GENETICS

Analysis of Restriction Fragment Length Polymorphism of Cytochrome P450 3A43 Gene and Evaluation of the Incidence of *CYP3A43*1B* Allele in Europeoid Residents of West Siberia

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 12, pp. 681-683, December, 2005
Original article submitted June 6, 2005

Analysis of restriction fragment length polymorphism is proposed for detecting *CYP3A43* gene c1047>T mutation. The incidence of this mutation (*CYP3A43*1B* allele) was evaluated in 102 europeoid residents of West Siberia and it was found to be 12.25%.

Key Words: *cytochrome P450 3A43; genetic polymorphism; analysis of restriction fragment length polymorphism*

Cytochrome P450 3A43 (CYP3A43) was discovered not long ago [2,5,12]. CYP3A43 belongs to the CYP3A subfamily, characterized by a wide substrate specificity, responsible for metabolism of endogenous (*e.g.* testosterone, progesterone, and hydrocortisone) and exogenous compounds, and participating in metabolism of more than 50% drugs used nowadays [10].

CYP3A43 gene is little studied. Data on its polymorphism were first reported in 2003 [1]. The study of *CYP3A43* sequence with the 5'-flanking region by the method of single-strand DNA conformation polymorphism in 48 europeoids in France revealed three mutations in the coding region of the gene: c.1047>T mutation (*CYP3A43*1B* allele) is a silent mutation located in exon 11 (the incidence of this allele according to Cauffiez is 10.4%), c.74delA (*CYP3A43×2A*) in exon 2 is a frame-shift mutation modifying the synthesis of CYP3A43 protein (incidence 5.2%), and c.1018C>G (*CYP3A43×3*) in exon 10 leads to P340A amino acid substitution (incidence 4.2%) [1]. By the present time this study is the only published report presenting the incidence of *CYP3A43* alleles.

We suggest a simpler and more convenient method of polymorphism detection for detecting c.1047C>T mutation and evaluating the incidence of *CYP3A43*1B* allele: analysis of restriction fragment length polymorphism using restriction endonuclease Bst2UI.

MATERIALS AND METHODS

Genome DNA was isolated from peripheral blood leukocytes of 102 West-Siberian europeoids by a previously described method [7]. All examined subjects were patients of Municipal Pediatric Hospital No. 1 aged 1.1-14 years (mean age 6.4 years), 44% boys and 56% girls. PCR was carried out on a Tertsik device using oligonucleotide primers [1], as a result of which a 354 b. p. fragment was ob-

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0007-4888/05/1406-0726

tained. The volume of reaction mixture was 30 μl: 0.1 mM each deoxynucleoside triphosphate, 0.1 μM each primer, and 100 U/ml Taq polymerase. PCR protocol included 35 cycles: 30 sec at 94°C, 20 sec at 62-50°C, and 12 sec at 72°C, including 5-min first denaturation and 7-min final elongation of the chain. The annealing temperature was gradually reduced from 62 to 50°C in every fifth cycle by the Touchdown principle [3], respectively. Enzymatic hydrolysis was carried out in optimal buffer for Bst2UI (SibEnzyme) at 60°C for 3 h, the products were separated by electrophoresis in 12% PAAG. The fragments were visualized by ethidium bromide staining and identified using marker DNA lengths in UV light.

The results were statistically processed using EpiInfo 6 software, the significance of differences was evaluated using χ^2 test with Yates correction.

RESULTS

Analysis of sequence of amplified *CYP3A43* gene exon 11 fragment (GenBank — AC011904) using Vector NTI software detected the position of restriction endonuclease recognition sites containing the studied substitution. For evaluation of *CYP3A43*1B* polymorphism we used the optimal, to our opinion, enzyme (Bst2UI restriction endonuclease, recognition site CCWGG, where W is A or T). The studied DNA fragment had three recognition sites for this restriction enzyme in positions 19-23, 235-239, and 259-263 for wild type sequences and two sites in positions 19-23 and 259-263 for mutant type sequences, forming fragments of 217, 93, 24, and 20 b. p. and 241, 93, and 20 b. p., respectively (Fig. 1).

The allele incidence in our study (Table 1) does not differ from previously reported data [1]: 79 of 102 examined subjects were wild type homozygotes, 21 heterozygotes, and 2 mutant type homozygotes. The distribution of genotypes corresponded to the expected in Hardy—Weinberg equilibrium (Table 2).

Of all *CYP3A*, *CYP3A4* is the predominant form for the liver. The highest levels of *CYP3A43* expression were demonstrated for the prostate, prostatic adenocarcinoma cells, and testicles, where the *CYP3A43* to *CYP3A4* ratio is >100, 20, and 20, respectively [12]. The level of *CYP3A43* expression in the liver in comparison with *CYP3A4* is 0.1% [11,12]. It is therefore most likely that CYP3A43 plays a minor role in the metabolism of drugs and other xenobiotics. Participation of CYP3A43 in 6β-hydroxylase activity (the main path of steroid metabolism with participation of all CYP3A) is negligible [5]. Presumably, its significance consists in metabolism of endogenous physiologically active compounds in organs with high level of its expression.

Analysis of functional manifestations of *CYP3A43* polymorphisms is presented in only one report [13] investigating association of haplotypes of *CYP3A43* and *CYP3A4* (*CYP3A4*1B* and *CYP3A43*3*) genes with the risk of prostatic cancer. *CYP3A4*1B* was found to be a potential risk factor for prostatic cancer [6,8,9]. A strong association of *CYP3A43*3* with the risk of this disease was detected in patients with a family history of this condition (odds ratio 5.86, confidence interval 1.10-31.16) [13]. The authors showed the absence of significant relationship between the *CYP3A4*1B* and *CYP3A43*3* combination and the risk of prostatic cancer in patients with

TABLE 1. Comparative Analysis of the Incidence of *CYP3A43* Gene Alleles in West Siberian and French Europeoids

Sampling	Allele				χ^2	p
	wild type		mutant type			
	abs.	%	abs.	%		
Europeoids in West Siberia, present study (*n*=102)	179	87.75	25	12.25	0.07	0.787
Europeoids in France, [1] (*n*=48)	88	89.6	10	10.4		

Note. *n*: number of examined subjects.

TABLE 2. Distribution of *CYP3A43* Genotype Incidences in West-Siberian Europeoids

c.1047 genotype	Number of genotypes in sampling (*n*=102)	Genotype incidence	*p* according to Hardy—Weinberg
Wild type homozygote	79	0.775	0.86
Heterozygote	21	0.206	
Mutant type homozygote	2	0.019	

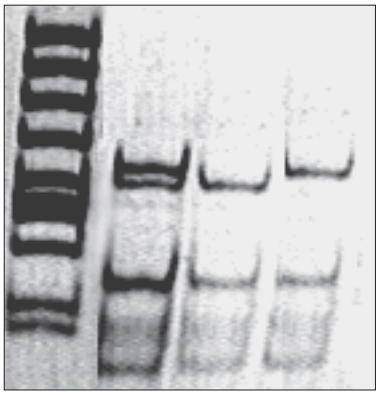

Fig. 1. Electrophoregram of Bst2UI restriction endonuclease hydrolysis. *1*) heterozygote; *2*) wild type homozygote; *3*) mutant type homozygote; *M*: DNA fragment length marker.

positive family history (odds ratio 3.62, confidence interval 0.34-38.88) [13]. These data indicate a special significance of *CYP3A43* polymorphism as a potential genetic factor of prostatic cancer risk and necessitate the development of methods for detecting these gene polymorphisms for the formation of groups at risk of this disease.

Hence, we developed a simple, rapid, and reproducible method for detecting c.1047C>T mutation in *CYP3A43* gene. Bst2UI restriction endonuclease was for the first time used for detecting *CYP3A43*1B* allele. The data on the incidence of *CYP3A43*1B* in West-Siberian europeoids are presented for the first time.

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