Objective: To report the clinical, functional, and in vivo microanatomic characteristics of a family with choroideremia with a deletion of the entire gene that encodes for the Rab escort protein 1 (CHM).

Methods: We performed clinical examination, flash electroretinography (ERG), light- and dark-adapted perimetry, and optical coherence tomography; reviewed medical records; and obtained the medical history of the proband and 3 other family members.

Results: At 4 years of age, the proband had a hypopigmented fundus and retinal pigment epithelium mottingling, and dark-adapted ERGs were reduced. Severe retinal pigment epithelium and choriocapillaris atrophy developed by 6 years of age, paralleled by a lesser ERG decline. Optical coherence tomography findings showed normal neural retinas overlying mild changes in the retinal pigment epithelium and thinned neural retina with impaired lamination, yet the neural retina was fairly preserved over retinal pigment epithelium and choriocapillaris atrophy. The carrier mother had diffuse elevation of 650-nm dark-adapted thresholds.

Conclusions: Deletion of the CHM gene causes severe choroideremia. Results of serial ERGs and fundus examinations documented progression first of rod and then of cone disease. Fundus appearance deteriorated rapidly, in excess of the severity of the ERG decline. Optical coherence tomography findings explained this observation, at least in part.

Clinical Relevance: To our knowledge, this is the earliest clinical, microanatomic, and ERG longitudinal phenotypic documentation in molecularly characterized choroideremia and the first documentation of impaired dark-adapted cone function in carriers. The preservation of the neural retina has mechanistic, prognostic, and therapeutic implications.

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et al.9 and Apáthy et al.10 In brief, red/green cone-mediated thresholds were measured after 40 minutes of dark adaptation (white standard background) with a 600-nm (orange) stimulus. Dark-adapted thresholds were determined in the light-adapted state (white standard background) as reported previously.7 The International Society for Clinical Electrophysiology of Vision "standard flash" was 0.32 log cd·s/m2. In individual V:1, responses were obtained with the person under full anesthesia (intravenous propofol infusion).

Monochromatic automated perimetry was performed as previously reported,7 based on the methods developed by Jacobson et al.11 Also, because in the latter study total retinal thickness measurements included the RPE hyperreflective band and the intervening thin yellow-white band above it. StratusOCT, which does not include the first hyperreflective band (shown in red in the false-color OCT maps) of approximately 20 to 25 µm attributable to photoreceptor inner and outer segments. For the purpose of comparing our findings more directly with those of Jacobson et al.11, measurements of total neural retinal thickness were conducted at the fovea and at 1.3 mm of eccentricity from the fovea on magnified views of the images over adjacent pixel columns at these locations. However, our total neural retinal thickness measurements included the first hyperreflective photoreceptor inner and outer segment band but not the second one, which is instead attributable to the RPE. The thickness of the outermost hyporeflective band in the OCT profile attributable to the outer nuclear layer (ONL) was also estimated. We performed identical measurements at 3.0 mm temporal to the fovea, in an area of neural retina overlying a large nummular area of RPE/CC atrophy. All measurements were averaged and are expressed as mean ± SD. Comparisons are given with our normal control image and the reference ranges that could be derived from Jacobson et al.11 Also, because in the latter study total retinal thickness measurements included the RPE peak,12 we enhanced comparability between their study and ours by reducing the published reference ranges by 30 µm, which is our estimated average thickness of the RPE hyperreflective band and the intervening thin yellow-white band above it.

**Molecular Genetics**

After obtaining informed consent, a whole-blood sample was collected by venipuncture from the proband, his 2 male siblings, and their mother. Genomic DNA was extracted with a commercially available kit (QIAamp 50 Maxi Kit; Qiagen Inc, Valencia, California) according to the manufacturer’s specifications. Molecular genetic analyses of the CHM gene were conducted at the University of Alberta. The CHM primers were modified from van Bokhoven et al.5 Reactions were performed in total volumes of 25 µL (2.5 µL 10× NEB buffer [New England Biolabs, Ipswich, Massachusetts], 2.5 µL of 2 mM deoxyribonucleotide triphosphates, 0.125 µL bovine serum albumin, 60-ng forward primer, 60-ng reverse primer, double-distilled water to volume, 50-ng genomic DNA, and 1 U Taq [New England Biolabs]). The cycling settings were as follows: 95°C for 5 minutes (95°C for 1 minute, χ°C for 1 minute, and 72°C for 1 minute) × 40 cycles, and 72°C for 7 minutes where the annealing temperature χ was 50°C (exons 2, 5B, 6, 7, 8, 9, 14, and 13), 52°C (exons 3, 5A, 11, 12, and 13), and 58°C (exons 1, 4, and 10). Polymerase chain reactions (PCRs) were run on 1% agarose gels with a 1-kilobase (kb) pair DNA ladder.

All research studies conducted in this investigation were approved by the institutional review boards of the participating institutions and were in compliance with the Declaration of Helsinki.

**Results**

**Proband (V:1)**

At baseline, visual acuity was 20/50 OD and 20/30 OS. Results of the posterior segment examination showed a hypopigmented fundus with normal optic discs, border-
line retinal vessels, coarse macular mottling, and punctate midperipheral RPE clumping (Figure 2A). Discrete RPE/CC atrophy was noticeable only in the peripapillary region and in small nummular lesions above and below the disc. Full-field flash ERGs (Figure 3) showed subnormal rod-driven responses (10% of our lowest reference limit) and attenuated mixed dark-adapted rod-cone a and b waves (about 45% and 60% of our lowest reference limits, to 0.32 log cd·s/m² and to 50% and 80% to 1.0 log cd·s/m² stimuli), with a relatively better preservation of the b waves. Light-adapted cone-driven ERGs were delayed in timing.

Findings were highly suggestive of early-stage chorroideremia. To verify this impression, the CHM exons were amplified from genomic DNA by means of PCR. No product could be obtained for any of the CHM exons amplified from the proband, whereas a control autosomic gene (VMD2) amplified normally (not shown). Although additional fluorescent in situ hybridization and protein expression studies could not be performed, these findings were consistent with a deletion of the whole CHM gene. This presumed deletion was further characterized, demonstrating that it incorporated a 57-kb region upstream of exon 1, excluding marker pJ7.6B, and a downstream region of at least 2.9 kb, including marker WI-11849 (not shown). Consistent with her carrier status, a product could instead be obtained from the wild-type allele for every maternal exon. This finding confirmed the clinical diagnosis of choroideremia in the proband and predicted no REP-1 transcript for affected males and in all clones harboring the mutated allele in female carriers.

At 5 years of age, symptoms of night blindness had developed in the proband, accompanied by a remarkable increase in the extension of the lesions with large irregular and confluent patches of RPE/CC atrophy in the midperiphery and in the peripapillary and parapapillary areas (Figure 2B). The RPE mottling and clumping had increased in coarseness in most retinal quadrants. Repeated ERG testing (Figure 3) showed a decline in both the size and quality of the dark-adapted responses. Mixed ERG responses were significantly reduced in amplitude compared with baseline, with an approximately equal reduction in a and b waves (about 30% of the lowest limit of normal). There was no change in photopic ERGs.

By 6 years of age, visual acuity was unchanged, but widespread coalescent areas of severe nummular RPE/CC atrophy had developed, sparing only the centermost macular region (Figure 2C). Results of further serial testing (Figure 3) demonstrated an additional decline in dark-adapted ERG amplitudes and sensitivity. At this stage, cone ERG amplitude loss was also apparent, especially to transient stimuli. Despite functional evidence of disease progression, ERG responses remained remarkably well preserved compared with the deterioration in fundus appearance. This finding suggested 2 possible and not mutually alternative explanations. First, the neural retina may be better preserved than what was suggested by the ophthalmoscopic appearance and, specifically, the neural retina overlying areas of clinically overt RPE/CC atrophy may be largely intact, a possibility that has already been suggested by previous clinical observations. Second, the better-than-expected b wave preservation may result from a compensatory increase in the gain of postreceptorial retinal neurons, a phenomenon that is often observed clinically in retinal dystrophies and that has been shown to occur at the animal level in rats harboring the P23H mutation of the rhodopsin gene (RHO). To test the former hypothesis, the in vivo microanatomy of the posterior pole of the proband was studied.

Figure 2. Composites of the fundus in the proband (individual V:1) at ages 4 years (A), 5 years (B), and 6 years (C). Progressive development of confluent areas of nummular atrophy of the retinal pigment epithelium and choriocapillaris can be appreciated.
RPE mottling that is evident in the red-free image, measurements at the fovea showed normal thickness of the retina and a minimally thinned ONL. At 1.3 mm of eccentricity, further RPE dropout and thinning can be seen in the red-free image. At this location, retinal thickness was again within the reported reference range. The ONL was clearly discernible, as in the normal example, and at the lowest reference limit in thickness. At 3.0 mm of eccentricity, a large area of nummular RPE/CC atrophy can be seen ophthalmoscopically and is identified by intense back-scattering in the OCT image (Figure 4). At this location, total retinal thickness was reduced only to 90% of the lowest reference limit. However, consistent with what has recently been reported by Jacobson et al., retinal lamination was irregular and the limits of the ONL were not clearly distinguishable, precluding the measurement of its thickness at this location.

**FEMALE CARRIER (IV:2)**

The 39-year-old obligate carrier showed a classic fundus appearance with streaky patches of coarse RPE mottling, deep retinal pigmentary deposits, and peripapillary RPE/CC atrophy (Figure 5A). Her only symptom was difficulty seeing at night. The ERG testing, however, showed responses (Figure 3) that were at or above the 97th percentile in amplitude and normal timing (not shown) at all flash intensities.

To determine whether the night vision problem was accounted for by patches of retinal dysfunction in the dark-adapted state that were too small to affect the mass retinal response of the ERG, light- and dark-adapted thresholds were measured. Light-adapted red/green cone-mediated thresholds were essentially within reference limits throughout the entire visual field (Figure 5B). Unexpectedly, 500-nm dark-adapted thresholds were within reference limits except for elevations at a few peripheral loci in the left eye, whereas 650-nm fully dark-adapted thresholds were markedly elevated at all tested loci in both eyes, in the left more so than in the right eye, and especially at extramacular loci (by about 1.0 and 1.5–2.0 log U at macular and extramacular loci, respectively).

**OTHER MALE SUBJECTS IN THE FAMILY**

The affected males in generation III (individuals III:2, III:4, III:6, and III:7), who were in the seventh and eighth decades of life when examined, experienced night blindness and peripheral visual field loss by the middle to late...
third decade and were severely affected, with light perception vision only or otherwise legal blindness. Individuals III:2 and III:6 retained sufficient vision to be able to drive a car until aged 40 to 45 years, whereas individuals III:4 and III:6 were reportedly never able to drive. Individual III:1 died young but was reportedly already legally blind by the middle of the third decade of his life.

Individuals V:2 and V:3, the proband’s brothers, were first examined at 4 and 3 years of age, respectively, at which time they had normal visual acuity, a hypopigmented fundus with nonspecific RPE mottling, and no overt sign of choroideremia (not shown). At subsequent yearly examinations, the mottled appearance of the RPE was unchanged, there was an increase in the myopic refraction but no deterioration in visual acuity, and there were no symptoms or fundus changes suggestive of choroideremia. The provisional clinical diagnosis of unaffected status for these brothers was confirmed at the molecular level by normal PCR amplification of all CHM exons (not shown).

**REVIEW OF SYSTEMS**

Because the deletion encompassed regions upstream and downstream from CHM, and because complex syndromic choroideremia phenotypes in association with deletions of the X chromosome have been previously reported, a detailed review of systems was performed in the affected male members and the female carrier participating in this study. With the exception of an unusu-
ally high rate of twin pregnancies in obligate carriers (Figure 1), none of the aforementioned subjects in this family showed any contributory systemic finding, thereby indicating that the deletion affecting the CHM gene in this family was not associated with any clinically overt systemic manifestation.

COMMENT

To our knowledge, this is the earliest clinical, microanatomic, and ERG longitudinal phenotypic documentation in molecularly characterized choroideremia and the first study of impaired dark-adapted cone function in carriers. Consistent with the predicted complete absence of REP-1 associated with a deletion of the entire CHM gene and the findings of Ponjavic et al in another such family, despite the initial preservation of rod and cone function that both studies documented, the entire absence of the CHM gene is associated with severe choroideremia. Serial ERGs in our proband also demonstrated progression of rod disease early on, followed by cone disease, consistent with the hypothesis of greater rod than cone vulnerability to compromised REP-1 function in affected males.

The deterioration in fundus appearance was strikingly rapid and exceeded the severity of the ERG losses. The clinical impression that the retina overlying areas of RPE/CC atrophy may be well preserved in choroideremia, already suggested by Sieving and supported by our ERG findings, was corroborated by the OCT studies. The OCT findings showed progressive retinal thinning and abnormal laminar proportionality to the severity of RPE damage, the latter always being ophthalmoscopically visible in areas with normal retinal thickness and microanatomic structure, and remarkable preservation of neural retinal tissue in areas of complete RPE/CC atrophy. In these latter areas, however, retinal lamination was already compromised, the limits of the ONL were uncertain, and some degree of thinning could be documented, which is consistent with the findings of Jacobson et al in children older than our proband. Shortening of the photoreceptor inner and outer segments in areas corresponding to RPE/CC atrophy is also likely, whereas no significant changes in the OCT variables were seen at locations of ophthalmoscopically apparent RPE disturbance.

Impaired cone performance in the dark-adapted state is the most plausible explanation for the symptom of poor night vision in the female carrier. Although abnormal thresholds had been reported before, to our knowledge, impaired dark-adapted cone-driven function in a choroideremia carrier documented across the visual field with monochromatic automated perimetry had not been previously reported. Cone dysfunction as the cause for nystagmus has been documented, although in these cases it was due to anomalous rod-cone interactions to flickering stimuli in the dark-adapted state in the absence of threshold abnormalities to static stimuli. Degenerate retinal cones in female carriers have been observed. However, to our knowledge, the molecular basis for cone disease in choroideremia carriers is unknown.

The differences that we observed between the affected proband and the female carrier suggest that the mechanism of retinal disease in choroideremia carriers may not necessarily be the same as in affected males. In addition, it must be remembered that the RPE and the neural retina are embryologically distinct tissues. In females, who are mosaics for the disease allele, the clones of RPE and photoreceptor cells expressing the mutant allele will not always correspond to one another. Therefore, the fundus of females can show either one of the following: (1) wild-type RPE clones juxtaposed to wild-type photoreceptor clones; (2) wild-type RPE juxtaposed to mutant photoreceptors; (3) mutant RPE juxtaposed to wild-type photoreceptors; or (4) RPE and photoreceptor mutant clones. These observations may help explain why, in human female carriers or in mouse heterozygote null CHM females, photoreceptor and RPE disease appear to occur independent of one another. Hence, extrapolation of female data to male disease must be approached with caution. In very young boys, based on our serial clinical and functional findings and the OCT data, changes in the RPE appear to precede degeneration of the overlying neural retina. This is consistent with findings from a zebra fish choroideremia model and largely also with the findings of Jacobson et al.

Despite the thinning and impaired retinal lamination seen in our proband and by Jacobson et al at sites of complete RPE/CC atrophy, ERG responses suggest that these portions of the neural retina are still contributing to the signals and are therefore still viable. We find this apparent ability of the neural retina to survive in these areas of clearly compromised RPE/CC integrity remarkable and of therapeutic and prognostic relevance. Notwithstanding the foreseeable future role of gene therapy, we suggest (1) that treatments that could restore the integrity of the RPE or compensate for its compromise may also represent an effective treatment strategy for choroideremia and (2) that retinal areas overlying complete RPE/CC atrophy may still be salvaged, at least at disease stages as early as that of our proband. Examples of such treatments may be RPE transplants and/or sustained intraocular delivery of pigment epithelium–derived factor, a molecule that has been shown to have potent neuroprotective effects on photoreceptors deprived of the support of RPE and to prevent the Muller cell changes resulting from this deprivation. Muller cell changes are now postulated to play a role in neural retinal remodeling also in choroideremia. Likewise, other agents capable of supporting photoreceptor health in the absence of the RPE may also be suitable treatment strategies for choroideremia. Preclinical studies to test these mechanistic and therapeutic hypotheses appear to be in order.

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