Letters to the Editor

Am. J. Hum. Genet. 62:1248–1252, 1998

Segregation of a *PRKCG* Mutation in Two RP11 Families

To the Editor:

Retinitis pigmentosa (RP) is a group of inherited neurodegenerative disorders of the retina. RP patients experience night blindness and tunnel vision (constricted visual fields) at an early stage and may become completely blind in the advanced stages of the disease (Bird 1995). RP is both clinically and genetically heterogeneous. The autosomal dominant subgroup of RP (adRP) can be caused by mutations in at least two genes, *rhodopsin* (3q21) and *peripherin* (6p12), and in seven other loci mapped by linkage analysis (Xu et al. 1996). We mapped the chromosome 19q locus (also known as "RP11" [MIM 600138; http://www.ncbi.nlm.nih.gov/htbin-post/Omim]; McKusick 1994) in four British families (Al-Maghtheh et al. 1996). A Japanese family (Xu et al. 1995) and three American families (McGee et al. 1997) also had linkage to this locus, suggesting that RP11 is a major locus for adRP (Al-Maghtheh et al. 1996). Interestingly, the phenotype in these families is characterized by "bimodal expressivity"; symptomatic RP patients have a relatively early onset of the disease, whereas some obligate disease-gene carriers are indistinguishable from normal individuals. Recombination events in the families with linkage have refined the localization of the RP11 gene to a 5-cM interval between markers D19S572 and D19S418, in the telomeric region of chromosome 19q13.4 (McGee et al. 1997). Among the positional candidate genes and expressed sequence tags mapping in this region is the gene *PRKCG,* a member of the protein kinase C (PKC) gene family (Hug and Sarre 1993).

PKC is a multifunctional family of closely related serine/threonine protein kinases. PKCs function in a wide variety of cellular processes, such as membrane-receptor signal transduction and control of gene expression. Various PKC isoenzymes are expressed in a tissue-specific manner (Hug and Sarre 1993). The PKC gene family has been shown to be expressed widely in the retina, although it is not clear which isoenzymes are involved in any particular retinal cell type (Newton and Williams 1993; Ohki et al. 1994). The observation by Newton and Williams (1991; 1993) that PKC phosphorylates rhodopsin in a light-dependent manner suggests the involvement of these kinases in desensitization of the photopigment. In *Drosophila* phototransduction, a PKC isoenzyme known as "eye-PKC" was found to be exclusively expressed in photoreceptor cells (Schaeffer et al. 1989). A mutation in eye-PKC has been shown to be responsible for the recessive *ina*C (inactivation no after potential C) *Drosophila* mutant, which exhibits photoreceptor deactivation and retinal degeneration (Smith et al. 1991). However, the specific-tissue expression and function of the PKCγ isoenzyme in the human retina is not yet clearly defined. Several reports have shown PKCγ to be expressed in rabbit, rat, frog, and goldfish retinas (Osborne et al. 1992). This expression of PKCγ is confined to amacrine and ganglion cells. No expression has been detected in the photoreceptors. This does not exclude the possibility that PKCγ expression in photoreceptors is at levels below the sensitivity of the detection method. Therefore, in addition to its being a positional candidate, there is circumstantial evidence to implicate *PRKCG* as a candidate for the RP11 gene. We have recently characterized the genomic structure of the *PRKCG* gene. The gene has 18 exons, and the intron-exon boundaries, including splice-site consensus sequences, have been defined. Amplification primers for each exon are listed in table1.

In this study, we screened the *PRKCG* gene for mutations, in RP11 families. All 18 exons and the 1.5-kb 5′ UTR were screened by both heteroduplex analysis (Keen et al. 1991) and direct genomic sequencing. We identified a point mutation that segregates with RP in two families, RP1907 (Al-Maghtheh et al. 1996) and ADRP24 (denoted as "family 2" by Moore et al. 1993; also see the legend to fig. 3). Another two isolated RP patients with a family history indicating dominant disease were also found to have the same mutation. This mutation was a C→A transversion, which substitutes a serine for an arginine residue at codon 659 of the *PRKCG* gene within the C4 catalytic domain (fig. 1). This residue is conserved in all known *PRKCG* genes, including human, bovine, and rat genes. The presence and segregation of this mutation (the sequence of which is shown in fig. 2) was also confirmed by digestion of the PCR product of exon 18 by the restriction enzyme

Table 1

Primer Sequences (5′–3′) Used in Amplification of the 18 *PRKCG* Exons, and PCR Conditions for each Amplification

Forward Primer	Reverse Primer	[Mg^{++}], Annealing Temperature
1F, agaaaggcaggatcctggtc	1R, cggcgtgataggagtctgca	1.5, 65°C
2F, ttggacacctgggccctgc	2R, ctgagggtcccaggagcc	1.5, 65°C
3F, gctggactaatccatgcctc	3R, aggagaaattgggacggacg	1.3, 60°C
4F, gctgacctagagagcaaggc	4R, gctttggaagggccctggca	1.5, 65°C
5F, tgaggtgctacccgcagctt	5R, acaagtgccttgggtcagcc	1.5, 64°C
6F, ctctaacccgtcacactctt	6R, tctgtcagctgtcattgcct	1.5, 60°C
7F, gccatgagctcggctctgca	7R, gtaatattgcgctccatcccc	1.5, 65°C
8F, tgcctctcccatgggtgc	8R, aaggccagctctgaaccgt	1.5, 60°C
9F, ctatctatcgccatggct	9R, aactgcctccattcaacg	1.5, 58°C
10F, gagcatttccttatcggctg	10R, aaccagaaatctgaccttccc	1.3, 55°C
11F, aggtcctgtaccactgggtt	11R, atcccaacgcagatgtccag	1.5, 65°C
12F, gtagatggatcccgcctcta	12R, acgtcagaaggtcagtggct	1.0, 58°C
13F, agccactgaccttctgacgt	13R, gtgttgagttcagcagtctag	1.5, 60°C
14F, ctagactgctgaactcaacac	14R, taagggatctcaaagcgtg	1.5, 56°C
15F, gcacttaacgtgggtagcg	15R, tagccaagccagcttctcc	1.5, 56°C
16F, gcatgtccctgactctctat	16R, agtgacttcaggaatgggag	1.5, 60°C
17F, atgtacctgtccggcact	17R, accaggtttttgttgcctgg	1.5, 55°C
18F, ctcggagctgcttaactttcc	18R, acgttggggacacctagtgg	1.5, 64°C

*Aci*I (fig. 3). This mutation was absent from 500 normal control chromosomes. Haplotype analysis in these two families and in the two single adRP cases with markers flanking the *PRKCG* gene reveals a founder effect, with disease-gene carriers of each pedigree sharing the same haplotype over an interval >5 cM, including the entire RP11 region (this haplotype would be expected to have a frequency of 10^{-5}, on the basis of allele frequencies in the U.K. population; see fig. 3). This could imply that the mutation on the ancestral chromosome took place relatively recently, although no genealogical link exists between these families during the past 160 years.

Close genetic association between the RP11 phenotype in these families and the Arg659Ser mutation is clearly evident. In conjunction with the involvement of an eye-PKC gene in retinal degeneration in *Drosophila* (Smith et al. 1991), this suggests that this mutation could be a cause of RP in these families. As stated above, one form of PKC is known to phosphorylate rhodopsin, perhaps as part of an adaptation mechanism. If the enzyme

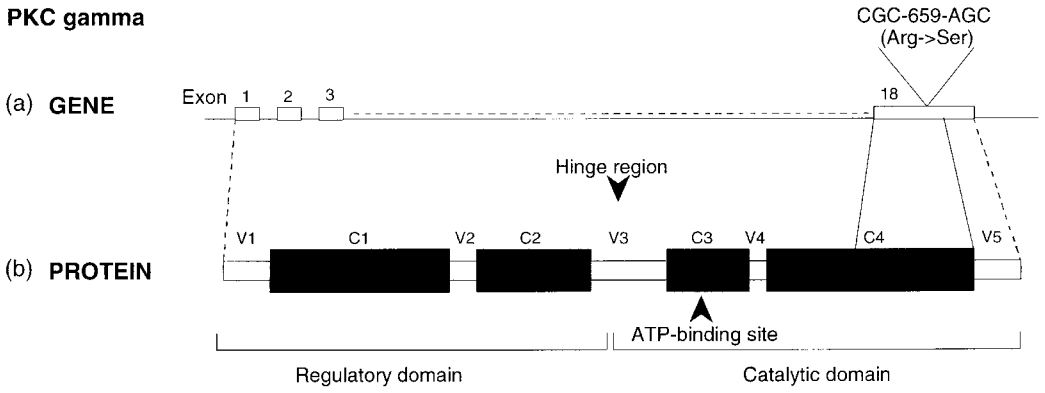

Figure 1 Diagrammatic representation of the position of the Arg659Ser mutation within exon 18 of (*a*) the DNA sequence and (*b*) the domain structure of PKCγ, as described by Hug and Sarre (1993). C1–C4 are the conserved domains, which are flanked and separated by the variable domains, V1–V5. V1–C2 represents the regulatory domain, which is separated from the catalytic domain (C3–V5) by V3 or the hinge region. C3 contains the ATP-binding site.

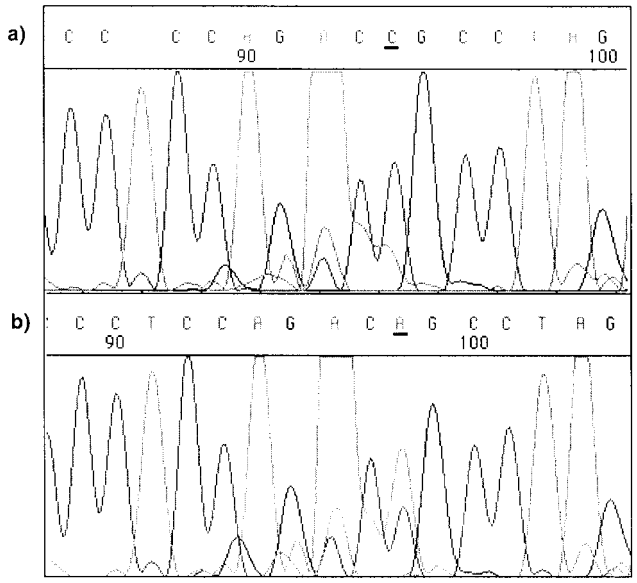

Figure 2 Comparison of sequencing data from (*a*) a normal and (*b*) a heterozygous mutant *PRKCG* exon 18 sequence. A C→A transversion (*underlined bases*) changes codon 659 from arginine (CGC) to serine (AGC).

involved were *PRKCG,* a mutation in this gene might be expected to have an effect on photoreceptors' adaptation or recovery in response to light flashes or to high or low light levels. Mutations in rhodopsin kinase and arrestin, both similarly involved in the restoration of resting potential in photoreceptors after light stimulus, have been shown to cause a rare recessive form of congenital stationary night blindness (CSNB), known as "Oguchi disease" (Fuchs et al. 1995; Yamamoto et al. 1997). As in the case of Oguchi disease, one might expect a mutation in PKCγ, an enzyme, to cause a recessive rather than a dominant phenotype. However, a mutation that affects the ability of the photoreceptor to restore rhodopsin sufficiently to an inactive state might lead to a background level of constitutive activation, which has been hypothesized, for several rhodopsin mutations, as being the cause of a dominant form of CSNB (Rao et al. 1994). Furthermore, a mutation in the β-subunit of phosphodiesterase (*PDE*β), an enzyme, was also found to cause a dominant form of CSNB (Gal et al. 1994). Mice deficient in PKCγ have been created by use gene-knockout technology. These animals exhibit mild deficits in spatial and contextual learning, but no mention is made, in the published description, of defective vision (Abeliovich et al. 1993; Chen et al. 1995). If *PRKCG* is the RP11 gene in these families, then it could be argued that these mice are not a true model for a human *PRKCG* missense mutation—in which the presence of the abnormal protein is likely to cause the disease, rather than the absence of normal enzyme activity. Alternatively, given the incomplete penetrance of the RP11 phenotype in some patients, this may be the result of a genetic background that protects against retinal degeneration in the mouse inbred strain.

Nevertheless, the entire *PRKCG* gene has been sequenced in three other families with RP11 linkage, and no disease-causing mutation has been found. Southern blots digested with various restriction enzymes and hy-

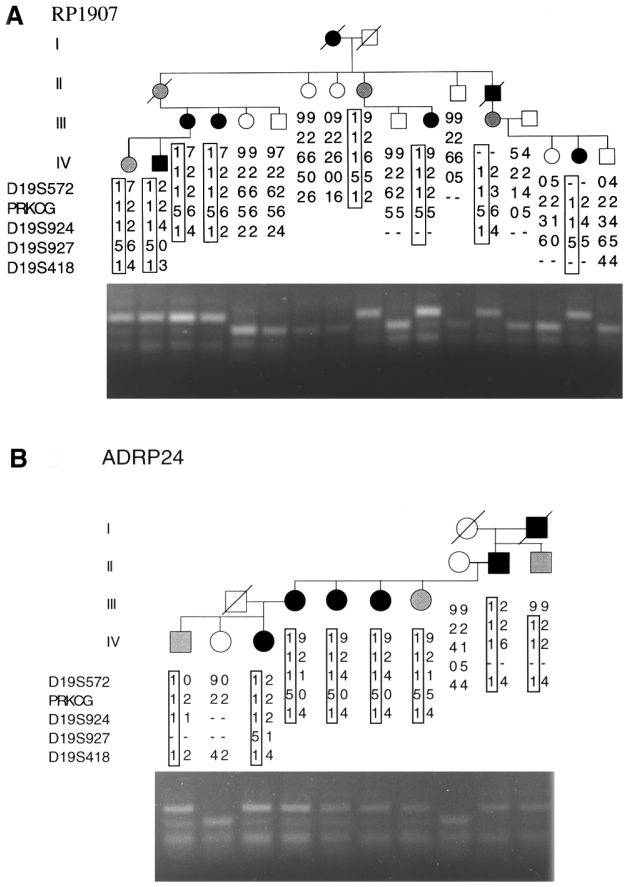

Figure 3 Pedigrees of (*a*) RP1907 and (*b*) ADRP24. (On the basis of the characteristic bimodal expressivity phenotype, this family was thought to be an RP11 family, but it gave a nonsignificant LOD score [0.6 at recombination fraction 0] with 19q markers.) Blackened symbols denote affected individuals; unblackened symbols denote normal individuals; and gray-shaded symbols denote asymptomatic disease-gene carriers, on the basis of haplotype analysis. The linked haplotype for 19q markers, including the *PRKCG* mutation, are shown below the pedigrees. Allele 1 for *PRKCG* represents the C→A mutation at codon 659, whereas allele 2 represents the normal sequence. Restriction digests of exon 18 PCR products, which demonstrate absence of the *Aci*I site in mutated alleles, are also shown, with fragment sizes of 110 bp (for the mutated allele), 81 bp (for the normal allele), and 49 bp.

bridized with a *PRKCG* cDNA probe also failed to disclose any rearrangements in these families. It is possible that, in these families, another, probably common, disease-causing mutation could have been missed or could lie in either upstream or downstream promoter/regulatory regions or introns that have not yet been fully characterized. A search for other RP patients carrying the Arg659Ser or other mutations in the *PRKCG* gene, in other laboratories involved in RP research, would further substantiate the involvement of this gene in this common form of dominant RP. Alternatively, the absence of causative mutations in a proportion of RP11 families might imply microheterogeneity, a hypothesis proposed to explain the apparent lack of mutations in *RPGR* in >50% of families with RP3 linkage (Fujita et al. 1997). Nevertheless, it also remains possible that the Arg659Ser is a rare allelic variant of the *PRKCG* normal sequence and is in linkage disequilibrium with the disease allele in these families. A search for other RP patients carrying this change may reveal a wider founder effect, which could further refine the locus for this common form of dominant RP.

Finally, a number of apparently non–disease-causing *PRKCG* polymorphisms were also detected by this study. One particularly interesting change in exon 18 (TTT647TTC) was found to segregate perfectly with the disease phenotype in another RP11-linked family (data not shown). Despite the fact that this change was absent from the 500 normal control chromosomes, it does not alter the amino acid sequence. Other neutral nucleotide-sequence changes (AAT189AAC and GGC411GGT), non–disease-causing amino acid changes (R141C, H415Q, and A523D), and a T-nucleotide insertion in intron 16, 39 bp downstream of exon 16, were also found. None of these changes were found to segregate with the disease phenotype in the families in which they were found, and each was also present in normal individuals.

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Diversity of Cystic Fibrosis Mutation-Screening Practices

To the Editor:

As the most common lethal recessive disorder in North America, cystic fibrosis (CF) has been discussed as a potential target of nationwide carrier screening ever since the cloning of the causative *CFTR* gene in 1989 (Kerem et al. 1989). Practical implementation of such proposals has been impeded, however, by the extreme mutational heterogeneity of *CFTR* alleles within the carrier population, with upwards of 600 different mutations identified thus far (Zielenski and Tsui 1995). Aside from the most prevalent mutation, ΔF508, most of these alleles are extremely rare or even "private," although approximately half a dozen account for 1%–3% each of carriers in the general Caucasian population, and other subsets are relatively more common in other ethnic groups. Therefore, analysis of numerous mutations is required to reach satisfactory carrier detection levels. Studies in the general Caucasian population have revealed that at least 15–20 mutations must be tested to detect >80% of obligate carriers (Cystic Fibrosis Genetic Analysis Consortium 1994). An exception is the Ashkenazi Jewish community, in which the analysis of only seven mutations can detect ~97% of carriers, making high-sensitivity carrier screening more readily attainable (Eng et al. 1997). The range of mutations tested has steadily increased over the years, but there are still no authoritative national guidelines specifying a minimum number of CF mutations acceptable in a population screening panel (i.e., screening for unknown mutations in individuals with no family history and thus no index case with a known mutation). In the early months shortly after the cloning of the *CFTR* gene, screening for four to seven of the most common mutations was the norm, but as the total number of cataloged *CFTR* mutations has expanded, and additional ethnic-specific mutations have been identified, the size of available test panels also has increased.

The only way (theoretically) to detect all possible mutations would be to sequence the entire gene, but the cost of that approach would be prohibitive for population screening. Therefore, laboratories developing *CFTR* mutation tests have had to be creative in their choice of technique and their selection of appropriate mutation panels. Techniques used have included PCR amplification with electrophoresis and/or restriction endonuclease digestion of the products, dot blot hybridization with allele-specific oligonucleotide (ASO) probes, reverse dot blots, pooled ASO and probe-elution strategies, conformational analysis, and (still under development) various types of oligonucleotide microarrays (DeMarchi et al. 1994; Ravnik-Glavac et al. 1994; Wall et al. 1995; Shuber et al. 1997;). In the absence of guidelines, the choice of number and type of mutations in CF test panels has been left to the discretion of the individual laboratories.

Like the mutations themselves, the number of laboratories offering such testing has been increasing over the years, yet there has been no systematic survey of the range of CF mutations being tested across the country. For several years, the American College of Medical Genetics/College of American Pathologists (ACMG/CAP) Biochemical and Molecular Genetics Resource Committee has been administering a molecular genetics proficiency testing program for CF and other disorders, providing a means to collect such data. The 45 laboratories currently participating in the CF challenges represent most of those offering such testing in the United States.

As part of our March 1997 proficiency challenge, we included a survey questionnaire to ascertain the range of mutations tested by the laboratories offering cystic fibrosis mutation screening. Forty-three (96%) of the 45 participating laboratories subscribing to the CF challenge responded to this survey. Of those, there was a wide range in the number of mutations tested, from just 1 to 70. One laboratory offers testing for ΔF508 only, and one or two laboratories each offer testing for 5, 7,

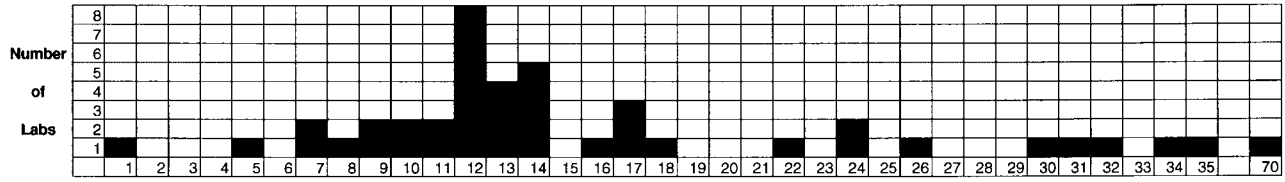

Figure 1 Number of CFTR mutations screened by participating laboratories.

8, 9, 11, 16, 17, 18, 22, 24, 26, 30, 31, 32, 34, or 70 mutations, with the median number centering around 12–14 mutations (fig. 1).

Aside from the absolute number of mutations, other notable findings in some of the laboratories' screening panels emerged from this survey. For example, 13 of the laboratories do not test for R117H, which many would feel is one of the relatively more common and important mutations, associated with both classical CF and congenital bilateral absence of the vas deferens (Jezequel et al. 1995). And only two of the other laboratories specifically indicated that they include testing for the intronic 5/7/9T polymorphism that markedly affects phenotypic expression of R117H and some other *CFTR* mutations (Kiesewetter et al. 1993; Chillon et al. 1995). Three laboratories do not include the prevalent W1282X Ashkenazi Jewish mutation, which would seem essential for any test panel directed at a North American urban population. Some of the laboratories included written comments that their panels cannot distinguish between mutations ΔF508 and ΔI507 (both 3-nucleotide deletions of adjacent codons) or G551D and R553X (two of the more common point mutations), which our ACMG/CAP committee already suspected, based on the results of our earlier CF challenges.

It is important to note that our survey addressed neither which laboratories are using their panels for testing of known mutations in at-risk relatives, as opposed to random proband or population screening, nor which particular ethnic groups, if any, are being targeted. These more limited and predefined applications would allow for more narrow test panels. In the same vein, it is possible that some of the laboratories in our survey perform CF mutation analysis primarily for research purposes. (The confidential structure of the CAP survey program precludes identification or contact of the individual laboratories by the resource committee.) Finally, although we believe this survey to be fairly comprehensive, there are undoubtedly some additional academic and/or commercial laboratories involved in CF testing that did not participate or respond.

In April 1997, the NIH convened a Consensus Conference on Cystic Fibrosis Testing. The consensus panel recommended that population-based CF screening be offered to all pregnant couples and those contemplating pregnancy in a program to be phased in over time (NIH Consensus Statement 1997). Without specifying a precise number of mutations, they stated that any test panel used should be capable of detecting $\geqslant 90\%$ of Caucasian carriers while achieving the best available sensitivity in other ethnic groups. For certain homogeneous ethnic groups, as few as five mutations would be sufficient to meet this criterion. But for general population screening in a country as heterogeneous as the United States, it is clear that many more mutations will need to be included.

In the absence of commercial test kits, setting up in-house multiplex testing for large numbers of *CFTR* mutations has proven to be a challenging and expensive exercise for most routine diagnostic molecular genetics laboratories, especially for those whose test volume does not lend itself to large-scale automation of the PCR and hybridization steps. A number of these laboratories have already abandoned CF mutation screening and refer their cases out to large reference laboratories that test as many as 70 or more mutations. The NIH panel's recommendation should exert even more pressure in this direction, although it might also inspire manufacturers to develop marketable *CFTR* mutation test kits. While most of the mutations beyond the first 10 or 20 are extremely rare, it could be argued that screening for fewer than 10–20 does not represent the current standard of care, unless the laboratory is restricting its testing to particular well-characterized ethnic groups such as Ashkenazi Jews. In any case, our experience suggests that more explicit guidelines specifying particular mutations and minimal test panel sensitivities for each ethnic population being screened would be prudent and helpful. Such guidelines could be recommended by professional practice organizations like the ACMG and CAP or through the NIH consensus conference process. In the meantime, this survey by the ACMG/CAP Biochemical and Molecular Genetics Resource Committee provides a valuable snapshot of the state and extent of CF mutation testing in the country at present and one that the broader medical genetics community should consider as

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-

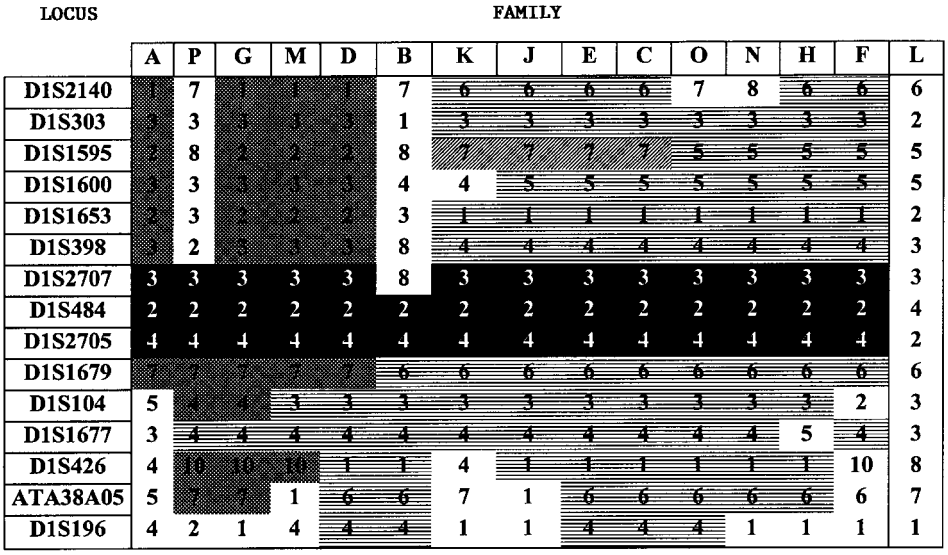

Figure 1 Disease-associated haplotypes of each of the 15 families. The oldest R59W-mutation chromosome in each family was used. The patterned sections indicate the regions conserved between the affected haplotypes of the different families.

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).

Table 1

World Distribution of the MTHFR Mutation

Country	No.	C/C	C/T	T/T	T Allele Frequency (%)	95% Confidence Range (%)
Europe:						
United Kingdom	94	45	42	7	18.6	13.0–25.9
Africa:						
Central African Republic						
Bantu	44	36	8	0	9.1	3.9–17.9
Pygmies	8	7	1	0	6.25	.16–34.8
Gambia	24	21	3	0	6.25	1.29–18.26
Kenya	61	55	6	0	4.9	1.80–10.7
Madagascar	97	84	13	0	6.7	3.6–11.4
Total	234	203	31	0	6.6	4.5–9.4
Middle East:						
Yemen	46	31	14	1	17.4	9.9–28.2
Asia:						
French Polynesia (Chinese ancestry)	64	38	25	1	21.1	13.9–30.7
Hong Kong (Chinese)	47	22	19	6	33.0	22.4–46.8
Maewo, Vanuatu	71	60	10	1	8.5	4.4–14.8
Mongolia	36	13	20	3	36.1	23.6–52.9
Palembang, Indonesia	61	42	18	1	16.4	10.0–25.3
Total	279	175	92	12	20.8	17.2–24.9
Asia Minor:						
Sri Lanka	67	61	6	0	4.5	1.6–9.7
Australasia:						
PNG Highlanders	85	77	8	0	4.7	2.0–9.3
Americas:						
Nu-Chah-Nulth	37	25	10	2	18.9	10.3–31.7
Brazilian Amerindians	39	12	19	8	44.9	31.2–62.4
Total	76	37	29	10	32.2	23.8–42.6
Grand Total	881					

The correlation between the frequency of myocardial infarction and neural tube defects with the allele frequencies presented here is consistent with the hypothesis that the C677T mutation is a risk factor for these diseases. Further study about the genetic, medical, and nutritional factors affecting the MTHFR polymorphism, as well as a better understanding of human demographic history, is needed to explain its high frequency and widespread distribution.

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Mutational Mechanisms for Generating Microsatellite Allele-Frequency Distributions: An Analysis of 4,558 Markers

To the Editor:

Genomewide linkage searches have been facilitated by the development of panels of microsatellite markers that are widely distributed throughout the genome and are highly polymorphic. Population geneticists have investigated mutational mechanisms for generating new microsatellite alleles, considered infinite allele models (Shriver et al. 1993), single-step mutation models (Shriver et al. 1993; Valdes et al. 1993; Di Rienzo et al. 1994; Deka et al. 1995), and multistep mutation models (Di Rienzo et al. 1994; Kimmel and Chakraborty 1996; Chakraborty et al. 1997), and compared theoretical predictions of various parameters such as number of alleles and degree of heterozygosity with real-world observations. These studies have, in general, examined relatively few markers with data derived from several populations. Here we report an analysis of the extensive, publicly available data for the Généthon $(AC)_n$ microsatellite markers (Dib et al. 1996) to investigate (1) the distribution of allele frequencies and (2) the distribution of size differences between alleles, for an idealized microsatellite marker, and to examine the implications of these observations for the underlying mutational model.

The Fondation Jean Dausset–CEPH database (version 8.1) was downloaded from the FTP server (ftp.cephb.fr). Five thousand sixty-three autosomal Généthon $(AC)_n$ microsatellite markers were identified by the nominal prefix "AFM;" 329 markers were eliminated from subsequent analysis, since the difference in size in base pairs between alleles was not an exact multiple of two. The genotypes of 22 unrelated founders of families 1332, 1347, 1362, 1413, and 1416, all of which originate in Utah, were then compiled for subsequent analysis. Markers were grouped by the number of different-sized alleles found in the sample of 44 Utah chromosomes; alleles were then ranked by size (bp) and the mean frequencies of the ranked alleles for 4,558 markers with between 3 and 11 alleles are plotted in figure 1*A*. The frequency distribution traces a distinctive, asymmetrical pattern that follows a function of the number of alleles. As expected, the mode allele lies midway in rank, and its frequency decreases with the total number of alleles. More noteworthy, we observe that the frequency distributions are all positively skewed (coefficients of skewness range from 0.074 to 0.211), the data are significantly different ($P < .01$) from a random sample drawn from a normal distribution using the Kolmogorov test (performed using the SAS UNIVARIATE procedure [SAS Institute 1990]). The frequency distributions for an additional 176 markers with between 12 and 22 different alleles are not shown, because there were insufficient numbers within each size class, leading to excessive variability.

Computer simulation studies were performed to explore plausible mutational mechanisms that underlie the asymmetrical distribution of mean frequencies of the ranked alleles. Models were based on the Fisher-Wright genetic drift model, in which $2N$ chromosomes were sampled with replacement from a diploid population of size N. Mutations at a rate ν were assigned that replace an allele of size S (measured as number of dinucleotides) with a larger or smaller allele. Markers were assumed to be unlinked and in linkage equilibrium. We examined a single-step mutation model (SSMM) in which newly mutated alleles have size $S + 1$ or $S - 1$ with equal probability (i.e., one dinucleotide repeat motif larger or smaller). We also examined multiple-step mutation models (MSMM) in which new alleles have size $S + n$ or

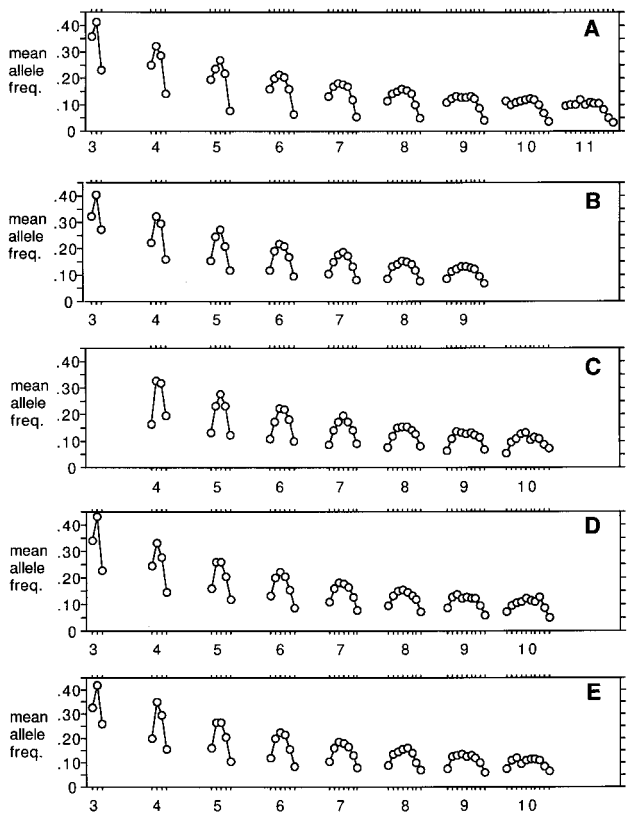

Figure 1 Distributions of mean frequencies for ranked alleles for microsatellite markers that are grouped by the number of alleles (indicated on the abscissa). *A*, Fesults from an analysis of 4,558 Généthon $(AC)_n$ microsatellite markers. *B*, Results for a simulation study for a multiple-step mutation model (MSMM) in which the initial number of repeat units (start size *S*) is five (no mutational bias). *C*, Results for a similar simulation study in which the start size *S* is 50 (no mutational bias). *D*, Results for a MSMM simulation study with a 2:1 mutational bias in favor of larger alleles in which the start size *S* is 5; *E*, Results for a similar simulation study in which the start size *S* is 50.

$S - n$ (where n is the number of dinucleotide repeat motifs) with alternatively equal probabilities (.5:.5) or with unequal probabilities (.666:.333) to introduce a mutational bias in favor of larger alleles. The relative mutation rates for step size n were modeled by a standard geometric distribution in which the relative mutation rate (ν_n) is defined as $\nu_n = m/(m+1)^n$, which has the useful property, when $m > 0$, that

$$\sum_{n=1}^{\infty} \frac{m}{(m+1)^n} = 1 \ .$$

These models define a natural lower "absorbing boundary" for the number of dinucleotide repeats contained within a microsatellite allele (i.e., mutations to zero or negative sizes are not permitted, so $n \geqslant 1$). Each simulation exercise commenced with a single allele of size *S*, where *S* ranged from 1 to 50. As the Généthon markers were selected to ensure that they were highly polymorphic, replicates were discarded if the heterozygosity for the marker at the end of the simulations did not exceed 50%. Simulations were carried out for a range of values of $4N\nu$, number of generations, and allele start size (*S*) for both the SSMM and the MSMM (and m for the MSMM simulations).

Figure 1 shows the mean frequencies of the ranked alleles in a random sample of 44 chromosomes following a simulation study of 5,000 markers over 20,000 generations under the MSMM with no mutational bias, with $4N\nu = 4$ and $m = 2$ and with the start size $S = 5$ (fig. 1*B*) or $S = 50$ (fig. 1*C*). It is evident by inspection that the simulated distribution with a start size $S = 5$ is positively skewed (fig. 1*B*), but when $S = 50$ there is virtually no skewness (fig. 1*C*). This demonstrates that asymmetry can result from a simple absorbing boundary model provided that the start size *S* is close to the boundary; other simulations with a range of start sizes indicate that the degree of skewing is roughly inversely proportional to *S*. Figures 1*D* and 1*E* show the results from a MSMM with a 2:1 mutational bias in favor of larger alleles with the start size $S = 5$ (fig. 1*D*) or with the start size $S = 50$ (fig. 1*E*). These latter results show that a mutational bias model (fig. 1*E*) can result in skewed allele frequency distributions. However, we note that even the simulation with the start size $S = 5$ and mutational bias (fig. 1*D*) does not appear to be as skewed as the Généthon data (fig. 1*A*).

The parameter $4N\nu$ was selected to model a randomly mating population of size 2,000 and a mutation rate $\nu = .0005$. This latter value is consistent with microsatellite mutation rate estimates reported in the literature which range from 10^{-2} to 10^{-5}. The simulated distributions of the number of alleles and heterozygosity were broadly comparable to those observed in the Généthon data (further details and results from additional simulations are available on the homepage http://well.ox.ac.uk/~mfarrall/microsatellite.html).

We have also examined the distribution of size differences of alleles, expressed as the number of dinucleotide repeat units, between adjacently ranked alleles, and we list the results from our analysis of the Généthon data and the SSMM and MSMM simulation studies for 20,000 generations in table 1. We note that, by inspection, the distribution of the size differences between adjacent ranked alleles in the Généthon sample is very closely matched by the MSMM (with no mutational bias) simulated data (where $m = 2$), while the SSMM underestimates the frequency of large differences in size between adjacently ranked alleles.

Table 1

Frequency Distribution of Size Differences between Microsatellite Alleles

No. of Repeats	Généthon	SSMM	MSMM
1	.812	.936	.810
2	.111	.047	.126
3	.036	.011	.037
4	.016	.004	.014
5	.011	.001	.007
6	.005	.001	.003
7	.003	.000	.002
8	.002	.000	.001
9	.001	.000	.001
10	.001	.000	.000

NOTE.—"No. of Repeats" indicates the difference in the number of dinucleotide repeats between adjacent alleles that have been ranked by their absolute size. SSMM = single-step mutation model. MSMM = multiple-step mutation model ($m = 2$), no mutational bias.

We have found that microsatellite allele frequency distributions tend to be positively skewed in favor of longer alleles, which is in agreement with an analysis of CAG repeats in the Huntington disease gene (Rubinsztein et al. 1994) and the unpublished results of W. Amos and D. Rubinsztein (cited in Rubinsztein et al. 1995). Our computer simulation results suggest that the underlying mutational model for generating new microsatellite alleles is likely to be asymmetrical and multistep. MSMM models with an absorbing boundary or with a mutational bias in favor of larger alleles can generate allele distributions that closely resemble those observed in the Généthon data. Models of directional evolution that result from mutational bias have been recently discussed (Rubinsztein et al. 1995; Primmer et al. 1996). Finally, the empirical mean frequencies of the ranked alleles derived from the Généthon analysis provide useful prior distributions to those applying Bayesian smoothing techniques (Lange 1997) to $(AC)_n$ microsatellite allele frequency estimates.

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Some Underlooked Properties of the Multifactorial/ Threshold Model

To the Editor:

Some years ago, when the multifactorial/threshold (MFT) model was beginning to be recognized (by some) as a useful way of thinking about the causes of common congenital malformations, I noted one of its implications. "It follows from the MFT model that in conditions appearing more often in one sex than the other, the sex ratio should change as the frequency changes" (Fraser 1971, p. 90). I suggested that such changes in the sex

ratio of a condition might be a more sensitive indicator of changes in its frequency than direct counts would be. Since then, there have been several relevant examples of changes in sex ratio with differences in frequency of a trait. These have evoked a number of explanations that, though ingenious, may be unnecessary; the answer may lie in the above-mentioned property of the MFT model.

The MFT model postulates a continuous distribution of "liability" to a particular defect and a threshold separating the continuous distribution into discontinuous parts, with only those individuals falling beyond the threshold having the defect. Cleft palate in the mouse is a classic example, with substantial experimental support (Fraser 1976). In this case, liability is a reflection of the developmental stage at which the palate shelves move toward closure. The threshold is the latest stage at which closure is still possible. Embryos in which shelf movement occurs later than this have cleft palate. Thus, the embryos with later closure are more susceptible, or liable (Fraser 1980*a*).

A number of predictions can be made from the MFT model, relating recurrence risk to sex of proband, severity of defect, number of affected relatives, degree of relationship to proband, and population frequency of the trait. These have been discussed extensively elsewhere (Fraser 1976, 1980*b*). Note that all these properties relate to how the frequency of a trait changes as the position of the liability distribution changes relative to the threshold and not to whether the distribution is normal (polygenic) or multimodal (several genes with low penetrance).

Two other predictions of the MFT model, to be discussed in this article, have been largely overlooked. The first of these addresses how changes in the sex ratio of a MFT trait can reveal changes in liability. For instance, the American Society for Human Genetics Statement on Behavioral Genetics (Sherman et al. 1997) uses emotional stability as an example of how sex-ratio differences are exaggerated at the tails of the frequency distribution in conditions where the distribution differs in the two sexes. For this multifactorial trait, males have a somewhat higher mean emotional stability score than females, but at the upper 1% of the distribution there is a striking excess of males. Figure 1 illustrates why. The dashed curve represents the distribution of "liability to stability" scores for males, and the solid curve that for females. The (arbitrary) threshold, T2, demarcates the upper 1% of the population, who could be considered those with the "ultrastability" trait. Note that in this group there is an obvious excess of males; that is, there are many more males than females in the area under the curve beyond T2. If the threshold is set at (say) the 5% level (T1), there will be an increase in frequency of the trait, but the excess of males beyond the T1 level is not as great as it was beyond the T2 threshold (i.e., the proportion of "ultrastable" males is smaller). Thus, the sex ratio of "ultrastable" individuals shifts toward equality as the frequency of the trait (number of individuals beyond the threshold) increases.

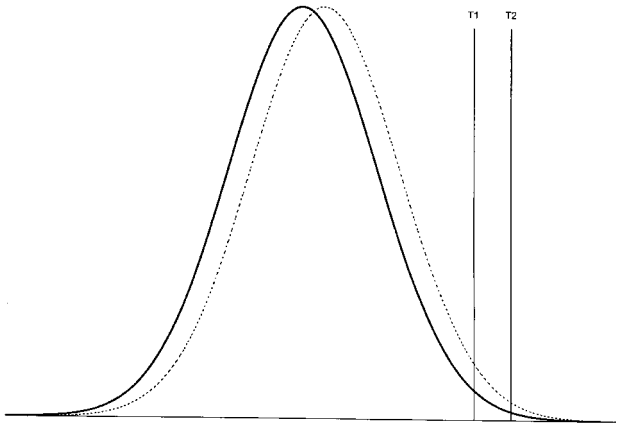

Figure 1 Multifactorial threshold model for a condition in which males and females have different frequencies. For details, see text .

For congenital malformations, one cannot see the distribution of liability for the trait but only the proportion of individuals who fall beyond the threshold (the frequency of the trait). In cleft lip, with or without cleft palate (CLP), for example, more males than females are affected, so we infer that the liability distribution for males is farther to the right than for females. Figure 1 now represents the distributions of liability to CLP for males (*dashed line*) and females (*solid line*); the threshold separates those with the trait (in the tail of the distribution) from those without it. Again, if the distribution of liability shifts to the right, so that the frequency increases, the sex ratio shifts toward equality. (For simplicity's sake, fig. 1 illustrates this by shifting the thresholds to the left, rather than the distributions to the right).

CLP provides several examples of this effect. For example, one would expect liability to be lower in families with one affected individual (simplex) than in those with more than one (multiplex), so the sex ratio should be closer to 1 in the multiplex families—and it is. The sex ratio in simplex versus multiplex families is reported as 2.0 versus 1.5 by Fraser (1980*b*) and as 3.2 versus 1.4 by Ray et al. (1993). An explanation is suggested by Ray et al. Perhaps "the loading of environmental factors predisposing to CLP is higher in multiplex families and results in a higher frequency of the less susceptible sex (females) becoming affected" (Ray et al. 1993, p. 1010). Possibly, but the simplest explanation is that the sex ratio shift is just what is expected for an MFT trait, with different liability distributions for the two sexes.

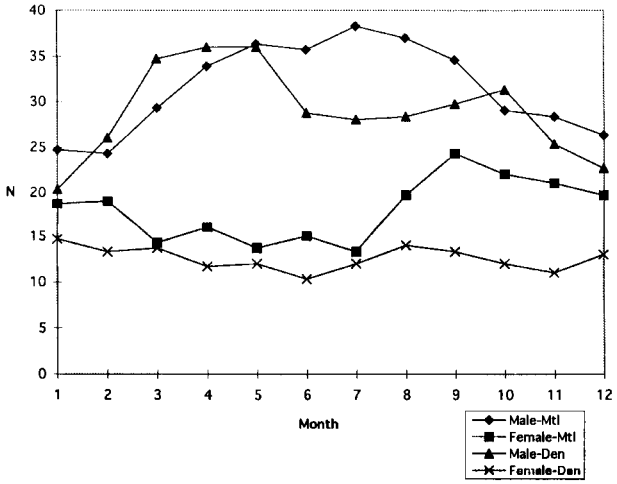

Figure 2 Month of birth for children with CLP, by sex, for Montreal and Denmark. For details, see text. The top two graphs represent males.

One might also expect that the liability distribution of CLP would be farther to the right in cases with associated malformations than in "isolated" cases, since, for one thing, this group would include some cases with syndromes in which CLP is a recognized component. For nonsyndromic, or "isolated," cases of cleft lip, the liability distribution would cross the threshold at a point where ~1 case in 1,000 would fall beyond the threshold, this being the frequency of CLP. For "syndromic" CLP (with reduced penetrance for CLP, of course) the proportion of patients with the syndrome who have CLP would be much higher than this, or else CLP would not be recognized as part of the syndrome. For cases in which the associated malformations were both caused by a teratogen, the liability to CLP in exposed embryos would also presumably be higher than in the general population. Thus, cases with associated malformations should have a liability distribution farther to right, and therefore a lower sex ratio, than those without them. This has been reported by Källen and Harris (1996), who found a sex ratio of 1.3 for those with versus 1.9 for those without associated malformations. The authors suggest that mutants with associated malformations consist of two subgroups: one equivalent to the children with isolated facial clefts and another with a normal sex ratio. This is a possibility, but one does not need another explanation; the MFT model predicts it.

This phenomenon is not limited to CLP. Lubinsky (1997), in an interesting article on sex-biased anomalies, points out that the sex ratio shifts toward equality in patients with versus those without additional malformations in the case of anencephaly, spina bifida, encephalocele, single umbilical artery, and diaphragmatic hernia. He postulates that these malformations may have complex and heterogeneous origins and that "with dual origins 'strong' events could increase the involvement of otherwise minor second processes with opposite bias from the primary disturbance" (p. 227). This is ingenious, but would it escape Occam's razor? The MFT model provides a simpler explanation.

A final example comes from my own experience. The gene for the autosomal dominant Van der Woude, or lip-pit syndrome, produces CLP in some carriers and isolated cleft palate (CP) in others. I thought it would be interesting to see if the cases of CLP and CP in families with this syndrome showed the same excess of males in CLP and females in CP patients that they do in nonsyndromic patients. To my surprise, they did not; both sex ratios shifted toward equality. "But of course, stupid," I said to myself. Both malformations have a higher penetrance in the syndrome than they do in isolated cases, so the shift in sex ratio toward equality is just what one would expect.

Another overlooked prediction from the MFT model, for traits with sex ratios deviating from 1, is that epidemiological variations are more manifest in the sex with the higher frequency. Observe in figure 1 that as the distribution is moved to the right (or the threshold to the left) and the frequency increases, the slope of the curve at the threshold increases. It is much steeper at T1 than at T2, but more so for males (dotted curve) than for females. This means that a change in liability for a rare trait, where the threshold is near the tail of the distribution, should result in a smaller change in frequency than it would for a more frequent trait, where the threshold is farther to the left relative to the distribution. Thus, anything altering liability would lead to a greater change in frequency in the sex with the higher frequency. So, in a search for factors that alter liability, and thus change the frequency, any change would be more discernible in the sex with the greatest frequency. Thus, one would expect the variation in frequency with various epidemiological factors to be more apparent in females in the case of anencephaly (which is more frequent in females), and more apparent in males in the case of CLP.

Since neural-tube defects show variations with season of birth, which could be in part a result of seasonal variations in maternal vitamin intake, I wondered whether CLP would also show an association with season of birth, since there is growing evidence that liability to CLP varies with maternal vitamin intake (Tolarova and Harris 1995; Czeizel et al. 1996; Shaw et al. 1997). For the above reasons, such an effect should be more evident in males. Previous studies of variation in season of birth for CLP, with sexes combined, have been inconsistent. A review of cases of CLP seen at the Montreal

Children's Hospital from 1950 to 1996 does indeed show a significant variation in males (fig. 2), with a peak around July–August, which is not present in the females (Fraser and Gwynn 1998). Figure 2 also shows data on season of birth from Fogh-Andersen's classic monograph on CLP (1942), which we have analyzed by sex. There is a significant variation in males, with a peak in April–May but not in females (F. C. Fraser and X. N. Rahnema, unpublished data). A similar peak for CLP (sexes combined) is reported from Finland (Rintala 1983), and it would be interesting to see whether this variation was more evident in males in this and other populations. The reasons for these variations remain to be clarified. There is some preliminary evidence that the effect may have diminished or disappeared in recent years. But, whatever the explanation, the point here is that for threshold traits that are more frequent in one sex than the other, epidemiological variations may be revealed more effectively by examining the sexes separately.

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