KCNV2-associated Retinopathy: Genetics, Electrophysiology and Clinical Course – KCNV2 Study Group Report 1,

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Abstract

**Purpose:** To investigate genetics, electrophysiology and clinical course of *KCNV2*-associated retinopathy in a cohort of children and adults.

**Study design:** Multicenter international clinical cohort study.

**Methods:** Review of clinical notes and molecular genetic testing. Full-field electroretinography (ERG) incorporating the international standards were reviewed and quantified and compared with age and recordings from control subjects.

**Results:** In total 230 disease-associated alleles were identified from 117 patients, corresponding to 75 different *KCNV2* variants, with 28 being novel. The mean age of onset was 3.9 years old. All patients were symptomatic before the age of 12 years (age range: 0-11 years). Decreased visual acuity was present in all patients, and four other symptoms were common: reduced color vision (78.6%), photophobia (53.5%), nystagmus (43.6%), and nystagmus (38.6%). After a mean follow of 8.4 years, the mean best corrected visual acuity (BCVA, ±SD) decreased from 0.81 LogMAR (0.27 LogMAR) to 0.90 LogMAR (0.31 LogMAR). Full-field ERGs showed pathognomonic waveform features. Quantitative assessment revealed a wide range of ERG amplitudes and peak times, with a mean rate of age-associated reduction indistinguishable from the control group. Mean amplitude reductions for the DA 0.01 ERG, DA 10 ERG a-wave, LA30Hz and LA3 ERG b-wave were 55%, 21%, 48% and 74% respectively. Peak times showed stability across 6 decades.

**Conclusion:** In *KCNV2*-retinopathy full-field ERGs are diagnostic, and consistent with largely stable peripheral retinal dysfunction. Report No.1 highlights the severity of the clinical phenotype and established a large cohort of patients, emphasizing the unmet need for trials of novel therapeutics.
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Introduction

*KCNV2*-associated retinopathy (OMIM #610356) was first described by Gouras et al in 1983 as cone dystrophy, with nyctalopia and supernormal rod responses.\(^1\) “Cone dystrophy with supernormal rod response” (CDSRR) was later linked to a 1.5 Mb region on chromosome 9p24, and variants in the *KCNV2* gene (OMIM #607604).\(^2\) *KCNV2* encodes a modulatory subunit (Kv8.2) of a voltage-gated potassium channel (Figure 1).\(^2\) *In situ* hybridization demonstrated *KCNV2* expression in human rod and cone photoreceptors.\(^2\) Abundant Kv8.2 (*KCNV2*) expression is also reported in the photoreceptor layer of the mouse retina.\(^3\) The Kv8.2 subunit interacts with different Kv2 channels in rods and cones, giving rise to potassium currents which shape the photoreceptor membrane potential.\(^4\)

*KCNV2*-retinopathy represents an uncommon autosomal recessive retinal disorder and a leading cause of inherited cone-rod dystrophy.\(^5\) Disease-causing variants in *KCNV2* are present in a substantial fraction (2-5%) of different cohorts with the broad clinical diagnoses of a cone dysfunction/dystrophy, suggesting that CDSRR may be underdiagnosed.\(^6\)-\(^8\) In total, we identified 114 cases described in the literature, in 22 studies and case reports.\(^9\) Clinically, *KCNV2* is characterized by variable age of onset, usually in infancy or early childhood, color vision defects (most commonly in the red-green axis), impaired adaptations to different light conditions, mild photophobia and nyctalopia.\(^10\)-\(^15\) In young patients clinical presentation can be variable, the most common presentation being abnormal head position, head shaking and nystagmus that improved with time.\(^10\) The photopic full-field electroretinogram (ERG) shows evidence of generalised cone system dysfunction, with scotopic ERGs revealing unusual rod system involvement, whereby responses to dim flashes are attenuated and markedly delayed, and ERG b-waves to strong flashes being relatively large, with a characteristic strong flash ERG waveform shape. The ERG abnormalities are pathognomonic; with directed molecular genetic testing confirming the diagnosis.\(^12\), \(^16\)-\(^22\) Many of the aforementioned findings are based on single reports and small cohorts, and questions about the ERG spectrum of the disease, stability over time and clinical presentation need further investigation, given the inherent variability in inherited retinal diseases.\(^5\), \(^23\)

Herein, we present the first report of a multicenter international collaborative retrospective cohort investigation of 117 individual adults and children with disease-causing variants in *KCNV2*. The current report provides a detailed description of the genetics, electrophysiology and clinical course of the disease. This information is of particular importance for improving genetic counselling and advice on prognosis, and provides a crucial step towards the design of a prospective natural history study and therapeutic clinical trial for *KCNV2*-retinopathy. The study also identifies a cohort of molecularly confirmed patients who may be suitable candidates for treatment, and further investigations of disease natural history.
Methods
The study protocol adhered to the tenets of the Declaration of Helsinki and received approval from all local ethics committees of the participating institutions. Informed consent was obtained from all adult subjects, whereas informed consent and assent were obtained from parents and children, respectively.

Patient Identification
Inclusion criteria for the current study were the molecular and/or phenotypic confirmation of KCNV2-associated retinopathy. A search was performed in the genetics database of Moorfields Eye Hospital, London, UK, the RetDis Biobank and database of the Center for Ophthalmology, University of Tübingen, Germany, and major referral centers across the globe were contacted for participation in the study.

Molecular Diagnosis
A combination of direct Sanger sequencing and next generation sequencing, including panels of retinal dystrophy genes, whole exome sequencing (WES) and whole genome sequencing (WGS), was used to identify variants in KCNV2, in the different referral centers. All recruited patients were reassessed for their detected variants. Minor allele frequency for the identified variants in the general population was assessed in the Genome Aggregation Database (gnomAD) datasets (http://gnomad.broadinstitute.org/). The Combined Annotation Dependent Depletion (CADD) score was calculated for all variants; a score greater than 15 is usually considered as mildly pathogenic and a score above 20 is strongly indicative. The deleterious annotation of genetic variants using neural networks (DANN) score was calculated for single nucleotide variants. A DANN score above 0.9 is considered suggestive of pathogenicity. The evolutionary conservation of the affected amino acid residues was evaluated with Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/). Classification of all detected variants was also performed based on the guidelines of the American College of Medical Genetics and Genomics (ACMG).

Clinical Assessment
All patients were seen by inherited retinal disease specialists at referral sites. Available clinical notes were reviewed, including: best corrected visual acuity (BCVA), refraction, fundoscopy and slit-lamp biomicroscopy findings. BCVA analysis was performed using the average of right and left eye and included cross-sectional and longitudinal analysis. Spherical equivalent was calculated for refractive errors. Mean myopic/hyperopic spherical equivalents for both eyes, were classified as: mild ≠0 diopters (D) to −/+3.0 D, moderate −/+3.0 D to −/+6.0 D, and high for −/+6.0 D or more.

Electrophysiological Assessment
The quantitative ERG analysis was restricted to recordings from a single referral centre (Moorfields Eye Hospital, London, UK), in order to avoid variability caused by different test protocols and/or use of different types of recording electrodes. Full-field ERG and pattern electroretinography (PERG) were performed using gold foil electrodes and incorporated the International Society for Clinical Electrophysiology of Vision standards (ISCEV), except in 4 young children who underwent ERG testing with skin
electrodes using modified protocols. Photopic On-Off ERGs were additionally recorded (stimulus duration 200ms) in the adults. The patient data were compared with ERGs from a control group of healthy subjects (age range 10 – 79 years) which included validated recordings for DA 0.01 (n = 117), DA 10.0 (n = 141), LA 3.0 30 Hz (n = 131), and the LA 3.0 (single flash cone) ERG (n = 109).

**Statistical Methods**
Statistical analysis was carried out using SPSS Statistics for Windows (Version 22.0, Armonk, NY: IBM Corp.). Significance for all statistical tests was set at P < 0.05. The Shapiro-Wilk test was used to test for normality for all variables.
Results

Demographics
In total, 117 patients with likely disease-causing variants in KCNV2 were ascertained for phenotyping, from 12 tertiary referral centres in 9 countries (UK, Germany, Spain, the Netherlands, UAE, Israel, Japan, USA and Canada). The cohort included 59 females (50.4%) and 58 males (49.6%). Data relating to 68 patients were previously reported in part, in at least one study (Supplementary Table 1). The baseline age and the follow-up time is indicated below on each individual assessment.

Molecular Genetics
Ninety-six families were identified. Twenty-one families contributed two patients each, with the remaining 75 patients being single cases. In 113 patients (96.6%), two KCNV2 disease-causing variants were identified: 52 (44.4%) patients were compound heterozygotes and 61 (52.1%) patients harboured homozygous variants. For four patients only one variant was identified (3.4%); however, since the patients were previously reported, and had a characteristic phenotype, they were included in the analysis. Supplementary Table 1 summarizes the molecular findings of each patient.

In total 230 disease-causing alleles were identified, corresponding to 75 different KCNV2 variants. Supplementary Table 2 presents all the variants identified in the cohort, their minor allele frequencies in the general population (Kaviar, gnomAD, Tommo, KRGDB, and HGVD databases) and their predicted effect. More than one third of the alleles (35.7%), presented as one of four recurrent variants: i) c.427G>T p.(Glu143*) (n=29, 12.61%), ii) c.1381G>A p.(Gly461 Arg) (n=26, 11.30%), iii) c.778A>T p.(Lys260*) (n=17, 7.39%), and iv) c.325C>T p.(Gln109*) (n=10, 4.34%). The aforementioned alterations were identified in 45 families (46.9%). The variant p.(Glu143*) was identified in 12 families from only two referral centers (Moorfields Eye Hospital, London, UK and Cleveland Clinic Abu Dhabi, Abu Dhabi, UAE), with 11 of the families being of Arabian Peninsula origin. The other three common variants were identified in various referral centers. Table 1 summarizes the most frequent KCNV2 variants, and Supplementary Table 2 presents the number of alleles identified and the frequency of all the alterations identified.

Out of 75 alterations identified, 28 (37.3%) were novel to the best of our knowledge (Supplementary Table 3). The majority of the variants (n=28, 37.3%), were missense, followed by nonsense (n=18, 24%) and frame-shifting indels (n=18, 24%). The missense variants identified in our cohort were not evenly distributed throughout the full length of the gene. Ten missense variants (35.7%) were clustered in the highly conserved tetramerization domain (N-terminal A and B box, NAB) that facilitates interaction between compatible Kv subunits. Interestingly, seven variants (25%) were identified in the extracellular link between the NAB and the first transmembrane domain (S1), and one (3.6%) in the intracellular part before the NAB. Only five variants (17.9%) were localized in the six transmembrane domains (S1-S6), and one variant (3.6%) in one of the intracellular loop segments. In the ultra-conserved potassium selective motif in the pore-forming loop between S5-S6 (P loop), four variants (14.3%) were identified, including the most common missense variant c.1381G>A p.(Gly461Arg). Other commonly encountered alleles also clustered within highly conserved domains (e.g.
c.427G>T p.(Glu143*) and c.325C>T p.(Gln109*) within the NAB domain. Figure 1 presents a graphical representation of the gene and its domains. Supplemental Figure 1 demonstrates the evolutionary conservation of the affected amino acid residues. Table 2 summarizes the variants by type and frequency.

Disease Onset
Age of onset was available for 95 patients, for 65 of them the age was recorded in years, and for 30 of them as 'infancy' (0-2 years old), 'early childhood' (3-8 years old) and 'middle childhood' (9-11 years old). Table 3 summarizes the age of onset by developmental stage for all 95 patients. The mean (±SD, median) age of onset for the patients with available age (n=65) was 3.9 years old (±3.0, 3 years). Twelve patients (18.5%) were symptomatic at birth. All patients were symptomatic before the age of 12 years (age range: 0-11 years). Age at baseline examination is detailed in the BCVA section.

Symptoms and Clinical Examination Findings
One hundred and one patients had recorded symptoms at disease onset. A universal finding was decreased VA (100%). The clinical presentation varied (Table 3) but common symptoms included; reduced color vision (78.6%), photophobia (53.5%) and nyctalopia (43.6%), with nystagmus occurring in a large proportion (38.6%). Twenty-nine patients (28.7%) had photophobia and nyctalopia, with or without other symptoms. Patients with nystagmus tended to be younger, and the nystagmus was observed to reduce with increasing age. Beyond the five common symptoms, five young children had strabismus, and three infants had head shaking.

On clinical examination, all patients had clear ocular media. On fundoscopy the peripheral retina was normal in most, including 4 with a tigroid appearance, but 4 patients showed subtle pigment mottling. Macular appearance was variable, being normal in a minority, but in most cases it ranged from an absent or reduced reflex to a bull's-eye appearance, and in advanced cases RPE hyperpigmentation and atrophic changes were common.

Visual Acuity
BCVA was assessed cross-sectionally and longitudinally. One hundred and two patients had BCVA available at one or more visits. None of the patients had any other vision limiting disease. The mean age (±SD, median, range) of the group was 22.1 years (±15.5, 18, 3-68 years), and their mean BCVA (±SD, median, range) was 0.83 LogMAR (±0.29, 0.88, 0.22-1.9 LogMAR) at baseline. Figure 2A presents all the available cross-sectional data. There was a weak statistically significant correlation between the mean BCVA for right and left eye, and the baseline age (r=0.20, P=0.04, Spearman). The weak correlation can reflect the early severe decrease in BCVA early in life and a further slow decline with age.

Seventy-five patients had available longitudinal data. The mean age (±SD) at baseline and follow up visit, was 19.5 years (±13.5 years) and 27.9 (±15.2 years) respectively. After a mean follow-up of 8.4 years (±SD, range; ±7.3, 1-31 years), the mean BCVA (±SD) decreased from 0.81 LogMAR (±0.27 LogMAR) to 0.90 LogMAR (±0.31 LogMAR). Figure 2B presents the longitudinal BCVA data. Despite the overlap
of the scatter baseline and follow-up data, the follow-up BCVA was significantly worse ($P = 0.005, z=2.78$, Wilcoxon Signed Rank matched-pairs test).

**Refraction**

Refraction data was available for 60 patients, with all of them being phakic, and all except one having a refractive error. The mean age of refraction ($\pm$SD, range, median) was 21.6 years ($\pm14.6, 3-58, 17.5$ years). The mean spherical equivalent was -3.57 D (range; -14.75 D to +2.75 D) in the right eye and -3.60 D (range, -16.25 to +3.75 D) in the left eye. The median spherical equivalent was -3.75 D and -3.19 D for the right and left eyes respectively. **Table 3** categorizes mean spherical equivalent of both eyes for all patients. High myopia (-6.0 D or more) was a common finding (31.7%).

**Electrophysiological Assessment**

ISCEV dark-adapted (DA) ERGs showed a pathognomonic combination of features in all subjects tested ($n=45$, age range: 4-59 years), and all had abnormal light-adapted (LA) ERGs. **Figure 3** presents a typical example and description of the features, in comparison to a normal control. Median peak times and amplitudes for standard ERG components (DA 0.01 ERG; DA 10 ERG a and b-waves; LA 30Hz ERG and LA 3 ERG a- and b-waves) were significantly different compared with the control group (**Table 4**). The mean peak time differences (delays) for the DA 0.01 ERG and DA 10 ERG a- and b-waves were 61ms, 12ms and 6ms respectively, and the mean delay in LA 30Hz ERG peak time was 9ms. The mean amplitude reductions for the DA 0.01 ERG, DA 10 ERG a-waves, LA 30Hz and LA 3 ERG a- and b-waves were 55%, 21%, 48%, 47% and 74% respectively; the DA 10 ERG b-waves showed a mean increase of 18% compared with the mean for the control group, but there was marked variation (range -24% to +70%).

The DA 0.01 ERG and DA 10 ERG a- and b-wave amplitudes tended to be larger in younger *KCNV2* cases, but there was wide variability and the mean rate of age-related decline was indistinguishable from that seen in the control group (**Figure 4A, 4C and 4E**). The DA ERG peak times were similar at all ages (**Figure 4B, 4D, and 4F**). The LA 30Hz and LA 3 ERG amplitudes and peak times showed no evidence of worsening with increasing age (**Figure 4G, 4H, 4I and 4J**).

Of the 4 young children tested using peri-orbital skin electrodes, noisy ERG recordings in the youngest (aged 4 years) were equivocal; in the others (aged 5, 5 and 10 years) the ERGs showed features consistent with *KCNV2*-retinopathy (**Supplementary Figure 2**).
Discussion
This international multicenter investigation explores the clinical phenotype and aspects of the natural history of KCNV2-retinopathy, in a large cohort of molecularly proven patients, over a wide range of ages. The findings of the current report (No.1) confirm the early onset of disease associated with severe visual impairment and pathognomonic ERG features, and suggest a high degree of functional stability over time. The study establishes a cohort of candidates that could potentially benefit from the development of novel therapeutics, such as gene replacement therapy, gene-editing and stem-cell based therapies.

Genetics and Disease Epidemiology
The genetic background of CDSRR is uniform and strictly associated with biallelic variants in KCNV2. Early genetic investigations - before the mapping of the disease locus - speculated upon an association with PDE6H variants. A later study (and the current report), supported a unique genotype-phenotype correlation related to KCNV2 variants, and PDE6H variants are now considered a rare cause of achromatopsia. All the patients in the current study had pathognomonic ERG findings and molecular confirmation of variants in KCNV2. The four patients with only one heterozygous variant identified were previously reported, and no further more sensitive methods for identification of the second variant have been performed so far. Robson et al published a cohort of 24 patients with 18 of them molecularly confirmed, subsequently a further five of them had genetic testing, with all yielding biallelic KCNV2 variants (Supplementary Table 1). The current study reported a further 28 novel KCNV2 variants which is a significant addition to the 95 previously reported variants.

With the recognition of the pathognomonic ERG phenotype, targeted KCNV2 screening is of high yield as previously suggested. In the same study KCNV2-retinopathy was identified as the second most common cause (11.3%) of paediatric inherited retinal disease (IRD) in an Emirati cohort of 71 patients. The increased prevalence of the disease in the aforementioned study may be a result of a founder effect. The variant c.427G>T p.(Glu143*) was the second most common in the cohort, with 11 of the 12 families identified being of Arabian Peninsula origin. The variant was also the most common variant in a previously reported Saudi cohort. KCNV2-retinopathy accounts for 0.7% and 0.25% of families with molecularly confirmed IRD in the United Kingdom and Germany, respectively. In the United States (US) disease frequency was calculated to 1/850,000 and an estimated yield of five new cases per year. We identified four variants (Table 1) which are involved in half of the affected families. KCNV2 study group highlights the worldwide distribution of the disease, without being able to account for its prevalence. RPE65-associated Leber congenital amaurosis (LCA), the disease with the first FDA approved adeno-associated virus (AAV) based-gene therapy treatment and a genotype investigated in detail, has an estimated frequency of 1/576,667, with an estimated seven new cases annually in the US. KCNV2 is a two exon gene encoding for 545 amino acids, that can thus also be accommodated in AAV vectors, making it an attractive target for gene therapy.
Clinical Presentation and Disease Course

*KCNV2*-retinopathy is a severe early onset disease. We identified *KCNV2*-retinopathy as a childhood onset disease, with more than half of the patients being symptomatic before the age of 3 years. One in ten patients was symptomatic at birth, with non-specific signs such as nystagmus and head shaking, which pose a diagnostic challenge given the similarity to cone dysfunction syndromes, including achromatopsia, and forms of LCA, or even non-retinal conditions, such as spasmus nutans when the fundus appearance is grossly normal, illustrating the importance of ERG testing and genetic screening for definitive diagnosis. Despite the lack of quantification, high frequency pendular nystagmus tends to decrease over the span of several years, as previously reported. The combination of photophobia and night vision difficulties was present in one third of our cohort and is an important diagnostic clue, that can help differentiate the disease from other cone dysfunction syndromes and early stage cone-rod dystrophies (not associated with night blindness). Light sensitivity and nyctalopia may be targeted for the development of relevant potential end-points for a future clinical trial. Photoaversion can be quantified, and nyctalopia can be tested with mobility assessments at different light levels.

BCVA was severely reduced in all patients from an early age (Figure 2A), and slowly worsened with age (Figure 2B). Halt or slowing of BCVA loss may not be ideal as a primary endpoint for clinical trials of short duration, given that the mean annual change of 0.01 LogMAR corresponds to one ETDRS letter every two years. Also, the severely reduced BCVA from early childhood (Figure 2), the early onset of disease and previously documented evidence of macular atrophy with increasing age, underline the need for early intervention. An interesting finding is the universal presentation of refractive error; no specific error was associated with the disease (Table 3), although over 30% had high myopia (-6.0 D or greater). The clinical characteristics have been previously described in smaller cohorts of 1 to 24 patients. We were able to establish the frequency of symptoms, elaborate on the age of onset and BCVA natural history in a much larger number of patients.

Electrophysiology

Full-field ERG findings are specific for the disease (Figure 3) in spite of variability in the absolute peak times and amplitudes of the main ERG components, as detailed in this study (Figure 4). In *KCNV2*-retinopathy, the scotopic dim flash ERG (DA 0.01) is delayed in all cases and is subnormal in the majority. The strong flash (DA10.0) ERG a-wave may be of mildly subnormal to normal amplitude and the b-wave is relatively large compared with dim flash responses; the b-wave amplitude often falls within the normal range, with a minority being abnormally large ("supernormal"). Comparison of the main ERG components with age over 6 decades shows a mean age-associated decline in amplitude at a rate similar to that in the unaffected control group (Figure 4). This is consistent with relatively stable peripheral retinal dysfunction and is in keeping with previous published evidence of non-progressive peripheral retinal dysfunction over 15 years, in spite of worsening macular atrophy.

All patients in the current cohort had detectable but abnormal LA ERGs indicative of generalised cone system dysfunction, including a child tested with skin electrodes (Supplementary Figure 1). A previous report of skin ERGs in a child highlighted the
possibility of unusually severe cone system dysfunction in association with homozygous deletion of \textit{KCNV2}.\textsuperscript{52} In contrast, Zobor et al did not observe a genotype functional phenotype-correlation in patients with no protein expression (n=3) or residual protein expression.\textsuperscript{19} Electrophysiological data from a cohort of ten patients were reported as being consistent with a post-phototransduction, but pre-inner nuclear layer, dysfunction.\textsuperscript{11} Another study (n=6) suggest altered function of the inner retina, based on the reduced oscillatory potentials.\textsuperscript{19} Stockman et al. psychophysically characterized the disease and suggested an intact phototransduction process.\textsuperscript{53}

\textbf{Limitations-Future Directions}

Our study has many strengths, including the size of the cohort, which is the largest to date evaluating \textit{KCNV2}-retinopathy, the age range of patients and that of the ERG control group, allowing age-associated evaluation of retinal function. In addition to clinical diagnosis and/or ERG, all included patients were molecularly confirmed with pathogenic variants in \textit{KCNV2}. The study includes patients from referral centers with wide geographic distribution, being less susceptible to selection bias of study population, and proving the benefits of international collaboration in rare diseases. Inherent limitations to the study relate to its retrospective nature; not all data were available for all patients and many of the available data were acquired by different protocols and methods (\textit{e.g.} ERG, genetic testing). The aforementioned limitations were ameliorated with, per protocol analysis of the collected data. Where no reporting protocol could be employed retrospectively for certain aspects of the clinical examination (\textit{e.g.} fundoscopy), the data were presented descriptively. ERG amplitude is reduced and peak time increased, with increasing myopia, and our cohort had a negative mean spherical equivalent. Case-control approach, with matching age and spherical equivalent, will be of value for comparing the responses of the patients to the responses of normal controls, in future prospective studies.

Report No.1 aimed to present the genetics, electrophysiology and clinical presentation. The genetic data in the current study provided a patient population that can be considered for future gene augmentation trials, insight into the disease genetic background and novel variants. Electrophysiology assessment further established the pathognomonic ERG phenotype in the context of phenotypic and age-associated variability, consistent with relatively stable peripheral retinal disease and suggesting a wide therapeutic window. The clinical presentation described further facilitates clinical diagnosis and highlights disease severity. The detailed investigation of the retinal phenotype and of structural meaningful end-points for future trials, were beyond the scope of Report 1. A wide range of fundus autofluorescence (FAF) abnormalities including ring-like or bull’s-eye changes, central atrophy, or increased foveal AF, have all been reported in the disease,\textsuperscript{11, 12, 45, 51} and OCT can show a variable degree of changes in the outer retina, ranging from ellipsoid zone disruption to diffuse outer retinal atrophy.\textsuperscript{10, 15, 22, 45, 54, 55} In the \textit{KCNV2} knock-out mouse, approximately 80% of cones are still intact by six months of age as compared to wild type, which if similar to humans, may allow for relatively late photoreceptor-directed treatment.\textsuperscript{56} However, further clinical and pre-clinical research, including prospective natural history studies, are needed to establish the optimal window for intervention, appropriate structural and functional (both retinal and visual) end-points to monitor both safety and efficacy, and identify
participants most likely to benefit. Report 2 will investigate longitudinal retinal imaging and end-points.

**Conclusions**
The current study is the first in-depth analysis and long term longitudinal study of *KCNV2*-associated retinopathy. Despite its retrospective nature, we recruited more patients than the total number of patients published in the literature, empowering our study to provide novel insights into disease natural history. This investigation (Report No.1) highlighted the early disease-onset, the severity of the clinical phenotype, the genetic background, the ERG stability, and established a cohort of patients with a wide geographic distribution, indicating the unmet need for trials of novel therapeutics.
Legends

**Figure 1: KCNV2 Protein and Domains**
The schematic diagram shows the *KCNV2* encoded protein structure, the alpha-subunit of the potassium channel (Kv8.2), and its domains. It consists of: (i) a highly conserved tetramerization domain; N-terminal A and B box (NAB); (ii) 6 transmembrane domains (S1-S6); (iii) extracellular and intracellular loop segments; and (iv) an ultra-conserved potassium selective motif in the pore-forming loop between S5-S6 (P loop). The distribution of the missense variants identified, are detailed in the results.

**Figure 2: Best Corrected Visual Acuity (BCVA) Assessment**
(A) Cross-sectional assessment of BCVA based on data from 101 patients. The dashed line marks the mean of the cohort (0.83 LogMAR). More than two-thirds of the patients (68.2%) had a BCVA between 0.54-1.12 LogMAR (shadowed area between lines marking ± standard deviation). (B) Longitudinal assessment of BCVA on data from 75 patients. The dashed lines mark the mean age and the mean BCVA at baseline; 19.5 years and 0.81 LogMAR, respectively. The continuous lines mark the mean age and mean BCVA at follow-up; 27.9 years and 0.90 LogMAR respectively. After a mean follow of 8.4 years, the mean BCVA (±SD) decreased by 0.09 LogMAR. The annual rate for the cohort was 0.011 LogMAR/year.

**Figure 3: Full-field ERG and Pattern ERG Recordings in a Case of KCNV2-retinopathy.**
The dark-adapted (DA) responses (top panels) show the pathognomonic features; to the dimmest flash the (DA 0.002) ERG was undetectable and the DA0.01 ERG delayed and subnormal. As flash strength increased up to 3 cd.s.m⁻² there was abnormal increased enlargement of the ERG. The DA10 (strong flash) ERG a-wave trough had a characteristic broad shape with a late negative component and the b-wave was disproportionately large relative to the attenuated dim flash responses. Light adapted 30Hz flicker (LA30Hz) and single flash cone (LA3) ERGs were delayed and subnormal (bottom panels). The photopic On-Off ERG (stimulus duration 200ms) showed a delayed and markedly reduced b-wave (an electronegative ON- response) and delay and mild reduction of the d-wave (the OFF-response). The S-cone ERG was simplified and reduced. The pattern ERG P50 component was undetectable in keeping with severe macular dysfunction, typical of the disorder. Representative control recordings from an unaffected individual are shown for comparison (N). All patient recordings showed a high degree of inter-ocular symmetry, are shown from one eye only, and are superimposed to demonstrate reproducibility, with the exception of the DA0.14 ERG (single recording). Broken lines replace eye movement artefacts seen after the b-waves for clarity. LE; left eye, DA; dark adapted, LA; light adapted, N; normal control, PERG; pattern ERG
Figure 4: Scatter Plots for Electrophysiological Parameters and Age.
The major full-field ERG component peak times and amplitudes in KCNV2 patients (filled circles) and unaffected control subjects (grey circles) are plotted against age. Data are shown for the DA0.01 ERG (A, B); DA10.0 ERG a- and b-waves (C, D, E, F); LA 30Hz ERG (G,H) and for the amplitude of the LA 3 ERG a- and b-waves (I, J). Regression lines are shown for the KCNV2-retinopathy (solid line) and control (grey broken line) data. See text for details. LE; left eye, DA; dark adapted, LA; light adapted, N; normal control, PERG; pattern ERG

Supplemental Figure 1: Multiple alignment of ten species of KCNV2
Evolutionary conservation of the affected amino acid residues in the KCNV2 gene was evaluated with Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) and the amino-acid-sequence alignment was numbered in accordance with the Homo sapiens KCNV2 sequence (ENSP00000371514). An asterisk indicates complete conservation across the ten species. The positions of variant residues are shown, and a highly conserved tetramerization domain; N-terminal A and B box (NAB) and an ultra-conserved potassium selective motif in the pore-forming loop between S5-S6 (P loop) are highlighted with boxes.

Supplemental Figure 2: Full-field ERG and Pattern ERG Recordings in a Paediatric Case of KCNV2-retinopathy.
Full-field ERG and pattern ERG (PERG) recordings obtained with lower eyelid skin recordings from the left eye of a child aged 5 years-old, showing diagnostic ERG waveforms. Recordings showed a high degree of inter-ocular symmetry. Representative control recordings from an unaffected child are shown for comparison (N). The PERG was recorded before mydriasis and was undetectable, in keeping with severe macular dysfunction. Note that in this case there is a 20ms pre-stimulus delay in the DA and LA single flash full-field ERGs. Patient traces are superimposed to demonstrate reproducibility. RE; right eye, LE; left eye, DA; dark adapted, LA; light adapted, N; normal control, PERG; pattern ERG
Financial Disclosures

Acknowledgement:
Michel Michaelides, Eberhart Zrenner, Susanne Kohl, and Camiel Boon are members of the European Reference Network for Rare Eye Diseases (ERN-EYE).
References

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<th>Variant</th>
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<th>Protein Effect</th>
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<th>Frequency (%)</th>
<th>Times Identified (n= )</th>
<th>Frequency (%)</th>
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<td><strong>46.9</strong></td>
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* reference sequence: NM_133497.4
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Table 3: Clinical Findings in Study-117

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<th>Age of Disease Onset (n=95)</th>
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<td>Infancy (birth to 2 years old),</td>
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<tr>
<td>Early childhood (3 to 8 years old)</td>
<td>45, 47.4%</td>
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<td>Middle childhood (9 to 11 years old)</td>
<td>25, 21.1%</td>
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<th>Common Symptoms and Findings (n=101)</th>
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<th>Frequency</th>
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<tbody>
<tr>
<td>Reduced BCVA</td>
<td>101, 100.0%</td>
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<tr>
<td>Reduced Color Vision*</td>
<td>55, 78.6%*</td>
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<tr>
<td>Photophobia</td>
<td>54, 53.5%</td>
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<tr>
<td>Nyctalopia</td>
<td>44, 43.6%</td>
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</tr>
<tr>
<td>Nystagmus</td>
<td>39, 38.6%</td>
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<td>Nyctalopia, Photophobia, Reduced Color Vision</td>
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<td>Reduced Color Vision and Photophobia</td>
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<tr>
<td>Reduced Color Vision</td>
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<tr>
<td>Nyctalopia and Photophobia</td>
<td>7, 6.9%</td>
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<td>Nystagmus and Nyctalopia</td>
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<tr>
<td>Nystagmus and Reduced Color Vision</td>
<td>5, 5.0%</td>
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<tr>
<td>Nyctalopia</td>
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<tr>
<td>Nystagmus and Photophobia</td>
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<tr>
<td>Nystagmus, Nyctalopia and Photophobia</td>
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<td>Photophobia</td>
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<td>Mild (0 D to −3.0 D)</td>
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<td>Moderate (−3.0 D to −6.0 D)</td>
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<td>High (−6.0 D or more)</td>
<td>19, 31.7%</td>
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<td>Hyperopic:</td>
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<tr>
<td>Mild (0 D to +3.0 D)</td>
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<td>Moderate (+3.0 D to +6.0 D)</td>
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<td>0 D Spherical Equivalent</td>
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*Only 70 of the 101 patients, had specific documented color vision test.

BCVA; best corrected visual acuity, D; diopters
Table 4: The amplitudes and peak times (median and percentiles) of the main full-field ERG components in a control group compared with KCNV2 retinopathy.

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<thead>
<tr>
<th>Stimulus</th>
<th>Component</th>
<th>Parameter</th>
<th>Control 5th</th>
<th>Control Median</th>
<th>Control 95th</th>
<th>KCNV2 5th</th>
<th>KCNV2 Median</th>
<th>KCNV2 95th</th>
<th>P Value</th>
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<tr>
<td>DA 0.01</td>
<td>b-wave</td>
<td>Amplitude</td>
<td>110</td>
<td>210</td>
<td>370</td>
<td>51</td>
<td>114</td>
<td>350</td>
<td>1.58 x 10^{-8} *</td>
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<tr>
<td></td>
<td></td>
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<td>77</td>
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<td>113</td>
<td>130</td>
<td>153</td>
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<td>319</td>
<td>435</td>
<td>166</td>
<td>271</td>
<td>365</td>
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<td></td>
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<td>9</td>
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<td>797</td>
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<td>25</td>
<td>50</td>
<td>85</td>
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Amplitudes are in microvolts (µV) and peak times are in milliseconds (ms). Statistical significance was established using Mann-Whitney U test.

*Statistically significant P value following Bonferroni correction

LA: light adapted, DA: dark adapted
Highlights

- The current study established the largest and most characterised cohort of molecularly confirmed patients with KCNV2-associated retinopathy.
- Report No.1 highlights the genetic background, evidence of ERG stability over a broad age range, and the severe phenotype of the disease.
Table 4: The amplitudes and peak times (median and percentiles) of the main full-field ERG components in a control group compared with KCNV2 retinopathy.

<table>
<thead>
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<th>Stimulus</th>
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Amplitudes are in microvolts (µV) and peak times are in milliseconds (ms). Statistical significance was established using Mann-Whitney U test.

*Statistically significant P value following Bonferroni correction

LA: light adapted, DA: dark adapted
**Figure 2**

**A**

Cross-sectional Visual Acuity Assessment (n=102)

![Cross-sectional Visual Acuity Assessment](Fig2_VA_KCNV2.tif)

**B**

Longitudinal Visual Acuity Assessment (n=75)

![Longitudinal Visual Acuity Assessment](Fig2_VA_KCNV2.tif)