Long-Term Follow-Up of the Human Phenotype in Three Siblings with Cone Dystrophy Associated with a Homozygous p.G461R Mutation of KCNV2

Christoph Friedburg,1,2,3 Bernd Wissinger,4,5 Maria Schambeck,2 Michael Bonin,5 Susanne Kobl,4 and Birgit Lorenz1,2

PURPOSE. To provide an up to 14-year overview of the early ocular phenotype in siblings with a homozygous p.G461R mutation in the KCNV2 gene.

METHODS. Two brothers and their sister were followed-up clinically from ages 5 years, 4 years, and 2 months, respectively, including complete ophthalmological examinations. Goldmann visual fields, two-color-threshold (2CT) perimetry, color vision testing, optical coherence tomography (OCT), fundus autofluorescence (FAF), and Ganzfeld electroretinograms (ERGs) were performed according to age-related capabilities. Genetic analyses included whole genome linkage analysis, homozygosity mapping, and candidate gene sequencing.

RESULTS. All three siblings were homozygous for the p.G461R mutation. At 5 months, the younger brother had no nystagmus and Teller-acute of 3.2 cyc/deg. At older age, all three presented nystagmus, increased light sensitivity, reduced color discrimination, and relative central scotomas. Visual acuities ranged from 20/200 to 20/70. The macula developed minor irregularities of the RPE, thinning in optical coherence tomography, and a ring of increased FAF. Scotopic (rod) sensitivity was reduced by 2 log and photopic sensitivity by 1 log in two-color-threshold perimetry. ERG responses were markedly delayed. Photopic amplitudes were severely reduced. Scotopic b-waves rose steeply with flash intensity, but for the standard flash supernormal amplitudes were only reached in the girl.

CONCLUSIONS. FAF was similar to that in cone-rod dystrophy. Although cone dysfunction was accompanied by rod dysfunction, and scotopic ERGs in patient 2 deteriorated, no patient demonstrated any unequivocal sign of rod degeneration. Grossly delayed b-waves with a steep response-versus-intensity relationship rather than supernormal amplitudes should remind clinicians of this specific condition. (Invest Ophthalmol Vis Sci. 2011;52:8621–8629) DOI:10.1167/iovs.11-8187

Larger than normal amplitudes of the electroretinogram (ERG) should not be confused with good function—on the contrary, they may point to serious pathology. Although this constellation is apparently relatively rare, it has been described in a variety of diseases including maculopathy, cone dystrophy, pigment epitheliopathy, optic neuropathy, vascular occlusion, and intoxication.1

Amplitudes of Ganzfeld (full-field) flash-ERGs depend on a number of factors, some of them controlled by using standard parameters for recordings.2 Because electrode types and, in the case of DTL-electrodes, positioning of the fiber may cause significant differences, acquisition of normal values is compulsory for each individual laboratory. With all these precautions in place, the range of normal amplitudes still is quite large, covering a factor of approximately two. It is therefore conceivable that a significant number of cases with moderate bilateral amplitude increase are misjudged as normal unless other parameters prove remarkable.

In 1983, Gouras et al.3 reported a retinal cone dystrophy with supernormal rod b-wave amplitudes to bright flashes but reduced amplitudes to dim flashes. Peak times in both situations were substantially delayed. Subsequently, others reported similar cases of cone dystrophy with supernormal rod ERG.4–11

The ERG is a cumulative potential arising from various groups of retinal cells.12 While the rising phase of the rod ERG a-wave is mainly elicited by electrical changes in the photoreceptor outer segments, the peak of the a-wave occurs due to the addition of further retinal signals, mainly from ON-bipolar cells.13 It is therefore extremely difficult to attribute changes in size of a- or even the b-waves to single retinal components. In a detailed investigation of rod activation and deactivation in patients with the above condition, including fitting of a model of the rising phase, Hood et al.14 concluded that there is no change of the phototransduction process. They located the site of disease to be beyond the photoreceptor outer segment in the inner nuclear layer.

In 2006, Wu et al.15 identified mutations of the KCNV2 gene in patients presenting with the above condition. The corresponding protein is only expressed in the outer retina,15 and is thought to act as a silent modulatory subunit (K₈.2) of a voltage-gated tetramer K⁺-channel. In vitro K₈.2 is able to assemble with K₂.1 to form heteromeric channels with altered properties that feature a narrowed membrane potential for activation and slowed inactivation kinetics. Such kinetics eventually leads to transient hyperpolarization overshoots on rapid changes in inward currents.16 These properties are reminiscent of the I₄(A) current first described in amphibian rod photoreceptors, which contributes to their transient augmented hyperpolarization at the onset of light or rapid change.
in light intensities. Yet the link between the molecular function and properties of Kv8.2 and the retinal phenotype caused by its defect is still elusive.

We provide a longitudinal, detailed description of the ocular phenotype in three siblings in whom we identified a homozygous mutation in KCNV2. The youngest was investigated as early as 5 months after birth, at which time he appeared clinically unaffected. Our study aimed to identify early and specific clinical markers and to search for disease progression or even degeneration during the observational period of up to 14 years.

**METHODS**

**Subjects**

A boy (IV:1 in Fig. 1) and his younger sister (IV:2) were referred with nystagmus first detected in the outpatients' department at the age of 5 and 3.5 years, respectively. The initial tentative diagnosis was achromatopsia. They, and later a younger brother (IV:3) from age 5 months on, were followed-up and reinvestigated after identification of the KCNV2 mutation in all three. Another brother born later carries no mutation. No other affected relatives are known. Both parents had ophthalmologic findings within normal limits, are of Caucasian origin, and were unaware of any consanguinity. DNA samples could also be obtained from the mothers of both parents (II:2 and II:6) who lived 5 miles apart.

Approval from the local Ethics Committee was obtained. All investigations conformed to the tenets of the Declaration of Helsinki and informed consent was obtained. The patients' wish to omit some investigations at their last visit, especially high intensity ERG and a follow-up standard ERG according to the International Society for Electrophysiology in Vision (ISCEV) in IV:3, was respected. Fundus autofluorescence (FAF) conditions (10 cd m\(^{-2}\) background for at least 10 minutes).

**Clinical Examination**

A standard ophthalmologic examination was performed on all visits, including refraction, best-corrected visual acuity, slit-lamp biomicroscopy, and funduscopy. Ocular alignment and possible nystagmus were judged including refraction, best-corrected visual acuity, slit-lamp biomicroscopy, and funduscopy. Ocular alignment and possible nystagmus were judged by experienced ophthalmologists and orthoptists. Additional tests included Goldmann kinetic visual fields, Ishihara and Ichikawa color plates, color vision arrangement tests (Farnsworth Panel D-15, Lanthynergic desaturated panel D-15) and an anomaloscope (Heidelberg Anomaloscope; Oculus, Wetzlar, Germany).

**Two-Color Threshold Perimetry**

To assess the spatial distribution of rod and cone mediated sensitivity, 2CT perimetry was performed as reported previously (original description by Jacobson et al.): A field analyzer (Humphrey Model 640, Carl Zeiss Meditec Inc., Dublin, CA, USA) was modified by Frederick Fitzke of the Institute of Ophthalmology in London to measure sensitivity to red and blue (peak luminance at 650 nm and 500 nm, respectively) both in scotopic (after 45 minutes in the dark) and photopic conditions (10 cd \(\text{m}^{-2}\) background for at least 10 minutes).

Scotopic sensitivity was considered rod mediated if sensitivity to red was at least 2 dB lower than to blue. A 2.0 log neutral density filter adapted the system’s range for this physiological difference. Rod sensitivity loss (RSL) was the difference in dB for scotopic blue versus the 10th percentile of 8 controls aged 20 to 30 years. If scotopic sensitivity was <9 dB lower for red than for blue, detection was considered cone mediated. Photopic, cone sensitivity loss (CSL) was reported as the threshold difference for red stimuli of the individual versus the 10th percentile of controls.

**Imaging**

Fundus photographs taken with a conventional fundus camera (Carl Zeiss Meditec AG, Jena, Germany) and later with a digital camera (Topcon TRC 50X; Tokyo, Japan) were digitally aligned with soft borders using commercially available software. FAF was recorded with a confocal scanning laser ophthalmoscope (Heidelberg Retina Angiograph; HRA; Heidelberg Engineering, Heidelberg, Germany) and single frames were averaged as reported previously (using the Heidelberg Eye Explorer). Optical coherence tomography (OCT) of cross-sections of the fovea was obtained using a time-domain OCT (Stratus OCT 3, Carl Zeiss Meditec AG, Jena, Germany). Eye movements could only be suppressed partially and degraded imaging quality. Horizontal or 30° tilted scans of 6 mm length that clearly illustrated the foveal pit were selected and retinal thickness was analyzed with the built-in retinal map analysis module (version 4.0.1) and with map diameters of 1, 3, and 6 mm.

**Electroretinogram**

ERGs were recorded with a Ganzfeld stimulator and recording equipment (Nicolet Spirit; Nicolet, Madison, WI) with DTL electrodes according to the 2004 standards of the ISCEV. Respecting the parents' and patients' wish to limit light exposure, no flash brighter than the ISCEV standard flash (1.5 to 3 cd \(\text{m}^{-2}\) \(\text{s}^{-1}\)) was used. Signals were sampled at 0.4 ms intervals (2500 Hz) after passing a Butterworth- and a 50 Hz-notch filter with artifact rejection in place. Curves shown are averages from 3 to 6 recordings. Baseline drift due to blinking had to be removed in a few data sets as judged by the preflash isoelectric line. Patient results were regarded as normal if within the 5th and 95th percentile of results from 23 subjects aged 20 years or younger.

**Molecular Genetic Analysis**

DNA was isolated from peripheral blood according to Miller et al. Initially, short tandem repeat (STR) marker-based linkage analyses for gene loci and candidate gene screening including CNGA3, CNGB3, GNAT2, PDE6C, SLC24A2, and GRK7 were done as previously described. For a whole genome linkage analysis and homozygosity mapping we used samples from the three affected siblings, the unaffected sibling, and the parents that were hybridized on single nucleotide polymorphism (SNP) arrays (GeneChip Human Mapping 100K Set, Affymetrix, Santa Clara, CA). Data analysis was performed with the dChip software program and macro applications (Microsoft Excel 2007, Microsoft, Redmond, WA). Mutation screening of KCNV2 was done by DNA sequencing of PCR amplified genomic DNA fragments.

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**Figure 1.** (A–C) Patients’ clinical results (top to bottom) at ages provided: Goldmann visual fields; Farnsworth Panel D-15 color test; measured by Rayleigh match with Heidelberg Anomaloscope for patient IV:1 and IV:3; and two-color-threshold (2CT) perimetry: blue and red stimuli were of 1.7° diameter equivalent to Goldmann target V and presented for 200 ms using the 30-2 program. Negative values denote sensitivity losses with severity of loss. Incomplete photopic data for IV:3. (D) Best corrected visual acuities. L, left; logMAR, log(minimum angle of resolution) = –log(visual acuity); R, right; y, years.

**Figure 2.** Retinal imaging from patients IV:1 and IV:2. (Top) Time domain optical coherence tomography (OCT) scans of 6 mm length, degraded by nystagmus. Infrared reflectance (inset) of the macula was reduced. Retinal thickness analyzed from horizontal or 30° tilted scans with the built-in retinal map analysis module and provided in Table 1 was clearly below normal except for the outer temporal areas. Autofluorescence on the right of OCTs showing a ring with increased fundus autofluorescence (FAF) around the fovea. (Bottom) Fundus pictures, some mounted from single shots, borders softened. L, left; R, right; y, years.
covering the coding parts of exons 1 and 2, and the flanking untranslated region (UTR) and intron sequences. All sequencing reactions were carried out using terminator chemistry (BigDye; Applied Biosystems, Darmstadt, Germany) and sequencing products were separated on a DNA sequencer (ABI3100; Applied Biosystems).

RESULTS

Clinical Phenotype

At the time of referral, patients IV:1 (5 years) and IV:2 (3 years) had a conjugated, jerk nystagmus of high frequency and small amplitude with some head nodding (clinical data summarized in Table 1) noticed by the parents shortly after birth. At 5 months of age, patient IV:3 had a normal binocular visual acuity with preferential looking of 3.2 cdeg/deg (normal 99% age-correlated confidence interval 1.5–8 cdeg/deg). He presented neither nystagmus nor glare sensitivity under repeated clinical observation up to the age of 16 months. From 22 months on nystagmus was noticeable. Visual acuity, as in the siblings, remained fairly constant between 0.1 and 0.25 (Fig. 1D). Being hypermetropic initially, patients IV:1 and IV:2 shifted to myopia by 9 and 15 diopeters respectively, while myopization in their brother IV:3 over the period observed was only 3 diopeters. Despite relative central scotomas, reduced binocular vision of 200 to 100 arc seconds could be demonstrated with Titmus Stereo Test.

Confusion of colors along the red/green axes was obvious from reading Ishihara color plates (IV:1 and IV:2, each at age 6) and in the Farrows Panel D-15 test (Fig. 1). Rayleigh matches obtained from patient IV:1 at age 19 years and IV:3 at 12½ years even revealed complete scotopization. The sister (patient IV:2) clearly had better color discrimination than both her brothers. Especially at age 17, she hardly confused any of the color probes with her left eye. In all 3 patients, the extent and number of confusions showed no clear trend of deterioration with age. Blocking filters of various types provided no relevant positive effect. Symptoms in this family were consistent with both rod dysfunction disorders and cone dystrophies.

Visual Fields and Scotopic versus Photopic Sensitivity

Using Goldmann visual fields targets III/4e, II/4e, and I/4e, patients IV:1 and IV:2 both had a central scotoma and peripheral concentric constriction (Fig. 1). The peripheral constriction did not progress over a period of 9 and 6 years, respectively. No central scotoma was detected in patient IV:3 at age 7 years, possibly due to age-related limited cooperation.

Rod and cone system sensitivity losses were examined with 2CT perimetry (Fig. 1). Rod sensitivity was always considerably later than normal, especially at low flash intensity, their slopes were about normal, but due to prolonged peak time, the peaks of a- and b-wave were supernormal. On a photopic background, responses to 30 Hz-flicker (Fig. 3C) were delayed and reduced to about a third of normal. For single flashes (SF and slightly more intense, Fig. 3D) very shallow slow responses were found that could arise from remaining rod function, which the standard background may not have fully suppressed.

An equivalent measurement for the scotopic standard flash regarding b-wave size and peak time had been obtained at age 5 years (Fig. 4A). At age 15 years (Fig. 4C), a- and b-waves in response to the standard flash were of normal size but only due to their delayed peak time. Their size, compared with the earlier two measurements, had decreased by one third and fell within the lower range of normal (Fig. 5). Patient IV:3 had about the same response to this flash intensity (Figs. 4F, 5). Patient IV:2 at age 11 and 17 years had quite comparable results with supernormal, delayed SF-responses (Figs. 4D, 4E).

Figure 5A provides an overview of b-wave peak size (Fig. 5A) and peak time (Fig. 5B) as a function of flash intensity. The response-versus-intensity relation (Fig. 5A) was steeper than normal in all three patients (i.e., responses ranked lower for flashes 2 or 0.75 log dimmer than SF than for SFs). Supernormal peak b-waves were only seen in patient IV:2, whereas patient IV:1 (and IV:3) would have to be defined as approximately normal or subnormal. Peak times (Fig. 5B), however, were always considerably later than normal, especially at low flash intensities.

<p>| Table 1. Stratus OCT Retinal Thickness (μm) with Map Diameters of 1, 3, and 6 mm for Patients IV:1 and IV:2 |
|-------------------------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|</p>
<table>
<thead>
<tr>
<th>Patient</th>
<th>Eye</th>
<th>Temporal</th>
<th>Inner Temporal</th>
<th>Central</th>
<th>Inner Nasal</th>
<th>Nasal</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV:1</td>
<td>Right</td>
<td>180</td>
<td>197</td>
<td>161</td>
<td>208</td>
<td>211</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>195&lt;sup&gt;a&lt;/sup&gt;</td>
<td>199</td>
<td>166&lt;sup&gt;a&lt;/sup&gt;</td>
<td>209</td>
<td>212</td>
</tr>
<tr>
<td>IV:2</td>
<td>Right</td>
<td>194&lt;sup&gt;b&lt;/sup&gt;</td>
<td>191</td>
<td>163</td>
<td>211</td>
<td>216</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>180</td>
<td>200</td>
<td>161</td>
<td>195</td>
<td>218</td>
</tr>
<tr>
<td>Children</td>
<td>Mean ± 2 SD</td>
<td>229 ± 29</td>
<td>263 ± 32</td>
<td>200 ± 31</td>
<td>279 ± 35</td>
<td>261 ± 34</td>
</tr>
<tr>
<td>Adult</td>
<td>Mean ± 2 SD</td>
<td>218 ± 28</td>
<td>262 ± 29</td>
<td>202 ± 36</td>
<td>274 ± 31</td>
<td>262 ± 32</td>
</tr>
</tbody>
</table>

All values were below the mean minus twice the standard deviation of 20 normal children’s eyes. Those few within the normal range of 34 adult eyes are labeled<sup>a</sup> (see Table 1 in Chopovska et al.<sup>37</sup>). Parentheses enclose the value for which automatic layer separation failed.

Imaging

A fine granular appearance of the macula was noted from 5 years on in the two boys (Fig. 2, not apparent at age 22 months in patient IV:3), and from 10 years on in the girl. FAF in IV:1 and IV:2 was clearly increased in a small ring around the fovea (testing refused by IV:3). Central infrared reflectance was reduced. On time-domain OCT patient IV:2 displayed some cystic changes especially in the fovea. Retinal thickness (from the inner limiting membrane to the RPE, Fig. 2) was reduced considerably compared with normal values (Table 1).

Electroretinogram

Complete results of Ganzfeld-electroretinogram (ERG) testing for patient IV:1 compared with a normal recording are shown in Figure 3. In the dark (Figs. 3A, 3B), responses were markedly prolonged. At equal times post flash, a- and b-waves elicited with flashes of the lower 3 intensities (starting from the top) were of subnormal size, but reached normal peak height later (i.e., their slopes were shallower). At standard flash intensity, their slopes were about normal, but due to prolonged peak time, the peaks of a- and b-wave were supernormal. On a photopic background, responses to 30 Hz-flicker (Fig. 3C) were delayed and reduced to about a third of normal. For single flashes (SF and slightly more intense, Fig. 3D) very shallow slow responses were found that could arise from remaining rod function, which the standard background may not have fully suppressed.

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satellite marker segregation analysis within the family. We
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GRK7
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known to be associated with cone dysfunction disorders, no-
For patient IV:1 we performed a mutation screening of genes
Molecular Genetics
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alby CNGA3, GNG3, GNAT2, and PDE6C, as well as cone-
specific expressed candidate genes, notably SLC24A2 and
GRK7, but no pathogenic mutation was detected. Moreover,
we excluded the CNGB3, GNAT2, and PDE6C loci by micro-
satellite marker segregation analysis within the family. We
therefore carried out a whole genome linkage analysis and homozygos-
ity mapping applying the Affymetrix 100k SNP ar-
array. This analysis revealed six regions on chromosomes 1, 2, 5,
9, 13, 16, and 17 with concordant segregation pattern (sugges-
tive of linkage) for the three affected siblings. However, only
two of these regions (on chromosome 5p13 and on chromo-
some 9p24) contained homozygosity intervals of substantial
length. The homozygous interval between rs4014085 and
rs6477148 on 9p24 covered a region of 5.1 megabase (Mb) and
included the KCNV2 gene. On sequencing of the coding exons
of KCNV2 we detected a homozygous c.1381G>A mutation
that causes a p.G461R substitution at the protein level. The
mutation was present in homozygous state in all affected sib-
lings and was absent in the unaffected youngest brother. Both
parents (III:3 and III:4) and the grandmothers (II:2 and II:6)
were heterozygous for the mutation (Fig. 6).

**DISCUSSION**

This study presents the first detailed investigation on the early
ocular phenotype of the homozygous p.G461R mutation of
KCNV2 in three young siblings spanning a period of 13 to 15
years. So far this mutation has been reported in two other
patients. In addition, this mutation was present in a number of
compound heterozygotes. The p.G461R mutation affects the third residue of the ultraconserved -GYG-

![Figure 3](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933459/) (A) Scotopic responses to single flashes of increasing intensity (relative to ISCEV standard flash (SF): SF-2,75 log; SF-2 log; SF-0,75, and SF). Steepness of a-wave and absolute position of peak of b-wave are approximately normal. (B) Scotopic oscillatory potentials. (C) Reduced photopic response to single flashes (SF and SF)

![Figure 4](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933459/) Change of scotopic ERG response with age in patients. Patient IV:1: RE at (A) 5 years, 10 months, standard flash SF only, average of 3; (B) 13½ years; (C) 19 years. Patient IV:2: LE at (D) 12 years; (E) 17½ years; and comparison with patient IV:3 at (F) LE 7 years. Flash intensity matched those in Figure 3A. In the first ERG session of patient IV:1 (A), only the SF provided any reliable measure-
ment. In later measurements significant changes were observed: Ini-
tially (A), a- and b-waves were of normal size. Later (B), the a- and
b-waves increased to a supernormal size with low b/a-ratio. Finally (C),
the a-wave decreases to about the initial value again, reestablishing a
normal b/a-ratio. Through this process, the peaks of the a- and b-wave
are delayed by approximately 50%-50%. Also note the similarities of
the response to SF in timing but not amplitude for (A) versus (D) and
(B) versus (E). This implies that patient IV:1 is more affected than
patient IV:2. Although patient IV:3 is the youngest, his responses (E)
were smaller than the latest ones of his siblings.
Patient IV:1, RE (myopization as in IV:1 and IV:2 is a common finding in many previous and the three reported here) had nystagmus. Strong decade of life nystagmus is reported in half of the patients. All triple peptide motif that acts as an ion selectivity filter in the K⁺ channel’s pore.33 The recessive nature of the mutation suggests a loss of function effect. Still, the exact effect of this or the other KCNV2 mutations on the photoreceptors and the visual system remains unclear.

Early diagnosis of this disease is possible but there is an overlap of symptoms with other cone dystrophies and cone dysfunction disorders. Many patients with KCNV2 mutations, including ours, experience glare sensitivity, highly reduced visual acuity (Fig. 1D) and nystagmus,15,18,19,20,34 but as in IV:3 not necessarily during the first year of life. Within the second decade of life nystagmus is reported in half of the patients. All four patients with the homozygous p.G461R mutation (one previous and the three reported here) had nystagmus. Strong myopization as in IV:1 and IV:2 is a common finding in many retinal dystrophies.36,37 Its pathophysiology is still poorly understood, but it is possibly linked to retinal blur.38 We also identified some peripheral visual field constriction and depression of rod sensitivity.

An important diagnostic sign to distinguish this disease from typical, complete achromatopsia is the variation of color vision with eccentricity. Whereas central color vision measured with the anomaloscope shows a complete scotopic match, color discrimination in our patients improved if the testing method allowed for more peripheral presentation (e.g., as in the Farnsworth Panel D15 color vision test). This demonstrates larger macular rather than diffuse deterioration and this is consistent with macular pathology on infrared images and reduced retinal thickness in OCT (Fig. 2; Table 1). The better color discrimination in patient IV:2 compared with IV:1 is compatible with the smaller and more localized CSL with 2CT perimetry.

The overall phenotype of patients with the homozygous p.G461R mutation appears relatively severe with consistent, early onset of nystagmus and visual glare, rather poor visual acuity (logMAR in Fig. 1D) and visual field constriction. However, patients with two nonsense mutations performed even worse regarding visual acuity alone compared with those with at least one p.G461R missense mutation.19

One of the main open questions regarding KCNV2 mutations is the extent of retinal degeneration versus dysfunction of the cone and rod system. Long-term observations on subjective measures previously focused mainly on visual acuity,11,19,20 which tentatively became poorer at higher age (Fig. 1D). Interpretation of this parameter alone is difficult due to considerable variation by even minor changes of fixation or by silencing of the nystagmus. Our study added further subjective (psychophysical) measures of cone function. Photopic central sensitivity as measured by 2CT perimetry deteriorated in patient IV:2 over a period of approximately 6 years (Fig. 1B). At the same time color mismatching for the Farnsworth Panel D15, presumably a function of more than just the macula as stated above, remained at least as good in IV:2 and was approximately stable in patients IV:1 and IV:3 (Fig. 1). Objective measures of cone structure and function were also obtained. Macular changes in our young patients were in the lower range of previous reports,14,18–20,34 and did not progress significantly. Ganzfeld-ERG with either 30 Hz flicker or photopically presented single flashes were significantly delayed and reduced but did not reveal conclusive deterioration over the period observed.

Infrared and OCT imaging of the macula (Fig. 2) demonstrated thinning of this cone-dominated region and FAF was enhanced in a ring concentric to the fovea in all our patients. At the earlier follow-ups these three methods had not yet been available. However, the ring of enhanced FAF in itself is an early, but nonspecific sign for progression and similar patterns are found in a number of progressive cone-rod dystrophies39,40 but not stationary disease. Three out of seven patients aged 18 years or younger reported by Robson et al.15 (their Fig. 4, 1C, 1G; one homozygous, one heterozygous mutation in KCNV2; no mutation identified in the third patient) had a similar pattern and all seven had fairly mild macular changes. In contrast, of those six patients aged 38 and older (2 homozygous, 1 heterozygous, and 3 unclear) only two had mild macular changes while the other four clearly showed macular atrophy. While loss of retinal pigment epithelium (RPE) is the main reason for reduced FAF besides disturbed rhodopsin recycling (e.g., in mutations of RPE6524 or vitamin A deficiency), increased FAF has been shown to correlate with still functioning outer retina and RPE in the proximity of degenerating areas.30–32 Robson et al.15 argued that the correlation of the inner ring diameter with age in their cross-sectional analysis combined with the sum of currently available data speaks
for a slow centrifugal deterioration of cone function in KCNV2-associated retinopathy. Its severity possibly depends on the actual genotype.

Even though labeled a cone dystrophy, it was the rod dysfunction that initially led to the identification of this distinct disease. The reduction of scotopic sensitivity at low intensities was experienced as nyctalopia by all our patients early in life. It measured approximately 15 dB in 2CT perimetry (i.e., lights needed to be 30 times brighter) similar to the 15 to 30 dB reported previously in some but not all patients (dark adapted threshold by Gouras et al.3; 2CT perimetry by Michaelides et al.10; dark adaptation by Wissinger et al.19; borderline dark adapted threshold by Foerster et al.6). Rod dysfunction is not limited to the posterior pole surrounding the ring of increased FAF. It extends into the periphery as illustrated by the reduced thresholds in outer areas of 2CT perimetry and by concentric constriction in Goldmann perimetry (Fig. 1) similar to previous results.10,14 Over a period of 5 to 9 years, rod function judged by first and last Goldmann visual fields for targets III/4 and I/4 did not change significantly in our patients. Equally and potentially more reliable, rod sensitivity in dark-adapted 2CT perimetry, in contrast to cone sensitivity, demonstrated no significant changes over a period of 6 years in patient IV:2.

Objective assessment of rod function by recording scotopic ERGs provided no consistent trend of amplitudes of a- or b-wave. Peak times, however, especially for lower intensity flashes, did increase within this period of 5 1/2 years in both patients IV:1 and IV:2. Because the response-intensity relation is disturbed and because the peak delay of the b-wave is the hallmark of this disease this long-term change in peak time is a significant indication of a mild progression of rod dysfunction. This must not be confused with rod degeneration for which unequivocal signs such as bone spicules or peripheral loss of RPE have not yet been reported in older, genetically confirmed patients.11

Most publications on patients with (presumed) KCNV2 mutations so far stress supernormal amplitudes. While all our patients exhibited an abnormal increase of scotopic b-wave amplitude with flash intensity (rvi), those of patients IV:1 and IV:3 at standard flash intensity remained within upper normal range. Normal ERG amplitudes may be as large as approximately 150% of the normal mean.1-4,13 Interpreting peak amplitudes in disease is even more challenging because signals originate from very different retinal sources,12,13 making it difficult even with elaborate isolation techniques or pharmacological approaches to identify or quantify any single source. Hood et al.14 calculated that the underlying photoreceptor component in patients presumably affected by mutations of KCNV2 was not larger but actually reduced to about half of the normal mean, and that the sensitivity of that response was normal. It normally peaks around 100 to 150 ms.44-45 A significant delay of the b-wave in itself, as found consistently in patients with mutations of KCNV2, leads to a larger fraction of the negative photoreceptor component being unmasked and to an apparent increase of the a-wave as in Figures 4B, 4D, and 4E. If the delayed positive (mainly bipolar) components then rise quickly, the b-wave also appears supernormal, though apart from a time shift no component has changed its size.

The especially long delay for dim, scotopic flashes (see Fig. 5; compare with Fig. 1c in Hood et al.14) has led to the suggestion that the dysfunction of KCNV2 imposes a gating mechanism between outer segment and postreceptorial cells. Under photopic conditions, a situation in which the interaction of ERG components is even more complex, the delay is also apparent (Figs. 3C, 3D). The dysfunction may either be due to changes in the rod pathway itself or to changes of rod-cone interaction in progressive cone disease.

In conclusion, our study provides a whole battery of parameters regarding the early phenotype of three siblings with homozygous p.G461R mutation of KCNV2. The fact that all our patients had nystagmus (though with delayed onset in one), impaired visual acuity, and glare sensitivity within 2 years after birth, possibly indicates a rather severe phenotype. Early detection should be possible by checking for changes in infrared reflectance, retinal thickness, and for a ring of increased FAF.

**FIGURE 6.** DNA sequence electropherogram illustrating the homozygous c.1381G>A mutation that causes a p.G461R substitution in KCNV2. As shown in the pedigree, all three patients IV:1, IV:2, and IV:3 were homozygous (−/−) for the mutation. Both parents and their mothers were heterozygotes (+/−), and the unaffected sibling IV:4 did not carry the mutation (+/+).
Though nonspecific for KCNV2, these signs, as well as (peri-)foveal changes in RPE and deterioration of color vision, distinguish this disease from (so-called stationary) cone dysfunction. The characteristic ERG features are greatly prolonged scotopic b-waves with an abnormally steep response-versus-intensity relationship and delayed photopic single flash and 50 Hz-flicker responses. Supernormal ERG amplitudes are most likely due to the changed response-versus-intensity relationship and delayed rod responses. Supernormal ERG amplitudes are most likely due to the changed response-versus-intensity relationship and delayed rod ERG components. They are neither specific1 nor present in all patients with this mutation. If the supernormal amplitude needs to be named at all, we strongly recommend using the term cone dystrophy with supernormal and delayed rod-ERG as suggested by Hood et al.14 While there is some indication of long-term cone deterioration, we also noticed increasing delays and potentially amplitude reduction in rod function that, given the combination of small morphologic changes with severe dysfunction, suggest a primary functional deficit rather than an indirect or secondary consequence of rod degeneration.

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References


