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Molecular Heterogeneity of Acute Intermittent Porphyria: Identification of Four Additional Mutations Resulting in the CRIM-negative Subtype of the Disease

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Summary

Four mutations of the porphobilinogen (PBG) deaminase gene that result in cross-reacting immunological material (CRIM)–negative forms of acute intermittent porphyria (AIP) have been identified by in vitro amplification of cDNA from patients and by cloning of the amplified products in a bacterial expression vector. One mutation is a single base deletion which causes a frameshift and which is expected to result in the synthesis of a truncated protein. Two other mutations consist of single base substitutions and lead to amino acid changes. The fourth mutation is a single base substitution producing an aberrant splicing and resulting in an mRNA which would encode a protein missing three amino acids. DNAs from 16 unrelated CRIM-negative AIP patients were screened for the presence of these four mutations, by hybridization with oligonucleotides specific for each of the mutations, but none of the four mutations was identified in additional patients. The results indicate that mutations responsible for CRIM-negative AIP are highly heterogenous.

Introduction

Acute intermittent porphyria (AIP) is an autosomal dominant disease defined by a partial deficiency of porphobilinogen (PBG) deaminase (hydroxymethylbilane synthase; E.C.4.3.1.8), the third enzyme of the heme biosynthetic pathway. The clinical manifestations are characterized by acute attacks of neurological dysfunctions with abdominal pain, hypertension, tachycardia, and peripheral neuropathy (Kappas et al. 1989).

The molecular heterogeneity of the mutations producing AIP was suggested by studies of the PBG deaminase gene locus which demonstrated that AIP mutations were associated with different restriction haplotypes (Lewellyn et al. 1987; Lee et al. 1988). Moreover, several phenotypic subtypes at the protein level have been described. In the more common forms of AIP, in which the PBG deaminase deficiency occurs in all cell types, different investigators have documented the existence of two subgroups of patients, based on the measurement of cross-reacting immunological material (CRIM) in erythrocytes; these subgroups are referred to as *CRIM-negative* and *CRIM-positive* phenotypes (Desnick et al. 1985; de Rooij et al. 1987; Kappas et al. 1989).

More recently, the genetic basis of AIP mutations has begun to be clarified. The mutations producing the CRIM-positive subtype of AIP have been shown to be heterogenous. Of nine patients studied, one had a point mutation in exon 12 of the PBG deaminase gene which resulted in a defective splicing and which led to the formation of a shortened mRNA (Grandchamp et al. 1989*a*), four had a transition G→A in exon 10 leading to an arginine-to-glutamine substitution at position 149 of the protein, and two had a G→A transition leading to an arginine-to-glutamine substitution at position 155 of the protein (Delfau et al. 1990). Similarly, elsewhere we have described two

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different mutations accounting for a subgroup of AIP families in which the expression of the enzymatic defect is restricted to nonerythropoietic cells (Grandchamp et al. 1989*b*, 1989*c*). Until now, only one mutation resulting in a CRIM-negative form of AIP has been reported (Scobie et al. 1990).

Here we report the determination of four additional mutations which result in the CRIM-negative form of the disease. The amplification products of PBG deaminase cDNA from four patients were cloned and sequenced. Subsequently, the genomic DNAs from 16 other unrelated patients were screened for the presence of these mutations. None of the four mutations was found in any of these patients, indicating the great heterogeneity of this subtype of AIP.

Patients and Methods

Patients

Twenty patients with CRIM-negative forms of AIP were studied. The diagnosis was established on the basis of typical clinical symptoms accompanied by increased urinary excretion of porphobilinogen and aminolevulinic acid and by normal fecal porphyrin excretion. Diminished activity of PBG deaminase was also observed in erythrocytes of all patients.

CRIM Determination

The CRIM phenotype was determined using erythrocytes from one or two members of 20 families with AIP as previously described (de Rooij et al. 1987).

RNA and DNA Isolation

Total cellular RNA was isolated from lymphoblastoid cells (Grandchamp et al. 1989*a*), and DNA was prepared from peripheral blood as previously described (Saiki et al. 1988).

Amplification of cDNA

One microgram of total cellular RNA was reverse transcribed. The resulting cDNA was amplified in vitro as previously reported (Delfau et al. 1990) using modified primers to introduce a *Nco*I restriction site in the 5′ primer (PHE1 *Nco*) and a *Hin*dIII site in the 3′ primer (PHE15 *Hin*d).

Cloning and Sequencing of the Amplified cDNA

The amplified cDNAs were phenol extracted, ethanol precipitated, digested with *Nco*I and *Hin*dIII, and purified by chromatography on Sephacryl S200 by using spin columns (IBF, Clichy, France). The purified fragments were ligated into the corresponding sites of the plasmid expression vector PKK 233 (Pharmacia, Uppsala) and were subcloned in *Escherichia coli* DH5.

Cloned cDNAs were sequenced using a sequenase sequencing kit (USB Corp., Cleveland) according to the instructions of the manufacturer. Two of the sequencing primers hybridized to the vector, one on each side of the insertion site (PKK1 and PKK2), and four other primers hybridized to either the coding (PHEI *Nco*, PHE6, or PHE10) or the noncoding (PHE15 *Hin*d) strands of the cDNA (table 1).

Bacterial Expression System

For each patient, 20 to 30 bacterial clones carrying a recombinant plasmid with a human PBG deaminase cDNA insert were tested for the expression of PBG deaminase activity. In brief, bacteria were grown overnight in 5 ml of LB medium, harvested by centrifugation, washed with Tris-HCl buffer (10-mmol/liter, pH8.0), and disrupted by sonication in the same buffer. The PBG deaminase activity was determined as previously described (Delfau et al. 1990).

Lysates of *E. coli* containing the recombinant plasmid PKK 233 with either normal or mutated PBG deaminase cDNAs were analyzed by western blot using specific antibodies against human PBG deaminase

Table 1

Oligonucleotides Used in Present Study

Primer	Nucleotide Sequence
Within exons:	
PHE1NCO	GAGCCATGGCTGGTAACGGCAATGC
PHE6	ATGCCCTGGAGAAGAATGA
PHE10.....	GGAAGCTTGACGAGCAGCAGGA
PHE11.....	AGCATACATGCATTTCTCA
PHE15HIND ...	CCCAAGCTTCTGTGCCCCACAAACCA
PHE15.....	CAGCAGCAACCCAGGCATCT
Within introns:	
PHI9	CCGACACTGTGGTCCTTAGCAA
PHI9AS.....	ACCCGTCACTCTTCTAAAAAGG
PHI10.....	TGAACGTATATCGCTTTCACAC
Within plasmid PKK 233:	
PKK1.....	TGGAATTGTGAGCGGATAAC
PKK2.....	CTTCTCTCATCCGCCAAAAC
ASO:	
446N.....	CTCCCTGCGAAGAGCAG
446M	CTCCCTGCAAAGAGCAG
734N.....	GACTCTGCTTCGCTGCA
734M	GACTCTGCGTCGCTGCA
900M	ACCATCCAGTCCCTGCC
IVS10-1M	GGCCCTACATGCCC

as previously described (Delfau et al. 1990). In some instances, immunoprecipitation of the proteins reacting with the antibodies was carried out prior to the western blot analysis, in an attempt to concentrate small amounts of immunoreacting material.

Direct Sequencing of Amplified Products

The DNA fragment between intron 9 (PHI9) and exon 11 (PHE11) was amplified by PCR, yielding a 457-bp fragment. One percent of the product was reamplified in order to generate single-stranded DNA by asymmetric PCR, as previously described (Dicker et al. 1989), using nested primers which hybridized to genomic sequences in exon 10 (PHE10) and intron 10 (PHI10), allowing the amplification of a 200-bp segment. Each primer was independently used as a limiting primer, in order to generate single-stranded DNA corresponding to one or the other strand of the genomic fragment. The PCR fragment was washed three times with 2 ml of H_2O in a Centricon 100 membrane filtration (Amicon, Danvers, MA). Of 50 µl collected, 7 µl were used for sequencing using the same primer as that which was limiting during the PCR.

Amplification of Genomic DNA

DNA amplifications were carried out according to the method of Saiki et al. (1988), in the presence of *Taq* polymerase Biolabs, Beverly, MA). With a thermocycler (Hybaid, Teddington, United Kingdom), two fragments were amplified independently. A 1,200-bp fragment between exon 6 (PHE6) and intron 9 (PHI-9AS) was obtained after 30 cycles, as follows: at 95°C for 15 s, at 48°C for 30 s, at 53°C for 1 s, and at 72°C for 1 min 30 s. A 1,900-bp fragment between intron 9 (PHI9) and exon 15 (PHE15) was amplified by 30 cycles, as follows: at 95°C for 15 s, at 54°C for 30 s, at 59°C for 1 s, and at 72°C for 2 min 30 s.

Results

We recently devised a new strategy to determine the mutations responsible for AIP in CRIM-positive patients (Delfau et al. 1990). The totality of the coding sequence of cDNA was amplified in vitro and was cloned in a prokaryotic expression vector. Because patients are heterozygous for the PBG deaminase–deficient allele, two types of clones are expected to occur: those with the normal cDNA, which express human PBG deaminase activity, and those with the mutated cDNA.

To extend our study to patients with a CRIM-negative phenotype, four patients were studied. For each patient, PBG deaminase activity was determined from 20 to 30 bacterial clones. In all the cases, 50% to 60% of the clones failed to express the human PBG deaminase activity and were considered likely to con-

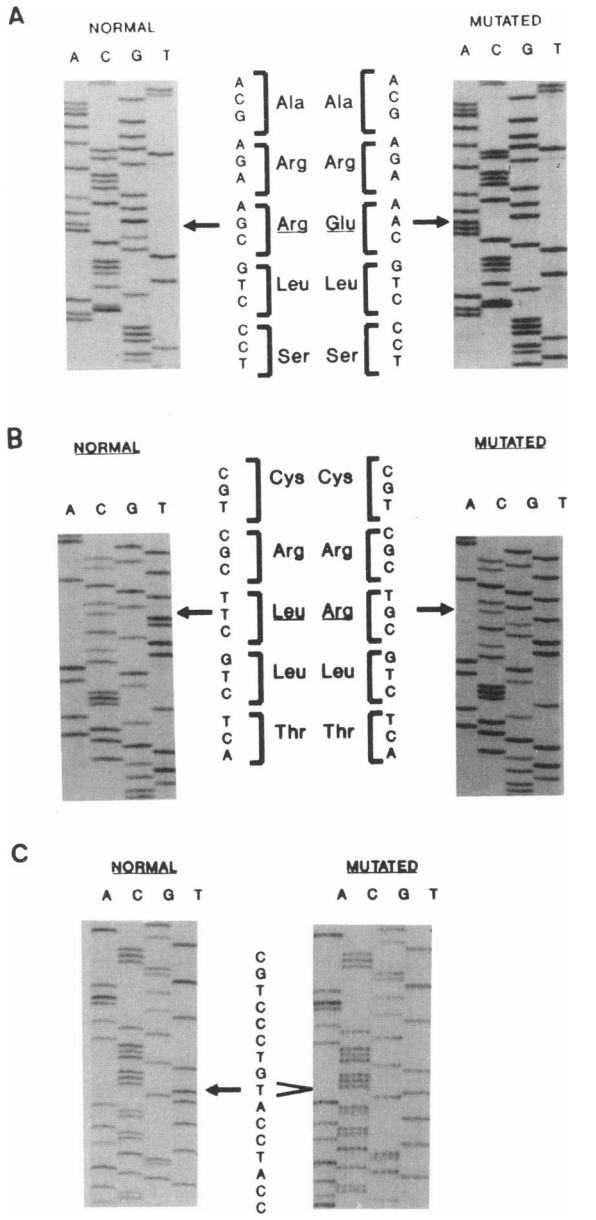

Figure 1 Sequences of different single base mutations. Partial sequences of the cDNA corresponding to normal and mutated alleles from the patients bearing the mutations 446 (*A*), 734 (*B*), and 900 (*C*) are shown. Arrows indicate the mutated bases. Amino acid changes are indicated.

tain the mutated cDNA. Three or four of these clones were sequenced for each patient. Mutations were confirmed on at least two independent clones. Some clones contained "sporadic" base changes which were attributed to errors introduced by the *Taq* polymerase during the PCR, as previously reported (Tindall et al. 1988).

Four different mutations were found:

1. A G→A transition at position 446 of the cDNA from the translation initiation codon ATG, occurring in exon 9, which leads to an arginine-to-glutamine substitution in the theoretical protein (fig. 1*A*).
2. A T→G transversion at position 734, occurring in exon 12, leading to a leucine-to-arginine substitution (fig. 1*B*).
3. A single base deletion of a thymine at position 900, in exon 14, resulting in a stop codon located 15 codons downstream from the deletion (fig. 1*C*).
4. A deletion of the last 9 bp of exon 10 (data not shown), resulting in a new *Bam*HI restriction site in the coding sequence. This mutation was directly confirmed by *Bam*HI digestion of amplified cDNA (data not shown). In order to determine the genomic mutation responsible for this abnormality, we decided to directly sequence an amplification product from genomic DNA containing the donor splice site of exon 10. After a first amplification of a 457-bp fragment between intron 9 and exon 11, a 200-bp fragment containing the junction segment was asymmetrically reamplified (fig. 2*A*). Direct sequencing of the amplification product showed a G→T substitution of the last base of the exon 10 (IVS 10–1) (fig. 2*B*).

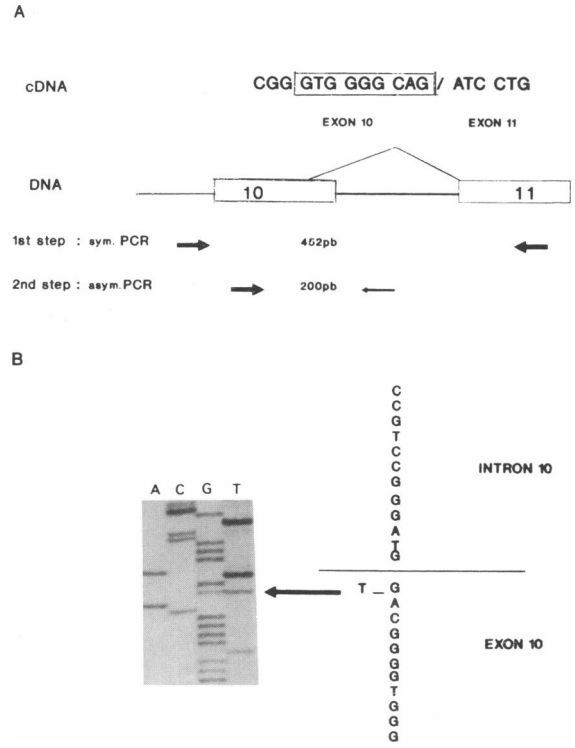

Figure 2 Genomic abnormality responsible for splicing defect. *A*, Asymmetric amplification of exon-intron 10 junction. A first amplification between intron 9 and exon 11 produced a 452-bp fragment which was reamplified in a second asymmetric PCR step, in order to generate a 200-base single-strand fragment. *B*, Direct sequencing of PCR products. The PCR products were washed three times using Centricon 100, and 7 µl of template was used for sequencing. The mutation is clearly revealed on the same line as the normal base.

The relative abundance of the abnormally spliced mRNA was determined by quantitative PCR after reverse transcription. A 122-bp cDNA fragment was amplified from mRNA of the patient. After PCR, the products from the normal mRNA and from the defective mRNA were separated by gel electrophoresis. As shown in figure 3, the two bands were of equal intensity, and the two products contained the same amount of radioactivity (data not shown), suggesting that the stability of the abnormally spliced mRNA was not diminished.

To confirm the presence of the different mutations at the genomic level and to study their prevalence

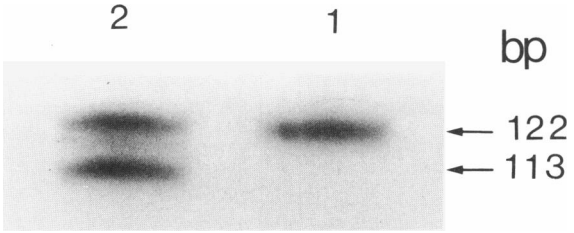

Figure 3 Quantitative analysis of abnormally spliced mRNA resulting from ISV10–1 mutation. PBG deaminase mRNAs from the patient with ISV10–1 mutation and from a normal control were reverse transcribed and amplified using oligonucleotides PHE10 and PHE11. Thirty cycles were performed as follows: at 94°C for 20 s, at 47°C for 30 s, at 55°C for 1 s, and at 72°C for 20 s. Five microcuries of ^{32}P-dCTP were added in each reaction in presence of 50 µmol each dNTP/liter. After PCR, 10-µl aliquots of each reaction were run on a 6% denaturing acrylamide gel. The DNA was fixed with 8% acetic acid, the gel was dried, and autoradiography was performed. The arrows indicate the sizes of the amplified fragments. Lane 1, Normal control. Lane 2, Patient with IVS10–1 mutation.

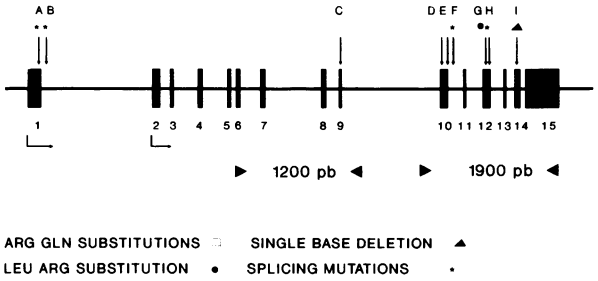

Figure 4 Schematic representation of PBG deaminase gene, with location of different mutations and position of primers used for amplification. The different mutations are indicated on a representation of the PBG deaminase gene. Exons are marked by dark boxes and numbered. Mutations C, F, G, I, were found in the present study; mutation A is from Grandchamp et al. (1989*c*); mutation b is from Grandchamp et al. (1989*b*); mutations D and E are from Delfau et al. (1990); and mutation H is from Grandchamp et al. (1989*a*).

among AIP patients, two fragments of PBG deaminase gene were amplified (fig. 4). The first fragment, between exon 6 and intron 9, contained the 446 mutation (C). The second, between intron 9 and exon 15, included the three other mutations (F, G, and I) identified in the present study. Mutations were revealed by hybridization of the corresponding amplified fragment with mutation-specific oligonucleotides. All the oligonucleotides, except that used for detecting the IVS10–1 mutation, were 17 bases long and centered on the position of the mutation. Their orientations were chosen in order to obtain the most destabilizing mismatch (Ikuta et al. 1987). The oligonucleotide used for the IVS10–1 mutation screening was 14 bases long, therefore excluding an intragenic DNA polymorphism (G or T; author's unpublished data) situated 6 bases upstream from the mutation. Despite its

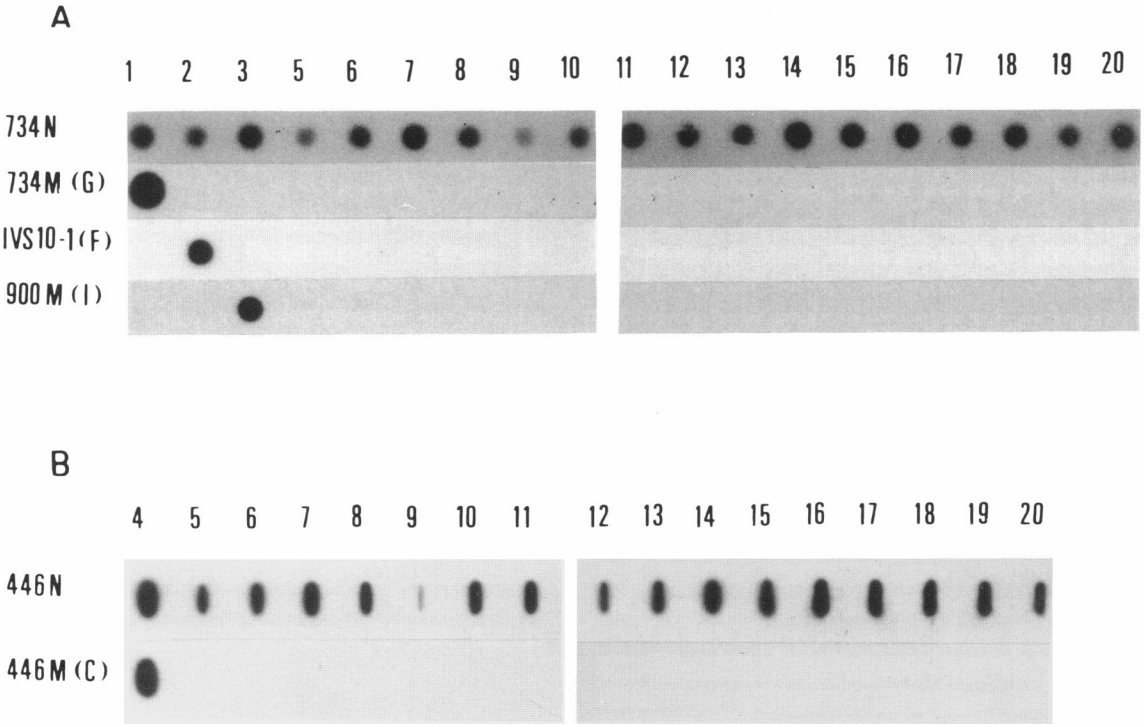

Figure 5 Dot or slot blot hybridization of amplified genomic DNA with mutation specific oligonucleotides. Twenty patients numbered 1–20, including the four patients bearing the mutations determined in the present study (patients 1–4), were screened for the presence of the different mutations, using mutation-specific oligonucleotides. *A,* Amplified fragments corresponding to sequence of 1,900 bp from intron 9 to exon 15 were applied to a zeta-probe membrane in quadruplicate and then were hybridized to four probes: 734N and 734M, corresponding, respectively to oligonucleotides matching either the normal (N) or the mutated (M) sequence centered on mutation 734 (G in fig. 1); and ISV10–1M and 900M, corresponding, respectively, to the oligonucleotides matching the sequence centered on either the mutation ISV10–1 or the mutation 900 (F and I, respectively, in fig. 1). *B,* 1,200-bp amplified fragments between exon 6 and intron 9, screened for mutation 446 (C on fig. 1), as described above, with oligonucleotides matching either the normal (446N) or the mutated sequence (446M).

small length, this oligonucleotide recognizes a single sequence in the amplified fragment, as assessed by computer analysis. The results of these studies confirmed the data obtained with the cDNA fragments (fig. 5).

Detection of the abnormal protein was attempted using specific immunoprecipitation and western blot analysis from lysates of *Escherichia coli* strains containing the mutated cDNAs, using specific antibodies against normal human PBG deaminase (de Rooij et al. 1987). Although the proteins synthetized in this bacterial system from a normal cDNA and from abnormal cDNAs corresponding to patients with a CRIM-positive phenotype were readily detectable (Delfau et al. 1990), similar experiments failed to show the presence of any protein product from the abnormal cDNAs characterized in the present study.

Sixteen additional patients with CRIM-negative AIP were screened for the presence of each of the four mutations. None of the mutations identified in these studies was found in additional patients (fig. *5A* and *B*).

Discussion

Most of the carriers with the AIP trait remain asymptomatic. Early detection of these individuals is important in the prevention of acute attacks, which are often precipitated by additional factors such as drugs, alcohol, and caloric deprivation (Kappas et al. 1989). Because asymptomatic carriers do not consistently excrete excess amounts of heme precursors, the usual screening method in AIP families is based on the determination of PBG deaminase activity in erythrocytes. Enzymatic activity levels, however, are affected by additional factors such as erythrocyte age and the presence of other diseases, resulting in some overlap between values for normal individuals and those for AIP patients (Grandchamp and Nordmann 1988; Kappas et al. 1989). These limitations prompted us to undertake the study of the mutations responsible for AIP at the DNA level.

The strategy that we have used in the present study to determine the mutations responsible for CRIM-negative forms of AIP relied on cloning PCR-amplified cDNAs in a bacterial expression vector. Starting from mRNA allows the amplification of the entire coding sequence in one step and focuses the search for mutations on approximately one 10th of the PBG deaminase gene. The success of this approach supposes that the mutated gene is transcribed and that the resulting mRNA is present and stable in the cells. All CRIM-positive and many CRIM-negative mutations could be detected in this way, including most splicing mutations and amino acid substitutions which result in protein instability.

Since patients with AIP are heterozygous for the mutation, the use of an expression vector allows an easy distinction between clones derived from the mutant mRNA and those derived from the normal mRNA, based on the determination of human enzymatic activity present in *E. coli,* and therefore it facilitates the interpretation of the sequencing data. The fact that 50% to 60% of the clones obtained from each patient failed to express the human PBG deaminase activity suggests that the mutated alleles were expressed at a level similar to that of the normal ones.

Several different types of mutations resulting in the CRIM-negative form of AIP were identified: In two cases, a single base change resulted in amino acid substitutions. One of these two mutations resulted from a G→A transition in a dinucleotide CG. Such mutations are thought to arise from oxidative deamination of methylated cytosines and have previously been detected with high frequency in several genetic diseases (Youssoufian et al. 1988).

The third mutation was a point deletion. This is an infrequent type of mutation but has been previously described (Weatherall et al. 1989).

The fourth mutation resulted in abnormal splicing of intron 10. Analysis of a genomic fragment containing the donor site of exon 10 showed that the patient was heterozygous for a point mutation G→T at the last position of exon 10 (table 2). Such a mutation has been previously described in a CRIM-positive form of AIP, at the last base of exon 12, but resulted in the absence of exon 12 in the mRNA (Grandchamp et al. 1989*a*). Here a cryptic site is activated, three codons upstream of the normal site, yielding a stable abnormal mRNA. The functional consequence of the mutation described here may appear surprising, since the mutated 5′ splice site still contains a six-nucleotide match with the consensus sequence. It is interesting,

Table 2

Study of 5′ Splice Site Sequence of Intron 10

Consensus	CAG/GTAAGT A G
Normal site	CAG/GTAGGG
Mutated site	CAT/GTAGGG
Cryptic site	CGG/GTGGGG

however, to note that the cryptic site also has a six-nucleotide match with the consensus sequence, in particular the G/GT highly conserved sequence (table 2). Pairing of U1 SnRNA to the mutated splicing site would create a C/A mismatch, more destabilizing than U/G matching which exists when the cryptic site is used.

No mutated protein produced from mRNA containing these mutations is detectable immunologically either in erythrocytes or in recombinant *E. coli* strains bearing the mutated cDNAs. This is not surprising for the single base deletion which leads to a truncated protein, but the single base substitutions and the splicing mutation affect neither the reading frame nor the sequences used for translational control. A single amino acid substitution responsible for an enzymatic defect with an unstable protein has been previously described (De Verneuil et al. 1986). Moreover, it has been demonstrated that PBG deaminase activity requires the covalent binding of a cofactor, a dipyrrolmethane, and it was suggested that this cofactor may protect the enzyme from degradation (Umanoff et al. 1988). The mutations determined here could prevent binding of the cofactor, leading to an unstable protein. These mutations, however, do not change the cysteine residue 261 of the human protein which is highly conserved through evolution. Cysteine 242 located at a homologous position in the *E. coli* enzyme has indeed been shown to covalently bind the cofactor (Miller et al. 1988). The absence of immunoreactive protein is compatible with the possibility that the enzyme is present but that its three-dimensional structure is sufficiently altered to prevent the recognition by antibodies.

Both previous work at the protein level (de Rooij et al. 1987) and RFLP studies (Lewellyn et al. 1987; Lee et al. 1988) indicated that acute intermittent porphyria is a heterogenous disease at the molecular level and that point mutations rather than large deletions are likely to account for most of the cases. Heterogeneity of CRIM-positive forms of AIP has been previously reported (Delfau et al. 1990). Here we show that in the CRIM-negative form, a phenotypically homogeneous subtype of AIP, many different mutations are responsible for the disease. The molecular heterogeneity of CRIM-negative forms seems to be more important than that of CRIM-positive forms, since two CRIM-positive mutations accounted for the disease in six of eight studied patients. On the contrary, each of the CRIM-negative mutations described here has a frequency lower than 1/20. The large number of different molecular lesions responsible for AIP may suggest that mutations at the PBG deaminase locus have been well tolerated in the human organism through evolution. This is perhaps not surprising, since heterozygous gene carriers infrequently express the disease phenotype. A larger epidemiological study is necessary to precisely define the frequency of the different mutations.

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References

Delfau MH, Picat C, de Rooij FWM, Hamer K, Bogard M, Wilson WHP, Deybach JC, et al (1990) Two different point mutations G to A in exon 10 of the porphobilinogen deaminase gene are responsible for acute intermittent porphyria. J Clin Invest 86:1511–1516

de Rooij FWM, Hamer CM, Wilson JPH (1987) Heterogeneity in acute intermittent porphyria: biochemical for family related variation in the mutant enzyme porphobilinogen deaminase. In: Topi GC, D'Alesandro Gandolfo L (eds) Proceedings of the International Meeting on Porphyrins and Porphyrias. Ist Dermatol S Gallicano 13:175–180

Desnick RJ, Ostasiewicz LT, Tishler PA, Mustajoki P (1985) Acute intermittent porphyria: characterization of a novel mutation in the structural gene for porphobilinogen deaminase. J Clin Invest 76:865–874

De Verneuil H, Grandchamp B, Beaumont C, Picat C, Nordmann Y (1986) Uroporphyrinogen decarboxylase structural mutant (Gly281→Glu) in a case of porphyria. Science 234:732–734

Dicker AD, Volkenandt M, Adamo A, Barreda C, Bertino J (1989) Sequence analysis of human gene for drug resistance: a rapid method for manual and automated direct sequencing of products generated by the polymerase chain reaction. Biotechniques 7:830–836

Grandchamp B, Nordmann Y (1988) Enzymes of the heme biosynthetic pathway: recent advances in molecular genetics. Sem Hematol 25:303–310

Grandchamp B, Picat C, de Rooij F, Beaumont C, Deybach JC, Nordmann Y (1989*a*) A point mutation G/A in exon 12 of the PBG deaminase gene results in exon skipping and is responsible for acute intermittent porphyria. Nucleic Acids Res 17:6637–6649

Grandchamp B, Picat C, Kaupinen R, Mignotte V, Peltonen L, Mustajoki P, Romeo PH, et al (1989*b*) Molecular analysis of acute intermittent porphyria in a Finnish family

with normal erythrocyte porphobilinogen deaminase. Eur J Clin Invest 19:415–418

Grandchamp B, Picat C, Mignotte V, Wilson JPH, Te Velde K, Sandkuyl L, Romeo PH, et al (1989*c*) Tissue specific splicing mutation in acute intermittent porphyria. Proc Natl Acad Sci USA 86:661–664

Ikuta S, Takagi K, Wallace RB, Itakura K (1987) Dissociation kinetics of 19 base paired oligonucleotide-DNA duplexes containing different single mismatched base pairs. Nucleic Acids Res 15:797–811

Kappas A, Sassa S, Galbraith RA, Nordmann Y (1989) The porphyrias. In: Stanbury JB, Wyngaarden JB, Fredrickson DS, Goldstein JL, Brown MS (ed) The metabolic basis of inherited disease, 6th ed. McGraw-Hill, New York, pp 1305–1365

Lee JS, Anvret M, Lindsten J, Lannfelt L, Gellerfors P, Wetterberg L, Floderus Y, et al (1988) DNA polymorphisms within the porphobilinogen deaminase gene in two Swedish families with acute intermittent porphyria. Hum Genet 79:379–381

Lewellyn DH, Elder GH, Kalsheker NA, Marsh OW, Harrisson PR, Grandchamp B, Picat C, et al (1987) DNA polymorphism of human porphobilinogen deaminase gene in acute intermittent porphyria. Lancet 2:706–708

Miller AD, Hart GJ, Packman LC, Battersby AR (1988) Evidence that the pyrromethane cofactor of hydroxylmethylbilane synthase (porphobilinogen deaminase) is bound to the protein through the sulphur atom of cystein-242. Biochem J 254:915–918

Saiki KR, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, et al (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491

Scobie GA, Llewellyn DH, Urquhart AJ, Smyth SJ, Kalsheker NA, Harrison PR, Elder GH (1990) Acute intermittent porphyria caused by a C→T mutation that produces a stop codon in the porphobilinogen deaminase gene. Hum Genet 85:631–634

Tindall KR, Kunkel TA (1988) Fidelity of DNA synthesis by the *Thermus aquaticus* DNA polymerase. Biochemistry 27:6008–6013

Umanoff H, Russel CS, Cosloy SD (1988) Availability of porphobilinogen controls appearance of porphobilinogen deaminase activity in *Escherichia coli* K-12. J Bacteriol 170:4969–4971

Weatherall DJ, Clegg JB, Higgs DR, Wood WG (1989) The hemoglobinopathies. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolis basis of inherited disease, 6th ed. McGraw-Hill, New York, pp 2281–2339

Youssoufian H, Antonarakis SE, Bell W, Griffin AM, Kazazian HH (1988) Nonsense and missense mutations in hemophilia A: estimate of the relative mutation rate at CG dinucleotides. Am J Hum Genet 42:718–725