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Influence of Age and Gender on the Clinical Expression of Acute Intermittent Porphyria Based on Molecular Study of Porphobilinogen Deaminase Gene Among Swiss Patients

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Abstract

Background: Acute intermittent porphyria (AIP) is an inherited disorder in the heme biosynthetic pathway caused by a partial deficiency of porphobilinogen (PBG) deaminase. Clinically, AIP is characterized as acute neurovisceral attacks that are often precipitated by exogenous factors such as drugs, hormones, and alcohol. An early detection of mutation carriers is essential for prevention of acute attacks by avoiding precipitating factors. This study was aimed at analyzing genetic defects causing AIP among Swiss families to further investigate aspects concerning the clinical expression of the disease.
Materials and Methods: The PBGD gene of index patients from 21 Swiss AIP families was systematically analyzed by denaturing gradient gel electrophoresis of polymerase chain reaction (PCR) amplified DNA fragments and direct sequencing.
Results: Five new mutations $insA^{503}$, del L170, T190I, P241S, and R321H, as well as three known mutations (R26H, R173Q and W283X) were detected. Twelve of the 21 index patients (57%) carried the prevalent mutation W283X previously found among the Swiss AIP population. Family-specific mutations were then screened among relatives of the index patients. Among the 107 studied individuals, 58 carried a PBGD gene mutation—30 were overt AIP patients and 28 were asymptomatic carriers. The apparent rate of overt disease in the study cohort was 52%, which is significantly higher than the previously reported penetrance of 10–20%. To further examine the clinical expression of AIP, the cumulative life-time risk was calculated among 58 mutation-positive individuals after stratifying for age. The result shows a linear increase of the percentage of the symptomatic patients with age, reaching up to 75% among carriers aged over 60. Moreover, statistical analysis of the gender distribution among patients and asymptomatic carriers indicated that the disease was more frequently expressed among females than males (Fisher's exact test two sided, $p = 0.001$).
Conclusions: This comprehensive search for genetic defects in the PBGD gene confirmed the existence of a prevalent mutation W283X among Swiss AIP patients, as well as a number of family-private mutations. Genetic analysis laid a groundwork for further studies such as the effects of gender and age on the clinical expression of AIP.

Introduction

Acute intermittent porphyria (AIP) is an inborn error of heme biosynthesis resulting from the half-normal activity of porphobilinogen deaminase (EC 4.3.1.8; PBGD). This enzyme, also known as hydroxymethylbilane synthase, catalyzes the head-to-tail condensation of four molecules of porphobilinogen (PBG) to form preuroporphyrinogen. Clinically, AIP manifests as acute attacks of abdominal pain, often associated with further gastrointestinal and neurologic dysfunctions. AIP is an autosomal dominant disorder with incomplete penetrance, as illustrated by family studies showing that roughly 50% of the relatives of index patients have a defective enzyme (1). In a retrospective study, however, only 15–20% of the affected individuals were symptomatic during a mean observation period of 10 years (2). To date, no information is available to answer the question as to what percentage of mutation carriers will become symptomatic.

Both endogenous and exogenous elements are involved in the clinical expression of the disease. Because women are much more prone to clinically overt disease than men, and children before puberty are rarely symptomatic, female sex hormones are incriminated as an endogenous component. Furthermore, the acute attacks are often triggered by exogenous factors such as drugs, alcohol, fasting, intercurrent infections, and mental stress. For this reason, attacks are thought to be preventable to a great

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extent. After early detection of latent mutation carriers, affected individuals can be counseled to avoid these triggers (1). Systematic testing of family members of newly diagnosed AIP patients may be an efficient tool to serve this purpose. Before molecular analysis of the PBGD gene became available, the diagnosis of mutation carriers usually relied on the enzymatic activity measurement. However, the enzyme assay has a sensitivity of approximately 90% and, owing to the overlap between high pathologic and low normal values, its diagnostic reliability is limited (3).

Since the publication of the cDNA sequence of the PBGD gene 10 years ago, knowledge of the genetic defects underlying AIP has greatly increased (4,5). More than 130 different mutations in the PBGD gene have so far been described among AIP patients of different races and ethnic groups. Most of these mutations are family specific, reflecting a high molecular heterogeneity of the defects causing AIP (6). Sixty percent of all known mutations are located in exons 10, 12, and 14 of the PBGD gene. Despite the generally heterogeneous nature of the PBGD gene mutations, prevalent mutations R116W, R173W, and W198X have been identified in Dutch, Swedish, and Nova Scotian (Canadian) AIP populations, respectively, and have been attributed to "founder effects" in these populations (7–9). Recently, we detected mutation W283X, with a prevalence of 72%, among 18 unrelated Swiss AIP families (10).

Despite this progress, the acquired molecular-genetic information has yet to be implemented into clinical investigations. In this study, we report the molecular analysis of a large cohort of Swiss AIP patients and their relatives, including 107 individuals from 21 unrelated families. With the results of the mutation analysis, we investigated two aspects of clinical importance in AIP epidemiology. First, the gender distribution amongst symptomatic and asymptomatic AIPs was reanalyzed based on the data of DNA diagnosis instead of enzymatic or biochemical analyses. Second, the cumulative lifetime risk for developing overt AIP was calculated among all carriers of a PBGD gene mutation in this cohort. The surprisingly high penetrance found in our study population will be discussed.

Material and Methods

Patients

A total of 107 subjects from 21 unrelated families mostly from the German-speaking part of Switzerland took part in this study (some family members live in southern Germany close to the Swiss border). The size of the families were as large as 21 members spanning over three generations. Among the 21 families, 30 individuals suffered clinically from AIP. Their diagnoses of AIP were established either in Kantonsspital Basel or in Stadtspital Triemli in Zürich.

Clinical and Laboratory Diagnoses

The diagnosis of AIP in patients was based on the typical clinical symptoms and laboratory measurements of decreased PBG deaminase activity and elevated concentrations of urinary δ-aminolevulinic acid (ALA) and PBG (11,12). The clinical onset of AIP among patients was at a mean age of 29.1 years for 23 females (ranging from 13–54 years) and 35.0 years for 7 males (ranging from 18–52 years). Peripheral blood samples were collected from all 107 participants of this study with informed consent.

Mutation Analysis

Mutation analysis of the PBGD gene was performed according to the previously described method (6,10). Genomic DNA was extracted from peripheral blood using a QIAamp blood kit (Qiagen, Hilden, Germany). The 15 coding exons and their flanking intronic sequences were analyzed in the index patient of each family. Briefly, the target sequences of the PBGD gene were amplified by polymerase chain reaction (PCR) using an Expand High Fidelity PCR System kit purchased from Roche Diagnostics Ltd. (Basel, Switzerland) and primers described in our previous work (10). The PCR products were then subjected to denaturing gradient gel electrophoresis (DGGE) analysis in a D Gene System (BioRad, Hercules, C, USA) (10).

DNA fragments showing an abnormal DGGE pattern were purified with the Qiaquick PCR purification kit (Qiagen) and directly sequenced using the Thermo Sequenase radiolabeled dye terminator (α-^{33}P ddNTP) cycle sequencing kit (Amersham Life Science, Cleveland, OH, USA) and previously described primers (10) to identify the exact mutations. Once the mutation of an index patient was known, the rest of the family were screened specifically for the mutation either by DGGE analysis or by restriction enzyme analysis (the restriction enzymes were purchased from Roche Diagnostics Ltd.).

Statistical Analysis

The statistical analysis was performed using the computer software Stata 6.0 (13). The study design was approved by the ethical committees from both Kantonsspital Basel and Stadtspital Triemli.

Results

Mutation Analysis in the Index Patients

Thirty individuals in this study have experienced at least one episode of acute abdominal pain accompanied by additional clinical symptoms characteristic of AIP. During or immediately after the attacks, urinary ALA and PBG levels were increased to greater than 2.5-fold of the normal value (data not shown). Twenty-eight of the 30 patients also showed an approximately 50% decrease of PBG deaminase activity in the red blood cells. The enzyme activity of the remaining 2 patients were within the reference range of a normal population (Table 1 and Fig. 3).

Table 1. Clinical data and mutations identified in the PBGD gene from the AIP affected individuals

	Clinical Data					PBGD Gene Mutation		
Family	Symptomatic	Asymptomatic	Sex	Age	PBG Deaminase Activity (Normal Range: 75–150 U)	Novel	Known	Sequence Modification
1	Index patient		F	58	45		$G^{77}\rightarrow A$	R26H[a]
		Son	M	35	51		$G^{77}\rightarrow A$	R26H
2	Index patient		F	50	50		$G^{77}\rightarrow A$	R26H
		Daughter	F	25	48		$G^{77}\rightarrow A$	R26H
3	Index patient		F	44	52	**ins A^{503}**		**Frameshift**
	Sister		F	49	65	**ins A^{503}**		**Frameshift**
	Aunt (paternal)		F	78	73	**ins A^{503}**		**Frameshift**
		Cousin (paternal)	M	49	101	**ins A^{503}**		**Frameshift**
		Aunt (paternal)	F	77	66	**ins A^{503}**		**Frameshift**
		Brother	M	52	52	**ins A^{503}**		**Frameshift**
		Niece (fraternal)	F	23	21	**ins A^{503}**		**Frameshift**
		Nephew (fraternal)	M	14	32	**ins A^{503}**		**Frameshift**
		Cousin (paternal)	M	42	53	**ins A^{503}**		**Frameshift**
		Cousin (paternal)	F	44	50	**ins A^{503}**		**Frameshift**
		Niece (fraternal)	F	23	46	**ins A^{503}**		**Frameshift**
		Nephew (fraternal)	M	25	48	**ins A^{503}**		**Frameshift**
4	Index patient		F	37	69	**del $CTC^{508\text{-}10}$**		**del L170**
		Father	M	68	59	**del $CTC^{508\text{-}10}$**		**del L170**
		Sister	F	34	60	**del $CTC^{508\text{-}10}$**		**del L170**
5	Index patient		F	18	53		$G^{518}\rightarrow A$	R173Q[b]
		Father	M	50	67		$G^{518}\rightarrow A$	R173Q
		Grandfather (paternal)	M	75	47		$G^{518}\rightarrow A$	R173Q
		Uncle (paternal)	M	55	49		$G^{518}\rightarrow A$	R173Q
6	Index patient		M	54	44		$G^{518}\rightarrow A$	R173Q
		Daughter	F	21	46		$G^{518}\rightarrow A$	R173Q
		Son	M	17	42		$G^{518}\rightarrow A$	R173Q
7	Index patient		F	71	44	$C^{569}\rightarrow T$		**T190I**
8	Index patient		F	33	57	$C^{721}\rightarrow T$		**P241S**
	Brother		M	35	60	$C^{721}\rightarrow T$		**P241S**
	Mother		F	73	58	$C^{721}\rightarrow T$		**P241S**
		Brother	M	36	54	$C^{721}\rightarrow T$		**P241S**
		Brother	M	40	57	$C^{721}\rightarrow T$		**P241S**
9	Index patient		F	45	51	$G^{849}\rightarrow A$		W283X[c]
		Brother	M	39	49	$G^{849}\rightarrow A$		W283X
		Nephew (fraternal)	M	7	45	$G^{849}\rightarrow A$		W283X
10	Index patient		F	42	68	$G^{849}\rightarrow A$		W283X
11	Index patient		F	61	54	$G^{849}\rightarrow A$		W283X
	Sister		F	62	62	$G^{849}\rightarrow A$		W283X
12	Index patient		F	44	73	$G^{849}\rightarrow A$		W283X
	Sister		F	39	52	$G^{849}\rightarrow A$		W283X
	Father		M	72	48	$G^{849}\rightarrow A$		W283X
	Uncle (paternal)		M	77	37	$G^{849}\rightarrow A$		W283X
13	Index patient		F	39	48	$G^{849}\rightarrow A$		W283X
	Daughter		F	13	61	$G^{849}\rightarrow A$		W283X
14	Index patient		M	69	58	$G^{849}\rightarrow A$		W283X
15	Index patient		M	56	103	$G^{849}\rightarrow A$		W283X
16	Index patient		F	55	52	$G^{849}\rightarrow A$		W283X
17	Index patient		F	63	50	$G^{849}\rightarrow A$		W283X
		Daughter	F	31	45	$G^{849}\rightarrow A$		W283X
		Daughter	F	37	60	$G^{849}\rightarrow A$		W283X
		Grandson	M	10	46	$G^{849}\rightarrow A$		W283X
		Grandson	M	6	39	$G^{849}\rightarrow A$		W283X
18	Index patient		F	55	52	$G^{849}\rightarrow A$		W283X
19	Index patient		F	43	36	$G^{849}\rightarrow A$		W283X
		Brother	M	42	51	$G^{849}\rightarrow A$		W283X
		Nephew (fraternal)	M	11	49	$G^{849}\rightarrow A$		W283X
20	Index patient		F	38	56	$G^{849}\rightarrow A$		W283X
21	Index patient		M	36	80	$G^{962}\rightarrow A$		**R321H**

[a]See Llewelly et al. (14).
[b]See Delfau et al. (15).
[c]See Chen et al. (16).

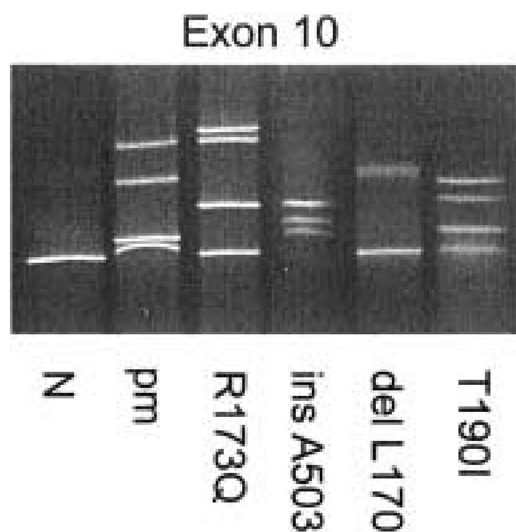

Fig. 1 DGGE analysis of exon 10 of the PBGD gene. Four different mutation namely, R173Q, ins A^{503}, delL170, and T190I were identified in the exon 10 of the PBGD gene among the AIP cohort of this study. N, a negative control sample; pm, polymorphism 6479G/T. The four mutations as well as the 6479G/T polymorphism all appeared as unique patterns in the DGGE gel.

Mutation analysis was performed in the PBGD gene of 21 index patients by DGGE analysis of individual exons in combination with direct sequencing. A total of eight different mutations were identified among them, $insA^{503}$, del L170, T190I, P241S, and R321H were new mutations (Table 1). Twelve index patients, all apparently unrelated, carried W283X, the prevalent mutation previously found among the German-speaking AIP population in Switzerland (10). Both mutations R26H and R173Q were shared by two families. As shown in Figure 1, four of the eight mutations were located in exon 10, a common target for mutations in the PBGD gene.

Mutations R26H, R173Q, and W283X have been reported in different populations so far (14–16). Both R26H and R173Q are common European mutations. Among the five newly found mutations, insertion of an "A" at position 503 causes a frameshift that generates a stop codon (TGA) 40 amino acids after the insertion. The truncated protein, with only 60% of the length of the intact enzyme is probably inactive. Leucine 170 is situated between R167 and R173–two of the four arginine residues that bind to the acetate and propionate side groups of the dipyrromethane cofactor in the active site of the enzyme (17). Deletion of L170 will most likely cause steric disruption in this region and therefore impair the enzyme activity. In addition, $insA^{503}$, del L170, and P241S, being the only mutation in the PBGD gene of the respective families, cosegregated with the low erythrocyte PBG deaminase activity through at least two generations (Table 1). These facts support the notion that the gene defects identified in this study were directly causative of the disease. Although index patient 21 had an erythrocyte PBG deaminase activity value of 80 units, which was slightly above our cut-off value of 75 units, the mutation identified in his PBGD gene involved R321, an amino acid residue that is highly conserved from bacterial to human PBG deaminases and, therefore, it likely plays a major role in the enzyme (17). Moreover, like both mutations G^{77}→A (R26H) and G^{518}→A (R173Q), the G^{962}→A (R321H) mutation occurred at CpG dinucleotides that are known hotspots of mutations (18).

Table 2. Alternations in restriction enzyme cleavage sites

Exon Affected	Mutation	Amino acid Alteration	Restriction Enzyme[a]
3	G^{77}→A	R26H	*Aci* I
10	$insA^{503}$	Frameshift	. . .
10	del $CTC^{508\text{-}10}$	del L170	. . .
10	G^{518}→A	R173Q	*Hpa* II
10	C^{569}→T	T190I	. . .
12	C^{721}→T	P241S	*Nde* II
14	G^{849}→A	W283X	*Hinf* I
15	G^{962}→A	R321H	*Mae* III

[a]The mutations result in an elimination of the respective restriction enzyme cleavage sites.

Molecular Screening of Families

Molecular screening of the family-own mutation among relatives of the index patients was carried out either by DGGE analysis or, if possible, by restriction enzyme analysis (Table 2). An example of restriction enzyme analysis in an AIP family carrying mutation W283X is shown in Figure 2. Thirty symptomatic AIP patients and 28 asymptomatic mutation carriers

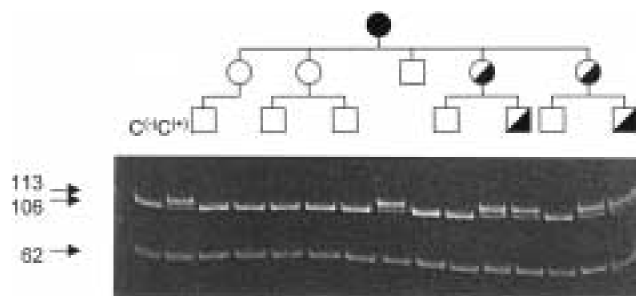

Fig. 2. Molecular screening of mutation W283X in the PBGD gene of a Swiss AIP family by restriction enzyme analysis. PCR amplified DNA fragments of exon 14 of the PBGD gene were subjected to restriction enzyme *Hinf* I digestion. A control sample with a known W283X mutation ($C^{(+)}$) appeared in three fragments of 113 bp, 106 bp, and 62 bp, whereas a control sample without the mutation ($C^{(-)}$) appeared in two fragments of 106 bp and 62 bp in an agarose gel after *Hinf* I digestion. In the pedigree, one symptomatic patient and four asymptomatic mutation carriers are indicated by solid and half-filled symbols, respectively.

were identified in this study cohort. The presence of the family-own mutation was excluded in the remaining 49 individuals. By comparing PBG deaminase activity measurements with molecular analysis (as the "gold standard"), all but three values from the mutation-positive individuals were beneath the lower limit of reference of 75 pmol uroporphyrin*mg^{-1} hemoglobin*h^{-1}, and all but three values from the mutation-negative (normal) individuals were above this cut-off value (Fig. 3). The respective sensitivity and specificity of the enzymatic assay were calculated as 94.8% and 93.9%.

Effect of Gender on Disease Expression

In this study, 23 of the 30 symptomatic AIP patients were female. On the other hand, among the 28 asymptomatic carriers, 9 were female and 19 were male (Table 3). The difference in sex ratios between symptomatic and asymptomatic AIP mutation carriers was significant ($p < 0.001$; Fisher's exact test, two-sided).

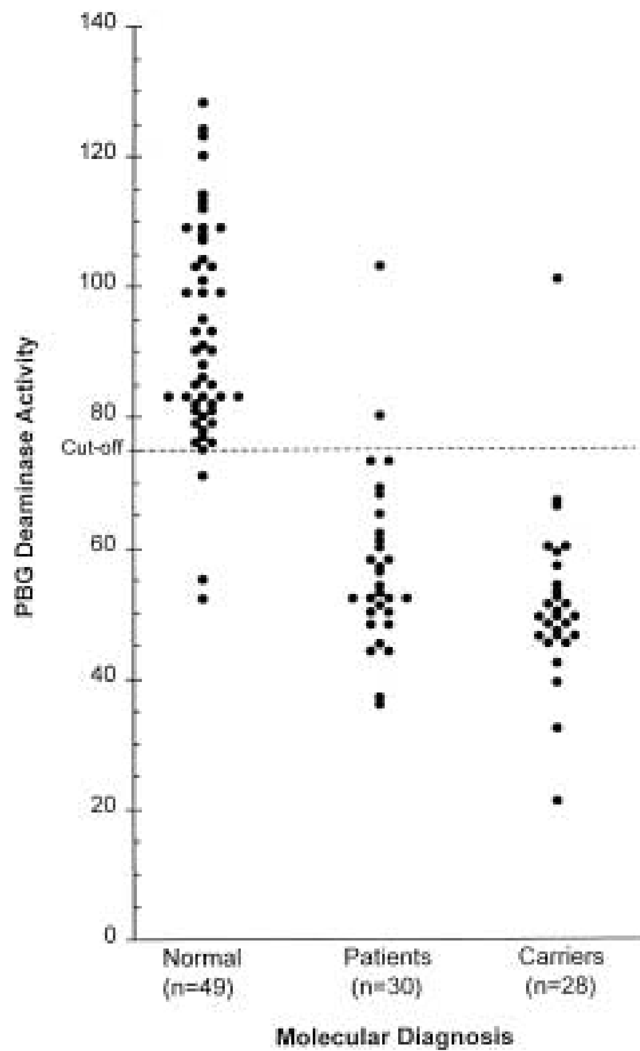

Fig. 3. Comparison between the PBG deaminase activity assay and mutation analysis in diagnosis of AIP. The 107 study subjects were categorized into three groups of normal ($n = 49$), patients ($n = 30$), and carriers ($n = 28$) based on the results of molecular analysis and clinical data. The mean ± SD of PBG deaminase activities of these groups were 92 ± 17 units, 57 ± 14, units and 51 ± 14 units, respectively. The cut-off value for the enzymatic assay was 75 units.

Table 3. The "gender effect" in AIP

	Patient	Carriers
Female	23	9
Male	7	19

Fisher's exact test (two-sided), $p < 0.001$.

Estimated Penetrance of AIP During Life Time

To study the effect of age on the clinical penetrance of AIP, the 58 mutation-positive individuals including 30 patients and 28 asymptomatic gene carriers, were divided into four groups based on their age at the time of the analysis (in 1999): 0–20 years, 21–40 years, 41–60 years, and 61–80 years. Two out of 8 individuals were symptomatic in group 0–20 years; 7 out of 19 individuals were symptomatic in group 21–40 years; 12 out of 19 individuals were symptomatic in group 41–60 years, and 9 out of 12 individuals were symptomatic in group 61–80 years. The percentages of symptomatic patients of the total number of mutation-positive individuals from each of the four age groups are depicted in Figure 4. A nearly linear increase of the percentage of patients with respect to the age is evident, starting from 25% in the lowest age group to 75% in the highest age group. The

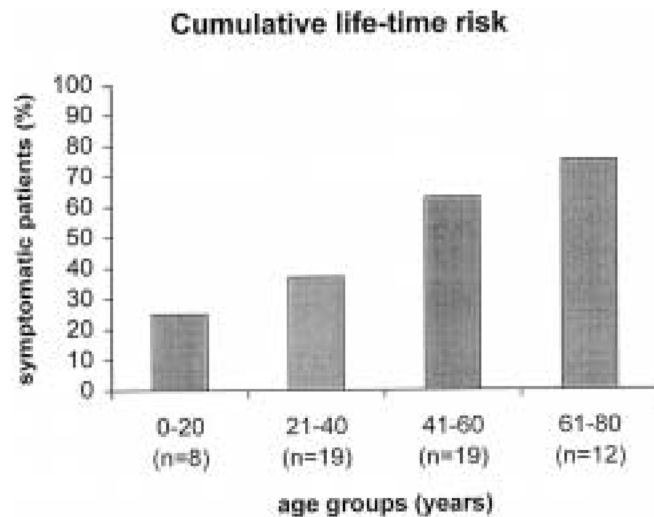

Fig. 4. Cumulative lifetime risk for developing clinical AIP. All AIP affected individuals including 30 AIP patients and 28 carriers were divided into four groups based on their age: 0–20 years, 21–40 years, 41–60 years, and 61–80 years. In each group, the symptomatic patients are shown in percentage in relation to the total number of patients and carriers.

increase in penetrance was not due to differences in the gender distributions among the groups; 2 out of 8 individuals in group 0–20 years, 12 out of 19 in group 21–40 years, 11 out of 19 in group 41–60 years, and 7 out of 12 in group 61–80 years were female.

Discussion

Molecular-Genetic Diagnosis of AIP

PBG deaminase is encoded by a single gene localized to chromosome 11 (11q24.1 → q24.2). The entire gene of 10-kb including 15 exons and intronic regions, as well as the 5′ regulatory and 3′ untranslated regions have been sequenced (19,20). Two distinct promoters generate housekeeping and erythroid-specific transcripts by alternative splicing of exon 1 and 2 (21). Three-dimensional structure of the human PBG deaminase has been resolved based on the structure of the bacterial enzyme, which facilitated the understanding of the catalytic mechanism of the enzyme reaction as well as the effect of different mutations on the enzyme activity (17).

In this study, we detected four novel mutations as well as three previously described mutations in the PBGD gene causing the classical form of AIP in 30 Swiss patients from 21 apparently unrelated families. In addition, molecular analysis identified 28 asymptomatic mutation carriers among relatives of the patients. Although more than 130 different mutations have so far been identified in the PBGD gene, novel mutations continue to emerge emphasizing the molecular heterogeneity underlying AIP. Besides being diagnostic markers, mutations in the PBGD gene obtained from AIP phenotypes are an important source of information for studying structure–function relationships of the human enzyme as discussed.

Together with the results from our recent study, a total of 87 individuals affected by AIP, including both patients and asymptomatic carriers, from 39 Swiss families were diagnosed by molecular genetic analysis (10). The prevalence of unambiguously identified mutation carriers in Switzerland is therefore calculated as 1–1.5 individuals per 100,000 inhabitants, a number that is in accordance with the published incidence of gene carriers (1).

Confirmation of the Prevalent Mutation W283X in Switzerland

In our recent publication, mutation W283X was identified in 13 apparently unrelated AIP patients all coming from the canton of Zürich (10). The origins of the 12 newly identified W283X families were not restricted to the Zürich canton, but were confined to the German-speaking Swiss population in the northern part of Switzerland. Based on the current genetic data, families with the W283X mutation comprise 64% of all 39 DNA-diagnosed AIP families in Switzerland. The genealogical data available could neither rule out nor substantiate the possibility that individuals carrying the W283X mutation were distantly related to each other. Further research is required to examine whether all carriers of mutation W283X share a common ancestral "founder."

Sensitivity and Specificity of PBG Deaminase Activity in Relation to Molecular Diagnosis

In AIP, the acute neurologic attacks are provoked by various endogenous and exogenous factors. Most, but not all, of these precipitating factors can cause the depletion of the hepatic free heme pool and, in turn, lead to an induction of 5-aminolevulinate synthase, the first and rate-limiting enzyme in the heme biosynthetic pathway. As a result, a large amount of ALA and PBG are synthesized in the liver. Either the excessive amount of porphyrin precursors or the lack of the endproduct heme, or the combination of both, may lead to the clinical symptoms of acute attacks (1). An accurate diagnosis of asymptomatic carriers of AIP is a prerequisite for an effective prevention of acute attacks by avoiding potential precipitating factors. However, the overlap between pathologic and normal values in the enzyme activity measurement poses diagnostic uncertainties for some mutation carriers and unaffected individuals with values near the cut-off (Fig. 3) (22). Although the results from the enzymatic assay presented in this study are fairly satisfactory, with both the sensitivity and specificity close to 95%, molecular analysis can ensure a 100% accuracy provided a particular mutation were identified in an AIP family.

Confirmation of an Increased Disease Expression in Women

Based on clinical observations, it is evident that women are more frequently affected by overt AIP than men despite the autosomal dominant inheritance in AIP (1,23). The assessment of the gender distribution among carriers based on conventional analyses was often impaired by the indeterminate diagnosis of latent AIP in some individuals. Our study determined the latent carrier status unequivocally by molecular genetic analysis and confirmed that the disease expression was strongly enhanced by female gender. Endocrine factors are known to play a major role in the induction of AIP in some patients. The acute attacks rarely occur before puberty and its frequency and severity decline in women after menopause. In fact, a subset of female patients experience regular, cyclical, premenstrual exacerbations of their disease. However, the pathogenic mechanism by which female gender predisposes to disease expression remains largely undefined.

Cumulative Lifetime Risk

The age of clinical onset of AIP is reported to be after puberty and most commonly between 20 and 40 years (1). In a study published in 1970, Stein and

Tschudy (24) documented 42 AIP patients. The age of onset in the 11 males ranged from 16–56 years with a mean of 36 years. In 31 females, the onset of disease symptoms ranged from 13–43 years with a mean of 25 years. Our study showed a similar age spectrum of disease onset among symptomatic patients with the mean age of 35.0 and 29.1 years for male and female patients, respectively.

We chose to analyze our cohort in a cross-sectional manner to calculate the risk of an individual with proven mutation becoming symptomatic (at least one attack) during a lifetime. The accurate DNA diagnosis of mutation carriers enabled us to calculate this cumulative lifetime risk. After stratification for age, the risk increased 3-fold, from approximately 25% in the first two decades of life to 75% in the 7th and 8th decades, a figure that is unexpectedly high. It is obvious that the overall rate of overt disease in our study cohort of 52% is much higher than what is generally described in the literature, namely, 10–20% (1,3). However, the penetrance of 10–20% has been questioned by two recent studies (25,26). As reported by Siervi et al., 40% of the 240 individuals with a low PBG deaminase activity were symptomatic in the Argentinean AIP population (25).

In a study of Swedish AIP, Andersson et al. compared the clinical penetrances among carriers of three PBGD gene mutations W198X, R173W, and R167W (26). A penetrance of 44% and 50% were observed for mutation W198X and R173W, respectively. On the other hand, only 13% of the carriers of mutation R167W had clinical manifestations of AIP. The predominance of W283X in our study cohort with a penetrance of 68% certainly contributed to the overall high clinical penetrance. The genotype-phenotype correlation deserves more attention in future AIP research.

It should be mentioned that the overall clinical penetrance of 52% in this study should not be interpreted as an absolute term. This study was designed in such a way that all relatives of an AIP family were contacted with the same information provided (in form of a letter). Symptomatic individuals within in a family might be more willing to take part in this study than those without any clinical symptoms. In seven families, only the index patient was recruited. In other words, a bias favoring inclusion of symptomatic over asympotomatic individuals may exist in this study. However, this bias is difficult to eliminate because participation is merely facultative.

In summary, molecular diagnosis of AIP paved the way for in-depth investigations on the pathophysiology of the disease as it has been demonstrated in our study. The somewhat provocative observations presented here will hopefully stimulate renewed interest in the clinical research of AIP, which has been to some extent overlooked in the past decade. Studies such as this can not only update our knowledge about the disease but also be beneficial to the AIP families. Currently, efforts should focus on providing comprehensive counseling to patients and latent gene carriers, as well as an effective treatment of acute attacks so that both the frequency and the severity of acute attacks in AIP can be reduced.

Acknowledgments

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