

Letters to the Editor

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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-Magthteh 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-Magthteh 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 *inaC* (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 PKC γ isoenzyme in the human retina is not yet clearly defined. Several reports have shown PKC γ to be expressed in rabbit, rat, frog, and goldfish retinas (Osborne et al. 1992). This expression of PKC γ is confined to amacrine and ganglion cells. No expression has been detected in the photoreceptors. This does not exclude the possibility that PKC γ 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 table 1.

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-Magthteh 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

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, aggagaaattgggacggagc	1.3, 60°C
4F, gctgacctagagagcaaggc	4R, gctttggaaggccctggca	1.5, 65°C
5F, tgaggtgctaccgcagctt	5R, acaagtgccttgggtcagcc	1.5, 64°C
6F, ctctaaccctgcacactctt	6R, tctgtcagctgtcattgctt	1.5, 60°C
7F, gccatgagctcggtctgca	7R, gtaatatggcctccatcccc	1.5, 65°C
8F, tgcctctccatgggtgc	8R, aaggccagctctgaacctg	1.5, 60°C
9F, ctatctatcgccatggct	9R, aactgcctccattcaacg	1.5, 58°C
10F, gagcatttccttateggctg	10R, aaccagaaatctgaccttccc	1.3, 55°C
11F, aggtcctgtaccactgggtt	11R, atcccaacgcagatgtccag	1.5, 65°C
12F, gtatagtgatcccgcctcta	12R, acgtcagaaggctcagtggtc	1.0, 58°C
13F, agcactgaccttctgagct	13R, gtgttgagttcagcagctag	1.5, 60°C
14F, ctgactgctgaactcaaac	14R, taaggatctcaaagcgtg	1.5, 56°C
15F, gcacttaacgtggtagcg	15R, tagccaagccagcttctcc	1.5, 56°C
16F, gcattgctcctgactctctat	16R, agtgacttcaggaatgggag	1.5, 60°C
17F, atgtacctgtccggcact	17R, accaggtttttgttgcctgg	1.5, 55°C
18F, ctcggagctgcttaactttcc	18R, acgttggggacacctagtgg	1.5, 64°C

is shown in fig. 2) was also confirmed by digestion of the PCR product of exon 18 by the restriction enzyme *AclI* (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 involved were *PRKCG*, a mutation in this gene might be expected to have an effect on photoreceptors' ad-

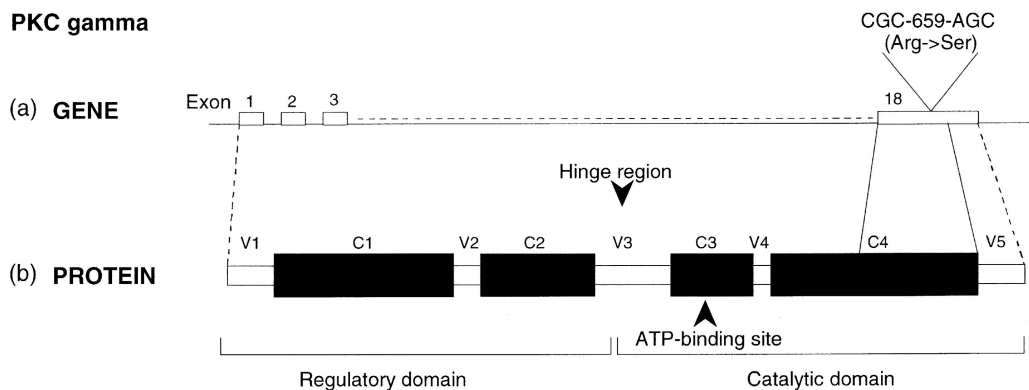


Figure 1 Diagrammatic representation of the position of the Arg659Ser mutation within exon 18 of (a) the DNA sequence and (b) the domain structure of PKC γ , as described by Hug and Sarre (1993). C1-C4 are the conserved domains, which are flanked and separated by the variable domains, V1-V5. V1-C2 represents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

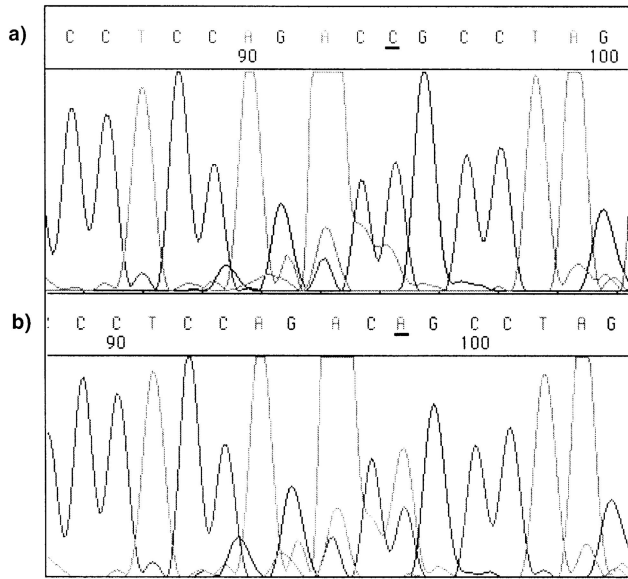


Figure 2 Comparison of sequencing data from (a) a normal and (b) a heterozygous mutant *PRKCG* exon 18 sequence. A C→A transversion (underlined bases) changes codon 659 from arginine (CGC) to serine (AGC).

aptation 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 PKC γ , 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 PKC γ 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 phe-

notype 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 hybridized 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

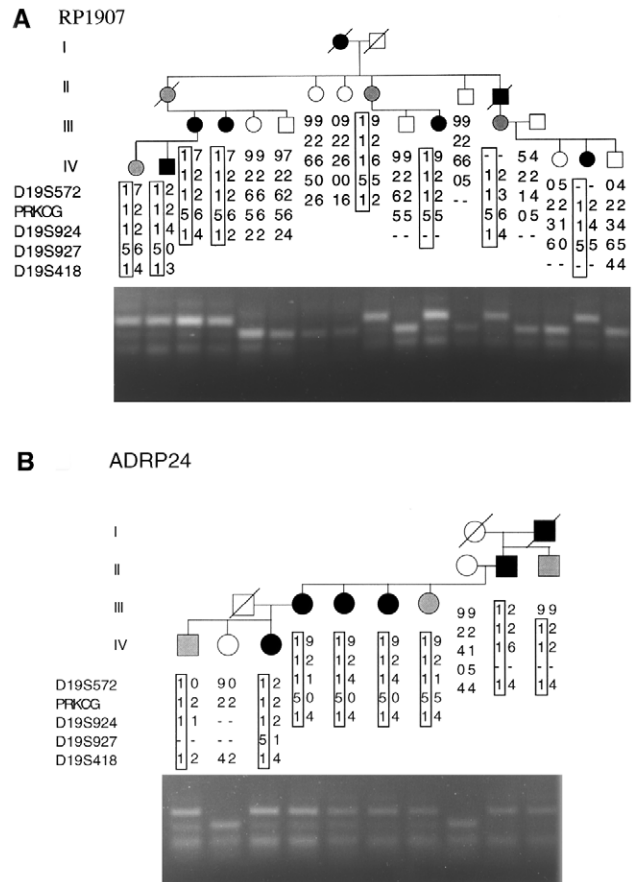


Figure 3 Pedigrees of (a) RP1907 and (b) ADRP24. (On the basis of the characteristic bimodal expressivity phenotype, this family was thought to be an RP11 family, but it gave a nonsignificant LOD score [0.6 at recombination fraction 0] with 19q markers.) Blackened symbols denote affected individuals; 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 *Acl*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.

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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References

- Abeliovich A, Paylor R, Chen C, Kim JJ, Wehner JM, Tonegawa S (1993) PKC γ mutant mice exhibit mild deficits in spatial and contextual learning. *Cell* 75:1263–1271
- Al-Maghteh M, Vithana E, Tardtlin E, Jay M, Evans K, Moore T, Bhattacharya SS, et al (1996) Evidence for a major retinitis pigmentosa locus on 19q13.4 (RP11), and association with a unique bimodal expressivity phenotype. *Am J Hum Genet* 59:864–871
- Bird AC (1995) Retinal photoreceptor dystrophies. *Am J Ophthalmol* 119:543–562
- Chen C, Kano M, Abeliovich A, Chen L, Bao S, Kim JJ, Hashimoto K, et al (1995) Impaired motor coordination correlates with persistent multiple climbing fiber innervation in PKC γ mutant mice. *Cell* 83:1233–1242
- Fuchs S, Nakazawa M, Maw M, Tamai M, Oguchi Y, Gal A (1995) A homozygous 1-base pair deletion in the arrestin gene is a frequent cause of Oguchi disease in Japanese. *Nat Genet* 10:360–362
- Fujita R, Buracznska M, Gieser L, Wu WP, Forsythe P, Abrahamson M, Jacobson SG, et al (1997) Analysis of the *RPGR* gene in 11 pedigrees with the retinitis pigmentosa type 3 genotype: paucity of mutations in the coding region but splice defects in two families. *Am J Hum Genet* 61:571–580
- Gal A, Orth U, Baehr W, Schwinger E, Rosenberg T (1994) Heterozygous missense mutation in the rod cGMP phosphodiesterase beta-subunit gene in autosomal dominant stationary night blindness. *Nat Genet* 7:64–68
- Hug H, Sarre TF (1993) Protein kinase C isoenzymes: divergence in signal transduction. *Biochem J* 291:329–343
- Keen J, Lester DH, Inglehearn CF, Curtis A, Bhattacharya SS (1991) Rapid detection of single base mismatches as heteroduplexes on hydrolink gels. *Trends Genet* 7:5
- McGee TL, Devoto M, Ott J, Berson EL, Dryja TP (1997) Evidence that the penetrance of mutations at the RP11 locus causing dominant retinitis pigmentosa is influenced by a gene linked to the homologous RP11 allele. *Am J Hum Genet* 61:1059–1066
- McKusick VA (1994) Mendelian inheritance in man: a catalog of human genes and genetic disorders. Johns Hopkins University Press, Baltimore
- Moore AT, Fitzke F, Jay M, Arden GB, Inglehearn CF, Keen TJ, Bhattacharya SS, et al (1993) Autosomal dominant retinitis pigmentosa with apparent incomplete penetrance: a clinical, electrophysiological, psychophysical and molecular genetic study. *Br J Ophthalmol* 77:473–479
- Newton AC, Williams DS (1991) Involvement of protein kinase C in the phosphorylation of rhodopsin. *J Biol Chem* 266:17725–17728
- (1993) Rhodopsin is the major *in situ* substrate of protein kinase C in rod outer segments of photoreceptors. *J Biol Chem* 268:18181–18186
- Ohki K, Yoshida K, Imaki J, Harada T, Matsuda H (1994) The existence of protein kinase C in cone photoreceptors in the rat retina. *Curr Eye Res* 13:547–550
- Osborne NN, Barnett ND, Morris NJ, Huang FL (1992) The occurrence of three isoenzymes of protein kinase C (α , β , and γ) in retinas of different species. *Brain Res* 570:161–166
- Rao VR, Cohen GB, Oprian DD (1994) Rhodopsin mutation G90D and a molecular mechanism for congenital night blindness. *Nature* 367:639–641
- Schaeffer E, Smith D, Mardon G, Quinn, W, Zuker C (1989) Isolation and characterization of two new drosophila protein kinase C genes, including one specifically expressed in photoreceptor cells. *Cell* 57:403–412

- Smith DP, Ranganathan R, Hardy RW, Marx J, Tsuchida T, Zuker CS (1991) Photoreceptor deactivation and retinal degeneration mediated by a photoreceptor-specific protein kinase C. *Science* 254:1478–1484
- Xu S, Nakazawa M, Tamai M, Gal A (1995) Autosomal dominant retinitis pigmentosa locus on chromosome 19q in a Japanese family. *J Med Genet* 32:915–916
- Xu SY, Schwartz M, Rosenburg T, Gal A (1996) A ninth locus (RP18) for autosomal dominant retinitis pigmentosa maps in the pericentromeric region of chromosome 1. *Hum Mol Genet* 5:1193–1197
- Yamamoto S, Sippel KC, Berson EL, Dryja TP (1997) Defects in the rhodopsin kinase gene in the Oguchi form of stationary night blindness. *Nat Genet* 15:175–178

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