it ponders how best to extend these services to larger populations.

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Linkage Disequilibrium Analysis in a Recently Founded Population: Evaluation of the Variegate Porphyria Founder in South African Afrikaners

To the Editor:

Variegate porphyria (VP; MIM 176200) is relatively rare in most populations, but it is one of the most common autosomal dominant genetic disorders in South Africa (Dean 1971). The disease is characterized by a diversity of symptoms, including a variable picture of skin symptoms and acute attacks. By means of genealogical studies, the history of VP in South Africa can be traced back to the marriage of a Dutch couple in the Cape of Good Hope in 1688 (Dean 1971). This, along with the high prevalence of VP in South Africa, has promoted the founder-gene hypothesis for VP in this country.

Mutations in the protoporphyrinogen oxidase gene (PPOX), the seventh enzyme in the heme biosynthetic pathway, have been shown to be causative of VP (Deybach et al. 1996; Meissner et al. 1996; Warnich et al. 1996*b;* Lam et al. 1997). This gene has been mapped to chromosome 1q22 by FISH (Taketani et al. 1995), and the position has been confirmed by linkage analysis (Roberts et al. 1995). Three mutations have been described in South African VP patients, but one of these, a C→T transition at nucleotide position 452 (R59W), was found in ~90% of patients (Meissner et al. 1996; Warnich et al. 1996*b*). This mutation spanned a CpG dinucleotide, and, to exclude the possibility of a recurrent mutation, intragenic haplotype studies were undertaken. Mutation R59W was shown to be associated with one of four potential haplotypes defined by two diallelic polymorphisms in exon 1 (Warnich et al. 1996*b*), thus supporting the founder hypothesis. However, this was not totally conclusive evidence, since the alleles associated with the R59W mutation are also the common alleles in the normal population for each of the polymorphisms (L. Warnich, unpublished data).

If the high incidence of a genetic disease in a particular population is due to a founder effect, most cases studied

Table 1

Allele Frequencies and LD Results for the Most Common Alleles of the 15 R59W-Mutation Chromosomes and for 88 Normal Chromosomes

Distance to Next Marker Locus[a] (cM)	Marker Locus	Frequency of Chromosome		χ^2 (P)[d]	P_{excess}
		Normal[b]	Disease[c]		
	D1S2140	.34	.47	.8805 (.3481)	.1908
.7					
	D1S303	.60	.87	3.892 (.0485)	.6648
.0					
	D1S1595	.18	.33	1.8126 (.1782)	.1852
3.0					
	D1S1600	.17	.53	9.7301 (.0018)	.4374
.0					
	D1S1653	.09	.53	19.1183 (<.0001)	.4867
1.5					
	D1S398	.33	.53	2.3122 (.1284)	.3040
2.8					
	D1S2707	.30	.93	21.2613 (<.0001)	.9044
1.2					
	D1S484	.30	.93	21.9526 (<.0001)	.9054
1.2					
	D1S2705	.28	.93	22.9604 (<.0001)	.9069
.0					
	D1S1679	.16	.67	18.4746 (<.0001)	.6036
4.8					
	D1S104	.18	.73	20.1534 (<.0001)	.6741
.0					
	D1S1677	.38	.80	9.4091 (.0022)	.6800
2.2					
	D1S426	.10	.53	17.2799 (<.0001)	.4802
.6					
	ATA38A05	.15	.47	11.7399 (.0006)	.4524
3.0					
	D1S196	.26	.47	2.6167 (.1057)	.2779
		.30	.47	1.7253 (.1890)	.2430

[a] Obtained from the Généthon and CHLC databases and from the sex-averaged map of the Marshfield Medical Research Foundation (http://www.marshmed.org/genetics/).

[b] Includes chromosomes from 27 individuals who were relatives of the families by marriage, as well as 34 normal chromosomes of affected parents.

[c] Calculated by counting, with use of the oldest R59W-mutation chromosome in each family.

[d] Calculations for statistical significance of data were done for the most common allele of each marker, in a pairwise manner using the χ^2 test with 1 df and no correction (Dawson-Saunders and Trapp 1990, pp. 150–151).

should have preserved alleles at closely linked loci, presenting the original founder chromosome (Hästbacka et al. 1992). In recently founded populations, comparable to the South African Afrikaner population, a conserved area of ~5–20 cM can be expected (Houwen et al. 1994). In the present study we have used linkage disequilibrium (LD) and haplotype analyses to investigate the single-founder hypothesis for VP in South Africa and to evaluate the use of the Afrikaner population for future LD mapping studies.

In the current study, 15 nuclear families with the R59W mutation (Warnich et al. 1996*b,* 1996*c*) were extended to include 132 members, 58 of whom were affected. The 15 families were unrelated to the second-degree and included one four-generation, seven three-generation, and seven two-generation pedigrees. A sequence-tagged site (STS) at the 3′ end of the PPOX gene was used to screen the CEPH YAC libraries. The primers used were D38537-F (5′-GGG AGT TGC TGT TAA TGA CTG T-3′) and D38537-R (5′-GCA ATT TTT ATT TTC ATG AAT GAG-3′). One of the positive YAC clones, 910_C_8, showed an unambiguous hit for two microsatellite markers, D1S2705 and D1S484. Thirteen other microsatellite markers flanking these markers (listed in table 1) and spanning ~21 cM, were subsequently selected from the Généthon (http://gdbwww.gdb.org) and Cooperative Human Linkage Center (CHLC; http://www.chlc.org) databases.

Haplotypes were constructed in each family under the assumption that there were the minimum number of recombinations. Disease-associated haplotypes were identified from alleles that were transmitted from af-

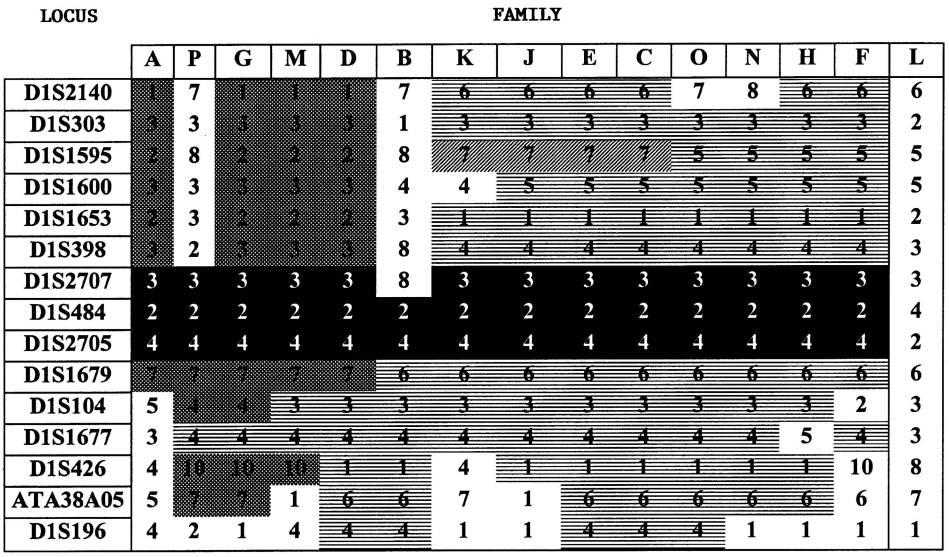

Figure 1 Disease-associated haplotypes of each of the 15 families. The oldest R59W-mutation chromosome in each family was used. The patterned sections indicate the regions conserved between the affected haplotypes of the different families.

fected parent to affected offspring, in each pedigree. Crossover events on the disease-associated chromosomes of two different individuals placed the PPOX gene telomeric of marker D1S2707 in one of them and centromeric of marker D1S2705 in the other. These observed recombinations delimit the location of the PPOX gene to a 2.4-cM region between markers D1S2705 and D1S2707, and they thus represent the highest-resolution genetic mapping of the gene yet.

LD studies were done by calculation of the statistical factor P_{excess} for the dominant disease-associated allele of each marker (Hastbäcka et al. 1992). The number of generations since the introduction of the VP gene was taken, on the basis of available genealogical records, as 12. The data generated are shown in table 1. The strongest association was observed at D1S2707 (allele 3), D1S484 (allele 2), and D1S2705 (allele 4), yielding P_{excess} values of .9044, .9054, and .9069, respectively. Two-point linkage analysis (data not shown) also illustrated close linkage of the disease locus to these three markers, with LOD scores of 9.37, 12.68, and 10.74 at recombination fraction (θ) values of .031, .023, and .014, respectively.

The extended haplotype associated with the R59W mutation in each family is shown in figure 1. Allele 3 was found to be conserved for marker D1S2707 in all of the families with the R59W mutation, except family B. Alleles 2 and 4 were detected for the markers D1S484 and D1S2705 in all of the families, except family L. Since the latter two markers are the nearest to the gene, we propose that family L most likely has an independent R59W mutation. It could thus be deduced that a small percentage of families with the R59W mutation will represent either recurrent mutations at the CpG hot spot or recent importations of the gene. It is interesting to note that the haplotype of family L could not be distinguished from the haplotypes of the other families when diallelic intragenic markers were used (Warnich et al. 1996*a,* 1996*b*). As shown in figure 1, two distinct subhaplotypes were observed surrounding the core haplotype—namely, the haplotype represented by families A, P, G, M, and D and the haplotype depicted by families B, K, J, E, C, O, N, H, and F. It is thus expected that variations in these subhaplotypes can be ascribed to earlier historical recombination events and/or mutations at some loci. We thus believe that, although there are apparently two groups of haplotypes that differ in flanking markers, they both descend from the same founder, because they share the same core haplotype, as has also been found in other founder-related studies (e.g., Labuda et al. 1996). There is also no geographical or genealogical evidence for two independent introductions of the VP gene in South Africa. Furthermore, a contiguous area of 10 cM (spanned by markers D1S2707 and ATA38A05) displayed highly significant ($P < .005$) LD values (table 1). These results are in agreement with data from other populations, in which the historical age of the founder effect was estimated to be 12 (Labuda et al.

1996), 8–12 (Puffenberger et al. 1994), and 5–12 generations (Houwen et al. 1994). In two other studies based on South African families, a conserved region of ~8 cM was found in two long-QT families with continuing genealogical studies already extending back through nine generations (de Jager et al. 1996), whereas an ancestral haplotype of 11 cM was found in 11 of 14 South African families with keratolytic winter erythema (Starfield et al. 1997).

Large shared segments are expected around disease genes in recently founded populations such as the Afrikaner population, and it was thus predicted that genome searches for these segments could be performed with only a few hundred markers (Houwen et al. 1994). This potentially powerful approach of LD mapping has, however, not been widely used in the past, one of the reasons being the scarcity of suitable founder populations. The next phase of gene mapping—namely, the mapping of complex traits—may especially benefit from conserved-haplotype detection and LD mapping in isolated populations (Lander and Schork 1994). Although the Afrikaner population is known to have founder effects for a number of genetic disorders (Jenkins 1990), it has rarely been exploited for the actual mapping of genes in the past.

From the results of the present study we conclude that the high frequency of the R59W mutation in South Africa could probably be ascribed to a common ancestor and is not due to multiple mutation events on a common haplotype. The current study thus not only provides the first firm molecular evidence for a founder hypothesis for VP but also shows that the South African Afrikaner population is a valuable candidate population for future mapping studies using LD analyses.

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Worldwide Distribution of a Common Methylenetetrahydrofolate Reductase Mutation

To the Editor:

Methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is needed for methionine synthase to convert homocysteine to methionine. A reduction in MTHFR activity, such as that caused by the C→T missense mutation at position 677 of the MTHFR cDNA (C677T), which produces a thermolabile form of the enzyme, results in increased plasma homocysteine (Frosst et al. 1995). Homozygotes for the C677T mutation may have an increased risk of cardiovascular disease (Frosst et al. 1995) and neural tube defects (Wilcken 1997).

Folate is an important cofactor in the conversion of homocysteine to methionine; therefore, C677T homozygotes may require more folate for thermolabile MTHFR to function adequately. Insufficient folate intake during pregnancy can cause neural tube defects (Smithells et al. 1980); however, the role of folate in vascular disease is not well established.

Previous studies of the C677T mutation have concentrated on European populations. The allele frequency in Europeans is 24%–40% (van der Put et al. 1997), 26%–37% in Japanese populations (Papapetrou et al. 1997; Sohda et al. 1997), and ~11% in an African American population (Stevenson et al. 1997). We have screened 881 unrelated individuals from 16 worldwide populations for the presence of the C677T polymorphism (table 1). The populations studied were chosen to complement the existing data set of the worldwide C677T allele frequency. The samples used in this study are anonymous and have been collected for ongoing studies of human genetic diversity. New primers used in this study (forward: 5′-TTT GAG GCT GAC CTG AAG CAC TTG AAG GAG-3′; and reverse: 5′-GAG TGG TAG CCC TGG ATG GGA AAG ATC CCG-3′) gave a PCR product of 173 bp and fragments of 125 and 48 bp after digestion with *Hin*fI.

The MTHFR polymorphism was found in every population tested. Unlike other mutations, such as factor V Leiden (Rees et al. 1995), Δccr5 (Martinson et al. 1997), and the HLA-H C282Y and H63D hemochromatosis mutations (Merryweather-Clarke et al. 1997), which are common only in Europe, the C677T mutation has a relatively high frequency throughout the world.

The prevalence of the C677T mutation is lowest in Africa (6.6%) compared with Europe and Asia, although there are unexpected findings such as 44.9% in an indigenous Brazilian population and 4.5% in a group of Sri Lankans. All of the populations in this study were in Hardy-Weinberg equilibrium.

Both myocardial infarction (Murray and Lopez 1996) and neural tube defects (Sever 1982) are believed to be more prevalent in Europeans than in Africans. In developed countries where most people are of European origin, the incidence of myocardial infarction is >5 times greater than in sub-Saharan Africa, and the prevalence rate for neural tube defects in whites is 1.5 times higher than in blacks in U.S. populations. Although environmental factors and other genetic factors clearly play an important role, the geographical pattern of the C677T allele frequency supports the hypothesis that it is a risk factor for vascular disease and neural tube defects.

The high frequency of the C677T mutation worldwide is surprising if homozygotes have an increased risk of disease. One possible explanation is that either heterozygous or homozygous mutant genotypes may, in certain circumstances, have a selective advantage over normal individuals. Two such theories have been suggested: a decreased risk of C677T homozygotes for colon cancer (Chen et al. 1996) and a beneficial effect to heterozygotes during times of starvation (Engbersen et al. 1995). In the second hypothesis, the thermolabile form of MTHFR is believed to decrease homocysteine remethylation so that the 1-carbon moieties of derivatives remain available for the vital synthesis of purines and thymidine.

The increased incidence of disease caused by the C677T mutation may only have been mildly deleterious to human populations. This could allow the C677T mutation to behave as an effectively neutral polymorphism so that demographic effects such as genetic drift could outweigh slight negative selection. Populations that had high frequencies of the C677T mutation and have been small in the past would be most susceptible to this effect (Thompson and Neel 1997).