A Chilean boy with severe photosensitivity and finger shortening: the first case of homozygous variegate porphyria in South America

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Summary

A 7-year-old Chilean boy presented with severe photosensitivity, blistering, erosions and scarring on sun-exposed areas of the body since the age of 6 months. Additionally, he showed a short stature and shortening of the fingers. Laboratory examination revealed greatly elevated protoporphyrin levels in the blood. Such biochemical findings can be observed in homozygous variants of usually autosomal dominantly inherited acute porphyrias such as variegate porphyria (VP) and hereditary coproporphyria, which usually do not become manifest before the second or third decade of life in heterozygotes. Using polymerase chain reaction-based techniques we identified a missense mutation in exon 7 on the paternal allele and a frameshift mutation in exon 13 on the maternal allele of the protoporphyrinogen oxidase gene that harbours the mutations underlying VP. This is the first homozygous case of VP in South America. As VP represents the most frequent type of acute porphyria not only in Chile but also in South Africa, more such cases could be expected in the future, particularly because a founder mutation for this disease has already been described in the Chilean and South African population.

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Variegate porphyria (VP) (OMIM 176200), one of the acute hepatic porphyrias, is characterized by a deficiency in the activity of protoporphyrinogen oxidase (PPOX), the penultimate enzyme in the pathway of haem biosynthesis. Affected individuals show increased photosensitivity, blistering of sun-exposed areas and abnormal skin fragility. Photosensitivity may occur alone or in combination with acute neurovisceral symptoms such as abdominal pain and hemiplegia. As these symptoms are also observed in other forms of acute porphyrias, biochemical studies are mandatory for establishing the correct diagnosis.[1]

VP is inherited as an autosomal dominant trait, displaying incomplete penetrance, as not all individuals carrying a mutation in the *PPOX* gene develop clinical symptoms. The human *PPOX* gene is located on chromosome 1q23 and contains one noncoding and 12 coding exons. To date, several different mutations in the *PPOX* gene have been reported, including a founder effect in the Chilean population.[2]

In heterozygote patients, PPOX activity is decreased by approximately 50% and symptoms do not usually appear before puberty. By contrast, cases of homozygous VP rarely occur and worldwide, only 11 such patients have been published previously, originating from consanguineous as well as nonconsanguineous families.[3–6] Clinically, homozygous VP shows a more severe phenotype with an onset of characteristic skin lesions already in early childhood. Further clinical features include clinodactyly, short stature and mental retardation.[7] However, no acute neurovisceral crises have been described in these patients.

We describe the first patient with homozygous VP in South America and characterize the molecular genetic basis in this unusual case.

Case report

The index patient, a 7-year-old Chilean boy, developed severe photosensitivity at the age of 6 months and, shortly thereafter, blisters and erosions recurrently developed during the first year of life. Blood porphyrin analysis performed at the age of 1 year according to Heller *et al.*[8] revealed raised levels of blood total protoporphyrin of 248 μg dL^{-1} (reference value for male individuals: 61 μg dL^{-1}). Based on the cutaneous symptoms,

age at onset and initial biochemical studies, erythropoietic protoporphyria was presumed at that time by the attending dermatologist, although blisters are not commonly observed in this disease. In subsequent clinical examinations at the age of 3, 4 and 7 years, the patient revealed not only skin fragility, blistering, erosions and pronounced scarring in sun-exposed body areas, including perioral radial linear scarring, but also a short stature of 112 cm (2 SD below the reference height of males at age 7 years) and shortening of the fingers (brachydactyly) (Fig. 1a,b). No mutilation or erythrodontia was observed. He attended school regularly and did not reveal mental retardation or any neurological disturbance. Biochemical blood porphyrin analyses were likewise performed at the age of 3, 4 and 7 years, and always revealed raised protoporphyrin concentrations, which ranged from four- to eightfold higher than the corresponding normal value for male individuals. At the age of 7 years, porphyrin analyses in urine and stool showed raised faecal protoporphyrin and coproporphyrin concentrations, with protoporphyrin levels of 161 μg g^{-1} dry weight (normal ≤30) being higher than the coproporphyrin levels of 53 μg g^{-1} dry weight (normal ≤20). In the urine, elevated concentrations for uroporphyrin of 63 μg in 24 h (normal ≤15) were measured. By contrast, a normal urinary coproporphyrin concentration of 88 μg in 24 h (normal ≤176) was detected as well as normal values for the porphyrin precursors δ-aminolaevulinic acid of 3·3 mg in 24 h (normal ≤8·2) and porphobilinogen of 0·1 mg in 24 h (normal ≤1·2).

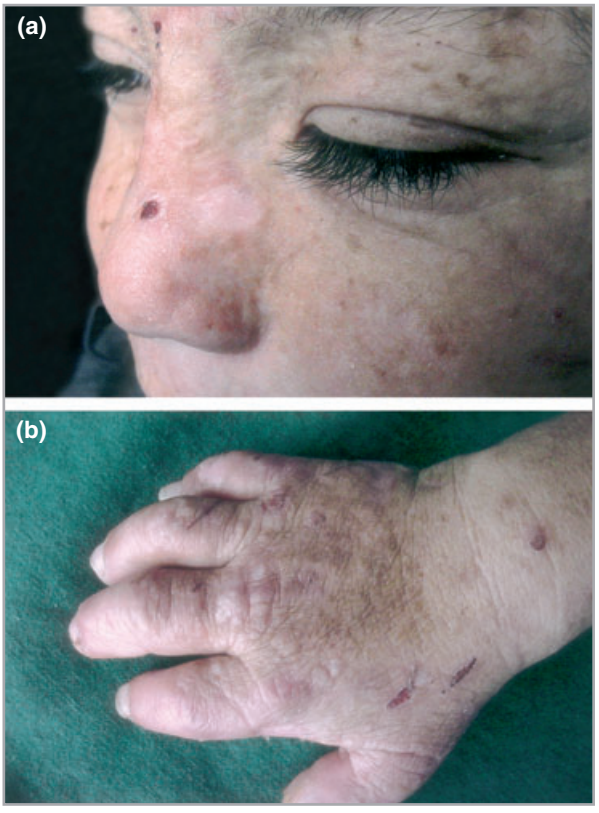

Fig 1. (a) Erosions and hyperpigmented and hypopigmented scars on the face of the index patient. (b) Erosions and hyperpigmented and hypopigmented scars on the back of the right hand of the index patient. Note the finger shortening.

His parents are nonconsanguineous and of Chilean origin. Whereas the father is healthy and has never experienced neurological or cutaneous symptoms, the mother has a long medical history of recurrent mild abdominal pain since the age of 18 years and shows discrete erosions and hypertrichosis in the face. No other relatives are affected. Both the father and mother revealed normal values for blood, stool and urinary porphyrins as well as for the porphyrin precursors δ-aminolaevulinic acid and porphobilinogen in the urine, although the mother showed discrete clinical signs of VP.

Blood samples were collected from the index patient, his parents and 150 Chilean controls in tubes containing ethylenediamine tetraacetic acid. All individuals provided informed consent for inclusion in the study. Genomic DNA was isolated according to standard techniques.[9]

A mutation detection strategy was developed, consisting of polymerase chain reaction (PCR) amplification of all *PPOX* exons using PCR primers that were published previously.[2] For mutation detection, PCR products were subjected to automated sequencing, using an ABI Prism 310 Genetic Analyser from Applied Biosystems Inc. (Foster City, CA, U.S.A.). To verify the sequence variations detected in exon 7 and exon 13 of the *PPOX* gene, a combination of heteroduplex analysis using conformation-sensitive gel electrophoresis, restriction enzyme digestion with the restriction endonuclease MnlI (New England Biolabs, Beverly, MA, U.S.A.), and automated sequencing was used, as previously described in detail.[2,10]

The cutaneous symptoms observed in the index patient in combination with the characteristic biochemical stool porphyrin profile showing elevated protoporphyrin and coproporphyrin concentrations, the protoporphyrin levels being higher than those of coproporphyrin, were indicative of VP. Because the skin symptoms had already developed in the first year of life and were much more severe than usually observed in heterozygotes with VP, we made the presumptive diagnosis of homozygous VP and subsequently sought to verify this diagnosis on the molecular genetic level.

In the patient, automated sequence analysis revealed two different mutations, in exon 7 and exon 13 of the *PPOX* gene. The mutation in exon 7 consisted of a G-to-A transition at nucleotide position 694 of the *PPOX* cDNA (counting the first base of the initiating methionine as number 1). This base substitution leads to a previously unreported missense mutation, consisting of an amino acid conversion from glycine to serine at position 232, designated G232S (Fig. 2a). The mutation in exon 13 consisted of a 2-bp deletion at position 1330 of the *PPOX* cDNA, designated 1330delCT (Fig. 2b).

As mutation G232S introduces a novel restriction site for the restriction endonuclease MnlI (recognition site CCTC), we used enzymatic digestion to study 150 unrelated Chilean controls for absence of the mutation and excluded this sequence variation as a common polymorphism. To verify mutation

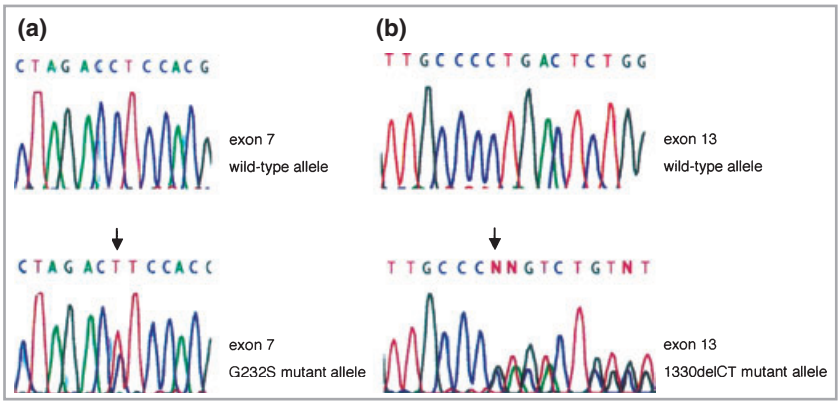

Fig 2. Results of mutation analysis in the index patient. For each mutation, the corresponding wild-type alleles are depicted in the upper panel. (a) Missense mutation G232S in exon 7 of the *PPOX* gene, consisting of a heterozygous G-to-A transition (lower panel), indicated by an arrow. Note that in the electropherogram the mutation is depicted in the 3′-to-5′ direction due to presence of an intronic polymorphism in intron 6 close to the splice-acceptor site. (b) Frameshift mutation 1330delCT in exon 13 of the *PPOX* gene, consisting of a 2-bp deletion (lower panel), indicated by an arrow.

1330delCT we used a combination of heteroduplex analysis and automated sequencing as previously described.[2]

Hereby, the patient was shown to be a compound heterozygote for mutations G232S and 1330delCT, confirming the presumed clinical and biochemical diagnosis of homozygous VP. DNA analysis in the parents revealed that the father carried mutation G232S and the mother mutation 1330delCT in the heterozygous state, respectively.

Discussion

The Chilean patient described herein represents the first case of homozygous VP in South America. To date, only 11 such patients have been reported worldwide, indicating that this variant of VP is very rare.

In Chile, VP is the most frequent form of acute porphyria.[11] This phenomenon is, in part, most likely to be attributable to a founder mutation in the *PPOX* gene, designated 1239delTACAC, that was recently described in the Chilean population.[2] Due to another founder mutation in the *PPOX* gene, designated R59W, VP also represents the most frequent type of acute porphyria in South Africa. The situation in Chile and South Africa stands in contrast to other countries in the world where acute intermittent porphyria is usually the most frequently observed acute porphyria.[12]

In both Chile and South Africa the founder mutations identified results in either absent (1239delTACAC) or very low (R59W) residual PPOX activity.[2,13] Therefore, true homozygosity for either of these mutations would probably not be compatible with life. Consequently, in both countries only compound heterozygotes with mild mutations that preserve some residual PPOX activity in *trans* to the respective founder mutation or another null mutation (1330delCT), as in our patient, are likely to survive. This notion is supported not only by our patient but also by previous reports of homozygous VP in which mutations severely abolishing enzyme activity were always heteroallelic to a milder one.[3,14] Although the exact frequency of VP in Chile is unknown, we speculate that the number of unrelated individuals carrying a *PPOX* gene mutation, even if clinically asymptomatic, is sufficient to originate a homozygous form of the disease in the Chilean population.

In homozygous VP, affected individuals carry mutations on both alleles of the *PPOX* gene, leading to a severe enzymatic defect with an earlier clinical onset and a more severe phenotype.[3–7,15] Already in the first year of life our patient showed a pronounced photosensitivity with subsequent development of typical scarring in photoexposed areas of the body. Further, we also observed other clinical features that have been reported in previous cases of homozygous VP such as brachydactyly and short stature. Interestingly, he does not reveal any neurological abnormalities and his intelligence is normal. In accordance with the cases reported previously, neurovisceral crises have not been observed in our patient to date. Still, strict avoidance of porphyrinogenic drugs was counselled to the family.

The paternal missense mutation G232S leads to the substitution of a nonpolar glycine residue by an uncharged polar serine residue at position 232 of the encoded protein. This glycine is evolutionarily strictly conserved in several species, including humans and mice. Thus, such an amino acid change will most probably perturb proper protein function. The importance of this glycine residue at position 232 is further supported by a previous report on a French patient with VP. In that patient, a different nucleotide deviation at the same amino acid position led to the substitution of the glycine residue by an arginine residue, designated G232R.[16]

Interestingly, the maternal mutation 1330delCT has already been identified by us in four other Chilean families (P. Poblete-Gutiérrez, unpublished data), making it tempting to speculate that the recurrent occurrence of this mutation also contributes to the fact that VP is the most frequent type of acute porphyria encountered in Chile. Currently, we are performing extensive

haplotype studies to elucidate if this mutation possibly represents a second founder mutation in VP in the Chilean population.

As increased protoporphyrin levels in the blood are the diagnostic hallmark in erythropoietic protoporphyria, the first biochemical analysis in this case initially led to a wrong diagnosis. Elevated blood protoporphyrin levels were previously also reported in cases of homozygous VP, homozygous porphyria cutanea tarda and homozygous hereditary coproporphyria.[17–19] Thus, raised erythrocyte levels of protoporphyrin appear to be a general consequence of severe recessively inherited enzymatic defects in the porphyrin–haem biosynthetic pathway as is the case in, for example, congenital erythropoietic porphyria and δ-aminolaevulinic acid dehydratase deficiency porphyria, which are always inherited in a recessive fashion.[19]

Due to the discordance of the clinical phenotype and the biochemical findings and with the intent to establish the precise diagnosis, we subsequently decided to study this family on the genetic level. Elucidation of the genetic basis in this family was also important for genetic counselling, considering that for the parents there is a probability of 50% for having a child with at least one mutated allele and 25% for a further offspring with two mutated alleles and a severe phenotype. Therefore, we believe that the case presented herein underlines the overall importance of genetic studies in the porphyrias.

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