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New Mutations of Porphobilinogen Deaminase Gene in Polish Families with Acute Intermittent Porphyria

Nowe mutacje genu deaminazy porfobilinogenu w polskich rodzinach z ostrą przerywaną porfirią

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Abstract

Background. Acute intermittent porphyria (AIP) is an autosomal dominantly inherited disorder of low clinical penetrance. A 50% lower activity of porphobilinogen deaminase (PBGD) stands for the metabolic error in AIP. The PBGD gene is composed of 15 exons of 39–438 bp in length and it is mapped to 11q24.1–24.2 chromosome. So far over 300 various mutations of the PBGD gene responsible for AIP have been described.
Objectives. Description of molecular background of AIP in patients from Polish population diagnosed based on biochemical analyses. Molecular diagnosis of AIP in family members.
Material and Methods. Biochemical study, such as porphobilinogen (PBG) and δ-aminolevulinic acid (ALA) excretion with urine and PBGD activity in erythrocyres have been examined in all probands and family members. AIP patients and their family members underwent further molecular diagnosis.
Results. Among 16 families with AIP diagnosis made on biochemical tests 10 new PBGD gene mutations have been discovered not yet described. Missense (83G>T, 89T>G, 281T>G, 293A>C, 500G>C and 796G>C); and frameshift (470delT, 716-725delACGATCCCGA, 723-724insCC and 969delT) mutation types have been revealed.
Conclusions. Similar to other populations, 10 new mutations revealed in Polish population can suggest heterogeneity of mutatios responsible for AIP. Biochemical methods can sometimes fail to diagnose the disease. Molecular analysis is much more reliable method to diagnose AIP in asymptomatic members of probands (**Adv Clin Exp Med 2010, 19, 4, 497–501**).

Key words: acute intermittent porphyria, mutations.

Streszczenie

Wprowadzenie. Ostra przerywana porfiria (AIP) jest chorobą uwarunkowaną genetycznie, dziedziczoną w sposób autosomalny dominujący o niskiej penetracji klinicznej. Błędem metabolicznym w AIP jest obniżona ok. 50% aktywność deaminazy porfobilinogenu (PBGD). Gen PBGD jest zbudowany z 15 eksonów o długości 39–438 bp i umiejscowiony na chromosomie 11q24.1–24.2. Dotychczas na świecie opisano ponad 300 różnych mutacji genu PBGD odpowiedzialnych za AIP.
Cel pracy. Poznanie podłoża molekularnego odpowiedzialnego za AIP w populacji polskiej u pacjentów, u których rozpoznano porfirię na podstawie badań biochemicznych. Diagnostyka molekularna członków rodzin z AIP.
Materiał i metody. U wszystkich probandów i członków ich rodzin wykonano badania biochemiczne: wydalanie porfobilinogenu (PBG) i kwasu δ-aminolewulinowego (ALA) w moczu oraz aktywność PBGD w erytrocytach. W celu dalszej diagnostyki rodzin z AIP u wszystkich pacjentów wykonano badanie DNA.
Wyniki. Wśród 16 polskich rodzin, u których rozpoznanie AIP postawiono na podstawie badań biochemicznych w genie PBGD stwierdzono 10 nowych mutacji nigdzie nieopublikowanych. Mutacje te to typu missens: 83G>T, 89T>G, 281T>G, 293A>C, 500G>C, 796G>C; typu frameshift: 470delT, 716-725delACGATCCCGA, 723-724insCC, 969delT.
Wnioski. Stwierdzenie wśród 16 polskich rodzin 10 nowych mutacji w genie PBGD może świadczyć o heterogennej naturze mutacji odpowiedzialnych za AIP w populacji polskiej, podobnie jak w różnych krajach świata.

Metody biochemiczne nie zawsze pozwalają jednoznacznie rozpoznać AIP. Analiza molekularna jest najdokładniejszą metodą pozwalającą na postawienie prawidłowego rozpoznania porfirii u bezobjawowych członków rodzin probandów (**Adv Clin Exp Med 2010, 19, 4, 497–501**).

Słowa kluczowe: ostra przerywana porfiria, mutacje.

Acute intermittent porphyria (AIP) is an autosomal dominantly inherited disorder with low clinical penetrance. The metabolic error in AIP consists in a 50% lower activity in porphobilinogen deaminase (PBGD), or hydroxymethylbilane (HMBS) (EC 4.3.1.8), catalyzing the third stage of heme biosynthesis [1].

The PBGD gene, 10 kb long, with the locus on chromosome 11q24.1–24.2, is composed of 15 exons, 39–438 bp long. Located in the gene are two promoters in the 5'flanking region and intron 1, which, respectively, generate housekeeping (containing exons 1 and 3–15) and erythroid-specific (containing exons 2–15) transcripts by alternative splicing of exons 1 and 2 [2–4].

Over 300 mutations of the PBGD gene responsible for AIP have been identified in different countries [5]. They show high heterogeneity and are confined to one or just a few families. They are mostly mutations with the so-called classical AIP with both isozymes affected. In approximately 5% of subjects PBGD activity in erythrocytes is normal but decreased in other tissues. The mutation is found in exon or intron 1, affecting only the so-called housekeeping enzyme variant [6].

Clinical AIP expression varies; approximately 90% of heterozygous patients remain asymptomatic throughout their lives. AIP aggravation or acute attack is precipitated by multiple factors, i.e. exo- and endogenic hormones, stress, some medication, infections, calorie deficits. An acute attack manifests as abdominal pain and neuropsychiatric symptoms [7].

During the attack, the AIP patients excrete significant amounts of porphobilinogen (PBG), δ-aminolevulinic acid (ALA) and porhyrins in the urine. Approximately 70% of patients without previous attacks show normal excretion of heme precursors [8, 9].

Material and Methods

Seventy patients (45 women, 25 men), aged 28–68 years, were members of 16 unrelated families from different parts of Poland. The initial diagnosis of porphyria was established by determining urinary porphyrin precursors, i.e. δ-aminolevulinic acid (ALA) and porphobilinogen, and PBGD activity in erythrocytes (Table 1). Thirty-one patients had manifest porphyria with at least one previous acute attack with typical AIP findings, i.e. increased urinary ALA and PBG excretion, decreased PBGD activity in erythrocytes determined on remission. Latent porphyria was diagnosed in 18 patients with decreased PBGD activity; in most subjects, the urinary porphyrin excretion was normal. The diagnosis was not feasible from biochemical findings from 30 family members with normal urinary ALA and PBG excretion and the PBGD activity showing an intermediate value, between that characteristic of AIP and the reference value (the so-called "non-certain").

The control group included 100 healthy subjects with AIP excluded in biochemical findings.

Biochemical Analysis

Urinary δ-aminolevulinic acid (ALA) and porhyrins were determined with Mauzerall and Granick's method [10], and PBGD activity in erythrocytes, using the method by Magnussen et al. [11].

Genetic Testing

DNA was isolated from EDTA-containing peripheral blood samples. PCR reaction for exons from 3–15 was carried out using primers without GC-clamp as described by Puy et al. [12].

PCR conditions were as follows: a 50 µl reaction mixture contained 0.1 µg genomic DNA, 5pM of each primer, 200 µm dNTPs, 10X PCR buffer (10 mM Tris-HCl, 50 mM KCl, 1 mM EDTA; 0.1% triton Mm-100; 50% glycerol v/v; pH 8), 2 mM MgCl2, 1U Taq polymerase, (Biotools). Amplification was performed for 35 cycles of 30 sec at 95°C, 30 sec at the annealing temperatures for particular exons, and 30 sec elongation at 72°C.

Sequencing was performed using the Big Dye Terminator Cycle Sequencing Kit v 1.1 on Hitachi 3730 Analyser (Applied Biosystem, USA). Each assessed fragment was sequenced towards sense and antisense. The resulting sequence was compared with the reference cDNA sequence from the GenBank accession no M95623, numbered from the ATG sequence of translation-initiation housekeeping codon of enzyme isoform where A is nucleotide +1.

Table 1. New PBGD gene mutations in Polish families with AIP diagnosed on biochemistry

Tabela 1. Nowe mutacje genu PBGD w polskich rodzinach, w których rozpoznanie AIP postawiono na podstawie badań biochemicznych

Family no (Nr)	Mutation (Mutacja)	Mutation consequence (Sekwencja)	Exon (Ekson)	Mutation type (Rodzaj)	Clinical Status/patent No (Stan kliniczny)	PBGD activity nmol porf./ml erytr./h	Urinary PBG µmol/24h	Urinary ALA µmol\24h
1	83G>T	S28I	3	MS	acute/5	10.3–14.9	330–1311.9	231–1062
	83G>T	S28I	3	MS	latent/2	14.1–19.7	8.3–198.3	23.7–148.5
2	83G>T	S28I	3	MS	acute/1	17.5	1225	997.8
	83G>T	S28I	3	MS	latent/3	12.6–16.2	5.8–15.8	16.6–48.8
3	83G>T	S28I	3	MS	acute/2	16.1–19.1	380–580	308–462.1
	83G>T	S28I	3	MS	latent/1	15.4	5	20.8
	83G>T	S28I	3	MS	uncertain/1	26	28.4	63.3
4	83G>T	S28I	3	MS	acute/1	16.1	456.6	202.7
	83G>T	S28I	3	MS	latent/1	11.8	6.2	23.2
	83G>T	S28I	3	MS	uncertain/1	26.5	5.4	12.2
5	89T>G	M30S	4	MS	acute	18.7	64.6	75.9
	89T>G	M30S	4	MS	latent	16.8	263.9	189.5
6	281T>G	V94G	7	MS	acute/3	17.3–25.3	602–652	432–1061.5
7	293A>C	K98T	7	MS	acute/5	16.9–22.8	358.1–923	185.5–365.3
	293A>C	K98T	7	MS	latent/3	11.9–22.2	17.7–147	52.6–92.7
	293A>C	K98T	7	MS	uncertain/1	26.7	76	107
8	470delA	stop+98	9	FS	acute/2	15.4–18.9	386.8–604	338.6–508
9	500G>C	R167P	10	MS	acute/1	17.9	315	511
	500G>C	R167P	10	MS	latent/2	10–15.3	4.4–11.5	12.8–54.4
10	500G>C	R167P	10	MS	acute/2	14.8–22.8	267.1–322.5	146–245.4
11	500G>C	R167P	10	MS	acute/1	18.9	250.6	180.5
12	716–725del ACGATC-CCGA	cod stop+13	12	FS	acute/1	18.1	372	407.2
	716–725del ACGATC-CCGA	cod stop+13	12	FS	latent/1	14.1	4.4	20.8
13	723–724in-sCC	cod stop+14	12	FS	acute/2	11.1–18.6	271.6–327	251–260.5
	723–724in-sCC	cod stop+14	12	FS	uncertein/1	26	11.1	26.8
14	796G>C	A266P	13	MS	acute	22.1	519	327
	796G>C	A266P	13	MS	latent/3	19.2–23.8	7.8–142.9	37.9–112.7
15	969delT	cod stop+21	15	FS	acute/1	8.5	431	252.4
16	969delT	cod stop +21	15	FS	acute/1	26	882	630
Normal (Norma)						29.34–39.18	0.95–13.3	1.1–30.5

PBGD – deaminase porphobilinogen, ALA – δ-aminolewulinic acid, PBG – porphobilinogen.
PBGD – deaminaza porfobilinogenu, ALA – kwas δ-aminolewulinowy, PBG – porfobilinogen.

Results and Discussion

Among the assessed 135 Polish families 61 different mutations were identified, including 16 novel ones [13, 14, unpublished data]. In 16 unrelated Polish AIP families ten different mutations, not yet reported, were detected: six missense, four frameshift mutations (Table 1).

Mutation 83G>T (S281) was detected in four families; nine patients had clinical manifestations, and seven were with latent form of porphyria. Four patients, from three families, with previous acute attacks, and two patients from families with latent porphyria, had mutation 500G>C (R167P). The remaining missense mutations were present only in single families. Mutation 89T>G (M30S) was found in two related patients with manifest porphyria. Mutation 281T>G (V94G) was identified in three patients with manifest porphyria. Mutation 293A>C (K98T) was detected in five patients with manifest AIP and in three patients with latent porphyria. Mutation 796G>C (A266P) was identified in a proband with manifest porphyria, and in three relatives with latent porphyrias.

Mutation 470delT gave rise to stop codon + 97, detected in two patients with manifest AIP in one family. Mutation 969delT resulted in the formation of stop codon + 21, carried by two patients with manifest AIP from unrelated families. A deletion of ten nucleotides: ACGATCCCGA at site 716-725 resulted in stop codon + 13; the mutation was detected in a patient with manifest porhyria and in a relative with latent porhyria. Insertion of CC at site 723-724 gave rise to stop codon + 14; the mutation was identified in two related patients with a history of transient attacks.

Substitutions of single aminoacids in the polypeptide chain are responsible either for enzyme deficiency resulting from the effect exerted on important residues at the catalytic site, or they impair protein folding. The frameshift mutations produce a premature stop codon and truncated enzyme.

All the novel mutations reported are responsible for porphyria since in none of the patients with mutations any other changes were detected in the PBGD gene in exons 3–15 and borderline sequences. Neither are they polymorphisms since they were not found in 100 healthy subjects.

Only three mutations were detected in two to four families, the others were identified in single families (Table 1). The presence of ten different mutations in 16 families indicates a high defect heterogeneity in the PBGD gene in Poland, confirming the results of previous studies of 25 different mutations identified, including 16 novel ones [13, 14]. A high mutation heterogeneity of the gene occurs also in other populations [12, 15–17]. Also in Finland, in spite of the largely isolated population, the disease is genetically heterogenous. Most mutations are family-specific, found only in one or two families, and country-specific [18].

The presence of mutation 83G>T in four families from the vicinity of Bydgoszcz may be associated with the so-called founder effect of the W283X mutation in patients from the German-speaking part of Switzerland [19, 20], G111R in Argentinians of Italian and Spanish ancestry [21], W198X, identified in northern Sweden [22], R173W, detected in Nova Scotia (Canada) [23].

All own study patients with the PBGD gene mutation showed a 50% decrease in the PBGD activity in erythrocytes and were heterozygous. The mutation was detected in probands and relatives with manifest and latent porphyria. In 30 family members the diagnosis based on biochemical analysis was not feasible; the mutation was found only in three patients, in 26 subjects it was absent; the latter, therefore, were considered to be healthy.

Although the urinary ALA and PBG determination is the simplest examination to detect AIP during an acute attack, molecular analysis is the only reliable method to correctly diagnose asymptomatic relatives, i.e. those with normal urinary excretion of heme precursors [24].

The sequencing technique is the most accurate method yielding 90–100% sensitivity [12, 25].

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