Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 10912–10915, December 1991
Genetics

Identification of the most common mutation within the porphobilinogen deaminase gene in Swedish patients with acute intermittent porphyria

(nonsense mutation/mutation screening/dideoxy method)

JIN-SUNG LEE* AND MARIA ANVRET

Department of Clinical Genetics, Karolinska Hospital, S-104 01 Stockholm, Sweden

Communicated by Jan G. Waldenström, September 6, 1991

ABSTRACT **Acute intermittent porphyria (AIP) is a metabolic disorder characterized by a partial deficiency of the porphobilinogen deaminase (PBGD, EC 4.3.1.8) activity. Previous haplotype analysis combined with genealogical data suggested a common origin of the PBGD gene mutation in the AIP families originating from northern Sweden (Lappland), where the highest prevalence of the disease (1 in 1500) is observed. An AIP family from Lappland consisting of two patients and two unaffected subjects was investigated. The genomic DNA fragments of the PBGD gene were amplified by polymerase chain reaction (PCR) and directly sequenced, and the sequence of the coding region was compared with the normal sequence to identify the mutation. A base substitution, G to A, in exon 10 of the PBGD gene was identified. The mutation changes the codon for Trp^{198} to a stop codon (nonsense mutation) and creates a recognition site for the restriction enzyme *Nhe* I. Screening of 33 Swedish AIP families showed that 15 had this mutation. Genealogical data revealed that 12 of the 15 families were related to the northern family. This finding supports the hypothesis of a "founder effect" of the mutation in the families originating from Lappland. In addition, a method is described for detection of specific sequences in the genome by one-sided PCR using *Taq* polymerase. This method is simple, fast, and economical and can be substituted for hybridization analysis using allele-specific oligonucleotides.**

Acute intermittent porphyria (AIP) is an autosomal dominant disorder caused by a defect in the gene encoding porphobilinogen deaminase [PBGD; porphobilinogen ammonia-lyase (polymerizing), EC 4.3.1.8.], an enzyme in the heme biosynthetic pathway (1). The highest prevalence of the disease, 1 in 1500, has been observed in northern Sweden (Lappland) based on urinary excretion of δ-aminolevulinic acid and porphobilinogen (2).

Most carriers of the AIP gene are clinically latent and the complexity of symptoms during the acute attack often poses a diagnostic problem. Therefore, correct diagnosis is a prerequisite for preventing acute attacks and for proper treatments. Determination of the PBGD activity in erythrocytes is today the most useful method for clinical diagnosis of the disease, even though there is an overlap of the enzyme activity between carriers and normal subjects. Four intragenic restriction fragment length polymorphisms (*Msp* I, *Pst* I, *Bst*NI, and *Apa*LI) in the PBGD gene have been successfully used for haplotype analysis including carrier detection in AIP families (3–5). Several point mutations of the PBGD gene have been identified in phenotypically different AIP patients (6–9). However, none of the mutations so far identified has been found to occur more frequently than any of the others.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Table 1. Sequencing primers used in different sites of the PBGD gene

Primer	Sequence (5′ to 3′)	Strand	Location
JS4	GCTCAGTGTCCTGGTTACTGCAG	+	Exon 2
PBi4	CCATCATGAATCGTAGCACAG	+	Intron 4
PBi4As	CACTGTGCTACGATTCATGATGG	−	Intron 4
PBi6	ATGCTCCTGAGATCTTGAG	+	Intron 6
PBi7	CCAGTGAGTTGGCCAATCGAG	+	Intron 7
JS131*	CCTCGAGCTGCTGTCTCCGTCACT-CTT	−	Intron 9
PBi10	CTTAGTACCCATACAGCCATTC	+	Intron 10
PBi12	GTGATGTCCTCAGGTCTGTGGTC	+	Intron 12
PBi12As	ACCACAGACCTGAGGACATCAC	−	Intron 12
JS129*	TCTCGAGCAGCAACCCAGGCAT-CTG	−	Exon 15

Other sequencing primers used in this study were described elsewhere: JS5 (7), JS6 (15), Phi9 and Phi10 (11).
*Primers designed to create *Xho* I sites at 5′ end.

Haplotype data in conjunction with screening for the previously known mutations (7, 8) indicate a "founder effect" in AIP families originating from Lappland and the presence of several different individual mutations in AIP families from other parts of Sweden (5). The same haplotype segregating with the disease was identified in the Lapplandic families, and none of the previously reported mutations was detected.

To identify the PBGD mutation in the Lapplandic families, direct sequencing of PCR-amplified genomic DNA fragments was performed (10). The greater part of the coding region, exons 2–15, can easily be obtained by PCR since these exons are clustered within two regions of the gene (11).

A nonsense mutation in exon 10 was identified. This mutation can be screened by restriction enzyme digestion of amplified DNA fragments. All AIP families originating from Lappland were found to have the same mutation. In addition, genealogical data suggested that this is the most common mutation in Swedish patients with AIP. Once the mutation was identified, direct carrier detection was possible without access to other biochemical data of those Swedish AIP patients.

A method of screening was developed and tested in the present study. This technique was a modification of the dideoxy DNA sequencing of the PCR-amplified double-stranded templates (10). Only one primer, 5′-end-labeled, is used in this reaction, which is designed to obtain the first chain termination at the mutation site in the presence of a proper dideoxynucleotide so that the mutation can be located by electrophoresis in a denaturing polyacrylamide gel.

Abbreviations: AIP, acute intermittent porphyria; CRIM, crossreacting immunological material; PBGD, porphobilinogen deaminase.

*To whom reprint requests should be addressed at present address: Department of Pediatrics, Yonsei University College of Medicine, C.P.O. Box 8044, Seoul, Korea.

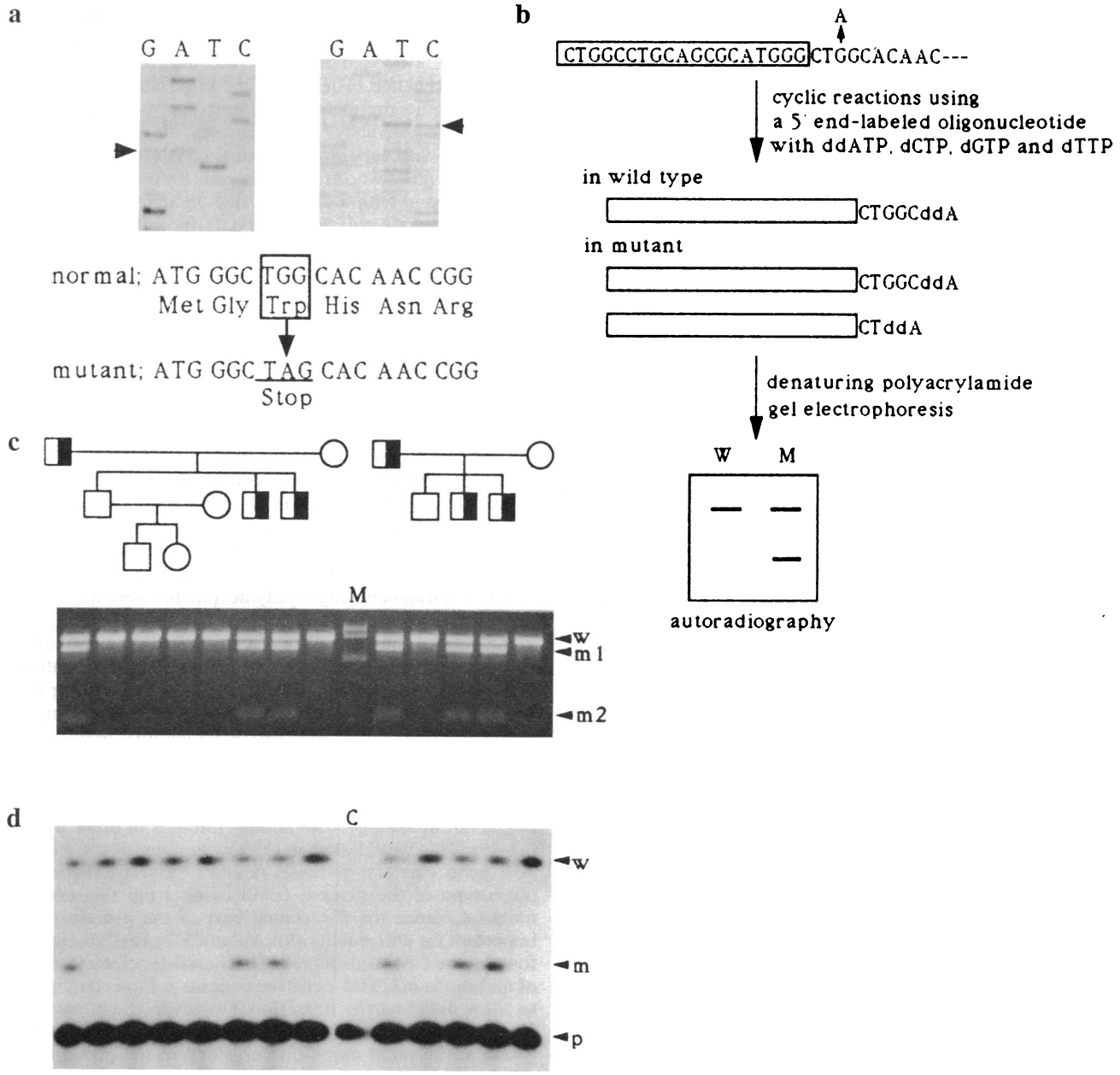

FIG. 1. (*a*) Direct sequencing of amplified genomic DNA shows a base substitution in sense strand (G to A) (*Left*) and antisense strand (C to T) (*Right*). (*b*) Strategy of the method for detection of a single base difference in the genome by using cyclic reactions. The sequence within the square is that of the primer used for detection of the mutation (Lapp10). The primer was designed to obtain the first chain termination by ddATP at the mutation site. ddA, Dideoxyadenosine. In heterozygotes (M), two different fragments terminating at adenine can be expected and then separated according to size in denaturing polyacrylamide gels. Samples homozygous for the wild-type allele (W) give a single band. (*c*) Carrier detection by enzymatic cleavage of PCR products from DNA of two nuclear families originating from northern Sweden. The 0.9-kb genomic DNA fragment was amplified with the primers Phi9 (sense primer in intron 9; ref. 14) and Phi11As (antisense primer in intron 11), 5'-GGC-ATA-TGT-CAC-CAT-GTG-GCT-T-3', with the PCR program including 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C. The amplified product was digested with *Nhe* I (Pharmacia) for 1 hr as recommended by the supplier and the fragments were separated in a 1% agarose gel. Phage λ DNA cleaved with *Hin*dIII and *Eco*RI was used as a length marker (lane M). The bands originating from the wild-type allele (w) and the mutant allele (m1 and m2) are indicated. (*d*) Results of screening for the mutation. The same amount of labeled primer (p) without reaction was run as a control (lane C). The signals from the wild-type allele (w) and the mutant allele (m) are indicated.

PATIENTS AND METHODS

Patients. An AIP family consisting of two patients and two unaffected subjects was investigated. Genealogical data revealed that this family originated from Lappland. The patients showed the crossreacting immunological material (CRIM)-negative phenotype (the ratio of enzyme concentration/enzyme activity being about 1:1) (12), which was measured by ELISA (5).

PCR Amplification and DNA Sequencing of the Genomic DNA. High molecular weight DNA from the patients was prepared as described (4). Genomic DNA of the PBGD gene was amplified into two fragments by PCR under the conditions described by Lee *et al.* (13), with a minor modification of the final Mg^{2+} concentration to 1 mM. Two sets of primers, JS4 with JS131 and Phi9 (14) with JS129, were used to amplify those fragments, which were 2.3 kilobases (kb) and 1.9 kb long, respectively. The 2.3-kb fragment contained the ge-

nomic DNA sequence from exons 2–9, while the 1.9-kb fragment spanned exons 10–15. The PCR-amplified genomic DNA fragments were directly sequenced by cyclic reactions using *Taq* polymerase in the presence of [α-[^{35}S]thio]dATP with a single primer, as described previously (10). The primer sequences used for preparation of templates and sequencing are shown in Table 1. The sequences of coding regions (exons 2–15) from the index cases were compared with the normal sequence (14), and possible mutation sites were excluded when the sequence was identical with the normal sequence.

Screening for the Mutation. A genomic DNA fragment containing the mutation site was amplified by PCR using the primers Phi9 and Phi11As. The PCR program was 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C for 35 cycles under the same conditions as described above. The samples were digested for 30 min with *Nhe* I (Pharmacia) as recommended by the supplier. Restriction enzyme-digested samples were electrophoresed in 1% agarose gels containing ethidium bromide and were observed under UV light. A total of 33 nuclear families with AIP were screened for the mutation.

A screening method using cyclic reactions was developed and tested for identification of the mutation. The principle of the method is shown in Fig. 1*b*. The template for the reaction was amplified by PCR with the primers Phi9 and Phi11As, and purified using Geneclean (Bio 101, La Jolla, CA). The primer (Lapp10) was 5′-end-labeled using [γ-^{32}P]ATP (Amersham) with T4 polynucleotide kinase and purified by Sephadex G-50 spin column chromatography (16). The cyclic reaction was performed in a 10-μl reaction mixture containing 5 ng of PCR-amplified templates, 1 μl of 10× PCR buffer, 1 μl of "A" termination mix (5 μM dCTP, dGTP, dTTP, and ddATP), 5 ng of labeled primer ($\approx 3 \times 10^6$ cpm), and 0.5 unit of *Taq* polymerase (Perkin–Elmer/Cetus). The program of the cyclic reaction was 1 min at 94°C and 1 min at 60°C for 15 cycles. After addition of 4 μl of stop solution (95% formamide/0.01% bromphenol blue/0.01% xylene cyanol), 2.5 μl of reaction sample was heated for 3 min at 92°C and before electrophoresis in denaturing 10% polyacrylamide gel.

RESULTS AND DISCUSSION

Identification of the mutation responsible for AIP in families originating from Lappland is clinically important in Sweden because of its large proportion of AIP patients. Previous haplotype analysis suggested a common origin for the AIP mutation in those families. All patients showed the CRIM-negative phenotype, only one haplotype (*Msp* I/*Pst* I/*Bst*NI/*Apa*LI, 2:1:1:2) segregating with the disease was found, and none of the carriers in the families demonstrated any of the previously known mutations in screening (5).

In the present study, part of the PBGD gene (from exon 2 to exon 15) from two patients was sequenced and the genomic DNA sequence of the gene was obtained (11). The sequence of the coding region was compared with the normal sequence. A point mutation was suspected when two bands with similar intensities appeared at the same level in different lanes on the autoradiogram. The mutation was confirmed when found at the same position in genomic DNA from the two patients.

A base substitution, G to A, was identified in exon 10, resulting in a change of the Trp^{198} (TGG) codon to a stop codon (TAG) (nonsense mutation) (Fig. 1*a*). This mutation also creates a restriction site for the enzyme *Nhe* I and can therefore easily be screened for (Fig. 1*c*). After screening of 33 Swedish AIP families, 15 were found to have this mutation. Genealogical data revealed that 12 of the 15 families were indeed related to the Lapplandic family mentioned above. The remaining 3 families have not been genealogically characterized. All 28 AIP gene carriers and none of 37 noncarriers in the 15 families were found to carry the mutation.

The screening method described in the present study is simple, fast, and economical because only one oligonucleotide probe is needed to detect the normal as well as the mutated sequence. The reaction can be automated using a programmable thermal cycler, and many samples can be tested simultaneously. Furthermore, the reaction can be performed with very small amounts of DNA and radioactive probes. Subjects who are homozygous for mutations will only have one band with a different size compared with the wild type. Two bands will appear in heterozygotes, whereas the wild type will show only a single band. The method is sensitive enough to detect one base difference between the wild type and the mutant by using an appropriate concentration of polyacrylamide gel. It can also be used for identification of other mutations in the genome, and fluorescent labeling can substitute for 5′-end-labeling of a primer with a radioactive isotope.

Southern blot analysis of the AIP families has indicated that a large deletion of the gene is not likely to be an important mechanism causing AIP. Actually, several point mutations within the PBGD gene have been identified in AIP patients with different phenotypes (6–9). However, many of those mutations appear to be unique for a family. Since all the polymorphic sites of the PBGD gene are clustered within a 1.5-kb fragment in the first intron, which spans about 3 kb, restriction fragment length polymorphisms were not useful to differentiate the mutant allele from the normal one. Expression study of the gene after plasmid cloning in prokaryotic cells has been used to identify plasmid clones containing mutated cDNA sequences (8, 11). Direct sequencing of the PCR-amplified double-stranded genomic DNA fragment of the gene could be an alternative to identify heterozygous mutations, as shown in the present study.

The mutation identified in this study is supposed to result in a truncated protein that probably is inactive and easily degraded, in analogy with similar findings in other diseases (17–19). This would explain the low activity of the PBGD in AIP gene carriers in these families. The CRIM-negative phenotype of the disease could result from this nonsense mutation, since the C-terminal part of the protein may be important for antigenicity (9). About 85% of AIP patients are found to be CRIM-negative (12). Therefore, characterization of mutations in CRIM-negative patients is important in order to allow direct carrier detection. However, the mutations so far identified, in different families, are very heterogeneous. In Sweden, the point mutation described in the present study appears to be the major mutation with respect to frequency. The mutation can easily be detected by restriction enzyme digestion of amplified products. This allows a clinical diagnosis within a day without access to other biochemical data for families related to the Lapplandic AIP families.

We thank Professors Jan Lindsten and Bo Lambert for their review of the manuscript. The work was supported by grants from the Swedish Medical Research Council (9108), the Torsten and Ragnar Soderbergs Foundation, and the Gadelius minnes Fond. J.-S.L. received financial support from the Swedish Institute and Karolinska Institute.

1. Kappas, A., Sassa, S., Galbraith, R. A. & Nordmann, Y. (1989) in *The Metabolic Basis of Inherited Disease*, eds. Scriver, C. L., Beaudet, A. L., Sly, W. S. & Valle, D. (McGraw-Hill, New York), Vol. 1, pp. 1320–1329.
2. Wetterberg, L. (1967) *A Neuropsychiatric and Genetical Investigation of Acute Intermittent Porphyria* (Stockholm Univ. Books, Stockholm), p. 63.
3. Llewellyn, D. H., Elder, G. H., Kalsheker, N. A., Marsh, O. W. M., Harrison, P. R., Grandchamp, B., Picat, C., Nordmann, Y., Romeo, P. H. & Goossens, M. (1987) *Lancet* ii, 706–708.
4. Lee, J.-S., Anvret, M., Lindsten, J., Lannfelt, L., Gellerfors,

P., Wetterberg, L., Floderus, Y. & Thunell, S. (1988) *Hum. Genet.* **79,** 379–381.
5. Lee, J.-S., Lundin, G., Lannfelt, L., Forsell, L., Picat, C., Grandchamp, B. & Anvret, M. (1991) *Hum. Genet.* **87,** 484–488.
6. Grandchamp, B., Picat, C., Mignotte, V., Wilson, J. H. P., Te Velde, K., Sankuyl, L., Romeo, P. H., Goossens, M. & Nordmann, Y. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 661–664.
7. Grandchamp, B., Picat, C., de Rooij, F. W. M., Beaumont, C., Deybach, J. C. & Nordmann, Y. (1989) *Nucleic Acids Res.* **17,** 6637–6649.
8. Delfau, M. H., Picat, C., de Rooij, F. W. M., Hamer, K., Bogard, M., Wilson, J. H. P., Deybach, J. C., Nordmann, Y. & Grandchamp, B. (1991) *J. Clin. Invest.* **86,** 1511–1516.
9. Scobie, G. A., Urquhart, A. J., Elder, G. H., Kalsheker, N. A., Llewellyn, D. H., Smyth, J. & Harrison, P. R. (1990) *Hum. Genet.* **85,** 631–634.
10. Lee, J.-S. (1991) *DNA Cell Biol.* **10,** 67–73.
11. Lee, J.-S. (1991) *Molecular Genetic Investigation of the Human Porphobilinogen Deaminase Gene in Acute Intermittent Porphyria.* (Reproprint AB, Stockholm), pp. 1–50.
12. Desnick, R. J., Ostasiewicz, L. T., Tishler, P. A. & Mustajoki, P. (1985) *J. Clin. Invest.* **76,** 865–874.
13. Lee, J.-S., Anvret, M. & Lindsten, J. (1990) *Hum. Genet.* **84,** 241–243.
14. Gu, X. F., Lee, J.-S., Delfau, M. H. & Grandchamp, B. (1991) *Nucleic Acids Res.* **19,** 1966.
15. Raich, N., Romeo, P. H., Dubart, A., Beaupain, D., Cohen-Solal, M. & Goossens, M. (1986) *Nucleic Acids Res.* **14,** 5955–5968.
16. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
17. Adams, J. G. & Coleman, M. B. (1990) *Semin. Hematol.* **27,** 229–238.
18. Baserga, S. & Benz, E. J. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2056–2060.
19. Cutting, G. R., Kasch, L. M., Rosenstein, B. L., Tsui, L.-C., Kazazian, H. H. & Antonarakis, S. E. (1990) *N. Engl. J. Med.* **323,** 1685–1688.