High Symmetry of Visual Acuity and Visual Fields in RPGR-Linked Retinitis Pigmentosa

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PURPOSE. Mutations in retinitis pigmentosa GTPase regulator (RPGR) cause 70% to 90% of X-linked retinitis pigmentosa (XLRP) cases, making this gene a high-yield target for gene therapy. This study analyzed the utility of relevant clinical biomarkers to assess symmetry and rate of progression in XLRP.

METHODS. A retrospective, cross-sectional analysis of 50 XLRP3 patients extracted clinical data including visual acuity (VA), visual fields (IHe and IIIe targets), foveal thickness, and ERG data points alongside molecular genetic data. Symmetry was assessed by using linear regression analysis. Kaplan-Meier survival curves (KMCs) and generalized linear mixed model calculations were used to describe disease progression.

RESULTS. Ninety-six percent of patients exhibited a rod–cone phenotype, and 4% a cone–rod phenotype. Open reading frame 15 (ORF15) was confirmed as a mutational hotspot within RPGR harboring 73% of exonic mutations. Significant variability, but no clear genotype-phenotype relationship, could be shown between mutations located in exons 1–14 versus ORF15. All biomarkers suggested a high degree of symmetry between eyes but demonstrated different estimates of disease progression. VA and foveal thickness, followed by perimetry IIIe, were the most useful endpoints to evaluate progression. KMC estimates predicted a loss of 6/6 vision at a mean of 34 years (+2.9; 95% confidence interval).

CONCLUSIONS. XLRP3 affects retinal structure and function symmetrically, supporting the use of the fellow eye as an internal control in interventional trials. VA and kinetic visual fields (IIIe) seem promising functional outcome measures to assess disease progression. KMC analysis predicted the most severe decline in vision between the third and fourth decade of life.

Keywords: retinitis pigmentosa, retinitis pigmentosa GTPase regulator (RPGR), gene therapy, disease progression
presents as a rod–cone degeneration pattern, although a minority of patients can present with a cone–rod phenotype.\textsuperscript{24} Multiple previous reports\textsuperscript{1} find that patients with \textit{ORF15} mutations present with a cone–rod pattern of degeneration. This diversity can partly be explained through allelic heterogeneity, but the variability persists in patients with the same mutation\textsuperscript{22} and has even been described in (dizygotic) twins.\textsuperscript{25} Genetic modifiers have been proposed to account for this, and two specific single nucleotide polymorphism (SNP) changes may be associated with severe disease.\textsuperscript{22}

Owing to the high prevalence of \textit{RPGRORF15} mutations in XLRP, the gene represents a high-yield therapeutic target. Several promising preclinical trials to replace \textit{RPGRORF15} in animal models have already been published.\textsuperscript{1,24–26} To achieve optimal application when potential treatments are brought to clinical trials, it is necessary to thoroughly analyze patient cohorts to determine their pretreatment characteristics. This study aimed to answer three questions: (1) Is XLRP symmetrical between eyes and can the contralateral eye therefore be used as an internal control in an intervention trial? (2) Which parameter is the most sensitive and robust outcome measure to reliably detect treatment safety and/or efficacy? (3) And lastly, is it possible to characterize the dynamic of disease progression to determine the optimal therapeutic window?

**MATERIALS AND METHODS**

**Patient Characteristics**

In this retrospective, cross-sectional study, 100 eyes of 50 patients with XLRP resulting from mutations in the \textit{RPGR} gene were analyzed. Patients were referred to and seen at the University Eye Hospital Tübingen and the Oxford Eye Hospital between 2006–2015 and include all genetically confirmed \textit{RPGR} patients evaluated during this interval at both locations. A systematic review of both institutions databases was performed and data points pertaining to visual acuity (VA), visual fields, electroretinography (ERG), and foveal thickness were extracted. Patients were subdivided into rod–cone phenotype, cone–rod phenotype, or rod–cone phenotype with split fovea where the centrotemporal degeneration encroaches on the fovea. The study was performed in accordance with the tenets of the Declaration of Helsinki 1975 (1983 revision). Institutional review board approval was obtained for genetic testing.

**Molecular Assessment**

All genetically tested participants gave written consent, approved by the local research and ethical review boards. Genomic DNA was extracted from peripheral blood samples by using standard protocols. Genetic testing was performed at the Centre for Genetics and Transcriptomics (CeGaT GmbH), as well as the molecular genetics laboratories in Tübingen, Regensburg, and München. Research and diagnostic genetic set-ups in the aforementioned laboratories included single-strand conformation polymorphism analysis, high-resolution melting curve analysis, Sanger sequencing, and next-generation sequencing. Sanger sequencing of PCR-amplified genomic DNA confirmed mutations.\textsuperscript{5,20} Analysis extent and depth was variable, ranging from Sanger sequencing of only \textit{ORF15} of the \textit{RPGR} gene, analysis of all coding exons of \textit{RP2} and \textit{RPGR} (including \textit{ORF15}), as well as panel sequencing of all inherited retinal dystrophy genes.\textsuperscript{5,20,27} Mutations were categorized as missense, nonsense, insertions, deletions, gross deletions, and splice defects. Exonic mutations were further characterized by mutation location, with \textit{ORF15} mutations considered distinct from mutations occurring in exons 1–14.

**Clinical Examinations**

**Visual Acuity.** Measurement of VA was performed by using Snellen charts. If VA improved with pinhole, the pinhole-corrected data point was used. Decimal Snellen VA was converted to logMAR by using the formula $\text{logMAR} = -\log$ (decimal acuity).\textsuperscript{28}

**Visual Fields.** Visual fields were tested with semiautomated kinetic perimetry with an Octopus 900 or Goldmann perimeter (Haag-Streit, Koeniz, Switzerland) as described previously.\textsuperscript{29,30} The peripheral visual field boundary and blind spot were assessed by using I4e and III4e targets. For the patient cohort from Tübingen, the visual field area (degree)\textsuperscript{2} for I4e and III4e targets was calculated by using the proprietary software’s built-in measurement tool. The visual fields of Oxford patients were scanned and assessed with ImageJ (version 2.0.0–rc-30/1.49s; http://imagej.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA). The outlines of I4e and III4e targets were traced and the area was calculated in degree\textsuperscript{2}. Previous work has shown that both methods come to comparable conclusions,\textsuperscript{31} but to test comparability in our setting, one representative patient’s visual field area (degree)\textsuperscript{2} was calculated by using both methods. Only 1% difference in the calculated area (degree\textsuperscript{2}) between the two methodologies was found and thus the results of the methodologies were deemed comparable in our setting.

**Foveal Thickness.** To assess foveal point thickness, all patients in both locations were scanned with spectral-domain optical coherence tomography (SD-OCT) using the Spectralis HRA+OCT platform (Heidelberg Engineering, Heidelberg, Germany) with its follow-up mode as described previously.\textsuperscript{32} Point measurement of retinal thickness in the central fovea was done with the caliper tool. This reports the thickness of the outer nuclear layer combined with the inner and outer segment length, that is, the photoreceptor layer, and has been shown to correlate with VA in patients with central serous chorioretinopathy.\textsuperscript{33} Some evidence for a correlation between foveal point thickness and VA has also been reported for RP (Güven, et al. IOVS 2016;57:ARVO E-Abstract 0519). For SD-OCT, multiple high-speed B-scans were recorded to assess central retinal architecture and quantify thickness of the remaining neuroretinal tissue. To improve signal to noise ratio (SNR), $n \geq 9$ scans were averaged for each B-scan recording, whereby SNR improved by the square root of $n$. Foveal thickness (micrometers) was measured by using an in-built measurement tool of the Eye Explorer software (Heidelberg Engineering).

**Electroretinography.** Each patient’s ERG measurements were assessed by using Espion (Diagnosys LLC, Lowell, MA, USA) following International Society for Clinical Electrophysiology of Vision (ISCEV) standards.\textsuperscript{24} Amplitudes values of dark-adapted (DA) 0.01 cd’s, DA 3.0 cd’s, DA 10.0 cd’s, and light-adapted (LA) 3.0 cd’s single flash recordings and 30-Hz flicker were extracted.

**Statistical Analysis**

Bivariate correlation, histograms, generalized linear mixed model analysis, and Kaplan-Meier survival curves were created by using Statistical Package for Social Sciences (SPSS) version 21 by IBM (SPSS, Inc., Chicago, IL, USA) for Windows.

**Correlation Between Left and Right Eyes.** Normality of target values distribution was assessed from the first measurement data of each patient by using superimposition of normal distribution line on histograms. Spearman’s $p$ analysis was performed to quantify the correlation between left and right eyes in nonnormally distributed values, using the first measurement of each patient.
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Progression Rate Analysis. Owing to the large variety in both the number of follow-up measurements and the temporal spacing between repeated follow-up measurements, the progression rate was estimated by using generalized linear mixed model (GzLMM) analysis. Linear mixed models (LMMs) provide a framework for the analysis of longitudinal data with complex interactions and multilevel grouping of measurements and allow for the quantification of relationships between a continuous dependent variable and one or more predictor variables. Clustered data, such as left and right eyes of the same patients as well as repeated measurements, can be appropriately fitted, accounting for interactions due to data originating from the same subjects or groups. In contrast to repeated-measures analysis of variance, LMMs allow inclusion of more heterogenic data, which makes it possible to include patients with an unequal number of repeated measurements in the analysis. GzLMMs add an additional layer of flexibility by allowing analysis of binary, ordinal, and count variables as well as nonlinear relationship functions.

In the following GzLMM, patient number was used as the subject. The number of “years since first visit” value was calculated for each repeated measure to account for temporal differences in measure repeats and was used in the model as “repeated measure” variable. The fixed effects were set to include the intercept and the “patient age at visit,” the latter specifying the age of the patient at the day of the follow-up measurement. The patient number was also used as a random effect. When left and right eyes were highly correlated, only the data from left eyes were used as target variable in order to simplify the model. Both linear model and gamma regression were assessed as link functions and the best-fitting mixed model was selected on the basis of the lowest information criteria values (Akaike corrected and Bayesian). When both link functions produced equal results, the model with more significant coefficients (lower P value) was preferred. Mixed model residuals distribution was assessed for normality as secondary measure of model suitability.

Vision Survival Analysis. Kaplan-Meier survival curve analysis was used to estimate cumulative survival of 6/6 vision, reading ability (defined as <6/15), and legal blindness (defined as <6/60) at different ages. Owing to wide variation in frequency of patient visits, all analyses were done by using both the complete set of patient visits as well as one visit per patient in order to avoid patients with many visits skewing the results. Both results are noted throughout the article.

Results

Patient Cohort Characterization

The cohort was comprised of 50 male patients with a clinical and genetically confirmed diagnosis of XLRP3, ranging in age from 7 to 69 years. Between 1 and 17 visits were recorded per patient. Sixteen patients were seen only once; 34 patients were seen between 2 and 17 times. Of patients with a follow-up appointment, the average number of follow-up visits was 2.4 per patient (median = 1 visit). Follow-up time ranged from 2 to 72 months, with an average of 22 months in between visits (median = 14 months). Perimetry and ERG analysis were used to distinguish between the rod-cone phenotype, seen in most cases, and the much less frequent cone-rod phenotype.23 Forty-seven patients presented with a rod-cone phenotype, two with a cone-rod phenotype, and one patient showed a rod-cone phenotype with split fovea. The two patients presenting with a cone-rod phenotype had a deletion (c.2405_2406delAG; p.E802Gfs*32) and a nonsense mutation (c.2689G>T; p.E897*) in ORF15, while the patient with the rod-cone phenotype with split fovea was hemizygous for c.3077_3080delAG; p.E1026Gfs*62. Though these numbers were not sufficient for a subgroup analysis, different phenotypes were color coded throughout analysis.

Eighteen percent of our patients were 20 years or younger, and in this cohort 44% were 10 years or younger at the age of onset. Of the nine patients who presented before the age of 20 years, five had mutations in exons 1–14 and four had ORF15 mutations. Of patients who presented when younger than 10 years, three had a mutation in exons 1–14 and one had an ORF15 mutation. None of these patients aged 20 years or younger were related and all were carriers of a different mutation.

Molecular Assessment

All patients were genetically analyzed, and mutations in RPGR were identified before this study (Fig. 1A; Table 1). The 50-patient RPGR-XLRP cohort comprised 41 different mutations, 19 of which were novel. Two mutations, c.2405_2406delAG and c.2256_2257delGA, occurred seven and four times, respectively. Even though both deletions occur within highly repetitive clusters of A and G repeats, the sequence following either deletion is not identical to the deleted repeats, making the alignment between the shifted nucleotides suboptimal.

Location of Mutations. Seventy-one percent of exonic mutations (i.e., excluding splice mutations and gross deletions) were present on exon ORF15, even though the number of ORF15 nucleotides account for only 49% of the exonic RPGR sequence (Figs. 1B, 1C). In the present patient cohort, this clustering of mutations on ORF15 was even more pronounced than previously noted.35,20 Missense mutations made up 20% of the total mutations and were clustered to the first half of the RPGR gene, with 80% occurring in exons 1 to 14, and only 20% in ORF15 (Figs. 1B–D). Conversely, deletions, which represented by far the largest percentage of total mutations, occurred in the C-terminus of the exonic RPGR sequence in 85% of cases and were observed in the N-terminal half in only 15% of cases (Figs. 1B–D). This trend has been reported previously.35,36 With insertions, deletions (in/dels), and duplications clustered in the repetitive, purine (A/G)-rich regions of ORF15, and other substitutions, such as missense mutations, occurring predominantly in the first 14 exons of the gene.

Correlation of Location With Severity of Phenotype.

To understand the impact of mutation location on phenotype, VA and perimetry (III4e target intensity) of patients with mutations in exons 1–14 were contrasted with VA and perimetry of patients with ORF15 mutations. Data of right and left eye are dependent upon one another, so use of both right and left eye data points might have skewed the data distribution. Since analysis showed high symmetry between both eyes for VA and perimetry, only the right eye data set was used. Analysis was done by using one visit per patient. Significant variability, but no significant difference, in VA or perimetry could be shown between mutations located in exons 1–14 and mutations on ORF15 (VA P = 0.90, perimetry P = 0.58) (Supplementary Fig. S1). These results were confirmed by the analysis of a selected age group (30–40 years) to control for difference in progression as a potential confounder. Since the two patients with cone-rod phenotypes were not shown to be outliers in disease progression (Fig. 3), they were included in the before mentioned analysis (see below).

Analysis of Disease Symmetry Between Eyes

The functional endpoint foveal thickness indicated the least robust degree of symmetry (ρ = 0.75, n = 35, P < 0.001). In contrast, the effect of degeneration on central visual function was highly symmetrical between right and left eye (ρ = 0.85, n
Perimetry showed an even higher degree of symmetry using isopter III4e ($q = 0.96, n = 38, P < 0.001$, Fig. 2C). Correlation of isopter I4e values was even higher ($q = 0.97, n = 30, P < 0.001$), yet likely represents an overestimation, as 40% of I4e measurements were (close to) zero in both eyes, hence creating a floor effect. As objective measure of retinal function, ERG b-wave amplitudes were symmetrical overall, with the highest symmetry without confounding floor effect found in DA single-flash responses following a 3 cd*s stimulus ($q = 0.98, n = 32, P < 0.001$; Fig. 2D) and in 30-Hz flicker (Spearman’s $q = 0.95, n = 32, P < 0.001$). Noteworthily, patients with cone–rod phenotype or rod–cone phenotype with split fovea did not present as outliers in symmetry analysis.

Analysis of Disease Progression

When analyzing the same structural and functional endpoints regarding their value in assessing disease progression, VA (Fig. 3A) and foveal thickness (Fig. 3B) proved to be the most useful parameters. Their relationship to age is best described by a natural logarithmic function, with logarithmic progression rates as reported in Table 2. Assessing disease progression by perimetry (isopter III4e) only makes sense within the first two decades of life, as the progressive visual field loss is (near-)complete as early as age 20 to 25 years (Fig. 3C; Supplementary Table S1). This creates a floor effect and results in limited discriminatory power for any efficacy analysis. ERG data (Fig. 3D; Supplementary Table S1) show no correlation with age. Again, patients with cone–rod phenotype or rod–cone phenotype with split fovea did not present as outliers in disease progression regardless of the outcome measure.

Subgroup Analysis of Disease Progression

To determine whether a homogenous disease progression in patients with the same mutations could be shown, two subgroups of patients carrying the same mutation were analyzed.

**RPGR Mutation c.2405_2406delAG.** The first subgroup comprised seven patients carrying the c.2204_2205delAG; p.E802Gfs*52 mutation (Supplementary Fig. S2). Patient No. 49 and patient No. 50 were second-degree relatives. Despite the identical mutation, no correlation of disease progression with age could be shown. The variability was further underscored...
by the cone–rod phenotype of patient No. 11, whereas the other six patients exhibited a rod–cone phenotype.

**RPGR Mutation c.2236_2237delGA.** The second subgroup comprises four patients with the c.2236_2237delGA, p.E746Rfs*23 mutation (Supplementary Fig. S5). In contrast to the c.2405_2406delAG mutation, c.2236_2237delGA showed a homogenous disease progression when looking at VA ($R^2 = 0.58$, $n = 4$, $P = 0.11$) and foveal thickness ($R^2 = 0.96$, $n = 4$, $P =$...
ERG endpoints exhibited $\rho$ values ranging from 0.50 (DA 3.0 cd*s) to 0.81 (LA 3.0 cd*s). $R^2$ for 30-Hz flicker was 0.79 ($n = 4$, $P < 0.01$). In perimetry, neither target (I4e or III4e) showed a homogenous disease progression. This is the result of patient No. 48 being an outlier in the I4e target perimetry (III4e not available), and patient No. 7 showing a relatively well-preserved III4e target perimetry.

**Kaplan-Meier Survival Curve**

Loss of VA is perhaps the most relevant outcome measure for patients. To estimate the decline of VA despite large phenotypic variability between patients, a Kaplan-Meier survival curve was calculated (Fig. 4) by using three cutoff points: loss of 6/6 vision (0.0 logMAR), loss of reading ability (0.4 logMAR), and a drop of vision under the limit for legal blindness (1.0 logMAR). Data from right and left eyes were calculated separately and again showed high degree of symmetry.

The most severe loss of VA was predicted to occur in the third and fourth decade of life. At age 20 years, over 80% of patients retained 6/6 vision and over 90% of patients were predicted to retain their ability to read. By age 40 years over 20% of patients were anticipated to be legally blind, with over 50% predicted to have lost their ability to read, and only 30% to retain 6/6 vision. In general, the mean estimated survival time for 6/6 vision was 34 years ($\pm 2.9$; 95% confidence interval), with a loss of reading ability occurring at 39 years ($\pm 2.6$) and reaching the limit for legal blindness at 48 ($\pm 1.6$) years.

**DISCUSSION**

The severity of XLRP due to RPGR mutations is evident in the early onset, with patients presenting as early as 7 years of age and nine patients presenting before the age of 20 years. Of the four patients who presented while under the age of 10 years, three had mutations in exons 1–14, which might hint at a trend of mutations in exons 1–14 presenting at a younger age, but the statistical power is not reliable owing to the small sample size.

ORF15 was confirmed as a sequence particularly vulnerable for mutation in RPGR, with 73% of mutations occurring in this terminal exon. However, ORF15 mutations did not demon-
strate a different degree of severity or phenotypic pattern (rod–cone versus cone–rod dystrophy) than mutations occurring in exons 1–14. These results differ from those of Sharon et al. 6 and Fahim et al. 22 who have demonstrated a milder disease phenotype in patients with ORF15 mutations than in patients with mutations in the N-terminal RCC1-like domain. It has been speculated that such a genotype/phenotype correlation could be explained by residual function of the truncated protein, since nonsense mutations in the terminal exon (ORF15) are not expected to undergo nonsense-mediated mRNA decay. 6,37 On the other hand, Hong et al.38 have shown a gain-of-function mutation in ORF15 that causes more severe disease progres-
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Massof et al.,40 who have analyzed VA and perimetry (V4e) of 60 genetically unclassified RP patients, report a high rate of bilateral symmetry in their probably genetically heterogeneous patient cohort. Of the patients who were diagnosed by clinical observation that the assumption of symmetry holds true for disease progression in XLRP3. 45–47 Birch et al.45 have demonstrated that loss of EZ width at the transitional zone between healthy and diseased retina correlates to loss of visual field in XLRP patients. EZ width has a low repeat variability, is not subject to floor effects in the assessment of disease progression, and is an anatomic measure that reflects the functional outcome of field sensitivity.

As subgroup analysis shows, a uniform disease progression or phenotype cannot be guaranteed even in patients carrying the same mutation. The conflicting evidence on variability in cohorts carrying the same mutation may be due to low statistical power of small cohorts and/or to unknown genetic modifiers and environmental circumstances influencing disease progression. Fahim et al.22 have found two SNPs that are associated with variable severity of retinal genetic disease.22 A continued search for potential genetic modifiers might help explain variability of genotype–phenotype relationship and eventually help to predict prognosis for individual patients. Since disease progression between patients can differ so greatly from each other, an interindividual control cannot be reliable. This again emphasizes the value of an intraindividual control in the form of the contralateral eye in an interventional trial, given of course, that the intervention is not applied systemically.

Kaplan-Meier survival curves showed the most prominent decline in VA throughout the third and fourth decade of life. The median age to reach legal blindness in our patient cohort
was compatible with the results of Sandberg et al. who calculate a median survival age of 45 years for XLRP3 patients. In a clinical trial in which efficacy is defined as lack of progression, patients should be selected before or at the beginning of this rapid rate of decline. On the other hand, a clinical trial designed to show efficacy in form of VA gain might need to choose patients toward the end or after the period of sharp decline. Beltran et al. recently have shown in a canine dog model of XLRP3 that treatment during intermediate stage of disease is able to substantially and significantly slow and arrest the progression of disease, a finding that potentially broadens the therapeutic window of intervention in patients with XLRP3.

In summary, our results indicated that degeneration between eyes is symmetrical, and therefore the contralateral eye can be used as an internal control in an interventional trial. Sensitivity was the greatest when using VA and foveal thickness as outcome measures. Perimetry with III-4e may be a useful endpoint in younger patients with less advanced stage of disease. Even individual mutations showed variability in disease progression and presenting phenotype (rod-cone versus cone-rod). Contrary to previous analysis, mutation location in or outside of ORF15 could not be correlated with severity of phenotype. Kaplan-Meier analysis showed the strongest decline in VA during the third and fourth decade of life. The median age for patients to reach legal blindness was 48 years. A prospective observational trial of genetically confirmed XLRP3 patients, using standardized outcome measures such as perimetry, multifocal ERG, OCT, and VA, would be needed to further explore the utility of these endpoints for efficacy studies.

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**References**