Running title: Genetic polymorphisms of POR in Japanese

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Abstract

Cytochrome P450 oxidoreductase (POR) transfers electrons from NADPH to all microsomal cytochrome P450 (CYP) enzymes and is necessary for microsomal CYP activities. In this study, to find genetic variations and to elucidate the haplotype structures of *POR*, we have comprehensively screened the genetic variations in the 5'-flanking region, all the exons and their flanking introns of *POR* for 235 Japanese subjects. Seventy-five genetic variations including 26 novel ones were found: 7 were in the 5'-fkanking region, 2 in the 5'-untranslated region (5'-UTR, non-coding exon 1), 16 in the coding exons (10 nonsynonymous and 6 synonymous), 45 in the introns, 4 in the 3'-UTR and 1 in the 3'-flanking region. Of them, 4 novel nonsynonymous variations, 86C>T (T29M), 1648C>T (R550W), 1708C>T (R570C) and 1975G>A (A659T), were detected with allele frequencies of 0.002. We also detected known nonsynonymous SNPs, 683C>T (P228L), 1237G>A (G413S), 1453G>A (A485T), 1508C>T (A503V), 1510G>A (G504R) and 1738G>C (E580Q) with the frequencies of 0.002, 0.009, 0.002, 0.434, 0.002 and 0.002, respectively. Based on the linkage disequilibrium (LD) profiles, the analyzed region could be divided into two LD blocks. For Block 1 and 2, 14 and 46 haplotypes were inferred respectively, and 2 and 6 common haplotypes found in more than 0.03 frequencies accounted for more than 81% of the inferred haplotypes. This study provides fundamental and useful information for the pharmacogenetic studies of drugs metabolized by CYPs in Japanese population.

Key Words

POR, genetic polymorphism, haplotype, Japanese, nonsynonymous variation,

Introduction

Cytochrome P450 oxidoreductase (POR) is a flavoprotein that transfers electrons from NADPH to all microsomal cytochrome P450 (CYP) enzymes.[1] The human genome contains 50 microsomal CYP enzymes including 15 genes principally for drug metabolism, and all of these microsomal CYPs require POR activity for catalysis. Mutations leading to disrupt POR activities have been known to cause autosomal recessive genetic diseases, ambiguous genitalia, congenital adrenal hyperplasia, Antley-Bixler syndrome, and polycystic ovary syndrome.[2-4]

Human POR, 77 kDa protein with 680 amino acids contains one flavin adenine dinucleotide (FAD) and one flavine adenine mononucleotide (FMN) molecules. Electrons from NADPH pass through the FAD to the FMN, and then to the CYP.[1] The POR-CYP interaction is formed by electrostatic power: the CYP-interacting surface of POR is charged by negative produced by acidic amino acids, and POR-binding site of CYP is positively charged by basic residues (Lys and Arg).

POR gene consists of 16 exons (including non-coding exon 1) spanning approximately 72 kb at chromosome 7q11.2.[5] Using human liver bank, 18.3, 16.5 and 3.39-fold interindividual variations were observed in POR mRNA levels, protein levels and cytochrome C reductase activities, respectively.[6] Since its activity is necessary for CYP functions, genetic variations in *POR* might affect the functions of broad ranges of CYPs that are involved in the drug metabolism. Many genetic variations have been already reported in *POR* gene for diverse populations,[6-8] and 41 alleles/haplotypes were publicized in Home Page of the Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee (http://www.cypalleles.ki.se/por.htm). Huang et al. (2008) sequenced 218 African-Americans, 260 European-Americans, 179 Chinese-Americans and 185 Mexican-Americans, and detected 140 distinct nucleotide variations including 15 nonsynonymous ones.[9] They also examined functional significance of these 15

variations using bacterial expression systems. Six and five variations were associated with >50% reduced *V*max/*K*m values for cytochrome C reduction activities and NADPH oxidation activities, respectively, when compared to those of wild-type. But when assayed with expressed P450c17, only four variations decreased 17α-hydroxylase and 17, 20-lyase activities by >50%. The authors also extended their study for effects of 35 POR nonsynonymous variations on CYP1A2 and CYP2C19 activities, in which 10 POR variant proteins was associated with no detectable catalytic activities and 8 including P228L had >50% reduced *V*max/*K*m values for both enzymes, when compared to those of the wild-types.[10] Recently, Gomes et al. (2009) comprehensively screened the effects of POR and CYP genetic variations as well as patients' non-genetic factors on 10 CYP catalytic activities using 150 Caucasian surgical liver samples, and found that three intronic polymorphisms were significantly associated with the altered CYP3A4 activity (IVS3+88G>A), CYP1A2, CYP2C8, CYP2C19 and CYP3A4 activities (IVS4+89C>T), and CYP2C19 and CYP3A4 activities (IVS11+20G>A), although their mechanisms for activity changes have not been revealed.[6] The most common polymorphism A503V (Minor allele frequency [MAF] = 0.303) had negligible functional effects[6], as reported previously.[9, 10]

Although many studies were conducted as above, reports are lacking on *POR* genetic polymorphisms in Japanese population. Here we sequenced the 5'-flanking region, all exons and their flanking regions of *POR* from 235 Japanese subjects.

Materials and Methods

Human genomic DNA samples

A total of 235 unrelated Japanese cancer patients administered paclitaxel were participated in this study. The ethical review boards of the National Cancer Center and the National Institute of Health Sciences approved this study. Written informed consent was obtained from all participating patients. Genomic DNA for sequencing analysis was extracted from blood leukocytes collected from the subjects prior to the paclitaxel administration.

PCR conditions for sequencing

The GenBank accession number, NT_079595.2 (genome) and NM_000941.2 (mRNA) were used for primer design and as the reference sequences. For sequencing, a set of four multiplex long-range PCR was performed to amplify all 16 exons from 150 ng of genomic DNA using 0.04 units/μl of LA-Taq in GC buffer I (Takara Bio Inc., Shiga, Japan) using primer sets (0.2 μM) designed in the 5'-flanking or intronic regions as listed in "1st PCR" of Table 1. The first PCR conditions were 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 60°C for 1 min, and 72°C for 2 min, and then a final extension at 72°C for 7 min. Next, short targeted regions for sequences, except for 5'-flanking region and exon 1, were amplified separately in the 2nd PCR using the 1st PCR product as a template by Ex-Taq (0.02 units/μl, Takara Bio Inc.) with primers (0.2 μM) listed in "2nd PCR" in Table 1. Because of a high GC content, 5'-flanking region and exon 1 were amplified using 0.04 units/μl of LA-Taq in GC buffer I with 0.2 μM of the primers listed in Table 1. The second PCR conditions were same as the 1st PCR. Thereafter, the PCR products were treated with a PCR Product Pre-Sequencing Kit (USB Co., Cleveland, OH, USA) and directly sequenced on both strands using an ABI BigDye Terminator Cycle Sequencing Kit ver. 3.1 (Applied Biosystems, Foster City, CA, USA) with the primers

listed in "Sequencing" of Table1. The excess dye was removed by a DyeEx96 kit (Qiagen, Hilden, Germany). The eluates were analyzed on an ABI Prism 3730xl DNA Analyzer (Applied Biosystems). All the rare detected variations were confirmed by repeating the PCR from the genomic DNA and sequencing the newly generated PCR products.

Linkage disequilibrium (LD) and haplotype analysis

Hardy-Weinberg equilibrium, LD analysis and diplotype configurations (a combination of haplotypes) were analyzed with SNPAlyze software ver. 7 (Dynacom Co., Chiba, Japan). Pairwise LD between variations with minor allele frequency (MAF) of greater than 0.03 was analyzed using r^2 and |D'| values. Nomenclature for the haplotypes were based on the Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee and these haplotypes were tentatively named as numbers (Arabic numbers for known and novel unambiguously determined haplotypes as defined by the Committee, and Roman numbers for ambiguously estimated ones with nonsynonymous variations) plus small alphabetical letters in this study. The haplotypes inferred in single subjects (ambiguous ones) are described with haplotype names and a question mark in Table 3, since the predictability for these very rare haplotypes is known to be low in some cases. Of these ambiguous haplotypes, the ones without amino acid changes were put together into "**1 others*" and with 1508C>T (A503V) into "**28 others*".

Results and Discussion

***POR* genetic variations found in a Japanese population**

The 5'-flanking regions (up to 39968 bases upstream of the translational start site, 1077 bases upstream of the transcriptional start site) , all the 16 exons and their flanking introns of *POR* were sequenced in 235 Japanese subjects. Seventy-five genetic variations, including 26 novel ones, were detected: 7 were in the 5'-fkanking region, 2 in the 5'-untranslated region (5'-UTR, non-coding exon 1), 16 in the coding exons (10 nonsynonymous and 6 synonymous), 45 in the introns, 4 in the 3'-UTR and 1 in the 3'-flanking region (see Table 2). All of the detected variations were found in Hardy-Weinberg equilibrium ($P \geq 0.11$).

All of the four novel nonsynonymous variations, 86C>T (T29M), 1648C>T (R550W), 1708C>T (R570C) and 1975G>A (A659T), were found as individual heterozygotes at 0.002 frequencies (Table 2). The T29 is located in transmembrane anchoring domain, and the latter three substitutions are within NADP(H)-binding domain, based on the crystal structure of rat POR which has 92% homology to human POR.[11] Note that corresponding position of human A659 is Thr in rat POR. The other three substitution positions are conserved between both species. Using PolyPhen program (http://genetics.bwh.harvard.edu/pph/) to predict the functional effect by the amino acid substitution, two substitutions, R550W and R570C seem to cause probably damaging on protein function based on the PSIC (position specific independent count) profile score differences derived from multiple alignment (The predictability of this program was checked by 12 known *CYP2C9* alleles with reduced enzymatic activities *in vitro*, and found that the successful predictablity was 0.583 [7/12].). The effects of T29M and A659T were predicted to be benign. Functional significance of these 4 novel variations should be clarified in the future. We also detected six known nonsynonymous variations, 683C>T (P228L), 1237G>A (G413S), 1453G>A (A485T), 1508C>T (A503V), 1510G>A (G504R) and

1738G>C (E580Q) with the frequencies of 0.002, 0.009, 0.002, 0.434, 0.002 and 0.002, respectively. Of these, P228L variant enzyme was shown to render over 60% reduced activities (*V*max/*K*m values) of CYP1A2 and CYP2C19.[10] In contrast, the effects of G413S, A485T and G504R substitutions were minimal for both CYP enzymes.[10] The frequency (0.434) of the most frequent nonsynonymous variation 1508C>T (A503V) was higher than those in Chinese-Americans (0.367), Mexican-Americans (0.310), European-Americans (0.264) and African-Americans (0.191) although its functional effects were reported to be very mild.[9, 10]

In addition to nonsynonymous variations, three intronic variations, IVS3+88G>A, IVS4+89C>T and IVS11+20G>A, were reported to be significantly associated with altered CYP activities, although their mechanisms were not clarified.[6] IVS3+88G>A was not detected in our Japanese population. IVS4+89C>T was found at 0.028 frequency in Japanese, which is much lower than those in European-Americans (0.363), Chinese-Americans (0.138), African-Americans (0.135) and Mexican-Americans (0.129).[9] The last one, IVS11+20G>A was detected at 0.389 frequency, similar to those in Chinese-Americans (0.360) and Mexican-Americans (0.407), but slightly and much lower frequencies were reported in European-Americans (0.317) and African-Americans (0.194), respectively.[9]

Linkage disequilibrium (LD) analysis

Using the 26 genetic variations detected with ≥0.03 frequencies, LD analysis was performed by the r^2 and |D'| statistics, and the pairwise values of both are shown with a 10-graded blue color in Fig. 1. |D'| is used to assess the probability for past recombinations, and r^2 is used as a parameter for the linkage between a pair of variations.

For r^2 values, perfect linkage (r^2 =1) was detected among IVS8+116C>T, IVS8-35C>T and 1508C>T (A503V), among IVS8-68G>C, IVS8-55A>G, IVS10-66C>T, IVS12-173A>G,

IVS12-34C>T, IVS12-33T>G and 1455C>T (A485A), and between IVS11+12C>T and IVS11+20G>A. Relatively strong linkages were observed among 387A>G (P129P), IVS7+225G>A, IVS7-187C>T, IVS10-97C>T, IVS11+12C>T and IVS11+20G>A ($r^2 \geq 0.69$), among IVS6-72A>G, IVS10-13G>C and above perfect linkages of IVS8-68G>C ~ 1455C>T (A485A) ($r^2 \geq 0.93$),among IVS10+101G>C, IVS12-108C>G and IVS13+33C>T ($r^2 \geq 0.91$). In addition 2349 (*306)G>A was strongly linked with above perfect linkages of IVS8+116C>T ~ 1508C>T (A503V) ($r^2=0.80$). On the other hand, only weak linkages ($r^2 \leq 0.31$) were observed between -38856A>C and rest of the variations. For |D'| values, strong LD (|D'|≥0.8) were observed in 97.3% (292/300) combinations of 25 variations from 387A>G (P129P) to 2415 (*372)G>A. Between -38856A>C and 387A>G (P129P), only 40% (10/25) of combination showed strong LD (|D'|≥0.8).

Based on the above results, we divided the analyzed region of *POR* into two LD blocks as indicated in Fig. 1. Block 1, spanning at least 1.3 kb, included 13 variations from -39914A>G in the 5'-flanking region to -38576C>T in intron 1. Block 2, which includes the 62 variations from 15A>G (G5G) to 2417+165 (*374+165)C>T was ranging approximately 33.0 kb. Six variations from exon 2 to intron 4 were tentatively included in Block 2, since the distance (26.4 kb) from 387A>G (P129P) to 15A>G (G5G) are closer than that (38.6 kb) from -38576C>T to 15A>G (G5G). The 2417+165 (*374+165)C>T was also included into Block 2.

Haplotype estimation

We then analyzed haplotype structures of *POR* for each block. The haplotypes in each block inferred by SNPAlyze software and their frequencies were shown in Table 3A and 3B. Using all the 13 and 62 variations, 14 and 46 haplotypes were inferred in Block 1 and 2, respectively. The diplotype configurations were obtained at probabilities over 0.99 for 99%

and 91% of the 235 subjects in Blocks 1 to 2, respectively. Of all the estimated haplotypes, 2 in Block 1 and 23 in Block 2 were detected only one chromosome ambiguously. Common haplotypes were defined as ones with more than 0.03 frequencies in this study.

Block 1 only includes variations detected in 5'-transcriptional regulatory region, non-coding exon 1 and intron 1. The most dominant haplotype was **1a* at frequency of 0.717, which was followed by **1b* at 0.202 frequency. The frequencies of the other haplotypes were less than 0.03. Although many haplotypes were predicted, these 2 common haplotypes (**1a* and **1b*) accounted for 92% of all the inferred haplotypes.

Block 2 covers all the coding exons. In Block 2, 11 haplotype groups (**1*, **28*, **35*, **36*, **42*, **43*, **I*, **II*, **III*, **IV* and **V*) were inferred. The **28*, **35*, **36* (known haplotypes), **42*, **43* (novel unambiguous haplotypes), **I*, **II*, **III*, **IV* and **V* (novel ambiguous haplotypes) harbor one of the nonsynonymous variations, 1508C>T (A503V), 1738G>C (E580Q), 683C>T (P228L), 1237G>A (G413S), 1975G>A (A659T), 1453G>A (A485T), 1510G>A (G504R), 1648C>T (R550W), 1708C>T (R570C), and 86C>T (T29M). Despite the finding from one subjects, the **43a* haplotype was unambiguous since this subject had a heterozygous 1975G>A (A659T) as sole heterozygous site on the basis of homozygous **1a* haplotypes. The most common haplotypes was **1a* (frequency: 0.336), followed by **28a* (0.177), **1b* (0.117), **28b* (0.089), **28c* (0.045) and **28d* (0.043). These 6 common haplotypes accounted for 81% of all the inferred haplotypes. Gomez et al. reported the haplotype frequencies of *POR* (from exon 2 to 3'-UTR) in the Caucasian populations, in which the group haplotype frequencies were 0.690 for **1*, 0.292 for **28*, 0.001 for **36* and 0.006 for **37* (1508C>T [A503V] and 1891G>A [V631I].[6] The **28* group haplotype was more prevalent in the Japanese (frequency = 0.430) than in the Caucasians, and **37* haplotype was not detected in the Japanese (Table 3B).

In conclusion, we identified 75 genetic variations including 26 novel ones from 235

Japanese subjects in *POR* gene. Four novel variations resulted in amino acid substitutions. Based on the LD profile, the analyzed region was divided into 2 blocks and their haplotype structures were inferred. This is the first report to comprehensively analyze *POR* gene and to estimate its haplotype structure in a Japanese population. This information is useful for pharmacogenetic studies to investigate the relationship between the interindividual differences in drug metabolism by CYPs and *POR* haplotypes.

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Legends for Figures

Linkage disequilibrium (LD) analysis of *POR*. Pairwise LD is expressed as r^2 (upper right) and |D'| (lower left) values (from 0 to 1) by 10-graded blue colors. A denser color represents closer linkage.

Table 1 Primer sequences used for amplification and sequencing of *POR*

Usage	Region	Forward primer	Reverse primer	Amplified length
1st PCR	5'-flanking - Exon 1	CTTCTTTGGACAGGTATTGTGCCC	TAGGACCATTTCAGACAAGTGCCA	5,943
	Exon 2	AGTCAGTGGCTGTCATTTTCTCTG	ATACCTGACCTCTCCCAAACGAAA	8,577
	Exon 3	TCATCCTCCTCCATCGGGTAATCA	TGAGTTAGAGGGAGGGTCTGTTCT	9,986
	Exons 4 - 16	GGATGTAAGTATCTATGGGAGGTC	AAATGCCTCCTCCCTGCTTAGTTC	8,345
2nd PCR	5'-flanking*	AAAAGATGGAGAAGGGGCTCTG	GCGAAAGATAGCACTCACCG	1,195
	Exon 1	CCACGCACTTTCATTTCTCT	ATACCGAGCCCTAACCCTCA	556
	Exon 2	TGAGTGAGCCCCTTCTCCTA	CCCAAGAGTCACCCCAAAAT	463
	Exon 3	AGCCCTGGTGTTGGATTAGA	GTTAGGCAAGAATGACTCCC	399
	Exon 4	ACAGTGAGAAGCAAGTCCCA	TGGGTTTGGTTTGGGAGATG	581
	Exon 5	CCCTCCGTGTTGTTACTTCT	AGTCGTCCAGCCAGACCTTT	565
	Exon 6	GTCAACCAGATGAAGCCTCT	TCTGTGTTGGAGGTGCGTGT	432
	Exon 7	CCTGATGCTCTGGGTTTATG	ACCCTATGACGGAGTGTCTT	361
	Exon 8	CCCTGCTTCTTGTCGTATGT	ATGAGCCCTTCTGCCAAAGA	538
	Exons 9 - 10	CTGAGATTCCCTGTGCTTTG	ACTATGACAGTGACGGGGTA	662
	Exons 11 - 12	TGTGTCAGACCGTGTAGTGT	GCTGGACAGATGCTGAGAAT	921
	Exons 13 - 14	TCGGGCTGGCTTGTGAGATT	TCTCACCTTGTGGGACTGCT	709
	Exons 14 - 16	GACGCTGCTGTACTACGGCT	CCAGAGGAGTCTTTGTCACT	675
	Exon 16	GTGGACTACATCAAGAAACT	GGTCTCTTCTATTCTCCCTT	657
Sequencing	5'-flanking	AAAAGATGGAGAAGGGGCTCTG	GTCTCGCTATGATGCCCAGGTT	
		AACCTGGGCATCATAGCGAGAC	CAGAGAAATGAAAGTGCGT	
	Exon 1	CCACGCACTTTCATTTCTCT	GTGGAAAAGTCGACCCTCAG	
	Exon 2	TGAGTGAGCCCCTTCTCCTA	CCCAAGAGTCACCCCAAAAT	
	Exon 3	AGCCCTGGTGTTGGATTAGA	GTTAGGCAAGAATGACTCCC	
	Exon 4	ACAGTGAGAAGCAAGTCCCA	TGGGTTTGGTTTGGGAGATG	
		AGAGGAACTTAGAAGGGACT	TTGGTTTGGGAGATGTGGCG	
	Exon 5	CCCTCCGTGTTGTTACTTCT	AGCCAGACCTTTCTTGCCT	
	Exon 6	GTCAACCAGATGAAGCCTCT	TCTGTGTTGGAGGTGCGTGT	
	Exon 7	CCTGATGCTCTGGGTTTATG	ACCCTATGACGGAGTGTCTT	
	Exon 8	CCCTGCTTCTTGTCGTATGT	ATGAGCCCTTCTGCCAAAGA	
	Exons 9 - 10	CTGAGATTCCCTGTGCTTTG	ACTATGACAGTGACGGGGTA	
	Exons 11 - 12	TGTGTCAGACCGTGTAGTGT	TGCAGGATGGCCAGGATGTG	
		CCCAATCAGCCCCATCTCAC	GCTGGACAGATGCTGAGAAT	
	Exons 13 - 14	TCGGGCTGGCTTGTGAGATT	TCTCACCTTGTGGGACTGCT	
	Exons 14 - 16	GACGCTGCTGTACTACGGCT	CCAGAGGAGTCTTTGTCACT	
	Exon 16	GTGGACTACATCAAGAAACT	GGTCTCTTCTATTCTCCCTT	

*LA-Taq with GC buffer I was used for amlification in 2nd PCR because of high GC contents.

Table 2. Summary of *POR* variations detected in this study

SNP ID				Position				Allele frequency (n=235)	
This Study	dbSNP (NCBI)	Reference	Location	NT_079595.2	From the translational initiation site or from the end of the nearest exon	Nucleotide change	Amino acid change		95% Confidence interval
MPJ6_POR_001[a]			5'-flanking	777673	-39914	cagctagtaataA/Gtagtagtagtag		0.011	0.001 - 0.020
MPJ6_POR_002[a]				778455	-39132	ccggcccggtgaC/Ttgcagggtccga		0.011	0.001 - 0.020
MPJ6_POR_003	rs72553984	9)		778485	-39102	agaagccgcagcC>Ggccgtctccagg		0.006	0.000 - 0.014
MPJ6_POR_004	rs12537282	9)		778488	-39099	agccgcagccgcC>Ggtctccaggcga		0.004	0.000 - 0.010
MPJ6_POR_005[a]				778512	-39075	actccgccacccC>Gcggaaccacgca		0.006	0.000 - 0.014
MPJ6_POR_006[a]				778540	-39047	tcatttctctgcC>Ggggcgacccagc		0.002	0.000 - 0.006
MPJ6_POR_007	rs72553972	9)		778544	-39043	ttctctgccgggC>Agacccagccgag		0.019	0.007 - 0.032
MPJ6_POR_008[a]			5'-UTR (Exon 1)	778710	-38877	aggcggtggtagC>Tgcctcagtggtg		0.002	0.000 - 0.006
MPJ6_POR_009	rs3823884	9)		778731	-38856	ggtgtgggcctgA>Cgccctgcccagg		0.255	0.216 - 0.295
MPJ6_POR_010[a]			Intron 1	778825	-38762	gtcgggccccaaG>Aactcgggtttg		0.002	0.000 - 0.006
MPJ6_POR_011	rs3735508			778910	-38677	tgggcactgcgcG>Atctggcccgacg		0.009	0.000 - 0.017
MPJ6_POR_012[a]				779010	-38577	gcctgagggtcgA>Gcttttccacagc		0.002	0.000 - 0.006
MPJ6_POR_013[a]				779011	-38576	cctgagggtcgaC>Ttttccacagct		0.006	0.000 - 0.014
MPJ6_POR_014	rs10262968	6, 8, 9)	Exon 2	817601	15	gatcaacatgggA>Ggactcccacgtg	Gly5Gly	0.011	0.001 - 0.020
MPJ6_POR_015[a]				817672	86	ttttcagcatgaC>Tggacatgattct	Thr29Met	0.002	0.000 - 0.006
MPJ6_POR_016	rs10225188	6)	Intron 3	842950	IVS3-95	gggggcggcctgG>Tagggcccccgcc		0.028	0.013 - 0.042
MPJ6_POR_017	rs10239977	6, 9)	Intron 4	843262	IVS4+89	gcaggggggaggcC>Tggcagggagtgg		0.028	0.013 - 0.042
MPJ6_POR_018	rs72554000	9)		843420	IVS4+247	cccctgagtgccG>Agctgccctcctg		0.013	0.003 - 0.023
MPJ6_POR_019[a]				843818	IVS4-115	cacgacactcagA>Ccatccctggcct		0.002	0.000 - 0.006
MPJ6_POR_020	rs1135612	6, 8, 9)	Exon 5	843953	387	gagcagcctgccA>Ggegatcgacaac	Pro129Pro	0.440	0.396 - 0.485
MPJ6_POR_021[a]			Intron 5	844115	IVS5+33	tatgggctcccgG>Atggcctgcggtg		0.013	0.003 - 0.023
MPJ6_POR_022	rs72555505	9)		844124	IVS5+42	ccggtggcctgcG>Agtgcctccctgg		0.013	0.003 - 0.023
MPJ6_POR_023[a]			Intron 6	845032	IVS6-79	gatggggtgggggT>Acggggcgtgcct		0.002	0.000 - 0.006
MPJ6_POR_024	rs2286819	8)		845039	IVS6-72	tggggtcggggcA>Gtgcctggcacca		0.140	0.109 - 0.172
MPJ6_POR_025	rs2286820	6, 8, 9)		845084	IVS6-27	ctcccctgagccG>Actcccccctctcc		0.009	0.000 - 0.017
MPJ6_POR_026	rs17853284	6, 9)	Exon 7	845152	683	agcagttctggcC>Tggccgtgtgtga	Pro228Leu	0.002	0.000 - 0.006
MPJ6_POR_027	rs10954732	6, 9)	Intron 7	845425	IVS7+225	aggacacatcgcG>Atcgggctctgtg		0.417	0.372 - 0.462
MPJ6_POR_028[a]				845427	IVS7+227	gacacatcgcgtC>Tgggctctgtggc		0.002	0.000 - 0.006
MPJ6_POR_029	rs2286821	6)		845631	IVS7-187	gcagctccacgcC>Tgcctccctcctt		0.419	0.375 - 0.464
MPJ6_POR_030[a]				845736	IVS7-82	aaggccatgcacG>Cgtctcccctgta		0.002	0.000 - 0.006
MPJ6_POR_031[a]				845736	IVS7-82	aaggccatgcacG>Agtctcccctgta		0.004	0.000 - 0.010
MPJ6_POR_032	rs3815455	6, 8, 9)	Intron 8	846032	IVS8+116	ccagacccgtgC>Tcccgagtgggtg		0.434	0.389 - 0.479
MPJ6_POR_033	rs72557926	9)		846055	IVS8+139	tgtgagtgtccaC>Tgacctgtccacg		0.013	0.003 - 0.023
MPJ6_POR_034[a]				846211	IVS8+295	gatcctctttggC>Aagaagggctcat		0.013	0.003 - 0.023
MPJ6_POR_035	rs13223707	8)		847046	IVS8-68	tgcaaccagaagG>Cgtccttggagac		0.134	0.103 - 0.165
MPJ6_POR_036	rs13240147	8)		847059	IVS8-55	gtcctggagacA>Ggagactcagatc		0.134	0.103 - 0.165
MPJ6_POR_037	rs41301394	6, 8)		847079	IVS8-35	agatcaaagcccC>Tggccgctcactg		0.434	0.389 - 0.479
MPJ6_POR_038[a]			Intron 9	847260	IVS9+30	caccccctgaacC>Gctcactctgggc		0.004	0.000 - 0.010
MPJ6_POR_039[a]			Intron 10	847471	IVS10+21	cacagtcagggcG>Accctgccgggct		0.002	0.000 - 0.006
MPJ6_POR_040	rs41301400	9)		847551	IVS10+101	gcctgaagccccG>Cgtgcctgggagg		0.098	0.071 - 0.125
MPJ6_POR_041	rs4732514	8)		848274	IVS10-97	ggcacctgttgcC>Tgcagagctggcc		0.419	0.375 - 0.464
MPJ6_POR_042[a]				848301	IVS10-70	aggtgtcaccccC>Ttcctgccgcagc		0.002	0.000 - 0.006
MPJ6_POR_043	rs4732515	8)		848305	IVS10-66	gtcaccccctccC>Tgccgcagccacc		0.134	0.103 - 0.165
MPJ6_POR_044	rs4732516	8, 9)		848358	IVS10-13	agtcctgcctgtG>Ctcttcctgcag		0.132	0.101 - 0.163
MPJ6_POR_045	rs41301424	6, 9)	Exon 11	848540	1236	ggcctcctcctcC>Tggcgagggcaag	Ser412Ser	0.002	0.000 - 0.006
MPJ6_POR_046		3, 6)		848541	1237	gcctcctcctccG>Agcgagggcaagg	Gly413Ser	0.009	0.000 - 0.017
MPJ6_POR_047	rs72557931	9)	Intron 11	848563_848571	IVS11+11_19	aggtgcgcccccTCAGCCCCC/- gcaacctccgcc		0.006	0.000 - 0.014
MPJ6_POR_048	rs2286822	6, 8, 9)		848564	IVS11+12	ggtgcgcccccC>Tagcccccgcaac		0.389	0.345 - 0.433
MPJ6_POR_049	rs2286823	6, 8, 9)		848572	IVS11+20	ccctcagccccG>Acaacctccgccc		0.389	0.345 - 0.433
MPJ6_POR_050	rs41301427	8, 9)	Intron 12	848833	IVS12+32	tgccagccacacG>Actggaggcccag		0.011	0.001 - 0.020
MPJ6_POR_051[a]				848993	IVS12-180	tcagcatctgtcC>Tagccccggtccc		0.002	0.000 - 0.006
MPJ6_POR_052	rs6961174			849000	IVS12-173	ctgtccagccccA>Ggtccccagaacc		0.134	0.103 - 0.165
MPJ6_POR_053	rs2302429	6)		849053	IVS12-120	tctgaggtttggG>Atgccaggtgggc		0.323	0.281 - 0.366
MPJ6_POR_054	rs2302430			849065	IVS12-108	gtgccaggtgggC>Gtggaagaggccc		0.100	0.073 - 0.127
MPJ6_POR_055	rs2302431	8, 9)		849139	IVS12-34	caagggcctcggC>Tgtggcggtggag		0.134	0.103 - 0.165
MPJ6_POR_056	rs2302432	8, 9)		849140	IVS12-33	aagggcctcggtT>Gtggcggtggagc		0.134	0.103 - 0.165
MPJ6_POR_057	rs72557947	9)	Exon 13	849227	1453	tacgagaccaagG>Actggccgcatca	Ala485Thr	0.002	0.000 - 0.006
MPJ6_POR_058	rs2228104	6, 8)		849229	1455	cgagaccaaggCc>Tggccgcatcaac	Ala485Ala	0.134	0.103 - 0.165
MPJ6_POR_059	rs1057868	3, 6, 8, 9)		849282	1508	ccaaggagcctgC>Tcggggagaacgg	Ala503Val	0.434	0.389 - 0.479
MPJ6_POR_060		3, 6)		849284	1510	aaggagcctgccG>Agggagaacggcg	Gly504Arg	0.002	0.000 - 0.006
MPJ6_POR_061[a]				849422	1648	ttcatccaggagC>Tgggcctggctgc	Arg550Trp	0.002	0.000 - 0.006
MPJ6_POR_062	rs2302433	9)	Intron 13	849476	IVS13+33	gagaggggggtgaC>Tgactgggagccc		0.091	0.065 - 0.118
MPJ6_POR_063[a]			Exon 14	849555	1708	tactacggctgcC>Tgccgctcggatg	Arg570Cys	0.002	0.000 - 0.006
MPJ6_POR_064	rs1057870	6, 8, 9)		849563	1716	ctgccgccgctcG>Agatgaggactac	Ser572Ser	0.028	0.013 - 0.042
MPJ6_POR_065		4)		849585	1738	tacctgtaccggG>Caggagctggcgc	Glu580Gln	0.002	0.000 - 0.006
MPJ6_POR_066	rs72557949	9)		849611	1764	gttccacagggaC>Tggtgcgctcacc	Asp588Asp	0.002	0.000 - 0.006
MPJ6_POR_067	rs72557952	9)	Intron 14	849670	IVS14+8	acaaggtgagacG>Aggcgggcaccac		0.004	0.000 - 0.010
MPJ6_POR_068[a]				849708	IVS14+46	gaggctggcaggG/- ccacagccacag		0.002	0.000 - 0.006
MPJ6_POR_069	rs72557953	9)		849712	IVS14+50	ctggcagggccaC>Gagccacagtgcc		0.028	0.013 - 0.042
MPJ6_POR_070[a]			Exon 16	850007	1975	gccatggagcacG>Acgcaggcggtgg	Ala659Thr	0.002	0.000 - 0.006
MPJ6_POR_071[a]			3'-UTR	850225_850227	2193_2195 (*150_*152)[b]	gggtgcatcctcCTC/- agcccccaggcc		0.006	0.000 - 0.014
MPJ6_POR_072	rs41302348	6, 8)		850282	2250 (*207)[b]	gcccagggcctgC>Gatgggggcaccg		0.045	0.026 - 0.063
MPJ6_POR_073	rs17685	6, 8)		850381	2349 (*306)[b]	cagcccctccacG>Atgatttccagtg		0.385	0.341 - 0.429
MPJ6_POR_074	rs2286824	6)		850447	2415 (*372)[b]	gttctgttttctG>Atatttgcctggt		0.045	0.026 - 0.063
MPJ6_POR_075[a]			3'-flanking	850614	2417+165[c] (*374+165)[b]	agggcactgggcC>Tcaggtcttcctt		0.002	0.000 - 0.006

[a] Novel variations detected in this study.

[b] Positions are shown as * and bases from the translational termination codon TAG.

[c] Position was shown as the last base of exon 16 (2417) and bases downstream of this base.

Table 3A *POR* Block 1 haplotypes

Reagion			5'-flanking							Exon 1 (5'-UTR)			Intron 1	Number	Frequency
Nucleotide change[a]			-39914 A>G	-39132 C>T	-39102 C>G	-39099 C>G	-39075 C>G	-39047 C>G	-39043 C>A	-38877 C>T	-38856 A>C	-38677 G>A	-38576 C>T		
Amino acid change															
Haplotypes[b,c]	*1	*1a												337	0.717
		*1b									■			95	0.202
		*1c							■		■			9	0.019
		*1d		■							■			5	0.011
		*1e	■								■			5	0.011
		*1f										■		4	0.009
		*1g					■							3	0.006
		*1h			■						■			3	0.006
		*1i											■	3	0.006
		*1j				■								2	0.004
		*1k								■				1	0.002
		*1l						■						1	0.002
		*1 others[d]												2	0.004
														470	1.000

[a] A of the translational start codon of *POR* is numbered 1. NT_079595.2 was used as the reference sequence.
[b] Major allele, white; minor allele, gray
[c] The haplotypes are described as numbers plus small alphabetical letters.

[d] The haplotypes inferred in only one chromosome ambiguously are put together into "others" and variations found only in these ambiguous haplotype were not included in this table.

Table 3B *POR* Block 2 haplotypes

Reagion			Exon 2		Intron 3	Intron 4		Exon 5	Intron 5		Intron 6			Exon 7	Intron 7			Intron 8						Intron 9	Intron 10			
Nucleotide change[a]			15 A>G	86 C>T	IVS3 -95 G>T	IVS4 +89 C>T	IVS4 +247 G>A	387 A>G	IVS5 +33 G>A	IVS5 +42 G>A	IVS6 -79 T>A	IVS6 -72 A>G	IVS6 -27 G>A	683 C>T	IVS7 +225 G>A	IVS7 -187 C>T	IVS7 -82 G>A	IVS8 +116 C>T	IVS8 +139 C>T	IVS8 +295 C>A	IVS8 -68 G>C	IVS8 -55 A>G	IVS8 -35 C>T	IVS9 +30 C>G	IVS10 +101 G>C	IVS10 -97 C>T	IVS10 -66 C>T	IVS10 -13 G>C
Amino Acid Change			G5G	T29M				P129P						P228L														
Haplotypes[b, c]	*1	*1a																										
		*1b																										
		*1c																										
		*1d																										
		*1e																										
		*1f																										
		*1g																										
		*1h																										
		*1i																										
		*1 others[d]																										
	*28	*28a																										
		*28b																										
		*28c																										
		*28d																										
		*28e																										
		*28f																										
		*28g																										
		*28h																										
		*28i																										
		*28j																										
		*28k																										
		*28 others[d]																										
	*35	*35a																										
	*36	*36a?[e]																										
	*42	*42a																										
		*42b?[e]																										
	*43	*43a																										
	*I	*Ia?[e]																										
	*II	*IIa?[e]																										
	*III	*IIIa?[e]																										
	*IV	*IVa?[e]																										
	*V	*Va?[e]																										

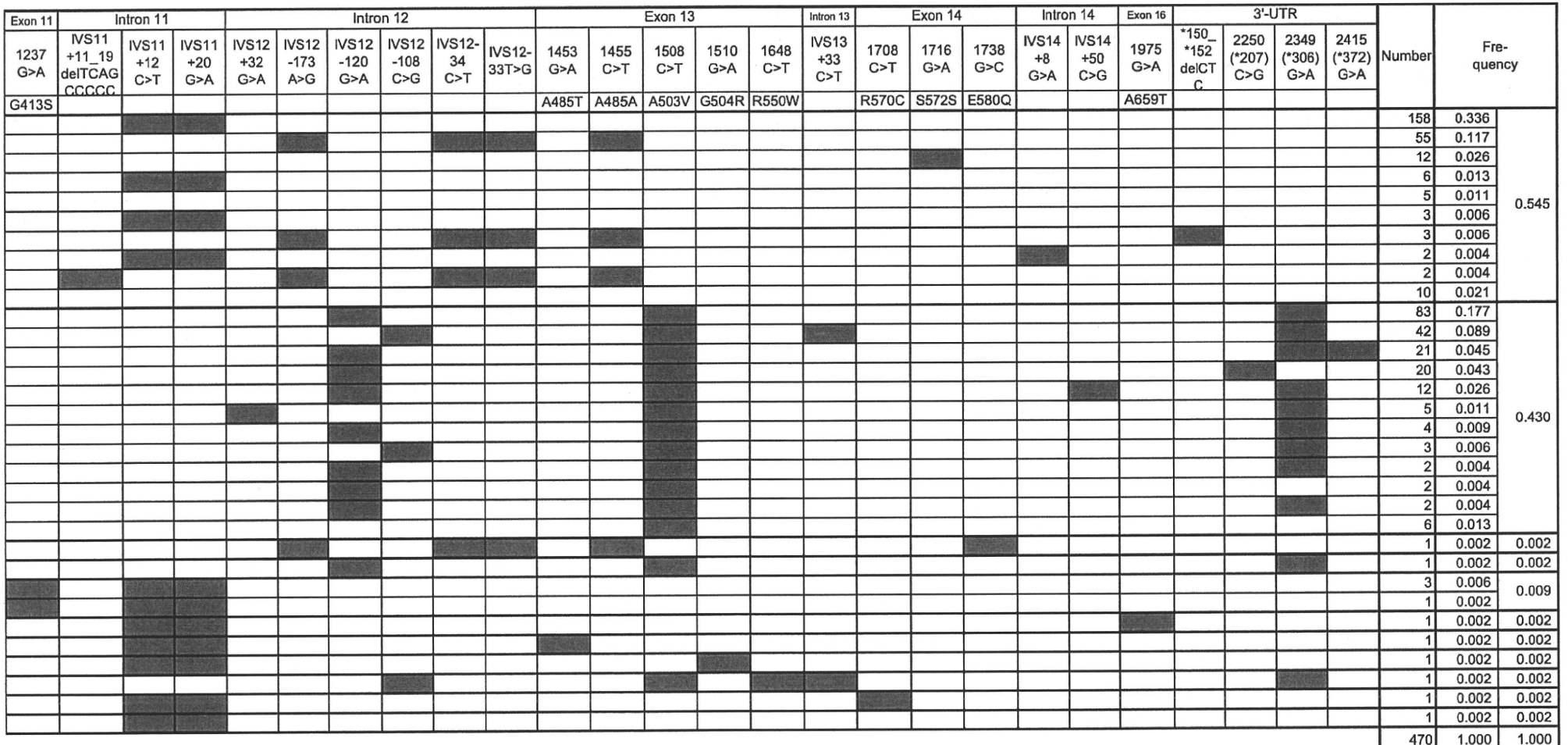
[a] A of the translational start codon of *POR* is numbered 1. NT_079595.2 was used as the reference sequence.

[b] Major allele, white; minor allele, gray

[c] The haplotypes are described as numbers plus small alphabetical letters.

[d] The haplotypes inferred in only one chromosome ambiguously are put together into "others" and variations found only in these ambiguous haplotype were not included in this table.

[e] The haplotypes inferred in only one subject are described with haplotype names and a question mark.



Exon 11	Intron 11			Intron 12						Exon 13					Intron 13	Exon 14			Intron 14		Exon 16	3'-UTR				Number	Frequency	
1237 G>A	IVS11 +11_19 delTCAG CCCCC	IVS11 +12 C>T	IVS11 +20 G>A	IVS12 +32 G>A	IVS12 -173 A>G	IVS12 -120 G>A	IVS12 -108 C>G	IVS12-34 C>T	IVS12-33T>G	1453 G>A	1455 C>T	1508 C>T	1510 G>A	1648 C>T	IVS13 +33 C>T	1708 C>T	1716 G>A	1738 G>C	IVS14 +8 G>A	IVS14 +50 C>G	1975 G>A	*150_ *152 delCT C	2250 (*207) C>G	2349 (*306) G>A	2415 (*372) G>A			
G413S										A485T	A485A	A503V	G504R	R550W		R570C	S572S	E580Q			A659T							
																										158	0.336	0.545
																										55	0.117	
																										12	0.026	
																										6	0.013	
																										5	0.011	
																										3	0.006	
																										3	0.006	
																										2	0.004	
																										2	0.004	
																										10	0.021	
																										83	0.177	0.430
																										42	0.089	
																										21	0.045	
																										20	0.043	
																										12	0.026	
																										5	0.011	
																										4	0.009	
																										3	0.006	
																										2	0.004	
																										2	0.004	
																										2	0.004	
																										6	0.013	
																										1	0.002	0.002
																										1	0.002	0.002
																										3	0.006	0.009
																										1	0.002	
																										1	0.002	0.002
																										1	0.002	0.002
																										1	0.002	0.002
																										1	0.002	0.002
																										1	0.002	0.002
																										1	0.002	0.002
																										470	1.000	1.000