GENETICS

Restriction Fragments Length Polymorphism in the *NAT1* Gene of Europeans in West Siberia

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 136, No. 11, pp. 544-546, November, 2003
Original article submitted September 2, 2003

Restriction fragment length polymorphism in the *NAT1* gene was assayed to reveal 7 mutations (97C>T; 190C>T; 350,351G>C; 402T>C; 752A>T; D^{1105}; D^{1025}) in 74 Europeans from West Siberia. New methods for detecting mutations 350,351G>C, 402T>C, 752A>T, Δ^{1105}, and Δ^{1025} were proposed.

Key Words: *genetic polymorphism; NAT1; restriction endonucleases; polymerase chain reaction*

In humans, N- and O-acetylation of aromatic and some heterocyclic amines is catalyzed by arylamine-N-acetyltransferases (EC 2.3.1.5) NAT1 and NAT2 that are characterized by genetic polymorphism [3]. Human arylamine-N-acetyltransferase-1 gene (870 b.p.) is localized in the p22 region of chromosome 8, does not contain introns, and encodes a 290-amino acid residue protein [3]. *NAT1* was initially described as a monomorphic enzyme. As differentiated from *NAT2*, the degree of acetylation of specific *NAT1* substrates was characterized by monomorphic distributions in various populations. Further molecular and genetic studies revealed 28 mutations, their combinations determine 26 alleles of this gene [4].

Arylamine-N-acetyltransferase plays an important role in acquired predisposition to ecological diseases (*e.g.*, tumors) [3]. Therefore, the study of this gene is of considerable importance. The individual risk of diseases associated with *NAT1* and/or *NAT2* genotypes is low. However, the risk markedly increases when considering these genes in combination with other genes and/or exposure to aromatic and heterocyclic amines. Moreover, high frequency of functionally important mutations in *NAT1* and *NAT2* determine high populational risk.

Polymorphism in *NAT1* and *NAT2* is characterized by ethnic differences. However, genetic polymorphism of *NAT1* in Russian Europeans received little attention. The restriction fragment length polymorphism (RFLP) analysis is widely used for identifying mutations in recognition sites for various restriction endonucleases.

Here we determined the frequency of mutations in the *NAT1* gene and selected restriction endonucleases for their detection.

MATERIALS AND METHODS

Genotyping was performed in 74 healthy Europeans living in Novosibirsk. DNA was isolated from peripheral lymphocytes as described elsewhere [5]. Polymerase chain reaction (PCR) on an AMP-105 amplifier (Bis) with oligonucleotide primers N-376 and N1177 [6] was performed to obtain a 1552-b.p. product. The reaction was conducted in 100 μl. The reaction mixture contained 50 μM deoxynucleoside triphosphates, 0.2 μM N-376, 0.2 μM N1177, and 20 U/ml Taq DNA polymerase in buffer for PCR. Thirty cycles of PCR were performed: 1 min at 94°C, 2 min

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0007-4888/03/1365-0482$25.00

TABLE 1. Restriction Endonucleases Used to Detect Mutations in the *NAT1* Gene by the RFLP Assay

Mutation, restriction endonuclease		Fragment length	
		"wild-type" allele	mutant allele
190C>T	Sse9I	226, 27, 51, 99, 26, 358, 110, 73, 139, 289, 103, 46, 5	226, 27, 51, 99, 26, 133, 225, 110, 73, 139, 289, 103, 46, 5
	Sse9I+VspI	81, 145, 27, 51, 99, 26, 358, 110, 73, 139, 289, 103, 46, 5	81, 145, 27, 51, 99, 26, 133, 225, 110, 73, 139, 289, 103, 46, 5
350,351G>C	PspN4I	813, 739	724, 89, 739
752A>T	BglII	1126, 426	1552
	Bst4CI	879, 194, 479	879, 194, 53, 426
97C>T	BglII	1126, 426	469, 657, 426
402T>C	Bst2UI	584, 968	584, 195, 773
Δ^{1105}	Bst2UI	584, 968	584, 901, 66
Δ^{1025}	Bst4CI	879, 194, 479	879, 194, 333, 145

at 60°C, and 2 min at 72°C. The first denaturation and final elongation stages lasted 4 and 6 min.

Enzymatic hydrolysis of the amplified DNA fragment was performed in an optimal buffer at various optimum temperatures for each restriction endonuclease over 3 h.

Restriction products were separated by gel electrophoresis in 2% agarose gel and Tris-acetate buffer. After electrophoresis DNA bands were visualized with ethidium bromide and identified in UV light by comparing with marker fragments. Restrictases BglII, Bst2UI, Bst4CI, PspN4I, Sse9I, and VspI (Research and Production Company Sib-Enzim) were used to detect 5 mutations in the encoding gene region and 2 mutations in the non-encoding gene region (97C>T; 190C>T; 350, 351G>C; 402T>C; 752A>T; Δ^{1105}; Δ^{1025}) [4].

RESULTS

Localization of restriction sites for various endonucleases in the primary sequence of the *NAT1* gene (GenBank, Acc. No. X17059) was estimated by means of Dnasis software to use optimal restriction endonucleases (Table 1). Mutation 350,351G>C (GGCA GG^{351}>$GGCACC^{351}$) was identified using PspN4I endonuclease (recognition site GGNNCC; N: any base). BanI endonuclease (GGYRCC; Y: T or C; R: A or G) was previously proposed to detect this mutation [2]. In our opinion PspN4I is more preferential for this purpose, since the restriction digest less significantly depends on nucleotides in positions 348 and 349 compared to BanI.

752A>T substitution was detected with BglII [1] and Bst4CI endonucleases (Fig. 1, 2). The RFLP assay is based on the disappearance of a certain site from the primary sequence or appearance of a new site due to mutation. In this instance, the mutation results in disappearance of the recognition site for BglII endonuclease consisting of 6 nucleotides. Theoretically, characteristics of hydrolysis will be identical with the appearance of mutations in any of the six positions and, therefore, they cannot be distinguished by this method. Bst4CI endonuclease allows us to localize mutations by the appearance of a new recognition site (Fig. 1). It was observed previously in studies of arylamine-N-acetyltransferase. Mutation 191G>A resulting in the substitution of arginine in position 64 for glutamine was first revealed in the *NAT2* gene with MspI endonuclease. Its recognition site is violated in the presence of this mutation (nucleotides 189-192). Further experiments revealed mutation 190C>T resulting in substitution of arginine for tryptophan. When MspI endonuclease was used to identify mutation in position 191, several samples could include an unknown mutation in position 190.

97C>T substitution was detected with BglII endonuclease [7]. Bst2UI and Bst4CI endonucleases were used to identify mutations in the *NAT1* gene by PCR. Mutations led to the appearance of a new site for Bst2UI (402T>C and Δ^{1105}) and Bst4CI (Δ^{1025}). 190C>T sub-

Mutant allele 5'-^{746}ATACAG**T**TCTAA, Bst4CI (ACNGT)

"Wild-type" allele 5'-^{746}ATACAG**A**TCTAA, BglII (AGATCT)

Fig. 1. Detection of mutation 752A>T in the *NAT1* gene. Recognition sites for restriction endonucleases are underlined. Bold font: nucleotides at position 742.

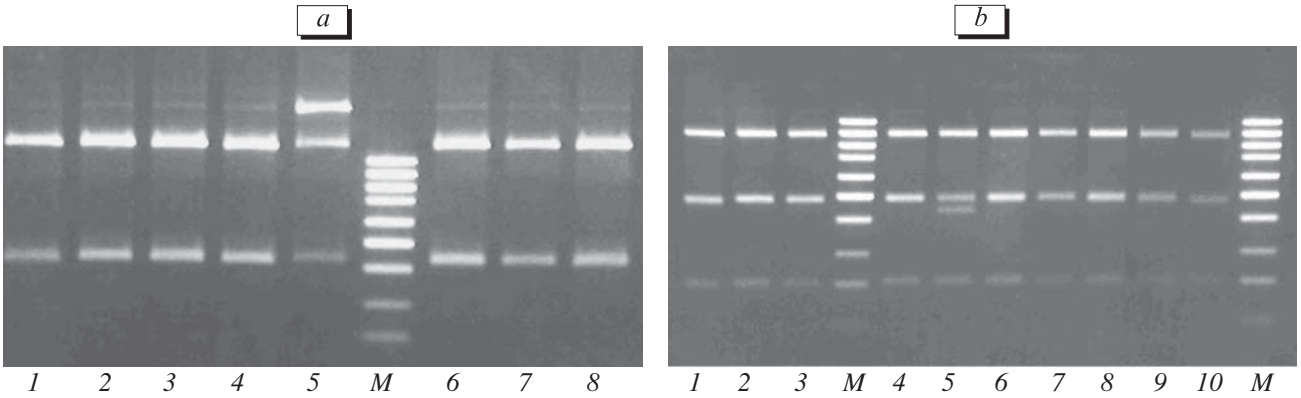

Fig. 2. Electrophoregram of hydrolysis with BglII (*a*) and Bst4CI restriction endonucleases (*b*). Track 5: heterozygote mutation 752A>T. *M*, marker for the precise length of DNA fragments (100-1000, 100-nucleotide steps).

TABLE 2. Frequency of Mutations in the *NAT1* Gene of Europeans in West Siberia

Mutation	Mutation frequency	
	experimental results	published data
97C>T	0	0 [7]
190C>T	0	0.001 [1]
350,351G>C	0	0 [7]
402T>C	0	—
752A>T	0.00676	0.004 [1]
Δ^{1105}	0	—
Δ^{1025}	0	—

Note. «—», not found in available literature.

stitution was detected with Sse9I endonuclease, which acts as an isoschizomer of Tsp509I endonuclease used to reveal this mutation [1]. A 358-b.p. fragment plays an important role in identification of this mutation and yields fragments of 133 and 225 b.p. (Table 1). Electrophoresis in 2% agarose gel revealed no differences in mobility of detected fragments and fragments with lengths of 139 and 226 b.p., respectively. To avoid superposition of fragments, we performed incubation with VspI endonuclease having a single site in the *NAT1* gene. A 226-b.p. fragment yielded 2 fragments of 81 and 145 b.p. In this case, mutation 190C>T can be easily identified.

We revealed 1 allele with mutation 752A>T. Other 6 mutations were not identified (rare mutations, Table 2). The frequency of mutations in Europeans of West Siberia is consistent with published data. However, it is necessary to increase the sample size to obtain real values.

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