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Seventy genetic variations in human microsomal and soluble epoxide hydrolase genes (*EPHX1* and *EPHX2*) in the Japanese population

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Abstract Human microsomal and soluble epoxide hydrolases (mEH and sEH) are enzymes that metabolize xenobiotic molecules. We screened DNA from 48 Japanese individuals for single-nucleotide polymorphisms (SNPs) in both genes by direct sequencing of the entire genomic regions containing *EPHX1* and *EPHX2*, except for repetitive elements. This approach identified 33 SNPs in the *EPHX1* gene; 6 of them were located in the 5′ flanking region, 17 in introns, 8 in exons, and 2 in the 3′ flanking region. In the *EPHX2* gene, we identified 36 SNPs, including 4 in the 5′ flanking region, 24 in introns, 5 in exons, and 3 in the 3′ flanking region, as well as one insertion/deletion polymorphism in the 5′ flanking region. These variants may contribute to a more precise understanding of the nature of correlations between genotypes and disease-susceptibility phenotypes that have been postulated in regard to human microsomal and soluble epoxide hydrolases.

Key words Single-nucleotide polymorphism (SNP) · Human microsomal epoxide hydrolase gene (*EPHX1*) · Human soluble epoxide hydrolase gene (*EPHX2*) · Xenobiotic-metabolizing enzymes · Genotype

Introduction

Epoxide hydrolases (EH, EC3.3.2.3) constitute a family of enzymes that function to hydrate simple epoxides to vicinal diols and arene oxides to *trans*-dihydrodiols. Epoxide hydrolases that are associated with drug metabolism are classified into two xenobiotic-metabolizing forms, microsomal (mEH) and soluble (sEH) (Skoda et al. 1988; Beetham et al. 1993); mEH can be distinguished from sEH on the basis of physical properties (Beetham et al. 1995).

The microsomal form of epoxide hydrolase (mEH), localized predominantly in the endoplasmic reticulum, is encoded by the *EPHX1* gene on chromosome 1q42.1 (Hartsfield et al. 1998). It catalyzes the conversion of a broad spectrum of highly reactive cytotoxic arene oxides and aliphatic epoxides to less toxic *trans*-dihydrodiols (Raaka et al. 1998). Polymorphisms that have been identified in the *EPHX1* gene are thought to affect its enzymatic activity (Gaedigk et al. 1994; Hassett et al. 1994). For example, two variant *EPHX1* alleles have been associated with altered mEH activity in Caucasians; in those subjects, substitution of histidine for the more common tyrosine at codon 113 in exon 3 decreased mEH activity, whereas substitution of arginine for histidine at codon 139 in exon 4 increased this activity, probably because those variants affected the stability of the mEH protein or increased the enzyme's affinity for substrates. However, when both mutations were present, the enzymatic activity was approximately normal (Hassett et al. 1994). In addition to these polymorphisms in the coding region, any variants present in the 5′ flanking region would also be likely to influence this enzyme's activity in humans (Raaka et al. 1998).

In contrast, the soluble form of epoxide hydrolase (sEH) is confined mainly to cytoplasm. This enzyme, encoded by the *EPHX2* gene on chromosome 8p12-p21 (Larsson et al. 1995), participates in the metabolism of xenobiotic molecules, with a preference for *trans*-substituted epoxides, such as *trans*-stilbene oxide (Bjelogrlic et al. 1994; Bauer et al. 1995). Recently, Sandberg et al. (2000) identified two variant sites in the *sEH* gene, resulting, respectively, in the substitution of arginine for glutamine in exon 8 and an insertion of arginine between codons 402 and 403 in exon 13. Although the activity of the glutamine-287 variant was similar to that of the wild type, the protein containing the arginine insertion exhibited strikingly lower activity.

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A number of investigators have examined potential associations of mEH genotypes with altered susceptibilities to various diseases. Results from these investigations suggest that certain mEH genotypes may, indeed, be associated with syndromes that include hepatocellular carcinoma (McGlynn et al. 1995), ovarian cancer (Lancaster et al. 1996), colon cancer (Harrison et al. 1999), smoking-associated cancers (Benhamou et al. 1998; Jourenkova-Mironova et al. 2000), and certain pulmonary disorders (Smith and Harrison 1997). However, the molecular basis for variations in mEH activity has not been characterized well.

To investigate the nature of apparent genotype/phenotype correlations for mEH and sEH more precisely, we began by searching for additional single-nucleotide polymorphisms (SNPs) in the *EPHX1* and *EPHX2* genes, including their promoter regions and entire introns, and report here a total of 70 genetic variations, of which 47 had not been reported before.

Materials and methods

Amplification of samples

Total genomic DNAs were isolated from peripheral leukocytes of 48 unrelated Japanese individuals by the standard phenol/chloroform extraction method. Exon-intron boundaries of the *EPHX1* gene were defined by comparison of genomic sequences (accession number, AC058782.8 in the GenBank database) with cDNA sequences (accession number, NM_000120.2 in the GenBank database); those of the *EPHX2* gene were defined by comparison of genomic sequences (accession number, AC010856.3 in the GenBank database) with cDNA sequences of *EPHX2* (accession number, AF233334.1 in the GenBank database). On the basis of the Genbank sequence information, we designed polymerase chain reaction (PCR) primers to amplify DNA fragments from both genes, excluding repetitive elements, by invoking the REPEAT MASKER computer program in the manner described by Seki et al. (2000). We used 60 ng of DNAs from three individuals, mixed in equal amounts, for each PCR experiment. The reactions were performed as follows: initial denaturation at 94°C for 2 min, followed by 35 cycles of amplification at 94°C for 30 s, annealing at 60°C for 1 min, and extension for 1 min.

Direct sequencing and detection of polymorphism

Products obtained from the PCR experiments were used as templates for direct sequencing and detection of SNPs, by the fluorescent dye-terminator cycle sequencing method (ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction kit; Perkin Elmer, CA, USA). These procedures were carried out according to methods we have described elsewhere (Ohnishi et al. 2000; Seki et al. 2000; Yamada et al. 2000). Allelic frequencies of nonsynonymous SNPs in the *EPHX1* and *EPHX2* genes were calculated individually from 93 unrelated Japanese individuals, respectively.

Results

By screening 96 Japanese chromosomes by direct DNA sequencing, we identified 33 SNPs in the *EPHX1* gene, and 36 SNPs and one insertion/deletion polymorphism in the *EPHX2* gene. Figure 1 illustrates the locations of these SNPs; detailed information about nucleotide positions and substitutions is summarized in Table 1 for *EPHX1* and in Table 2 for *EPHX2*.

Of the 33 SNPs found in *EPHX1*, 6 were present in the 5′ flanking region, 17 in introns, 8 in exons, and 2 in the 3′ flanking region; 25 of the total were transitions and the remaining 8 were transversions. Of the 8 SNPs found in exons, 2 caused substitutions of amino acids (Tyr113His in exon 3 and His139Arg in exon 4). Both of these variants had been reported previously (Hassett et al. 1994).

Of the 36 SNPs detected in *EPHX2*, 4 were located in the 5′ flanking region, 24 in introns, 5 in exons, and 3 in

Table 1. Summary of SNPs detected in the *EPHX1* gene

SNP no.	Location	Position[a]	SNP
1	5′ Flanking	−699	C/T[b]
2	5′ Flanking	−613	T/C[b]
3	5′ Flanking	−362	G/A[b]
4	5′ Flanking	−290	G/T[b]
5	5′ Flanking	−259	T/C[b]
6	5′ Flanking	−200	T/C[b]
7	Exon 1	66	G/A(5′UTR)[c]
8	Intron 1	110	C/T
9	Intron 1	143	G/A
10	Intron 1	1097	T/G
11	Intron 1	1717	T/C
12	Intron 1	1772	G/T
13	Intron 1	2054	A/G
14	Intron 1	2114	C/T[d]
15	Intron 1	2287	C/G[d]
16	Intron 2	1414	G/T
17	Intron 2	1514	A/G[d]
18	Exon 3	154	T/C(Tyr113His)[d,e]
19	Exon 3	174	G/A(Lys119Lys)
20	Intron 3	6583	G/C
21	Exon 4	52	A/G(His139Arg)[e]
22	Intron 4	34	G/A
23	Intron 4	63	C/T
24	Intron 5	154	G/A
25	Intron 5	276	T/C
26	Exon 6	130	C/T(Pro284Pro)
27	Exon 8	31	C/T(Asn357Asn)[c]
28	Intron 8	206	C/A
29	Intron 8	353	A/G
30	Exon 9	82	G/A(Lys416Lys)[c]
31	Exon 9	184	G/C(Ser450Ser)[c]
32	3′ Flanking	212	A/G[d]
33	3′ Flanking	708	A/G

SNP, single nucleotide polymorphism

[a] For SNPs in the 5′ flanking region, intron region, or 3′ flanking region, nucleotide positions are counted from the first intronic nucleotide at the exon/intron junction (for SNPs in the exon region, nucleotide positions are counted from the first exonic nucleotide at the exon/intron junction)

[b] SNPs previously reported by Raaka et al. (1998)

[c] SNPs previously reported in the GenBank database

[d] SNPs previously reported in the dbSNP database

[e] SNPs previously reported by Hassett et al. (1994)

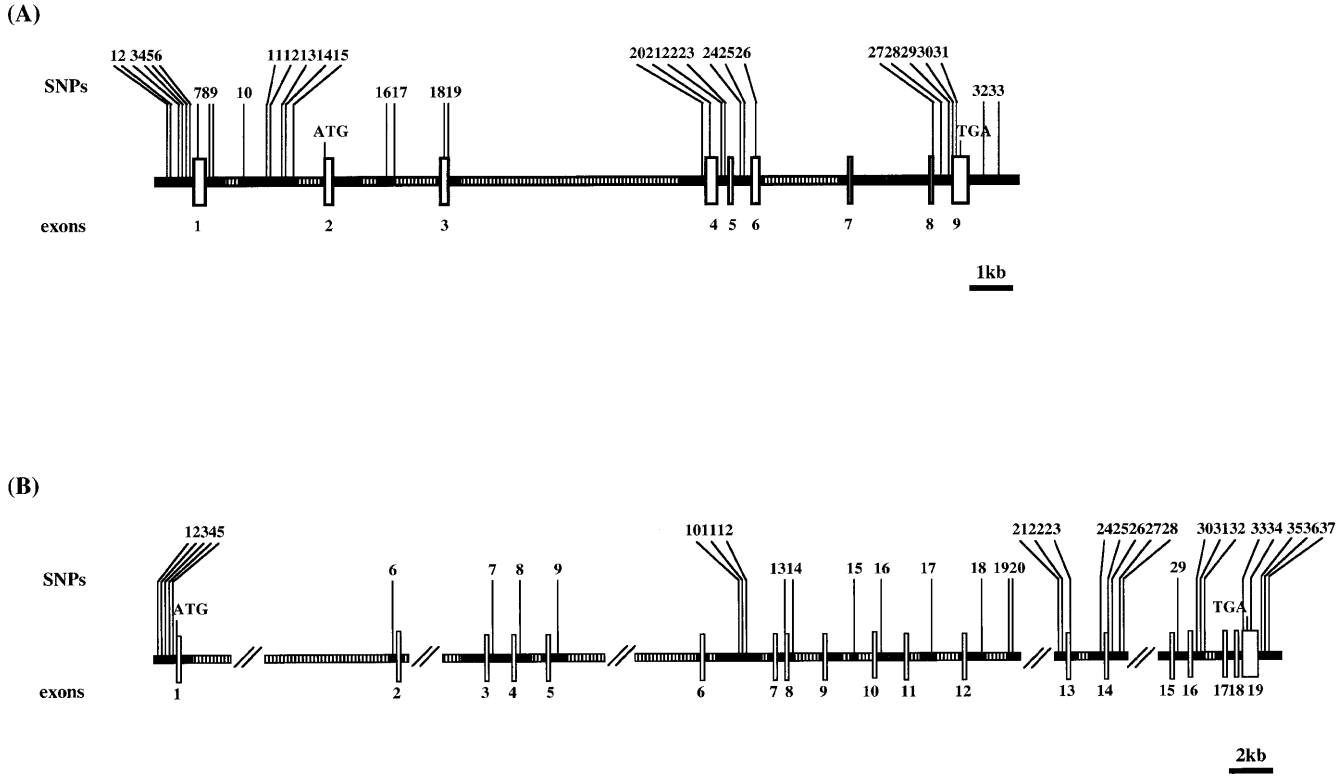

Fig. 1A,B. Locations of single-nucleotide polymorphisms (*SNPs*) in **A** the *EPHX1* and **B** *EPHX2* genes, indicated by *vertical lines*. *Open boxes* represent exons; *hatching* on the chromosomes indicates regions of repetitive elements. *ATG* and *TGA*, Initiation and stop codons, respectively

the 3′ flanking region; 27 were transitions and 9 were transversions. Among the five variant sites present in exons, two would cause amino acid substitutions, Arg287Gln in exon 8 and Ser407Ile in exon 13.

Discussion

We identified 33 SNPs in the *EPHX1* gene, of which 17 were novel. Two of these 33 SNPs would substitute amino acids, Tyr113His in exon 3 and His139Arg in exon 4, but both of these variants had been reported previously (Hassett et al. 1994). Recently, Belmahdi et al. (2000) reported two additional nonsynonymous polymorphisms in the *EPHX1* gene among 150 French individuals; one caused an Arg49Cys substitution in exon 2 (the frequency of the minor allele was 0.67%) and the other, an Arg454Gln substitution in exon 9 (the frequency of the Gln allele was 0.73%). We found neither of these variants among the 93 Japanese individuals we screened. Although the biological significance of the French polymorphisms is unclear, they must be considered very rare substitutions.

On the basis of evidence that, in two of the variant *EPHX1* alleles, substitutions of histidine for tyrosine at codon 113 and arginine for histidine at codon 139 were associated with altered mEH activity in Caucasians (Hassett et al. 1994), potential associations between mEH genotypes and susceptibility to certain diseases have been investigated in various laboratories. Although some findings have suggested an association with risk for carcinomas of liver, ovary, colon, and lung, as well as emphysema (McGlynn et al. 1995; Lancaster et al. 1996; Smith et al. 1997; Benhamou et al. 1998; Harrison et al. 1999; Jourenkova-Mironova et al. 2000), no conclusive results have emerged. Because the molecular basis for genetic variations in mEH activity has not yet been well characterized, additional studies to examine potential relationships between mEH allelic status and susceptibility to certain diseases will be required to clarify any potential associations. The novel SNPs in *EPHX1* published here will contribute to investigations of that kind.

Among the 37 genetic variations found in the *EPHX2* gene, 30 variants, including a nonsynonymous SNP (Ser407Ile) were novel. Recently, Sandberg et al. (2000) reported eight variants in *EPHX2* cDNA, of which two led to amino-acid substitutions (Arg287Gln and $Arg^{402–403ins}$). The sEH protein containing the arginine insertion exhibited a strikingly lower activity, but the activity of the 287Gln variant was similar to that of the wild type. In the Japanese population, we found no $Arg^{402–403ins}$ in any of the 92 individuals examined; instead, we found a novel Ser407Ile substitution in only 1 of these 184 chromosomes (allelic frequency of 0.54%) although its biological effect is unknown. We consider this variant to be a rare substitution in the Japanese population.

Table 2. Summary of genetic variations detected in the *EPHX2* gene

SNP no.	Location	Position[a]	SNP
1	5′ Flanking	−(523-522)	insC
2	5′ Flanking	−522	T/C
3	5′ Flanking	−521	C/T
4	5′ Flanking	−516	G/C
5	5′ Flanking	−515	C/G
6	Intron 1	−74	T/C
7	Intron 3	72	T/C
8	Intron 4	473	A/G
9	Intron 5	276	C/T
10	Intron 6	2150	G/A[b]
11	Intron 6	2181	G/A[b]
12	Intron 6	2185	A/G[b]
13	Exon 8	29	G/A(Arg287Gln)[b,c]
14	Intron 8	8	T/C
15	Intron 9	1573	C/T
16	Intron 10	207	T/C
17	Intron 11	1173	G/C[b]
18	Intron 12	911	G/T
19	Intron 12	2425	C/T
20	Intron 12	2460	G/A
21	Intron 12	−281	T/C
22	Intron 12	−268	T/G
23	Exon 13	50	G/T(Ser407Ile)
24	Intron 13	1739	G/T
25	Exon 14	33	G/A(Ala425Ala)
26	Intron 14	314	T/C
27	Intron 14	878	C/T
28	Intron 14	948	T/C
29	Intron 15	259	C/T
30	Intron 16	459	G/C
31	Intron 16	645	G/A
32	Intron 16	985	G/A
33	Exon 19	4	A/C(Pro531Pro)[c]
34	Exon 19	114	G/A(3′UTR)[c]
35	3′ Flanking	12	T/C
36	3′ Flanking	374	C/T
37	3′ Flanking	544	G/A

SNP, single nucleotide polymorphism

[a] For SNPs in the 5′ flanking region, intron region, or 3′ flanking region, nucleotide positions are counted from the first intronic nucleotide at the exon/intron junction (for SNPs in the exon region, nucleotide positions are counted from the first exonic nucleotide at the exon/intron junction)

[b] SNPs previously reported in the dbSNP database

[c] SNPs previously reported by Sandberg et al. (2000)

In addition to the five genetic variations in the 5′ flanking region of *EPHX2*, we also found a CA microsatellite polymorphism (data not shown). Because variants that are present upstream of either gene may effect transcriptional efficiency, they, and the other SNPs published here, should be useful for examining the relationships between an individual's EH genotype and his or her susceptibility to certain diseases.

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