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Extended haplotype studies in South African and Dutch variegate porphyria families carrying the recurrent p.R59W mutation confirm a common ancestry

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Summary

Background Variegate porphyria (VP) is due to a partial deficiency of protoporphyrinogen oxidase (PPOX), the seventh enzyme in the haem biosynthetic pathway. Clinically, VP is characterized by photosensitivity and acute neurovisceral attacks that can manifest separately or together in affected individuals. The disease is inherited in an autosomal dominant fashion with incomplete penetrance and *PPOX* gene mutations associated with VP are usually unique to patients and their families. In South Africa, however, VP is highly prevalent as the result of a founder mutation, designated p.R59W. Previous genealogical and haplotype studies showed a link between South African and Dutch carriers of p.R59W and it was suggested that this mutation was introduced to South Africa by Dutch settlers at the end of the 17th century.

Objectives To perform extended haplotype analysis in six South African and Dutch VP families with the p.R59W mutation.

Methods Haplotyping of 13 microsatellite markers flanking the *PPOX* gene on chromosome 1q22-23 and five informative single nucleotide polymorphisms within and around the gene.

Results A core haplotype cosegregated in all families studied.

Conclusions Our data deliver further confirmation that the South African and Dutch VP families carrying mutation p.R59W shared a common ancestor.

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Conflicts of interest
None declared.

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Variegate porphyria (VP; OMIM 176200) is a hereditary metabolic disorder that results from a partial dysfunction of protoporphyrinogen oxidase (PPOX; E.C. 1.3.3.4.), the seventh enzyme in the haem biosynthetic pathway.[1–4]

VP can present with cutaneous and/or neurovisceral symptoms.[1] Cutaneous symptoms are restricted to the sun-exposed areas of the body and comprise photosensitivity and increased skin fragility. Acute neurological attacks can manifest with characteristic neurovisceral and (in severe cases) motor symptoms arising from a biochemically associated autonomic and motor neuropathy. Typically these include abdominal pain, hypertension and tachycardia. These attacks are frequently precipitated by exposure to certain drugs or other porphyrinogenic factors that induce haem synthesis.[1]

VP is an autosomal dominant trait caused by mutations in the *PPOX* gene on chromosome 1q22-23.[1–4] The disease shows incomplete penetrance and mutations are usually unique to individual patients and their families. In South Africa, however,

VP is common due to a single recurrent missense mutation in exon 3 of the *PPOX* gene, designated p.R59W.[5,6] In South African patients with VP, this mutation was shown to be associated with an identical core haplotype defined by two allelic polymorphisms in exon 1 and several microsatellite markers flanking the *PPOX* gene, indicative of a founder effect.[6,7] Further haplotype analysis with two unspecified markers confirmed a link between South African and Dutch carriers of p.R59W, suggestive of a common ancestral background.[8] Genealogical studies linked this ancestral event back to Gerrit Jansz van Deventer and Adriaantje Jacobs van den Berg from Rotterdam, who both came to the Cape of Good Hope from the Netherlands in the 17th century.[9–11] In this study, we performed for the first time an extended haplotype analysis in six South African and Dutch VP families carrying p.R59W and show that a core haplotype cosegregates with the mutation in these families.

Materials and methods

Patients and families

We studied three South African VP families comprising 21 individuals and three Dutch VP families consisting of 15 individuals. Of these, 13 South African and 11 Dutch individuals carried the p.R59W mutation. All participants in this study provided informed consent, in accordance with ethical guidelines set forth by the Human Research Committee of Stellenbosch University, the Maastricht University Medical Center and the Declaration of Helsinki principles.

Genomic DNA (gDNA) was extracted from ethylenediamine tetraacetic acid-anticoagulated blood samples according to standard techniques, as previously described.[12,13]

Analysis of microsatellite markers

Genotyping of 13 proximal or distal microsatellite markers, flanking the *PPOX* gene on chromosome 1q22-23 (Ensembl Gene ID: ENSG00000143224), was performed as previously reported (Table 1).[7,14,15] These markers spanned an interval of approximately 12 cM on the Marshfield Genetics map (http://research.marshfieldclinic.org/genetics/GeneticResearch/compMaps.asp). Polymerase chain reaction (PCR) and fragment length analysis were performed as previously described[14] although in this study fluorescently labelled PCR probes were used and fragment analysis was performed by capillary electrophoresis on an automated sequencer (ABI 3100; Applied Biosystems, Carlsbad, CA, U.S.A.).

Single nucleotide polymorphism marker analysis

In addition to the p.R59W mutation, five informative intra- and extragenic single nucleotide polymorphisms (SNPs), spanning the *PPOX* gene locus (Fig. 1), were genotyped in all individuals using PCR–restriction fragment length polymorphism analyses (Table 2). The SNPs were selected according to their prevalence in caucasian individuals, as reported in the HapMap Project database (http://hapmap.ncbi.nlm.nih.gov/) and by Warnich *et al.*[15] All PCR amplification reactions were prepared to a final

Table 1 Haplotype analysis in the South African (VP-SA-1–3) and Dutch (VP-NL-1–3) families shows a core haplotype in linkage with mutation p.R59W that spans approximately 36 kb

Markers/locus	VP-SA-1	VP-SA-2	VP-SA-3	VP-NL-1	VP-NL-2	VP-NL-3	
D1S303 (155·64 Mb)	177	181	181	181	181	181	
D1S2140 (155·69 Mb)	255	231	251	250	255	255	
D1S1595 (155·69 Mb)	283	259	279	278	283	283	
D1S1653 (157·93 Mb)	96	100	96	100	100	100	
D1S398 (159·64 Mb)	175	151	155	155	155	155	
D1S484 (160·77 Mb)	123	123	123	123	123	123	
D1S2705 (160·86 Mb)	152	142	142	150	150	150	
rs12031437 C/G (161·127 Mb)	**C**	**C**	**C**	**C**	**C**	**C**	
rs2502806 G/A (161·136 Mb)	**G**	**G**	**G**	**G**	**G**	**G**	
rs2301287 c.88-47G/C (intron 2)	**C**	**C**	**C**	**C**	**C**	**C**	*PPOX* gene (161·136–161·141 Mb)
p.R59W (exon 3)	**T**	**T**	**T**	**T**	**T**	**T**	
rs4575098 G/A (161·155 Mb)	**G**	**G**	**G**	**G**	**G**	**G**	
rs4233367 C/T (161·163 Mb)	**C**	**C**	**C**	**C**	**C**	**C**	
D1S1679 (162·36 Mb)	**161**	165	**161**	**161**	**161**	**161**	
D1S1677 (163·56 Mb)	198	198	198	194	194	194	
D1S104 (163·64 Mb)	154	154	154	154	154	154	
D1S426 (165·31 Mb)	128	128	128	137	137	137	
D1S38A05 (- Mb)	175	175	175	173	159	175	
D1S196 (167·60 Mb)	269	269	269	273	269	273	

An extended haplotype in five of the six studied families is shown in bold. An additional core haplotype is shared by families VP-SA-1–3 (underlined). Families VP-NL-1–3 share an additional core haplotype that is shown in italic. The respective values in each cell reflect the size of the allele (in bp) segregating with the mutation at each microsatellite marker. The *PPOX* gene is boxed.

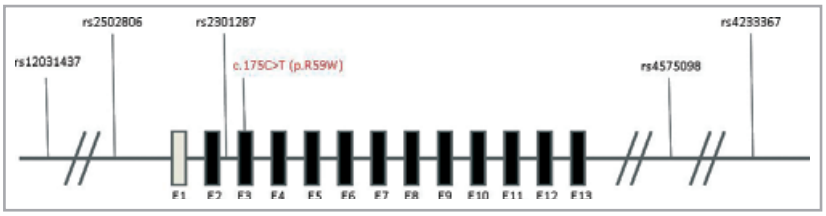

Fig 1. The informative single nucleotide polymorphism markers that were genotyped and their position relative to the *PPOX* gene. All variants are labelled such that +1 corresponds to the first A of the ATG start codon and −1 corresponds to the proximal nucleotide.

volume of 25 μL, containing 15 ng of gDNA, a final concentration of 1× buffer, 1·5 mmol L^{-1} $MgCl_2$, 0·4 mmol L^{-1} dNTPs, 0·4 μmol L^{-1} of each primer, and 0·5 U of BIOTAQ™ DNA polymerase. All reagents were supplied by Bioline (London, U.K.). All amplification cycle reactions were performed as previously described by Warnich *et al.*[15]

Results

In all families studied a common haplotype was detected (Table 1). The core haplotype in the six South African and Dutch families spanned at least 36 kb between the extragenic SNPs, rs12031437 and rs4233367 (Table 1). Furthermore, in five of the six families we found an extended core haplotype that spanned approximately 1·23 Mb between SNP rs12031437 and marker D1S1679 on chromosome 1q23.3 (Table 1, shown in bold).

Haplotyping in two of three South African VP families revealed that mutation p.R59W cosegregated with a core haplotype that spanned approximately 6·47 Mb between extragenic SNP rs12031437 and marker D1S196 (Table 1, underlined). The same core haplotype is present in the third South African family (VP-SA-2, Table 1), except for a different allele at marker D1S1679. In the three Dutch VP families, haplotyping revealed that all mutation carriers shared a common core haplotype spanning approximately 7·38 Mb between markers D1S1653 and D1S426 (Table 1, shown in italic).

Discussion

VP has a high prevalence in the European immigrant population of South Africa (0·003), as compared with the rest of the world.[7] Approximately 90% of all patients with VP in South Africa carry a c.175C>T transition in exon 3 of the *PPOX* gene that results in an arginine to tryptophan substitution, designated p.R59W.[5–7] This missense mutation has been shown to encode a protein with negligible catalytic activity as a result of the disruption of its flavin cofactor binding site.[5,16] As p.R59W occurs at a CpG dinucleotide and methylated cytosines are prone to C>T deamination, we and others sought to elucidate whether this mutation represents a mutational hotspot or if it is the result of a common ancestral event. Previous studies in the South African VP population were suggestive of the latter hypothesis to be true.[5–7] However, regarding the SNPs tested in the earlier studies, the alleles associated with mutation p.R59W were also the common alleles encountered in the South African population of European descent. With regard to microsatellite markers tested, Groenewald *et al.*[7] identified a common haplotype in the majority of the p.R59W carriers, indicating that the South African patients with VP shared a common ancestor.

In genealogical studies, this common ancestor was traced back to a marriage at the end of the 17th century, suggesting that the South African VP founder mutation was brought from Holland to South Africa.[10,11] In support of this notion, p.R59W has been reported during an international conference in 1997 to occur in Dutch VP families. Based on an analysis in these families of two unspecified markers, which cosegregated with the p.R59W mutation, the authors of the abstract deduced a true link between the South African and Dutch p.R59W carriers, supporting the founder effect hypothesis.[8] However, it is not possible to infer from the abstract how many individuals were studied, which markers were used, and where precisely these markers were located in relation to the *PPOX* gene, making interpretation of the findings difficult. Furthermore, a careful re-evaluation of the marker positions reported by Groenewald *et al.*[7] and Frank *et al.*[17] revealed that the genomic localization of the *PPOX* gene and the flanking microsatellite markers used in their studies have undergone several revisions due to physical genomic map updates. Thus, these previous data may need to be re-interpreted.

Based on these new clues, we studied three South African and three Dutch VP families carrying the p.R59W mutation, by extended haplotype analysis using 13 microsatellite markers and five informative SNPs. SNPs in exons 1, 2 and 7 [c.-418G/A, c.-414C/A (rs2301286), c.-413G/T, c.-318G/T, c.-295C/G (rs72714915), c.766C/G (rs12735723)] were also tested, but were not found to be informative in the examined families (data not shown). The data presented in Table 1 show that all families share a common core haplotype that spans at least 36 kb on chromosome 1q23.3. An extended haplotype that spans 1·23 Mb was found at marker D1S1679 in five of the six families studied. The difference in allele size observed in family VP-SA-2 with marker D1S1679 could represent a recent slippage mutational event. The occurrence of this slippage event is confirmed by the conserved haplotype in the South African families, which extends beyond this marker. Thus, we hypothesize that the core haplotype for all families studied indeed spans 1·23 Mb. The markers and SNPs used for haplotyping were all informative in our families, therefore, the coincidental sharing of identical alleles seems highly improbable. We strongly believe that these data indeed provide strong support for the Dutch origin of the South African p.R59W mutation. This notion is further supported by the fact that the three Dutch VP families studied all originate from a geographical region not far from the city of Deventer, the Netherlands (K.t.V.; personal communication).

Table 2 Polymerase chain reaction–restriction fragment length polymorphism assays utilized for the genotyping of the informative single nucleotide polymorphism markers, with corresponding conditions

Variant	rs number	Region	Primer sequence	Enzyme	Genotype	Size of fragment (bp)	MAF Dutch (n = 16)	MAF SA (n = 21)
c.8584C/G	rs12031437	5′ upstream	5′-CCT AGC GCC CAC TAC CAA AGC-3′	HaeIII	CC	271,104	0·313	0·175
			5′-TGC TTG CCT CAA ACC TCA GC-3′		CG	375, 271, 104		
					GG	375		
c.1079G/A	rs2502806	5′ upstream	5′-ACG TGC TGT TCT ACC AAC TG-3′	HaeIII	GG	370, 232	0·281	0·310
			5′-CAG TGT TTG TCA ACA GTG G-3′		GA	602, 370, 232		
					AA	602		
c.88-47G/C	rs2301287	Intron 2	5′-CTT CTG GAG CGC AGG TTG TC-3′	MspI	CC	166, 140, 87, 60, 49, 22	0·313	0·167
			5′-CCT CCC CTA AAC TCT ATT CC-3′		GC	306, 166, 140, 87, 60, 49, 22		
					GG	306, 87, 60, 49, 22		
c.1434+14425G/A	rs4575098	3′ downstream	5′-CCT GCC TGG GGA AGT TGT TC-3′	SspI	AA	200,199	0·219	0·071
			5′-GCC AGG GTT CCT GGA GTG TG-3′		AG	399, 200, 199		
					GG	399		
c.1434+22070T/C	rs4233367	3′ downstream	5′-GGC GTG GCT GTA GGA ACA GG-3′	SmaI	CC	279, 148	0·281	0·310
			5′-GGA AGG AAG GAG GGG CTC TG-3′		CT	327, 279, 148		
					TT	327		

MAF, minor allele frequency; SA, South African.

Estimation of the age of a founder is strongly influenced by assumptions made about recombination and mutation history, and mostly cannot be verified. Therefore, such calculations remain difficult and are often not very precise.[18] Genealogical studies in the South African families with the p.R59W mutation suggest that the founder mutation arose at the end of the 17th century. In line with this notion, van Schothorst *et al.*[19] identified a founder effect in the *PGL1* gene in Dutch families with hereditary paragangliomas of the head and neck. The common haplotype cosegregating with the *PGL1* founder effect spanned a region of approximately 2 Mb and the common ancestral mutational event was traced back to 1776, which is about 90 years later than the supposed origin of the South African VP founder mutation.[19,20] Considering the report by van Schothorst *et al.* and our experimental data, the smaller core haplotype of 1·23 Mb identified in the six VP families in our study would rather suggest that the South African *PPOX* founder mutation arose earlier than the 17th century. Thus, we propose that p.R59W is most likely to be older than hitherto assumed and indeed has arisen prior to the documented marriage of Gerrit Jansz van Deventer and Adriaantje Jacobs van den Berg in 1688.

Here, we were able to compare VP individuals from one country in which a conserved haplotype cosegregates with a recurrent mutation with the corresponding haplotype of VP families residing in the country in which the common ancestor probably lived. Our results and critical re-evaluation of previously published data from other groups deliver confirmatory evidence that the South African and Dutch carriers of mutation p.R59W share a common ancestor.

What's already known about this topic?

- It was assumed, based on historical data and pedigree analyses, that the South African and Dutch patients with variegate porphyria who carry the recurrent mutation p.R59W might share a common ancestor and that the mutation was introduced to South Africa by Dutch settlers.

What does this study add?

- A core haplotype cosegregates in all South African and Dutch variegate porphyria families studied here. Our data confirm that all carriers of mutation p.R59W indeed have a common ancestor and suggest that this mutation might be older than previously assumed.

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