Some Mutations of Exon-7 in Cytochrome P450 Gene 3A4 and Their Effect on 6β-Hydroxylation of Cortisol

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Analysis of variants of exon 7 sequences in cytochrome P450 gene *3A4* in a sample of Caucasoid persons was carried out. The effect of these variants on activity of CYP3A was assessed by the level of cortisol 6β-hydroxylation. Alleles *CYP3A4*5* and **17* were not detected: probably, these mutations are rare and consequently they have little effect on the character of polymorphic distribution of CYP3A4 activity in this population. The incidence of *CYP3A4*2* was 5.26%. The 6βOH-cortisol/cortisol ratio in an individual with *CYP3A4*2/*2* genotype was 7.408, which corresponded to "slow metabolizer" phenotype in this sample.

Key Words: *CYP3A4; genetic polymorphism; CYP3A4 activity*

Cytochrome P450 3A4 (CYP3A4) belongs to a large subfamily CYP3A, which includes about 30% total hepatic P450 cytochromes [14]. Apart from CYP3A4, this subfamily includes CYP3A5, CYP3A7, and CYP3A43. Cytochrome CYP3A is characterized by a wide substrate specificity. It is involved into the metabolism of structurally different exo- and endogenous substances and metabolizes more than 50% drugs [15]. CYP3A4 is a predominant form of all CYP3A cytochromes in the liver of adult humans, where its content attains 71-99.5% relatively to the total level of *CYP3A* transcripts [6]. Cytochrome CYP3A5 is detected in the liver of about 10-20% Caucasians and its content varies in different individuals [7]. Substrate specificity of cytochromes in the subfamily largely overlaps, so the pattern of expression in organs and its peculiarities in the ontogeny suggest that in most adult individuals CYP3A4 significantly contributes into the metabolism. Individual differences in activity of xenobiotic metabolism are largely (by ~80%) determined by genetic factors [10]. There are 4 mutations in exon 7 of *CYP3A4* leading to the following amino acid substitutions: c.554 C>G, Thr185Ser (allele **16A*); c.566 T>C, Phe189Ser (**17*); c.653 C>G, Pro218Arg (**5*); and c.664 T>C, Ser222Pro (**2*). We studied the incidence of alleles *CYP3A4*2*, **5*, **17* and possible effects of these mutations on cortisol 6β-hydroxylation in carriers.

MATERIALS AND METHODS

Genome DNA was isolated from peripheral blood leukocytes [8] in the population sample of Western Siberia Caucasians. CYP3A activity was characterized by the 6β-hydrocortisol/cortisol (6βOHCL/CL) ratio [1]. The sequence of exon 7 in *CYP3A4* was analyzed using Vector NTI software. Polymerase chain reaction (PCR) was performed on a Tertzik amplifier (DNK-Tekhnologiya). The same fragment was amplified with oligonucleotide primers for detection of alleles **5* and **17* [5] and allele **2* [2]. PCR yielded 448 and 124 b.p. fragments, respectively.

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The reaction mixture for detection of **5* and **17* alleles (50 μl) included 1.5 μM deoxyribonucleoside triphosphate (dNTP), 0.01 μM primers, and 100 U/ml Taq DNA-polymerase. The PCR program included 35 cycles: 30 sec at 94°C, 20 sec at 64-52°C, and 12 sec at 72°C, including the first denaturation (melting) at 95°C for 5 min and final chain elongation at 72°C for 7 min. Primer annealing temperature was gradually decreased from 64 to 52°C. For detection of *CYP3A4*5*, enzymatic hydrolysis was carried out in optimal buffer for BamHI (SibEnzyme) at 37°C for 16 h. The fragments were separated by electrophoresis in 2% agarose gel. The examined fragment of DNA wild allele contained one recognition site for BamHI yielding 197 and 251 b.p fragments. In case of heterozygote, 448, 251, and 197 b.p. fragments should appear, while mutant homozygote yields one 448 b.p fragment. For detection of *CYP3A4*17*, the reaction was performed in the optimal buffer for Hpy188III (New England Biolabs, Inc.), and the products were separated by electrophoresis in 12% PAAG. The amplified fragment of the wild allele contained one recognition site Hpy188III and formed fragments consisting of 396 and 52 b.p. The mutation leads to the appearance of two recognition sites forming 396, 281, 115, and 52 b.p. fragments in case of heterozygote and 281, 115, and 52 b.p. fragments in case of mutant homozygote.

The reaction mixture for detection of *CYP3A4*2* allele (30 μl) contained 1.5 μM dNTP, 0.01 μM each primers, 0.1 μM Mg^{2+}, 100 U/ml Taq DNA-polymerase. The PCR program included 35 cycles: 30 sec at 94°C, 20 sec at 61-53°C, and 12 sec at 72°C, including the first denaturation at 95°C for 5 min and final chain elongation at 72°C for 7 min. For detection of *CYP3A4*2* enzymatic hydrolysis was carried out in the optimal buffer for BsoMaI (SybEnzyme) at 55°C for 16 h, and the products were separated by electrophoresis in 12% PAAG. The examined fragments contained one recognition site yielding fragments 98 and 26 b.p. in case of wild-type homozygote, 124, 98, and 26 b.p. fragments in case of heterozygote, and 124 b.p. fragment in case of mutant homozygote (Fig. 1). The fragments were revealed by staining with ethidium bromide and identified with DNA length marker in transmitted UV-light. The specimens were essayed for the presence of *CYP3A4*2* (n=76), *CYP3A4*5* (n=75), and *CYP3A4*17* (n=71). The data were processed statistically using χ^2 test with Yates correction.

RESULTS

In a previous study including 169 persons 2 heterozygotes *CYP3A4*5* were found, in whom 6βOHCL/CL ratio attested to decreased activity of this enzyme [5]. The allele-forming mutation c.653 C>G determines amino acid substitution Pro218Arg, which modifies spatial structure of CYP3A4 within the binding site responsible for hydrophobic interaction with the substrates. In Caucasians, *CYP3A4*5* and **17* are extremely rare or absent [3,4]. The allozyme carrying Phe189Ser substitution is characterized by decreased (by 99%) V_{max} and CL_{max} for nifedipine and impaired (by 70%) metabolism of testosterone and insecticide chlorpyrifos in comparison with *CYP3A4*1* [3,9]. In this study, alleles *CYP3A4*5* and **17* were not found. Probably, these mutations are rare in Caucasoid persons of Western Siberia, so they have little effect on the patterm of polymorphic distribution of CYP3A4 activity in this population. For obtaining more information about incidence of these mutations and their effect on enzyme activity, larger sample is required. In a group of 55 Caucasoid persons, the incidence of the first detected *CYP3A4*2* was 2.7% [11]. The cited paper revealed a 6-9-fold decrease in activity of the allele-encoded enzyme in nifedipine metabolism *in vitro* and slight differences in the rate of 6β-hydroxylation of testosterone compared to wild-type allozyme. Other study [2] revealed no *CYP3A4*2* among 216 Caucasians in Russia. Our study (n=76) revealed 6 heterozygotes and 1 mutant homozygote by *CYP3A4*2* allele, the incidence of this allele being

TABLE 1. Comparative Analysis of Distribution of *CYP3A4*2* Allele Incidence in Caucasoid Persons of Western Siberia and Finland (χ^2=0.49, p=0.4852)

Region	Total number of alleles	Number of alleles			
		wild type		mutant type	
		abs.	%	abs.	%
Western Siberia	152	144	94.74	8	5.26
Finland [14]	110	107	97.27	3	2.72

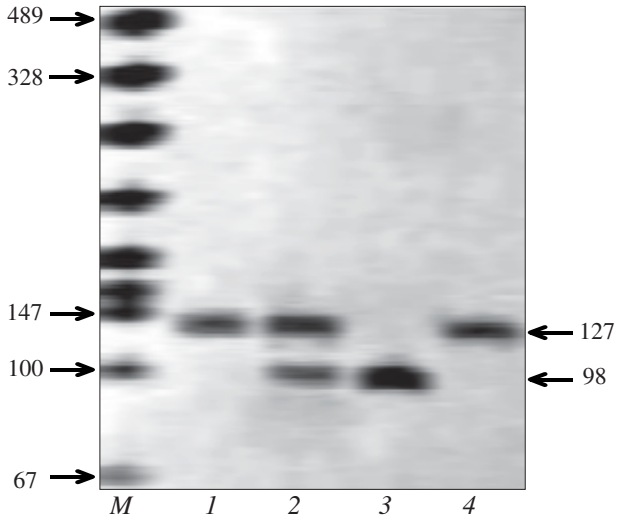

Fig. 1. Electrophoregramm of endonuclease hydrolysis of BsoMAI restriction. *1*) amplicon; *2*) heterozygote; *3*) wild-type homozygote; *M*: DNA length marker.

5.26%. The genotype distribution obeyed Hardy—Weinberg law. Comparative characteristics of the incidence of **2* alleles in Caucasians of Western Siberia and Finland is shown in Table 1. In our study, the incidence of this allele did not significantly differ from previous data [11]. In heterozygotes, the *CYP3A4*2*-controlled enzyme activity was not measured. In 12-year-old person **2* homozygote, enzyme activity assessed by 6βOHCL/CL was 7.408. This person belongs to the group of "slow metabolizers" [1], where the 6βOHCL/CL values ranged from 0.276 to 12.500 (n=28). Thus, our data showed that 664 T>C substitution in the homozygote state somewhat reduced enzyme activity.

Many researches showed that activity of CYP3A varies in the population by 40 and more times obeying the polymorphic or normal distribution. The search for genetic reasons located on the coding and regulatory domains of *CYP3A*, and genes involved in their regulation (the genes of pregnane, retinoid, and constitutive androstan receptors) explained only some aspects of this variability. Therefore, the data are important on the dramatic changes in CYP3A activity during the first 1.5-2 years of human life [1,13], which elucidate a possible role of factors of individual development in the early childhood in the formation of metabolic polymorphism.

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