Stop Mutations in Exon 6 of the Choroideremia Gene, CHM, Associated With Preservation of the Electroretinogram

Choroideremia (Mendelian Inheritance in Man [MIM] #303100) is an uncommon X-linked chorioretinal degeneration characterized by motting of the retinal pigment epithelium (RPE) and inexorable centripetal extension of areas of choroidal and outer retinal atrophy. Extensive rod and cone system dysfunction characteristically occurs within the first years of life. Eventually, the rod electroretinogram (ERG) becomes undetectable and cone responses are recordable only with computer averaging. Nystagmus and progressive field constriction typically become evident in the second decade with acuity loss eventually occurring because of macula involvement. Female carriers often exhibit “moth-eaten,” patchy pigmentation at the level of the RPE compatible with lyonization.

We describe the finding of normal or mildly subnormal ERGs in 3 young affected males (aged 8, 9, and 10 years) from 2 unrelated families with choroideremia (Figure 1). Direct sequencing of the choroideremia gene (CHM) identified a different nonsense (stop) mutation in each family. The mutations were only 14 codons apart in exon 6, suggesting that a common mechanism underlying these mutations may lead to a milder clinical and electrophysiological phenotype.

The patient representing case 1 was asymptomatic when evaluated at age 6 years. Visual acuity was 20/20-2 OD and 20/20 OS. Fundus examination disclosed fine, peppery RPE motting in the midperiphery but normal-appearing optic discs, retinal vasculature, and maculae (Figure 2A-C). Bone spicule formation and choroidal atrophy were absent. His maternal grandfather had advanced choroideremia (Figure 2D).

Electroretinograms (Figure 3A) performed to standards from the International Society for Clinical Electrophysiology of Vision demonstrated, at age 10 years, mildly subnormal responses of rods (67% of normal mean; lower limit, 83% of mean) and, to a greater degree, dark-adapted cones (44% of normal mean; normal lower limit, 76%) with normal amplitudes of light-adapted cones consistent with mild rod and scotopic cone dysfunction. Rod and cone implicit times were normal. Repeat ERGs at 12 years of age showed minimal further rod loss. Karyotyping was normal. Direct sequencing of CHM identified a nonsense mutation (R239X, CGA→CTA), also identified in the patient’s maternal grandfather.2,3

The patient representing case 2, who is from an unrelated family with choroideremia, was first evaluated at 9 years of age. He was asymptomatic with visual acuities of 20/20 OU. The results of color vision testing and Goldmann perimetry testing were normal. Fundus examination identified fine, peppery RPE motting in the midperiphery but nor-

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Figure 1. Pedigrees for case 1 (A) and cases 2 and 3 (B). Black squares indicate affected males; circles with black dots, known carrier females; gray squares, males affected by history.

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mal-appearing optic discs, retinal vasculature, and maculae (Figure 2E and F). Bone spicule–like pigment formations and choroidal atrophy were not observed. The ERG responses were normal (Figure 3B); responses from a repeat ERG at age 11 years remained normal.

The patient representing case 3 is the maternal cousin of the patient in case 2 and was also asymptomatic when evaluated at 8 years of age. Visual acuity was 20/25 OD and 20/20-1 OS. Color vision screening was normal. Goldmann perimetry showed mild superotemporal restriction to the II-2e test target. Fundus examination revealed diffuse mottling of the RPE throughout the posterior pole and midperipheral retina similar to the moth-eaten appearance of choroideremia carriers. Electroretinogram testing (Figure 3B) showed normal cone amplitudes and implicit times to single flash and 30-Hz flicker. Rod amplitudes were reduced approximately 40%. The maximal dark-adapted B-wave response was reduced approximately 50%. Direct sequencing of CHM in cases 2 and 3 identified a nonsense mutation (R253X, CGA→TGA).2,3

Comment. Choroideremia, like other X-linked retinal disorders, shows variability in the degree to which hemizygous individuals are affected. Typically, disparities are

Figure 2. Color fundus photographs of affected individuals from the affected families. A-C, Case 1. The right eye, left eye, and peripheral view of the left eye at ages 6, 10, and 12 years, respectively. D, His maternal grandfather at age 71 years. E and F, Case 2. The right eye and left eye, respectively.
greater between families than among members of the same family. For example, among Danish patients with various mutations in the CHM gene, Rosenberg and Schwartz found a 25-year age difference between individuals in the age at which time the peripheral visual field became unquantifiable by the Esterman method. Variation has also been reported in patients with choroideremia with the same mutation.5

Our patients are notable not only for their mild clinical phenotype but also for the mild nature of ERG abnormalities. The ERG in choroideremia has been studied by many investigators and is almost uniformly severely abnormal early in the course of the disease.1 Previously, Ponjovic et al reported mildly subnormal ERG responses in a 6-year-old boy in a family with choroideremia. Other affected members within this branch of the family had ERG responses that were more intact than those of more distant relatives, suggesting that factors other than the mutation itself may be determinants of severity and clinical course.3

Our observations highlight that the absence of markedly abnormal ERG responses at an early age does not necessarily exclude the diagnosis of choroideremia and serves to emphasize the need for follow-up testing in these patients.

The CHM gene encodes REP-1 (Rab escort protein-1), a ubiquitously expressed “housekeeping” protein. The protein REP-1 is essential for the regulation (isoprenylation) of Rab GAPases, which are then able to bind the cytoplasmic face of intracellular organelles. Rab GAPases appear to function as molecular switches activating “effector” proteins that guide intracellular vesicular transport.6 Mutations in REP-1 are not lethal because a related gene product, REP-2, serves the needs of all tissues except those of the eye.7 Although the precise mechanism by which chorioretinal degeneration occurs remains to be elucidated, histological analyses of choroideremia patients point toward the rod photoreceptors or RPE as the initial sites of pathologic abnormalities.8 Failure of vesicular transport of, for example, the Rab GAPase Rab27a (which has a greater affinity for REP-1 than REP-2) may lead to abnormal photoreceptor opsin transport, RPE phagocytosis, and lysosomal degradation or melanin granule transport.9

Both mutations in our families lie in a segment of the gene encoding the second “sequence conserved region” of the REP-1 protein. X-ray crystallographic studies of the bovine ortholog propose that this region facilitates tight molecular folding, and sequence analysis suggests that this domain may also mediate protein-protein interactions.8 Although nonsense mutations usually result in unstable messenger RNA molecules that are rapidly degraded, there is evidence that when stop mutations are located toward the 3’ end of the message, protein translation can proceed.10 However, the mutations in our cases fall within the middle of the gene. The cause for the mild phenotype early in the course of disease in these individuals is unknown but may arise because of environmental influences, epigenetic factors, or genetic modifiers that allow the transcriptional apparatus to read through the premature stop codon, resulting in a translation of some functional protein, or from sequence

Figure 3. Electroretinograms (ERGs) of affected individuals performed to International Society for Clinical Electrophysiology of Vision standards. A, Case 1. The normal ERG is a composite average of 22 controls who were 7 to 20 years of age. For case 1, the right and left eyes are superimposed. Testing included, in addition, a red stimulus that was balanced to give equal rod responses to the blue stimulus. This stimulus produced greater loss of the early dark-adapted cone responses (arrows) than later-occurring rod responses. B, Case 2 demonstrated normal cone and rod amplitudes and implicit times. Case 3 demonstrated normal cone responses but notably reduced rod responses. The left eye tracings are shown. The vertical scale is 100 µV per division. The error bars for the B waves show the 95% confidence ranges for normals. OPs indicates oscillatory potentials.
In recent years, photodynamic therapy (PDT) using verteporfin has emerged as an important method for treating certain subtypes of choroidal neovascularization (CNV) in age-related macular degeneration. Despite the significant beneficial effects, a 4% risk of acute severe vision decrease has been reported for patients undergoing PDT with verteporfin. An initial thermally enhanced photochemical impact on the macula appears to induce changes in the choriocapillaris to arrest the progression of the neovascularized complex without significant permanent damage to the choriocapillaris, retinal pigment epithelium, and photoreceptors. There have been a few cases, however, of transient choroidal ischemia, where normal choroidal vessels have shown evidence of transient delay. The ischemic change in these eyes evolves without clinical evidence of permanent damage. This is a report of a choroidal infarction observed in the treatment of CNV in age-related macular degeneration using standard methods of PDT with verteporfin.

**Report of Cases.**

**Case 1.** In an otherwise healthy 81-year-old woman diagnosed with age-related macular degeneration associated with subfoveal occult CNV in the right eye (Figure 1), PDT was performed according to a standard protocol using 6 mg of verteporfin per square meter of body surface area over 10 minutes (Visudyne; CIBA Vision Corp, Duluth, Ga). Five minutes after completion of the infusion, photocoagulation was started using light at a wavelength of 689 nm (Coherent Opal Photoactivator; Coherent Inc, Palo Alto, Calif). The visual acuity was 20/80 OD and 10/400 OS. In the past, the left eye underwent laser photocoagulation and PDT without any adverse effects. Approximately 60 seconds into the laser treatment, the patient began complaining of pain in the right eye. The pain escalated throughout the remainder of the treatment and persisted for hours. Immediately following completion of the treatment, visual acuity decreased to 10/400 OD; intraocular pressure measurement and anterior segment examination results remained normal. The first angiography was repeated after 3 weeks at which time the visual acuity was 10/400. Funduscopy showed an oak-leaf pattern of pigmentation in the macula. Fluorescein angiography (FA) revealed an area of choroidal nonperfusion within the macula that persisted on late frames (Figure 2A). Indocyanine green angiography confirmed a choroidal occlusion, and early- and late-phase angiograms showed continuous hypofluorescence in the macula (Figure 2B and C). After 3 months, macular pigmentation developed in the oak-leaf pattern previously seen on angiography (Figure 3A) and evidence of choroidal nonperfusion remained (Figure 3B). Visual acuity did not change from 10/400 OD at the latest follow-up. Furthermore, a new area of classic CNV developed at the edge of the choroidal insult.

**Case 2.** A 90-year-old woman diagnosed with age-related macular degeneration in the right eye was experiencing worsening metamorphopsia. Visual acuity was 20/100, and FA revealed occult CNV associated with confluent soft drusen (Figure 4). The patient underwent PDT with verteporfin followed immediately by an intravitreal injection of triamcinolone acetonide according to a previous protocol for treating CNV in age-related macular degeneration.

Rab GTPases and their substrates are involved in intracellular traffic and disease. Variation in the REP-1–interacting phenotypic spectrum that are associated with a milder phenotype within the first decade of life.

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**Figure 1.** Pretreatment late-phase fluorescein angiogram of the right eye from case 1 demonstrating choroidal neovascularization in the macula of the right eye.