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Mutations in the retinal guanylate cyclase (*RETGC-1*) gene in dominant cone–rod dystrophy

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The dominant cone–rod dystrophy gene *CORD6* has previously been mapped to within an 8 cM interval on chromosome 17p12–p13. The retinal-specific guanylate cyclase gene (*RETGC-1*), which maps to within this genetic interval and previously was implicated in Leber's congenital amaurosis, was screened for mutations within this family and in a panel of small families and individuals with various cone and cone–rod dystrophy phenotypes. A missense mutation (E837D) was identified in affected members of the CORD6 family, as well as a second missense mutation (R838C) in three other families with dominant cone–rod dystrophy. *RETGC-1* is only the fourth gene to be implicated in cone–rod dystrophy and this is the first report of dominant mutations in this gene.

INTRODUCTION

Cone–rod dystrophies belong to a group of eye disorders, the chorioretinal dystrophies, that are the most common cause of inherited eye disease. Cone–rod dystrophy is characterized by the initial degeneration of cone photoreceptor cells, causing early loss of visual acuity and colour vision, followed by the degeneration of rod photoreceptor cells leading to progressive night blindness and peripheral visual field loss (1). The disease displays phenotypic heterogeneity, and recent genetic studies have implicated a number of different gene loci in its aetiology (2–4). However, as yet, mutations have only been identified in three genes, *peripherin/RDS* (5–8), *CRX* (9) and *ABCR* (10).

We have recently localized a gene for a dominant cone–rod dystrophy (*CORD6*) to an 8 cM interval on chromosome 17p12–p13 (3) that includes the retinal-specific guanylate cyclase gene (*RETGC-1*) (11). Mutations in this gene have been shown to be responsible for Leber's congenital amaurosis (LCA1) (12,13), the most severe form of inherited retinopathy with total blindness or greatly impaired vision recognized at birth or in early infancy. These mutations show a recessive pattern of inheritance with no reported heterozygous effects.

A number of examples have now been reported where different mutations in the same gene result in clinically distinct inherited retinopathies (5–8,14–19) and where the pattern of inheritance also differs (20,21). It is possible, therefore, that a dominant phenotype is associated with mutations in *RETGC-1*, and we have, accordingly, screened the original CORD6 family and a panel of small families and individuals with various cone and cone–rod dystrophy phenotypes for *RETGC-1* mutations.

RESULTS

The pedigree of the four generation British CORD6 family in which the disease phenotype has been mapped to chromosome 17p12–p13 (3) is shown in Figure 1A. The first six individuals on the left side of the pedigree are additional members of the family who were recruited subsequent to its first publication. Cone–rod dystrophy in this family displays an early onset, with loss of central vision reported before 7 years of age and peripheral field loss by the fourth decade. A notable feature is marked photophobia, particularly when dark-adapted. Fundoscopy shows a 'bull's eye' maculopathy early in the disease (Fig. 2A), with later involvement of the peripheral retina. Electroretinography showed no detectable cone responses early in disease, with progressive abnormality of rod responses appearing later. A detailed clinical description of the disease in this and other families presented in this report will be given elsewhere (K. Gregory-Evans *et al*., in preparation).

Direct sequence analysis of all 18 coding exons of *RETGC-1* in the CORD6 family revealed a heterozygous alteration in exon 13 at nucleotide 2584 (G→C) in 10 affected individuals (Fig. 1A and C) that was absent in 10 unaffected individuals of the family. This sequence change results in the abolition of an *Hha*I

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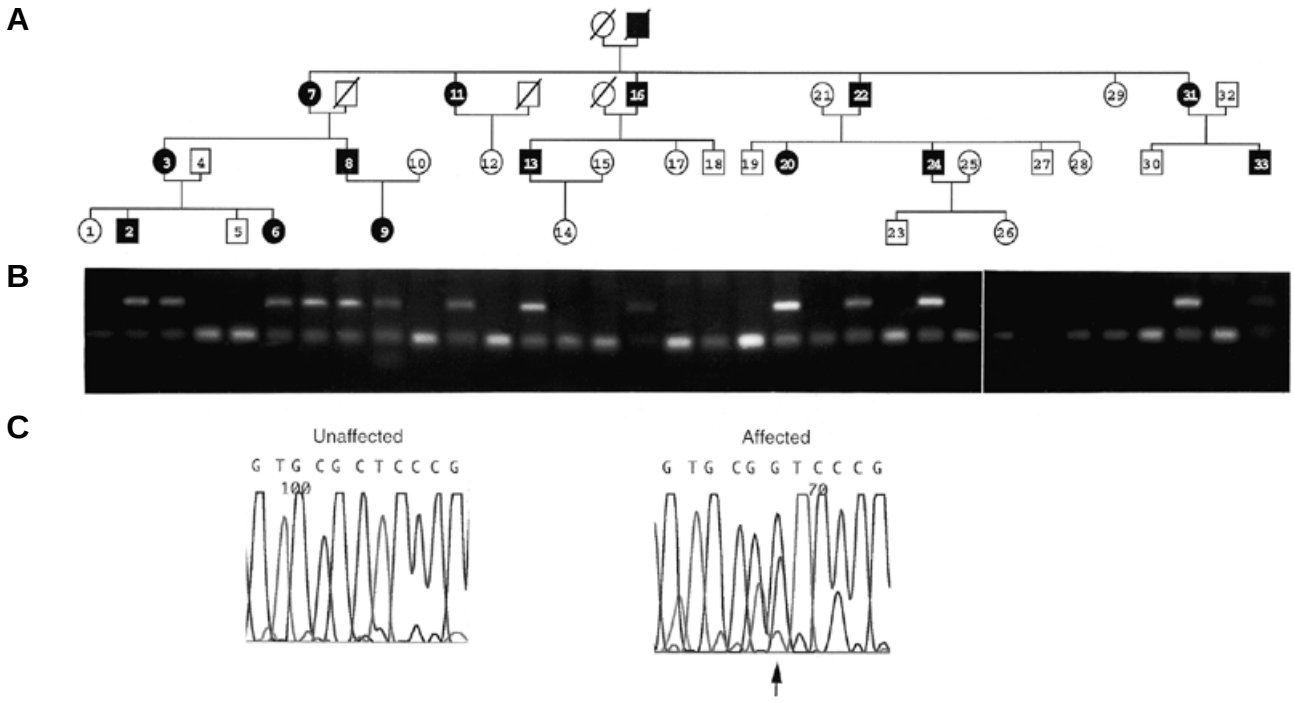

Figure 1. Co-segregation of the dominant G→C missense mutation in affected members of the CORD6 pedigree. (**A**) Pedigree of the CORD6 family. (**B**) Co-segregation of the abolished *Hha*I site in exon 13 with affected individuals of the family. Affected individuals exhibit the larger undigested DNA product (mutated allele) as well as the smaller digested doublet (bands not resolved on the gel) (normal allele). Unaffected individuals show only the doublet. (**C**) Reverse sequence of an unaffected individual (left) and an affected individual (right). The heterozygous G→C missense mutation in exon 13 at nucleotide 2584 (E837D) is indicated by an arrow.

restriction endonuclease site that co-segregates with all affected individuals (Fig. 1B). The loss of *Hha*I was not observed in any of the unaffected individuals within the pedigree, nor in >600 normal chromosomes from other British individuals (data not shown). The absence of the mutation in such a large number of normal chromosomes would imply that the G→C transversion is the disease-causing mutation. However, an alternate possibility is that this sequence alteration represents a very rare non-pathological polymorphism.

Approximately 50 additional small families or individuals with various cone or cone–rod dystrophy phenotypes were screened by *Hha*I digestion of exon 13. Heterozygous loss of this site was detected in affected individuals in the three additional cone–rod dystrophy families shown in Figure 3. Sequence analysis of all affected individuals revealed that the loss of the *Hha*I site in this case was the result of a C→ T transition at nucleotide 2585. Affected individuals in these families, although aware of poor vision in bright light from an early age, suffered loss of central vision in the late second or third decade, rather later therefore than in the CORD6 family. The fundus appearance of affected members of these families was very similar to the CORD6 phenotype (Fig. 2B). Electrophysiological testing revealed marked loss of photopic function by the mid-teens, with scotopic function becoming compromised later. Genealogical studies have failed to show that these families form a single large pedigree.

The G→C transversion in the CORD6 family results in the replacement of glutamate by aspartate at codon 837 (E837D) whereas the C→T transition in the other three families results in the replacement of arginine by cysteine at codon 838 (R838C). These amino acid changes occur within the putative dimerization domain of the RETGC-1 protein (22) (Fig. 4A). RETGC-1 is a member of a subgroup of membrane-bound guanylate cyclases which are expressed specifically in sensory tissues (24). Alignment of part of this domain (from codon 809 to 871) from human RETGC-1 and six other members of the subgroup shows that both Glu837 and Arg838 are fully conserved (Fig. 4B). The predicted secondary structure of this region of the protein is that of an α-helix and the cysteine substitution is likely to cause a steric change. Although the aspartate substitution in the CORD6 family is unlikely to destroy the formation of the predicted α-helix, the conservation of this amino acid at this site indicates that it may be critical for the proper functioning of the enzyme.

DISCUSSION

Guanylate cyclase is a critical component in the recovery process of phototransduction in the vertebrate retina. In both rod and cone photoreceptors, photoactivated rhodopsin stimulates cGMP phosphodiesterase activity via GTP/GDP exchange on the G protein transducin, with consequent hydrolysis of cGMP and the closure of cGMP-gated channels. The entry of Ca^{2+} through the channel is thereby blocked, but export continues, resulting in a hyperpolarization of the plasma membrane (25). In the recovery phase, the drop in Ca^{2+} concentration (26) leads to a stimulation of membrane-bound guanylate cyclase production via the activation of guanlylate cyclase-activating protein (27). The catalytic conversion by the cyclase of GTP to cGMP results in the restoration of cGMP levels to the dark state and the re-opening of the gated channels (28). Two isoforms of guanylate cyclase, encoded by separate genes, have been identified in the mam-

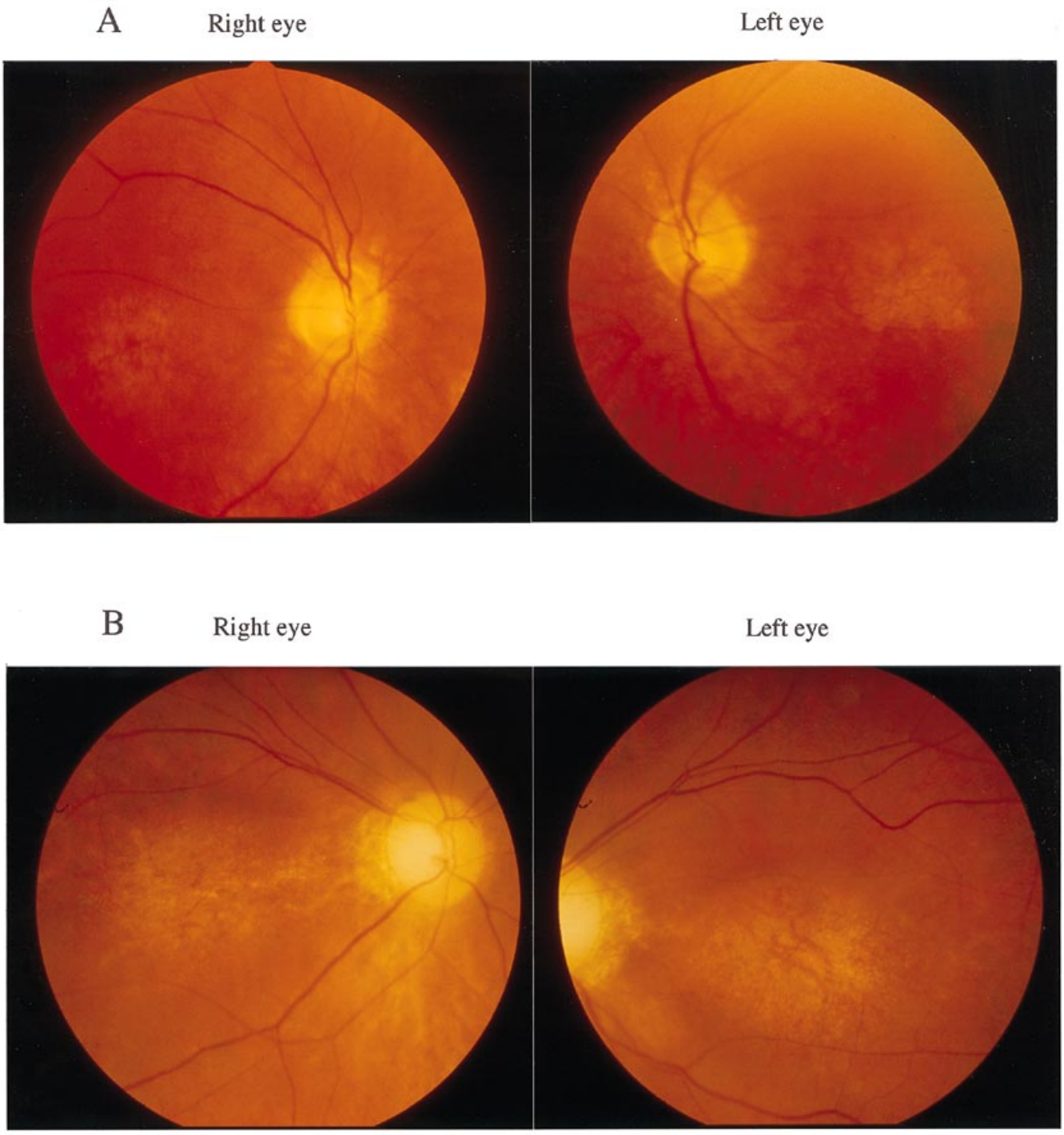

Figure 2. (**A**) Colour fundus photography of right and left eyes of a 48-year-old male from family 1 (CORD6) showing strong bilateral macular atrophy. (**B**) Colour fundus photography of right and left eyes of a 45-year-old female from family 2 (leftmost in Fig. A) showing similar macular atrophy.

malian retina (29). The active cyclase is a dimer (30,31) and although both are expressed in cone and rod photoreceptors, homomers between two identical subunits are formed preferentially *in vivo* (31). In the human retina, the two isoforms are encoded by *RETGC-1* and *RETGC-2*, with *RETGC-1* showing a higher levels of expression in cone than in rod cells (32–34). Since recessive mutations in *RETGC-1* are responsible for the severe blinding condition of LCA1, it is unlikely that RETGC-2 can compensate for the loss of RETGC-1 activity.

We have identified two new mutations in the *RETGC-1* gene that are associated with dominant cone–rod dystrophy, a E837D substitution that co-segregates with the dystrophy in the CORD6 family (3), and a R838C substitution that is present in three additional small families with cone–rod dystrophy. Neither

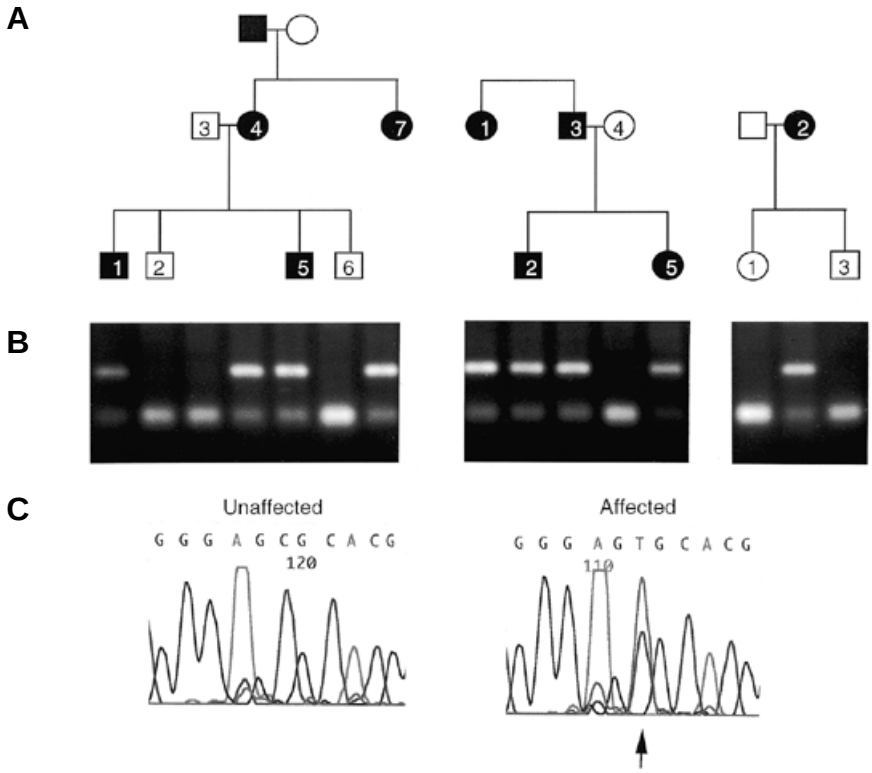

Figure 3. The dominant C→T missense mutation in affected members of the three other cone–rod dystrophy families. (**A**) Pedigrees of the three families, with numbers indicating the individuals for whom DNA samples were available for molecular analysis. (**B**) Co-segregation of the abolished *Hha*I site in exon 13 with the affected individuals of the families (see legend to Fig. 1B). (**C**) Sequence of an unaffected individual (left) and an affected individual (right). The C→T heterozygous missense mutation in exon 13 at nucleotide 2585 (R838C) is indicated by an arrow.

mutation is present in >600 normal chromosomes, indicating that the cone–rod dystrophies in the respective families appear to be caused by these mutations. However, until functional studies are undertaken, we cannot entirely rule out the possibility that these mutations are very rare non-pathological polymorphisms. The base changes are in adjacent nucleotides in the gene, suggestive of a mutation-prone region, although this will require the screening of additional cone–rod dystrophy patients to determine whether this is indeed the case.

The region of the gene where both mutations are located encodes the putative dimerization domain of the RETGC-1 protein (23). Significantly, recessive LCA1 mutations (12,13), although found throughout the catalytic and kinase-like domains, are notably absent from this region. This suggests a possible mechanism for the dominant action of the two cone–rod mutations. Substitutions in the dimerization domain may result in a steric change during dimer formation that affects the activity of both mutant–mutant and mutant–normal dimers. The resulting loss of functional enzyme would result in a reduction in activity below the 50% level expected in heterozygotes for recessive null mutations. Such a dominant-negative effect has already been demonstrated by *in vitro* mutagenesis in the rat orthologue, GC-E (31). The consequent inability to regenerate cGMP would account for the extreme photophobia exhibited by affected members of the CORD6 family.

To date, only three other genes have been implicated in cone–rod dystrophy, *peripherin/RDS* (5–8), *CRX* (9) and *ABCR* (10). The *RETGC-1* mutations are the first examples therefore of cone–rod dystrophy arising from structural changes in one of the enzymic components of the phototransduction process. However, the recent identification of a dominant mutation in the activator of retinal guanylate cyclase (*GUCA1A*) in dominant cone dystrophy (35) confirms the importance of a normal recovery phase in phototransduction to the maintenance of photoreceptor function.

MATERIALS AND METHODS

Isolation of DNA

DNA was extracted from EDTA-blood samples with a Nucleon II kit (Scotlab Bioscience). Genotyping was performed as described previously (22).

Mutation screening

The coding exons of the *RETGC-1* gene were amplified using the intronic primers and annealing temperatures essentially as described (12), except that the 5′ portion of exon 2 was amplified with primer pair 5′-TTACGGGGAGAACCCTAGGGGAGGCCG-3′ (forward) and 5′-AGAGAAGATGGGGTCGCAAG-3′ (reverse) at an annealing temperature of 68°C, the middle portion of exon 2 with primer pair 5′-CTCTCCGCCGTGTTCACGGT-3′ (forward) and 5′-GCGATCCCGGCTTCTTCGGC-3′ (reverse) at 60°C, and the 3′ portion of exon 2 with primer pair 5′-TCCGGTGAACCCTGCGGCCT-3′ (forward) and 5′-TGCCGGCAGGACCAGCCGAC-3′ (reverse) at 68°C. A different forward primer (5′-GCATTCTGG-

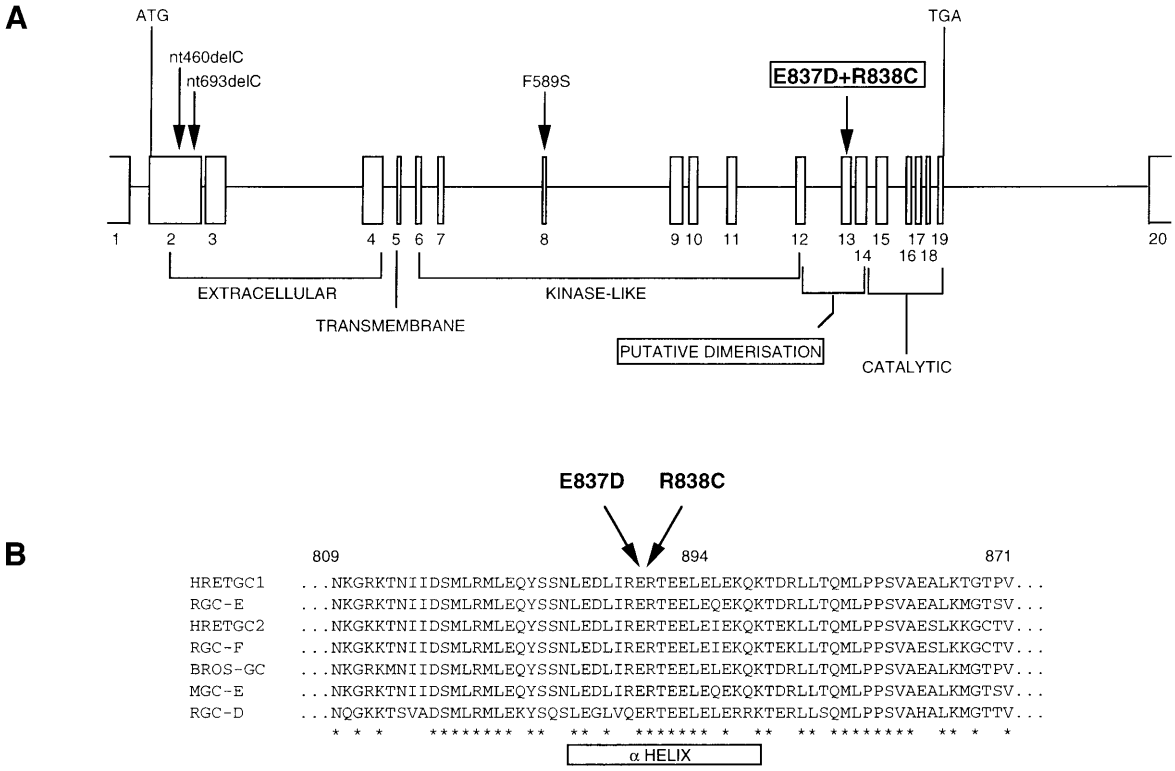

Figure 4. Structural and functional organization of guanylate cyclase. (**A**) Intron–exon structure of the human *RETGC-1* gene with protein domains shown underneath. E837D denotes the location of the G→C missense mutation within exon 13 in the CORD6 family. R838C denotes the location of the C→T missense mutation within exon 13 of the other three cone–rod dystrophy families. Both are situated within the putative dimerization domain of the protein. nt460delC, nt693delC and F589S denote published mutations which have been identified in exons 2 and 8 in three families with Leber's congenital amaurosis (12). (**B**) Amino acid sequence alignment of human RETGC-1, rat GC-E (29), human RETGC-2 (34), rat GC-F (29), bovine ROS-GC (36), mouse GC-E (29) and rat GC-D (37). Codon 894 (numbering from the start of translation) denotes the end of the putative dimerization domain and the start of the catalytic domain of the protein. Asterisks identify residues of identity, and the α-helical domain within this region is also indicated. Residues Glu837, which is replaced by Asp in the CORD6 family, and Arg838, which is changed to Cys in the three other cone–rod dystrophy families, are indicated by the arrows.

GACAGTGAGCC-3′) was used for exon 8. Exons 6 and 7 were amplified at an annealing temperature of 55°C, exon 11 at 68°C, exon 15 at 58°C and exon 17 at 68°C. PCR reactions (25 or 50 µl) were performed, each containing 1.5 mM $MgCl_2$, 0.4 mM each primer, 200 mM each dNTP, 16 mM $(NH_4)_2SO_4$, 67 mM Tris–HCl pH 8.8, 0.01% Tween-20 and 1 U of *Taq* DNA polymerase (Bioline). After an initial denaturation for 3 min at 94°C, 30 cycles of denaturation at 94°C for 1 min, annealing at the exon-specific temperature for 1 min, and extension at 72°C for 1 min were performed, with a final extension at 72°C for 3 min. Each exon was sequenced directly in both directions, using the PCR generation primers. Sequencing was performed using AmpliTaq FS polymerase cycle sequencing with dye-labelled dideoxyterminators, and the products were visualized on an Applied Biosystems Model 373 DNA Sequencer. Products obtained for exon 13 were digested with *Hha*I and analysed on 2% agarose gels.

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ABBREVIATIONS

ABCR, retina-specific ABC transporter gene; bovine *ROS-GC*, bovine rod outer segment guanylate cyclase gene; *CORD6*, cone–rod dystrophy 6 gene; *CRX*, photoreceptor-specific homeobox gene; *GUCA1A*, gene encoding the activator of retinal guanylate cyclase; *LCA1*, Leber's congenital amaurosis gene 1; mouse GC-E, mouse guanylate cyclase E; *peripherin/RDS*, peripherin retinal degeneration slow gene; rat GCE-D, GC-E and GC-F, rat guanylate cyclases D, E and F, respectively; *RETGC-1* and *RETGC-2*, human retinal guanylate cyclase genes 1 and 2, respectively.

REFERENCES

1. Moore, A.T. (1970) Cone and cone–rod dystrophies. *J. Med. Genet.*, **29**, 289–290.
2. Evans, K., Fryer, A., Inglehearn, C., Duvall-Young, J., Whittaker, J., Gregory, C.Y., Ebenezer, N., Hunt, D.M. and Bhattacharya, S. (1994) Genetic linkage of cone–rod retinal degeneration to chromosome 19q and evidence for segregation distortion. *Nature Genet.*, **6**, 210–213.

3. Kelsell, R., Evans, K., Gregory, C.Y., Moore, A.T., Bird, A.C. and Hunt, D.M. (1997) Localization of a gene for dominant cone–rod dystrophy (*CORD6*) to chromosome 17p. *Hum. Mol. Genet*., **6**, 597–600.
4. Kelsell, R.E., Evans, K., Gregory, C.Y., Jay, M.R., Moore, A.T., Bird, A.C. and Hunt, D.M. (1998) Localisation of a gene for dominant cone–rod dystrophy (CORD7) to chromosome 6q. *Am. J. Hum. Genet.*, in press.
5. Nakazawa, M., Kikawa, E., Chida, Y. and Tamai, M. (1994) Asn244His mutation of the peripherin/*RDS* gene causing autosomal dominant cone–rod degeneration. *Hum. Mol. Genet.*, **3**, 1195–1196.
6. Nakazawa, M., Kikawa, E., Chida, Y., Shiono, T. and Tamai, M. (1996) Autosomal dominant cone–rod dystrophy associated with mutations in codon 244 (Asn244His) and codon 184 (Tyr184Ser) of the *peripherin*/*RDS* gene. *Arch. Ophthalmol.*, **114**, 72–78.
7. Nakazawa, M., Naoi, N., Wada, Y., Kakazaki, S., Maruiwa, F., Sawada, A. and Tamai, M. (1996) Autosomal dominant cone–rod dystrophy associated with a Val200Glu mutation of the *peripherin*/*RDS* gene. *Retina*, **16**, 405–410.
8. Kohl, S., Christ-Adler, M., Apfelstedt-Sylla, E., Kellner, U., Eckstein, A., Zrenner, E. and Wissinger, B. (1997) *RDS/peripherin* gene mutations are frequent causes of central retinal dystrophies. *J. Med. Genet*., **34**, 620–626.
9. Freund, C. L., Gregory-Evans, C. Y., Furukawa, T., Papaioannou, M., Looser, J., Ploder, L., Bellingham, J., Ng, D., Herbrick, J.-A.S., Duncan, A., Scherer, S.W., Tsui, L.-C., Loutradis-Anagnostou, A., Jacobson, S.G., Cepko, C.L., Bhattacharya, S.S. and McInnes, R.R. (1997) Cone–rod dystrophy due to mutations in a novel photoreceptor-specific homeobox gene (*CRX*) essential for maintenance of the photoreceptor. *Cell*, **91**, 543–553.
10. Cremers, F.P.M., van de Pol, D.J.R., van Driel, M., de Hollander, A.I., van Haren, F.J.J., Knoers, N.V.A.M., Tijmes, N., Bergen, A.A.B., Rohrschneider, K., Blankenagel, A., Pinckers, A.J.L.G., Deutman, A.F. and Hoyng, C.B. (1998) Autosomal recessive retinitis pigmentosa and cone–rod dystrophy caused by splice site mutations in the Stargardt's disease gene *ABCR*. *Hum. Mol. Genet*., **7**, 355–362.
11. Oliveira, L., Miniou, P., Viegas-Pequignot, E., Rozet, J.-M., Dollfus, H. and Pittler, S.J. (1994) Human retinal guanylate cyclase (GUC2D) maps to chromosome 17p13.1. *Genomics*, **22**, 478–481.
12. Perrault, I., Rozet, J.M., Calvas, P., Gerber, S., Camuzat, A., Dollfus, H., Châtelin, S., Souied, E., Ghazi, I., Leowski, C., Bonnemaison, M., Le Paslier, D., Frézal, J., Dufier, J.-L., Pittler, S., Munnich, A. and Kaplan, J. (1996) Retinal-specific guanylate cyclase gene mutations in Leber's congenital amaurosis. *Nature Genet.*, **14**, 461–464.
13. Perrault, I., Rozet, J. M., Munnich, A. and Kaplan, J. (1997) Des mutations retrouvées pour la première fois dans une guanylyl cyclase (*retGC*) responsables d'une cécité néonatale: l'amaurose congénitale de Leber. *Médecine/Sciences*, **13**, 581–583.
14. Nichols, B.E., Sheffield, V.C., Vandenburgh, K., Drack, A.V., Kimura, A.E. and Stone, E.M. (1993) Butterfly-shaped pigment dystrophy of the fovea caused by a point mutation in codon 167 of the RDS gene. *Nature Genet.*, **3**, 202–207.
15. Weleber, R.G., Carr, R.E., Murphey, W.H., Sheffield, V.C. and Stone, E.M. (1993) Phenotypic variation including retinitis pigmentosa, pattern dystrophy, and fundus flavimaculatus in a single family with a deletion of codon 153 or 154 of the *peripherin*/*RDS* gene. *Arch. Ophthalmol.*, **111**, 1531–1542.
16. Wells, J., Wroblewski, J., Keen, J., Inglehearn, C., Jubb, C., Eckstein, A., Jay, M., Arden, G., Bhattacharya, S. and Bird, A.C. (1993) Mutations in the human retinal degeneration slow (*RDS*) gene can cause either retinitis pigmentosa or macular dystrophy. *Nature Genet.*, **3**, 213–218.
17. Reig, C., Alicia, S., Gean, E., Vidal, M., Arumi, J., De la Calzada, M.D., Antich, J. and Carballo, M. (1995) A point mutation in the RDS-peripherin gene in a Spanish family with central areolar choroidal dystrophy. *Ophthal. Genet.*, **16**, 39–44.
18. Allikmets, R., Singh, N., Sun, H., Shroyer, N.F., Hutchinson, A., Chidambaram, A., Gerrard, B., Baird, L., Stauffer, D., Peiffer, A. *et al.* (1997) A photoreceptor cell-specific ATP-binding transporter gene (*ABCR*) is mutated in recessive Stargardt macular dystrophy. *Nature Genet.*, **15**, 236–246
19. Martinez-Mir, A., Paloma, E., Allikmets, R., Ayuso, C., del Rio, T., Dean, M., Vilageliu, L., Gonzàlez-Duarte, R. and Balcells, S. (1998) Retinitis pigmentosa caused by a homozygous mutation in the Stargadt disease gene *ABCR*. *Nature Genet.*, **18**, 11–12.
20. McLaughlin, M.E., Sandberg, M.A., Berson, E L. and Dryja, T.P. (1993) Recessive mutations in the gene encoding the b-subunit of rod phosphodiesterase in patients with retinitis pigmentosa. *Nature Genet*., **4**, 130–134.
21. Gal, A., Orth, U., Baehr, W., Schwinger, E. and Rosenberg, T. (1994) Heterozygous missense mutation in the rod cGMP phosphodiesterase b-subunit gene in autosomal dominant stationary night blindness. *Nature Genet*., **7**, 64–68.
22. Kelsell, R., Godley, B.F., Evans, K., Tiffin, P.A.C., Gregory, C.Y., Plant, C., Moore, A.T., Bird, A.C. and Hunt, D.M. (1995) Localization of a gene for progressive bifocal chorioretinal atrophy (PBCRA) to chromsome 6q. *Hum. Mol. Genet*., **4**, 1653–1656.
23. Laura, R.P., Dizhoor, A.M. and Hurley, J.B. (1996) The membrane guanylyl cyclase, retinal guanylyl cyclase-1, is activated through its intracellular domain. *J. Biol. Chem*., **271**, 11646–11651.
24. Yang, R.-B., Fülle, H.-J. and Garbers, D.L. (1996) Chromosomal localization and genomic organization of genes encoding guanylyl cyclase receptors expressed in olfactory sensory neurons and retina. *Genomics*, **31**, 367–372.
25. Chabre, M. and Deterre, P. (1989) Molecular mechanism of visual transduction. *Eur. J. Biochem*., **179**, 255–266.
26. Polans, A., Baehr, W. and Palczewski, K. (1996) Turned on by Ca^2! The physiology and pathology of Ca^{2+}-binding proteins in the retina. *Trends Neurosci.*, **19**, 547–554.
27. Dizhoor, A.M., Lowe, D.G., Olshevskaya, E.V., Laura, R.P. and Hurley, J.B. (1994) The human photoreceptor membrane guanylyl cyclase, ret GC, is present in outer segments and is regulated by calcium and soluble activator. *Neuron*, **12**, 1345–1352.
28. Lolly, R.N. and Lee, R.H. (1990) Cyclic GMP and photoreceptor function. *FASEB J*., **4**, 3001–3008.
29. Yang, R.-B., Foster, D.C., Garbers, D.L. and Fülle, H.-J. (1995) Two membrane forms of guanylyl cyclase found in the eye. *Proc. Natl Acad. Sci. USA*, **92**, 602–606.
30. Thompson, D.K. and Garbers, D.L. (1995) Dominant negative mutations of the guanylyl cyclase-A receptor. Extracellular domain deletion and catalytic dominant point mutations. *J. Biol. Chem*., **270**, 425–430.
31. Yang, R.-B. and Garbers, D.L. (1997) Two eye guanylyl cyclases are expressed in the same photoreceptor cells and form homomers in preference to heteromers. *J. Biol. Chem*., **272**, 13738–13742.
32. Dizhoor, A.M., Lowe, D.G., Olshevskaya, E.V., Laura, R.P. and Hurley, J.B. (1994) The human photoreceptor membrane guanylyl cyclase, RetGC, is present in outer segments and is regulated by calcium and a soluble activator. *Neuron*, **12**, 1345–1352.
33. Liu, X., Seno, K., Nishizawa, Y., Hayashi, F., Yamazaki, A., Matsumoto, H., Wakabayashi, T and Usukura, J. (1994) Ultrastructural localization of retinal guanylate cyclase in human and monkey retinas. *Exp. Eye Res*., **59**, 761–768.
34. Lowe, D.G., Dizhoor, A.M., Liu, K., Gu, Q., Spencer, M., Laura, R., Lu, L. and Hurley, J.B. (1995) Cloning and expression of a second photoreceptor-specific membrane retina guanylyl cyclase (RetGC), RetGC-2. *Proc. Natl Acad. Sci. USA*, **92**, 5535–5539.
35. Payne, A.M., Downes, S.M., Bessant, A.A.R., Taylor, R., Holder, G.E., Warren, M.J., Bird, A.C. and Bhattacharya, S.S. (1998) A mutation in guanylate cyclase activator 1A (GUCA1A) in an autosomal dominant cone dystrophy pedigree mapping to a new locus on chromosome 6p21.1. *Hum. Mol. Genet*., **7**, 273–277.
36. Goraczniak, R.M., Duda, T., Sitaramayya, A. and Sharma, R.K. (1994) Structural and functional characterisation of the rod outer segment membrane guanylate cyclase. *Biochem. J*., **302**, 455–461.
37. Fülle, H.-J., Vassar, R., Foster, D.C., Yang, R.-B., Axel, R. and Garbers, D.L. (1995) A receptor guanylyl cyclase expressed specifically in olfactory sensory neurons. *Proc. Natl Acad. Sci. USA*, **92**, 3571–3575.