Cellular and Molecular Biology ™ **55**, N°2, 64-69
DOI 10.1170/T854

ISSN 1165-158X
2009 Cell. Mol. Biol.™

EVIDENCE FOR AN ANCESTRAL FOUNDER OF THE COMMON R116W MUTATION IN THE HYDROXYMETHYLBILANE SYNTHASE GENE IN ACUTE INTERMITTENT PORPHYRIA IN THE NETHERLANDS

F.W.M. DE ROOIJ ✉, F.G. KAVELAARS, H. KOOLE-LESUIS AND J.H.P. WILSON

Dept. of Internal Medicine, Section Vascular & Metabolic Diseases, Erasmus University Medical Center, Rotterdam, The Netherlands

✉ F.W.M. de Rooij , PhD, Dept. Internal Medicine, Room Bd299
Erasmus University Medical Center 230 's Gravendijkwal 3015 CE Rotterdam The Netherlands
E-mail : f.derooij@erasmusmc.nl

Received, May 31st 2009; Accepted May 31st, 2009; Published July 1st, 2009

Abstract – Introduction: Acute intermittent porphyria (AIP), the most common acute hepatic porphyria, is an autosomal dominant inborn disorder of heme biosynthesis caused by mutations in the porphobilinogen deaminase (PBGd) gene. The prevalence of AIP in Europe is estimated as 1/10.000-1/20.000. The majority of the known AIP mutations are restricted to only one or just a few AIP families, with the exception of the frequent occurring R116W mutation which is found in 19/80 Dutch AIP families. This mutation has also been reported in 6 other populations (Sweden, Norwegian, i.a.) Recent haplotype analysis of Norwegian and Swedish patients with the R116W mutation show high heterogeneity. The conclusion of that report is that this mutation is abundant due a high mutability of CpG dinucleotides. The Dutch R116W families are well documented with extended pedigrees (up to 1750) which makes it possible to study the haplotypes in these families.
Aim: To investigate haplotype heterogeneity in the Dutch R116W families.
Methods: To investigate the haplotype heterogeneity of the Dutch R116W families, intragenic single nucleotide polymorphism's (SNPs) which cover the whole PBGd gene of 8.6 kb were selected. In addition to the intragenic SNPs, microsatellite markers were selected, flanking the genomic region of the PBGD gene covering a distance of 7.48cM in chromosome 11.
The 7 SNPs were first analysed in 4 out of 19 R116W families selected for their most complete and informative pedigree. The 7 analysed SNPs revealed a distinctive R116W haplotype and were used to analyse the other 14 families in this study cohort, which mainly consisted of DNA from single patients or families with limited members.
Results: The informative SNPs reveal a distinctive haplotype which segregates with the R116W mutation present in the Dutch AIP families (-64T, 1345 G, 2479 G, 3581 G, 6479 T, 7064 C and 8578 A). SNP base nrs a less conserved microsatellite haplotype was observed in these AIP families.
Conclusion: This common R116W haplotype based on 7 SNPs strongly suggests that the relatively high frequency of the R116W mutation in Dutch AIP patients is due a founder effect (eldest parent in pedigree was born in 1750!!). The high mutability of CpG dinucleotides is not a likely explanation for the abundant presence of the R116W mutation, since it is only reported in a few western countries. The heterogeneity described in the Sweden and Norwegian patients and the homogeneity found in the Dutch R116W carriers is compatible with origin of the mutation in Scandinavia with later introduction into the Netherlands. Due to the high frequency of this R116W mutation within the Dutch AIP families it may be applied to refine estimations of the prevalence of AIP in The Netherlands.

INTRODUCTION

Acute intermittent porphyria (AIP; OMIM 176000) is an autosomal dominant disorder resulting from mutations in the hydroxymethylbilane synthase (HMB-synthase; EC 4.3.1.8) gene (1). HMB synthase was previously named uroporphyrinogen-I-synthase or porphobilinogen deaminase. Its activity is reduced to half normal in AIP. HMB-synthase is encoded by a single gene of 8.6 kb on chromosome 11 (11q24.1 → q24.2) and contains 15 exons (Fig 1A). AIP is the most common acute hepatic porphyria in most parts of the world and occurs in all races and ethnic groups (2). AIP is characterised by episodic attacks of abdominal pain, accompanied by neurological dysfunction. The acute attacks are often provoked by exogenous factors

such as certain drugs, alcohol, caloric restriction and endocrine factors.

At least 227 mutations in the HMB-synthase or PBGd gene have been reported so far; the majority of these mutations are restricted to only one or just a few AIP families (3). However five mutations with a founder effect have been reported from different parts of the world.

W198X is the most common mutation in AIP patients in Northern Sweden and Norway (4,5). A R173W founder mutation has been described from Nova Scotia, which on the basis of genealogical data is presumed to have derived from Southwestern Germany (6).

A G111R mutation was found in 12 out of 26 apparently unrelated propositi in Argentina, and shown by haplotype analysis with intragenic and flanking markers to be derived from an ancestral founder (7).

In Switzerland, the W283X mutation, which is present in nearly 60% of Swiss patients and in some French patients, has been calculated to arisen about 1000 years (40 generations) ago (8).

A 669_698del has been shown by haplotype analysis to be a founder gene in AIP patients living in Murcia in Southeastern Spain (9).

In the Netherlands, the most frequent mutation found is a missense mutation, R116W (10), which we have found in 19 out of 80 AIP families in the Netherlands. Although it has been suggested that this high frequency of R116W in the Dutch population might be due to a founder effect (7,10), formal haplotype analysis had not been performed and genealogical analyses of these Dutch R116W families with well extended pedigrees (up to 1750) failed to identify a common ancestor.

The R116W mutation has also been reported in other countries including Sweden (4, 5), Norway (5), France (10), Finland (11), Germany (12), Spain (13), South Africa (14) and even in an Afro-Caribbean patient living in Guadeloupe (15) and in a Japanese patient (16).

Recent analysis of unrelated Norwegian and Swedish families with the R116W mutation using intragenic single nucleotide polymorfisms (SNPs) in the HMB synthase gene and closely linked microsatellites flanking the HMB synthase gene in 11q23.3, showed high haplotype heterogeneity for the R116W mutation in these two populations (5). Each of the Norwegian and Swedish R116W families has its own specific haplotype co-segregating with the R116W mutation. The conclusion drawn by Tjensvoll and colleagues was that R116W is a recurrent mutation occurring at a mutation hotspot based on the high mutability of CpG motifs (5). To determine whether recurrent mutation or a founder effect was responsible for the high prevalence of the R116W mutation in the Dutch AIP population we decided to re-examine the Dutch families to determine whether they had a common haplotype or a variety of haplotypes.

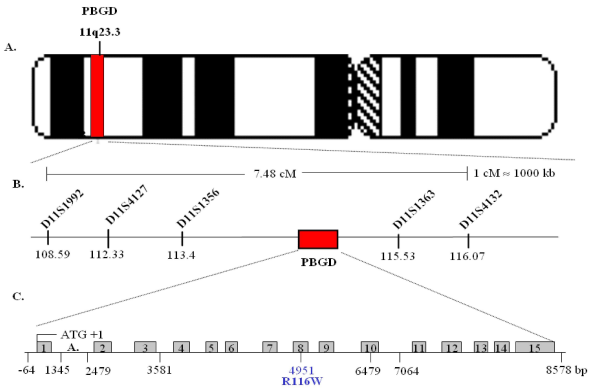

Figure 1. A. Location of the PBGd gene on chromosome 11. B. The location of the five microsatellite markers (D11S1992, D11S4127, D11S1356, D11S1363 and D11S4132) flanking the PBGD gene in the chromosomal region 11q23.3. C. Schematic representation of the PBGd gene. Genomic positions of seven SNPs (-64 C/T, 1345 A/G, 2479 A/G, 3581 A/G, 6479 G/T, 7064 C/A and 8578 G/T and the location of the R116W PBGd gene mutation, 4951, is also indicated.

PATIENTS AND METHODS

Subjects

Of the 80 families with AIP known to us, 19 have been found to have the R116W mutation. The diagnosis of AIP was based on a history of symptoms compatible with an acute attack, a low erythrocyte HMB synthase activity and raised plasma aminolevulinic acid and porphobilinogen levels in all cases [17]. DNA was available from 16 of the 19 unrelated R116W families. There were 9 single patients and 7 informative families. The pedigrees of the 7 R116W families include 15 R116W carriers and 9 relatives with wild type HMB synthase (Fig.1). For mutation and haplotype analysis genomic DNA was extracted from leukocytes isolated from peripheral whole blood or from cultured EB-virus transformed lymphoblastoid cells [16].

Analysis of the R116W mutation ($C^{4951} \rightarrow T$)

The presence (or absence) of mutation R116W in the HMB synthase gene of all 37 subjects was established by sequencing. The R116W mutation was established in the index patient by sequencing, the remainder of the family members were screened specifically for this R116W mutation by PCR with sequencing, using the following primers for exon 8: intron 7 coding gagaatagaggtgatctgaact and intron 9 non-coding cttgtctttttccttggctgca

Haplotype analysis using intragenic.SNPs

Seven known SNPs, most of them different from the Norwegian study, which cover the whole PBGD gene of 8.6 kb (Fig 1C), were analyzed in all 36 individuals from the 16 R116W families/patients. The relative frequency of the selected SNPs are given in table 1.

Haplotype analysis using microsatellite.markers

In addition to the intragenic SNPs, five microsatellite markers selected for their high heterozygosity, and allele size and flanking the genomic region of the HMB synthase gene covering a distance of 7.48 cM on chromosome 11q23, were analysed in all 36 individuals from the 16 R116W families. The allele distribution for the five microsatellite markers analysed is shown in fig. 3. The flanking microsatellites were amplified by PCR using the conditions in table 2.

Table 1. Intragenic SNPs in the Human PBGd gene.

Name	Position SNP			Frequency in CEPH[a]
rs589925	-64	5'-UTR	C	0,63
			T	0,37
rs10790281	1345	intron 1	G	0,58
			A	0,42
rs1799994	2479	intron 1	A	0,62
			G	0,38
rs17075	3581	intron 3	G	0,75
			A	0,25
rs1799995	6479	exon 10	G	0,69
			T	0,31
rs1784304	7064	intron 10	C	0,75
			A	0,25
rs640603	8578	3'-NTR	G	0,65
			A	0,35

[a] Calculated from 78 non-porhyric white unrelated families from the Centre d"Etude du Polymorfisme Human(CEPH).

Table 2. Microsatellites in the PBGd gene

microsatelite marker	percent heterozygosity	allele size	coding primer	dye	non-coding primer
D11S1992	72	149-170	tgtcaaactcctgtgctcaa	FAM	ataggggactccatctctgg
D11S4127	71	87-103	atgagaagtgccatccagc	FAM	actatgcccagtgtgtgtgc
D11S1356	86	193-213	gttgctcatctgttgctca	FAM	acctgccctgacttgc
D11S1363	59	242-252	gaaaatggtatttagaaaccaa	FAM	cccaagggcttacaac
D11S4132	75	176-214	gtgcaagttttggcttcgtc	HEX	actccagcctgggtgaaa

RESULTS

Seven SNPs were determined in the 36 individuals from the 16 R116W families. The allele bearing mutation R116W was characterised in 7 pedigrees namely A, B, C, D, E, F and G according to Mendelian inheritance (Fig.2 and table 3). Examination of the remaining 9 patients in this study cohort, revealed the presence of the same SNPs (data not shown). Their haplotype could not be completely resolved due to heterozygosity at certain SNPs. In the 11 healthy individuals (wild type for the HMB synthase R116W mutations) 11 SNP haplotypes were found which were all different from the mutant haploptypes.

In contrast to the SNP haplotypes, a considerable variability was observed among the

lengths of the microsatellites within the seven informative families (table 3). In the 11 healthy individuals (wild type for the HMB synthase R116W mutations) 19 microsatellite haplotypes were found which were all different from the mutant haploptypes. In one normal individual one haplotype was the same as the haplotype of the mutant allele of another family.

The haplotype of the PBGd gene obtained from the probands of the 7 AIP Dutch AIP families with an informative pedigree shows SNPs homogeneity for the R116W mutation. This haplotype homogeneity for the Dutch R116W families does not correspond with the haplotype heterogeneity in the Norwegian and Swedish R116W families.

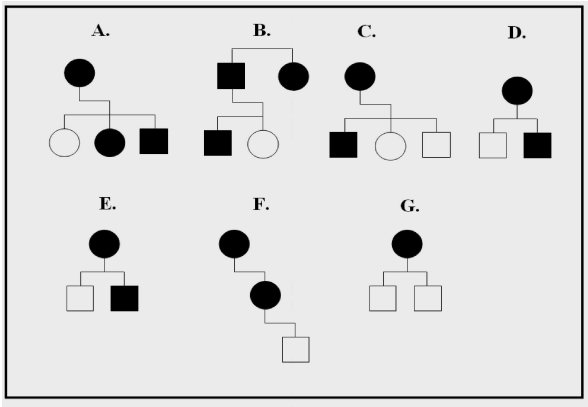

Figure 2. The allele bearing the R116W mutation was typified in seven pedigrees namely A,B,C,D,E,F and G according to Mendelian inheritance. R116W carriers and normal individuals are indicated by solid and empty symbols, respectively.

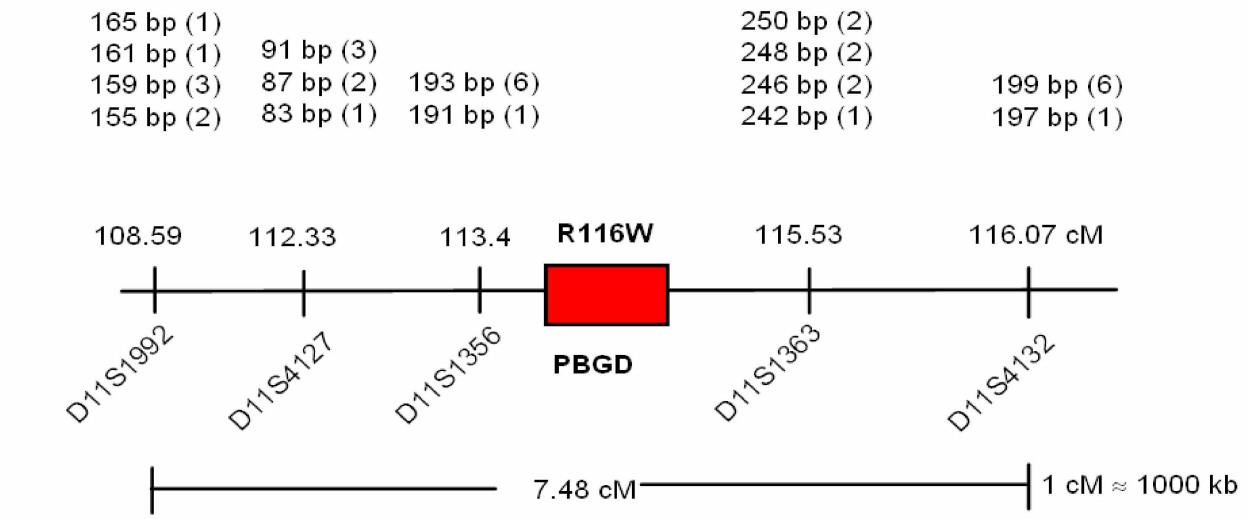

Figure 3. Allele distribution for the five analysed microsatellite markers in the 7 Dutch R116W AIP families.

Table 3. Haplotypes of the seven R116W allele among Dutch AIP families with an informative pedigree

		Family						
		A	B	C	D	E	F	G
Locus								
D11S1992 (108,59 cM)		165	159	159	159	155	155	161
D11S4127 (112,33 cM)		87	91	91	91	87	83	-
D11S1356 (113,40 cM)		191	191	191	191	193	191	191
PBGD gene	-64 C/T (5-UTR)	t	t	t	t	t	t	t
	1345 A/G (intron 1)	g	g	g	g	g	g	g
	2479 A/G (intron 1)	g	g	g	g	g	g	g
	3581 A/G (intron 3)	g	g	g	g	g	g	g
	4949 CGG→TGG R116W (exon 8)							
	6479 G/T (exon 10)	t	t	t	t	t	t	t
	7064 C/A (intron 10)	c	c	c	c	c	c	c
	8578 G/T(3'-NTR)	a	a	a	a	a	a	a
D11S1363 (115,53 cM)		250	250	248	246	248	242	246
D11S4132 (116,07 cM)		199	199	199	199	197	199	199

DISCUSSION

The haplotype homogeneity in the PBGd gene of the Dutch AIP families with the R116W mutation shows that the high prevalence of this mutation in the Netherlands is due to a founder effect. The difference in microsatellite haplotype indicates that the R116W mutation is a relative ancient mutation, which is supported by the failure to find a common ancestor even when pedigrees were traced back to 1750. Our findings are in contrast with those reported from Norway and Sweden (5), where each family with R116W has its own haplotype. Although different SNPs were used to haplotype the R116W families in both studies, this is problably not the reason for the difference in results. This R116W mutation occurs at a CpG dinucleotide, and at such a site the transition rate is five times the base mutation rate (18,19).

Two of the other mutations in AIP which have been found to have an ancestral founder also show in addition this widespread geographical occurrence and haplotype heterogeneity when other populations are examined. The R173W mutation which is a founder gene in Nova Scotia has also been reported from Japan, Sweden, Finland, France, United Kingdom and Spain (13, 19,20,21,22). The G111R mutation which as a founder gene is the most common mutation in AIP in Argentina, has been described in Spanish, Swiss, German, Polish, Czech, United Kingdom and Brazilian patients (13, 23, 24,25,26,27). In the only haplotype study performed in the G111R patients, the Swiss and German families shared partially an intragenic and extended microsatellite haplotype whereas the Polish family had a unique haplotype. Schneider-Yin and colleagues concluded that such recurrent CpG mutations in the AIP population could therefore either be of ancestral origins or derived from de novo events.

The haplotype homogeneity strongly suggests that the relatively high frequency of the R116W mutation in Dutch AIP patients is due a founder effect. Further studies comparing HMB synthase microsatellites and SNPs among the various families who share a specific disease-associated mutation to determine whether they might share an ancient ancestral founder. In view of the cultural and social ties it is possible for instance that the R116W mutation was inherited from Scandinavian countries into the Netherlands, and that there is an ancestral link between AIP families with the G111R mutation in Spain and Argentina. Comparison of the prevalence of haplotypes within a general population with that of the known AIP population could also be useful for estimating more accurately and also be used to refine estimates of the prevalence of AIP (28).

REFERENCES

1. Anderson, K.E.; Sassa, S.,; Bishop,D.F.; Desnick, R.J. In: the Metabolic and Molecular Basis of Inherited Disease. 8th edn: Scriver, C.R., Beaudet, A.L. Sly, W.S., Valle, D., Childs, B. Vögelstein, B. Eds.; McGraw-Hill: New York, 2001; Vol 11, Chapter 124, pp2991-3062
2. Elder GH. Molecular genetics of disorders of haem biosynthesis. *J Clin Pathol* 1993; **46**: 977-981
3. http://www.uwcm.ac.uk/uwcm/mg/
4. Lee JS, Anvret M. Identification of the most common mutation within the porphobilinogen deaminase gene in Swedish patients with acute intermittent porphyria. *Proc Natl Acad Sci U S A.* 1991;**88**:10912-5.
5. Tjensvoll K, Bruland O, Floderus Y, Skadberg O, Sandberg S, Apold J. Haplotype analysis of Norwegian and Swedish patients with acute intermittent porphyria (AIP): Extreme haplotype heterogeneity for the mutation R116W. *Dis Markers*. 2003-2004;**19**:41-6.
6. Greene-Davis ST, Neumann PE, Mann OE, Moss MA, Schreiber WE, Welch JP, Langley GR, Sangalang VE, Dempsey GI, Nassar BA. Detection of a R173W mutation in the porphobilinogen deaminase gene in the Nova Scotian "foreign protestant" population with acute intermittent porphyria: a founder effect. *Clin Biochem.* 1997;**30**:607-12.
7. De Siervi A, Rossetti MV, Parera VE, Astrin KH, Aizencang GI, Glass IA, Batlle AM, Desnick RJ. Identification and characterization of hydroxymethylbilane synthase mutations causing acute intermittent porphyria: evidence for an ancestral founder of the common G111R mutation. *Am J Med Genet*. 1999;**86**:366-75.
8. Schneider-Yin X, Hergersberg M, Goldgar DE, Rufenacht UB, Schuurmans MM, Puy H, Deybach JC, Minder EI. Ancestral founder of mutation W283X in the porphobilinogen deaminase gene among acute intermittent porphyria patients. *Hum Hered.* 2002; **54**:69-81.
9. Guillen-Navarro E, Carbonell P, Glover G, Sanchez-Solis M, Fernandez-Barreiro A. Novel HMBS founder mutation and significant intronic polymorphism in Spanish patients with acute intermittent porphyria. *Ann Hum Genet.* 2004; **68**:509-14.
10. Gu XF, de Rooij F, Lee JS, Te Velde K, Deybach JC, Nordmann Y, Grandchamp B. High prevalence of a point mutation in the porphobilinogen deaminase gene in Dutch patients with acute intermittent porphyria. *Hum Genet.* 1993; **91**:128-30.
11. Kauppinen R, von und zu Fraunberg M. Molecular and biochemical studies of acute intermittent porphyria in 196 patients and their families. Clin Chem. 2002;48:1891-900.
12. von Brasch L, Zang C, Haverkamp T, Schlechte H, Heckers H, Petrides PE. Molecular analysis of acute intermittent porphyria: mutation screening in 20 patients in Germany reveals 11 novel mutations. *Blood Cells Mol Dis.* 2004; **32**:309-14.
13. Solis C, Lopez-Echaniz I, Sefarty-Graneda D, Astrin KH, Desnick RJ. Identification and expression of mutations

in the hydroxymethylbilane synthase gene causing acute intermittent porphyria (AIP). *Mol Med*. 1999; **5**:664-71.
14. Ong PM, Lanyon WG, Hift RJ, Halkett J, Moore MR, Mgone CS, Connor JM. Detection of four mutations in six unrelated South African patients with acute intermittent porphyria. *Mol Cell Probes*. 1996; **10**:57-61.
15. Robreau-Fraolini AM, Puy H, Aquaron C, Bogard C, Traore M, Nordmann Y, Aquaron R, Deybach JC. Porphobilinogen deaminase gene in African and Afro-Caribbean ethnic groups: mutations causing acute intermittent porphyria and specific intragenic polymorphisms. *Hum Genet*. 2000; **107**:150-9.
16. Morita Y, Daimon M, Kashiwaba M, Yamatani K, Igarashi M, Fukase N, Ohnuma H, Ikezawa Y, Sugiyama K, Manaka H. A point mutation, C to T, in exon 8 of the porphobilinogen deaminase gene in a Japanese family with acute intermittent porphyria. *Jpn J Hum Genet* 1995; **40**; 207-213.
17. Krawczak M, Ball EV, Cooper DN. Neighboring-nucleotide effects on the rates of germ-line single-base-pair substitution in human genes. *Am J Hum Genet*. 1998; **63**:474-88.
18. Waters TR, Swann PF. Thymine-DNA glycosylase and G to A transition mutations at CpG sites. *Mutat Res*. 2000; **462**:137-47.
19. Tomie Y, Horie Y, Tajima F, Kitaoka S, Nanba E, Yuasa I, Kawasaki H. Mutation in the exon 10 (R173W) of the hydroxymethylbilane synthase gene in two unrelated Japanese families with acute intermittent porphyria. *Res Commun Mol Pathol Pharmacol.* 1998; **99**:5-15
20. Andersson C, Floderus Y, Wikberg A, Lithner F. The W198X and R173W mutations in the porphobilinogen deaminase gene in acute intermittent porphyria have higher clinical penetrance than R167W. A population-based study. *Scand J Clin Lab Invest*. 2000; **60**:643-8.
21. Kauppinen R, Mustajoki S, Pihlaja H, Peltonen L, Mustajoki P. Acute intermittent porphyria in Finland: 19 mutations in the porphobilinogen deaminase gene. *Hum Mol Genet.* 1995;**4**:215-22
22. Puy H, Aquaron R, Lamoril J, Robreau AM, Nordmann Y, Deybach JC. Acute intermittent porphyria: rapid molecular diagnosis. *Cell Mol Biol (Noisy-le-grand).* 1997; **43**:37-45.
23. Whatley SD, Woolf JR, Elder GH. Comparison of complementary and genomic DNA sequencing for the detection of mutations in the HMBS gene in British patients with acute intermittent porphyria: identification of 25 novel mutations. *Hum Genet* 1999; **104**:505-510.
24. Schneider-Yin X, Hergersberg M, Schuurmans MM, Gregor A, Minder EI. Mutation hotspots in the human porphobilinogen deaminase gene: recurrent mutations G111R and R173Q occurring at CpG motifs. *J Inherit Metab Dis.* 2004; **27**:625-31.
25. Gregor A, Schneider-Yin X, Szlendak U, Wettstein A, Lipniacka A, Rufenacht UB, Minder EI. Molecular study of the hydroxymethylbilane synthase gene (HMBS) among Polish patients with acute intermittent porphyria. *Hum Mutat.* 2002; **19**:310.
26. Rosipal R, Puy H, Lamoril J, Martasek P, Nordmann Y, Deybach JC. Molecular analysis of porphobilinogen (PBG) deaminase gene mutations in acute intermittent porphyria: first study in patients of Slavic origin. *Scand J Clin Lab Invest.* 1997; **57**:217-24.
27. Ribeiro GS, Marchiori PE, Kuntz Puglia PM, Nagai MA, Dos Santos ML, Nonoyama K, Hirata MH, Barretto OC. Porphobilinogen deaminase gene mutations in Brazilian acute intermittent porphyria patients. *J Clin Lab Anal.* 2002;**16**: 259-65.
Nordmann Y, Puy H, Da Silva V, Simonin S, Robreau AM, Bonaiti C, Phung LN, Deybach JC. Acute intermittent porphyria: prevalence of mutations in the porphobilinogen deaminase gene in blood donors in France. *J Intern Med.* 1997 Sep;**242** (3):213-7.