ABSTRACT:
The N-oxygenation of amines by the human flavin-containing monooxygenase (form 3) (FMO3) represents an important means for the conversion of lipophilic nucleophilic heteroatom-containing compounds into more polar and readily excreted products. Certain mutations of the human FMO3 gene have been linked to abnormal drug or chemical metabolism. For example, abnormal N-oxygenation of trimethylamine has been shown to segregate with mutations of human FMO3. To date, however, it is not known whether there is a pharmacogenetic basis for abnormal drug metabolism by human FMO3. The objective of this study was to estimate the allele and genotype frequencies at three variable DNA sites in the FMO3 gene in male and female blood bank donors representative of non-Hispanic Caucasians, non-Hispanic African Americans, Hispanics, and Asians sampled from the United States. The common polymorphisms at variable sites 158, 257, and 308 were experimentally determined using a high-throughput chip-based genotype variation detection method combining MassEXTEND and matrix-assisted laser desorption ionization time-of-flight mass spectrometry. We also compared the genetic variation of nonhuman primate FMO3 with the human FMO3 gene. Exon sequence analysis of the monkey FMO3 gene sequence showed that it was similar to the human gene sequence but differed from the human consensus sequence at 31 fixed positions. Compared with that of human, the chimpanzee exon sequence had one polymorphism that induced an amino acid change. The evolutionary history of the FMO3 gene was inferred from the pattern of haplotype relationships across different populations and species. Statistically significant heterogeneity in the relative frequencies of single and multiple site alleles, haplotypes, and genotypes of the human FMO3 among ethnic subdivisions suggests that population differences in the susceptibility of humans to abnormal metabolism or adverse drug reactions for chemicals metabolized by human FMO3 could exist.
Katchamart et al., 2000). Thus, rats treated with major chemopreventive indoles present in cruciferous vegetables (i.e., indole-3-carbinol or its acid condensation products likely to be produced by the acid contents of the stomach) significantly reduced the detoxication of nicotine. This result parallels that observed for two individuals suffering from trimethylaminuria that showed impaired nicotine N-1-oxygenation (Ayesh et al., 1988). For tamoxifen, inhibition of N-oxygenation could actually decrease the risk of humans developing toxic side effects if FMO-mediated N-oxygenation is responsible for tamoxifen-dependent covalent binding to protein (Umemoto et al., 1999). Previously, we reported that humans fed 300 g of Brussels sprouts/day led to a significant decrease in urinary TMA N-oxide formation, presumably from the inhibition of hepatic FMO3 (Cashman et al., 1999). It is possible that individuals with decreased human FMO3 activity have the potential for altering the toxicity of drugs or chemicals and inducing adverse drug reactions.

Mutations that are identified through individuals with extremely rare phenotypes may not be typical of variation in the gene in the population at large. Before determining whether variation of a gene contributes to susceptibility to disease in the population at large, the amount and organization of genetic variation in the gene must be evaluated in samples of individuals ascertained without regard to their health status. It is likely that many common genetic variants (i.e., least frequent allele greater than 1% in the general population) having smaller effects combine to determine the major fraction of variation in human disease susceptibility in the population at large (Risch and Merikangas, 1996). Previous studies examined the prevalence of two prominent human FMO3 polymorphisms in French Canadian and Australian Caucasians (Cashman et al., 2000b), European (Sachse et al., 1999), and Asian (Kang et al., 2000) populations. In the present study, we report the relative frequencies of single and multiple site allele configurations, haplotypes, and genotypes of the three major common polymorphisms of human FMO3 in samples of non-Hispanic Caucasians, non-Hispanic African Americans, Asians, and Hispanics ascertained without regard to health status from the United States. The DNA sites with the most variation in the gene were quantified using a high-throughput mutation detection method combining MassEXTEND coupled with mass measurement by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (Scheme 1). Our study suggests that population differences may exist in the susceptibility of humans to abnormal metabolism or adverse drug reactions for chemicals metabolized by human FMO3 due to the frequency of the variants observed.

Materials and Methods

Chemicals. All chemicals and reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) in the highest purity commercially available. Buffers and other agents were purchased from VWR Scientific, Inc. (San Diego, CA). D-Trimethylamine (99 atom D %) (“D-TMA) was purchased from Isotech, Inc. (Mansburg, OH). Oasis solid-phase extraction cartridges were purchased from Waters (Milford, MA).

Genomic DNA and Study Population. Genomic DNA was obtained from blood bank donors in California and Utah. After institutional review board ethics approval, the frequencies of common human FMO3 single nucleotide polymorphisms were experimentally determined from DNA obtained from independent individuals. The ethnicity of the individuals was defined by a self-report questionnaire that indicated the race of both parents. One hundred and seventy nine non-Hispanic Caucasians (i.e., 89 males and 90 females) were from Utah (1% Amish pedigree, 2% Venezuelan pedigree, 2% French pedigree, and 95% Utah pedigree). Ninety non-Hispanic African Americans (i.e., 45 males and 45 females) were from California. The 85 Hispanic samples (i.e., 43 males and 42 females) were composed entirely of Mexican origin. The 66 Asians (i.e., 33 males and 33 females) were composed of 5% Vietnamese, 8% Asian, not further specified, 13% Korean, 31% Japanese, and 43% Chinese. The pools broadly represent the populations in the United States. Another group of 92 non-Hispanic Caucasians was examined for the presence of the rare human FMO3 P153L mutation that is the cause of trimethylaminuria. Using the MassEXTEND technology also showed that all of the samples were
negative for the E305X mutation. Genotyping for FMO3 P153L and E305X served as a positive control that the samples were not from individuals with severe trimethylaminuria.

**Primate DNA.** Genomic DNA from baboon (i.e., Papio cynocephalus, Papio anubis, Papio ursinus, and Papio hamadryas) and genomic DNA from chimpanzee (i.e., Pan troglodytes) was generously provided by Professor David Smith (University of California, Davis, CA). CDNA from monkey (i.e., Macaca mulatta) was prepared from liver obtained from the University of Washington Primate Center (Seattle, WA) tissue-sharing program.

**PCR Amplification.** Oligonucleotides were synthesized at Operon, Inc. (Alameda, CA). Nonbiotin-labeled primers (-bio) were obtained unpurified, and biotin-labeled primers were obtained as high-pressure liquid chromatography-purified materials from Operon, Inc. The sequences of the PCR primers were as follows: exon 4, FMO3-329-bio: d(GCC AGT TAT GTG GCT AGC M from Invitrogen (Carlsbad, CA), and d(AAC GCC AGG AGG CAT ATC AC), FMO3-311: d(CAG TTC CAG AAG TGG CTC CT), exon 7, FMO3-743-bio: d(CCA AAC TGA AGG GGA CCT TG) and FMO3-341: d(CCA GCA TTC TGT GTC GCA TT). PCR was carried out with Hotstart Taq obtained from QIAGEN (Valencia, CA). The PCR cocktail was composed of 1× PCR QIAGEN Buffer [Tris-HCl, (NH₄)₂SO₄, 15 mM MgCl₂], 200 μM dNTPs, 1 U of final concentration Hotstart Taq, forward and reverse primers both at a 0.04-μM final concentration, and high-pressure liquid chromatography-purified water to a total reaction volume of 45 μl. The final addition was 5 μl of 5 ng/μl DNA template. PCR cycling was carried out in an MJ Research thermal cycler (Watertown, MA). PCR-cycling conditions were: an initial hold of 15 min at 95°C, followed by 44 cycles of 5 s at 95°C, 20 s at 56°C and 30 s at 72°C, and a final hold at 4°C. Biotinylated amplicons were transferred to a microtiter plate well containing 0.1 mg of streptavidin-coated Dyna beads (Dyna, Inc., Oslo, Norway) that usually had 150 l of serum or an internal standard in water. The analyte was eluted with 700 l of acetonitrile/water (5:95, v/v). The eluant was separated and analyzed for the presence of TMA by MassEXTEND also showed the 305X truncation was not observed under reliable quality-control conditions. PCR was done with the same primer pairs as that for the human FMO3 gene because a FMO3 158-primer was converted to a single product consistent with the frequency estimates of nucleotide substitution (FENS) program as outlined previously (Zhang et al., 1999).

**Results**

The primer FMO3 DNA Sequencing experiments to quantify potential sequence variations in exons 4, 6, and 7 were developed for the human FMO3 gene. Scheme 1 shows a schematic representation of the procedure. The primers were used to compare the summary statistics. Under neutrality, the two estimates should be equal and D = 0. Pair-wise linkage disequilibrium was measured with the linkage disequilibrium parameter D, calculated as D = pᵢj - pᵢpᵢ. The frequency of the most common gametic type for a pair of sites and pᵢ were the frequencies of the nucleotide alleles in the haplotype (Hartl and Clark, 1997).

**Data Analysis and Statistics.** The statistics D and D' were used to compare the frequency estimates of nucleotide substitution (FENS) program as outlined previously (Zhang et al., 1999).

**MALDI-TOF MS.** Matrix preloaded Spectro chips from Sequenom, Inc. (San Diego, CA) were prepared with 5 to 12 nl of sample with a piezoelectric nanoplotter. MALDI-TOF mass spectrometry was carried out on a Sequenom instrument. Data acquisition and mass spectrometry and genotyping were done concomitantly in an automated mode with SpectroTyper software from Sequenom, Inc. (San Diego, CA). The sequence data were assembled using external calibration, and accurate mass values were determined using internal calibra-
It has previously been observed that the human FMO3 P153L mutation is rare and is linked to the rare inborn error of metabolism called trimethylaminuria (Dolphin et al., 1997; Treacy et al., 1998). The human FMO3 E158K genotype distribution and allele frequency of the major common polymorphisms of human FMO3 were determined in DNA samples obtained from a population of male and female non-Hispanic Caucasians, non-Hispanic African Americans, Hispanics, and Asians sampled from a United States population. The common polymorphisms at alleles 158, 257, and 308 were examined by the MassEXTEND method described above. Table 2 lists the analysis of relative allele frequencies for sites 158, 257, and 308 of the human FMO3. For the human FMO3 158 and 257 alleles, Caucasians, African Americans, and Hispanics had similar allelic frequencies, but Asians were significantly different. For the FMO3 308 allele, Caucasians, Asians, and Hispanics possessed similar allelic frequencies, but African Americans were significantly different (Table 2). For male and female Caucasians, African Americans, and Hispanics, the 158KK genotype frequencies were 0.17, 0.14, and 0.13, respectively, whereas Asians had a considerably lower genotype frequency; only 0.02 were homozygous for FMO3 158KK (Table 3). The human FMO3 V257 MM genotype was only observed in Caucasians, whereas for African Americans, Hispanics, and Asians, the 257 MM genotype was not observed (Table 3).

The final common human FMO3 polymorphism examined was at allele 308. The human FMO3 E308G allelic frequency for Caucasians, Asians, and Hispanics was 0.16, 0.14, and 0.12, respectively (Table 2). In African Americans for the population examined, the 308G allele frequency was 0.04 and showed that the African American 308G allelic frequency was low. Table 3 showed that the 308GG allele frequency was 0.05, 0.02, and 0.02, respectively, for Caucasians, Asians, and Hispanics. For the African American samples examined, there was no human FMO3 308GG genotype observed.

We examined the prevalence of the eight haplotypes for the three common FMO3 polymorphisms described above. The results were listed in Table 4. Two of the eight possible haplotypes (i.e., E-M-G, and K-M-E) were not observed in the populations examined. The K-M-G haplotype was only observed in Hispanics. Of the populations examined, a fourth haplotype (i.e., E-V-G) was quite rare, appearing only in African Americans and Hispanics with relative haplotypes of 0.0056 and 0.0059, respectively. Compared with the wild type haplotype (E-V-E), some of the haplotypes were quite prevalent (Table 4). Thus, the relative frequencies of the E-M-E haplotype was 0.0726.

---

**Table 1**

<table>
<thead>
<tr>
<th>Substitution</th>
<th>Exon</th>
<th>MassEXTEND Product</th>
<th>Length</th>
<th>Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>E158K</td>
<td>4</td>
<td>Termination: ddA</td>
<td>15</td>
<td>4459</td>
</tr>
<tr>
<td>Primer</td>
<td></td>
<td>cccacctacaaaaa</td>
<td>16</td>
<td>4756.2</td>
</tr>
<tr>
<td>K158</td>
<td>6</td>
<td>Termination: ddA</td>
<td>17</td>
<td>5085.4</td>
</tr>
<tr>
<td>E158</td>
<td></td>
<td>cccacctacaaaaa</td>
<td>18</td>
<td>5480.6</td>
</tr>
<tr>
<td>V257M</td>
<td>6</td>
<td>Termination: ddA</td>
<td>19</td>
<td>5777.8</td>
</tr>
<tr>
<td>Primer</td>
<td></td>
<td>atctctgactgtgtgta</td>
<td>22</td>
<td>6740.4</td>
</tr>
<tr>
<td>M257</td>
<td></td>
<td>atctctgactgtgtgta</td>
<td>20</td>
<td>6169.8</td>
</tr>
<tr>
<td>V257</td>
<td></td>
<td>atctctgactgtgtgta</td>
<td>22</td>
<td>6825.4</td>
</tr>
<tr>
<td>E308G</td>
<td>7</td>
<td>Termination: ddA</td>
<td>19</td>
<td>5885.8</td>
</tr>
<tr>
<td>Primer</td>
<td></td>
<td>aacgtgaaggaatccacq</td>
<td>20</td>
<td>6169.8</td>
</tr>
<tr>
<td>E308</td>
<td></td>
<td>aacgtgaaggaatccacq</td>
<td>22</td>
<td>6825.4</td>
</tr>
</tbody>
</table>

---

**Fig. 1.** MALDI-TOF MS of the human FMO3 gene with a common sequence at codon 158.
0.0667, 0.1969, and 0.07059 in Caucasians, African Americans, Asians, and Hispanics, respectively. In contrast to the E-M-E relative haplotype frequency that was large in Asians, the relative frequency of the K-V-E haplotype was quite low in Asians (i.e., 0.00757) but quite prevalent in the other populations examined (i.e., 0.229, 0.3722, and 0.0667). The frequency of the K-V-G haplotype also varied considerably as a function of ethnic group. Thus, the prevalence of the K-V-G haplotype was quite low in Asians, the relative frequency of the K-V-E haplotype was quite high in Asians (i.e., 0.229, 0.3722, and 0.2353) in Caucasians, African Americans, and Hispanics, respectively. In contrast to the E-M-E relative frequency differences, linkage disequilibrium was also investigated. D was calculated for all pairs of sites in all four ethnic groups examined (Table 5). For each pair of sites, we calculated the statistic D, defined as \( p \cdot p - \hat{p} \cdot \hat{p} \), where \( p \cdot p \) are the frequencies of the haplotype with nucleotides \( i \) and \( j \) at the two sites, and \( \hat{p} \) and \( \hat{p} \) are the frequencies of the nucleotides \( i \) and \( j \), respectively. \( D \) values are dependent on allele frequencies. We also calculated the statistic \( D' \), \( D'' \) is standardized and is independent of allele frequencies, and the value is from 0 to 1.0. A test of overall linkage disequilibrium, based on the number of observed and expected signs of \( D \), showed a significant excess of cases of disequilibria in which rare alleles were associated.

The comparison of monkey \( FMO3 \) gene sequences to human was an important control because we wanted to compare human allelic variation with nucleotide variation in monkey. The homologous genomic exon region in monkey (\( M. \) mullatta) was compared with human and showed 31 sequence differences between the human consensus (GenBank accession NM006894) and monkey. The sequence differences included 54 nucleotide substitutions. The monkey \( FMO3 \) gene shared 94.2% sequence identity with that of the human (Figs. 2 and 3). The associated estimate of net sequence divergence between human and monkey that took into account human polymorphic variation was not statistically significant. Although the large number of nonsynonymous substitutions (i.e., 31) with a \( K_s \) value of 0.02498 ± 0.00492 compared with the number of synonymous substitutions (\( K_s \) value of 0.0668 ± 0.01486) appeared to be suggestive, there was no evidence for positive selection on these sequences. Because \( K_s \) was a good deal greater than \( K_a \), it is possible that a modest purifying selection was at work.

Comparison of the chimpanzee \( FMO3 \) gene to the human \( FMO3 \) gene showed that at least 12 single nucleotide variations and one large deletion were observed in the immediate intron regions. Five silent mutations were present in the exon regions (N285, L288, E424, 1486, and I525), and only one amino acid change was identified (i.e., H116R, CAT to CGT). However, for the five individual baboon DNA samples that we examined using the same conditions for the chimps, we could only amplify exon 8. In baboon \( FMO3 \) exon 8, one amino acid variant (K418R, AAA to AGA) was identified, and 20 single nucleotide variations were observed in the intron adjacent to exon 8.

We conclude that the intronic regions of baboon \( FMO3 \) differ substantially from that of human and chimpanzee.
Most genetic variation can be accounted for by a few prevalent alleles function of the enzyme (Motulsky, 1991; Kalow and Grant, 1995). Variations or small deletions/insertions that may lead to alteration in chemical metabolism generally result from single-nucleotide substitutions. Interindividual variation of enzymatic metabolic activity can result in significant differences in the biotransformation of drugs or xenobiotics. For example, genetic polymorphism of CYP2D6-mediated debrisoquine 4-hydroxylation varies considerably across different ethnic groups. For example, “poor metabolizers” make up approximately 5 to 10% of the Caucasian population but only about 0.1% of the Asian population (Wolf et al., 1992). For individuals with a particular CYP2D6 variant, the polymorphism may cause an exaggerated clinical response and side effects of debrisoquine administration (Tucker et al., 1977). Another example stems from the CYP2C19-mediated 4'-hydroxylation of (S)-mephentoin. In Caucasians, the prevalence of the CYP2C19 poor-metabolizer phenotype is approximately 0.2% but is significantly larger in Asians (i.e., 15–20%) (Goldstein and de Morais, 1994).

Human FMO3 is recognized as the prominent form of FMO present in the adult liver that contributes to the oxygenation of nucleophilic heteroatom-containing chemicals, drugs, and endogenous materials. Human FMO3 has a broad substrate specificity, and recent studies have shown that, barring steric limitations, human FMO3 is capable of oxygenating numerous N- and S-containing xenobiotics (Cashman, 2006a). In addition, human FMO3 has been shown to N-oxygenate several endogenous and dietary amines of significant physiological importance including biogenic amines (Lin and Cashman, 1997a,b).

### Table 3: Human FMO3 genotype frequencies

<table>
<thead>
<tr>
<th>Site</th>
<th>Relative Frequency</th>
<th>Test for Hardy Weinberg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Combined Caucasians</td>
<td>Africans</td>
</tr>
<tr>
<td>Site 158</td>
<td>0.39 (EE)</td>
<td>0.33 (EK)</td>
</tr>
<tr>
<td>Site 257</td>
<td>0.02 (VV)</td>
<td>0.04 (VM)</td>
</tr>
<tr>
<td>Site 308</td>
<td>0.35 (EE)</td>
<td>0.34 (EK)</td>
</tr>
</tbody>
</table>

### Table 4: Human FMO3 haplotype frequencies (sites 158–257–308)

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>Combined</th>
<th>Caucasians</th>
<th>Africans</th>
<th>Asians</th>
<th>Hispanics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative frequency (EVE)</td>
<td>0.5571</td>
<td>0.4222</td>
<td>0.5222</td>
<td>0.6515</td>
<td>0.57058</td>
</tr>
<tr>
<td>Relative frequency (KVE)</td>
<td>2.261</td>
<td>0.3722</td>
<td>0.00757</td>
<td>0.2353</td>
<td></td>
</tr>
<tr>
<td>Relative frequency (EME)</td>
<td>0.090047</td>
<td>0.0667</td>
<td>0.1969</td>
<td>0.07059</td>
<td></td>
</tr>
<tr>
<td>Relative frequency (KVG)</td>
<td>0.01933</td>
<td>0.01393</td>
<td>0.1117</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Relative frequency (KMG)</td>
<td>0.00119</td>
<td>0.00056</td>
<td>0.0059</td>
<td>0.0059</td>
<td></td>
</tr>
<tr>
<td>Relative frequency (EME)</td>
<td>0.01196</td>
<td>0.017561</td>
<td>0.1117</td>
<td>0.0059</td>
<td></td>
</tr>
</tbody>
</table>

### Table 5: Analysis of linkage disequilibrium

<table>
<thead>
<tr>
<th>Site</th>
<th>Combined data set n = 420</th>
<th>Site 158</th>
<th>Site 257</th>
<th>Site 308</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 158</td>
<td>0.01038</td>
<td>-0.10138</td>
<td>0.079643</td>
<td></td>
</tr>
<tr>
<td>Site 257</td>
<td>0.02984</td>
<td>0.03089</td>
<td>0.0286</td>
<td></td>
</tr>
<tr>
<td>Site 308</td>
<td>0.0211</td>
<td>0.02703</td>
<td>0.0286</td>
<td></td>
</tr>
</tbody>
</table>

### Discussion

Variation in genes that encode enzymes responsible for drug or chemical metabolism generally result from single-nucleotide substitutions or small deletions/insertions that may lead to alteration in function of the enzyme (Motulsky, 1991; Kalow and Grant, 1995). Most genetic variation can be accounted for by a few prevalent alleles (i.e., alleles with a frequency of greater than 1% in the population), although the frequency of these alleles can vary wildly across populations. Interindividual variation of enzymatic metabolic activity can result in significant differences in the biotransformation of drugs or xenobiotics. For example, genetic polymorphism of CYP2D6-mediated debrisoquine 4-hydroxylation varies considerably across different ethnic groups. For example, “poor metabolizers” make up approximately 5 to 10% of the Caucasian population but only about 0.1% of the Asian population (Wolf et al., 1992). For individuals with a particular CYP2D6 variant, the polymorphism may cause an exaggerated clinical response and side effects of debrisoquine administration (Tucker et al., 1977). Another example stems from the CYP2C19-mediated 4'-hydroxylation of (S)-mephentoin. In Caucasians, the prevalence of the CYP2C19 poor-metabolizer phenotype is approximately 0.2% but is significantly larger in Asians (i.e., 15–20%) (Goldstein and de Morais, 1994).

Human FMO3 is recognized as the prominent form of FMO present in the adult liver that contributes to the oxygenation of nucleophilic heteroatom-containing chemicals, drugs, and endogenous materials.
In normal individuals, TMA is very efficiently N-oxygenated to the polar and nonodorous metabolite TMA N-oxide to the extent of at least 97%. Individuals with trimethylaminuria have a diminished ability to N-oxygenate TMA and excrete a large amount of odorous TMA. Individuals with a severe phenotype possess defective human \(FMO3\) and are afflicted with trimethylaminuria or "fish-like odor syndrome" that can also lead to psychosocial consequences. Trimethylaminuria has been documented in the British (Dolphin et al., 1997), Australian (Treacy et al., 1998), and North American (Akerman et al., 1999) populations. Although rare, some mutations segregate with the most severe trimethylaminuria phenotype, and these include the P153L mutation and the E305X truncation mutation (Cashman et al., 1997; Dolphin et al., 1997; Treacy et al., 1998).

In addition to mutations that cause severe trimethylaminuria, we previously reported evidence for allelic variation within the human \(FMO3\) that influence drug responsiveness (Cashman et al., 2000b). However, in the present study, no TMA could be detected in the plasma by highly sensitive LCMS analysis, and this prevented a correlation of phenotype to genotype because urine samples were unavailable. The lack of TMA present in the plasma underscores the apparent efficiency of urinary excretion of TMA, and the extremely high reuptake of TMA in the kidney.

The first major conclusion from the data presented here is that human \(FMO3\) shows a frequency of single nucleotide variation that is somewhat higher than the average reported values previously reported (Cashman et al., 2000) for Canadian and Australian populations. For populations with a significant number of poor metabolizers, it is possible that individuals may be more susceptible to adverse drug reactions or exaggerated clinical response for drugs that are metabolized by \(FMO3\). Altered substrate activities have been observed for human \(FMO3\) and may be responsible for mild trimethylaminuria (Zschocke et al., 1999). For example, the \(N\)-oxidative detoxication of amphetamine and methamphetamine by human \(FMO3\) may be under pharmacogenetic control (Cashman et al., 1999). In a previously studied cohort of Australian trimethylaminuria patients, several patients manifested hypertension and adverse reactions from tyramine, other amines, and sulfur-containing medications (Treacy et al., 1998).

A second major conclusion from the data in this report is that the frequency of single nucleotide variants differ widely across ethnic groups. The human \(FMO3\) haplotypes described here for Utah Caucasians have similarity to that of a German population (Sachse et al., 1999) and a Canadian population (Cashman et al., 2000b) previously reported. In addition, the data reported here are in agreement with that of a study of a Korean population that used ranitidine as a test substrate to correlate \(FMO3\) genotype and phenotype (Kang et al., 2000). The observation of differences of human \(FMO3\) haplotypes between ethnic groups is intriguing because it may suggest that \(FMO3\)-dependent metabolism may vary considerably across ethnic groups. Larger and more diverse populations must be examined before a definitive statement along these lines can be made. Ultimately, however, if the metabolism and detoxication of a drug or chemical is dependent on human \(FMO3\), an individual with impaired \(FMO3\) may be at risk for exaggerated clinical response, and this could lead to adverse reactions.

Knowledge of \(FMO3\) polymorphisms could lead to the develop-
ment of high-precision DNA diagnostics for pharmacogenetics purposes. For large numbers of samples, the MALDI-TOF technology provides a high-throughput and analytically robust method for assessing polymorphism. We developed MassEXTEND assays and optimized them for the three common polymorphic variants of human \textit{FMO3}, and the products were analyzed by MALDI-TOF mass spectrometry. The MassEXTEND assay clearly differentiates known codon variations of different human \textit{FMO3} genotypes because the mass values are specific. In some cases, multiplexing was possible, and a duplex reaction was established for the simultaneous analysis of variation in codons 153 and 158. High-quality data presented here can provide information about possible forces that acted to shape the genetic variation observed.

Evolutionary comparisons with sequences from a near species relative, the monkey, can illuminate features about human genetic variability. The monkey sequences indicated which nucleotides that segregate in humans might be ancestral. Such sites may be conserved by chance or because the variation was not deleterious enough to be eliminated from the population via selection. Although it is tempting to speculate that selection has maintained certain genetic variation in some populations, our statistical analysis does not suggest any evidence for positive selection of these genes. It is possible, of course, that demographic forces have played a role in creating haplotype differences rather than selection. It is interesting to note that, compared with human \textit{FMO3}, the monkey \textit{FMO3} variation was spread somewhat uniformly over the exons with the exception of the amino terminus and some of the consensus sequences of flavin adenine dinucleotide and NADPH-binding domains at positions 9 to 14 and 190 to 196, respectively. In addition, the region between amino acids 240 and 290 was also devoid of variation with the exception of the common polymorphism at position 257. It is possible that the monkey was heterozygotic at position 257. This is relatively common in human populations and probably without significantly deleterious metabolic consequences. Of note is that the monkey possesses the FATGY sequence at position 327 that is present in all mammalian and plant (Zhao et al., 2001) \textit{FMOs} observed to date.

In summary, the use of the high-throughput MassEXTEND methodology to rapidly genotype populations of healthy individuals from Californians and Utah has provided considerable insight into the relative allelic frequencies of human \textit{FMO3} haplotypes in individuals ascertained without regard to their health status. Human \textit{FMO3} may play a role in the ethnic group-dependent metabolism of drugs and chemicals, and a complete description of the metabolic distribution of such a chemical should take this into account.

**Acknowledgments.** We thank Sara Hamon and Professor Charles Sing of the Department of Human Genetics (University of Michigan, Ann Arbor, MI) for performing statistical evaluation of the data. We thank Quyen Tran for technical assistance. We are grateful to Professor David Smith (University of California, Davis, CA) for the baboon and chimpanzee genomic DNA samples. We also acknowledge the stimulating discussions with Professor Nicholas Schork (University of California, San Diego, CA).

**References**


Downloaded from dmd.aspetjournals.org at ASPET Journals on June 21, 2017