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IDENTIFICATION OF A RECURRENT MUTATION IN THE *PROTOPORPHYRINOGEN OXIDASE* GENE IN SWISS PATIENTS WITH VARIEGATE PORPHYRIA: CLINICAL AND GENETIC IMPLICATIONS

A. M. VAN TUYLL VAN SEROOSKERKEN [1, 4] *✉, X. SCHNEIDER-YIN [5] *, R. J. SCHIMMEL [6], R. S. BLADERGROEN [1, 2, 4], J. BARMAN [5], P. POBLETE-GUTIÉRREZ [1, 4], M. VAN GEEL [1, 2, 4], J.FRANK [1, 4] ° AND E. I. MINDER [5] °

[1]Department of Dermatology and [2]Maastricht University Center for Molecular Dermatology (MUCMD) and [3]Euregional Porphyria Center Maastricht and [4]GROW - School for Oncology and Developmental Biology, Maastricht University Medical Center (MUMC), The Netherlands; [5]Zentrallabor and Swiss Porphyrin Reference Laboratory, Stadtspital Triemli, Zürich, Switzerland; [6]Medical school, University Medical Center Utrecht, The Netherlands
Anne Moniek van Tuyll van Serooskerken, M.D. Department of Dermatology,University Hospital Maastricht
P. Debyelaan 25; Postbus 5800, 6202 AZ Maastricht; The Netherlands
Phone: 31-43-3857292, Fax: 31-43-3877293, E-mail: am.van.tuyll@mumc.nl
*These authors contributed equally to this manuscript
°These authors share senior authorship

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Abstract – Variegate porphyria (VP), one of the acute hepatic porphyrias, results from an autosomal dominantly inherited deficiency of protoporphyrinogen oxidase (PPOX), the seventh enzyme in heme biosynthesis. Affected individuals can develop both cutaneous symptoms and potentially life-threatening neurovisceral attacks. Thirty unrelated VP index patients and families are currently known in the Swiss Porphyrin Reference Laboratory in Zürich. In 16 of a total of 24 genetically tested families, we detected a recurrent mutation in the *PPOX* gene, designated 1082-1083insC, reflecting a prevalence of 67%. Haplotype analysis revealed that 1082-1083insC arose on a common genetic background and, thus, represents a novel founder mutation in the Swiss population. Knowledge on the carrier status within a family does not only allow for adequate genetic counseling but also for prevention of the potentially life-threatening acute porphyric attacks. Hence, future molecular screening in Swiss VP patients might be facilitated by first seeking for mutation 1082-1083insC.

Key words: Porphyria; variegate porphyria; protoporphyrinogen oxidase gene; recurrent mutation; Switzerland; haplotyping; founder mutation

INTRODUCTION

The porphyrias are a clinically and genetically heterogeneous group of metabolic disorders characterized by hereditary or acquired partial deficiencies of enzymes controlling the biosynthesis of heme and its precursors. Variegate porphyria (VP; OMIM 176200), one of the acute hepatic porphyrias, is the result of dysfunction of protoporphyrinogen oxidase (PPOX), the seventh enzyme in the heme biosynthetic pathway.[1,2]

In VP, both skin symptoms and life-threatening neurovisceral attacks can occur and for this reason, the disease is also classified as neurocutaneous porphyria. The cutaneous signs include increased skin fragility, photosensitivity, blisters, erosions, crustae, miliae, scars, hyper- and hypopigmentation, and hypertrichosis on the sun-exposed body sites. The neurological manifestations comprise colicky abdominal pain, tachycardia, nausea, vomiting, constipation, muscle weakness, para- and tetraplegia. This symptom complex is often accompanied by hyponatremia and can be precipitated by various porphyrinogenic factors. Of note, clinical symptoms may be variable and usually do not develop before puberty.[1-3]

To establish a diagnosis of VP, biochemical analyses are mandatory since the cutaneous symptoms encountered cannot be differentiated from those seen in porphyria cutanea tarda or hereditary coproporphyria[1-5]. Further, neurovisceral attacks may also occur in other forms of acute hepatic porphyrias such as acute intermittent porphyria (AIP) and hereditary

coproporphyria (HCP). Enzymatic assays may complement the biochemical studies.[1-3,5]

VP is inherited as an autosomal dominant trait with incomplete penetrance, since not all carriers of a heterozygous mutation in the *PPOX* gene will develop clinical symptoms.[1-3] To date, a broad number of *PPOX* mutations, including missense, nonsense, frameshift, and splice site mutations, have been reported in different ethnic groups.[6] These sequence deviations are usually unique to one or a few families. Exceptions to this rule are recurrent mutations that have been reported in South Africa[7-10] Chile[11], Sweden[12], and Finland[13], reflecting either hotspot mutations or founder effects.

Recently, a recurrent *PPOX* gene mutation, designated 1082-1083insC, was identified among Swiss VP patients.[14,15] In an effort to elucidate whether this mutation arose independently on distinct genetic backgrounds or as the result of a single ancestral mutational event, we performed haplotype studies among both previously and newly diagnosed VP patients and families from Switzerland.

MATERIALS AND METHODS

Patients and families

We studied 35 individuals from 16 apparently unrelated Swiss VP families. In 22 of them, the VP diagnosis was made based on a combination of characteristic biochemical findings, including plasma fluorescence emission scanning, urinary and stool porphyrin measurement, and DNA mutation analysis, as previously described.[16] Of note, not all mutation carriers showed clinical symptoms. All individuals provided informed consent for inclusion in the study, in accordance with guidelines set forth by the local institutional review board and the declaration of Helsinki principles.

Clinical material, DNA extraction, PCR amplification and mutation analysis

Blood samples from affected individuals and family members were collected in tubes containing EDTA as anticoagulant. Genomic DNA was isolated by using the QIAampTM blood kit (Qiagen, Hilden, Germany). The 12 coding exons and adjacent splice sites of the *PPOX* gene were PCR amplified using primers as previously described.[15] Automated DNA sequencing analysis was performed on an ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, CA).

Haplotype analysis

Haplotypes were constructed using 14 microsatellite markers flanking the *PPOX* gene on chromosome 1q22-23 in both centromeric and telomeric directions, D1S303, D1S2140, D1S1595, D1S1653, D1S398, D1S2707, D1S484, D1S2705, D1S1679, D1S1677, D1S104, D1S426, D1S38A05, D1S196 (Table 1).[10] These markers span a distance of approximately 12 cM on the Marshfield Genetics map, indicating that they are tightly linked to the *PPOX* gene (http://www.marshmed.org/genetics). PCR and fragment length analysis were performed as previously described.[11]

RESULTS

Recurrent mutation in the PPOX gene

Of the 35 individuals studied, 22 individuals were found to carry an identical frameshift mutation in the PPOX gene. This sequence deviation consists of a 1 base pair insertion at position 1082 of the PPOX cDNA (counting the first base of the methionine initiation codon as +1), designated 1082-1083insC, that leads to a premature termination 18 bases downstream of the insertion site.

Haplotype analysis

Haplotyping revealed that in all 22 mutation carriers from the 16 unrelated families, the 1082-1083insC mutation co-segregated with a core haplotype on chromosome 1 (Table 1; shaded in dark grey). This haplotype spans approximately 0.37 cM on the long arm of chromosome 1. Additionally, 14 individuals from 10 families share an extended “1082-1083insC” haplotype that spans approximately 3.49 cM (Table 1; shaded in light grey).

DISCUSSION

Thirty unrelated VP index patients and families are currently registered in the Swiss Porphyrin Reference Laboratory and Porphyria Outpatient Clinic in Zürich, Switzerland. Up to now, DNA analysis has been performed in 24 of these families. Here, we studied a Swiss cohort of 35 individuals from 16 unrelated VP families by haplotype analysis.

Table 1. Haplotype analysis reveals a core haplotype shared by all individuals studied. Additionally, an extended haplotype is shared by 14 individuals from 10 families.

Markers/Loci	Position on ***Chromosome 1 (bp)***	VP I	VP II	VP III	VP IV	VP V	VP VI	VP VII	VP VIII	VP IX	VP X	VP XI	VP XII	VP XIII	VP XIV	VP XV	VP XVI
D1S303 (153.90 cM)		3	2, 4	4, 5	3, 4	4, 6	2	4	2, 4	4	2	3, 4	4, 6	4	2, 4	6	4
D1S2140 (153.95cM)	127’050’098-127’050’353	4	3, 4	2, 3	4, 5	2	6	2	4, 5	4	2, 3	4, 5	2	1	4	4	4, 5
D1S1595 (153.95 cM)	127’050’098-127’050’384	3	3, 4	2, 3	4, 6	2	8	2	5, 7	4	2, 3	4, 6	2	1	4	4	4, 6
D1S1653 (156.2 cM)	129’290’751-129’290’854	1, 3	3	2, 3	1, 3	1, 3	3	1	3	2	2	2, 3	2	3	1,2	1	3
D1S398 (157.91 cM)	130’996’288-130’996’466	2	1	3, 6	2, 4	3	1	2, 3	1	1	1	2, 5	1	1	2 ,6	2	3
D1S2707 (158.34 cM)	131’429’566-131’429’720	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	3, 7	3, 7	**6**	**6**	n.d.	7	**6**
D1S484 (159.03 cM)	**132’123’732-132’123’862**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
D1S2705 (159.12 cM)	**132’214’259-132’214’412**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**
PPOX (159.40 cM)	**132'493’137-132'497’953**	**1082-1083insC mutation**															
D1S1679 (160.62 cM)	133’606’635-133’606’781	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	3	6
D1S1677 (161.83 cM)	134’804’939-134’805’141	**3**	**3**	**3**	**3**	4	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	2	3	2
D1S104 (161.90 cM)	134’882’097-134’882’258	2	2	4, 5	2	4	2	4, 6	2	2	4, 5	1, 4	2	2	1, 4	5	2, 4
D1S426 (163.57 cM)	136’553’915-136’554’076	3, 8	2	3, 8	2	2, 3	3	3, 10	2	3	2, 3	3, 6	3, 8	2	3, 5	2, 3	3
D1S38A05		2	5	3, 5	3, 5	2	2, 5	1, 2	2	6	2, 3	3	5	2	4, 5	n.d.	3
D1S196 (165.87 cM)	138’850’205-138’850’471	3, 4	1, 5	1, 5	1, 3	5	1	1, 6	3	5	5	5	3	3	1	4	6

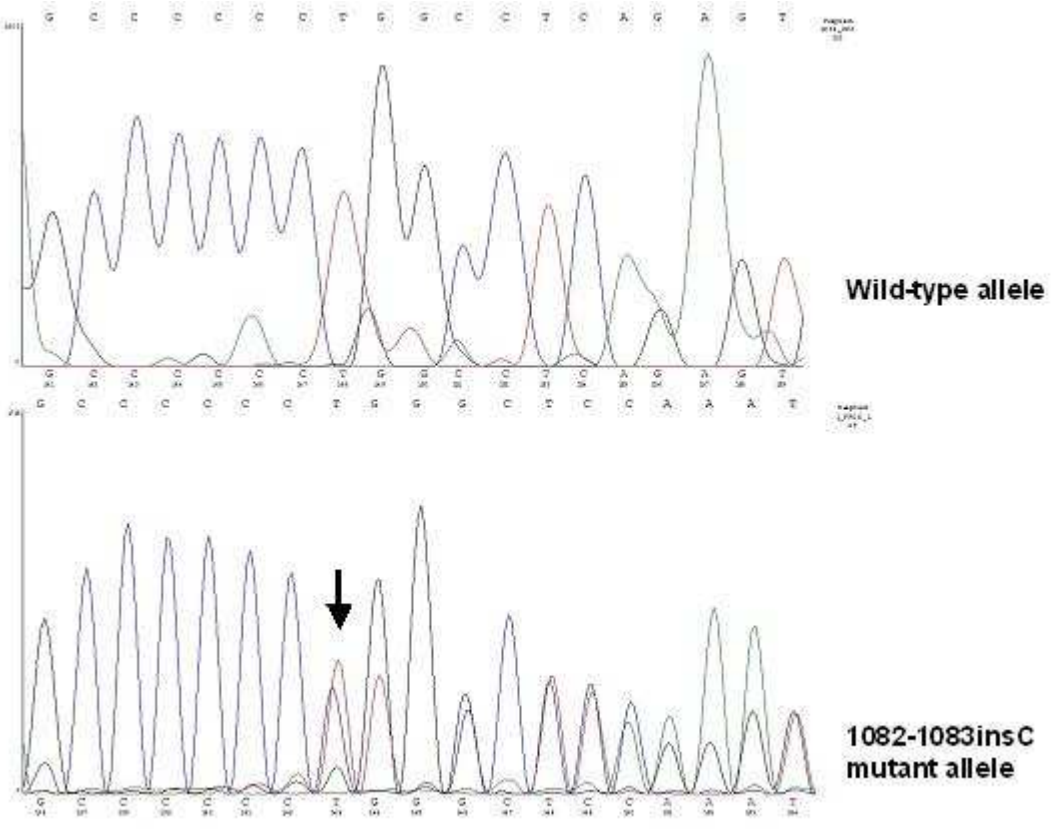

Figure 1. Recurrent frameshift mutation in exon 10 of the *PPOX* gene identified in this study. The sequence alteration consists of a heterozygous 1 base pair insertion, designated 1082-1083insC (lower panel; indicated by an arrow). The upper panel depicts the corresponding wild-type allele.

Making a diagnosis only based on clinical symptoms is difficult since VP is a rare disorder and its symptoms can mimic those of other diseases. Disease penetrance is low and thus, mutation carriers may present different phenotypes. They either (i) manifest solely skin symptoms, (ii) show only acute neurological attacks, or (iii) display both cutaneous and neurovisceral symptoms.[1,3] Laboratory techniques such as plasma fluorescence scanning, fecal porphyrin analysis, and enzyme activity measurement are usually available in specialized laboratories and often not sufficiently sensitive to detect clinically asymptomatic carriers.[17] Therefore, DNA analysis should be considered as the gold standard i.e., the most stringent technique to confirm the diagnosis of VP.[18]

To date, more than 150 different *PPOX* gene mutations have been reported in VP.[6,15,19-22] Although the majority of these sequence deviations affects only single families, recurrent mutations have been detected on a few occasions. In 1996, a recurrent missense mutation, designated as R59W, was reported in the South African population.[7-10] The R59W mutation had a high prevalence of 95% among South African VP families. According to the genealogical data, carriers of R59W most likely shared a common ancestor who immigrated from the Netherlands to South Africa at the end of the 17th century.[7-10] Since then, additional recurrent *PPOX* mutations have been described in VP families from Chile,[11] Finland, [12] and Sweden,[13] reflecting additional founder mutations or, in the case of R168H, a hotspot mutation.

A previous study found that mutation 1082-1083insC in exon 10 of the *PPOX* gene was prevalent in the Swiss population.[14,15] Up to now, this mutation has been identified in 16 of a total of 24 unrelated VP families which underwent genetic testing, giving a prevalence of 67%.

In order to elucidate whether the mutation arose independently on different genetic backgrounds or originated from a common ancestor we performed haplotype analysis. Our data show that all Swiss families studied here share a common core haplotype that spans approximately 0.37 cM, between marker D1S484

and the *PPOX* gene (Table 1). An additional extended haplotype of approximately 3.49 cM, defined by markers D1S2707 and D1S1677, is shared by 10 families (Table 1). Although the unambiguous identical-by-descent sharing of this haplotype cannot be demonstrated without the genotyping of additional married-in-individuals who were unavailable for this study, the coincidental identical-by-state sharing of alleles at up to five different microsatellite markers in perfect concordance with the segregation of the same 1-base pair insertion mutation in 16 unrelated families seems improbable. The markers used for haplotyping were all highly polymorphic and, thus, there is enough evidence to suggest that 1082-1083insC arose on a common genetic background in a single ancestor and represents a novel founder mutation for VP in the Swiss population.

The mutation carriers identified in this study displayed different phenotypes although they share the same underlying genetic defect. This is in line with previous reports on VP from other countries such as South Africa, Finland, and Spain.[17,21,22] The reasons for the different phenotypes even among individuals within the same family, are not yet well understood.[17,21] However, our previous observation of simultaneous disease manifestation in Swiss monozygotic twins with VP who also carried mutation 1082-1083insC, indicates the important role of genetic background in the manifestation of overt disease.[14] Hence, elucidation of these yet unidentified modifying genetic factors will be a major future challenge in porphyria research. We strongly believe that the identification of putative modifier genes could best be accomplished in a cohort of VP patients carrying the same mutation that arose on a common genetic background, as is the case in the families described here.

Interestingly, mutation 1082-1083insC was also identified by Whatley et al. in several French VP patients who only had cutaneous symptoms.[6] While the Swiss twins with VP previously described by us likewise only revealed skin findings, several of the mutation carriers in this study also developed acute porphyric attacks.[6,14,15,19] Hence, our results support the previous notion of an apparent absence of genotype-phenotype-correlations in VP. However, we have previously identified within the cohort of Swiss VP patients a female carrier of mutation 1082-1083insC who also developed hepatocellular carcinoma (own unpublished data). Patients with acute hepatic porphyria, including VP, apparently have an increased risk to develop this type of primary liver cancer. Of note, mutation 1082-1083insC has recently also been reported in a French VP patient with hepatocellular carcinoma.[23] Hence, the possibility of mutation 1082-1083insC being associated with a high incidence of HCC and, thus, a previously unrecognized or underestimated genotype-phenotype correlation, certainly deserves further consideration.[23,24]

To date, a comparison among different types of clinical manifestation in large cohorts of VP families has been reported from South Africa, Great Britain, France, and Finland.[6,17,22] Whereas in the latter three countries a heterogeneous spectrum of mutations underlies the disease, VP in South Africa is mainly attributable to one single founder mutation, R59W. In the South African population, 69% of the patients had only cutaneous symptoms, 15% solely developed acute porphyric attacks, and 16% revealed both manifestations.[6,25] Among the British and French VP patients the distribution pattern was 59%, 20%, and 21%[6], respectively; and in the Finnish cohort it was 40%, 27%, and 14%, respectively.[6,22] These data are in line with the distribution of clinical symptoms among the group of Swiss mutation carriers studied here, indicating that a significant difference in clinical variability does not exist when comparing genetically heterogeneous VP populations such as, Great Britain, France, and Finland, with the more homogeneous ones such as, South Africa and Switzerland.[6,15,22,26]

In VP and in other types of acute hepatic porphyrias as well, the knowledge on carrier status within a family does not only allow for adequate counseling but also for the prevention of potentially life-threatening acute porphyric attacks, as shown in previous studies from other countries in which recurrent *PPOX* gene mutations prevail. Therefore, molecular screening in Swiss VP patients might be facilitated in the future by first seeking for mutation 1082-1083insC.

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