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Highly polymorphic human CYP4A11 gene

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Abstract The cytochrome P450 (CYP) is a monooxidase, which regulates metabolism of drugs and fatty acids in the liver and kidney. Among isoforms of the CYP4A subfamily, CYP4A11 is a major lauric acid (medium-length fatty acids) omega hydroxylase and is involved in the balance of lipids in the human liver. We performed direct DNA sequencing in 24 unrelated Korean individuals in the whole gene, including the 1-kb upstream region of *CYP4A11*. Seventy sequence variants were identified: six in exons, including two non-synonymous SNPs; 60 in introns; and four in 3'UTR. In comparison with SNPs enrolled in the SNP database (dbSNP) of the National Center for Biotechnology Information (NCBI), 26 novel polymorphisms (24 in introns and two in 3'UTR) were identified in Korean subjects ($n=24$). The distributions of polymorphisms confirmed were significantly different from those in the dbSNP of the NCBI. Information clarified in this study would provide valuable for further studies, including genetic association studies for various diseases and drug responses.

Keywords *CYP450* · *CYP4A11* · Single nucleotide polymorphism

Introduction

The cytochrome P450 (CYP) genes are a superfamily of monooxidases that catalyze biotransformation of various xenobiotics, including drugs and chemical pollu-

tants. P450 genes are involved in many important endogenous biochemical processes, including the steroid hormone prostaglandin and leukotriene biosynthesis. P450-mediated reactions are primarily detoxification processes whereas certain substrates are metabolically activated, resulting in the generation of reactive intermediates with increased toxicity or mutagenicity (McKinnon and Nebert 1994; Shimada et al. 1994). P450 transforms lipid-soluble drugs into the water-soluble form by adding the hydroxyl group so that compounds such as drugs and poisonous substances can be easily excreted from the body.

The CYP4A subfamily, one of 18 subfamilies in the CYP4 family, is mainly involved in the metabolism of medium- and long-chain fatty acids such as the fatty acid omega-hydroxylase enzyme (Okita and Okita 2001). The metabolism of short-, medium-, and long-chain fatty acid is regulated by the peroxisomal and mitochondrial beta oxidation system. The CYP4As are important to maintain balance of fatty acids and membrane integrity by reducing the rapid accumulation of fatty acids because inharmonious function of the systems result in the accumulation of toxic, free fatty acids (Adas et al. 1999). The CYP4As are regulated by peroxisome proliferator activated receptor alpha (*PPARA*) as a transcriptional mediator in the liver and, consequently, are involved in the reduction of lipid levels in the liver (Johnson et al. 2002; Yu et al. 2003).

Among isoforms of the CYP4A subfamily, CYP4A11 is a major lauric acid (medium-chain fatty acid) omega hydroxylase in human liver and kidney (Adas et al. 1999; Imaoka et al. 1993; Powell et al. 1996). CYP4A11 functions to convert arachidonic acid to 20-hydroxyeicosatetraenoic acid (20-HETE) involved in the regulation of blood pressure with CYP4F2 in the kidney (Lasker et al. 2000), and it is also believed to be associated with hypertension caused by elevation of cadmium (Cd) in the kidney (Baker et al. 2003). It was reported that a functional variant of *CYP4A11*, +8590T>C [+8610T>C (F434S) in this study], was associated with hypertension due to its role as a poly-

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genic determinant of blood pressure control in humans (Gainer et al. 2005).

Despite its functional importance, genetic variants of *CYP4A11* have not been fully examined. In an effort to identify the information of polymorphism(s) in genes in which variant(s) might be implicated in fatty-acid metabolism, disease association, and drug response, we scrutinized the genetic polymorphisms in *CYP4A11* by direct sequencing. Here, we present 70 genetic variants in highly polymorphic *CYP4A11* identified in the Korean population.

Materials and methods

We sequenced the whole gene of *CYP4A11*, including 1,000 bp upstream of the promoter region, with 24 unrelated Korean DNA samples using ABI PRISM 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). Thirty primer sets of the amplification and sequencing analysis were designed based on GenBank sequences (reference sequence of *CYP4A11*:

NM_000778.2). Specific primers for amplification of *CYP4A11* were designed to maximize the difference between *CYP4A11* and *CYP4A22* showing highly homologous to each other. Information regarding the primers used is available on our Web site ([http://www.snp-genetics.com/reference/supplementary information to CYP4A11.doc](http://www.snp-genetics.com/reference/supplementary%20information%20to%20CYP4A11.doc)). The ABI prism big dye terminator cycle sequencing ready reaction kits (Applied Biosystems) were used for comparative sequencing in accordance with the manufacturer's recommendation. Sequence variants were verified by chromatograms. Minor allele frequencies of variants identified in this study were compared with the SNP database (dbSNP) of the National Center for Biotechnology Information (NCBI) using the chi-square test. Deviations from Hardy-Weinberg equilibrium were estimated using chi-square tests. We examined a widely used measure of linkage disequilibrium between all pairs of biallelic loci, Lewontin's D' ($|D'|$) (Hedrick 1987). Haplotypes were inferred using the algorithm (Haploview) that searches for a spine of strong $|D'|$ running from one marker to another (Barrett et al. 2005). Phase probabilities of each site were calculated for each individual by this software.

Fig. 1 Gene maps and polymorphisms identified in *CYP4A11* on chromosome 1p33 (Ref. Genome Seq. NT_032977.7). **a** Map of *CYP4A11*. Coding exons are marked by black blocks and 5'- and 3'UTRs by white blocks. The first base of the translational start site is denoted as nucleotide +1. Details for polymorphisms in *CYP4A11* are shown in Table 1. **b** Linkage disequilibriums ($|D'|$) among *CYP4A11* polymorphisms. Asterisks (*) indicate haplotype-tagging SNPs

Results and discussion

P450 comprise a superfamily of enzymes pivotal in the metabolism of innumerable substrates of both endogenous and exogenous origin (McKinnon and Nebert

A. Map of *CYP4A11*

B. LDs ($|D'|$) among *CYP4A11* polymorphisms

Table 1 Frequencies of the human CYP4A11 gene polymorphisms^a. *rs#* refSNP ID, *rs build* ref SNP build, *NCBI* National Center for Biotechnology Information, *HWE* Hardy–Weinberg equilibrium, *ND* not determined

Locus	Position	Exon-offset	rs#	rs build	Amino acid change	Freq. in Korean subjects (<i>n</i> = 24)	Freq. in dbSNP of NCBI			Heterozygosity ^b	HWE ^b
							Caucasian	African	Asian		
+ 927A > G	Intron 1	195 + 732	rs9332982	121		0.042	–	–	–	0.080	0.978
+ 928C > T	Intron 1	195 + 733				0.292	0.042**	0.136**	–	0.413	0.154
+ 933G > A	Intron 1	195 + 738				0.042	–	–	–	0.080	0.978
+ 951C > A	Intron 1	195 + 756				0.042	–	–	–	0.080	0.978
+ 1027T > A	Intron 1	195 + 832				0.042	–	–	–	0.080	0.978
+ 1028G > A	Intron 1	195 + 833	rs9332983	119		0.042	–	–	–	0.080	0.978
+ 1051C > T	Intron 1	195 + 856				0.042	–	–	–	0.080	0.978
+ 1098A > G	Intron 1	195 + 903				0.042	–	–	–	0.080	0.978
+ 1126G > A	Intron 1	195 + 931				0.042	–	0.042	–	0.080	0.978
+ 1156C > T	Intron 1	195 + 961				0.417	0.333**	0.136**	–	0.486	0.306
+ 1178A > C	Intron 1	195 + 983	rs4287122	119		0.042	–	–	–	0.080	0.978
+ 1193T > C	Intron 1	195 + 998				0.042	–	–	–	0.080	0.978
+ 1197T > C	Intron 1	195 + 1002				0.042	–	–	–	0.080	0.978
+ 1203C > T	Intron 1	195 + 1008				0.042	–	–	–	0.080	0.978
+ 1342A > G	Intron 1	195 + 1147				0.042	–	–	–	0.080	0.978
+ 1345A > T	Intron 1	195 + 1150	rs12047326	120		0.042	–	–	–	0.080	0.978
+ 1453A > G	Intron 1	195 + 1258				0.457	ND	ND	ND	0.496	0.598
+ 1510A > C	Intron 1	195 + 1315				0.196	0.071**	0.174	–	0.315	0.333
+ 1525C > A	Intron 1	195 + 1330				0.196	0.071**	0.174	–	0.315	0.333
+ 1536A > G	Intron 1	195 + 1341				0.196	0.068**	0.174	–	0.315	0.333
+ 1566T > C	Intron 1	195 + 1371	rs9332987	119		0.196	0.068**	0.174	–	0.315	0.333
+ 1567G > A	Intron 1	195 + 1372				0.196	0.068**	0.174	–	0.315	0.333
+ 1639T > C	Intron 1	195 + 1444				0.196	0.068**	0.167	–	0.315	0.333
+ 1644T > C	Intron 1	195 + 1449				0.196	0.068**	0.208	–	0.315	0.333
+ 1656C > A	Intron 1	195 + 1461				0.196	0.068**	0.167	–	0.315	0.333
+ 1658A > G	Intron 1	195 + 1463	rs9332993	119		0.196	0.068**	0.167	–	0.315	0.333
+ 1679C > T	Intron 1	195 + 1484				0.196	0.068**	0.167	–	0.315	0.333
+ 1687G > T	Intron 1	195 + 1492				0.196	0.068**	0.167	–	0.315	0.333
+ 1698T > G	Intron 1	195 + 1503				0.196	0.068**	0.167	–	0.315	0.333
+ 1977G > T	Intron 1	196 + 1320				0.022	–	–	–	0.043	0.994
+ 2920A > G	Intron 1	196 + 377	rs9332998	100		0.214	0.196	0.326***	–	0.337	0.406
+ 3288T > C	Intron 1	196 + 9				0.109	–	–	–	0.194	0.843
+ 5640A > G	Intron 4	511 + 147				0.400	0.196	0.326***	–	0.480	0.535
+ 5682T > A	Intron 4	511 + 105				0.400	0.196	0.333***	–	0.480	0.535
+ 6083A > G	Intron 5	635 + 172				0.214	0.065**	0.167***	–	0.337	0.458
+ 6701C > T	Intron 6	791 + 174	rs9333010	119		0.105	–	–	–	0.188	0.877
+ 6778A > G	Intron 6	791 + 97				0.132	0.043**	0.042**	–	0.229	0.804
+ 7139T > C	Exon 8	969			H323H	0.400	–	–	–	0.480	0.012
+ 7226T > C	Exon 8	1056			H352H	0.429	–	–	–	0.490	0.254
+ 7227G > A	Exon 8	1057			G353S	0.429	–	–	–	0.490	0.254
+ 7289T > C	Intron 8	1088 + 31	rs2405593	100		0.024	–	–	–	0.046	0.994
+ 7436C > A	Exon 9	1170			G390G	0.452	ND	ND	ND	0.495	0.826
+ 7499T > C	Intron 9	1222 + 11				0.381	ND	ND	ND	0.472	0.625
+ 7515C > T	Intron 9	1222 + 27				0.425	ND	ND	ND	0.489	0.337
+ 7519G > C	Intron 9	1222 + 31				0.350	–	–	–	0.455	0.907
+ 7527G > A	Intron 9	1222 + 39	rs2897179	101		0.425	ND	ND	ND	0.489	0.337
+ 7561T > A	Intron 9	1222 + 73				0.425	–	–	–	0.489	0.337
+ 7567G > A	Intron 9	1222 + 79				0.425	ND	ND	ND	0.489	0.337
+ 7605C > A	Intron 9	1222 + 117				0.425	ND	ND	ND	0.489	0.337
+ 8072G > A	Intron 9	1223 + 315				0.208	0.114	0.062	–	0.330	0.436
+ 8193G > A	Intron 9	1223 + 194	rs9333016	119		0.205	–	–	–	0.325	0.483
+ 8363C > G	Intron 9	1223 + 24				0.364	–	–	–	0.463	0.704
+ 8610T > C	Exon 11	1301				0.188	0.087**	0.354**	–	0.305	0.528
+ 8826G > A	Intron 11	1364 + 153				0.023	–	–	–	0.044	0.994
+ 8878C > T	Intron 11	1364 + 205				0.182	–	–	–	0.298	0.581
+ 9120C > T	Intron 11	1364 + 447	rs911904	86		0.022	–	–	–	0.043	0.994
+ 9121G > A	Intron 11	1364 + 448				0.043	0.314**	–	–	0.083	0.977
+ 9140G > A	Intron 11	1364 + 467				0.043	–	–	–	0.083	0.977
+ 9516T > A	Intron 11	1364 + 843				0.043	–	–	–	0.083	0.977
+ 9520T > G	Intron 11	1364 + 847				0.043	–	–	–	0.083	0.977
+ 10965C > T	Intron 11	1365 + 159	rs9333030	119		0.286	0.132**	0.045**	–	0.408	0.954
+ 10981A > G	Intron 11	1365 + 143				0.262	0.132**	0.045**	–	0.387	0.819
+ 10989T > C	Intron 11	1365 + 135				0.119	–	0.227	–	0.210	0.826

Table 1 (Continued)

Locus	Position	Exon-offset	rs#	rs build	Amino acid change	Freq. in Korean subjects (<i>n</i> = 24)	Freq. in dbSNP of NCBI			Heterozygosity ^b	HWE ^b
							Caucasian	African	Asian		
+10991T>A	Intron 11	1365 + 133	rs9333033	119		0.262	0.132**	0.045**	–	0.387	0.819
+11122A>T	Intron 11	1365 + 2				0.023	–	–	–	0.044	0.994
+11133C>T	Exon 12	1374	rs9333035	119	I458I	0.432	0.136**	0.333**	–	0.491	0.632
+11373T>A	Exon 12	1614	rs9333037	119		0.023	–	0.208**	–	0.044	0.994
+11374C>G	Exon 12	1615				0.023	–	–	–	0.044	0.994
+11489C>A	Exon 12	1730				0.091	–	–	–	0.165	0.896
+12216A>G	Exon 12	2457	rs12041693	120		0.286	ND	ND	ND	0.408	0.389

^aCalculated from translation start site

^bHeterozygosity and Hardy–Weinberg equilibrium were calculated in Korean subjects

** *P* < 0.0001; *** *P* < 0.05 (difference of minor allele frequencies between this study and NCBI dbSNPs)

1994). Among P450, the CYP4A are important to maintain balance of fatty acids and membrane integrity (Adas et al. 1999), and CYP4A11 is involved not only in fatty-acid metabolism in the liver but also blood pressure regulation in the kidney (Lasker et al. 2000; Powell et al. 1996). Although *CYP4A11* polymorphisms and their function have not been fully understood as yet, *CYP4A11* is likely to be highly polymorphic.

In the present study, we identified 70 polymorphisms in Korean subjects: six in exons (two nonsynonymous and four synonymous SNPs), 60 in introns, and four in 3'UTR (Fig. 1a). Minor allele frequencies of variants identified in this study were compared with the dbSNP of the NCBI using the chi-square test (Table 1).

Among polymorphisms identified in *CYP4A11*, 33 are newly discovered, and 37 listed in the dbSNP of the NCBI are confirmed in Korean subjects. Among novel polymorphisms, 24 are located in introns and two in 3'UTR. The difference of frequencies of confirmed polymorphisms reported in the dbSNP of the NCBI were analyzed using the chi-square test. Among 37 SNPs confirmed, most showed significant differences in minor allele frequencies compared with Caucasians and Africans (Table 1). In the LD ($|D'|$) analysis, polymorphisms in *CYP4A11* might be tightly linked to one another, and there were 17 haplotype-tagging SNPs (Fig. 1b).

Several polymorphisms in several CYP genes among the P450 gene family have been reported to be associated with various diseases. *CYP1A1* polymorphisms (*CYP1A1**3) are associated with increased inducibility and increased lung cancer susceptibility (Anttila et al. 2001; Kawajiri et al. 1990). *CYP1B1* polymorphisms may be associated with significant changes in estrogen metabolism, which results in increased risk of breast cancer associated with estrogen-mediated carcinogenicity (Hanna et al. 2000). *CYP2E1* polymorphism is associated with abnormal liver function among vinyl chloride monomer (VCM)-exposed workers (Huang et al. 1997). Nine variants of *CYP4A11* were previously identified (Gainer et al. 2005), and among nine variants, only three polymorphisms [+5670A>T, +7119C>T and 8590T>C (+5682T>A, +7139T>C and

+8610T>C in this study, respectively)] were also identified in this study. The association between *CYP4A11*+8590T>C [+8610T>C (F434S) in this study] and essential hypertension varied according to hypertension comorbidity (Gainer et al. 2005).

In summary, the *CYP4A11* gene was highly polymorphic, and minor allele frequencies of polymorphisms were quite different from those in the dbSNP of the NCBI. Novel polymorphisms in Korean subjects are mainly discovered in introns and 3'UTRs. In addition to abundant studies for drug responses and disease associations with *CYP1*, 2, and 3 families, the *CYP4A11* variations clarified in this study would provide valuable information for further studies, including genetic association studies for various diseases and drug responses.

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