New Mutation, P575L, in the GUCY2D Gene in a Family With Autosomal Dominant Progressive Cone Degeneration

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Objectives: To clinically characterize the retinal abnormalities and identify the mutation causing an autosomal dominant cone degeneration in an African American family.

Methods: Clinical characterization of family members using fundus photography, fluorescein angiography, and electrophysiological testing. Standard molecular genetic methods were used, including segregation analysis and DNA sequencing of candidate genes. Genetic mutation screening was performed in 20 individuals: 10 clinically unaffected and 10 affected.

Results: The affected family members had findings consistent with a primary cone degeneration. A novel mutation, P575L, was found in exon 8 of the GUCY2D gene in 12 members of this family.

Conclusions: In addition to finding a previously undescribed mutation in GUCY2D, 2 of the family members who were thought to be unaffected through routine clinical examinations also had this mutation. These findings suggest that autosomal dominant cone degeneration in this family demonstrated age-dependent penetrance, which appears incomplete. This is the first African American family reported with a mutation in GUCY2D. Because the disease in this family and the one we previously described is primarily a cone degeneration, this disease should be more properly classified as cone degeneration and be called cone degeneration 2.

Clinical Relevance: This study helps to expand the phenotype of the disease and help clinicians identify patients with cone degenerations.

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Cone dystrophies are a heterogeneous group of retinal disorders characterized by widespread loss of cone function with relative preservation of rod function. The classic triad of symptoms is photophobia, dyschromatopsia, and decreased central vision. Peripheral visual fields and night vision are typically preserved. Color vision is usually impaired early in the disease course with elevation of protan, deutan, or tritan thresholds. Funduscopy results can vary from mild pigment granularity to a discrete atrophic lesion in the center of the macula. Electrophysiologic testing is important to confirm the diagnosis. Pertinent electroretinogram (ERG) characteristics include a reduction in the single-flash photopic and 30-Hz flicker response. The rod-isolated responses are normal in the early stages; however, as the disease progresses, rod function may also be slightly impaired. Cone degeneration can be inherited as an autosomal dominant, autosomal recessive, or X-linked recessive trait with the most common variant being autosomal dominant.1-31

In the past decade, much progress has been made in characterizing the genetics of cone dystrophies. Several genetic loci have been identified in association with cone and cone-rod dystrophies, including peripherin/the human retinal degeneration slow gene (RDS), guanylate cyclase activator 1A gene (GUCA1A), and guanylate cyclase 2D gene (GUCY2D [Crx]). Other cone and cone-rod dystrophy loci have been mapped to chromosomes 6q, 18q, 19q, and 17p.32-59 CORD5, which is an autosomal dominant, almost pure cone degeneration, was mapped to chromosome 17p13-12 by Small et al and later found to be caused by mutations in GUCY2D.51-59 GUCY2D mutations were initially described in the autosomal recessive disease, Leber congenital amaurosis.60-69 We describe an African American family with autosomal dominant cone degeneration CORD5 with a previously undescribed mutation in GUCY2D.
Clinical data collected included medical and ocular histories, family history with pedigree, and external and slitlamp examination, refraction, and indirect and direct ophthalmoscopy findings. Best-corrected Snellen visual acuities were obtained. Examinations of 20 participants were performed in the ophthalmology office or at the participants' homes.

**ELECTROPHYSIOLOGY TESTING**

The ERGs were performed on a full-field (Ganzfeld) combination, single flash-averaged unit on 5 participants. Our normal laboratory values and analyses of data for age and sex effects have been previously determined. The standard International Society of Clinical Electrophysiology in Vision protocol was followed. A 2-channel recorder was used for electrooculography testing. The maximum response in the light was divided by the minimum response in the dark and multiplied by 100 and expressed as a percentage (Arden ratio).

The final rod threshold was determined by dark adapting the patient for 45 minutes and then testing the threshold of light perception at 11° above fixation with a Goldmann-Weekers dark adaptometer. A Nagel anomaloscope was used for color vision screening. Goldmann visual field tests, fluorescein angiography, and fundus photography were performed in the standard manner on 4 members of the family.

**GENETIC ANALYSIS**

We ascertained a family of 4 generations with cone degeneration. The family is African American and comprises 24 living individuals (Figure 1). The inheritance pattern of the cone-rod degeneration phenotype in this family was consistent with an autosomal dominant trait. Nineteen individuals from the family were examined and characterized by the criteria previously described for CORDS. We obtained institutional review board approval and voluntary informed consent from all participating individuals before enrolling them in the study. Blood was obtained by venipuncture for DNA analysis from 20 participants. The DNA was extracted using the Puregene protocol (Gentra Systems, Minneapolis, Minnesota). The family's pedigree (Figure 1) was constructed using Cyrillic software (Cherwell Scientific Inc, Oxford, England).

Genotyping with microsatellite markers was performed as described by Small et al to define the minimal candidate region. New polymorphic markers were identified by analyzing sequences from clones (GenBank AD005695). Flanking primers were designed using Primer 3 (Broad Institute, Cambridge, Massachusetts; http://www.genome.wi.mit.edu) and Celera (Rockville, Maryland; http://www.celera.com). We developed the following markers, which are deposited at the GDB Human Genome Database (http://www.gdb.org): CORD5NU8 (GDB No. 11505481), CORD5NU12 (GDB No. 11505496), CORD5NU15 (GDB No. 11505484), CORD5NU17 (GDB No. 11505497), CORD5NU19 (GDB No. 11505486), CORD5NU20 (GDB No. 11505487), CORD5NU26 (GDB No. 11505488), CORD5NU34 (GDB No. 11505489), CORD5NU35 (GDB No. 11505498), CORD5NU37 (GDB No. 11505491), and CORD5NU38 (GDB No. 11505492).

For DNA sequencing of the candidate genes, primers were synthesized more than 20 base pairs internal to the intronic region. After polymerase chain reaction amplification using patient and control DNA, the products were separated on agarose gel, 2%. The bands were cut from the gel and purified using the Puregene protocol (Gentra Systems, Minneapolis, Minnesota). Mutation screening for the P575L mutation was performed for being polymorphic by genotyping a total of 12 controls. The previously mentioned clones were aligned using the publicly available gene sequence from GenBank (Bethesda, Maryland; http://www.ncbi.nlm.nih.gov) and Celera (Rockville, Maryland; http://www.celera.com). We developed the following markers, which are deposited at the GDB Human Genome Database (http://www.gdb.org): CORD5NU8 (GDB No. 11505481), CORD5NU12 (GDB No. 11505496), CORD5NU15 (GDB No. 11505484), CORD5NU17 (GDB No. 11505497), CORD5NU19 (GDB No. 11505486), CORD5NU20 (GDB No. 11505487), CORD5NU26 (GDB No. 11505488), CORD5NU34 (GDB No. 11505489), CORD5NU35 (GDB No. 11505498), CORD5NU37 (GDB No. 11505491), and CORD5NU38 (GDB No. 11505492).

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**RESULTS**

**REPORT OF CASES**

**Case 1**

Case 1 (#116) was examined at the age of 22 years and was subsequently seen at her home in 1995 at age 38 years. She had had seizures since the age of 12 years. Her medical history was otherwise unremarkable except for visual problems. Photophobia and decreased visual acuity began at 7 years of age. At age 22 years, her visual acuity was 20/200 OD and 20/300 OS. Slitlamp examination detected no abnormalities and her intraocular pressure levels were normal. Fundoscopy revealed a bilaterally symmetrical central area of macular retinal pigment epithelium (RPE) atrophy (Figure 2A). The transmission defect visible on fluorescein angiography corresponded to the dropout of the macular RPE (Figure 2B). There was no evidence of choroidal hypofluorescence or “silent choroid.” The photopic ERG was unrecordable (Figure 3). The scotopic ERG and the dark-adaptation final rod threshold results were normal. The electrooculography results were unreliable owing to poor central fixation. On reexamination at age 38 years, the participant’s near visual acuity was J16 OU; fundus examination revealed pigmentary clumps, but otherwise there was no significant change from her prior examination. Color vision testing with Ishihara plates was 0/14 OU.
Case 2

The cousin of case 1 (#108) was examined at 32 years of age in 1980 and at 47 years in 1995. She had had seizures since the age of 7 months and was mentally retarded. Her medical history was otherwise unremarkable except for visual problems. The family history was negative for seizures, except for case 1, and was negative for mental retardation.

She had had decreased visual acuity since the age of 7 years. Visual acuity was unobtainable in her right eye owing to poor cooperation and 20/80 OS. Slitlamp examination revealed no abnormalities. The macular area had a granular appearance with circular RPE atrophy. Electroretinogram and visual field testing were not possible owing to the patient’s poor cooperation.

Case 3

The mother of case 1 (#1006) was examined at ages 54 and 69 years in 1980 and 1995. Her medical history was unremarkable except for visual problems.

Funduscopy performed when she was aged 54 years showed a well-demarcated macular lesion with atrophy of the RPE. The photopic ERG was unrecordable and the scotopic ERG had normal amplitudes with a slightly prolonged implicit time (Figure 3). On examination at 69 years, her near visual acuity with correction was J16 OU. Color vision examination results with Ishihara plates was 0/14 OU.

Case 4

Case 4 (#105) was examined at ages 21 and 38 years and at home once when he was aged 33 years. His medical history was unremarkable except for visual problems, which surfaced during his teenage years.

On examination at age 21 years, his visual acuity without correction was 20/400 OU. Funduscopy showed an area of discrete macular RPE atrophy. Fluorescein angiography revealed hyperfluorescence of a window defect in the area of the macular RPE loss. The photopic ERG was nearly extinguished, and the flicker fusion test revealed an abnormal amplitude but was recordable at 60 flashes/s OU (Figure 3). The scotopic ERG and the dark-adaptation final rod threshold results were normal. Color vision testing with a Nagel anomaloscope revealed an abnormally wide equation with a protanomalous axis and a severe luminosity loss. The Goldmann visual field results were within normal limits except for a central scotoma. On examination at 33 years, his corrected visual acuity was 20/200 OD and 20/100 OS. His fundus examination results were unchanged. Color vision testing results with Ishihara plates was 0/14 OU.

Case 5

Case 5 (#101) is the sister of case 4. Her medical history was unremarkable except for visual problems. She was examined at ages 27 and 39 years.

On examination at 27 years, her visual acuity was 20/200 OD and 20/300 OS. Funduscopy showed a pattern very similar to case 4, with macular RPE atrophy (Figure 4A). Fluorescein angiography demonstrated hyperfluorescence in the macular region (Figure 4B).
photopic ERG and the flicker fusion test results were abnormal in each eye. The scotopic ERG and dark-adaptation final rod threshold results were normal. Color vision testing with the Nagel anomaloscope revealed an abnormality identical to that seen in case 4. The Goldmann visual fields were within normal limits except for a central scotoma. On examination at age 39 years, the patient’s uncorrected visual acuity was 20/400 (20/200 pinhole) OD and 20/400 (20/200 pinhole) OS. Fundus examination revealed central atrophy, which demonstrated large choroidal vessels in each eye. She missed all 14 plates in each eye in the Ishihara color vision testing.

Case 6

Case 6 (#1002), aged 47 years, is the mother of cases 4 and 5. Her medical history was unremarkable except for visual problems, which started at the age of 33 years. She was examined at ages 48 and 60 years.

On examination at 48 years, her visual acuity was 20/100 OD and 20/200 OS. Funduscopy showed macular RPE atrophy with a sharp border (Figure 5A). Fluorescein angiography allowed visualization of the choroidal vessels in the area of macular RPE atrophy (Figure 5B). The photopic ERG revealed subnormal amplitude in each eye. The scotopic ERG and the dark-adaptation final rod threshold results were normal (Figure 3). Color vision testing with the Nagel anomaloscope showed an abnormality identical to that seen in cases 4 and 5. The Goldmann visual fields were significant for a central scotoma in each eye. On examination at age 60 years, her visual acuity was 20/200 pinhole N1 OD and 20/200 (20/300 pinhole) OS. Fundus examination revealed a discrete macular atrophy in each eye,
which appeared unchanged from the patient’s previous examination.

Case 7

This individual (#110) was examined during a home visit at age 29 years. At that time, she had no visual complaints and specifically denied photophobia. Her visual acuity without correction using a near vision card was 20/15 OD and 20/20 OS. She correctly identified 15 of 15 pseudoisochromic color plates. Dilated funduscopic examination results were normal. She was found to have the P575L mutation.

Case 8

This patient (#9001) was examined during a home visit at the age of 14 years. She had no visual complaints and specifically denied photophobia. Her uncorrected visual acuity with a near card was 20/20 OD and 20/20 OS. She correctly identified 13 of 15 pseudoisochromatic color plates. Dilated funduscopic examination results were normal. She was found to have the P575L mutation.

GENETIC ANALYSIS

Genetic mutation screening was performed on 10 clinically unaffected and 10 affected individuals. A novel mutation, P575L, was found in exon 8 of the GUCY2D gene in 14 members of this family (Figure 6). Two of the family members (#9001 and #110) who were thought to be clinically unaffected by examinations also carried this mutation. We tested 244 control chromosomes and found no such base-pair changes.

The heterozygous mutation in exon 8, C1797T, resulted in a missense mutation in codon 575 (P575L), which segregated with all affected family members. There were 2 unaffected family members who were found to have the mutation as well, suggesting incomplete penetrance. This is the second report of apparent incomplete penetrance with GUCY2D mutations. Penetrance is the probability of expressing the disease given that a mutation is present, and the degree of penetrance depends on the extent and type of clinical evaluations performed on the participants. Penetrance can also be age dependent, as seems to be the case in this family as well as our previous white CORD5 family.

The first individual exhibiting reduced penetrance (#9001) was a 14-year-old girl with no symptoms of photophobia, hemeralopia, or decreased visual acuity. Her vision was 20/20 OU, and her fundus examination results were unremarkable. She may have been too young to have any clinical manifestations yet. The second unaffected individual (#110) was a 29-year-old woman with a visual acuity of 20/15 OD and 20/20 OS. She had no symptoms and no clinical evidence of cone degeneration on ophthalmoscopy. Because of previous reports documenting age-dependent penetrance in CORD5, it is possible that these individuals will manifest the disease phenotype at a later age, though it is unlikely for the 29-year-old woman (#110). Previous cone degeneration studies by Small et al41 have documented an individual who was symptomatically normal until age 51 years. This study estimated the age-dependent penetrance to be 60% in the first decade of life, 80% within the first 2 decades of life, and 95% thereafter. If one defines the disease and its penetrance on routine clinical examination, as is typical (without additional testing), these 2 family members (#9001 and #110) likely represent incomplete penetrance. Some geneticists make the case, rightfully, that incomplete penetrance is the extreme form of variable expressivity. In this study, most of the clinically affected family members felt they were visually impaired all of their lives or developed symptoms at ages 8 to 16 years. Based on our previously described CORD5 family, participants who were asymptomatic had normal ERG results as well. When performing genetic linkage studies, it is important to account for this in the model (para-

![Genomic structure of GUCY2D showing the mutation P575L in a family with autosomal dominant progressive cone degeneration.](http://archopht.jamanetwork.com/pdfaccess.ashx?url=/data/journals/ophth/11918/)
metric analysis) of the disease as an age-dependant penetrance that is not complete (i.e., incomplete penetrance). This seems to be an important feature of GUCY2D mutations.

Five different mutations (E837D, R838C, R838H, G838S, and T839M in exon 13) in the GUCY2D gene causing cone-rod degeneration have been described previously.\textsuperscript{41-54} The functional consequences of these guanylate cyclase mutations leading to cone-rod degeneration have not been fully elucidated.\textsuperscript{60-63} However, it has been shown that activated retinal guanylate cyclase GUCY2D restores cyclic guanine monophosphate levels in photo-receptor cells in light-adapted states.\textsuperscript{61,62} GUCY2D is activated by guanylate cyclase activation protein (GCAP1), which is itself inhibited by increased intracellular Ca\textsuperscript{2+} levels, which occurs during dark-adapted states.\textsuperscript{61} A mutant in GCAP1 (Y99C) has been shown in autosomal dominant cone degeneration to constitutively activate GUCY2D even in dark-adapted states.\textsuperscript{62}

Missense mutations in codon 838 have been previously described and characterized in other CORD5 and CORD6 families.\textsuperscript{41,43,45,51,53} Molecular biological studies by Willke et al.\textsuperscript{53} have shown that changing arginine to any of these amino acid substitutions at codon 838—R838A, R838C, R838H, or R838S—results in altered GCAP1-stimulated cyclase activity. This activity is equal to or superior to wild-type at low Ca\textsuperscript{2+} concentrations; however, at high Ca\textsuperscript{2+} concentrations, these mutations resulted in a failure to inactivate cyclase activity. Additionally, codon 838 was found to lie within the dimerization domain that forms a coiled-coiled structure in the active protein.\textsuperscript{54} Different mutations at codon 838 have shown different disease severity and age at onset. Even within single families with the same mutations, there is a significant difference in disease severity and age at onset in affected individuals.

The GUCY2D gene was first found to have mutations in patients with the autosomal recessive disease, Leber congenital amaurosis.\textsuperscript{49,64} Most of these mutations causing Leber congenital amaurosis are in the first few exons and cause severe disruption of the entire protein. Therefore, in the 1 gene, there are mutations causing autosomal recessive and autosomal dominant diseases. We feel that autosomal dominant cone degeneration is a misnomer. We suggest that this disease should be referred to as a cone degeneration 2 and not CORD5 or CORD6 as previously reported. We have shown previously that CORD5 and CORD6 are actually the same disease, gene, and mutation. Both of our families with CORD5 showed signs of having primarily a progressive degeneration of the cones with little or no rod involvement. One form of cone degeneration has been reported as X-linked and is designated cone degeneration 1. The autosomal dominant cone degeneration reported herein should be designated cone degeneration 2.

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