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Genetic variations in NADPH-CYP450 oxidoreductase in a Czech Slavic cohort

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Abstract

Background—Gene polymorphisms encoding the enzyme NADPH–cytochrome P450 oxidoreductase (POR) contribute to inter-individual differences in drug response.

Aim—To estimate polymorphic allele frequencies of the *POR* gene in a Czech Slavic population.

Materials & Methods—The gene *POR* was analyzed in 322 Czech Slavic individuals from a control cohort by sequencing and HRM analysis.

Results—Twenty-five SNP genetic variations were identified. Of these variants, 7 were new, unreported SNPs, including two SNPs in the 5´flanking region (g.4965 C>T and g.4994 G>T), one intronic variant (c.1899 −20C>T), one synonymous SNP (p.20Ala=) and three nonsynonymous SNPs (p.Thr29Ser, p.Pro384Leu and p.Thr529Met). The p.Pro384Leu variant exhibited reduced enzymatic activities compared to wild type.

Conclusion—New POR variant identification indicates that the number of uncommon variants might be specific for each subpopulation being investigated, particularly germane to the singular role that POR plays in providing reducing equivalents to all CYPs in the endoplasmic reticulum.

Keywords

POR; CYP; P450 oxidoreductase; allele frequencies; haplotype; Czech Slavic population; pharmacogenetics

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Disclaimer: The authors had full access to the data and take responsibility for its integrity. All authors have read and agree with the manuscript as written.

Introduction

Cytochrome P450 oxidoreductase (POR, E.C. 1.6.2.4.) belongs to a small family of diflavin reductases that donate electrons to a wide spectrum of heme-containing enzymes. POR is located on the cytosolic side of the endoplasmic reticulum and is essential in several biochemical processes such as steroidogenesis, xenobiotic metabolism, catabolism of bile and fatty acids and heme and squalene oxygenation [1, 2]. As inferred from its name, the main redox partners of POR constitute a group of microsomal cytochrome P450 enzymes (CYPs) [3]. The human genome contains 50 genes coding for microsomal CYPs, among which are several steroidogenic and many drug-metabolizing proteins [1]. CYP enzymes from families 1 to 3 are responsible for the metabolism of ~80–90% of all phase I-dependent, clinically used drugs [4, 5]. Since POR functions as the unique electron donor to all microsomal CYPs, pharmacogenomics interest has focused on POR genetic variants in recent years.

Human POR is a ~79 kDa protein comprised of 680 amino acids. It contains two flavin cofactors, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The atomic structure of the soluble human POR protein has been described recently [6]. The molecule consists of three domains, a FMN-binding domain, a connecting domain and a FAD/NADP(H)-binding domain [6]. The connecting domain performs an important function as a flexible hinge, which brings two cofactor-binding domains into close proximity and thus facilitates the electron flow within the POR molecule [7, 8]. Electrons are transferred from NADPH through FAD and FMN to the respective acceptors and electron flow is controlled by the redox properties of the flavin cofactors [9], which are dictated by the protein structure. Molecular changes in the *POR* gene cause disruption in POR catalytic activities [10–18]. In mice, complete loss of the *Por* gene is embryonically lethal [19, 20]. Liver-specific knockout of *Por* generates phenotypically normal mice with seriously affected hepatic drug metabolism [21]. Initial studies of *POR* variants focused on catalytic assays with steroid-metabolizing CYPs [10–13], but recent interest has been on drug-metabolizing enzymes [14–17] and several *in vivo* studies have been performed [22–25]. With a growing number of POR assays becoming available, it has been demonstrated that the catalytic ability of one POR mutant with a particular CYP cannot predict its catalytic ability with another CYP [15, 17], or with another redox partner such as heme oxygenase [26]. POR function may also vary with CYP isoform [27] and the specific substrate metabolized [15, 17]. Thus, every single mutant must be individually assayed with the specific CYP of interest combined with the unique substrate being investigated.

The *POR* gene was identified by Shephard *et al*. [28] and it was localized to human chromosome 7q11.2. The gene contains 15 coding exons and a single untranslated exon termed 1U [29]. Molecular changes in the gene coding for POR were for the first time reported in 2004 [10, 30, 31]. Genetic variations identified to date are summarized on the official web site of *POR* polymorphisms (http://www.cypalleles.ki.se/por.htm) and, so far, 48 *POR* alleles/haplotypes have been published there. Recessive mutations in the *POR* gene have been associated with Antley-Bixler syndrome (ABS), disordered steroidogenesis, ambiguous genitalia, congenital adrenal hyperplasia (CAH) and polycystic ovary syndrome [32, 33]. Patients usually present with a combination of these symptoms and the syndrome

caused by defects in *POR* has been termed P450 oxidoreductase deficiency (PORD) [32]. To date, five population genetic studies addressing the distribution of *POR* genetic differences within various populations have been performed [12, 34–37]. Specific subgroups of the Caucasian population were represented in several of these reports [12, 34, 35]. This study was undertaken to further define *POR* allele frequencies in the, so far unreported, Caucasian subpopulation, specifically in the Czech Slavic population.

Methods

Ethics

The study was carried out in accordance with the Declaration of Helsinki of the World Medical Association and was approved by the Committee of Medical Ethics at the General University Hospital in Prague. Informed consent was obtained from all adult participants and from parents of underage individuals.

Samples

The study enrolled a total of 322 subjects, including 144 males and 178 females. 227 DNA samples of adults were acquired during the longitudinal collection of control samples of healthy individuals from Czech Slavic population in our laboratory. In addition, the DNA of 95 neonates was extracted from cord blood [38].

DNA analysis

Genomic DNA was extracted from peripheral blood samples anticoagulated with EDTA according to a standard protocol or from cord blood using previously described methods [38]. All 16 exons of the *POR* gene with surrounding exon/intron boundaries (more than 20 bp of their flanking regions) were amplified by PCR using specific primers [37]. DNA analysis (HRM analysis and DNA sequencing) were implemented according to previously described techniques [37] with minor changes. We extended the region of exon 1U (E1U) by using following primers: Fw 5´CGAAGGAGGAGGCTAGACCG-3´ and Rev 5´-AAGCTGTGGAAAAGTCGACCC-3´. The PCR products of E1U region were thus 651 bp long. The PCR reactions were carried out in a total volume of 12.5 μl, including 25 ng of genomic DNA, 0.1 μM of each primer, 8% DMSO and 1× Plain PP Master Mix (Top-Bio, Prague, Czech Republic). Initial denaturation was at 94°C for 2 min, followed by 33 cycles of 30 s denaturation at 94°C, 30 s annealing at 65 °C and 45 s elongation at 72°C, with a final extension at 72°C for 5 min.

Haplotyping studies

The linkage disequlibrium and haplotype block estimations analyses were performed as previously described [37]. In short, unphased genotype data of the whole cohort were entered into the Genotype Visualization and Algorithmic Tool (GEVALT) version 2 software [39]. Only polymorphisms passing the following thresholds were used for phasing and further steps: Hardy-Weinberg $p > 0.001$, minimum genotype % = 100%, minimum minor allele frequency = 0.001. Phasing, the linkage disequilibrium (LD) analysis and estimation of the haplotype block structure were performed utilizing the Genotype Resolution and Block Identification using the Likelihood (GERBIL) algorithm [40].

Comparative LD analysis was performed using the SNPs common to the current study and the previous report of *POR* polymorphisms in two Jewish cohorts [37].

Statistical method

Statistical analysis was performed by using software STATISTICA 10 (StatSoft, Czech Republic) and Pearson's chi-squared test.

Results

In order to determine the frequency of *POR* genetic variations in the normal population, all 16 exons and exon/intron boundaries were examined in 322 Czech individuals. With the view of identifying potential regulatory variants, we extended the sequence of exon 1U in the region containing SP1 binding sites, which have been shown to play a critical role in *POR* transcription [41]. We identified 25 distinct *POR* genetic variations (Table 1). Four of them were found in the 5´-flanking region, 7 in intronic regions and 14 in the protein coding regions (exons). From the 14 variations found in exons, 7 were synonymous, 6 were missense single nucleotide polymorphisms (SNP) and one SNP was found in the first untranslated exon. Of the 25 variants identified, 7 were new SNPs, not described previously, including two SNPs in the 5´-flanking region (g.4965 C>T and g.4994 G>T), one intronic variant (c.1899 −20 C>T), one synonymous SNP (p.20Ala=) and three nonsynonymous SNPs (p.Thr29Ser, p.Pro384Leu and p.Thr529Met). All 7 novel SNPs were found as individual heterozygotes at allele frequencies 0.002 (0.2%). All SNPs found in the cohort are summarized in Table 1.

Nine of the 25 identified SNP variants had allele frequencies greater than 10% and are considered as *common POR* SNPs. No significant differences in the allele frequencies of these variants in the Czech population and the allele frequencies obtained in the previous studies were found. Likewise, no statistically significant difference in allele frequencies of the common SNPs was observed between male and female groups (Table 2) except for the uncommon SNP rs72557941 with 6 heterozygous men (out of 144) and only one heterozygous woman (out of 178) ($p<0.05$). The most common SNP resulting in an amino acid change, p.Ala503Val (*POR*28*), was present at the allele frequency of 0.269 (26.9%). All but one uncommon variant occurred with a frequency lower than 0.01 (1%). We reported the relatively high frequency of SNP rs72557941 (more than 0.01), but in previous studies, only Gomes et al. [35] reported this SNP and with lower frequency (0.007). We have not detected any minor (G) allele of rs10262966, which has been described in the Caucasian population in all previous studies, with frequencies between 0.007–0.045 (0.7–4.5%) [12, 34, 35].

Haplotyping Studies

All of the 25 genotyped single nucleotide polymorphisms were used for LD assessment and haplotype block identification analyses as they passed the quality criteria (see **Methods**). Figure 1A depicts the relatively strong LD pattern across the *POR* gene, evident particularly from the markers with higher MAF. Four haplotype blocks with frequencies over 1% were inferred with indication of high values of multiallelic D´ between the blocks (Figure 1B).

Almost half of the studied cohort were carriers of the *POR*1* haplotype, *i.e.*, either corresponding to the reference sequence or its variants without amino acid changes. *POR*28* haplotypes were present in 150 individuals, the remaining haplotype block combinations were present in low frequencies.

Functional Analysis of the p.Pro384Leu POR Missense Variation

Sequencing of the *POR* gene revealed a novel *POR* genetic variation p.Pro384Leu, which is a one-base substitution (c.1151C>T) on one allele in exon 10. To investigate this new POR variant, we expressed and purified the variant protein, determined flavin content, cytochrome *c* reduction and several CYP-mediated hydroxylation activities. Wild type (WT) POR and the p.Pro384Leu variant were bacterially expressed and purified as full length proteins (Supplementary Figure 1). For quantitative analysis of the proteins expressed in *E. coli*, SDS-polyacrylamide gel electrophoresis (PAGE) was performed and molecular mass of the proteins were determined to 77 kDa. Both fractions had greenish-brown color, which was expected due to spectral contribution of oxidized FAD (yellow) and air-stable FMN semiquinone (blue-gray). 11 mg and 7.25 mg of purified protein were produced per liter of culture for WT and the p.Pro384Leu variant, respectively.

HPLC-based analysis of flavin content was performed to assess the protein:FAD:FMN ratio. POR protein concentration was quantified by oxidized flavin absorbance ($\Delta\varepsilon$ = 21.4 $mM^{-1}cm^{-1}$) [42]) and compared to the microbicinchoninic acid (BCA) protein assay (Pierce) according to standard protocol. The purity of each protein preparation was determined by SDS-polyacrylamide gradient gel electrophoresis. These analyses showed that the p.Pro384Leu variant had the full complement of FAD and FMN and the protein: FAD: FMN ratio was the same as in the WT (1:1:1) (Supplementary Figure 2).

The Km for NAPDH was measured using cytochrome c as the electron acceptor (Supplementary Figure 3) for both WT and p.Pro384Leu. No difference was observed between WT and p.Pro384Leu (K_m^{NADPH} for WT = 1 μM and p.Pro384Leu = 0.9 μM), suggesting that the mutation has no significant effect on the apparent NADPH binding. However, the observed Vmax for p.Pro384Leu was ~60% of the WT, suggesting a possible impairment of electron flow in the mutant. As CYPs are the physiological electron acceptors for POR, various CYP activities were measured to assess the possible effect(s) of the mutation reflecting a physiological scenario (Supplementary Table 1). CYP2E1 activity in the presence of p.Pro384Leu showed a similar rate compared to the WT protein as measured by the conversion of p-nitrophenol to 4-nitrocatechol, whereas a slight decrease in the rate of arachidonic acid metabolism was observed with the p.Pro384Leu variant. It is interesting to note that the metabolism of 7-benzyloxy-4-trifluoro-methylcoumarin (BFC), which can be attributed due to both CYP3A11 and CYP1A2, showed a marked decrease compared to wild type. Such findings reiterate the fact that the characterization of POR mutations found in humans requires more rigorous testing, using various substrates of interest and the outcome should not be generalized.

Discussion

POR is an essential component of several enzyme systems, including systems containing drug-metabolizing CYPs. Several studies have raised the question of the importance of common *POR* variations in drug metabolism [12, 17, 34, 43]. Five studies have investigated the role of *POR* gene variants in relation to pharmacogenetics [12, 34–37]. We decided to look for *POR* SNP frequencies in an unstudied Czech Slavic population. The present study confirmed several of the already reported common *POR* SNPs (>10%) and their allelic frequencies were not significantly different from frequencies found in other ethnic groups (see Table 1). In contrast to our previous report [37], we also sequenced the proximal promoter region of the *POR* gene containing three SP1 binding sites shown to be important in the transcription of *POR* [41]. We have identified several heterozygous SNPs in this region (Table 1), but none of them lie within the crucial SP1 binding sites.

Our results revealed six amino acid changing variants (Table 1), among which four were not reported previously (**p.The29Ser, p.Arg371His, p.Pro384Leu, p.Thr529Met**) (Figure 3, variant p.The29Ser is not a part of the model, which is based on Δ66 truncation wild-type POR). During preparation of this manuscript, we found that the amino acid variant p.Arg371His has been described for the first time in the database of an Exome Variant Server (http://evs.gs.washington.edu/EVS/), but its impact on the POR function was not yet studied. Of the remaining known SNPs, p.Ala503Val is a common polymorphism with an allele frequency of 0.191 – 0.434 [12, 34–37]. According to recent studies, p.Ala503Val is a likely contributor to a pharmacogenetic variation in drug and xenobiotic metabolism [15, 17]. Amino acid variant p.Val631Ile was discussed in our previous work [37]. It is not a PORD-causing SNP [11], but it severely decreases the activity with some CYPs [14]. Therefore, we considered it as a potential biomarker for future *POR* pharmacogenetics screening [37].

POR variant p.Pro384Leu was identified within this population study. The substitution of a cyclic amino acid, proline, for a hydrophobic amino acid, leucine, and the important location of the Pro384 residue within the connecting domain and in a helical sequence, led us to express and purify this protein variant. The results of biochemical assays showed reduced activities of the p.Pro384Leu variant compared to WT. The Pro384 residue is located within the connecting domain of the POR molecule, a globular domain that was shown to be involved in the movement and interaction of the FAD- and FMN-binding domains [7, 44]. It is surprising, therefore, that POR activity was not more severely affected by the p.Pro384Leu substitution. It is not unexpected, however, that a minor loss of function from a single allele had no apparent effect on the health of the individual. There is a possibility that individuals exhibiting this variant may possess compromised capabilities to metabolize drugs as a consequence of challenging the system.

Subtle genetic variations and differences in patterns of LD, even within a single geoethnic group (*e.g.*, Caucasian population) have been recognized as a potential source of complications in genome-wide association studies (GWAS). The biological and pharmacogenetic importance of rare variants arising from recently identified mutations emphasizes the importance of genetic characterization of relevant genes in distinct

subpopulations [45], thus addressing personalized medicine. In agreement with the single allele frequency data, our results provide further evidence for the strong LD pattern across the *POR gene* region as well as the substantial representation of *POR*1* and *POR*28* haplotypes that we and others reported previously in several geoethnic groups [15, 25, 37].

Our study cohort consisted of 227 DNA samples obtained from adults and 95 samples obtained from cord blood of neonates. Clinical features of POR deficiency manifest already in newborn infants with symptoms including adrenal insufficiency, skeletal deformities and neonatal presentation with disordered sex development [46]. Therefore, we do not consider the age variations of the cohort to have impact on the *POR* allele frequency.

Several studies confirmed that some commonly occurring *POR* polymorphisms [17, 35] or polymorphisms affecting POR activity, but not associated with PORD disease [34], might have significant effects on drug-metabolizing CYP activities. Therefore, it is of further research interest to study polymorphisms found in the general population due to their potential role as possible additional challenges to the drug-metabolizing capacity of these individuals due to drug-drug interactions or induction of CYPs by environmental agents.

Conclusion

This first investigation of the Slavic population shows that allele frequencies of commonly found SNPs in the Czech Slavic population are similar to those from other studies investigating the *POR* gene in another Caucasian population [12, 34, 35]. We have not found any mutations associated with *POR* deficiency, suggesting that these are rare variants not commonly represented in the normal population. We described four new amino acid-changing variants, none of which occurred more than once suggesting that these are rare variants. Haplotype analyses did not reveal any statistically significant findings. Biochemical investigation of the new amino acid variant p.Pro384Leu showed reduction of its activity compared to WT, indicating that some of the uncommon variants can alter POR activity without causing POR deficiency. Observation of new variants in a subpopulation clearly indicates that the number of POR variants will be different depending on the cohort chosen for such studies. It is critical to continue these efforts in search of variants of such an important enzyme that plays a critical role in both endo- and xeno-biotic metabolism, including the metabolism of more than 90% of therapeutic drugs. This will be an important contribution to the future of personalized medicine and help to reduce possible negative outcomes due to adverse drug reactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Future perspective

Pharmacogenetics is a rapidly evolving field that will clearly lead to the development of pharmacogenetic tests. Since POR is an important drug metabolizing enzyme, in the future, the *POR* gene may become essential in pharmacogenetic screening.

Executive summary

The role of NADPH–P450 oxidoreductase in pharmacogenetics

Polymorphisms in the gene encoding the enzyme NADPH–P450 oxidoreductase (POR) influence the activity of microsomal cytochromes P450 and thus modulate drug effects and contribute to inter-individual differences in drug response.

Population studies

To date, five population studies addressing the distribution of *POR* genetic differences within various populations have been performed. This study was undertaken to search for polymorphic variants of *POR* gene in the unstudied Czech Slavic population.

New *POR* variants in the Czech Slavic population

Analyzing of the *POR* gene in the Czech Slavic population showed a total of 25 different *POR* genetic variations, 7 of which were new, not described previously (two variants were found in flanking region: g.4965 C>T and g.4994 G>T, one intronic variant: c.1899 −20C>T and four exonic variants: p. 20Ala=, p.Thr29Ser, p.Pro384Leu and p.Thr529Met).

Biochemical characterization of the new p.Pro384Leu variant

Biochemical investigation of the new amino acid variant p.Pro384Leu showed reduction of its activity compared to WT, indicating that some of the uncommon variants can potentially alter POR activity without causing POR deficiency. This is particularly important due to the singular role that POR plays in providing reducing equivalents to all CYPs in the endoplasmic reticulum.

Haplotyping studies

Our data provide evidence for the strong LD pattern across the *POR gene* region as well as the substantial representation of *POR**1* and *POR**28* haplotypes.

Conclusion

The observation of new *POR* genetic changes indicates that the number of uncommon POR variants might be specific for each subpopulation being investigated.

Reduction in the activity of p.Pro384Leu variant provides evidence that some uncommon *POR* variants might alter drug metabolism and thus *POR* variants might be important for the future of personalized medicine.

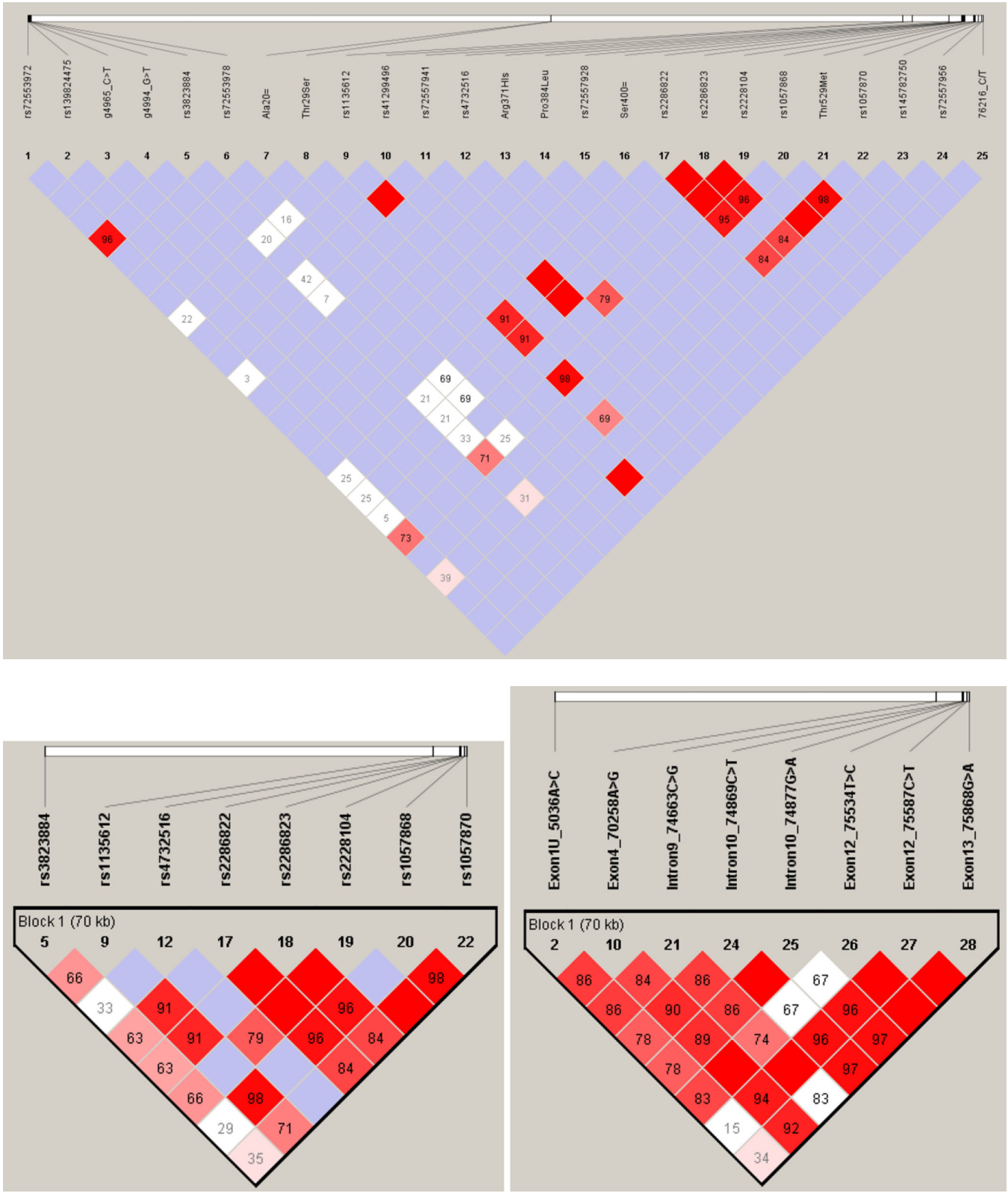

Figure 1. Linkage equilibrium analysis of *POR* in the Czech population

Linkage disequilibrium pattern of the analyzed polymorphisms across the *POR* gene in the Czech cohort (GEVALT v.2. software). The values of D' are indicated in number and by color (for LOD >2: bright red: D=1, shades of red/pink: D<1; for LOD <2: blue: D=1, white D<1).

„Data not shown": **LD plot derived from SNPs common to AJ/MJ study and the current one**.

LEFT: Czech cohort, RIGHT: combined sample of MJ and AJ. Similarly strong LD.

Figure 2. Inferred Haplotypes in Czech Population

The haplotype display of *POR* in the Czech cohort (GEVALT v.2. software, GERBIL algorithm) shows each haplotype in a block with its frequency in the respective population and connections from one block to the next (thin and thick lines refer to >10% and >50% chromosomes proceeding from the indicated haplotype on the left to the indicated haplotype on the right). In the crossing areas, a value of multiallelic D' (inversely related to the fraction of chromosomes that have experienced historical recombination) is shown. The numerical labels of the variants correspond to those shown in the Figure 1. Only haplotypes with frequency >1% in the Czech cohort are shown.

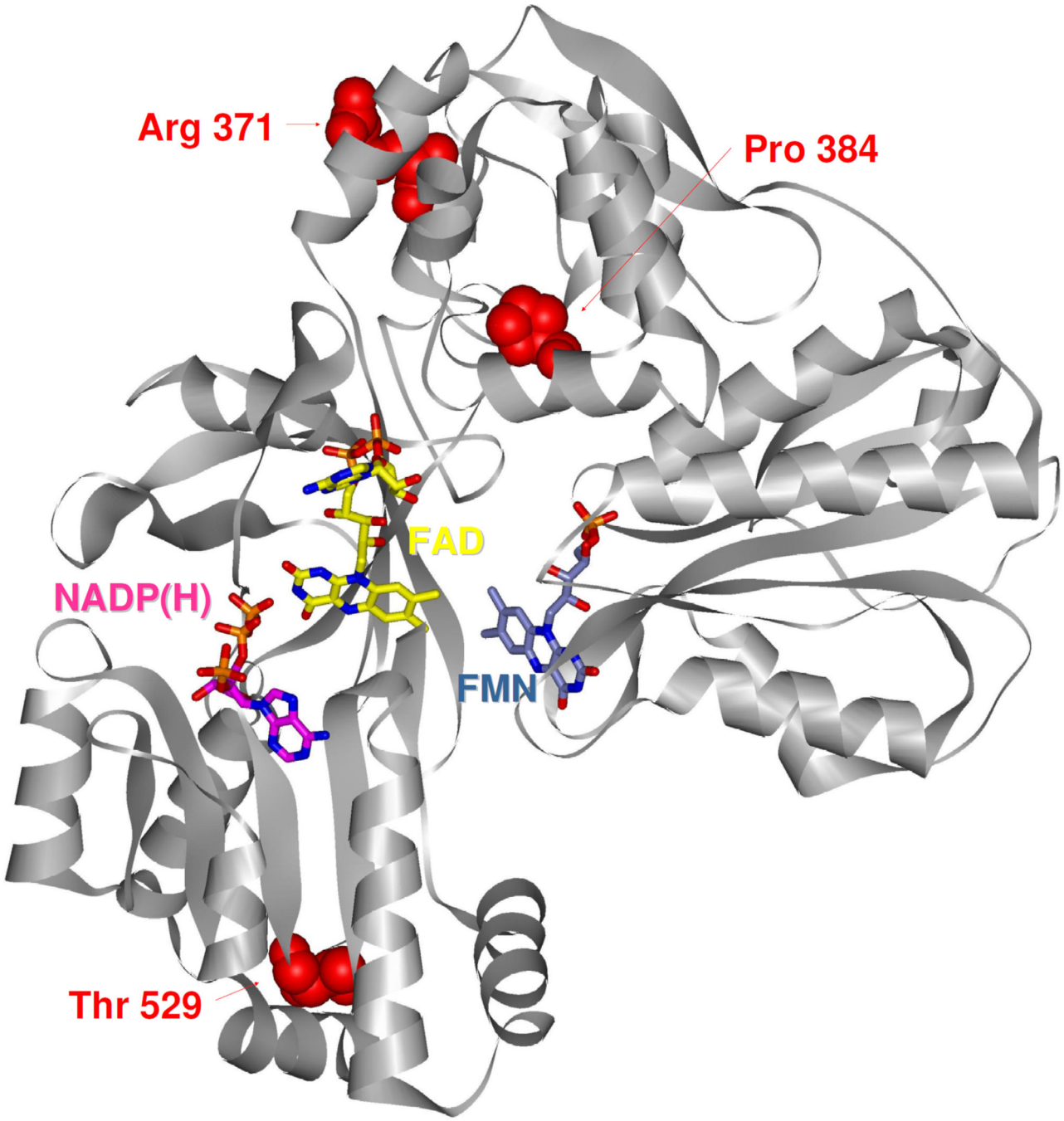

Figure 3.

Missense variants found in the Czech Slavic population. The human POR structure [6] is depicted, gray ribbons indicate the peptide backbone. FAD (yellow) and FMN (blue–gray) cofactors and NADP(H) coenzyme (only 2′,5′-ADP of NADP+ was structurally resolved, pink) are shown in stick configuration. Amino acid residues corresponding to Czech Slavic variants are shown in space-filling configuration (see inset labels). FAD: Flavin adenine dinucleotide; FMN: Flavin mononucleotide.

Table 1

Genetic variations in the *POR* gene found in the Czech Slavic population. Newly found variants are shown in red, previously described ones in yellow.

Exon	g.	c.	p.	SNP ID	CZ (644)	CZ	AJ	MJ	Hart	AA	CA	AS	ME	Gomes
5´-Flanking	4849 C>A			rs72553972	113	0,175	-	-	-	0,026	0,130	0,078	0,077	
5´-Flanking	4883 C>T			rs139824475	3	0,005	-	-	-	-	-	-	-	
5´-Flanking	4965 C>T				1	0,002	-	-	-	-	-	-	-	
5´-Flanking	4994 G>T				1	0,002	-	-	-	-	-	-	-	
Exon 1	5036 A>C	−47 A>C		rs3823884	159	0,247	0,224	0,221	-	0,812	0,266	0,311	0,377	
Intron 1	5099 C>T	−5 +21 C>T		rs72553978	5	0,008	-	-	-	0,000	0,009	0,000	0,003	
Exon 2	43951 C>T	60 C>T	Ala20=		1	0,002	-	-	-	-	-	-	-	
Exon 2	43976 A>T	85 A>T	Thr29Ser		1	0,002	-	-	-	-	-	-	-	
Exon 5	70258 A>G	387 A>G	Pro129=	rs1135612	183	0,284	0,318	0,360	0,212	0,083	0,268	0,419	0,420	0,260
Intron 5	70943 G>A	517 −4 G>A		rs41299496	1	0,002	-	-	-	-	-	-	-	
Exon 10	73673 C>T	984 C>T	Ala328=	rs72557941	7	0,011	-	-	-	-	-	-	-	0,007
Intron 10	74663 C>G	1067 −13C>G		rs4732516	634	0,984	0,991	0,938	0,953	0,609	0,959	0,870	0,869	
Exon 11	74721 G>A	1112 G>A	Arg371His		1	0,002	-	-	-	-	-	-	-	
Exon 11	74760 C>T	1151 C>T	Pro384Leu		1	0,002	-	-	-	-	-	-	-	
Exon 11	74800 G>A	1191 G>A	Ser397=	rs72557928	1	0,002	-	-	-	0,000	0,000	0,003	0,000	
Exon 11	74809 G>A	1200 G>A	Ser400=		1	0,002	0,000	0,004	-	-	-	-	-	
Intron 11	74869 C>T	1248 +12C>T		rs2286822	222	0,345	0,385	0,393	0,359	0,208	0,319	0,360	0,407	0,293
Intron 11	74877 G>A	1248 +20G>A		rs2286823	221	0,343	0,382	0,393	0,374	0,194	0,317	0,360	0,407	0,297
Exon 13	75534 T>C	1455 T>C	Ala485=	rs2228104	632	0,981	0,991	0,952	0,923	0,675	0,967	0,881	0,868	0,990
Exon 13	75587 C>T	1508 C>T	Ala503Val	rs1057868	173	0,269	0,294	0,206	0,219	0,191	0,264	0,367	0,310	0,303
Exon 13	75665 C>T	1586 C>T	Thr529Met		1	0,002	-	-	-	-	-	-	-	
Exon 14	75868 G>A	1716 G>A	Ser572=	rs1057870	222	0,345	0,306	0,335	0,309	0,182	0,378	0,138	0,132	0,363
Exon 15	76133 G>A	1891 G>A	Val631Ile	rs145782750	1	0,002	0,000	0,004	-	-	-	-	-	0,007
Intron 15	76153 G>T	1898 +13 G>T		rs72557956	1	0,002	-	-	-	0,000	0,008	0,003	0,003	
Intron 15	76216 C>T	1899 −20 C>T			1	0,002	-	-	-	-	-	-	-	

g. - Genomic position NG_008930.1
c. - Coding position NM_000941.2

p. - Amino acid change position NP_000932.3

The sign (+) represent nucleotides upstream the last base translated in the genomic sequence and the sign (−) represents nucleotides downstream the first base translated in the genomic sequence. MJ-Moroccan Jewish allele frequencies, AJ-Ashkenazi Jewish allele frequencies and AA-African American allele frequencies, CA-Caucasian American allele frequencies, AS-Chinese American allele frequencies, ME-Mexican American allele frequencies according to Huang *et al.* [12]. Frequency data were compiled from studies Hart *et al.* [34], Huang *et al.* [12], Gomes *et al.* [35], Saito *et al.* [36] and Tomkova *et al.* [37].

Table 2

Comparison of allele frequencies between male and female. Newly found variants are shown in red.

Exon	c.	p.	SNP ID	F	M	P value
5´-Flanking	4849 C>A		rs72553972	0,197	0,149	0,1165
5´-Flanking	4883 C>T		rs139824475	0,008	0,000	0,1184
5´-Flanking	4965 C>T			0,003	0,000	0,3680
5´-Flanking	4994 G>T			0,000	0,003	0,2659
Exon 1U	5036 A>C	−47 A>C	rs3823884	0,264	0,226	0,2618
Intron 1U	5099 C>T	−5 +21 C>T	rs72553978	0,003	0,014	0,1112
Exon 1	60 C>T	Ala20=		0,000	0,003	0,2659
Exon 1	85 A>T	Thr29Ser		0,003	0	0,3680
Exon 4	387 A>G	Pro129=	rs1135612	0,284	0,285	0,9774
Intron 4	517 −4 G>A		rs41299496	0,003	0	0,3680
Exon 9	984 C>T	Ala328=	rs72557941	0,003	0,021	0,0283
Intron 9	1067 −13C>G		rs4732516	0,989	0,979	0,3274
Exon 10	1112 G/A	Arg371His		0	0,003	0,2659
Exon 10	1151 C>T	Pro384Leu		0,003	0	0,3680
Exon 10	1194 G>A	Ser397=	rs72557928	0,003	0	0,3680
Exon 10	1200 G>A	Ser400=		0,003	0	0,3680
Intron 10	1248 +12C>T		rs2286822	0,346	0,344	0,9628
Intron 10	1248 +20G>A		rs2286823	0,343	0,344	0,9777
Exon 12	1455 T>C	Ala485=	rs2228104	0,983	0,979	0,7104
Exon 12	1508 C>T	Ala503Val	rs1057868	0,258	0,281	0,5159
Exon 12	1586 C>T	Thr529Met		0	0,003	0,2659
Exon 13	1716 G>A	Ser572=	rs1057870	0,362	0,323	0,2950
Exon 14	1891 G>A	Val631Ile	rs145782750	0	0,003	0,2659
Intron 14	1898 +13 G>T		rs72557956	0,003	0	0,3680
Intron 14	1899 −20 C>T			0	0,003	0,2659

c. - Coding position NM_000941.2
p. - Amino acid change position NP_000932.3

F – *POR* allele frequencies in female group
M – *POR* allele frequencies in male group