Clinical Characteristics of Rod and Cone Photoreceptor Dystrophies in Patients With Mutations in the C8orf37 Gene

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Purpose. To provide the clinical features in patients with retinal disease caused by C8orf37 gene mutations.

Methods. Eight patients—four diagnosed with retinitis pigmentosa (RP) and four with cone-rod dystrophy (CRD), carrying causal C8orf37 mutations—were clinically evaluated, including extensive medical history taking, slit-lamp biomicroscopy, ophthalmoscopy, kinetic perimetry, electroretinography (ERG), spectral-domain optical coherence tomography (SD-OCT), autofluorescence (AF) imaging, and fundus photography.

Results. In families A and D, respectively, one and three patients showed a classic RP phenotype with night blindness followed by concentric loss of visual field. Severe visual loss to light perception occurred early in the course of the disease. The symptoms initiated during infancy (family A) or adolescence (family D). Ophthalmoscopy revealed macular atrophy, bone spicules, attenuated vessels, and waxy pale optic discs. SD-OCT showed profound photoreceptor degeneration and AF demonstrated atrophy of the retinal pigment epithelium (RPE). ERG responses were nonrecordable in these patients. In families B and C, the patients were diagnosed with CRD. Initial symptoms were photophobia or loss of visual acuity and occurred during infancy (family B) or adolescence (family C). Ophthalmoscopy and AF revealed profound macular RPE atrophy and SD-OCT demonstrated macular photoreceptor degeneration. ERG responses were severely reduced in a cone-rod pattern or were nonrecordable. Interestingly, both patients in family B demonstrated polydactyly.

Conclusions. Mutations in C8orf37 give rise to an early or adolescent-onset autosomal recessive CRD or RP phenotype with early macular atrophy. The occurrence of postaxial polydactyly in one family suggests a syndromic phenotype, which may indicate C8orf37 has a ciliary function.

Keywords: clinical characteristics, C8orf37, retinitis pigmentosa, cone-rod dystrophy
substantially reduced cone responses, although rod responses will deteriorate as the disease progresses.

Mutations in many different genes have been associated with either CRD or RP. CRD and RP display all Mendelian modes of inheritance. Also, digenic and mitochondrial inheritance have been described for RP. Until now, mutations in 36 genes have been associated with nonsyn- dromic autosomal recessive (ar) RP. Proteins of these genes are involved in phototransduction, retinoid (vitamin A) metabolism, transport along the connecting cilium, intercellu- lar signaling or synaptic interaction, interphotoreceptor matrix, gene regulation, and phagocytosis. 

Characteristics of C8orf37-Associated Dystrophies

Clinical Analysis

Clinical data were collected from the medical records of these patients. Following the identification of causative C8orf37 mutations, all patients were reevaluated in addition to the data accumulated over the years. Medical history was registered with a focus on age of onset, initial symptoms, and overall course of the retinal disorder. Age of onset was defined as the age at which the initial symptom was first noticed by the patient. We asked all patients about the presence of syndromic features, which are generally present in 20% to 30% of retinal dystrophy patients. These questions concerned the presence of hearing and balance abnormalities, renal failure, cardiac and respiratory anomalies, polydactyly, obesity, cognitive impair- ment, fertility disorders, hypogonadism, and dental anomalies. The clinical examination included best-corrected visual acuity, slit-lamp biomicroscopy, ophthalmoscopy, and fundus photog- raphy. Goldmann (kinetic) perimetry was performed in six patients. In patients AIV:1 and D-IV:4 perimetry proved impossible due to severe visual impairment. Cross-sectional images of the central retina were obtained with a commercially available spectral-domain optical coherence tomography (SD-OCT) instrument (Spectralis; Heidelberg Engineering and Cirrus; Carl Zeiss Meditec) by performing a volume scan (15° × 20°) through the fovea. Central foveal thickness was measured at the foveola using imaging and vision software (Heidelberg Eye Explorer Software, version 1.6.4.0; Heidelberg Engineering) or vision software (Cirrus Software, version 5.1.1.6; Carl Zeiss Meditec). Fundus autofluorescence (FAF; Spectralis, Heidelberg Engineering) could be performed in six patients. A full-field ERG was performed in all patients except patient D-IV:4. ERGs were performed using Dawson-Trick-Litzkow (DTL) electrodes and a visual electrophysiology program (Espion Visual Electrophysiology System; Diagnosys LLC, Lowell, MA) in the Institute for Ophthalmic Research, Tuebingen, Germany; DTL-electrodes and the RETI-port system (Roland Consults, Stasche, & Finger GmbH, Brandenburg an der Havel, Germany) in the Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; Bipolar Burien Allen electrodes and the UTAS SunBurst Color LED Ganzfeld (LKC Technologies, Gaithersburg, MD) in the Goldschleger Eye Research Institute, Tel Hashomer, Israel; and monopolar corneal electrodes (Henkes-type, Medical Workshop B.V., Groningen, The Netherlands) and the computerized UTAS 3000 system (LKC Technologies) in the Hadassah–Hebrew University Medical Center, Jerusalem, Israel. ERGs were assessed according to local standard values. All centers followed the guidelines of the International Society for Clinical Electrophysiology of Vision.

Patients and Methods

Subjects and Genetic Analysis

Patients with inherited photoreceptor dystrophies were referred to specialized ophthalmic centers and examined at the Radboud University Nijmegen Medical Centre (by CBH and BJK), Hadassah-Hebrew University Medical Center in Jerusalem, Israel (by EB), the Goldschleger Eye Research Institute (by YR), or the Institute for Ophthalmic Research in Tuebingen, Germany (by SH).

Subsequently, genetic analysis was performed in all cases. After the discovery of a causative homozygous C8orf37 mutation using homozygosity mapping and targeted next- generation sequencing (NGS) in a German RP patient (A-IV:1), further genetic analysis was performed in approximately 400 families with arRP, CRD, or Leber congenital amaurosis. This resulted in three more families from The Netherlands or Israel with causative mutations in C8orf37. In total, four families including eight affected individuals were selected for this clinical study (Table).

We adhered to the tenets of the Declaration of Helsinki and informed consent was obtained from all participating patients prior to the collection of a blood sample and additional ophthalmologic examinations. Prior to this study, we obtained approval from the Institutional Ethics Committee from the Radboud University Nijmegen Medical Centre.

RESULTS

We included a total of eight patients from four families in this study. An overview of the clinical findings in these patients is provided in the Table. In families A and D, the affected individuals were diagnosed with RP whereas in the other families (B and C) the patients were diagnosed with CRD. Additionally, both patients from family B mentioned postaxial polydactyly in their medical history, including one additional finger and toe on the right hand and foot, respectively. In the other families, no extraocular abnormalities suggesting syn- dromic RP or CRD were observed.

In the RP families (A and D), the mean age of onset was approximately 12 years and ranged from infancy to the age of 18 years. The initial symptoms in these patients were either loss of visual acuity (n = 2) or night blindness (n = 1) in these patients, although both symptoms occurred within a year after
<table>
<thead>
<tr>
<th>Patient ID/#</th>
<th>Age of Onset, y/</th>
<th>Initial Symptom</th>
<th>Visual Acuity*</th>
<th>Lens Status</th>
<th>Ophthalmoscopy Results</th>
<th>ERG Results†</th>
<th>Goldmann Perimetry</th>
<th>OCT Results</th>
<th>Autofluorescence Results</th>
<th>Nonocular Findings</th>
<th>Homozygous Mutation</th>
<th>Dx</th>
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</thead>
<tbody>
<tr>
<td>A-IV/1/Infancy/57/M</td>
<td>Night blindness/loss of visual acuity</td>
<td>LP Clear</td>
<td>Profound panretinal choroidal atrophy, waxy pallor of the optic disc, severely attenuated vessels, irregular pigment clumps, bone spicules in the midperiphery</td>
<td>UTP</td>
<td>Scan approximately 500 μm temporal of macula. Retinal and choroidal thinning.</td>
<td>Macular hypoautofluorescence and numerous hypofluorescent lesions in the peripapillary region.</td>
<td>Diffuse hypofluorescent spots in peripapillary region. Hyperfluorescence in peripapillary region with hypofluorescent spots.</td>
<td>Polydactyly on right foot and right hand</td>
<td>c.1562A→G (splice defect)</td>
<td>CRD</td>
<td></td>
<td></td>
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<tr>
<td>B-V/17/41/F</td>
<td>Photophobia</td>
<td>CF Clear</td>
<td>Severe atrophy with RPE alterations and gloss in the macula, normal aspect of the optic disc, mild attenuation of peripheral retinal vessels; only peripheral pigmentation (both bone spicule-like as well as round), choroidal atrophy in the midperiphery.</td>
<td>LE Central scotoma RE: not reliable. Progressive constriction of VF.</td>
<td>Severe thinning of retina, loss of photoreceptors with preservation of RPE, severe thinning of the nerve fiber layer. CFT: 150 μm (RE), 160 μm (LE).</td>
<td>Hypoautofluorescence in the foveal region, panfoveal hyperautofluorescence, numerous irregular hypoautofluorescent spots in the peripapillary region.</td>
<td>Polydactyly on right foot and right hand</td>
<td>c.1562A→G (splice defect)</td>
<td>CRD</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>B-V/7/10/26/M</td>
<td>Photophobia</td>
<td>20/125 Clear</td>
<td>RPE alterations, atrophy, and gloss in the macula. Normal aspect of vascular and optic disc. Sporadic round pigmentation in periphery.</td>
<td>NR BE: Constricted VF</td>
<td>Severe thinning of retina, loss of photoreceptors with preservation of RPE, severe thinning of the nerve fiber layer. CFT: 150 μm (RE), 160 μm (LE).</td>
<td>None</td>
<td></td>
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<tr>
<td>C-II/1/Infancy/36/M</td>
<td>Loss of visual acuity</td>
<td>20/250 Clear</td>
<td>Macular atrophy with intraretinal pigment clumping, peripapillary atrophy, temporal optic disc pallor, attenuation of the retinal vessels.</td>
<td>NP</td>
<td>Severe thinning of the retina, loss of photoreceptors-RPE complex.</td>
<td>None</td>
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<tr>
<td>C-II/2/Infancy/30/M</td>
<td>Loss of visual acuity</td>
<td>20/60 Clear</td>
<td>Macular atrophy, peripapillary atrophy, temporal optic disc pallor, attenuated vessels, RPE changes in the RE following extracapsular surgery for retinal detachment.</td>
<td>NP</td>
<td>Severe thinning of the retina, loss of photoreceptors-RPE complex.</td>
<td>None</td>
<td></td>
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<tr>
<td>D-IV/1/8/58/F</td>
<td>Night blindness/loss of visual acuity</td>
<td>HM HM</td>
<td>Very mild PSC cataract in RE</td>
<td>NP</td>
<td>LE performed only, remaining VF of 5/5 with severe sensitivity loss.</td>
<td>Severe thinning of the retina, atrophy of the RPE and choriocapillaris.</td>
<td>None</td>
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<td>D-IV/3/17/45/F</td>
<td>Loss of visual acuity</td>
<td>LP IP</td>
<td>Yellowbrown atrophic lesion in the macula with pigment clumps, waxy optic disc pallor, attenuated vessels, gray atrophy along the vascular arcades, heavy bone spicule pigmentation in midperiphery.</td>
<td>NP</td>
<td>LE performed only, remaining VF of 5/5 with severe sensitivity loss.</td>
<td>Severe thinning of the fovea, loss of photoreceptor-RPE complex, intraretinal hyperreflective clumps, atrophy of choriocapillaris. CFT: 51 μm (RE), 65 μm (LE).</td>
<td>None</td>
<td></td>
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<tr>
<td>D-IV/4/10/40/F</td>
<td>Loss of visual acuity</td>
<td>LP IP</td>
<td>Brown atrophic lesion in the macula with pigment clumps, waxy optic disc pallor and peripapillary atrophy, attenuated vessels, grayish atrophic changes in the peripapillary region, heavy bone spicule pigmentation in the midperiphery.</td>
<td>NP</td>
<td>UTP</td>
<td>Severe thinning of the fovea, loss of photoreceptor-RPE complex, irregular hyperreflective intraretinal clumps, paravascular pseudodrusen in RE. CFT: 106 μm (RE), 95 μm (LE).</td>
<td>None</td>
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*BE, both eyes; CFs, counting fingers; CFT, central foveal thickness; Dx, diagnosis; F, female; HM, hand movements; LE, left eye; LP, light perception; M, male; NP, not performed; NR, nonrecordable; PIS, photoreceptor inner segment; POS, photoreceptor outer segment; PSC, posterior subcapsular cataract; RE, right eye; SR, severely reduced; UTP, unable to perform test, due to severe vision loss; VF, visual field.

†The upper line represents the right eye, the lower line represents the left eye. †ERG performed at the age of 19 and 37 years. Both rod and cone responses were nonrecordable at age of 19 years. §ERG performed at the age of 29 and 41 years. Scotopic responses were severely reduced and photopic responses were nonrecordable at age of 29. || Goldmann perimetry performed at age of 29 and 41 years. At an age of 29 years, there was no construction of the visual field. *Negative scotopic ERG.
the onset of the disease in these patients. In one patient (D-IV:1), the exact initial symptom could not be determined because night blindness and visual acuity loss occurred simultaneously. After the onset of the disease, visual acuity deteriorated to hand movements or light perception within the following two decades (range, 8–17 years). No nystagmus was observed. Early stages of posterior subcapsular cataract were present in the patients of family D, whereas the lens of patient A-IV:1 was clear. Ophthalmoscopy displayed the classic RP features of bone spicule pigmentation, attenuated vessels, and pallor of the optic disc as well as profound atrophic lesions in the macula, as well as bone spicule-like pigmentation in the midperiphery. (B) Fundus photograph composition of right eye of patient B-V:7 (age 26 years), revealing macular atrophy as well as slightly attenuated arterioles and areas of ongoing chorioretinal atrophy. One round pigmentation can be found along the inferior vascular arcade. (C) Fundus photograph of the central retina in the right eye of patient C-II:1 (age 36 years) showing profound macular atrophy and intraocular irregular pigmentation, as well as attenuated arterioles. (D) Fundus photograph composition of patient D-IV:1 at age 39 years (right eye) reveals macular atrophy and pigmentation clumps, as well as pallor of the optic disc, attenuated vessels, paravascular atrophy of the retinal pigment epithelium, and abundant bone spicules.

**Figure 1.** Fundus photographs of patients carrying mutations in C8orf37. (A) Fundus photograph of the central retina in the right eye of patient A-IV:1 (age 37 years) showing profound retinal degeneration, waxy pallor of the optic disc, attenuated vessels, irregular pigmentation in the macula, as well as bone spicule-like pigmentation in the midperiphery. (B) Fundus photograph composition of right eye of patient B-V:7 (age 26 years), revealing macular atrophy as well as slightly attenuated arterioles and areas of ongoing chorioretinal atrophy. One round pigmentation can be found along the inferior vascular arcade. (C) Fundus photograph of the central retina in the right eye of patient C-II:1 (age 36 years) showing profound macular atrophy and intraocular irregular pigmentation, as well as attenuated arterioles. (D) Fundus photograph composition of patient D-IV:1 at age 39 years (right eye) reveals macular atrophy and pigmentation clumps, as well as pallor of the optic disc, attenuated vessels, paravascular atrophy of the retinal pigment epithelium, and abundant bone spicules.

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In the two CRD families (B and C), the mean age of onset was approximately 7 years and, like that in the RP patients, ranged from infancy to the age of 18 years. Either photophobia \( (n = 2) \) or loss of visual acuity \( (n = 2) \) was mentioned as an initial symptom. In the three decades following the onset of the disease, all four CRD patients developed loss of visual acuity that gradually decreased to low vision levels of 20/60 to counting fingers. These patients showed eccentric fixation and no nystagmus or nystagmoid wandering eye movements were present. Biomicroscopy revealed no cataract was present in these patients. Ophthalmoscopy revealed macular RPE atrophy as well as (mild) attenuation of the vessels (mean age: 35 years; Figs. 1B, 1C). In patient C-II:2, pigment changes due to extraocular surgery for retinal detachment were observed.
Figure 2. (Auto)fluorescence imaging and corresponding SD-OCT examinations in patients with mutations in C8orf37. (A, B) Autofluorescence (A) and SD-OCT (B) image of the right eye of patient A-IV:1 (age 37 years) showing macular hypoautofluorescence and numerous hypoautofluorescent lesions in the perimacular region (A), as well as severe thinning of the retina temporal of the macula (B). (C, D) Autofluorescence (C) and SD-OCT (D) imaging of the right eye of patient B-V:7 (age 26) reveals foveal hypoautofluorescence, parafoveal hyperautofluorescence, numerous irregular hypoautofluorescent spots in the perimacular region (C), and severe thinning of the foveal thickness and profound loss of the photoreceptor layer with preservation of the RPE cells (D). (E, F) Fluorescence angiography (E) and OCT (F) image of the central retina in the left eye of patient C-II:2 at the age of 30 years (no autofluorescence was performed) showing hypofluorescence in the macula (E), and loss of photoreceptor outer segments, thinning of the retina, and atrophy of the choriocapillaris (F). (G, H) Autofluorescence (G) and OCT (H) image of the central retina in the right eye of patient D-IV:1 (age 39 years) reveals hypofluorescence in the macula, indicating atrophy as well as atrophic lesions along the vascular arcades (G) and severe thinning of the retina with profound loss of the photoreceptor–RPE complex (H). Green lines indicate the location of the corresponding OCT examination. Image quality varies as result of unstable eccentric fixation.
The presence of C8orf37 mutations as a cause of RP/CRD is low (1%). It is hypothesized that the level of dysfunctional ABCA4 protein is instrumental in the degeneration pattern, causing either Stargardt disease, CRD, or RP.

Interestingly, C8orf37 is among the few genes that can cause both autosomal recessive RP and CRD. Until now, this has been described in only two other genes: ABCA4 and CERKL. For ABCA4, it is hypothesized that the level of dysfunctional ABCA4 protein is instrumental in the degeneration pattern, causing either Stargardt disease, CRD, or RP [15-26]. Although the phenotype associated with CERKL mutations was originally characterized as RP, most patients that have been described until now are diagnosed with CRD [26-31]. This is in accordance with the observation that the CERKL protein is mainly localized in cone photoreceptors of mouse retinae [52]. The C8orf37 gene is expressed in both rod and cone photoreceptors [16]. This fits with the involvement of both types of photoreceptors, but does not explain the pattern of photoreceptor degeneration. Within the four families, we did not observe differences in degeneration patterns. This suggests a connection between the degeneration pattern and a genetic cause, given that family members carry identical C8orf37 mutations and are likely to share modifier alleles as well. Here, the correlation between phenotype and genotype is purely theoretical, given that the exact effects of the mutations on the C8orf37 protein and its function are not known. However, our previous study localized the protein to the ciliary rootlet of the connecting cilium in mouse photoreceptors and to the base of the primary cilia of human RPE cells, indicating a ciliary function [16].

Ciliopathies are diseases characterized by the dysfunction of the cilium, which may lead to either multigenerational or single-organ diseases. The presence of postaxial polydactyly in patients B-V:1 and B-V:7 is interesting in view of the probable ciliary function of C8orf37, because it is one of the cardinal features of Bardet–Biedl syndrome (BBS), a ciliopathy characterized by retinal degeneration, obesity, polydactyly, hypogonadism, renal dysfunction, and cognitive impairment [45]. Retinal degeneration is a hallmark of many syndromic ciliopathies [45,46], although mutations in retina-specific ciliary genes may also lead to nonsyndromic RP [57,58]. One third of nonsyndromic retinal dystrophies involve defects in a ciliary protein. Ubiquitously expressed ciliary genes, such as C8orf37 and, for example, RPGR, are more likely to cause syndromic phenotypes. Also in RPGR-associated disease,
most of the patients demonstrate isolated RP, although systemic symptoms have been identified occasionally.

Besides the postaxial polydactyly in individuals B-V:1 and B-V:7, we did not identify other syndromic features in these individuals. In the other patients no syndromic abnormalities were observed, although we could not perform detailed additional examinations to exclude subclinical symptoms. Polydactyly generally occurs in 5 to 19 per 10,000 live births. We cannot exclude coincidental coexistence but the polydactyly in this family occurred only in the siblings affected with CRD. This strengthens the hypothesis of associations between the retinal phenotype and polydactyly. In the remaining patients, we could identify other features previously linked to ciliopathies: electronegative ERG waveforms in patients C-II:1 and C-II:2 (Fig. 3), which have been described in BBS-associated BBS, and the early occurrence of macular atrophy. These data indicate that mutations in C8orf37 may be able to cause both syndromic and non-syndromic phenotypes, similar to other ciliary genes such as USH2A, BBS1, and RPRG.

In conclusion, mutations in C8orf37 cause autosomal recessive CRD or RP with early macular involvement. The dystrophies start in the first two decades of life and progress relatively fast to a common phenotype of end-stage retinal degeneration. Although the exact function of C8orf37 is not known yet, its immunolocalization, associations, and interactions with other proteins and the presence of polydactyly in one of our families would suggest a ciliary function.

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References


