# Progressive Cone Dystrophy Associated with Mutation in *CNGB3*

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**Purpose.** To determine the molecular basis for phenotypic variability in a three-generation consanguineous family containing a single individual with complete achromatopsia and three individuals with progressive cone dystrophy.

**Methods.** Four affected individuals underwent ophthalmic examination, electrophysiological assessment, color fundus photography, and psychophysical testing. Blood samples were obtained for DNA extraction and mutation screening of the cone-specific cGMP-gated (CNG) channel protein gene *CNGB3* was undertaken.

**Results.** The clinical findings in one family member were consistent with a diagnosis of complete achromatopsia, with nystagmus, photophobia, and poor visual acuity from early infancy and complete color-blindness, normal fundi, and absent cone responses with normal rod responses on electroretinography (ERG). Mutation analysis revealed her to be homozygous for the common *CNGB3* achromatopsia mutation, 1148delC (Thr383fs). In contrast, the three other symptomatic individuals in the family had findings consistent with progressive cone dystrophy. Their visual problems began later in childhood (ranging from 3 to 14 years of age) and there was evidence of progressive deterioration in cone function. All three had a marked tritanopic color vision defect and fundoscopy revealed bilateral macular atrophy. Electrophysiological testing of these three subjects demonstrated clear evidence of progressive deterioration of cone responses over time; rod responses were normal. All three individuals with this progressive phenotype were found to be compound heterozygotes for the 1148delC (Thr383fs) frameshift mutation and a novel Arg403Gln missense mutation in *CNGB3*.

**Conclusions.** Mutations in *CNGB3*, which have been shown to cause achromatopsia, are now shown to be associated with autosomal recessive progressive cone dystrophy. In this study, a novel Arg403Gln mutation was identified, located in the middle of the pore domain of the cone CNG cation channel β-subunit, which when associated with the nonsense mutation Thr383fs, resulted in progressive cone dystrophy. (*Invest Ophthalmol Vis Sci.* 2004;45:1975–1982) DOI:10.1167/iovs.03-0898

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Progressive cone dystrophies (PCDs) are a clinically heterogeneous group of disorders. In contrast to the stationary cone dystrophies, which present in early infancy and childhood, the PCDs are not usually symptomatic until late childhood or early adult life.[1] In the PCDs, photophobia is a prominent early symptom, and there is progressive loss of central vision and color vision, with nystagmus also being a common feature.[2,3] Fundus examination often reveals a typical bull's-eye maculopathy, although in some cases there may be only minor atrophy and pigmentation of the macular retinal pigment epithelium. The optic nerve head may show a variable degree of temporal pallor.

PCDs are usually characterized by a progressive loss of color vision, with all three classes of cone photoreceptor affected, thereby producing color vision defects along all three color axes and often progressing to complete loss of color vision over time.[2,3] Exceptions to this are cases in which there is a predominant involvement of L-cones leading to a protan color vision phenotype.[4–6] Autosomal dominant cone dystrophy pedigrees with early tritan color vision defects have also been reported.[7–9]

PCDs are genetically heterogeneous, with autosomal dominant, autosomal recessive, and X-linked inheritance all having been described. Several loci and causative genes have now been identified, including COD1, COD2, GUCA1A, and *RPGR*.[10–13]

In contrast, achromatopsia is a stationary cone dystrophy that appears at an earlier age and is inherited as an autosomal recessive trait. To date, three genes associated with achromatopsia have been characterized: *CNGA3*[14–16] and *CNGB*3,[17–19] located at 2q11 and 8q21, which encode the α- and β-subunits of the cGMP-gated cation channel in cone cells, respectively, and *GNAT2*, located at 1p13, which encodes the cone α-transducin subunit.[20,21]

Molecular genetic studies of patients with achromatopsia have revealed more than 50 disease-causing mutations in *CNGA3*,with most being missense mutations.[15,16] In contrast, only 10 different mutations of *CNGB3* have been identified to date, with seven of these being nonsense mutations.[18,19,22–24] The most frequent mutation of *CNGB3* currently identified is the single base-pair frameshift deletion, 1148delC (Thr383fs), which has been shown to be present in up to 84% (199/237) of the *CNGB3* mutant disease chromosomes in patients with achromatopsia (Kohl S. *IOVS* 2001;42:ARVO Abstract 1745).[18]

Missense mutations in *CNGA3* have been previously reported in two individuals with cone–rod dystrophy and in a single individual with a progressive cone dystrophy phenotype.[16] However, mutations in *CNGB3* have not been associated with PCD. We now report, in a consanguineous family,


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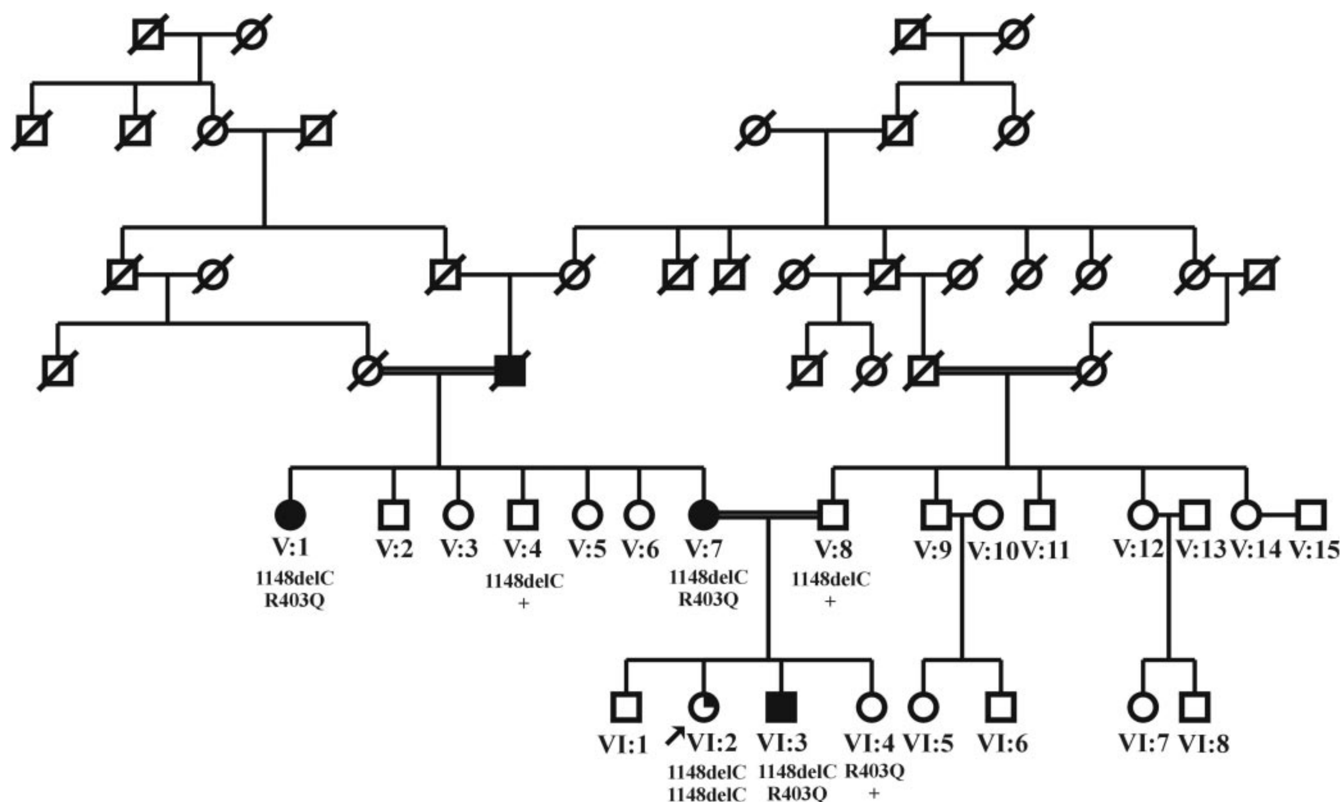


**FIGURE 1.** Family pedigree. *Solid symbols*: individuals with progressive cone dystrophy; *symbol with a single shaded quadrant*: subject with an achromatopsia phenotype; and *open symbols*: unaffected individuals. The segregation of the *CNGB3* sequence variants, Thr383fs and R403Q, is shown.

mutations in *CNGB3* that cause complete achromatopsia and an autosomal recessive progressive cone dystrophy phenotype.

## PATIENTS AND METHODS

A three-generation family with autosomal recessive cone dystrophy was ascertained. After informed consent was obtained, blood samples from affected and unaffected family members were taken for DNA extraction and subsequent mutation screening of *CNGB3*. The protocol of the study adhered to the provisions of the Declaration of Helsinki.

### Clinical Assessment

Four affected members of a consanguineous Pakistani family with cone dystrophy were assessed (Fig. 1). A full medical history was taken and an ophthalmic examination performed. Subjects also underwent color fundus photography and electrophysiological assessment, including electro-oculography (EOG) and full-field electroretinography (ERG), incorporating the recommendations of the International Society for Clinical Electrophysiology of Vision.[25,26] The testing conditions for rod-specific ERG were as follows: pupil dilatation, 20-minute dark adaptation, and Ganzfeld stimulation with a stimulus intensity of 2.5 log units below standard flash. For photopic ERG testing, conditions included: 10 minute preadaptation, background illumination of 28 $cd/m^2$, and single flash photopic response recorded using a standard flash of 2.8 $cd/m^2$ (Ganzfeld). Corneal Burian-Allen contact lens electrodes were used to record the ERGs.

Color vision testing using the enlarged Farnsworth D-15 (PV-16) and/or the Farnsworth-Munsell (FM) 100-hue test was performed in all four affected individuals. In two affected subjects (V:7 and VI:2) further color vision testing was possible, including the use of the Hardy-Rand-Ritter (HRR) plates (American Optical Company, New York, NY), Sloan achromatopsia plates, the Mollon-Reffin (M-R) minimal test,[27] and anomaloscopy. The PV-16, FM 100-hue test, Sloan achromatopsia plates and M-R test were all performed under CIE Standard Illuminant C from a MacBeth easel lamp.

### Molecular Genetic Analysis

The 18 coding exons of *CNGB3* were amplified by PCR in each individual by using primer sequences and conditions, as previously published.[18] Fifty-microliter PCR reactions were performed as follows: $1 \times NH_4$ reaction buffer (Bio-Line, London, UK), 1 mM $MgCl_2$, 200 $\mu$M each dNTP, 10 pmol each of the forward and reverse primers, 200 ng to 1 $\mu$g DNA, 1 U *Taq* (Bio-Line). After resolution on a 1% (wt/vol) agarose gel, products were excised and eluted. Direct sequencing of PCR products was performed on a gene analyzer (model 3100; Applied Biosystems [ABI], Foster City, CA), using the original PCR primers in the sequencing reactions. The sequence was examined for alterations, using DNA Sequence Analysis (ABI Prism) and GeneWorks (IntelliGenetics, Inc., Mountain View, CA) software.

GenBank sequences were used to construct an alignment of channel protein sequences and to analyze the evolutionary conservation of the corresponding amino acid positions (http://www.ncbi.nlm.nih.gov/Genbank; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD). The sequences used were the cGMP-gated (CNG) channel cone $\beta$-subunits of human (accession number AF272900), mouse (AJ243572) and dog (AF490511) and the rod $\beta$-subunits of human (AF042498) and rat (NM031809). Sequences were aligned using Clustal W[28] (provided in the public domain by the European Bioinformatics Institute, European Molecular Biology Laboratory, Heidelberg, Germany; available at http://www.ebi.ac.uk/clustalw).

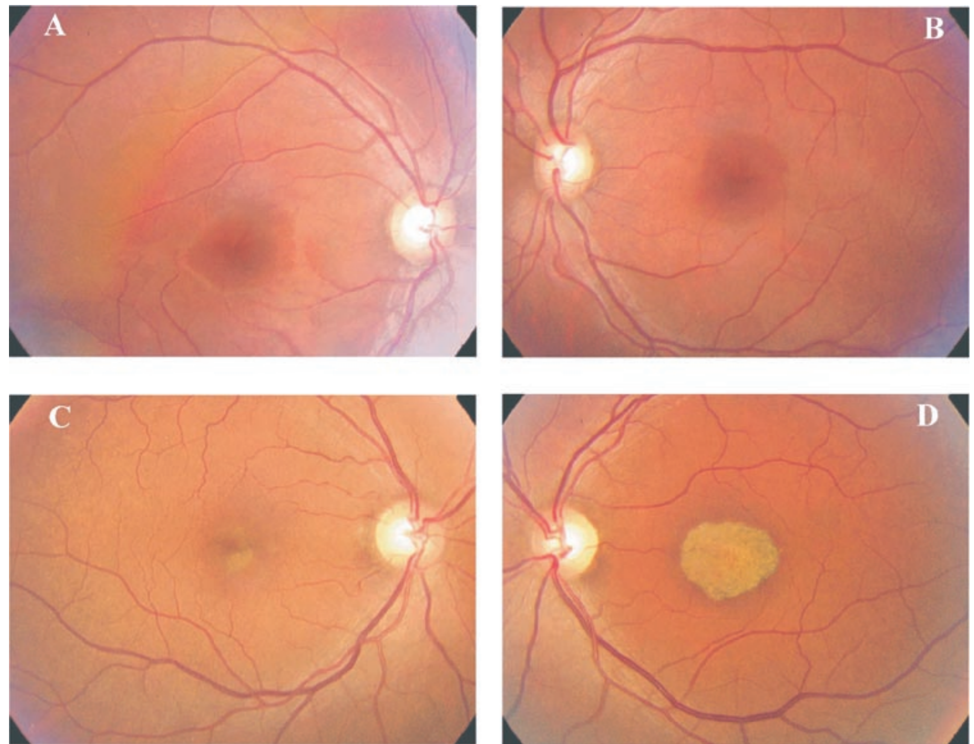


**Figure 2.** (**A**, **B**) Color fundus photographs of the index individual VI:2, showing a normal appearance. (**C**, **D**) Color fundus photographs of the index individual's mother, V:7, showing bilateral macular atrophy and pigmentation, worse in the left eye than in the right.

## Results

### Clinical Assessment

Four affected individuals of a three-generation, consanguineous Pakistani family were assessed (Fig. 1). The index case (VI:2) had a phenotype indistinguishable from complete achromatopsia, in that she had complete absence of color vision on detailed testing, prominent pendular nystagmus from early infancy, photophobia, and visual acuity of 3/60. Her fundi were found to be normal (Figs. 2A, 2B), and on ERG testing she had absent cone responses, with normal rod function (Fig. 3).

In contrast, the three other individuals in the family (V:1, V:7, and VI:3) presented with findings consistent with a progressive cone dystrophy phenotype. Their visual problems started later in childhood, ranging from 3 to 14 years of age, and a variable deterioration in visual acuity over time has been documented (Table 1). On examination, all three subjects were found to have residual color vision and bilateral well-demarcated pigmented macular atrophy (Figs. 2C, 2D). Psychophysical testing revealed a generalized dyschromatopsia affecting all three axes, with the color vision defect being much worse along the tritan than red-green axis. In individual V:7, color vision testing at two time points demonstrated progressive deterioration of cone function. At age 37, her FM 100-hue test total error score was 248; 12 years later it had increased to 400 (normal age-matched total error score is <100), with more tritan than red-green errors on both occasions (Fig. 4). Color vision testing of subject V:7 with HRR plates and the Mollon-Reffin minimal test confirmed the generalized dyschromatopsia, with the color vision defect being worst along the tritan axis. ERG testing performed at two time points, in two individuals (V:7 and VI:3) demonstrated a progressive deterioration in cone function (Fig. 3). Clinical findings are summarized in Table 1.

### Molecular Genetics

The 18 coding exons of *CNGB3* were screened for mutations in affected and unaffected family members. As shown in Figure 1, the index case with complete achromatopsia was found to be homozygous for the 1-bp frameshift deletion 1148delC (Thr383fs). The other three affected individuals were found to be compound heterozygotes, carrying the Thr383fs mutation and a novel missense mutation, Arg403Gln (a G→A change at nucleotide 1208; Fig. 5). Unaffected relatives were either homozygous wild-type (wt), Thr383fs/wt heterozygotes or Arg403Gln/wt heterozygotes. The segregation of these *CNGB3* mutations can be seen in Figure 1.

The Arg403Gln (R403Q) mutation was not detected in 100 ethnically matched control chromosomes, indicating that it is not a common polymorphism. The position and nature of this substitution is fully consistent with disease association. Arg403 is located in the middle of the pore domain of the CNG channel subunit. It is conserved in other mammalian (dog and mouse) cone $\beta$-subunits but replaced in rod $\beta$-subunits by Lys, another positively charged residue. Alignment of the pore region of cone and rod $\alpha$- and $\beta$-subunits (Fig. 6) demonstrates the high degree of sequence conservation in this region, particularly within each subunit class. The two classes differ, however, in the number of charged residues, with only one (Glu) in the $\alpha$-subunits but three in the $\beta$-subunits. The Glu in the $\alpha$-subunit is known to be responsible for $Ca^{2+}$ binding in homotetrameric CNGA channels,[29–32] and the loss of charge arising from the Arg403Gln substitution in the $\beta$-subunit is likely to be significant therefore for the functioning of the pore region.

## Discussion

Mutations in the gene encoding the cone-specific $\beta$-subunit of the cGMP-gated cation channel protein are a common cause of the stationary cone dysfunction syndrome, achromatopsia.[18,19,22–24] The novel Arg403Gln mutation reported herein, when associated with the frameshift mutation, Thr383fs results in PCD. To the best of our knowledge, this is the first demonstration of PCD caused by a mutation in *CNGB3*.

Two sequence variants have been identified in this pedigree: Thr383fs and Arg403Gln. Thr383fs, the most common

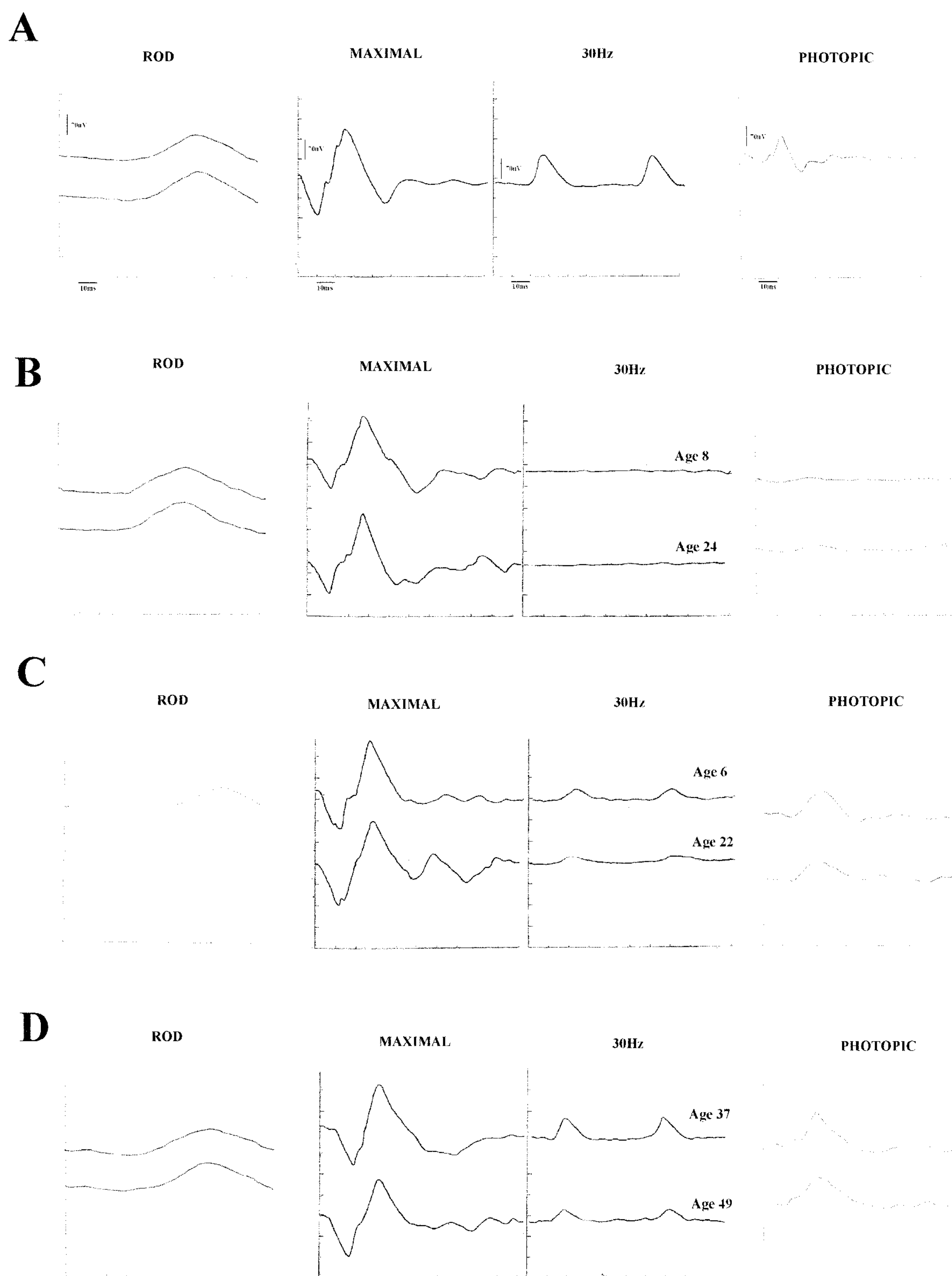


**FIGURE 3.** ERG data from three patients. (All ERGs scaled at 70 $\mu$V/division on the *y*-axis and 10-ms/division on the *x*-axis; as indicated on normal traces; **A**). ERG testing of the index case (VI:2) revealed absent cone responses and normal rod function. In contrast, ERG testing performed at two time points in two affected individuals (V:7 and VI:3), demonstrated progressive deterioration in cone function. (**A**) Normal ERG traces for comparison. (**B**) VI:2: normal rod but absent cone responses at ages 8 and 24 years. (**C**) VI:3: normal rod responses but a progressive reduction in cone responses between the ages of 6 and 22 years. (**D**) V:7: normal rod responses but a progressive reduction in cone responses between the ages of 37 and 49 years.

disease mutation identified in *CNGB3* (Kohl S. *IOVS* 2001;42: ARVO Abstract 1745),[18,24] would generate, if translated, a truncated channel subunit that lacks three important regions: the pore domain, the sixth $\alpha$-helical transmembrane region, and the cGMP-binding site. The index case with complete achromatopsia is homozygous for this null mutation, Thr383fs,

**TABLE 1.** Summary of Clinical Findings

| Patient | Age (y) | Visual acuity (OD OS) | Refractive Error (OD OS) | Nystagmus | ERG | Macula | Color Vision |
|---|---|---|---|---|---|---|---|
| VI:2 | 8 | 3/60 | +4.50/−1.0 × 90 | Pendular | Absent cone responses; normal rod function | Normal | Absent |
| | | 3/60 | +3.00/−1.0 × 90 | | | | |
| | 24 | 3/60 | +4.75 DS | Pendular | Absent cone responses; normal rod function | Normal | Absent |
| | | 3/60 | +3.25 DS | | | | |
| VI:3 | 6 | 6/24 | +2.50 DS | Manifest latent | Reduced cone responses; normal rod function | Bilateral pigmented macular atrophy OS > OD | Residual; D15 tritan axis |
| | | 6/24 | +2.00 DS | | | | |
| | 22 | 1/60 | +1.0 DS | Manifest latent | Markedly reduced cone responses; normal rod function | Bilateral pigmented macular atrophy OS > OD | Residual |
| | | 2/60 | +1.0 DS | | | | |
| V:7 | 37 | 6/24 | Plano/+2.0 × 90 | Latent | Reduced cone responses; normal rod function | Bilateral pigmented macular atrophy OS > OD | Residual; D15 tritan axis |
| | | 6/24 | Plano/+1.0 × 90 | | | | |
| | 43 | 6/36 | Plano/+2.0 × 90 | Latent | Reduced cone responses; normal rod function | Bilateral pigmented macular atrophy OS > OD | FM 100-hue: tritan axis with error score of 268 |
| | | 6/36 | Plano/+1.0 × 90 | | | | |
| | 49 | 6/36 | — | Latent | Markedly reduced cone responses; normal rod function | Increased bilateral pigmented macular atrophy OS > OD (Figs. 2C, 2D) | FM 100-hue: tritan axis with error score of 400 (Fig. 4) |
| | | 6/36 | | | | | |
| V:1 | 42 | 6/24 | −1.25 DS | Absent | — | Bilateral pigmented macular atrophy | — |
| | | 6/24 | −0.75 DS | | | | |
| | 56 | 6/36 | — | Absent | Reduced cone responses; normal rod function | Increased bilateral pigmented macular atrophy | Residual; FM 100-hue: tritan axis with error score of 584 |
| | | 6/36 | | | | | |

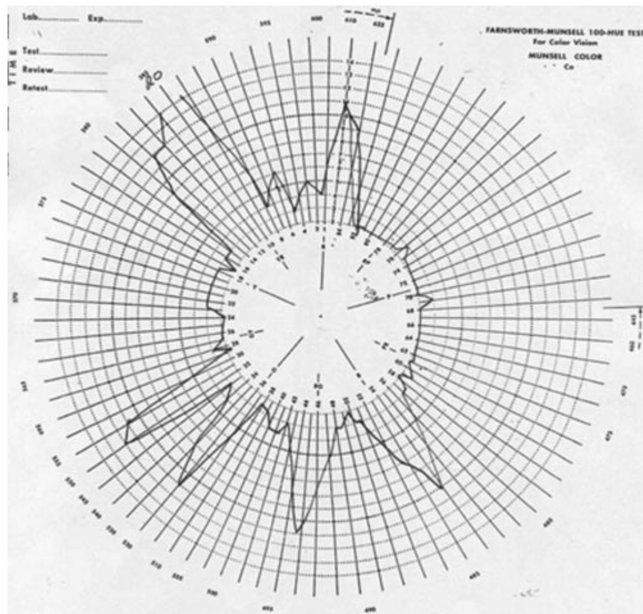

**FIGURE 4.** The Farnsworth Munsell 100-hue color vision test performed by V:7, demonstrating a tritan axis of confusion with a total error score of 400.

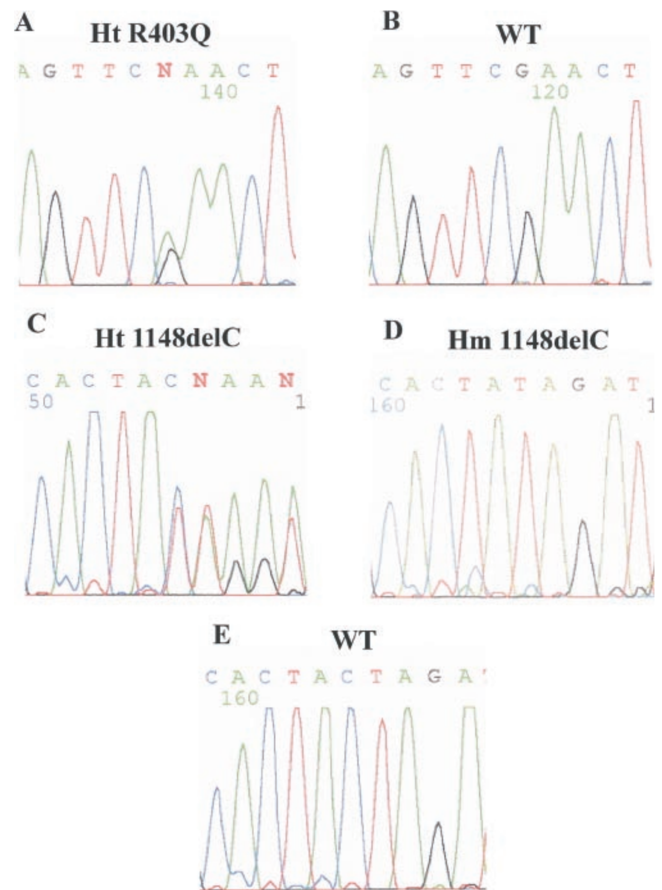


**FIGURE 5.** Sequence electropherograms of exon 11 (**A**, **B**) and exon 10 (**C–E**) of *CNGB3*. (**A**) Affected compound heterozygote showing the G→A change at nucleotide 1208 which results in the novel missense mutation, Arg403Gln; (**B**) unaffected subject; (**C**) heterozygous 1148delC mutation; (**D**) homozygous 1148delC mutation; (**E**) unaffected subject.

whereas the three other affected individuals with PCD are compound Thr383fs/Arg403Gln heterozygotes. The novel missense alteration we have identified, Arg403Gln (R403Q), is located in the middle of the pore domain of the CNG channel subunit at a site conserved in other cone $\beta$-subunits and occupied by another positively charged residue, Lys, in rod $\beta$-subunits. The mutation places neutral Gln into this site. This change in charge from a positively charged Arg to an uncharged Gln may affect cation transfer through the channel pore and thereby adversely affect channel function.

Negatively charged residues are known to be important for the binding of $Ca^{2+}$ to the pore[29–32]; replacement of the single charged Glu residue in the $\alpha$-subunit pore region (see Fig. 6) with various neutral residues abolishes high-affinity $Ca^{2+}$-binding.[30,31,33] Similar experiments have not been performed with the $\beta$-subunit, and so the precise role of Arg403 has yet to be established. However, the markedly reduced color vision and visual acuity present in compound heterozygotes indicates that the mutant Gln403 subunit supports only limited cation movement through the channel to account for the residual cone function in these patients. Nevertheless, this contrasts with the total loss of function and complete color-blindness associated with homozygosity for the Thr383fs frameshift mutation.[18,19,22,24]

The progressive nature of the disorder in the compound heterozygous patients is more difficult to explain. One possibility is that truncated mutant protein arising from the Thr383fs mutant allele accumulates over time, leading to progressive cone cell loss; however, it is most likely that the frameshift mutation is subject to nonsense mediated mRNA decay. Alternatively, it seems more probable that the progressive nature of the disorder arises from effects of the Arg403Gln missense mutation. The abnormal functioning of the mutant protein may alter the intracellular levels of $Ca^{2+}$ and/or cGMP. Such changes in cGMP are known to result in photoreceptor loss in the *rd* mouse[34] and may also underlie the cone–rod and cone dystrophies associated with dominant mutations in *GUCY2D*, the retinal form of guanylyl cyclase type 1,[35,36] and its activating protein, *GUCA1A* (GCAP1).[13,37]

The missense mutation Ser435Phe (S435F), identified in *CNGB3*, is believed to be the underlying cause of the unique high incidence (5%–10%) of achromatopsia in the Pingelapese people of the Eastern Caroline Islands in Micronesia.[18,19,38] Ser435Phe is located in the sixth transmembrane domain of the CNGB3 polypeptide. Although causing complete achromatopsia, the recent report that the Ser435Phe mutation, when coexpressed with human wt CNGA3 subunits in *Xenopus*

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Rod α (CNGA1)   VYSLYWSTLTLTTIGETPP
Cone α (CNGA3)  VYSLYWSTLTLTTIGETPP
                        **   ** ***   *
Rod β (CNGB1)    IRCYYWAVKTLITIGGLPD
Cone β (CNGB3)  LRCYYWAVRTLITIGGLPE
                         ↑
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**FIGURE 6.** Sequence alignment of the pore region of human cone and rod $\alpha$- and $\beta$-subunits of the CNG channels. *Arrow*: the position of the Arg403Gln substitution; *bold*: charged residues; *asterisks*: identity of residues conserved across both rod and cone subunits.

oocytes,[39] results in an increased affinity for cGMP, a decreased single channel conductance, and a decreased sensitivity to blockage by L-*cis*-diltiazem, demonstrates that mutant effects on $\beta$-subunit function have the potential to alter the balance between cGMP and $Ca^{2+}$ within the photoreceptor. Indeed, because Ser435Phe, located in a transmembrane domain, has been demonstrated to affect channel properties, it is certainly plausible that Arg403Gln, a mutation actually located within the pore region itself, may also affect channel conductance and thereby lead to a cone dystrophy phenotype. In contrast, coexpression of mutant Thr383fs $\beta$-subunits with wt CNGA3 subunits produced channels with properties indistinguishable from homomeric CNGA3 channels, consistent therefore with the complete nonfunctioning of the truncated protein.[39]

We report the first family with a progressive cone dystrophy phenotype associated with mutation in the cone-specific cGMP-gated channel gene *CNGB3*. It has been possible to establish a genotype–phenotype correlation in this pedigree. The homozygous Thr383fs genotype, is associated with complete achromatopsia, in which there is absent cone function. This contrasts with the compound heterozygote genotype, Thr383fs/Arg403Gln, in which it is likely there is residual mutant protein function, which results in a progressive cone dystrophy phenotype.

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