

## **Novel *KCNV2* mutations in cone dystrophy with supernormal rod electroretinogram**

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## INTRODUCTION

The cone dystrophies are progressive hereditary retinal disorders with electrophysiological or psychophysical evidence of cone dysfunction and degeneration<sup>1</sup>. There is considerable clinical and genetic heterogeneity and various phenotypes have been described including an unusual cone disorder associated with supernormal and delayed rod ERG b-waves<sup>2-8</sup>. This rare form of retinal dystrophy (OMIM # 610356) is characterised by autosomal recessive inheritance, onset in the first or second decade of life with poor visual acuity, marked photophobia, myopia, dyschromatopsia in the red-green axis, central scotoma and, later in the course of the disease, night blindness. There is often disturbance of the retinal pigment epithelium with increased autofluorescence in perifoveal or central macula areas, and no apparent retinal degeneration in the periphery<sup>8</sup>. A characteristic hallmark of the disease is the decreased and delayed dark-adapted response to dim flashes which contrasts with the supernormal response at the highest levels of stimulation. In addition, light-adapted responses to a bright flash or to 30-Hz flickers are delayed and markedly decreased.

Elevation in retinal cyclic guanosine monophosphate (cGMP) has been considered as a possible pathophysiological mechanism leading to photoreceptor cell degeneration in these patients based on the similarities of the ERG features observed between the patients and animal retinas showing increased cGMP. However, no convincing mutation was found in the cone cGMP-phosphodiesterase (PDE) gamma subunit gene (*PDE6H*)<sup>9</sup>. Mutation screening of *NR2E3*, whose mutations cause the enhanced S-cone syndrome (ESCS)<sup>10</sup>, featuring a large b-wave amplitude to stimulation with blue light, was also negative<sup>8</sup>.

By linkage analysis of a consanguineous family originating from the middle East, a group led by Webster and colleagues<sup>11</sup> recently showed that this disorder is due to mutations in *KCNV2*, a gene mapping on chromosome 9p24, and coding for a voltage-gated potassium channel subunit<sup>12</sup>. *KCNV2* is strongly expressed in heart and photoreceptors<sup>11,13</sup>. In photoreceptors, its gene product, Kv8.2, form heterotetrameres with Kv2.1, producing a permanent outward potassium current characteristic for the photoreceptor cells<sup>13,14</sup>. We describe here three additional families with the same condition whose affected members carry novel mutations in *KCNV2*.

## MATERIALS AND METHODS

### Families and clinical examination

Three families, originating from Southern France (# 1), Morocco (# 2) and Algeria (# 3) were recruited for this study. The families from Morocco and Algeria were identified as being consanguineous (Figure 1). There was no evidence of extra-ocular signs of disease. Blood was obtained from family members with their informed consent.

A standard ophthalmic exam (refractometry, visual acuity, slit-lamp examination, applanation tonometry, funduscopy) was performed. Colour vision was evaluated with the Lanthony's desaturated 15 Hue test. Kinetic visual fields were determined with a Goldman perimeter using targets V<sub>4e</sub>, II<sub>4e</sub> and I<sub>4e</sub>. OCT measurement of the macula was performed using an OCT-3 system (Stratus model 3000, Carl Zeiss Meditec, CA) with the software version 3.0. Autofluorescence measurements were obtained with the HRA2 confocal angiograph (Heidelberg, Germany) and fundus pictures were taken. Full-fields ERG was recorded using a ganzfeld apparatus (Metrovision, France) with a bipolar contact lens electrode on maximally dilated pupils, according to the ISCEV protocol<sup>15</sup>.

### DNA analysis

#### PCR reaction

Genomic DNA was extracted from 10-ml peripheral blood samples by a standard salting out procedure<sup>16</sup>. For the 2 coding exons of *KCNV2*, primers were designed to include flanking intronic sequences (Table 1). PCR reactions contained 10 pmoles of forward and

reverse primers, 100-150 ng of genomic DNA, 3 mM MgCl<sub>2</sub>, 200 μM of each dNTP, 1 U of *Taq* DNA polymerase (AmpliTaq Gold, Applied Biosystems) in a 50-μl volume with the appropriate buffer. Following the denaturation step at 95°C for 9 min, the amplification was carried out for 35 cycles at 95°C for 30 sec, the appropriate annealing temperature for 30 sec (Table 1), and 72°C for 1 min, ending with a final extension step at 72°C for 10 min. Amplicons were run on 2 % agarose gels in 1X TAE buffer to verify the quality and specificity of the PCR reaction.

#### *Sequencing*

The purified PCR products (QIAquick PCR purification Kit, QIAGEN) were sequenced in both directions using the BigDye Terminator Cycle Sequencing Ready Reaction kit V1.1 (Applied Biosystems) on an ABI PRISM 3130 capillary sequencer (Applied Biosystems). Sample sequences were aligned to the wild-type ones and to those of 50 control chromosomes originating from France and North Africa. They were analyzed with the Collection and Sequence Analysis software package (Applied Biosystems).

## **RESULTS**

### **Clinical findings**

Members from the 3 families had typical cone dystrophy with supernormal rod ERG (Table 2). In all families, the parents from affected patients were clinically normal. Photophobia was a constant finding and was associated with a decreased visual acuity ranging from 5/10 to 1/20. The oldest patient (54 years, II-2 from family 3) had light perception in one eye. An early onset nystagmus was often observed, and a few patients complained of mild night blindness. Fundus examination disclosed a loss of the foveal reflex (Figure 2, family 1), fine granular pigmentations of the fovea, and in oldest patients, a round-shaped foveal atrophy (Figure 2, family 3), suggesting that the disease slowly progresses towards the death of foveal photoreceptors and retinal pigment epithelium cells. In the 2 young affected sisters from family 1, fundus autofluorescence in the foveal area appeared slightly decreased and the OCT-3 scan already showed a significant thinning of the retina at the foveal pit ( $114 \mu\text{m} \pm 23$  vs  $161 \mu\text{m} \pm 19$  in age-matched controls) (Figure 2, family 1). Goldman perimetry revealed a slight constriction of the peripheral isopter and various degrees of central relative scotomas (Table 2). Dyschromatopsia was an almost constant finding, showing a red/green axis in most patients that tended to be scotopic in severe cases (III-2 from family 3). ERG findings were characteristic of the cone dystrophy with supernormal rod ERG (Figure 3). All patients had decreased light-adapted responses, with 30-Hz flicker amplitudes ranging from 54 to 15 μV (normal > 105 μV). In dark-adapted conditions, the amplitudes of the response at the highest level of stimulation ( $318 \text{ cd.s/m}^2$ ) were constantly supernormal, ranging from 469 to 690 μV (normal value 300-450 μV), in contrast with decreased amplitudes at the lowest level of stimulation ( $1 \text{ cd.s/m}^2$ ) ranging from 0 to 70 μV (normal value 100-200 μV) with a remarkable delay in the b-wave peak ranging from 171 to 180 ms (normal value < 130 ms). The intermediate level of stimulations at  $3.2 \text{ cd.s/m}^2$  showed no significant difference in amplitudes between affected and non affected patients (Table 3).

### **Genotypic findings**

*KCNV2* mutations were found in the 3 families. In family 1 (Figure 1), both affected sisters III-2 and III-3 were compound heterozygotes for the c.442G>T nonsense mutation in exon 1 that leads to a stop codon (p.Glu148Stop), and for the c.1381G>A missense mutation in exon 2 leading to an amino acid change (p.Gly461Arg) (Figure 1). The presence of only one heterozygous mutation in each unaffected parent indicated that they were carriers and confirmed the autosomal recessive inheritance of the disease. In family 2, the affected brother III-7 and sisters III-6 and III-8 were homozygotes for a c.1404delC mutation in exon 2 leading to a frameshift and a premature stop (His468fsX503) (Figure 1); DNA from affected

sister III-3 was not available. Unaffected brother III-2 and sisters III-1, III-4 and III-5 were either heterozygote carriers or had a wild type genotype (Figure 1). Both parents who were first cousins were heterozygote carriers. In family 3, only the DNA from the proband II-3 was available. We found that she was homozygotic for a c.1001delC mutation in exon 1 leading to a frameshift and a premature stop (Ala334fsX453) (Figure 1). Her parents were not known to being related but her affected sister II-2 made a consanguineous marriage with an unaffected male and had 3/6 affected children, suggesting that the proband inherited both alleles from a common ancestor. None of these mutations were present in 50 control chromosomes.

## DISCUSSION

We report here patients from three families showing cone dystrophy with supernormal rod ERG. They all carry mutations in *KCNV2*. Most patients from the 22 published families (including ours) have been found mutated in *KCNV2*<sup>11, 17</sup> except for one in which no mutation was found<sup>11</sup> and 2 for which only one allele was mutated<sup>11, 17</sup>.

All 4 mutations that we found presumably inactivate the function of *KCNV2* (Figure 4). Three of them are novel and truncate the protein either inside the N-terminal A and B boxes (Glu148Stop), before the 3<sup>rd</sup> transmembrane segment with the addition of 119 novel aminoacids (Ala334fsX453), or before the 6<sup>th</sup> transmembrane segment with the addition of 35 novel aminoacids (His468fsX503). The 4<sup>th</sup> mutation is recurrent and changes the 3<sup>rd</sup> aminoacid (Gly461Arg) of the highly conserved GlyTyrGly K<sup>+</sup> selective motif in the P loop, which forms with other subunits the pore of the channel<sup>13,14</sup>. To date, there have been 24 published mutations<sup>11, 17</sup>, of which 13 are truncating (with the 2 mutations p.Gly143X and p.Lys260X being recurrent) and 11 are amino acid changes (with the p.Gly461Arg mutation being reported in 5 families). Amino acid changes are found exclusively in the N-terminal fragment of the protein which includes the A and B boxes, and in the P-loop, indicating the importance of those regions for the function of *KCNV2*.

The description of the disease in our patients is well in accordance with what has been found before showing a slow and progressive degeneration of the macular area and a relatively mild impairment of visual acuity in young patients. A red-green axis of dyschromatopsia is frequently observed as usually found in cone dystrophies. Differences in the amplitude of the dark-adapted b-wave are remarkable. It is decreased at low levels of stimulation and increased at high levels of stimulation, implicating, as previously shown<sup>3</sup>, that there is an intermediate level where there is no difference. We found this point at 3.2 cd.s/m<sup>2</sup> (Table 3). However, the b-wave implicit time still remains highly augmented. Thus, the highly delayed b wave, even if the amplitude is normal, is a major criterion to recognize this condition.

The slow progression of the disease, the fact that the condition is probably linked to one gene with probably loss-of-function mutations, makes this disease an attractive choice for gene therapy. This will have to be tested on knock out animal models.

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The authors confirm that they are in compliance with their Institutional Review Boards (IRBs) as the Department of Ophthalmology of the Hospital of Montpellier has the authorization # 11018S from the French Ministry of Health for biomedical research in the field of physiology, pathophysiology, epidemiology and genetics in ophthalmology.

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## FIGURE CAPTIONS

**Figure 1 : Pedigrees and genotypic findings in 3 families segregating cone dystrophy with supernormal rod ERG.** In pedigrees, squares indicate males, circles indicate females, blackened symbols are affected individuals, and a double horizontal line between a mating pair indicates consanguinity. The mutation information (“+” means a wild-type allele) was indicated only for those individuals whose DNA samples were available for the study. Note that for family 2, delC corresponds to the His468fsX503 mutation. For each family, the electropherograms for the mutated (patient) and wild-type (normal) sequence are shown. The normal sequence is written in black italic and the mutated nucleotides are in red. The nucleotide mutation and the protein sequence are written on top of each electropherogram.

**Figure 2 : Fundus photographs, autofluorescence and OCT-3 scans in patients having cone dystrophy with supernormal rod ERG from families 1 and 3.** In both sisters III-2 and III-3 from family 1, the fundus shows a loss of the foveal reflex (left eye, OS) with a slightly decreased autofluorescence (AF) and with a thinned foveal photoreceptor layer at the OCT-3 scan (OCT). Fundus examination of both eyes (right and left eyes, OD and OS) of the proband II-3 and of her affected nephew III-2 from family 3 shows a foveo-macular atrophy that was more prominent in III-2.

**Figure 3 : ISCEV electroretinogram in patients having cone dystrophy with supernormal rod ERG from families 1, 2 and 3.** ERGs were recorded in affected members from families 1 (III-2, III-3), 2 (III-6, III-7) and 3 (II-2, III-2) and in non affected members (normal or carriers) from family 2 (II-1, III-4, III-5). In affected individuals, the low intensity response in dark adapted conditions ( $1 \text{ cd.s/m}^2$ ) is decreased and delayed while at higher intensities ( $101\text{-}318 \text{ cd.s/m}^2$ ) the response is supernormal. In light adapted conditions, the cone responses to single flash (cone) and to 30-Hz flickers are notably reduced in affected individuals compared to normal.

**Figure 4 : Schematic representation of the KCNV2 subunit of the  $\text{K}^+$  channel,** drawn from the *KCNV2* sequence deposited in GenBank # NM133497 and from the structure of Kv channels (reference 14), showing the N-terminal A and B boxes (NAB, in blue), the 6 transmembrane segments of the protein (S1-S6) and the P loop (in red) which forms the channel pore. Small numbers indicate the amino acid positions. The position and sequence of the 4 mutations found are indicated, in blue for the aminoacid change and in red for truncating mutations.