Journal of Bioenergetics and Biomembranes, Vol. 27, No. 2, 1995

Porphobilinogen Deaminase Gene Structure and Molecular Defects

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Received January 18, 1995; accepted February 1, 1995

Porphobilinogen deaminase (PBGD) is the third enzyme of the heme biosynthetic pathway. The half-normal activity of human PBGD causes acute intermittent porphyria (AIP), an autosomal dominant inherited disease. Two PBGD isoforms, one ubiquitous and one erythroid specific, are encoded by a single gene localized to chromosomal region 11q24.1–11q24.2. The 10-kb PBGD gene comprises 15 exons and two distinct promoters initiate the ubiquitous and the erythroid transcripts by alternative splicing. In AIP, diagnosis of asymptomatic heterozygotes is crucial to prevent occurrence of life-threatening acute attacks by avoiding known precipitating factors. Difficulties with the biochemical diagnosis could be overcome by the ability to identify the PBGD gene defects in AIP patients. Mutational analysis of the PBGD gene or the use of intragenic polymorphisms offer accurate identification of the gene carriers. To date, 58 mutations and 10 polymorphisms have been reported at the PBGD locus. The great heterogeneity of the mutations in AIP patients requires appropriate screening and diagnostic strategies to identify gene defects in AIP families.

KEY WORDS: Porphobilinogen deaminase; acute intermittent porphyria; mutations; polymorphisms; gene

INTRODUCTION

Porphobilinogen deaminase (PBGD, EC 4.3.1.8, also referred to as hydroxymethylbilane synthase or uropophyrinogen I synthase), the third enzyme of the heme biosynthetic pathway, catalyzes in the cytosol, the stepwise deamination and head-to-tail condensation of four molecules of porphobilinogen (PBG), resulting in the formation of the unstable linear tetrapyrrole 1-hydroxymethylbilane. Uroporphyrinogen III cosynthase then rapidly converts the intermediate into uroporphyrinogen III, a key intermediate in the biosynthesis of heme, chlorophylls, and corrins. PBGD occurs ubiquitously, and primary structures of the deaminases indicate that the enzyme is structurally conserved (around 50% amino acid sequence identity) throughout the species from bacterias to humans, including plants. Site-directed mutagenesis of the crystallized *E. coli* deaminase has underlined the role of invariant arginine residues, both for dipyrromethane cofactor binding and PBG polymerization reaction (Jordan and Woodcock, 1991; Lander *et al.*, 1991; Louie *et al.*, 1992; Jordan *et al.*, in this issue).

In humans, acute intermittent porphyria (AIP) is a dominantly inherited disease caused by the partial deficiency in the activity of PBGD (Strand *et al.*, 1970; Miyagi *et al.*, 1971; Meyer *et al.*, 1972).

MOLECULAR BIOLOGY OF THE HUMAN PBGD GENE

The human red cell PBGD has been isolated to homogeneity (Anderson and Desnick, 1980). PBGD has been shown to be a monomer of about 37 kilodaltons (kd). Multiple molecular forms of PBGD have been identified by isoelectrofocusing in human

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0145-479X/95/0400-0197$07.00/0

tissues; the electrophoretic patterns were different in erythrocytes from that observed in other tissues, and they were thought to correspond to enzyme-free-substrate and enzyme-substrate intermediates containing one to four PBG subunits (Meisler and Carter, 1980; Anderson *et al.*, 1981).

Chromosomal Localization

The gene locus encoding PBGD has been assigned to chromosome 11 through study of human/mouse somatic cell hybrids (Meisler *et al.*, 1980). Subsequently the gene assignment for human PBGD was made to chromosome regions 11q13–11qter then 11q23–11qter using somatic cell hybridization and immunological, electrophoretic, and cytogenetic techniques (Wang *et al.*, 1981; Meisler *et al.*, 1981). PBGD dosage effect in trisomy of 11qter supported assignment to the region 11q23.2–11qter (de Verneuil *et al.*, 1982). By *in situ* hybridization and by gene dosage studies in patients with monosomy or trisomy of 11qter, Namba *et al.*, (1991) refined the assignment to 11q24.1–11q24.2.

PBGD Isoforms and Corresponding cDNAs

Two distinct molecular forms of the PBGD enzyme have been identified in human cells: an ubiquitous isoform of 44 kd and an erythroid-specific isoform of 42 kd (Grandchamp *et al.*, 1987). Analysis of cell-free translation products directed by mRNAs from human erythropoietic spleen and from human liver demonstrates that the two PBGD isoforms were encoded by distinct mRNAs, and that the expression of the erythroid-specific mRNA was exclusive to erythroid cells.

cDNAs encoding the ubiquitous and erythroid specific isoforms have been cloned and sequenced (Raich *et al.*, 1986; Grandchamp *et al.*, 1987). The erythroid cDNA has an open reading frame of 1,038 base pairs (bp), coding for 344 amino acids. The ubiquitous cDNA exhibits a 1,320 bp stretch of almost perfect identity, but with different 5′ extremity: an additional in-frame AUG codon was found 52 bp upstream from the initiating codon of the nonerythropoietic cDNA. Thus, an additional 17 amino acid residues occurred at the NH_2-terminus of the ubiquitous PBGD isoform, which accounted for its higher molecular mass (361 amino acids). Southern analysis of human genomic restriction fragments indicated the existence of a single gene encoding PBG deaminase.

Organization of the Human PBGD Gene

A genomic clone has been isolated and promoter regions partially sequenced (Chretien *et al.*, 1988). The chromosomal gene has been shown to be split into 15 exons spread over 10 kb of DNA. The two distinct mRNAs were produced through alternative splicing of two primary transcripts arising from two promoters as depicted in Fig. 1A.

The upstream promoter is active in all tissues; the ubiquitous or housekeeping transcript initiated by this promoter is encoded by exons 1 and 3 through 15. The other promoter, located 3 kb downstream in intron 1, is active only in erythroid cells, and initiates a transcript encoded by exons 2 through 15. When introduced into nonerythroid cells, the erythroid promoter was completely inactive and deletion of the housekeeping promoter did not result in its activation, proving its strict erythroid specificity. The erythroid promoter displays some structural homology with the β-globin gene promoter, including a CAAC motif, a CAAT-like box, two GATA-1, and one NFE-2 binding sites, suggesting that some common trans-acting factors may coregulate the transcription of these genes during erythroid development (Chretien *et al.*, 1988; Mignotte *et al.*, 1989a, b; Frampton *et al.*, 1990). Although the erythroid promoter of human PBGD lacks a TATA box, transcription is initiated at a single nucleotide. An initiator element located around the initiation site is essential for accurate selection of initiation sites during erythroid differentiation (Beaupain *et al.*, 1990).

The complete genomic sequence, including the 5′ regulatory, 3′ untranslated, and intronic regions, has recently been published (Yoo *et al.*, 1993). The 15 exons ranged in size from 39 to 438 bp, and the 14 introns ranged from 87 to 2,913 bp. The gene has six *Alu* repetitive elements, one of the J and five of the Sa subfamilies. Additional putative housekeeping and erythroid regulatory elements are provided, but evaluation is required for their functional significance.

Polymorphisms in the PBGD Gene

To date, 10 intragenic polymorphisms have been mapped in the PBGD gene. Their locations, restriction sites, and alterations are indicated in Fig. 1B. Each polymorphic site gives rise to a codominant two-allele polymorphism and for all of them the distribution of homozygotes and heterozygotes was in Hardy–Weinberg equilibrium. A recent review by Astrin and Desnick (1994) gives compiled data on

the reported frequencies of these polymorphisms in various populations. The first four polymorphisms (*Msp*I, *Pst*I, *Bst*NI, and *Apa*LI) have been mapped within the first intron (Lee and Anvret, 1987; Llewellyn *et al.*, 1987b; Lee *et al.*, 1990b, 1991; Scobie *et al.*, 1990). The complete genomic sequence of the PBGD gene allows one to determine the precise locations, which are not initially known (1345G/A, 1500T/C, 2377C/A, and 2479A/G; Yoo *et al.*, 1993). In addition, conditions for the polymerase chain reaction (PCR) based amplification and analysis of most of the 10 polymorphisms are available (Lee *et al.*, 1991; Picat *et al.*, 1991; Gu *et al.*, 1991; Yoo *et al.*, 1993).

The reported allele frequencies of the four restriction fragment length polymorphisms (RFLPs) in the first intron range from 0.37 to 0.63 (Astrin and Desnick, 1994) with the exception of the Finnish population (Kauppinen *et al.*, 1990).

By haplotyping, strong linkage disequilibrium between the intron 1 RFLPs was found in all populations studied (Llewellyn *et al.*, 1987a, b; Lee *et al.*, 1988; Kauppinen *et al.*, 1990; Lee *et al.*, 1990b; Yoo *et al.*, 1993). Moreover, the intron 3 *Bsm*AI RFLP was also in strong linkage disequilibrium with the intron 1 RFLPs (Yoo *et al.*, 1993). Thus, it appears that to use in conjunction *Pst*1, *Msp*1, *Bst*N1 in intron 1 and *Bsm*A1 in intron 3 would be of little value for haplotype linkage studies. In contrast, the *Hinf*1 exon 10 RFLP was not in disequilibrium with either the intron 1 or 3 RFLPs (Yoo *et al.*, 1993). It has to be noticed

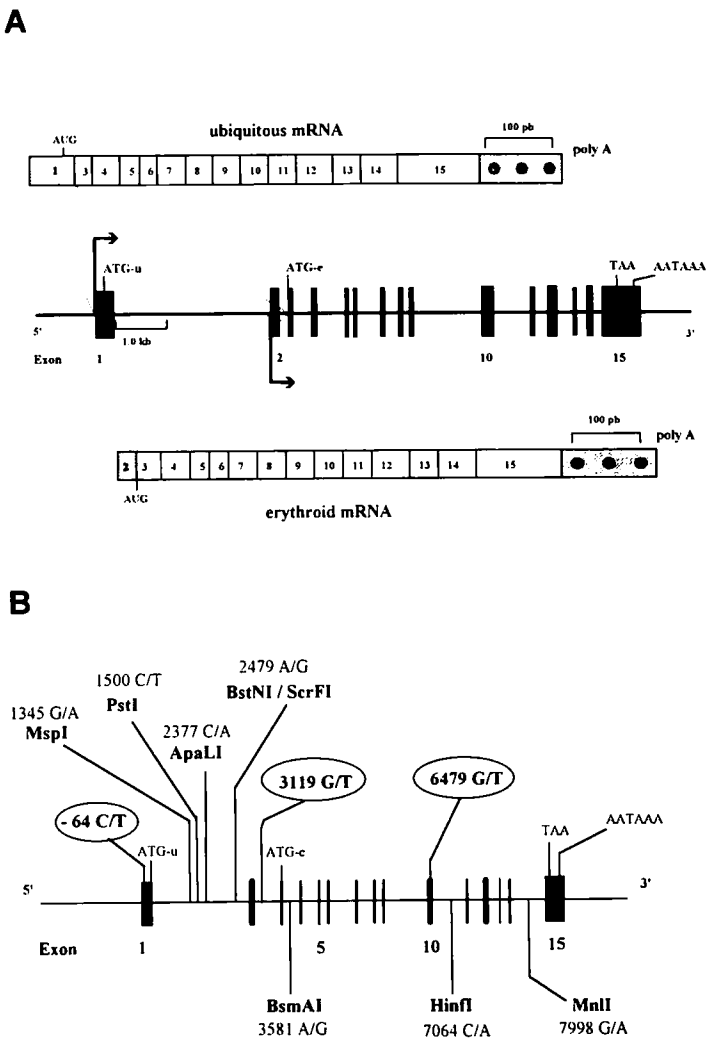

Fig. 1. Gene structure of the human PBGD gene, alternative splicing (A), and polymorphisms (B).

Table I. Reported Mutations in the PBGD Gene of Patients with AIP

	Position	Mutation	Sequence modification	R. Enz.	References
Exon 1	3	ATG → ATA	Initiation codon: translation impairment	Nla III	Chen *et al.* (1994)
	33	GCG → GCT	SD	Hph I	Grandchamp *et al.* (1989a)
Intron 1	33 + 1	gtg → atg	SD	—	Grandchamp *et al.* (1989c)
Exon 3	77	CGC → CAC	R26H	—	Llewellyn *et al.* (1993a)
Exon 4	91	CGCT → CACT	A31T	—	Gu *et al.* (1994)
	100	CAG → AAG	Q34K	—	Mgone *et al.* (1992)
	125	TTGT → TAG	L42X	—	Puy *et al.* (in press)
Exon 5	163	GCT → TCT	A55S	—	Gu *et al.* (1994)
	174	Del C	Frameshift (stop → cod + 40)	—	Gu *et al.* (1994)
	182	Ins G	Frameshift (stop → cod + 5)	—	Gu *et al.* (1994)
Intron 5	210 + 1	gta → ata	SD (Del exon 5)	Mac III	Gu *et al.* (1994)
Exon 6	218–219	Del AG	Frameshift (stop → cod + 9)	—	Gu *et al.* (1994)
Exon 7	277	GTT → TTT	V93F	—	Chen *et al.* (1994)
	287	TCC → TTC	S96F	—	de Rooij (personal communication)
	295	GAC → CAC	D99H	—	de Rooij (personal communication)
	331	CGGA → CAGA	G111R	Nla IV	Gu *et al.* (1993b)
Intron 7	345 − 1	cag → caa	SA (Del exon 8)	Bsa JI	Schreiber *et al.* (1994)
Exon 8	346	CGG → TGG	R116W	—	Lee *et al.* (1992); Gu *et al.* (1993a)
	347	CGG → CAG	R116Q	—	Mgone *et al.* (1994)
Exon 9	446	CGA → CAA	R149Q	Mbo II	Delfau *et al.* (1991)
	446	CGA → CTA	R149L	Mbo II	Gu *et al.* (1994)
	463	CAG → TAG	Q155X	—	Scbbie *et al.* (1990a)
	470	Ins A	Frameshift (stop → cod + 37)	—	Schreiber *et al.* (1994)
Intron 9	499 − 1	cag → caa	SA (Del exon 10)	—	Lundin *et al.* (1993, 1994)
Exon 10	499	CGG → TGG	R167W	—	Gu *et al.* (1992); Llewellyn *et al.* (1992)
	500	CGG → CAG	R167Q	—	Delfau *et al.* (1990); Llewellyn *et al.* (1992); Mgone *et al.* (1992)
	517	CGG → TGG	R173W	Msp I	Lee, J. S. (1991); Lundin *et al.* (1993)
	518	CGG → CAG	R173Q	Msp I	Delfau *et al.* (1990); Kauppinen *et al.* (1992)
	530	CTG → CGC	L177R	—	Mgone *et al.* (1992)
	593	TGG → TAG	W198X	Nhe I	Lee and Anvret (1991)
	601	CGG → TGG	R201W	Msp I	Lundin *et al.* (1994); Chen et al. (1994)
	604	Del G	Frameshift (stop → cod + 53)	Nci I	Schreiber *et al.* (1994)
	610	CAG → TAG	Q204X	—	Mgone *et al.* (1994)
	612	CAG → CAT	SD (Del 9 bp exon 19)	Bam HI	Delfau *et al.* (1991)
Exon 11	625	GAG → AAG	E209K	Mnl I	Grandchamp *et al.* (1993)
Intron 11	652 − 3	cag → gag	SA (Del exon 12)	—	Llewellyn *et al.* (1993b)
Exon 12	667	GAA → AAA	E223K	—	Gu *et al.* (1994)
	715–716	Del CA	Frameshift (stop → cod + 9)	—	Puy *et al.* (in press)
	730–731	Del CT	Frameshift (stop → cod + 6)	—	Mgone *et al.* (1993); Gu *et al.* (1994)
	734	CTT → CGT	L245R	—	Delfau *et al.* (1991)
	739	TGC → CGT	C247R	—	Mgone *et al.* (1993)
	740	TGC → TTC	C247F	Fnu 4HI	Chen *et al.* (1994)
	742	Ins 8 bp (TTCGCTGC)	Frameshift (stop → cod + 10)	—	Gu *et al.* (1994)
	748	GAA → AAA	E250K	—	Gu *et al.* (1994)
	754	GCC → ACC	A252T	—	Mgone *et al.* (1993)
	755	GCC → GTC	A252V	Eco NI	Mgone *et al.* (1993)
	766	CAC → AAC	H256N	—	Mgone *et al.* (1992)
	771	CTG → CTA	SD (Del exon 12)	Bst NI	Grandchamp *et al.* (1989b)
	771	CTG → CTC	SD (Del exon 12)	—	Daimon *et al.* (1993)
Intron 12	771 + 1	gta → ata	SD (Del exon 12)	—	Puy *et al.* (in press)
Exon 13	806	ACA → ATA	T2691	—	Mgone *et al.* (1994)
	820	GGG → AGG	G274R	—	Mgone *et al* (1994)
Intron 13	825 + 1	gta → ata	SD (Del exon 13)	—	Llewellyn *et al.* (1993b)
Exon 14	848	TGG →TAG	W283X	—	Mgone *et al.* (1994); Chen *et al.* (1994)

Table I. Continued

	Position	Mutation	Sequence modification	R. Enz.	References
	900	Del T	Frameshift (stop → cod + 15)	—	Delfau *et al.* (1991)
Intron 14	921 + 1	gta → ata	SD (Del exon 14)	Rsa I	Gu *et al.* (1993b)
Exon 15	913	Ins C	Frameshift (stop → cod + 1)	Nla III	Puy *et al.* (in press)
	1062	Ins C	Frameshift (stop → cod + 4)	Msp I	Daimon *et al.* (1994)

R. Enz.: restriction enzyme (mutation specific).
SD: splice donor site mutation.
SA: splice acceptor site mutation.
Del: deletion; ins: insertion.
(stop → cod + X): stop codon occurs X codons downstream.
Mutated bases are in bold.
Mutations occurring at CpG dinucleotide are underlined.

that the −64 C/T polymorphism in exon 1 could be detected by *Apa*1 restriction analysis using a 5′ primer with a mutated sequence to amplify the region (Picat *et al.*, 1991). In summary, 7 out of 10 polymorphisms might be used in conjunction for linkage analysis.

MOLECULAR GENETICS OF ACUTE INTERMITTENT PORPHYRIA

Acute Intermittent Porphyria

Acute intermittent porphyria, the most common type of acute hepatic porphyrias, is an inherited autosomal dominant disease with incomplete penetrance. Enzymatic studies have shown that a mean 50% decrease of PBGD activity is found in the cells from AIP patients as well as from asymptomatic carriers of the gene defect (Kappas *et al.*, 1989). However, the majority of gene carriers (90%) remain otherwise biochemically and clinically normal throughout their lives. Clinical expression of the disease occurs almost after puberty and more commonly in women than in men. Environmental or acquired factors (e.g., caloric deprivation, drugs, alcohol, steroid hormones) may precipitate acute attacks. These acute attacks are characterized clinically by neurological dysfunction which may affect the peripheral, autonomic, or central nervous systems, associated with abdominal pain, hypertension, and tachycardia. Clinical manifestations are accompanied by abnormal excretion of heme precursors, δ-aminolevulinic acid (ALA) and PBG, in urine (see Moore, 1993, for review). AIP has been reported in many races (Elder, 1993), but Lapland, Scandinavia, and United Kingdom populations are believed to have the highest incidence of AIP (Waldenstrom, 1956; Lee *et al.*, 1991). However, epidemiological data on AIP has mainly been based on urinary ALA and PBG determination, which greatly underestimates the prevalence of AIP since up to 80% of latent carriers have normal levels.

Molecular Defects in the Human PBGD Gene

Molecular heterogeneity of the mutations responsible for AIP was suggested by immunological studies of the defective enzyme. Several laboratories reported the existence of cross-reacting immunological material (CRIM)-negative and CRIM-positive mutations of PBG deaminase in patients with AIP (Desnick *et al.*, 1985). The former was found in around 85% of the affected individuals. In addition, some families with AIP, as judged by strict clinical and biochemical criteria, displayed no evidence of PBG deaminase deficiency in erythrocytes (Mustajoki, 1981; Wilson *et al.*, 1986). This variant AIP is the result of PBG deaminase deficiency in nonerythropoietic tissues. Molecular heterogeneity of the mutations responsible for AIP was also suggested by studies of the PBG deaminase gene locus, which showed that AIP mutations were associated with different restriction haplotypes (Lee and Anvret, 1987).

As of January 1995, 58 mutations responsible for AIP have been identified in the PBGD gene and are specified in Table I. This represents 22 additional mutations as compared to the latest published mutation update (Astrin and Desnick, 1994).

The variant AIP (normal erythrocyte PBGD) resulted from three mutations; two of them impaired exon 1 splicing and were the first mutations identified in the PBGD gene (Grandchamp *et al.*, 1989a, 1989c). The third one was recently described as a G-to-A

transition in the initiation codon which precluded translation of the ubiquitous mRNA but not the erythroid one (Chen *et al.*, 1994). From the 55 remaining mutations that cause classic AIP, 80% (44/55) are point mutations.

From all punctual mutations a G is involved in 66% (31/47), a C in 26% (12/47), a T in 8% (4/47), and never a A. This disequilibrium is only partially explained: among the mutations that modify a CpG dinucleotide, only 11 are due to the deamination of a methylated cytosine resulting, in the coding strand, in a G-to-A transition in 7 cases and a C-to-T transition in 4 cases (Table I). The repartition of each nucleotide (20 to 29%) is homogeneous along the coding sequence. The high frequency of mutated G (11/12) in consensus splice sites account for only a part of the disequilibrium.

The types of mutations are non-sense mutations 11% (5/47), missense mutations (amino acid substitutions) 62% (29/47), and splicing mutations 26% (12/47). The 11 frameshifts result from 6 small deletions, 4 small insertions and one 8-bp insertion.

Historically AIP, and subsequently PBGD mutations, were classified according to the CRIM status of the patients. It rapidly appeared that in the CRIM-positive phenotype, the heterogeneity of the mutations was limited, which in turn would facilitate the gene defect identification and subsequent family studies: on 18 CRIM-positive patients (Delfau *et al.*, 1990; Gu *et al.*, 1992; Kauppinen *et al.*, 1992; Llewelyn *et al.*, 1992) the R167Q mutation was found in 9, the R173Q in 4, and the R167W in 1. It is noteworthy that all occurred in exon 10, and that the only reported cases of homozygous AIP patients (Dutch brother and sister) had alleles with the R167Q and R167W mutations (Llewellyn *et al.*, 1992), and in a presumed homozygous case (Beukeveld *et al.*, 1990) the father had the R167Q and the mother the R173Q (Picat *et al.*, 1990). By homology to the *E. coli* enzyme, these arginines in human PBGD appear to be involved in the substrate binding (Jordan and Woodcock, 1991; Louie *et al.*, 1992). Finally, in 43 unrelated patients (French and Dutch origin) the A31T mutation in exon 4 was identified in 5 patients that were all CRIM-positive (Gu *et al.*, 1994).

On the contrary, in the CRIM-negative phenotype, which represent over 80% of AIP patients, there is increasing evidence for a high degree of allelic heterogeneity in the PBGD mutations except in Sweden and Holland where a high prevalence of some mutations has been reported: In Lapland (North Sweden) the W198X mutation was found in 12 of 15 families, but these were related to each other, suggesting a common founder (Lee and Anvret, 1991). In Holland, a R116W was found in 15 of 49 patients; 10 of the 15 families were from eastern Holland, but

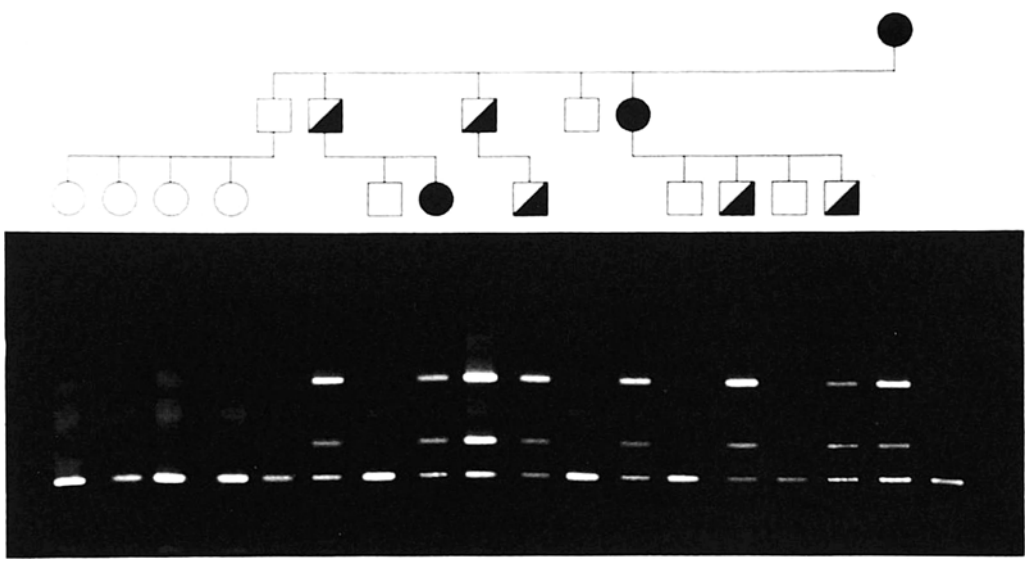

Fig. 2. DGGE analysis for the detection of gene carriers in an AIP family. Normal individuals showed the normal homodimerus band, whereas a pattern of three bands was observed in patients and asymptomatic gene carriers (author's personal communication).

no common relatives could be found from up to three or four generation analysis (Gu *et al.*, 1993a).

In order to compare data from different laboratories, the CRIM status assignment should obey strict biochemical criteria that are not always fulfilled (e.g., same tissues studied, antibodies specifications). It was the case for the exon 12 splicing defect (771G → A, Grandchamp *et al.*, 1989b) that led to a CRIM-positive status on lymphoblasts which was not further confirmed on erythrocytes (Grandchamp, B., personal communication). In addition, the recently published PBGD mutations were not associated with their respective CRIM status (Mgone *et al.*, 1994; Chen *et al.*, 1994). However, evaluation of the CRIM status remains important as a part of biochemical studies on protein structure/function or phenotype/genotype relationships.

Molecular Diagnosis in AIP Families

In AIP, usual biochemical tests have well-known limitations (Lamon *et al.*, 1979; Bonaitie-Pellie *et al.* 1984) and the molecular heterogeneity of the observed mutations in AIP complicates the development of standard procedures. The detection of gene carriers in a family with a known mutation should be performed using appropriate molecular techniques to identify specific mutations (Grompe, 1993). The analysis procedure has to be chosen depending on the type and location of the mutation. Screening methods such as denaturating gradient gel electrophoresis (DGGE) or single-strand conformation polymorphism (SSCP) seem useful for the rapid detection of unknown mutations in newly diagnosed AIP family (Kauppinen, 1992; Bourgeois *et al.*, 1992; Gu *et al.*, 1994). However, the sensitivity of these screening methods needs to be evaluated. In addition they do not preclude sequencing of the DNA sample in order to find the mutation responsible for the abnormal migration and in some cases to evaluate functional consequences or to eliminate a possible polymorphism. As illustrated in Fig. 2, we used DGGE to identify asymptomatic heterozygotes in one AIP family (variant form 33 + 1G → A).

Finally in rare cases for which PBGD activity and molecular investigations failed to detect the genetic defect, linkage analysis to track the AIP gene in affected families are still useful. Indeed, this

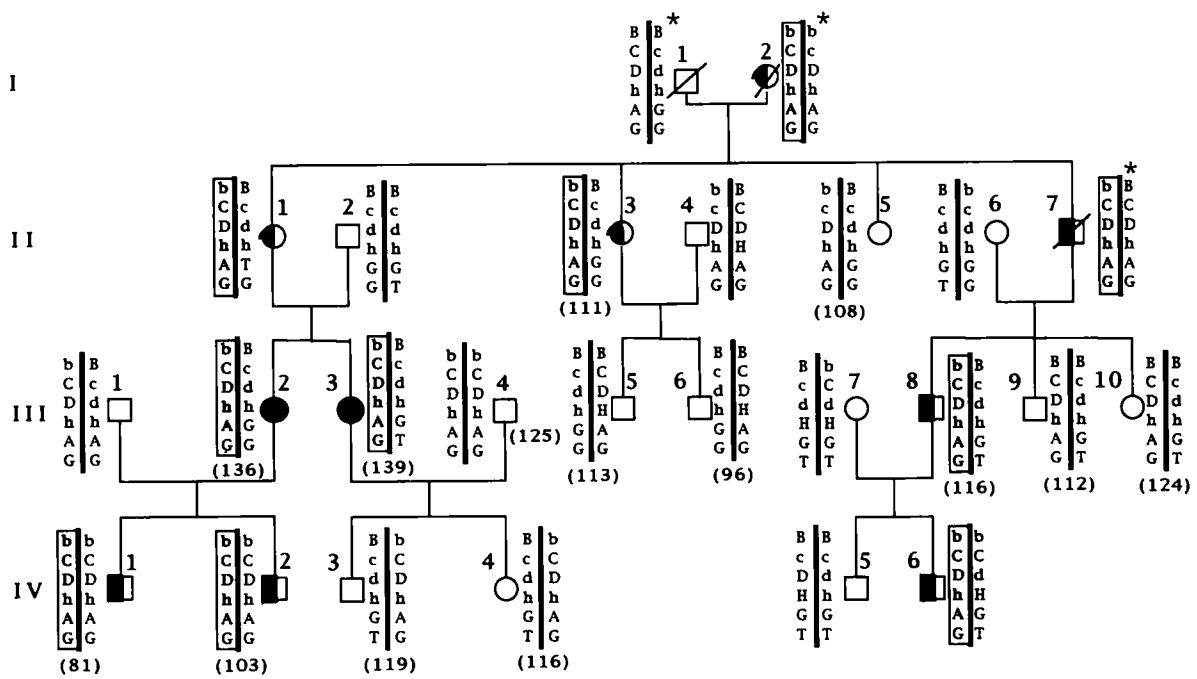

Fig. 3. Haplotyping in an AIP family with nonerythroid PBGD deficiency. In brackets, PBGD activity in pM/mg of hemoglobin × hour at 37°C. Polymorphism analysis was showed for *Apa*I (B/b) (−64 C/T exon 1, see text and Fig. 1B), *Apa*LI (C/c), *Bst*NI (D/d), *Hinf*I (H/h), A/G (intron 3), and G/T (exon 10). Black symbols indicate patients with overt AIP; half-shaded symbols, asymptomatic carriers; dashed symbols, deceased; (*) predicted haplotype. The framed haplotype bCDhAG was disease-associated (author's personal communication).

approach is confined to the families of patients who have both informative genotypes and sufficient unequivocally affected living relatives, to enable linkage to be established. In the pedegree shown in Fig. 3, we used six polymorphisms in the PBGD gene in an AIP family with two index cases; diagnosis was made on the strictest clinical and biochemical criteria; the erythrocyte PBGD was in the normal range in all members of the pedegree, and until now we failed to identify the genetic defect at the PBGD locus. In this family, linkage analysis allows one to identify 6 asymptomatic gene carriers and 7 normal individuals.

Available identified mutations, accurate and sensitive mutation screening methods, or the use of intragenic polymorphisms, allow asymptomatic carriers and normal subjects in AIP families to be identified with greater certainty than can be achieved by enzymatic methods. Structure/function relationship studies of the mutant enzymes will benefit from the availability of PBGD three-dimensional structure (*E. coli*, Louie *et al.*, 1992). Now one of the challenges will be to try to connect the various clinical expressions in AIP to the disease-causing mutations.

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