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Letter to the Editor

A recurrent mutation in variegate porphyria patients from Chile and Sweden: Evidence for a common genetic background?

Variegate porphyria (VP) (OMIM 176200), one of the acute hepatic porphyrias, is caused by a partial deficiency of protoporphyrinogen oxidase (PPOX), the seventh enzyme in the haem biosynthetic pathway. The clinical findings include increased photosensitivity, skin fragility, blistering, erosions, scarring and milia on the sun-exposed areas of the body and distinct neurovisceral symptoms, which comprise acute attacks of abdominal pain, nausea, vomiting, tachycardia, hypertension, muscle weakness, hemi- and tetraplegia, respiratory failure, and coma [1].

VP is inherited as an autosomal dominant trait with incomplete penetrance and caused by mutations in the *PPOX* gene on chromosome 1q22–23. Different mutations in the *PPOX* gene have been reported [2] (for an overview see the Human Gene Mutation Database at www.hgmd.cf.ac.uk), which are usually unique to individual VP families.

Here, we first studied on the genetic level four unrelated and newly ascertained Chilean VP families comprising 17 individuals. The diagnosis of VP was made on the basis of typical clinical symptoms in association with confirmatory biochemical findings. After collecting EDTA-anticoagulated blood samples we isolated genomic DNA and amplified by polymerase chain reaction (PCR) all coding regions and splice sites of the *PPOX* gene as previously described [3]. PCR products were directly sequenced using the BigDye deoxy terminator V3.1 cycle sequencing kit (Applied Biosystems Inc, Foster City, CA, USA) according to the manufacturer's instructions.

In 10 of the 17 Chilean individuals we detected a recurrent 2 bp-deletion in exon 13 of the *PPOX* gene, designated c.1330_1331delCT. The carriers showed different clinical symptoms and some were even asymptomatic, suggesting that there is no genotype-phenotype correlation for c.1330_1331delCT. This mutation leads to a premature termination 8 codons (p.L444DfsX8) downstream of the deletion site. Interestingly, the same frameshift mutation has been previously described in Swedish VP patients [4]. This raised the question whether these apparently unrelated VP families from two different countries share a common ancestor.

Subsequently, we compared by haplotype analysis the four Chilean VP families with the four unrelated Swedish VP families carrying mutation c.1330_1331delCT. The latter consisted of 36 individuals of whom 16 were carriers. Haplotypes were derived using 14 microsatellite markers proximal and distal of the *PPOX* gene as previously reported [3,5]. These markers spanned approximately 12 cM on the Marshfield Genetics map (http://www.marshmed.org/genetics). PCR and fragment length analysis were performed as previously described [3].

Haplotyping in the four Chilean VP families revealed that for all patients the mutation co-segregated with a core haplotype that spanned approximately 2.87 cM between markers D1S484 and D1S104. Additionally, families VP-C1, VP-C2 and VP-C3 shared an extended haplotype spanning 8 cM. The largest haplotype, covering approximately 11.96 cM, was found in families VP-C2 and VP-C3 (Table 1).

In the four Swedish VP families, haplotyping revealed that all mutation carriers shared a common core haplotype spanning approximately 1.59 cM between markers D1S484 and D1S1679 that was completely different from the Chilean core haplotype. Furthermore, families VP-S2, VP-S3 and VP-S4 shared an extended haplotype that spanned approximately 2.72 cM (Table 1).

The majority of mutations in the *PPOX* gene are specific for individuals or families. Nevertheless several recurrent mutations have already been described. The first recurrent mutation in VP reported is a missense mutation in exon 3 of the *PPOX* gene, designated p.R59W. Due to a founder effect this mutation is the prevailing genetic defect in the South African VP population [5]. The second recurrent missense mutation reported, p.R168H, was detected in three VP families from Germany, the USA, and Chile. Haplotype analyses showed that this mutation in exon 6 of the *PPOX* gene co-segregated with different haplotypes in each family, suggestive of a mutational hotspot [6]. Another recurrent missense mutation in exon 5 of the *PPOX* gene and was reported in 27 carriers from 11 apparently unrelated Finnish VP families [7]. This mutation, p.R152C, has also been described in VP patients from France, Sweden and the USA (Table 2).

Besides the aforementioned missense mutations, two recurrent frameshift mutations have been reported. In carriers of these mutations, c.1239_1243delTACAC and c.1082_1083insC, a common ancestral background has been confirmed [3,8]. The first mutation is located in exon 11 and was identified in Chilean VP patients, whereas the latter resides in exon 10 and was detected in 22 individuals from 16 apparently unrelated Swiss VP families. Of note, c.1082_1083insC has also been reported in VP patients from France, Italy, Argentina, Spain and Germany (Table 2). Thus, it might be interesting to elucidate if these patients from six different countries are distantly related. All recurrent *PPOX* gene mutations reported to date are summarised in Table 2.

Based on these previous reports and considering historical developments we wondered if the Chilean and Swedish VP patients carrying mutation c.1330_1331delCT might be distantly related. Chileans, as most of South American people, have a strong European ancestry since many Europeans have emigrated to South America during centuries, including Swedes. Furthermore, after the social-political developments in Chile in the early 1970s more than 18,000 Chileans emigrated to Sweden [9]. Here, we show within the four Chilean VP families a minimum shared haplotype spanning a chromosomal region of 2.87 cM (Table 1). Likewise, we detected a minimum shared haplotype of 1.59 cM in the Swedish cohort (Table 1). The markers used for haplotyping were highly polymorphic and the coincidental sharing of haplotypes in perfect segregation with the mutation at several microsatellite markers seems highly improbable. Interestingly, a comparison of the haplotypes co-segregating with c.1330_1331delCT in both countries showed that

Table 1
Haplotype analysis in the four Chilean VP families (VP-C) shows co-segregation of a core haplotype in linkage with mutation c.1330_1331delCT (shaded in dark grey). An additional extended proximal haplotype is shared by families VP-C1, VP-C2, and VP-C3 (shaded in light grey). Families VP-C2 and VP-C3 also share a common haplotype extending distally (shaded in middel grey). Only the haplotype in linkage with the mutation is shown. Haplotype analysis in the four Swedish VP families (VP-S) shows co-segregation of a core haplotype in linkage with mutation c.1330_1331delCT (shaded in blue). An additional extended proximal haplotype is shared by families VP-S2, VP-S3, and VP-S4 (shaded in light blue). The respective values in each cell reflect the size of the allele (in bp) segregating with the mutation at each microsatellite marker.

Markers/Locus	VP-C1	VP-C2	VP-C3	VP-C4	VP-S1	VP-S2	VP-S3	VP-S4
D1S303 (155.64 cM)	185	185	185	181	ND	ND	ND	ND
D1S2140 (155.69cM)	258	258	258	254	246	254	254	258
D1S1595 (155.69 cM)	286	286	286	282	196	196	196	196
D1S1653 (157.93 cM)	111	111	111	104	108	108	104	104
D1S398 (159.64 cM)	159	159	159	159	163	155	155	155
D1S2707 (160.07 cM)	155	155	155	149	143	155	155	155
D1S484 (160.77 cM)	129	129	129	129	126	126	126	126
D1S2705 (160.86 cM)	150	150	150	150	156	156	156	156
PPOX (161.14 cM)	**Mutation c.1330_1331delCT**							
D1S1679 (162.36 cM)	148	148	148	148	156	156	156	156
D1S1677 (163.56 cM)	192	192	192	192	274	282	282	286
D1S104 (163.64 cM)	156	156	156	156	162	162	162	162
D1S426 (165.31 cM)	139	141	141	141	141	141	141	139
D1S38A05	163	163	163	181	ND	ND	ND	ND
D1S196 (167.60 cM)	267	267	267	267	276	276	267	276

ND: not determined. UCSC build Feb. 2009 (GRCh37/hg19).

Table 2
Overview of recurrent *PPOX* gene mutations reported to date.

PPOX mutation	Location	Country of occurrence	Haplotype analysis performed	Estimated frequency	Reference
p.R59W	Exon 3	South-Africa, the Netherlands	Yes	~95% in South-Africa	[5], Warnich L, et al. Hum Mol Genet 1996;5:981–4. de Rooij FWMMG, et al. Acta Heamatol 1997;98:103. Meissner PN, et al. Am J Hum Genet 1998;62:1254–8.
p.R152C	Exon 5	USA, France, Finland, Sweden	No	~52% in Finland	[6,2,7,4]
p.R168H	Exon 6	Chile, USA, Germany, the Netherlands	In 3 carriers from Chile, the USA and Germany	Unknown	[6,3]
c.1082_1083insC	Exon 10	France, Italy, Switzerland, Spain, Argentina, Germany	Only in the Swiss population	~52% in Switzerland	[2,8], D'Amato M, et al. Hum Mutat 2003;21:448. Schneider-Yin X, et al. Swiss Med Wkly 2006;136:515–9. Lecha M, et al. J Eur Acad Dermatol Venereol 2006;20:974–9. Rossetti MV, et al. BMC Med Genet 2008;9:54. Hanneken S, et al. Int J Colorectal Dis 2009;24:127–8.
c.1239_1243delTACAC	Exon 11	Chile	Yes	Unknown	[3]
c.1330_1331delCT	Exon 13	Sweden, Chile	Yes	Unknown	[4], this study

they were different from one another. Therefore, it is very likely that this mutation arose independently in both countries.

Mutation c.1330_1331delCT reflects a microdeletion that occurred within the following sequence of the PPOX cDNA, TTGCCC**CT**GACTCT. The mutation site is imbedded in a 5 bp-nucleotide motif, C_4T, which recently has been recognized to be non-coincidentally associated with microdeletions. This and other short oligonucleotide motifs of 5–7 bp are overrepresented in the vicinity of microdeletions and reflect mutational hotspots within genes [10]. Such a mutational hotspot could explain why mutation c.1330_1331delCT arose independently in two VP cohorts from different countries.

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