% in Northern Europe [12], increases to 7–10% in the Mediterranean region [34,35], and become as high as 29% in Ethiopia [36], but in Sub-Saharan Africans the median of duplications/multiplications alleles is about 7% only [12]. In the present study, 87 of the 1020 volunteers (8.5%) were found to carry a duplication/multiplication allele, but only 2.6% of those alleles could be unambiguously assigned as functional alleles (CYP2D6*1×2, *1×3, *2×2, *35×2, *2×5).

In Brazil as in all continental groups, EM was the most common phenotype. Native South American individuals belong to either the UM or the EM class which predicts high metabolic capacity of these populations [12]. The prevalence of the PM phenotype appears to be higher among Europeans, whereas higher heterogeneity has been reported in the African continent where the prevalence of PMs ranged from 0 to 19% [2,9,12]. This phenotype is less frequent in Asian populations [12,37]. The frequency of predicted PMs in Brazilians was 2.5% independent of region or ancestry, which is similar to the prevalence of PMs reported in North American Latino populations (2.2%–6.6%) [9].

In Brazil, the second most common predicted phenotype was IM (7.1%). This finding is due, at least in part, to CYP2D6*17 (5.6%), CYP2D6*29 (3.4%), and CYP2D6*41 (5.5%) alleles.

Table 6. CYP2D6 predicted phenotype frequencies according to self-reported color and geographical region.

| Phenotype | North | Northeast | South | Total number of individuals |
|-----------|-------|-----------|-------|---------------------------|
| White     |       |           |       | 74                        |
| Brown     | 3.4   | 3.4       | 3.4   | 74                        |
| Black     | 1.4   | 3.4       | 3.4   | 74                        |
| Total     | 8.2   | 10.2      | 10.2  | 85                        |

The Multinomial Log-Linear analysis: self-reported skin color is not associated with the predicted phenotype (p = 0.089); region is not associated with the predicted phenotype (p = 0.467).

(Table 7). The IM predicted phenotype is associated with a higher proportion of African ancestry and a lower proportion of European ancestry in Brazilians.
Table 7. Genetic ancestry (mean ± standard deviation) proportions according to CYP2D6 phenotypes.

| CYP2D6 phenotype | Amerindian  | African     | European    |
|------------------|-------------|-------------|-------------|
| PM               | 0.145 ± 0.164 | 0.168 ± 0.222 | 0.687 ± 0.257 |
| IM               | 0.099 ± 0.098 | 0.387 ± 0.308 | 0.514 ± 0.318 |
| EM               | 0.117 ± 0.146 | 0.247 ± 0.265 | 0.636 ± 0.304 |
| UM               | 0.088 ± 0.118 | 0.242 ± 0.269 | 0.670 ± 0.298 |

p* = 0.314  0.001  0.013

*p-value for the Kruskal-Wallis One-Way ANOVA.

**Significantly higher mean in the pairwise comparisons. FDR adjusted p-values for the IM × PM, IM × EM, and IM × UM comparisons are, respectively: 0.0014, 0.011, and 0.048.

**Significantly higher mean in the pairwise comparisons with EM and UM phenotypes. FDR adjusted p-values for the IM × EM, and IM × UM comparisons are, respectively: 0.018, 0.027.

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Common decreased-function variants, CYP2D6*10, *17 and *41, led to higher number of IMs in East Asia, Africa and Middle East respectively [2,12]. In the admixed Brazilian population investigated herein this phenotypic class was associated with higher African and consequently with lower European ancestries proportions. Although no differences by race/color were observed among CYP2D6 phenotype frequencies we demonstrated that the influence of ancestry in Brazilians is better explained by the individual proportions of African and European ancestry as a continuous variable as previously shown in other pharmacogenetic studies in the Brazilian population [26,27,38]. However, this influence is restricted to the IM predicted phenotype.

Typical substrates for CYP2D6 are largely lipophilic bases and include some antidepressants, antipsychotics, antiarrhythmics, antiemetics, beta-blockers and opioids. In the Brazilian population, the frequency of PMs owing to CYP2D6 polymorphisms is 2.5% and 3.7% are UMs. Both groups have an altered capacity to metabolize some drugs and could thus potentially benefit from genotyping for CYP2D6 when treated with any of these drugs. Subjects with multiple gene copies will metabolize drugs more rapidly and therapeutic plasma levels will not be achieved at ordinary drug dosages, whereas individuals lacking functional CYP2D6 alleles metabolize CYP2D6 substrates at a lower rate, and the risk for adverse drug reactions is higher [39,40]. The CYP2D6 IMs constitute 7%, but these patients’ potential to benefit from genotyping for CYP2D6 is less clear. The importance of the IM phenotype is further complicated by drug specificity. CYP2D6*17, which is frequent in Brazilians, was described as being associated with reduced activity for several CYP2D6 substrates. However, CYP2D6*17 appears to be associated with normal activity for risperidone [41].

This work should be interpreted in the context of some limitations. First, we did not screen all described SNPs, but we investigated all alleles with frequencies higher than 1% in the Americas [42], except CYP2D6*12 and CYP2D6*82 but these alleles were found only in native Argentinian-Paraguayan and Mexican Amerindians respectively. Therefore, some alleles such as CYP2D6*6 were not observed and/or its tag SNP was not included because its global frequency is less than 1% including previous Brazilian samples [12,31–33], as well as the CYP2D6*13-like CYP2D7/2D6 hybrid genes, which are fairly rare (0.1–0.2%) in European and African populations [42]. Second, some alleles could not be unambiguously detected because their SNP combination did not match any known CYP2D6 alleles (Table S2). Although we ruled out genotyping errors employing new independent genotyping of these samples, we did not resequence them to eventually describe new variants because this procedure was beyond the scope of this exploratory study. Third, we estimated copy number based on only one assay that targets exon 9. Although some authors claim that this strategy has limitations and pitfalls [43,44], these limitations seem to be restricted to Asians where exon 9 conversions appear at higher frequencies. However, in other ethnic groups, one study compared four CYP2D6 regions (exon 1, intron 5, intron 6, and exon 9) in copy number determination and the four regions resulted in robust copy number assignments that were in agreement with genotyping, sequencing and extra-long PCR-based data [44]. Another investigation described that the TaqMan assay targeting exon 9 revealed high sensitivity and specificity [45]. Recently Fang et al. [46] compared all the three TaqMan real-time PCR assays for copy number determination (intron 2, intron 6, exon 9) and they showed high concordant results. The CYP2D6*5 allele was also confirmed by long-range PCR.

Implementing pharmacogenetics in clinical practice has proven to be a challenge worldwide, and it is expected that this would be an even greater challenge in admixed populations because of their heterogeneity and genetic diversity. The Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for codeine and tricyclic antidepressants therapies [28,47,48] strongly recommends CYP2D6 testing before codeine use, for other drugs the potential genotyping benefits are less clear. PM and UM classes did not vary among regions and/or ancestry proportions therefore unique guidelines for Brazilians are possible and could potentially avoid ineffective or adverse events outcomes.

Materials and Methods

Ethics Statement

The Ethics Committees of the Instituto Nacional de Cancer (INCA), Rio de Janeiro, Universidade Federal de Minas Gerais, Universidade Federal do Rio Grande do Sul, Universidade Federal do Ceará, and Universidade Federal do Pará approved the study as well as the written Informed Consent form. The samples were anonymized after collection. Each individual signed a written informed consent.

Subjects

The study cohort consisted of 1020 healthy adults recruited from the north, northeast, south and southeast regions of Brazil. Sample collection and the individual ancestry determination were fully described previously [24]. Briefly, each individual was asked to self-identify according to the classification scheme adopted by
| CYP2D allele | Nucleotide Position in the CYP2D6 gene | CYP2D6 duplication | Enzyme activity |
|--------------|----------------------------------------|--------------------|-----------------|
|              | -1584 31 100 1023 1846 2549 2615 2850 2988 3183 4180 |                    |                 |
| *1           | C G C C G A A C G G G G              | -                  | Normal          |
| *2           | G G C C G A A T G G G C              | -                  | Normal          |
| *3           | C G C C G G Del A C C G G G G        | -                  | None            |
| *4           | C G T C A A A C G G G C              | -                  | None            |
| *5<sup>b</sup> | -- -- -- -- -- -- -- -- -- -- -- -- | --                  | None            |
| *9           | C G C C C G A Del C G G G G          | -                  | Decr            |
| *10          | C G T C C G A A C G G G C            | -                  | Decr            |
| *17          | C G C T G A A T G G G C              | -                  | Decr            |
| *29          | C G C C G A A T G A C                | -                  | Decr            |
| *34          | C G C C G G A A T G G G G            | -                  | ND              |
| *35          | G A C C C G A A T G G G C            | -                  | Normal          |
| *39          | C G C C C G A A C G G G C            | -                  | Normal          |
| *41          | C G C C C G A A T A G C G            | -                  | Decr            |
| *1x          | C G C C C G A A C G G G G            | +                  | Incr            |
| *2x          | G/C G C C G A A A T G G G C          | +                  | Incr            |
| *4x          | C G T C A A A C G G G C              | +                  | None            |
| *17x         | C G C T G A A T G G G C              | +                  | ND              |
| *3x          | G A C C C G A A T G G G C            | +                  | Incr            |

Nucleotides in bold are the polymorphic sites.
Decre = decreased activity.
Incr = increased activity.
ND = activity not determined.
*According to The Human Cytochrome P450 (CYP) Allele Nomenclature Database [33].
*Deletion of the entire CYP2D6 gene.
SNPs ID numbers are listed in Table S2.
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the official Brazilian Census [49], which relies on self-perception of skin color. The subjects were distributed into the following three groups: white (n = 336), brown (n = 349), and black (n = 333).

Laboratory procedures

Genomic DNA was isolated from peripheral blood by standard procedures. CYP2D6 SNPs (Table 8; Table S3) were determined by allelic discrimination with TaqMan SNP Genotyping Assays according to the manufacturer’s recommended protocols. CYP2D6 gene deletion and/or duplications were identified by a TaqMan Copy Number Assay. Hs00010001_cn specificity: targets CYP2D6 exon 9 sequences and will not amplify CYP2D7 or CYP2D8 pseudogenes, or CYP2D6 alleles having CYP2D7 sequences in exon 9 (e.g. CYP2D6*36). An assay for Ribonuclease P RNA component H1 gene (RNase P, assay ID 440326) was used as reference to determine copy number. The duplication reaction was performed according to the manufacturer’s instructions. Samples carrying one and three copies were included in all plates as CYP2D6 copy number controls. The amplification products were analyzed with the CopyCaller software v2.0. Reactions were considered acceptable if confidence >95% and Z score <1.75.

Definition of alleles, genotypes and phenotype classes

Alleles were inferred using the software PHASE v.2.1. As parameters 100 burn-in steps followed by 10,000 Markov Chain Monte Carlo iterations were used [50,51]. The haplotypes generated by Phase were compared to the CYP2D6 allele nomenclature of The Human Cytochrome P450 (CYP) Allele Nomenclature Database [8]. For allele designation only perfect matches were considered. The CYP2D6*1 allele was set when no nucleotide change was observed in all genotyped SNPs (Table 8). When Phase software inferred a SNP combination that could not be assigned to a known allele, they were pooled as others (Table S3 shows more details about these alleles).

The prediction of enzyme activity corresponding to each haplotype was based on The Human Cytochrome P450 (CYP) Allele Nomenclature Database [8]. This classification of phenotypes is based on the assumption of dominance, in which the phenotype is determined by the most efficiant haplotype in the genotype. Based on these assumptions metabolic status-genotype (predicted phenotype) was defined as: ultrarapid metabolizer (UM): at least three active gene copies; extensive metabolizer (EM): one or two active alleles; intermediate metabolizer (IM): two reduced activity alleles or one reduced activity and one inactive allele; poor metabolizer (PM): two inactive alleles [13,41]. Other IM classifications could also be used such as the CPICs guidelines. But, these guidelines are gene/drug guidelines. They used a system IM classifications could also be used such as the CPICs guidelines.

Table 1 CYP2D6 Genotypes observed in Brazilians. (DOCX)

Table 2 Description of the alleles called Others. (DOC)

Table 3 Variants of the CYP2D6 genotyped and their SNP ID number. (DOC)

Author Contributions

Conceived and designed the experiments: MHH SDJP GSK. Performed the experiments: DCF JPG VAS. Analyzed the data: DCF [JG MEDM]. Contributed reagents/materials/analysis tools: AKRS MARS SDJP. Contributed to the writing of the manuscript: DCF JPG MHH GSK.

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Statistical analyses

Allele and genotype frequencies were estimated by gene counting. Genetic diversity based on allele frequencies was assessed by analysis of molecular variance (AMOVA) [52–54]. Two levels of diversity were assessed: among regions and among color/race strata. Inter-region variability was determined by FST and their 95% confidence intervals were estimated with diveRsity package [55] in the R language and environment [56]. The parameter used was 3,000 bootstraps.

Chi-square test was performed to compare the CYP2D6 allele and phenotypes among regions. Statistical associations between predicted phenotype and self-reported skin color or geographical region were inferred by fitting multinomial log-linear models. This procedure obviates the need for correction for multiple comparisons because the main effects and interaction terms are tested simultaneously within each regression context. Kruskal–Wallis test was performed to examine the association between the predicted phenotype and the genetic ancestry, followed by the Mann– Whitney U test for pairwise comparisons. The pairwise p-values were adjusted for the False Discovery Rate (FDR) [57]. Statistical analysis was performed using the SPSS18.0 statistical package for Windows, WinPEPI [58], and the Arlequin Software v.3.0 [59]. P-value<0.05 was considered significant in all analyses.

Supporting Information

1. Supporting Information CYP2D6 Genotypes observed in Brazilians. (DOCX)
2. Supporting Information Description of the alleles called Others. (DOC)
3. Supporting Information Variants of the CYP2D6 genotyped and their SNP ID number. (DOC)
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