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New *CYP2A6* gene deletion and conversion variants in a population of Black African descent

Abstract

Aims—Cytochrome P450 2A6 (*CYP2A6*) is a human enzyme best known for metabolizing nicotine and nitrosamine precarcinogens. Our aim was to discover and characterize new *CYP2A6* alleles in a population of Black African descent.

Materials & Methods—We used cloning, sequencing, and genotyping of genomic DNA to discover new variants, and *in vivo* nicotine pharmacokinetic phenotyping to characterize the functional effect of the new alleles.

Results—Four new *CYP2A6* alleles, *CYP2A6**4*G*, *4*H*, *1*B4*, and *1*L*, were discovered and characterized in a population of Black African descent. The two new deletion alleles, *CYP2A6**4*G* and *4*H*, are distinguished by different crossover junctions at 7.9kb and 7.8kb downstream of the *CYP2A6*+1ATG start site, respectively; their combined allele frequency is 1.6%. The new gene conversion alleles, *CYP2A6**1*B4* and *CYP2A6**1*L*, contain 27bp and 10bp of *CYP2A7* sequence in the *CYP2A6*3′-flanking region, respectively; their combined allele frequency is 7.3%. *CYP2A6**4 appears to associate with lower *CYP2A6* activity *in vivo*, while *CYP2A6**1*L* does not; however, *CYP2A6**1*L* confounds genotyping assays that use the 2A6R3 and 2A6R4 primers.

Conclusions—As new variants are discovered, the relationships between *CYP2A6* genotype, nicotine metabolism, smoking behaviors, and tobacco-related cancer risk will be further clarified.

Keywords

CYP2A6; deletion; gene conversion; nicotine; *trans*-3′-hydroxycotinine/cotinine ratio

1. Introduction

Summary Points

- *CYP2A6* is a human enzyme best known for metabolizing nicotine and nitrosamine precarcinogens.
- *CYP2A6* is highly polymorphic; the gene contains single nucleotide polymorphisms, deletions, duplications, gene conversions, etcetera (<http://www.cypalleles.ki.se/cyp2a6.htm>).
- Genetic variation can decrease, increase, or modify *CYP2A6* activity and/or substrate specificity.
- Generally, *CYP2A6* genotype is associated with slower nicotine metabolism, lighter cigarette consumption, shorter smoking duration, and lower lung cancer risk.

Cytochrome P450 2A6 (CYP2A6) is a human hepatic enzyme best known for metabolizing tobacco-related compounds, such as nicotine [1], cotinine [2], and nitrosamine precarcinogens [3]. The *CYP2A6* gene contains single nucleotide polymorphisms (SNPs), deletions, duplications, chimeras, and gene conversions. Genetic variation can decrease, increase, or modify CYP2A6 activity and/or substrate specificity [4]. The last several years have seen considerable advances in the identification of *CYP2A6* genetic variants. Currently, 37 numbered alleles, two duplication alleles, and a number of *CYP2A6*1* variants are named (<http://www.cypalleles.ki.se/cyp2a6.htm>).

In our previous publication describing *CYP2A6* genetics and *in vivo* nicotine pharmacokinetics in a population of Black African descent, we briefly reported two findings of interest; they are explored in detail in the present study. First, one of 281 genomic DNA samples failed to amplify in all of our *CYP2A6* PCR-based genotyping assays [5], suggesting that it may be homozygous for a novel *CYP2A6* gene deletion. Second, we reported a series of SNPs [5] that correspond to a stretch of *CYP2A7* (pseudogene) sequence in the 3'-flanking region of the *CYP2A6* gene (i.e. new gene conversion). The aim of the current study was to characterize the new deletion and conversion alleles through sequencing, genotyping, and association with *in vivo* nicotine pharmacokinetics. NonSNP genetic variation merits further attention as (a) gene conversions between *CYP2A6* and *CYP2A7* are proving to be a major challenge to gene- and allele-specific genotyping [6] and (b) genetic variation in *CYP2A6* contributes to nicotine pharmacology [7], smoking etiology [8], and tobacco-related cancer [9].

2. Methods

2.1. Overview

We conducted a *CYP2A6* genetic and nicotine pharmacokinetic study in current smokers (smoked at least 5 of 7 days) and nonsmokers (1 to 99 lifetime-cigarettes, nonsmoking during the study) from a Canadian population of Black African descent (N=281). The details of study recruitment, procedures, and pharmacokinetic analyses can be found in previous reports [5; 10]. In addition, we examined allele frequencies in American subjects, of different ethnicities (see Americans in Table 1), that are from another study [11]. The University of Toronto Research Ethics Board, Institutional Review Board Services (Canada), and Committee on Human Research at the University of California, San Francisco approved the studies.

2.2. Kinetic analysis

Kinetic measurements and analyses were performed in the Canadian population of Black African descent (N=281) as follows. Briefly, participants were interviewed regarding their smoking history and administered 4 mg of oral nicotine. The resulting nicotine, cotinine, and *trans*-3'-hydroxycotinine plasma levels were assessed by HPLC [12]. The *trans*-3'-hydroxycotinine/cotinine ratio (3HC/COT; at 270 min) and the estimated nicotine area under the concentration time curve (AUC; over 360 min) were proxy measures of CYP2A6 activity and nicotine clearance, respectively [10]. The *CYP2A6*1* group (N=124) is comprised of both *CYP2A6*1A* and **1B*. Individuals with alleles *CYP2A6*2*, **9*, **12*, **14*, **17*, **20*,

*21, *23, *24, *25, *26, *27, *28, *31, and *35 were excluded from the current kinetic analyses except for the compound heterozygote group (*CYP2A6**4/**X*; where **X* represents a *CYP2A6**6, *9, *17, or *23 allele). One participant with the genotype *CYP2A6**1/*4/**new hybrid allele* was excluded from the kinetic analyses.

2.3. Cloning and sequencing

2.3.1. Gene deletion variants—Segments of genomic DNA from two individuals with the new deletion alleles, approximately 8.6 kb in length, were amplified according to a one or two-step long PCR protocol [A] (Supplementary Tables 1, 2, and 3). Long PCR products were subcloned into a pCR-XL-TOPO vector (TOPOXL PCR Cloning® Kit, Invitrogen, Canada). Clones were fully sequenced or partially sequenced as follows: at least 500 bp from the 5′-region of the PCR insert (confirms *CYP2A7* sequence), at least 500 bp from the 3′-region of the PCR insert (confirms *CYP2A6* sequence), and at least 2000 bp surrounding the putative crossover region in the PCR insert (determines the point where the nucleotide sequence changes from *CYP2A7* to *CYP2A6*) (ACGT Corp, Canada). For further reference, we have defined the crossover junction as the point where the nucleotide sequence changes from *CYP2A7* to *CYP2A6*, and the crossover region as the 250 bp surrounding the junction.

2.3.2. Gene conversion variants—Genomic DNA from two individuals with the new gene conversion was amplified (9.2 kb), cloned, and sequenced according to a previously described long PCR protocol [5].

2.4. *CYP2A6* genotyping

Two new two-step PCR-based genotyping assays were developed, one to detect the new *CYP2A6**4*G* and *CYP2A6**4*H* deletion alleles and one to detect the new gene conversions that are characteristic of the *CYP2A6**1*B4* and *CYP2A6**1*L* alleles. The assay for *CYP2A6**1*B* was also modified to avoid the sequence variation identified in this study. Primers and conditions used for all new or modified genotyping assays are shown in Supplementary Tables 1, 2, and 3. Assays were developed using cosmids containing individual genomic clones of *CYP2A6*, *CYP2A7*, and *CYP2A13* as controls [13; 14].

2.5. Statistics

Mann-Whitney and Kruskal-Wallis tests were used to compare two or more groups, and Dunn's was used for multiple-comparison corrections when Kruskal-Wallis between multiple groups was $p < 0.05$. Statistical analyses were carried out using Prism (Prism 4 for Macintosh, Version 4.0c 2005. San Diego: GraphPad Software Inc.).

3. Results

Summary Points

- In the present study, four new *CYP2A6* alleles, *CYP2A6**4*G*, *4*H*, *1*B4*, and *1*L*, were discovered and characterized in a population of Black African descent.

- The two new deletion alleles, *CYP2A6**4*G* and *4*H*, have a combined allele frequency of 1.6%; *CYP2A6**4 appears to associate with lower *in vivo* nicotine metabolism.
- The new gene conversion alleles, *CYP2A6**1*B4* and *CYP2A6**1*L*, have a combined allele frequency of 7.3%. *CYP2A6**1*L* does not associate with *in vivo* nicotine metabolism; however, *CYP2A6**1*L* confounds some genotyping assays.

3.1. Gene deletion variants

Previously, we reported one of 281 genomic DNA samples failed to amplify in all of our *CYP2A6* genotyping assays [5]. It did amplify, however, in a PCR-based assay designed to detect *CYP2A7* in the 5′– and *CYP2A6* in the 3′–region of hybrid genomic DNA samples. Cloning and sequencing indicated that this null sample had two distinct deletion alleles, *CYP2A6**4*B* – an established allele [15] and *CYP2A6**4*G* – a new allele. Based on the *CYP2A6**4*G* sequence, we developed a genotyping assay that would identify the new deletion allele, as well as *CYP2A6**4*A*, *CYP2A6**4*D*, *CYP2A6**4*E*, and *CYP2A6**4*F* (Fig. 1). The new *CYP2A6**4 assay detects any deletion allele that has a crossover junction after intron 7 and before 8 255 bp from *CYP2A6*+1ATG; it will not detect *CYP2A6**4*B* (GenBank accession number **NG_000008.7**). Through genotyping, eight new deletion alleles were identified. We sequenced the deletion allele of an additional sample to confirm the genotyping assay, and this revealed a crossover junction that differed from *CYP2A6**4*G*. Differing deletion junctions indicate different evolutionary origins and thus distinct alleles; therefore, the second new deletion allele was named *CYP2A6**4*H*. For a detailed sequence alignment that illustrates the slightly different crossover junctions of *CYP2A6**4*G* and *4*H* see Figure 2. The frequency of the new deletions is 1.6% in this population of Black African descent. Table 1 shows deletion allele frequencies.

3.2 Gene conversion alleles

Previously, we reported a series of SNPs, 7073T>C, 7082G>C, 7094G>C, and 7099T>C [See supplementary material in reference 5]. Through cloning and sequencing, we demonstrate in the present study that these SNPs form a 27 bp stretch of *CYP2A7* sequence in the 3′–flanking region of *CYP2A6*. The new variant allele contains the 27 bp gene conversion (i.e. nucleotides 7073 to 7099) in haplotype with the 58 bp gene conversion that is found in all *CYP2A6**1*B* alleles; thus, the allele is named *CYP2A6**1*B4* (Fig. 3). Based on the *CYP2A6**1*B4* sequence, we developed a genotyping assay that identified several other samples with the new gene conversion. To confirm the genotyping assay, we sequenced the variant allele of an additional sample. This revealed a distinct new allele, named *CYP2A6**1*L*, with a smaller gene conversion (i.e. 10 bp; nucleotides 7073 to 7082) that is not in haplotype with the 58 bp gene conversion. For a detailed alignment that shows the nucleotide sequence of *CYP2A6**1*B4* and *1*L* see Figure 4. The frequency of *CYP2A7* sequence at position 7073 to 7082 or 7099 in the 3′–flanking region of the *CYP2A6* gene is 7.3% in the present study population. Table 1 shows the gene conversions' frequencies in several different ethn racial groups. Because the new *CYP2A6**1*B4* and *CYP2A6**1*L* gene conversions are located where the primers 2A6R3 and 2A6R4 are designed to anneal, we

modified the genotyping assay for *CYP2A6*1B*. The new *CYP2A6*1B* frequency can be found in Table 1.

3.3 *In vivo* nicotine pharmacokinetics

The *in vivo* nicotine pharmacokinetic phenotypes of 3HC/COT₂₇₀ and nicotine AUC₃₆₀ were significantly different between *CYP2A6*1*1*, *CYP2A6*1*4*, *CYP2A6*4*4*, and *CYP2A6*4*X* groups (Kruskal-Wallis, $p < 0.001$ [3HC/COT₂₇₀] and $p < 0.01$ [nicotine AUC₃₆₀]; Fig. 5A and B). However, nicotine pharmacokinetics were similar between *CYP2A6*1*1* and *CYP2A6*1*1L* groups (Mann-Whitney, $p = 0.19$ [3HC/COT₂₇₀] and $p = 0.81$ [nicotine AUC₃₆₀]; Fig. 5C and D) and similar between *CYP2A6*1*1*, *CYP2A6*1/*1B*, and *CYP2A6*1B*1B* groups (Kruskal-Wallis, $p = 0.60$ [3HC/COT₂₇₀] and 0.07 [nicotine AUC₃₆₀]; Fig. 5E and F).

4.0 Discussion

Summary Points

- The present study adds to the knowledge of nonSNP *CYP2A6* genetic variation in populations of Black African descent.
- As genotyping methods evolve, we expect that the relationships between *CYP2A6* genotype, nicotine metabolism, smoking etiology, and tobacco-related cancer risk will be further clarified.

The major findings of the present study are as follows. Four new alleles were discovered and characterized in this population of Black African descent. The two new deletion alleles, *CYP2A6*4G* and *CYP2A6*4H*, have distinct crossover junctions, and their combined allele frequency is 1.6% bringing the *CYP2A6*4* deletion allele frequency (excluding *CYP2A6*4B*) to 2.7% in this population. The two new gene conversion alleles, *CYP2A6*1B4* and *CYP2A6*1L*, contain different amounts of *CYP2A7* sequence at the same location in the 3'-flanking region of *CYP2A6*; together the gene conversions' frequency is relatively high at 7.3%.

To date, several different low frequency deletion alleles, namely *CYP2A6*4A*, **4B*, **4E*, **4F*, **4G*, and **4H* have been found in this population of Black African descent [5]. The number of distinct *CYP2A6*4* deletion alleles supports others findings that African populations show a greater diversity of deletion variants compared with other ethn racial groups [16]. *CYP2A6* plays a substantial role in nicotine and nitrosamine metabolism so identifying deletion alleles is important. *In vivo* *CYP2A6* activity should be lower in individuals with at least one deletion allele and accordingly, the average exposure to nicotine after oral administration (nicotine AUC₃₆₀) should be significantly higher [4]. However, three of the five *CYP2A6*1*4* individuals had unexpectedly high activity. One potential explanation could be if there was a duplication allele containing an active *CYP2A6* gene in haplotype with a *CYP2A6* deletion. In support, one individual with normal *CYP2A6* activity (data not shown) had the following genotype *CYP2A6*1A*4A*new hybrid allele*. The new hybrid allele remains to be fully characterized; however, it has *CYP2A6* sequence in the 5'-

region until intron 2, after which the sequence becomes *CYP2A7*. Overall, nine of the 12 individuals with kinetic data and a deletion allele had low *CYP2A6* activity.

Some of the allelic diversity in *CYP2A6* (e.g. *CYP2A6**26 [5]) may have originated from gene conversions between the *CYP2A6* gene and the *CYP2A7* pseudogene. Even if gene conversions do not result in amino acid changes, they are notable because they can create technical challenges in gene- and allele-specific genotyping [6]. The new gene conversions found in *CYP2A6**1B4 and *1L each result in a failure of primers, 2A6R3 and 2A6R4, to anneal to genomic DNA during PCR. As a result, the alternative allele is amplified and overestimated as homozygous, while the allele with the new gene conversion is underestimated. For example, if an individual had the actual genotype *CYP2A6**X/*1L (*CYP2A6**X denotes any named allele), then in any genotyping assays using the 2A6R3 or 2A6R4 primers the new gene conversions would prevent the amplification of the *CYP2A6**1L allele. The result would be an inferred genotype of *CYP2A6**X/*X and an overestimation of the frequency of *CYP2A6**X. A number of publications have used the 2A6R3 [17] or 2A6R4 [18] primers, which may have resulted in overestimations of some variant alleles [e.g., 6; 19].

*CYP2A6**1B is one of the more prevalent alleles in all ethnoracial groups [4]. Generally, this allele has a 58 bp gene conversion and nucleotides 6832A and 6835C in the 3'-UTR. Together, this specific sequence produces an mRNA transcript with greater stability [20]. *CYP2A6**1B is associated with faster nicotine metabolic clearance in European Americans and higher *CYP2A6* activity in Asian populations [6; 21]. Yet *CYP2A6**1B was not associated with faster activity in the present population. Because our PCR-based *CYP2A6**1B genotyping assay is designed to detect only part of the 58 bp gene conversion, we cannot be sure that the alleles have all of the specific sequence that is required for the greater transcript stability conferred by the *CYP2A6**1B allele. In support of this, we have described an allele, *CYP2A6**1B17, that has the 58 bp gene conversion but also has SNPs at positions 6832A>G and 6835C>A [5]. Based on *in vitro* studies, this allele should not produce greater transcript stability [20]. Therefore, a *CYP2A6**1B-type that is not an increase-of-function allele (e.g. like *CYP2A6**1B17) is noteworthy, if it predominates in populations of Black African descent, as it could contribute to the ethnoracial differences in nicotine metabolism and genotype associations [22].

The descriptor "of Black African descent" does not define a homogenous ethnic group, and African Canadians could be genetically distinct from African Americans due to genetic drift or gene flow. Populations of Black African descent from different geographic locations have different histories of ethnic admixture [23], and approximately 60% of the present African Canadian population self-identified with a Jamaican or Caribbean heritage [24]. To date, however, *CYP2A6* allele frequencies do not appear to differ substantively among populations of Black African descent [5; 21; 25]. Learning more about *CYP2A6* genetics in populations of Black African descent is important because compared to European Americans, African Americans have slower nicotine and cotinine metabolic clearance [22], smoke considerably fewer cigarettes per day [101], and experience greater morbidity from most smoking-related diseases [102]. *CYP2A6* genotype is associated with nicotine metabolism [26], smoking behavior [8], and lung cancer [9]. Thus, distinctive undiscovered genetic

variation in *CYP2A6* in populations of Black African descent may be a plausible biological explanation for some of these ethnoracial differences, and our findings will increase the precision of association studies. More generally, knowing *CYP2A6* genotype could allow for more accurate prediction of an individual's response to, or toxicity from, nicotine replacement therapy.

The PCR-based methods of the present study are advantageous because they amplify fairly large sections of genomic DNA. Consequently, precise haplotypes and crossover junctions can be established. A limitation of using primers and PCR-based assays is that there are virtually no areas in the 3'-flanking region of the *CYP2A6* gene that may be used with 100% confidence as gene specific primers.

Conclusions

Over the past five years, our group and others have demonstrated that there are new, specific, and interesting *CYP2A6* SNPs, indels, and copy number variants in populations of Black African descent [5; 27]. The present study adds to the knowledge of nonSNP *CYP2A6* genetic variation in populations of Black African descent. As genotyping methods evolve, we expect that the relationships between *CYP2A6* genotype, nicotine metabolism, smoking etiology, and tobacco-related cancer risk will be further clarified. Improving study design features, such as exclusion criteria, outcome measures, gene-gene interactions, and ethnicity, as well as precisely defining appropriate phenotypes will also be key to improving association studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

CYP2A6	Cytochrome P450 2A6
SNPs	single nucleotide polymorphisms
3HC/COT	<i>trans</i> -3'-hydroxycotinine/cotinine ratio
AUC	area under the concentration time curve

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Figure 1.

Schematic showing regions where deletion junctions are found. Region 1 is located within intron 8 or exon 9 (*CYP2A6**4*D*, [28]), region 2 is located in and just outside of the 3′–UTR (*CYP2A6**4*A*, *4*E*, and *4*F*; [5; 29]), region 3 is located in the near 3′–flanking region (*CYP2A6**4*G* and *4*H*), and region 4 is located in the far 3′–flanking region (*CYP2A6**4*B*; [15]). The first (1st) and second (2nd) amplification primers for both the old (captures deletion regions 1 and 2; [5]) and the new (captures deletion regions 1, 2, and 3) deletion two-step PCR-based genotyping assays are named and represented with arrows. The assays use common forward primers but the old assay uses the primers called 2A6R0 in the first and 2A6R4 in the second amplification [5], while the new assay uses the primers called 2A6R11 in the first and 2A6R12 in the second amplification. Exons are shown in black boxes.

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CYP2A6      TCTAGACAGATTCTTAAAAAGCACCTATTCTCAGTAAACAGCTTAGTATAGCAT...
CYP2A7      TCTAGACACATTCTTAAAAACCAACCCATTCTCAGTAAACAGCTTAGTATAGCAT...
CYP2A6*4G   TCTAGACACATTCTTAAAAACCAACCCATTCTCAGTAAACAGCTTAGTATAGCAT...
CYP2A6*4H   TCTAGACACATTCTTAAAAACCAACCCATTCTCAGTAAACAGCTTAGTATAGCAT...
*****
CYP2A6      ...ATAGTGAGTACAAAACCTTCTAGAAGATAATGTAGGAGAAAATCTAGATGACCTTAAG
CYP2A7      ...ATAGTGAGTACAAAACCTTCTAGAAGATAACGTAGGAGAAAATCTAGATGACCTTAAG
CYP2A6*4G   ...ATAGTGAGTACAAAACCTTCTAGAAGATAACGTAGGAGAAAATCTAGATGACCTTAAG
CYP2A6*4H   ...ATAGTGAGTACAAAACCTTCTAGAAGATAATGTAGGAGAAAATCTAGATGACCTTAAG
*****
CYP2A6      TTTGGTGATGGCTTATTAGATACAACACCAAAAGCAACATCCTTGAAGAAGCAATTGAT
CYP2A7      TTTGGTGATGACTTATTAGATACAACACCAAAAGCAACATCCTTGAAGAAGCAATTGAT
CYP2A6*4G   TTTGGTGATGACTTATTAGATACAACACCAAAAGCAACATCCTTGAAGAAGCAATTGAT
CYP2A6*4H   TTTGGTGATGGCTTATTAGATACAACACCAAAAGCAACATCCTTGAAGAAGCAATTGAT
*****
CYP2A6      AAGTTCTGTGTCATTAAAATTAGAAGCTTCTGCTCTGCAAAAGAATTGTCAAGAGAATGA
CYP2A7      AAGTTCTGTGTCATTAAAATTAGAAGCTTCTGCTCTGCAAAAGAATTGTCAAGAGAATGA
CYP2A6*4G   AAGTTCTGTGTCATTAAAATTAGAAGCTTCTGCTCTGCAAAAGAATTGTCAAGAGAATGA
CYP2A6*4H   AAGTTCTGTGTCATTAAAATTAGAAGCTTCTGCTCTGCAAAAGAATTGTCAAGAGAATGA
*****
CYP2A6      7913 bp from CYP2A6 +1ATG
CYP2A7      CAATACAAGCTACAGACTAGGAGAACATATTGACAGGGGACATACCTGATAAGGGACTTA
CYP2A6*4G   CAATATAAGCTACAGACTAGGAGAACATATTGACAGGGGACATACCTGATAAGGGACTTA
CYP2A6*4H   CAATACAAGCTACAGACTAGGAGAACATATTGACAGGGGACATACCTGATAAGGGACTTA
*****
CYP2A6      CATTCAAATACACAAAGAAGCTGTTAAACTCAA---TAGAAAAACAACCAATTAAAGAA
CYP2A7      CATTCAAATACACAAAGAAGCTGTTAAACTCAAACAGTAGAAAAACAACCAATTAAAGAA
CYP2A6*4G   CATTCAAATACACAAAGAAGCTGTTAAACTCAA---TAGAAAAACAACCAATTAAAGAA
CYP2A6*4H   CATTCAAATACACAAAGAAGCTGTTAAACTCAA---TAGAAAAACAACCAATTAAAGAA
*****

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Figure 2.

Alignment of genomic DNA sequence from *CYP2A6*, *CYP2A7*, *CYP2A6*4G*, and *CYP2A6*4H*. Underlined nucleotides represent *CYP2A7* sequence. *CYP2A6*4G* has a crossover junction 7913 bp from the +1ATG start site of *CYP2A6*, while *CYP2A6*4H* has a crossover junction 7760 bp from the +1ATG start site of *CYP2A6*. Clones were fully sequenced or partially sequenced as follows: at least 500 bp from the 5'-region of the 7.4 kb PCR insert (to confirm *CYP2A7* sequence), at least 500 bp from the 3'-region of the 7.4 kb PCR insert (to confirm *CYP2A6* sequence), and at least 1000 bp surrounding the putative crossover region in the PCR insert (to determine the point where the nucleotide sequence changes from *CYP2A7* to *CYP2A6*). Dots (.) each represent 100 bp of identical *CYP2A6* and *CYP2A7* sequence (a 600 bp stretch).

Figure 3.

Schematic showing multiple gene conversions in *CYP2A6*. Gene conversions contain *CYP2A7* sequence in the 3'-UTR and flanking region of *CYP2A6*. The annealing of primers 2A6R1, 2A6R2, 2A6R3, and 2A6R4, which are named and represented by arrows, are affected by gene conversions. The *CYP2A6**1B15 and *1B17 alleles prevent 2A6R2 primer annealing [5; 6], the *CYP2A6**1B16 allele prevents 2A6R1 and 2A6R2 primer annealing [6], the *CYP2A6**1B4 and *1L alleles prevent 2A6R3 and 2A6R4 primer annealing. Exons are shown in black boxes while open boxes represent *CYP2A7* sequence.

```

CYP2A6      ACCCCCGTGTACCTTTGTTCAAAAACCATTCACGCTCACCT-----AATTGCCACAAA
CYP2A7      ACCCCCGTGTACCTTTGTTCAAAAACCATTCACGCTCACCTTAATTGCCACAAA
CYP2A6*1B4  ACCCCCGTGTACCTTTGTTCAAAAACCATTCACGCTCACCT-----AATTGCCACAAA
CYP2A6*1L   ACCCCCGTGTACCTTTGTTCAAAAACCATTCACGCTCACCT-----AATTGCCACAAA
*****

CYP2A6      CCTCTGCGAAGGG-----CGTTCATGCCCATTTTACACGTGACAAAAGTGGGCTTAGA
CYP2A7      CCTCTGCGAAGGGGAAAGCGTTCATGCCCATTTTACACGTGACAAAAGTGGGCTTAGA
CYP2A6*1B4  CCTCTGCGAAGGG-----CGTTCATGCCCATTTTACACGTGACAAAAGTGGGCTTAGA
CYP2A6*1L   CCTCTGCGAAGGG-----CGTTCATGCCCATTTTACACGTGACAAAAGTGGGCTTAGA
*****

CYP2A6      AAGTTGCTCTCT---GATGTCTCACAAAACATAAGTGCCAGAAAATCTTTGAACACAGA
CYP2A7      AAGTTGGCTCTATCTGATGTCTCACAAAACATAAGTGCCAGAAAATCTTTGAACACAGA
CYP2A6*1B4  AAGTTGCTCTCT---GATGTCTCACAAAACATAAGTGCCAGAAAATCTTTGAACACAGA
CYP2A6*1L   AAGTTGCTCTCT---GATGTCTCACAAAACATAAGTGCCAGAAAATCTTTGAACACAGA
*****

CYP2A6      7073T>C 7082G>C 7094G>C 7099T>C
TCTGTGCCCATAGCCTTCTAGACAGATTCTTAAAAAGCACCTATTCTTCACGTAAAA...
CYP2A7      TCTGTGCCCATAGCCTTCTAGACACATTCTTAAAAACCCCATTCCTCAGTAAAA...
CYP2A6*1B4  TCTGTGCCCATAGCCTTCTAGACACATTCTTAAAAACCCCATTCCTCAGTAAAA...
CYP2A6*1L   TCTGTGCCCATAGCCTTCTAGACACATTCTTAAAAAGCACCTATTCTTCACGTAAAA...
*****

CYP2A6      ...TTGTAGACCTAAATGAATAGTGAGTACAAAACCTTCTAGAAGATAATGTAGGAGAAAA
CYP2A7      ...TTGTAGACCTAAATGAATAGTGAGTACAAAACCTTCTAGAAGATAACGTAGGAGAAAA
CYP2A6*1B4  ...TTGTAGACCTAAATGAATAGTGAGTACAAAACCTTCTAGAAGATAATGTAGGAGAAAA
CYP2A6*1L   ...TTGTAGACCTAAATGAATAGTGAGTACAAAACCTTCTAGAAGATAATGTAGGAGAAAA
*****

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Figure 4.

Alignment of the genomic DNA sequence of *CYP2A6*, *CYP2A7*, *CYP2A6*1B4*, and *CYP2A6*1L*. Underlined nucleotides represent *CYP2A7* sequence. *CYP2A6*1B4* contains a 27 bp stretch of *CYP2A7* sequence in the 3'-flanking region of *CYP2A6* at 7073 to 7099 bp from the +1ATG start site of *CYP2A6*. *CYP2A6*1L* contains a 10 bp stretch of *CYP2A7* sequence in the 3'-flanking region of *CYP2A6* at 7073 to 7082 bp from the +1ATG start site of *CYP2A6*. Dots (.) each represent 100 bp of identical *CYP2A6* and *CYP2A7* sequence (a 600 bp stretch).

Figure 5.

Nicotine kinetics of different genotype groups. 3HC/COT₂₇₀ and nicotine AUC₃₆₀ illustrate the impact of *CYP2A6**1/*1 (N=124), *CYP2A6**1/*4 (N=5), *CYP2A6**4/*4 (N=1), and *CYP2A6**4/*X (N=6) (A and B); *CYP2A6**1/*1 (N=124) and *CYP2A6**1/*1L (N=24) (C and D); and *CYP2A6**1A/*1A (N=74), *CYP2A6**1A/*1B1-17 (N=44), and *CYP2A6**1B1-17/*1B1-17 (N=6) (E and F). $\phi = p < 0.05$ and $\phi\phi = p < 0.01$ Dunn's multiple comparison test versus *CP2A6**1/*1 where $p < 0.05$ with the Kruskal-Wallis test. Each point represents a study participant and lines represent group means. Open squares have a *CYP2A6**4A, *4D, *4E, or *4F allele. Closed circles have a *CYP2A6**4G or *4H allele. Close squares have a *CYP2A6**1B4 allele.

Table 1

CYP2A6 alleles frequencies between different ethnic groups

	Canadian ^d		American ^b		Asian
	African	European	African	Asian	
	Alleles N (%)	Total alleles	Alleles N (%)	Total alleles	Alleles N (%)
CYP2A6*4A,*4D-F (established deletions only)	7 (1.2) ^c	562	-	-	-
CYP2A6*4A,*4D-H [†] (established and new deletions)	15 (2.7) ^{c,d}	562	0 (0.0)	166	1 (1.3)
CYP2A6*IB4	3 (0.5)	562	1 (0.6)	166	0 (0.0)
CYP2A6*IL [‡]	38 (6.8) ^e	562	20 (12.0) ^f	166	7 (9.2)
Old CYP2A6*IB1-17 [§]	100 (17.8)	562	-	-	-
New CYP2A6*IB1-17 [§]	86 (15.3)	562	47 (28.3)	166	15 (19.7)
				76	31 (31.6)

^a African Canadian study population was previously described [10].

^b American study populations were previously described [11].

^c Includes a sample with three alleles detected through sequencing (genotype: *1/*4*new hybrid allele not yet characterized)

^d Includes a sample with three alleles detected through genotyping (genotype: *1/*4*9). Through genotyping, one sample was determined to have a deletion allele with the old, but not the new, CYP2A6*4 assay.

^e Includes two samples with three alleles detected through genotyping (genotype: *1/*IB*IL).

^f Includes one sample with three alleles detected through genotyping (genotype: *1/*IB*IL).

^g Does not include CYP2A6*IB4 alleles.

[†] Allele frequencies differ across ethnic groups, p<0.05.

Note. CYP2A6*4D-H, CYP2A6*IB4, CYP2A6*IL, and CYP2A6*IB1-17 are in Hardy-Weinberg equilibrium in the African Canadian population.