Progression of Retinal Pigment Epithelial Alterations During Long-term Follow-up in Female Carriers of Choroideremia and Report of a Novel CHM Mutation

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**Objectives**: To report clinical and functional findings in 2 female carriers of choroideremia who were followed up for 11 and 17 years and who showed progression of fundus alterations; and to report a novel CHM mutation.

**Methods**: We performed follow-ups in 2 female carriers of choroideremia, including repeated clinical and electrophysiologic examinations and fundus autofluorescence. Molecular analysis of the CHM gene was done by direct sequencing of the coding exons.

**Results**: Follow-up of female carrier 327 took place during 17 years. A second female carrier (subject 869) with a novel gene mutation in CHM was followed up for 11 years. The 2 carriers showed marked pigmentary alterations in the periphery of the retina. At the initial visit, carrier 869 had multiple small, yellowish flecks in the macula. Both carriers developed subnormal 30-Hz flicker responses on full-field electroretinography during follow-up, whereas electrooculography responses were normal. In both carriers, progression of fundus alterations was noted. Fundus autofluorescence images showed multiple small flecks with reduced autofluorescence.

**Conclusions**: Over time, fundus alterations in female carriers of choroideremia are visible, and mild cone dysfunction might develop. Multiple yellowish flecks can exist in the macula. The typical mottled irregularity in fundus autofluorescence is a valuable diagnostic criterion that facilitates specific genetic testing.

**Clinical Relevance**: Fundus alterations in female carriers of choroideremia can progress over time and a mild generalized cone dysfunction can develop. Characteristic irregularities are seen in fundus autofluorescence imaging, which is helpful in identifying female carriers of choroideremia.


**Choroideremia** is a rare retinal dystrophy caused by mutations in the CHM gene (GenBank NM_000390), located on the chromosome Xq21.2, and usually only affects males. So far, more than 100 different pathogenic CHM mutations have been described, all leading to a complete loss of the gene product Rab escort protein 1. Expression of CHM has been shown in rods and retinal pigment epithelium (RPE) cells, indicating the origin of the disease in rods and/or RPE cells and the subsequent degeneration of the cones and the choroid as a secondary effect. Affected males show a progressive degeneration of the photoreceptors and the RPE, followed by a degeneration of the choroid, and finally a complete atrophy of the choroid, resulting in visibly bare sclera. Already early in life, full-field electroretinograms (ERGs) demonstrate distinct rod-cone dysfunction in those affected.

Manifesting female carriers of choroideremia with severe functional abnormalities are very rare. Without a positive family history, the diagnosis of choroideremia can be a challenge in females. In most cases, female carriers have no visual disturbances. Full-field ERG results are abnormal in only about 15% of carriers. Patchy fundus changes include various grades of mottled RPE alterations, RPE stippling, and spotty pigment atrophy in the periphery. The variable phenotype in female carriers can be explained by lyonization, ie, a random X chromosome inactivation. The mottled RPE changes cause typical patchy fundus autofluorescence (FAF) irregularities with multiple small spots of reduced FAF, which is another important diagnostic criterion for female carriers. We present morphologic and functional data of 2 female carriers who we followed up clinically for 11 and 17 years.
### Table. Clinical Data of the Female Choroideremia Carriers

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Carrier 327</th>
<th>Carrier 869</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual acuity, OD/OS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.2/1.2 1.2/1.2 1.2/1.2 1.25/1.25 1.2/1.2 1.2/1.0</td>
<td>0.3/0.5 0.5/0.7 0.4/0.4 0.5/0.7 0.6/0.7</td>
</tr>
<tr>
<td>Desaturated panel D-15 test, No. of errors, OD/OS</td>
<td>... ... 0/0 2/4 ... 0/0</td>
<td>1/2 ... 2/0 ... 1/0</td>
</tr>
<tr>
<td>Goldmann perimetry</td>
<td>Normal Normal Normal Normal ... ... Small central scotoma in the right eye (I/2) Small central scotoma in the right eye (I/2) ... ... Temporal midperiphery scotoma in each eye (I/3) and small central scotoma in the right (I/1) and left (I/2) eyes</td>
<td></td>
</tr>
<tr>
<td>EOG light rise, %, OD/OS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>... ... 370/... 297/... 253/254 ... ... 271/298 ... ... ... 329/268</td>
<td></td>
</tr>
<tr>
<td>Full-field ERG</td>
<td>Normal Normal Normal 30-Hz flicker response reduced to 86% 30-Hz flicker response reduced to 81% Normal Normal Normal ... 30-Hz flicker response reduced to 76%</td>
<td></td>
</tr>
<tr>
<td>Multifocal ERG</td>
<td>... ... ... ... ... ... ... Normal in the right eye; not performed in the left eye Normal in the right eye; not performed in the left eye ... Normal in the right eye; reduced paracentral nasal in the left eye</td>
<td></td>
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</tbody>
</table>

Abbreviations: EOG, electrooculography; ERG, electroretinography; ellipses, test not performed.

<sup>a</sup>Visual acuity is shown in decimal fractions.

<sup>b</sup>For normal values, see the “Methods” section.

## Methods

The patients were examined between 1986 and 2008 in the Department of Ophthalmology of the Charité, Berlin, Germany. Clinical examinations and phlebotomies for genetic analysis were conducted after informed consent was obtained. Our research adhered to the tenets of the Declaration of Helsinki, and investigational review board approval was obtained. Some data of carrier 327 have been published (not shown here) previously.

The patients underwent complete eye examinations. Color vision was tested with the desaturated panel D-15 test. Goldmann perimetry was performed. Fundus autofluorescence imaging was carried out using a Heidelberg Retina Angiograph (Heidelberg Engineering, Heidelberg, Germany) as described in detail elsewhere. Argon laser light (488 nm) was used to excite autofluorescence of the RPE. A wide bandpass filter with a cutoff at 500 nm was inserted in front of the detector. Optical coherence tomography (OCT) was performed with the Stratus OCT 3 (Carl Zeiss, Jena, Germany). Single horizontal scans of the macula were obtained. Dark adaptation was performed with a Goldmann-Weekers adaptometer under standard conditions as described previously.

Electrooculography was performed prior to the development of the International Society for Clinical Electrophysiology of Vision (ISCEV) standards using an older method with available normal values (normal light rise, 200%-400%). Since 1992, a ramp test method (normal light rise >160%) was used according to ISCEV standards as described elsewhere.

From 1986 to 1991, standardized protocols for full-field ERG had not yet existed. However, normal values were available for this method. This older ERG method was used during the first 3 visits of carrier 327 and the first visit of carrier 869. Since 1992, recording of full-field ERG was done according to the regularly updated ISCEV standards. The recording protocols have been described in detail elsewhere.

Recording of the multifocal ERG (mfERG) was done in accordance with the ISCEV guidelines. Until 2003, mfERG was recorded and analyzed using the VERIS system (EDI, San Mateo, California); since 2005, the RetiScan system has been used (RolandConsult, Brandenburg, Germany). The recording protocols have been described in detail elsewhere.

Genomic DNA was isolated from peripheral blood lymphocytes in patient 869 using standard extraction procedures. For mutational analysis, coding exons of CHM were amplified with exon-flanking primers and purified using enzymatic digestion (exonuclease I and alkaline phosphatase). (All primer sequences and polymerase chain reaction conditions for the sequence analyses are available from B.H.F.W. on request.) Direct sequencing of both forward and reverse strands was performed with the BigDye Terminator Kit 1.1 (Applied Biosystems, Foster City, California) according to the manufacturer’s instructions. Sequencing reactions were separated electrophoretically on an ABI3130xl automated sequencer (Applied Biosystems). Electropherograms were analyzed with the SeqPilot software (JSI Medical Systems, Kippenheim, Germany).

## Results

### Female Carrier 327

Owing to unavailability of a DNA sample, mutational analysis of carrier 327 was not possible. However, her affected brother was known to carry a deletion of exon
1 in the CHM gene; her son was affected; and her mother was a manifesting carrier, as reported previously. This indicates that carrier 327 is an obligate carrier of the pathogenic mutation.

The carrier was reexamined several times between the ages 20 and 37 years (Table). She had no visual disturbances and her visual acuity was normal in the presence of emmetropia. Dark adaptation, tested at the first 2 visits, showed normal rod adaptation. In the beginning, full-field ERG showed normal amplitudes of the standard combined response under scotopic conditions and of the single-flash cone and 30-Hz flicker response under photopic conditions. However, when she was examined at 28 and 37 years of age, 30-Hz flicker responses were subnormal.

The subject’s anterior segments were normal. Ophthalmoscopy revealed multiple hypopigmented flecks and fine pigment clumps, predominantly in the periphery of the retina (Figure 1). Her retinal vessels and optic discs were normal. She had peripapillary annular atrophy of her RPE and choroid.

During follow-up, the pigmentary irregularities changed. In the right eye, some pigment clumps became smaller. In the left eye, the pigment clumps became larger and confluent, with a pattern resembling a honeycomb (Figure 1). Her peripapillary atrophy worsened slightly.

Fundus autofluorescence imaging, available at last examination, showed patchy irregularities inside and outside the vessel arcades. Multiple flecks of reduced and single flecks of increased FAF were visible. In the peripapillary atrophy zone, FAF was absent (Figure 2).

The family history of carrier 869 revealed neither visual impairment nor fundus changes in her brother, parents, or grandparents. Between 26 and 37 years of age, the subject’s existing visual problems were stable: disturbed night vision (since childhood), photophobia, and reduced visual acuity. Her hyperopia ranged between +5.75 and +6.75 spherically and −1.0 and −1.5 cylindrically in both eyes during follow-up. Visual acuity fluctuated during follow-ups and was maximally 0.7 (Table). The full-field ERG results were normal at all recording conditions; however, there were subnormal amplitudes of the 30-Hz flicker response at the last visit. Multifocal ERG revealed normal amplitudes in the subject’s right eye. Her left eye, tested at the last visit, showed amplitude reduction in the paracentral nasal area (Figure 3).

At initial examination, the subject’s fundus had marked pigmentary stippling in the periphery. Within the vessel arcades, smaller and larger areas with slightly hyperpigmented or hypopigmented RPE were visible as were

![Figure 1. Fundus photography of the left eye from carrier 327 during long-term follow-up at 21, 28, 35, and 37 years of age. An increase in the number of pigment clumps can be seen especially in the nasal inferior area.](image1)

![Figure 2. Fundus autofluorescence of the left eye from carrier 327 at 37 years of age. Mottled irregularities are seen inside and outside the vessel arcades. There are multiple small flecks of reduced and single flecks of increased autofluorescence.](image2)

![Figure 3. Multifocal electroretinogram from carrier 869 at 37.8 years of age. The right eye shows normal amplitudes; the left eye shows reduced amplitudes in the paracentral nasal area. deg indicates degrees.](image3)
multiple small, yellowish, drusenlike flecks (Figure 4). The negative family history and the yellowish flecks in the macula rendered questionable the conclusion that she was a carrier of CHM. Nevertheless, the similarity of FAF findings between carrier 327 and carrier 869 led us to initiate molecular analysis of CHM. Subsequently, a heterozygous point mutation in intron 10, which results in an exchange of adenine to guanine at position c.1350-2, was found (Figure 5).

Retinal vessels and the optic disc were normal. As in carrier 327, carrier 869 had peripapillary atrophy of the RPE and choroid, which slightly enlarged over the years. During follow-up, the peripheral pigmentary clumps grew paler (Figure 6). Comparing the subject’s left macula at 26 years with that at 32 years of age, we found that a few yellowish flecks had lost their brightness, whereas other flecks became more yellowish (Figure 4). In the right macula, subtle changes in the brightness of the juxtafoveal single yellowish flecks could also be seen.

As in carrier 327, multiple small flecks of reduced FAF were seen inside and outside the vessel arcades in car-

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**Figure 4.** Fundus photography, fundus autofluorescence, and optical coherence tomography from carrier 869 during long-term follow-up at 26, 32, and 37.8 years of age. Smaller and larger areas with slightly hyperpigmented or hypopigmented retinal pigment epithelium and multiple small, drusenlike, yellowish flecks are visible. Fundus autofluorescence shows multiple dark small flecks and parafoveal flecks with very bright fundus autofluorescence. Subtle irregularities of the integrity of the retinal pigment epithelium are visible with optical coherence tomography (single horizontal scan through the macula).

**Figure 5.** Electropherogram of the intron 10/exon 11 boundary of the CHM gene. The intronic sequence is in lower case; the exonic sequence in upper case. Detection of a heterozygous nucleotide change at position c.1350-2 from adenine (A) to guanine (G) (arrow) in the carrier, resulting in a loss of the acceptor splice consensus sequence.
carrier 869 (Figure 4). In addition, small flecks with very bright FAF were in a parafoveal location. However, the fundus initially had many more bright yellowish flecks than FAF imaging. Some flecks were bright at the fundus and in FAF; however, most of the bright flecks in FAF did not correspond to the bright flecks seen funduscopically but rather to hypopigmented areas (Figure 4). Fundus autofluorescence was missing in the peripapillary zone of atrophy. A single horizontal OCT scan through the fovea demonstrated subtle irregularities at the RPE level (Figure 4).

Follow-up data on female carriers are limited in the literature. Kärnä,8 who performed 1 of the largest studies about choroideremia to date, which included 114 female carriers, reported a general increase in fundus alterations with age—comparing younger and older age groups—and slow progression in few carriers observed up to 11 years. In our study, changes like increased or diminished pigment clumps and slight enlargement of the peripapillary atrophy were seen in both carriers during long-term follow-up, indicating a slow progression of retinal and/or RPE abnormalities. Both carriers initially had quite different findings in the macula: only subtle irregularities in carrier 327 but distinct RPE changes and multiple yellowish spots in carrier 869 that resembled drusen. Kärnä also reported white drusenlike foci in female carriers of choroideremia.8 Most of the multiple drusenlike flecks in carrier 869 did not show an increased FAF. The different FAF behavior of the various flecks might indicate varied lipofuscin material with fluorophores that predominantly do not autofluoresce at the wavelength we used or varied metabolic activity of the RPE cells, causing different amount of lipofuscin to accumulate in the cells.

Fundus autofluorescence findings in female carriers have been reported in some studies.10,11,20 In all reported carriers, there were multiple similar small flecks of reduced FAF at the central fundus, indicating mottled missing of lipofuscin in these areas, a sign of RPE dysfunction or loss. In carrier 869, FAF images outside the large arcade vessels showed larger flecks with reduced FAF than in the center. To date, follow-up data of FAF in carriers are not reported. Carrier 869 was reexamined with FAF after 5 years and showed no obvious changes in the flecks with reduced FAF at the posterior pole but distinct changes in number and arrangement of the very bright spots.

There are studies reporting OCT findings in affected males,21,22 but none to our knowledge in female carriers. The OCT in carrier 869 demonstrated small irregularities in the integrity of the RPE level. These findings correspond to histopathologic studies that have reported abnormal RPE with irregular thickness, variable lipofuscin content, and pigment clumping.6,23

Although fundus changes showed progression over the years, we did not find a decrease in visual acuity during long-term follow-up. Kärnä8 assumed a very slow deterioration of central vision with age when comparing different age groups.

The light rise of electrooculography remained normal in our carriers. Comparing data of different age groups, Yau et al24 also reported maintenance of normal electrooculography results in carriers, whereas Pinckers et al25 reported a decreasing Arden ratio with increasing age.

Full-field ERG at the last visits showed subnormal 30-Hz flicker responses in both carriers. Sieving et al7 observed 2 carriers for 10 years and reported a significant reduction in amplitude to single flashes of white light. It remains unclear why, in contrast to affected males, the rod function stays normal whereas the cone response shows decreased ERG amplitudes, though most fundus changes are peripheral where mostly rods are present. The total number of rods, however, is quite larger than that of the cones, which may be an explanation, as suggested by Vajaranant et al.26 A histopathologic study recently described cone degeneration and a severe loss of blue cones in retinal areas of a female symptomatic carrier.27

Multifocal ERG was performed in carrier 869 and showed paracentral areas with reduced amplitudes in the left eye. Rudolph et al28 also reported reduced mfERG amplitudes in carriers. Vajaranant et al29 described their mfERG findings in carriers as a mosaiclike pattern of retinal dysfunction explained by random X-chromosome inactivation.

Mutation c.1350-2A>G, identified in carrier 869, has not been reported so far and is likely to result in defective splicing as it mutates the highly conserved dinucleotide acceptor consensus at the intron/exon border. Similar mutations that affect the acceptor splice site at position 2 in introns 3, 7, 11, and 12 of the CHM gene have repeatedly been described as causative for choroideremia.3,5 We therefore conclude that the fundus changes of carrier 869 are caused by this mutation. As no DNA samples from other family members were available, we could not evaluate whether this mutation occurred spontaneously or it was segregating in the pedigree.

In conclusion, both carriers showed slow progression of fundus changes and FAF alterations during the very long-term follow-up. Alterations on the level of the RPE were seen with OCT. Retinal function showed development of mild cone dysfunction in the full-field ERG.
and mfERG, whereas visual acuity and electrooculography results remained unchanged. Fundus autofluorescence is helpful in identifying female carriers of choroideremia and facilitates specific molecular genetic testing, which in our case led to the identification of a novel CHM gene-splice site mutation.

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REFERENCES