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CYP3A5 Variant Allele Frequencies in Dutch Caucasians

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Background: **Enzymes of the cytochrome P450 3A (CYP3A) family are responsible for the metabolism of >50% of currently prescribed drugs. *CYP3A5* is expressed in a limited number of individuals. The absence of *CYP3A5* expression in ~70% of Caucasians was recently correlated to a genetic polymorphism (*CYP3A5*3*). Because CYP3A5 may represent up to 50% of total CYP3A protein in individuals polymorphically expressing *CYP3A5*, it may have a major role in variation of CYP3A-mediated drug metabolism. Using sequencing, have been identified (Hustert et al. *Pharmacogenetics* 2001;11:773–9; Kuehl et al. *Nat Genet* 2001;27:383–91) variant alleles *2 through *7 for *CYP3A5*. Detection of *CYP3A5* variant alleles, and knowledge about their allelic frequency in specific ethnic groups, is important to establish the clinical relevance of screening for these polymorphisms to optimize pharmacotherapy.**

Methods: **In a group of 500 healthy Dutch Caucasian blood donors, we determined the allelic frequency of the *CYP3A5*2, *3, *4, *5, *6, and *7 alleles by use of newly developed PCR-restriction fragment length polymorphism assays.**

Results: **The frequency of the defective *CYP3A5*3* allele in the Dutch Caucasian population was 91%, followed by the *CYP3A5*2* (1%) and *CYP3A5*6* (0.1%) alleles. The *CYP3A5*4, *5,* and *7 alleles were not detected.**

Conclusions: **On the basis of its allelic frequency, screening for the *CYP3A5*3* allele in the Caucasian population is extremely relevant. In addition, screening for the *CYP3A5*2* allele may be taken into consideration in individuals heterozygous for the *CYP3A5*3* allele. The *CYP3A5*4, *5, *6,* and *7 alleles have low allelic frequencies that do not support initial screening.**

The CYP3A isoenzymes constitute the largest portion of cytochrome P450 protein in the liver and small intestine *(1–3)*. The four members of this subfamily, *CYP3A4*, *CYP3A5*, *CYP3A7*, and *CYP3A43 (4, 5)*, are located adjacent to each other on chromosome 7q21 and are responsible for the metabolism of 50–60% of all currently prescribed drugs. In addition, two pseudogenes, *CYP3AP1* and *CYP3AP2*, have been identified *(6)*. To date, CYP3A4 has been regarded as the most important enzyme of this subfamily because it forms the bulk of CYP3A protein and mRNA in the liver in most individuals *(1–3)*. Its catalytic activity may show up to 90-fold variation *(7)*. *CYP3A7* is expressed mainly during fetal life where it accounts for ~50% of the total CYP protein *(8)*. After birth, expression is usually silenced. For the recently described *CYP3A43*, no protein expression data are currently available *(9)*. CYP3A5 protein was previously detected in the livers of some, but not all, adult Caucasian individuals *(8, 10–14)*. The basis for this variation in expression was not known.

In a recent study, Kuehl et al. *(15)* demonstrated that only people with at least one *CYP3A5*1* allele actually expressed CYP3A5 protein. Using DNA sequencing, they identified the *CYP3A5*3* and *6* alleles. The single-nucleotide polymorphisms in these alleles produced alternative splicing and protein truncation and thus absence of CYP3A5 activity. In another study, the livers of 10% of Caucasian individuals expressed *CYP3A5* to a high extent (high expressers), whereas the remaining individuals showed on average nine times less activity (low expressers) *(7)*. The major determinant for this variation in expression was a single-nucleotide polymorphism at position 6986, which in the *g.6986G* allele (*CYP3A5*3*) led to alternative splicing of CYP3A5 transcripts and absence of CYP3A5 protein; the *g.6986A* allele (*CYP3A5*1*) correlated with high expression *(7, 15)*. Because CYP3A5 may represent up to 50% of the total hepatic CYP3A content in

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people expressing *CYP3A5*, this gene may be the most important genetic contributor to interindividual and interracial differences in CYP3A-dependent drug clearance. Detection of *CYP3A5* variant alleles, and knowledge about the allelic frequencies in the population, will be extremely useful in establishing the clinical relevance of *CYP3A5* genotyping to optimize pharmacotherapy. To date, several variant alleles have been identified (*CYP3A5**2 through *7) by sequencing (*7*, *10*, *15*, *16*). For screening purposes, we developed PCR-restriction fragment length polymorphism (PCR-RFLP) assays for the detection of *CYP3A5* variant alleles. We report here the allelic frequencies of *CYP3A5* variant alleles in a group of 500 healthy Dutch Caucasian volunteers.

Materials and Methods

SAMPLES AND DNA ISOLATION

After receiving informed consent, we obtained EDTA-whole blood from 500 healthy Dutch Caucasian volunteers. We isolated genomic DNA from 300 μL of blood with the GenomicPrep Blood DNA Isolation Kit (Amersham Pharmacia Biotech) and estimated DNA yields by measuring the absorbance at 260 nm (A_{260}). The Medical Ethical Committee of the University Hospital Rotterdam approved the study.

PCR-RFLP FOR *CYP3A5* VARIANT ALLELES

For a 50-μL PCR, we used ~50 ng of genomic DNA. The PCR mixture contained 1× buffer [10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, and 10 mg/L gelatin (Perkin-Elmer)], 0.2 mM each of the deoxynucleotide triphosphates (Roche), 1.25 U of AmpliTaq Gold (Perkin-Elmer), and 40 pmol each of the forward and reverse primers (Table 1). The underlined nucleotides are mismatches with the *CYP3A5* sequence, creating restriction sites in the PCR product. PCR conditions were as follows: 7 min at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 55 °C, and 1 min at 72 °C; and finally 7 min at 72 °C. The PCR product was sequenced, confirming that indeed only the *CYP3A5* gene was amplified. The PCR product (10 μL) was then digested with the appropriate restriction enzyme (Roche; Table 1) in a total volume of 15 μL for 2 h at 37 °C and subsequently analyzed on a 4% agarose/Tris-borate-EDTA gel with ethidium bromide staining. The fragments obtained for wild-type and variant alleles are indicated in Table 1. We confirmed the presence of variant alleles of *CYP3A5* by direct sequencing of the PCR product on an automated ABI 310 capillary sequencer (Perkin-Elmer), using the Big Dye Terminator Cycle Sequencing Kit (Perkin-Elmer).

Results

On the basis of the published *CYP3A5* variant alleles, we developed specific PCR-RFLP tests for *CYP3A5**2 through *7 and applied those in a large-scale genotyping study on samples obtained from 500 healthy Caucasian volunteers (Fig. 1). The frequency of individuals that were wild type, heterozygous, or homozygous for the variant alleles is depicted in Table 2. The *CYP3A5*3* allele was abundantly present in our population, with an allelic frequency of 91.7%. Only one individual was homozygous wild type for *CYP3A5*3*. The *CYP3A4*2* allele was found in 10 individuals, all heterozygotes, giving an allelic frequency of 1%. One individual was heterozygous for the *CYP3A5*6* allele. The *CYP3A5*4*, **5*, and **7* alleles were not detected in our study population. Among the 84 persons who were heterozygous or wild type for the *CYP3A5*3* allele, only one was also heterozygous for another variant allele (*CYP3A5**2). Because heterozygotes and wild types for *CYP3A5*3* may have CYP3A5 activity, we estimate that 83% of Dutch Caucasians do not have CYP3A5 enzymatic activity.

Discussion

CYP3A enzymes are the predominant cytochrome P450 proteins in the human liver and play an important role in

Table 1. PCR-RFLP primers, size of PCR product, restriction enzymes used, and sizes of fragments obtained with wild-type and variant alleles for *CYP3A5*.

CYP3A5 allele	Primers[a]	PCR product size, bp	Enzyme	Fragment sizes, bp	
				Wild-type allele	Variant allele
**2*	5′-CTGTTTCTTTCCTTCCAGGC-3′	269	*Tas*I	269	182, 87
	5′-CTCCATTTCCCTGGAGACTTG-3′				
**3*	5′-CATCAGTTAGTAGACAGATGA-3′	293	*Ssp*I	148, 125, 20	168, 125
	5′-GGTCCAAACAGGGAAGAAATA-3′				
**4*	5′-TCGACTCTCTCAACAATCCTC-3′	281	*Taq*I	261, 20	241, 20
	5′-AAAGTGTGTGAGGGCTCTCGA-3′				
**5*	5′-CCATGAAGATCACCACAACT-3′	240	*Nla*III	226, 14	189, 37, 14
	5′-CCTGTCCCCAGATTCATGC-3′				
**6*	5′-GTGGGGTGTTGACAGCTAAAG-3′	495	*Dde*I	230, 137, 103, 25	230, 137, 128
	5′-TGGAAGATGATTCAGCAGATAGT-3′				
**7*	5′-CTTCAATAGTACTGCATGGAC-3′	108	*Dde*I	61, 24, 22	41, 24, 22, 20
	5′-CTGTACCACGGCATCATAGCT-3′				

[a] Mismatches with the *CYP3A5* sequence are underlined.

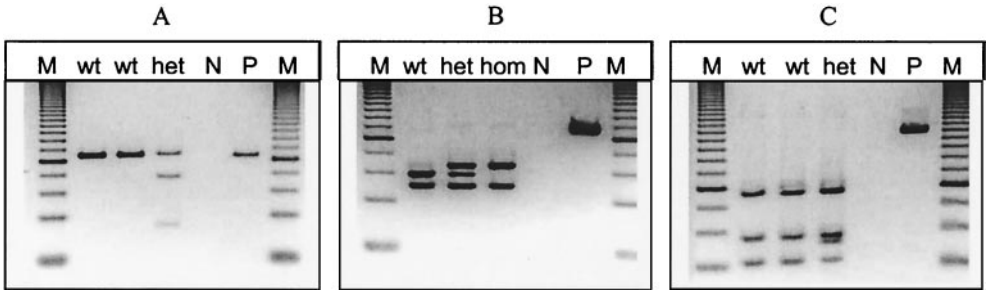

Fig. 1. PCR-RFLP analyses for *CYP3A5* variant alleles.

(*A*), *CYP3A5*2*; (*B*), *CYP3A5*3*; (*C*), *CYP3A5*6*. *M*, 50-bp DNA ladder; *wt*, wild type; *het*, heterozygote; *N*, negative control (water); *P*, undigested PCR product; *hom*, homozygous variant.

the metabolism of numerous exogenous and endogenous substrates. *CYP3A5* is polymorphically expressed and may account for up to 50% of CYP3A content *(15)*. Recently, six variant alleles were described *(7, 15)*. Homozygosity for the allele *g.6986G* was strongly correlated with decreased CYP3A5 activity [factor of 2–3 *(15)*], whereas the wild-type *CYP3A5*1* allele (*g.6986A*), present in either homozygous or heterozygous form, correlated with high CYP3A5 activity. In addition, individuals with a *CYP3A5*1* allele had threefold higher total CYP3A protein when compared with individuals homozygous for *CYP3A5*3 (15)*. Thus, *CYP3A5* expression may be the most important factor determining the total CYP3A content of the human liver.

In a large-scale screening for *CYP3A5* variant alleles among 500 healthy individuals, we conclude that the *CYP3A5*3* allele is abundantly present in the Dutch Caucasian population, displaying an allelic frequency of 91%. This finding is in agreement with the reported frequency of 95% found in 183 samples from Caucasian patients from Germany and Switzerland *(7)*. For comparison, the *CYP3A5*3* allele was detected in 73% of Chinese, 71% of Japanese, 70% of Korean, and 27% of African-American individuals *(7)*. The *CYP3A5*2* allele, encoding a *g.27289C→A* allele in exon 11, was found in 2% of the individuals (allelic frequency, 1%) and may thus be regarded as a genetic polymorphism in this population *(17)*.

Table 2. Allelic frequencies of *CYP3A5* variant alleles in the Dutch Caucasian population (n = 500).

CYP3A5 allele	AA[a] change	Wild type	Heterozygotes	Homozygotes	Allelic frequency
**2*	T398N	0.980	0.020	0.000	0.010
**3*	Splicing Defect	0.002	0.167	0.831	0.917
**4*	Q200R	1.000	0.000	0.000	0.000
**5*	Splicing Defect	1.000	0.000	0.000	0.000
**6*	Splicing Defect	0.998	0.002	0.000	0.001
**7*	Frameshift	1.000	0.000	0.000	0.000

[a] AA, amino acid.

A previous study involving 19 Caucasian individuals reported an allelic frequency of 5% (n = 19) *(10)*. This allele was not found when 45 African-American genomic DNA samples were investigated, suggesting that in this ethnic group the allelic frequency is <1% *(7)*. Heterozygosity for the *CYP3A5*2* allele, however, may potentially affect *CYP3A5* expression in individuals who are already heterozygous for *CYP3A5*3.* We found only one individual heterozygous for both the *CYP3A5*2* and **3* alleles, and this individual may thus lack CYP3A5 activity despite being heterozygous for the *CYP3A5*3* allele. The allelic frequencies of the *CYP3A5*4, *5, *6*, and **7* alleles were ≤0.1%, and these variant alleles are therefore regarded as less relevant for screening purposes in the Caucasian population. In African Americans, the *CYP3A5*6* allele (a *g.14690G→A* variant in exon 2, leading to the skipping of exon 7) has an allelic frequency of 13% (n = 45) *(7)*, suggesting a significant difference in the distribution of this allele between African Americans and Caucasians. This also holds for the *CYP3A5*7* allele, which encodes a single nucleotide (T) insertion at *g.27131* that leads to termination of the open reading frame; this allele was found in 9 of 45 African Americans, giving an allelic frequency of 10% *(7)*. Apparently, the *CYP3A5*6* and/or the *CYP3A5*7* alleles do not cosegregate with the *CYP3A5*3* allele in Caucasians, as was suggested for African Americans *(7)*. On the basis of the *CYP3A5* variant alleles detected in our group of 500 Dutch Caucasians, we deduce that 83% may have low expression of *CYP3A5*. This is in agreement with the finding that 10% of Caucasians were high expressers of *CYP3A5 (7)*.

In conclusion, we showed that screening for the *CYP3A5*3* alleles is relevant in the Caucasian population. Genotyping for the *CYP3A5*2* allele in *CYP3A5*3* heterozygotes may subsequently be performed. In addition, we presented simple DNA-based tests that can be used to investigate interindividual differences in *CYP3A5* expression. This will greatly facilitate studies on the relevance of pharmacogenetics for *CYP3A* genes with respect to disease risk and to the pharmacokinetics and pharmacodynamics of many drugs.

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