R primer, to screen 48 individuals (45 of whom were unrelated) whom we had previously identified as C282Y homozygotes. In this group, we identified one additional unrelated individual with the C282Y mutation and the 5569 G→A polymorphism. Closer examination of the assay on the basis of which the previous diagnosis was made in this individual revealed an extremely faint normal band that had been interpreted to result from partial digestion. In total, therefore, the polymorphism has been found in 8 of 202 unrelated individuals who were referred for HH testing. An estimate of the allele frequency can be made on the basis of the C282Y carrier frequency. We found these 8 polymorphism carriers among a total of 43 unrelated C282Y (non-H63D) carriers. Our estimated population frequency of this allele is, therefore, 8/43 (=.186). Consequently, in our population, this polymorphism had the potential to result in ~19% of C282Y heterozygotes being misidentified as homozygotes.

We identified the 8 polymorphism carriers, in addition to 44 unrelated C282Y homozygotes, from our total sample of 202 unrelated individuals referred for testing. If the assumption of homozygosity, along with access to parental genotypes, had been made in all individuals with the polymorphism, as well as in those with the homozygosity, this would have led to an estimate of ~8/52 (=.154) for nonparentage, of which half of these cases, or 8%, would have been assumed to result from nonpaternity. The frequency of this polymorphism is high enough to warrant concern that the interpretation of homozygosity in these cases will result in an overestimate of the C282Y-allele frequency, a misdiagnosis of this condition, and an incorrect assumption of nonpaternity in some families. In our hands, the polymorphism promoted misinterpretation of a restriction-digestion–based assay, but any form of analysis (including allele-specific oligonucleotide hybridization, ARMS, or direct sequencing) that incorporates the Feder et al. (1996) reverse primer is equally prone to misdiagnosis. It is recommended that all laboratories using the Feder et al. reverse primer to test for the C282Y mutation confirm C282Y-homozygote results by using a flanking primer set and *Mse*I digestion.

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No Mutations in the Coding Region of the *PRKCG* Gene in Three Families with Retinitis Pigmentosa Linked to the *RP11* Locus on Chromosome 19q

To the Editor:

Retinitis pigmentosa (RP) and allied degenerations of the retina are genetically heterogeneous, with well over 50 loci implicated so far through gene identifications or linkage-based chromosomal assignments. Among these genes, the dominantly inherited *RP11* locus (MIM 600138) on chromosome 19q is noteworthy because some carriers develop RP that is symptomatic at age <20 years, whereas others are asymptomatic and show no funduscopic or electroretinographic signs of disease even at age >70 years (Berson et al. 1969; Berson and Simonoff 1979; Evans et al. 1995; Nakazawa et al. 1996; McGee et al. 1997). On the basis of its chromosomal

assignment, the *PRKCG* gene is a candidate for *RP11*. This gene encodes a form of protein kinase C that is expressed in the retina. Last year, Al-Maghtheh et al. (1998) described two families with *RP11*-linked dominant RP in which a missense change (Arg659Ser) in *PRKCG* cosegregated with disease. Only one of these two families (RP1907) clearly exhibited asymptomatic, obligate carriers who transmitted the disease to offspring. The authors failed to discover a mutation in *PRKCG* in three other families with reduced penetrance showing linkage to this region. Nevertheless, the authors speculated that *PRKCG* could be the *RP11* gene.

In response to that report, we have undertaken an analysis of the *PRKCG* gene in three additional families with dominant RP with reduced penetrance. All three families have unaffected, obligate carriers, and we previously reported linkage data pointing to *RP11* as the cause of RP in these families (McGee et al. 1997). An affected individual from each family was chosen for the current study (the patients were individuals III-2 from family 1295, IV-8 from family 2474, and IV-34 from family 1562) (McGee et al. 1997), as well as DNA from an unrelated control individual without RP and without a family history of retinal degeneration. We amplified each of the 18 exons of the *PRKCG* gene individually, using PCR from leukocyte DNA obtained from these individuals. Primer pairs were the same as those reported elsewhere (Al-Maghtheh et al. 1998) except for exons 5, 10, and 11, for which we used primers (sense/antisense) as follows: exon 5, 5′ portion, TGAGGTGCTACCCGCAGCTT / CAGTTACGTGGATCTCATCT; exon 5, 3′ portion, AGGCTGCGAGATGAACGTGC / AGGCGAGGGGGCGGGGCCTC; exon 10, GGCTGTGTAAGGTCTAAGTG / CACAGGAGCCCAGTCTCTTC; exon 11, CTGGGTTCCCAACATGGACT / CTTGCCTCTCCCTAAACTCA. The amplified fragments were sequenced directly by means of standard methods.

None of the patients had a defect in codon 659. Furthermore, none of the patients had an abnormality in the coding region or the flanking-intron splice-acceptor or -donor sites, except for one patient who heterozygously carried a silent change in codon 24 (Ala24Ala, GCT→GCC). In all eight gene copies carried by the three patients and the control individual, the sequence of codons Phe19 and Ser148 in exons 1 and 5, respectively, was different from that published elsewhere (TTT instead of TTC, and TCC instead of TCT, respectively), suggesting that the previously reported sequence (GenBank accession number M13977) is an allelic variant, a sequencing artifact, or an error. The previously reported (Al-Maghtheh et al. 1998) silent polymorphism at codon Asn189 was encountered, with two of the three patients being heterozygotes (carrying the sequences AAT and AAC for that codon) and the third patient being a homozygote for the sequence AAT. We previously documented a recombination event in the vicinity of *RP11* in one branch of family 1562 (McGee et al. 1997); however, the intragenic polymorphisms were not informative in this branch of that family and did not allow us to determine on which side of the crossover the *PRKCG* locus lies.

Our analysis provides no evidence that *PRKCG* is the *RP11* gene. Although our data cannot exclude the pathogenicity of the Arg659Ser missense change, the possibility that it is a rare, nonpathogenic variant remains plausible, since none of the three families analyzed here and only two of the five families analyzed elsewhere (Al-Maghtheh et al. 1998) carry an anomaly that would change the sequence of the encoded protein. Another formal possibility is that there are two RP genes in this region, but it would be necessary that both RP loci exhibit reduced penetrance. In either case, it appears that an RP gene in this region remains to be identified.

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