SNP Communication

Five Novel Single Nucleotide Polymorphisms in the EPHX1 Gene Encoding Microsomal Epoxide Hydrolase

Kisho SHISEKI1, Masaya ITODA1, Yoshiro SAITO1,2, Yukiko NAKAJIMA1, Keiko MAEKAWA1,2, Hideo KIMURA3, Yu-ichi GOTO3, Osamu SAITO4, Masaaki KATO4, Teiichi OHNUMA4, Mitsuru KAWAI4, Kenji SUGAI4, Taisuke OHTSUKI4, Chieko SUZUKI4, Narihiro MINAMI3,4, Shogo OZAWA1,5 and Jun-ichi SAWADA1,2

1Project team for Pharmacogenetics, 2Division of Biochemistry and Immunochemistry, 3Division of Pharmacology, National Institute of Health Sciences, Tokyo, Japan, 4National Institute of Neuroscience, 5National Center Hospital for Mental, Nervous and Muscular Disorders, National Center for Neurology and Psychiatry, Tokyo, Japan

Summary: Five novel single nucleotide polymorphisms (SNPs) were found in the EPHX1 gene from 96 Japanese epileptic patients. The detected SNPs were as follows:

1) SNP, MPJ6_EX1009; GENE NAME, EPHX1; ACCESSION NUMBER, NT_004525.12; LENGTH, 25 bases; 5'-CCTCAGTTCACTGCAGTG-3'.

2) SNP, MPJ6_EX1013; GENE NAME, EPHX1; ACCESSION NUMBER, NT_004525.12; LENGTH, 25 bases; 5'-TCCGCAGGCCAGGCGAGCCG-3'.

3) SNP, MPJ6_EX1026; GENE NAME, EPHX1; ACCESSION NUMBER, NT_004525.12; LENGTH, 25 bases; 5'-GTCTCCTCCTGGACTGTTCGACC-3'.

4) SNP, MPJ6_EX1028; GENE NAME, EPHX1; ACCESSION NUMBER, NT_004525.12; LENGTH, 25 bases; 5'-AGGCAGGAGGACGACGAGCCGAGCAGC-3'.

5) SNP, MPJ6_EX1030; GENE NAME, EPHX1; ACCESSION NUMBER, NT_004525.12; LENGTH, 25 bases; 5'-TGGGTTCAAGTACAGGCGAGGGCTTCACTGCAGTG-3'.

The frequencies were 0.016 for MPJ6_EX1028 (IVS8 + 54G > A) and 0.005 for the other SNPs. The SNP MPJ6_EX1013 (30G > A) results in an amino acid alteration (E44Q). The other three SNPs in the coding region, MPJ6_EX1009 (30G > A), MPJ6_EX1026 (1056C > T), and MPJ6_EX1030 (1239G > A) result in synonymous changes (V10V, D352D, and V413V, respectively).

Key words: EPHX1; novel SNPs; non-synonymous alteration; drug metabolism

Introduction

Microsomal epoxide hydrolase (mEH) is a member of an α/β-hydroxylase family and catalyzes the hydrolysis of a wide range of arene and aliphatic epoxides by trans-addition of water to form glycols.1,2)

This enzyme has been reported to be localized at the cytosolic side of the endoplasmic reticulum membrane in all examined tissues, with the highest activity in the...
Fig. 1. Electropherograms (sense strands) for the novel EPHX1 SNPs. (A) MPJ6_EX1009 (wild-type 30G/W; variant 30G/A). (B) MPJ6_EX1013 (wild-type 130G/W; variant 130G/C). (C) MPJ6_EX1026 (wild-type 1056C/W; variant 1056C/T). (D) MPJ6_EX1028 (wild-type IVS8+54G/W; variant IVS8+54G/A). (E) MPJ6_EX1030 (wild-type 1239G/W; variant 1239G/A). Arrows indicate the variable nucleotide positions.

mEH activity has been extensively characterized with exogenous drug and chemical substrates. In these cases, the majority of epoxides are thought to be formed by cytochrome P450-mediated oxidation, such as CYP3A4-catalyzed 10,11-epoxidation of the antiepileptic drug carbamazepine. mEH also plays important roles in the detoxification of metabolically formed hazardous epoxides derived from chemicals.
such as styrene 7,8-oxide and benzo[a]pyrene 4,5-oxide. Thus, mEH is an important enzyme involved in drug efficacy and chemical carcinogenicity.

The rates of mEH-catalyzed hydrolysis of benzo[a]pyrene 4,5-oxide differ approximately 40-fold between individual human blood lymphocytes. This difference has been suggested to be attributed to EPHX1 genetic polymorphisms. Hassett et al. reported two polymorphisms (Y113H and H139R) and analyzed their effects on enzyme activity using the substrate benzodioxin.2) Thus, mEH is an important enzyme involved in drug efficacy and chemical carcinogenicity.

In this study, we sequenced all exons and surrounding introns of EPHX1. Saito et al. reported 33 EPHX1 polymorphisms, including 17 novel ones, using genomic DNA from 48 Japanese individuals.8) Recently, we have detected 5 non-synonymous single nucleotide (SNPs) polymorphisms, including Y113H and H139R, using DNA from 72 established cell lines from Japanese individuals, and characterized their functional effect on catalyzing the hydrolysis of cis-stilbene oxide.9) Two of the non-synonymous changes influenced the activities in vitro, but not dramatically. In this study, we sequenced all exons and surrounding introns of EPHX1 from 96 Japanese epileptic patients, and identified five novel SNPs.

Materials and Methods

Human DNA samples: DNA was extracted from the blood leukocytes of 96 Japanese epileptic patients. Both of the ethics committees of the National Center for Neurology and Psychiatry and the National Institute of Health Sciences approved this study. Written informed consent was obtained from all patients.

PCR conditions and DNA sequencing: PCR amplification and DNA sequencing were performed as described previously. All the detected novel SNPs were confirmed by repeating the PCR on genomic DNA and sequencing the newly generated PCR products.

Results and Discussion

We detected 5 novel SNPs, which are described below. The nucleotide sequences of the PCR-amplified DNA were completely identical to the EPHX1 reference sequence NT_004525.12, except for these SNPs.

1) SNP, MPJ6_EX1009; GENE NAME, EPHX1; ACCESSION NUMBER, NT_004525.12; LENGTH, 25 bases; 5′-GTTCCTCCTGGAC/TGACCTGCTGACC-3′.

2) SNP, MPJ6_EX1013; GENE NAME, EPHX1; ACCESSION NUMBER, NT_004525.12; LENGTH, 25 bases; 5′-AGGCAGGGGAGG/AGCCAGCTGGGA-3′.

3) SNP, MPJ6_EX1026; GENE NAME, EPHX1; ACCESSION NUMBER, NT_004525.12; LENGTH, 25 bases; 5′-GTAAAGTGGGT/AAGGTCAATAC-3′.

The electropherograms of the novel SNPs are shown in Fig. 1. The cDNA positions of these SNPs were 30 (A of the translational start codon is numbered as 1) (MPJ6_EX1009) and 130 (MPJ6_EX1013) in exon 2, 1056 (MPJ6_EX1026) in exon 6, 1239 (MPJ6_EX1028) in intron 8, and 1239 (MPJ6_EX1030) in exon 9. MPJ6_EX1028 was found in the homozygous and heterozygous state, with a frequency of 0.016. The other SNPs were heterozygous, with a 0.05 frequency.

Of these SNPs, MPJ6_EX1013 (102G>C) resulted in an amino acid alteration (E44Q). Codon 44 is distant from the catalytic site (D226, E404, and H431), but is adjacent to amino acid residue 43, when altered (R43T). Codon 44 was previously shown to increase hydrolyase activity to cis-stilbene oxide.2) The other three SNPs in the coding region (MPJ6_EX1009, MPJ6_EX1026, and MPJ6_EX1030) cause synonymous amino acid changes (V10V, D352D, and V413V, respectively). The biological significance of these changes is currently unknown.

Acknowledgments: The authors, Kisho SHISEKI and Masaya ITÔDA, contributed equally to this work. We thank Ms. Chie KNUDSEN for her secretarial assistance.

References


Novel SNPs in EPHX1


