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Swiss patients with variegate porphyria have unique mutations

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Summary

Background: Variegate porphyria (VP), also known as South African porphyria, is a low-penetrance, autosomal dominant disorder as the result of a partial deficiency of protoporphyrinogen oxidase (PPOX). Clinically, VP is characterised by photosensitivity and neurovisceral attacks whereby the two symptoms can appear separately or together in patients. VP is little known in Switzerland. In this study, we report a clinical, biochemical and mutational study of eight Swiss VP patients and their families.

Results: Six of the eight index patients presented with only skin symptoms, and one with only neurological symptoms. Another patient had both skin and neurological symptoms. Faecal porphyrin excretion was elevated in all patients thus enabling diagnosis. Four different mutations including three novel mutations (G11D, 1041-1042 ins T and 1262-1263 ins 22bp) were identified in this cohort. Mutation 1082-1083 ins C, which had been reported in the French VP population, was shared by five apparently unrelated patients of this study.

Conclusion: The novel *PPOX* gene mutations are apparently unique to the Swiss population. Both clinical and biochemical presentations varied considerably even among those patients who carried an identical mutation, which does not favour the existence of a genotype-phenotype correlation in VP.

Key words: variegate porphyria; protoporphyrinogen oxidase; faecal porphyrin excretion; mutation; genotype-phenotype correlation

Introduction

Variegate porphyria (VP, McKusick 176200) is a dominantly inherited disorder of haem biosynthesis resulting from a partial deficiency of protoporphyrinogen oxidase (EC 1.3.3.4, PPOX). PPOX, a membrane-embedded flavoprotein, catalyses the oxidation of protoporphyrinogen IX to protoporphyrin IX which is the penultimate step in the haem biosynthesis pathway. Variegate porphyria, as implied by its name, presents clinically in different ways – either with neurovisceral symptoms, photosensitivity, or both in variable degrees [1]. The neurovisceral symptomatology of VP ie abdominal pain, nausea, vomiting and pareses, is indistinguishable from that of other types of acute porphyria, acute intermittent porphyria (AIP) and hereditary coproporphyria (HC). Therefore, VP should be considered in the differential diagnosis of acute porphyria, especially if PBG deaminase (enzyme which is defective in AIP) activity is normal. In Switzerland however, the prevalence of VP is roughly 5 times lower than that of AIP according to our unpublished data. The cutaneous manifestation of VP i.e., blisters and fragility in light exposed skin areas, is similar to that of porphyria cutanea tarda (PCT). The differentiation of these two types of porphyria, which is of particular importance regarding treatments, can be achieved by biochemical assays including faecal porphyrin analysis and plasma fluorescence scanning [2, 3].

Not all individuals having a defective PPOX however, will show symptoms of VP. The penetrance of neurovisceral symptoms is lower in VP comparing to AIP i.e., acute attacks occur in approximately 20% of the individuals with a defective enzyme [4]. However, the remaining 80% of the PPOX-deficient individuals are at risk for development of acute neurovisceral crises if they are exposed to certain drugs, alcohol, or other provoking agents [4]. Since VP is an autosomal dominant disorder, statistically 50% of the siblings of an index patient will inherit the defective PPOX. Family screening is therefore indicated. As the sequence of human *PPOX* gene became available, mutation analysis has proven to be an efficient diagnostic procedure for VP, especially among asymptomatic individuals within VP families [5–7]. Up to now, *The Human Gene Mutation Database* recorded a total of 128 different mutations in the *PPOX* gene (Cardiff; www.hgmd.cf.ac.uk). In this first study of variegate porphyria in Switzerland, we performed mutational analysis in eight patients and their families who had been diagnosed VP by both clinical and biochemical analyses.

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Patients and methods

Patients: Eight apparently unrelated Swiss patients as well as some of their relatives were studied (table 1). The index patients had current or past symptoms suggestive of VP. The diagnosis of VP in the index patients was established by faecal porphyrin measurement or in combination with plasma fluorescence-emission spectroscopy. The study was approved by the ethical committee from the Stadtspital Triemli.

Biochemical analysis: Faecal porphyrin was analysed as described previously [8]. Fluorescence-emission spectroscopy of plasma was performed according to Poh-Fitzpatrick [9].

DNA analysis: Peripheral blood samples from all subjects were collected after appropriate informed consent. Genomic DNA was isolated from peripheral blood (EDTA-anticoagulated) by using the QIAamp™ blood kit (Qiagen, Hilden, Germany). All 13 coding exons of the *PPOX* gene as well as parts of the intron sequence adjacent to the exons were amplified by PCR using primers listed in table 2. Sequence analysis was performed on an ABI Prism 310 genetic analyser (Applied Biosystems, Foster City, CA).

Results

Faecal porphyrins, especially coproporphyrin III and protoporphyrin IX concentrations, and coproporphyrin III/I ratio were grossly abnormal in all symptomatic individuals. The ratio between coporporphyrin isomers III and I is a more reliable diagnostic parameter for VP than porphyrin concentrations. It enables discrimination between VP and acute intermittent porphyria that shows a normal coproporphyrin III/I ratio but occasionally elevated faecal porphyrin concentrations [10].

Mutational analysis was initiated among eight index patients. Direct sequencing of PCR-amplified *PPOX* gene fragments unveiled four different mutations in these patients. Subsequently, family members of the mutation-defined index patients were screened specifically for their family-own mutation.

Three of the four mutations identified in this study were novel mutations (table 1, figure 1). A G to A transition at nucleotide position 32 resulting in the substitution of Gly11 by an aspartic acid residue (G11D) was found in VP-1. Another missense mutation (G11S) that affected the same amino acid residue Gly11 was reported in an American VP patient by Frank et al. [11]. A T inserted between nucleotide 1041 and 1042 was identified in the index patient as well as her daughter in VP-2. The insertion causes frameshift that generates a stop codon, two codons away from the insertion site. Another novel frameshift mutation resulting from a 22-bp insertion between nucleotide 1262-1263 was found in VP-8. In fact, the 22-bp insertion was an exact duplication of the sequence at the intron11/exon 12 junction region of the *PPOX* gene as shown in figure 1D. Interestingly, mutation 1082-1083 insC which has been previously described in the French VP population, was identified in five apparently unrelated Swiss VP patients of this study [7].

Table 1

Clinical, biochemical and mutation data of Swiss VP patients/families.

patient/ families	age/sex	symptoms neuroskin	symptoms logical	faecal porphyrins				plasma fluorescence at 626 nm	mutation status
				copro I <20 nmol/g dry stool	copro III <12 nmol/g dry stool	ratio <2 nmol/g dry stool	proto <80 nmol/g dry stool		
VP-1	42/M	+	–	16	107	6.7	227	+	**G32→A, G11D**
VP-2									
index	42/F	+	–	84	750	8.9	640	+	**1041-1042 ins T, stop+2**
daughter	16/F	–	–	n.d.	n.d.		n.d.	n.d.	**1041-1042 ins T, stop+2**
son	13/M	–	–	n.d.	n.d.		n.d.	n.d.	negative
VP-3	69/F	+	–	14	57	4.0	260	n.d.	1082-1083 ins C, stop +18
VP-4	47/F	–	+*	24	130	5.4	400	n.d.	1082-1083 ins C, stop +18
VP-5	44/F	+	–	53	160	3.0	830	n.d.	1082-1083 ins C, stop +18
VP-6	48/F	+	–	11	66	6.0	120	n.d.	1082-1083 ins C, stop +18
VP-7									
index	60/F	+	+	48	210	4.4	370	+	1082-1083 ins C, stop +18
son	36/M	–	–	n.d.	n.d.		n.d.	–	1082-1083 ins C, stop +18
VP-8	58/F	+	–	34	150	4.4	360	n.d.	**1262-1263 ins 22bp, stop +21**

* neurological and psychiatric symptoms
n.d.: not determined; copro: coproporphyrin; proto: protoporphyrin; ratio: coproporphyrin III to I ratio
novel mutations appear in ***boldface***

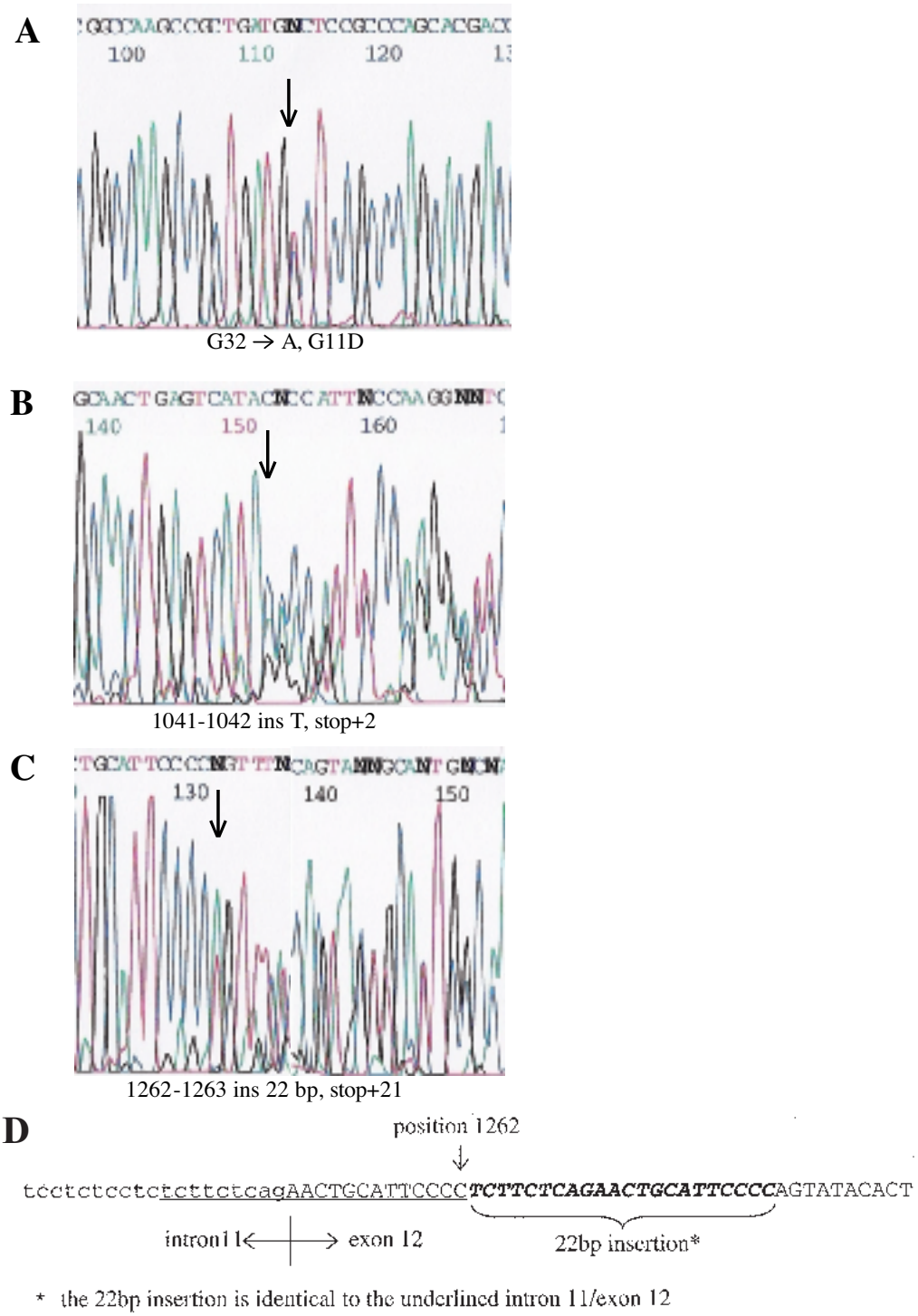
Table 2

Primer sequences used for PCR-amplification of the PPOX gene.

exon	forward primer	reverse primer
1	5'-gtc ccg cca atc cag atg tag-3'	5'-gcg agg tcc ccg tcc ag-3'
2	5'-agt gag tgg ccg gga tag aa-3'	5'-ggg cca gga tcc atc tag ctt-3'
3	5'-ggc cct ctg aat atg c-3'	5'-tac ttc ctc ccc taa act cta-3'
4	5'-agt tta ggg gag gaa gta tgt-3'	5'-gca gtg agc caa gat cgc-3'
5	5'-tgg agc tgg gga ggt atg tc-3'	5'-ggc atc ccc aaa tga tac aga-3'
6	5'-ggc ctt cat ttc cat ccg tca-3'	5'-ttg cag cga gcc gat ctg a-3'
7	5'-act gca tcc agc ctc aat gat-3'	5'-gcc cat gtc taa gta gct t-3'
8	5'-tct aca tag tca ccc aat ctc-3'	5'-ccc agg aag gta tag ctt-3'
9	5'-ccc aaa gag gac tga caa ctg-3'	5'-tct cga act cct gac ctt gtt-3'
10	5'-cct ttc ctt ctg acg cat ga-3'	5'-gaa cct ccc agc atc acc taa-3'
11	5'-ctc ctc tgt gct cca ttg tag-3'	5'-gcc ttg gct gac ata cag t-3'
12&13	5'-ggc cta gga cat caa ta-3'	5'-tta tgc cta tag gtg ata gaa-3'

Figure 1

Three novel *PPOX* gene mutations in Swiss patients with variegate porphyria,
A: mutation G11D in patient VP-1;
B: mutation 1041-1042 ins T, stop+2 in family VP-2 and
C: mutation 1262-1263 ins 22 bp, stop+21 in patient VP-8.
D: a portion of the *PPOX* gene indicating the sequence of the 22bp insertion between nucleotide position 1262 and 1263.



Discussion

The diagnosis of VP in all patients was based on a typical faecal porphyrin pattern. Plasma fluorescence, which has recently been demonstrated as the most sensitive diagnostic tool for VP, was however performed only among some individuals of this study [2]. As shown in table 1, positive plasma fluorescence was observed in all individuals who presented with VP symptoms. The son of VP-7, currently being asymptomatic although carrying a mutation, was negative for the plasma scan.

As mentioned earlier, the symptoms which a VP patient presents could be solely cutaneous, solely neurological, or a combination of both. The frequencies of these three types of clinical presentation were found to be 69%, 15% and 16%, respectively among South African patients and 59%, 20% and 21%, respectively among English and French patients [4, 12]. Although the number of individuals of the present study is rather small, a similar distribution of the three clinical types is observed namely, six of the eight patients had only skin symptom, one patient had only neurological symptom and one patient had both skin and neurological symptoms.

It is worth mentioning that patient VP-4, who never experienced any skin symptoms, suffered from depression since the age of 21 and was under paroxetine treatment for many years. Paroxetine is classified as "probably porphyrinogenic (PRP)" in the Drug Database for Acute Porphyria (www.drugs-porphyria.org). At the age of 42, the patient presented for the first time symptoms of an acute porphyric attack that included abdominal pain, vomiting, constipation. The acute attack was confirmed as a symptom of VP with the demonstration of elevated precursor levels (table 1). The porphyric symptom was accompanied by further psychiatric disturbances such as anxiety and insomnia. Generally, psychiatric illness is believed not to be associated with VP [4]. In the case of patient VP-4, the pre-existing psychiatric problem and as a result the yearlong drug treatment may have triggered the porphyric attack.

As shown in table 1, five female patients who carried the 1082-1083 insC mutation exhibited variable VP symptoms including all three clinical types as mentioned earlier. The biochemical abnormality i.e., the amount of porphyrin excreted in faeces varied also considerably among the five patients. In a previous study published by Whately et al, mutation 1082-1083 insC was identified in six French VP patients. All six patients were reportedly to have only skin lesions [7]. In our study the clinical phenotype of the 1082-1083 insC mutation also included acute porphyric attacks. In other words, mutation 1082-1083 insC is not associated with any particular clinical type.

In a VP population with a high degree of heterogeneity in terms of the *PPOX* gene mutations, the genotype-phenotype correlation could be examined by dividing the patients into groups based on the type of mutations they carry (missense, splice site, frameshift and nonsense mutations) as Whatley et al. did in their study of 108 VP patients from 104 families. A comparison between the mutation types and clinical types i.e., skin lesions alone, acute attack alone and both together in that large study cohort did not show statistical significance [7]. The lack of genotype-phenotype correlation in VP has also been reported among South African patients. Although this disease population is relatively homogenous with predominantly R59W – the South African founder mutation, the variability in clinical symptomology does not differ significantly from that of heterogeneous VP populations [12, 13]. In general, the genotype does not appear to be the only determinant of clinical presentations. Current evidence from South Africa suggests that most patients with VP do not have acute attacks unless exposed to unsafe drugs. Additional genetic factors as well as environmental influences may all contribute to the variable expression of VP.

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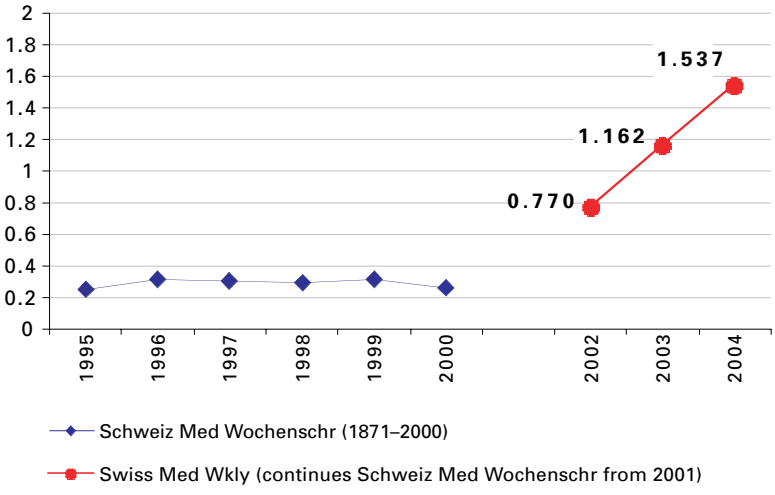

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