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SHORT COMMUNICATION

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Thirteen single-nucleotide polymorphisms (SNPs) in the alcohol dehydrogenase 4 (*ADH4*) gene locus

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Abstract The human alcohol dehydrogenase 4 (*ADH4*) gene encodes the class II ADH4 pi subunit, which contributes to the metabolization of a wide variety of substrates, including ethanol, retinol, other aliphatic alcohols, hydroxysteroids, and lipid peroxidation products. Here we report the results of systematic screening for single-nucleotide polymorphisms (SNPs) in the *ADH4* gene by means of direct sequencing combined with a polymerase chain reaction method. A total of 16 genetic variations including 13 SNPs were found; 4 in the 5′ flanking region, 4 in the 5′ untranslated region, and 8 within introns. No variation was found in coding, 3′ untranslated, or 3′ flanking regions. Eight of the 13 SNPs were not reported in the NCBI dbSNP database or any previous publications. Our SNP map presented here should provide tools to evaluate the role of ADH4 in complex genetic diseases and a variety of pharmacogenetic effects.

Key words Single-nucleotide polymorphisms (SNPs) · Insertion–deletion polymorphisms · High-density SNP map · Alcohol dehydrogenase 4 (*ADH4*) gene · Japanese population

Introduction

Alcohol dehydrogenases (ADHs, EC1.1.1) are key enzymes that catalyze oxidative conversion of various alcohols to the corresponding aldehydes in a reversible fashion (Jörnvall and Höög 1995; Lieber 1997). Enzymatic analysis of catalytic properties, gene expression pattern, and comparison of DNA sequences of ADHs in various vertebrate species have caused them to be classified them into seven distinct classes (Duester et al. 1999). One of the family members, the human alcohol dehydrogenase 4 (*ADH4*) gene (OMIM: 103740), encodes a class II pi-ADH that catalyzes oxidoreduction of a broad range of alcohols and the corresponding aldehydes (Höög et al. 1987; Ditlow et al. 1984; Sellin et al. 1991). The gene spans an approximately 21-kb genomic region and consists of nine exons (von Bahr-Lindström et al. 1991). The expression of mRNA was detected mainly in liver, and a low amount of expression was observed in small intestine, pancreas, and stomach (Estonius et al. 1996).

We previously constructed high-density single-nucleotide polymorphism (SNP) maps of the regions encoding eight alcohol dehydrogenase genes in the Japanese population (Iida et al. 2001). In this study, we systematically screened 48 Japanese volunteers for SNPs in an additional member of this gene family, *ADH4*, and constructed a fine-scale SNP map of the 26.9-kb region containing the entire gene by direct sequencing combined with a polymerase chain reaction (PCR) analysis.

Subjects and methods

Blood samples were obtained with written informed consent from 48 healthy Japanese volunteers for this study, which was approved by the ethical committee of the RIKEN SNP Research Center. On the basis of genomic sequences from the GenBank database (accession number: AP002026), we designed primers to amplify the *ADH4* gene in its entirety, as well as 2 kb upstream from the first exon and downstream from the last exon. Most of the regions corresponding to human repetitive sequences were excluded in this study. The methods used to screen for SNPs are available from our website (http://snp.ims.u-tokyo.ac.jp/).

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Results and discussion

A total of 13 SNPs among the 48 volunteers were identified in the 26.9-kb region covering the entire *ADH4* gene: 4 in the 5′ flanking region, 4 in the 5′ untranslated region, and 5 within introns. No variation was found in coding, 3′ untranslated, or 3′ flanking regions. In addition to SNPs, we identified three insertion–deletion polymorphisms in introns 1, 5, and 7, of which that in intron 1 was a mononucleotide repeat polymorphism. A fine-scale physical map of the *ADH4* gene locus (Fig. 1) was constructed, and detailed information on the genetic variations found is summarized in Table 1. A comparison of our data with a previous report by Edenberg et al. (1999) and also with SNPs deposited in the dbSNP database in the U.S. National Center for Biotechnology Information (NCBI) showed that 8 of the 13 SNPs detected by us were novel (Table 1) (as of mid-July 2001). Among the 13 SNPs, 8 were located in the 5′ flanking and 5′ untranslated regions (Fig. 1). According

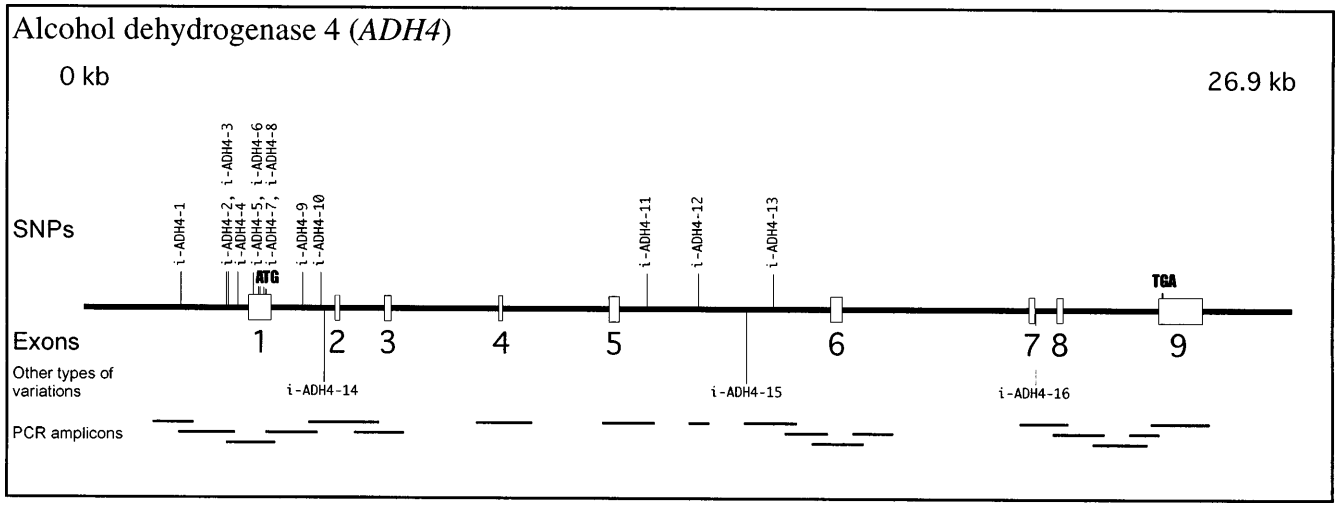

Fig. 1. Genomic organization and localizations of single-nucleotide polymorphisms (*SNPs*) in human alcohol dehydrogenase 4 (*ADH4*). A total of 16 kb (60%) excluding repetitive-sequence-rich segments in the 26.9-kb genomic region corresponding to the *ADH4* gene was screened; the polymerase chain reaction (PCR) *amplicons* are drawn with *horizontal lines* below the gene map. *Exons* and introns are represented by *rectangles* and *horizontal lines*, respectively. Thirteen SNPs identified in this study are indicated above the gene (designations correspond to those in the left-most column in Table 1). The three insertion–deletion variations are also shown below the gene

Table 1. Characterization of variations from the *ADH4* gene locus

ID	Region	Position[a]	Flanking sequence[b]	Variation (5′ to 3′)[c]	Flanking sequence[b]	Identity to dbSNP	Reference
i-ADH4-1	5′ Flanking region	−1487	aactaaaaaaaactcatgca	T/C	tgcattggggaaaacagttt	rs2226896	
i-ADH4-2	5′ Flanking region	−482	acagccagagacccagaacc	A/G	tcagggctggttgatggact		
i-ADH4-3	5′ Flanking region	−437	catcaggtgggacaaaaaga	G/A	tagctccttagcagtgacta		
i-ADH4-4	5′ Flanking region	−234	actcaagcatatgtgcaacc	A/G	agtacatgaaaagaatttgt		
i-ADH4-5	5′ Untranslated region	−361	ggtaagttaaatgggcgatt	C/G	tgaggagtagaaatttcctt		
i-ADH4-6	5′ Untranslated region	−253	ttcaataaaagaaaaaagaa	T/A	ttaaaaaatcttggagctca		Edenberg et al. 1999
i-ADH4-7	5′ Untranslated region	−220	ggagctcactgggagcaatg	A/G	ggtttgcagctgaagtccaa	rs1800761	Edenberg et al. 1999
i-ADH4-8	5′ Untranslated region	−136	cagcaacaaaggagaaaagg	A/C	agtgattggagaattaagca	rs1800759	Edenberg et al. 1999
i-ADH4-9	Intron 1	707	ttatatttgaaattaaaaat	A/G	taatttgaggctagaaaaaa		
i-ADH4-10	Intron 1	1109	catctaccttattcaaactc	C/A	ataaatctattctctctgtt	rs2032348	
i-ADH4-11	Intron 5	619	tcaaagagggatctcacaat	T/C	ggacatctcaacctgcttat		
i-ADH4-12	Intron 5	1755	tttacgcacacaattactca	T/C	taataaaaaatttaaaaaat		
i-ADH4-13	Intron 5	3425	actgagactctggagcaata	T/C	attaagaatcatactgaaca		
i-ADH4-14	Intron 1	1181–1189	ggtaaactttaatacacctg	$(T)_{9–11}$	caagaaataaaaaatgtaat		
i-ADH4-15	Intron 5	2828	tccagtcaaagtcgacctaa	A/del	tttccaggagttgttcttcc		
i-ADH4-16	Intron 7	15	ttggtggtcagttttttttt	T/del	cttcatagctttaaattctt		

del, deletion polymorphism
[a] Nucleotide numbering is according to the mutation nomenclature (den Dunnen and Antonarakis 2000)
[b] Both 5′ and 3′ flanking sequences to each variation are denoted by small letters
[c] Variation is shown by capital letters

to the report (Edenberg et al. 1999), a −75A>C substitution (i-ADH4-8) is likely to have a dramatic effect on promoter activity; the promoter activity of the DNA sequence containing an A at position −75 was more than double that of the DNA sequence containing a C at −75. Since the rate of ethanol oxidation largely depends on ADH activity, the difference in the amount of *ADH4* expressed in liver should influence the metabolization of ingested ethanol. Ultimately, it is possible that the SNP at this position may affect a person's risk for alcoholism by modulating alcohol metabolism. Furthermore, the remaining four SNPs in the 5′ flanking region might also have some effect on promoter function.

The SNP collection reported here will provide useful information for genetic studies and accelerate certain aspects of human genomic and pharmacogenetic research.

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