Progression in X-linked Retinitis Pigmentosa Due to ORF15-RPGR Mutations: Assessment of Localized Vision Changes Over 2 Years

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PURPOSE. To determine the progression rate and the variability of rod and cone sensitivities in patients with X-linked retinitis pigmentosa (XLRP) caused by mutations in ORF15-RPGR.

METHODS. ORF15-RPGR-XLRP patients (n = 15) were studied prospectively over 2 years with static perimetry sampling the visual field under dark-adapted and light-adapted conditions on a 12° square grid covering 168° width and 84° height. Natural history of rod and cone sensitivity loss and test-retest variability were estimated. Data were analyzed pointwise as well as averaged across small regions of neighboring loci of approximately 80 mm² (900 deg²) in size representing the likely extent of localized gene therapy injections.

RESULTS. Retinal loci with mild to moderate loss of sensitivity tended to be in the mid- to far-peripheral retina in most patients. When averaged across small regions, dark-adapted rod vision progressed at an average of 2 dB per year with a coefficient of repeatability (CR) of 6.3 dB, and light-adapted cone vision with white stimulus progressed at an average of 0.9 dB per year with a CR of 3.8 dB. For an average patient enrolled in an early-phase clinical trial, significant (α = 0.05) progression would be predicted to occur with 80% power in 4.5 years for rod vision and 6.1 years for cone vision. Localization of regions in the temporal hemifield and grouping of results from multiple patients would permit trial designs of shorter duration.

CONCLUSIONS. Measurement of rod sensitivity under dark-adapted conditions averaged across a small region showed the greatest potential for detectability of progression in the shortest period.

Keywords: coefficient of repeatability, cone sensitivity loss, natural history, progression rate, retinal degeneration, rod sensitivity loss, test-retest variability

Treatments for inherited retinal diseases (IRDs) are being considered along different strategic paths involving cellular, genetic, and optogenetic therapies, pharmacological compounds, and electronic implants.1-3 The aims of the different treatments vary depending on the pathophysiology of the retinal disease, the mechanism of action of the intervention, its route of delivery, and the severity of the condition at the time of intervention.4 Beyond the primary safety assessment, the overarching goals of experimental interventions are either “better vision” involving improvement of an aspect of visual function in the short term, or “retained vision” resulting in a positive change to the natural trajectory of visual function loss in the long term. Hypothetically, treatments could aim to tackle both goals in rare IRDs demonstrating a complex disease with both dysfunction and degeneration components, such as the RPE65 form of Leber congenital amaurosis (LCA)5; however, evidence from gene therapy results suggest modification of the natural history of degeneration will be challenging.6,7

One of the important outcome measures relevant to visual function of many IRDs is computerized perimetry, which estimates topography of light sensitivity for rods and cones.8-10 Large-magnitude localized improvements in visual function occurring over the short term have already been demonstrated with perimetric outcomes in RPE65-LCA patients undergoing gene therapy.6,7,11 In most other IRDs with a goal of modifying the loss of vision, the magnitude of changes is expected to be smaller and occur over longer periods of time while strongly interacting with a variable natural history of disease. Reliable detection of such small and slow changes, and differentiation of the effects of an intervention from the variability of the natural history of disease, remains challenging.

A common IRD is X-linked retinitis pigmentosa (XLRP) caused by ORF15 mutations in the RPGR gene, which accounts for approximately 60% of RPGR mutations.12-15 Preclinical studies of gene augmentation therapy in RPGR-mutant dogs and mice have shown efficacy.16-20 and early-phase clinical trials have already started (NCT03116113, NCT03252847, and NCT03316560 at clinicaltrials.gov). Perimetry is one of the likely outcome measures to evaluate changes in visual function corresponding to extracentral retinal regions to be treated with gene therapy in ORF15-RPGR-XLRP.15 Perimetric changes may occur in the short term corresponding to improvements (efficacy) or decrements (toxicity) in sensitivity, or in the long term due to the natural history of disease, or both. Statistical
Informed consent was obtained. The study was approved by the institutional review board. Patients were a subset of a larger group of 70 prospectively enrolled into a 2-year natural history study (Table 1). Results from the standard visual field grid (120° width by 84° height) were used in the current work to allow comparison. Included were all eyes with ultra-wide visual field testing at the first visit were considered. This approach also helped minimize the effects of scattered light under dark-adapted testing conditions. Additional data with white (W, broadband) stimuli were available to estimate dark-adapted sensitivity losses (DASL-W) in retinal regions with severe vision loss (defined as RSL-B > 50 dB); photoreceptor mediation at these loci was indeterminate.

### Perimetry Test Protocols

For each patient, perimetry with the five different test conditions was assessed at two separate visits with a median follow-up period of 1.9 years (range 1.5–2.1 years; Table 1). Both eyes of most patients (14 of 15) were tested sequentially. At the first visit, sensitivity estimates were obtained on a 12° grid of 102 loci distributed across the visual field of 168° width by 84° height.\(^8\) The ultra-wide visual field extent sampled provides a better understanding of the retained function in the mid- and far-periphery in ORF15-RPGR-XLRP.\(^{15\text{*}}\) Evaluation of central function at higher spatial resolution\(^{22}\) was not included. To avoid fatigue, visual field testing was partitioned into three grids (Supplementary Fig. S1A). Results from the standard visual field grid (120° horizontal extent) were combined with additional nasal and temporal patterns (24° horizontal extent each) to produce the composite ultra-wide visual field map of 168° width. A 24° eccentric fixation target was used to test the nasal and temporal patterns. In addition, the subject’s head was rotated approximately 20° nasally when testing the nasal field to avoid the near blocking the inferonasal stimuli. Most eyes had reduced visual acuities but retained foveal fixation (Supplementary Table S1) as determined by optical coherence tomography before visual field testing; P48/F32 was the only exception with eccentric fixation. Subject fixation during visual field testing was monitored via the infrared video imaging. Normal data were obtained using the nonoverlapping grid pattern (Supplementary Fig. S1A).

At the second visit of the natural history study, composite ultra-wide visual field testing was slightly modified with the incorporation of built-in test-retest sampling (Supplementary Fig. S1B, bold squares). This efficient approach was developed to avoid repeating all testing within the same visit, which would be prohibitive due to the prolonged testing time required. Instead, there were 12 overlapping points chosen at retinal locations with high likelihood of retaining measurable rod and cone function\(^{15\text{*}}\); six loci were in the temporal far-peripheral field along a crescent-shaped arc at an eccentricity of approximately 70°, and another six in the nasal mid-peripheral field at an eccentricity of approximately 50°.

### Table 1. ORF15-RPGR-XLRP Patients

<table>
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<tr>
<th>Patient/Family*</th>
<th>Age, y</th>
<th>Follow-up, y</th>
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<tbody>
<tr>
<td>P1/F1</td>
<td>24</td>
<td>1.9</td>
</tr>
<tr>
<td>P6/F4†</td>
<td>18</td>
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</tr>
<tr>
<td>P10/F6</td>
<td>28</td>
<td>1.8</td>
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<tr>
<td>P11/F7†</td>
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<td>P62/F37</td>
<td>19</td>
<td>1.9</td>
</tr>
<tr>
<td>P69/F44</td>
<td>42</td>
<td>2.0</td>
</tr>
<tr>
<td>P70/F45</td>
<td>34</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* Patient/Family identifiers as previously published in Charng et al.\(^{15}\).
† No variability data in both eyes.
‡ Progression only in right eye, variability in both eyes.

In all patients, static threshold perimetry was performed in fully dilated eyes under dark- and light-adapted conditions using the modified Humphrey field analyzer (Carl Zeiss Meditec, Dublin, CA, USA).\(^8,15,21,22\) Dark-adapted test conditions included monochromatic blue (B, 500 nm) and red (R, 650 nm) stimuli (Goldmann V size, 200 ms duration) used to determine photoreceptor mediation. For loci demonstrating rod mediation with the B stimulus, rod sensitivity loss (RSL-B) was obtained by subtraction of the B sensitivity from mean normal value at each locus. To minimize floor effects, only loci with mild to moderate rod sensitivity loss (defined as RSL-B ≤ 30 dB) at the first visit were considered. This approach also helped minimize the effects of scattered light under dark-adapted testing conditions. Additional data with white (W, broadband) stimuli were available to estimate dark-adapted sensitivity losses (DASL-W) in retinal regions with severe vision loss (defined as RSL-B > 50 dB); photoreceptor mediation at these loci was indeterminate.

Light-adapted testing was performed with orange (O, 600 nm) and white (W, broadband) stimuli presented on a 10 cd.m\(^{-2}\) white background. Both stimuli are considered to provide an estimate of the local cone sensitivity loss (CSL-O or CSL-W) under light-adapted conditions; however, there are advantages and disadvantages with each stimulus. The W stimulus has the advantage of providing 12.6 dB greater dynamic range, thus allowing measurement of sensitivity losses at retinal loci with greater severity of disease compared with that available with the O stimulus. The disadvantage of the W stimulus is the potential for rod intrusion previously demonstrated in primary cone diseases.\(^{23,24}\) Similar to rod function, to minimize floor effects, only loci with mild to moderate cone sensitivity loss (defined as CSL-O and CSL-W ≤ 15 dB) at the first visit were considered.
combined (C) across the whole (composite) visual field. Linear mixed-effects models were used to assess variability and progression. All analyses were done using decibel (dB) values. Random effects terms for subject and eye (nested), and location were included in the models to account for the correlation structure within the data. Variability was quantified using a coefficient of repeatability (CR), calculated as $1.96 \times r$, where $r$ is the SD of the difference in sensitivity loss at the overlapping crescent locations; differences were obtained by subtracting the standard grid values from the peripheral grid values. For progression, annual rates of change were obtained from two visits approximately 2 years apart.

RESULTS

Ultra-Wide Visual Testing Across Time With Multiple Conditions

Right eye of P70/F45 at age 34 is a representative example of baseline results on enrollment in the 2-year natural history study (Fig. 1A). In the central approximately 60-degree-diameter region covering the macular and perimacular regions, there is severe loss of rod sensitivity. Milder sensitivity losses are distributed across the mid- and far-peripheral visual field and show a range of RSL-B values from nearly normal to larger losses (Fig. 1A, left). CSL-O and CSL-W values are similarly distributed across the central, mid- and far-peripheral fields, and show a range of losses in cone-based day vision (Fig. 1A, middle and right). The mean difference between CSL-O and CSL-W is 0.7 dB, which suggests that both test conditions comparably probe cone function in this eye.

When P70/F45 was evaluated again 2 years later (Fig. 1B), RSL-B values showed an average increase of 1.9 dB, supporting a tendency for a small deterioration; at individual loci there were a range of changes from $-0.5$ dB (improvement) to $+18$ dB (deterioration) (Fig. 1C, left). CSL-O and CSL-W values are similarly distributed across the central, mid- and far-peripheral fields, and show a range of losses in cone-based day vision (Fig. 1A, middle and right). The mean difference between CSL-O and CSL-W is 0.7 dB, which suggests that both test conditions comparably probe cone function in this eye.

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Progressive Loss of Rod- and Cone-mediated Sensitivities

Natural history of disease progression was evaluated within small retinal regions similar in size to those to be treated with localized gene therapy. First, in each of 29 tested eyes of 15
patients, regions with six neighboring samples corresponding to a retinal area of approximately 80 mm² (900 deg²) were selected. There were four regions selected in each visual field quadrant of each eye (representative examples are shown in Supplementary Fig. S2A). The regions were chosen to be as close to the central retina as possible (more likely to be reached surgically) while retaining at least three samples with mild to moderate vision loss. Topographic distribution of retinal regions chosen across all eyes (Supplementary Fig. S2B) tended to follow the distribution of retained function in mid- to far-peripheral retina.

Over the 2-year follow-up period (median 1.9, range 1.5–2.1 years), pointwise analysis of all loci within the selected regions showed annual progression rates of 2.1, 0.7, and 1.0 dB per year for RSL-B, CSL-O, and CSL-W, respectively (Fig. 2A, Table 2). With regional analysis, where sensitivity losses were averaged across each region, the progression rates were similar to pointwise values (Fig. 2B, Table 2) and interocular differences were not significant for the three conditions (P = 0.5, 0.6, and 0.5 for RSL-B, CSL-O, and CSL-W, respectively). Rates in the temporal hemifield regions tended to be smaller than the rates in the nasal hemifield (Table 2).

Test-Retest Variability of Rod- and Cone-mediated Sensitivities
Interpreting the significance of changes in sensitivity loss over time requires the understanding of test-retest statistics. Across the cohort, test-retest differences were available from 26 eyes of 13 patients (Table 1) and were quantified with the CR estimated across individual testing loci (pointwise) or averaged across neighboring loci (regional). The test-retest differences did not show significant bias (P > 0.1) for any of the conditions tested.

Pointwise CRs were 9.6, 5.1, and 4.7 dB for RSL-B, CSL-O, and CSL-W, respectively (Fig. 3A, Table 2). Regional CRs were smaller (6.3, 3.8, and 3.8 dB for RSL-B, CSL-O, and CSL-W, respectively) than pointwise CRs for all the test conditions (Fig. 3B, Table 2), as expected. There was a tendency for greater variability in the nasal hemifield compared with the temporal hemifield under most conditions, both with pointwise and regional analyses (Table 2).

Implications for the Duration of Clinical Trials Involving Localized Interventions
Outcomes of localized interventions, such as subretinal gene therapy, may depend on comparison of the treated region to an untreated control region (selected intraretinally or contralaterally or both) either in individual patients or small cohorts. If the aim of the treatment is to locally modify the natural history of progression, an important consideration is the observation duration that will be necessary to detect significant change with a chosen method in a patient with a specific retinal disease. For perimetric measures, the estimates of progression and test-retest variability presented here can be used to design

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Two-year progression rates of rod and cone function loss in ORF15-RPGR-XLRP. (A, B) Estimated progression rates of RSL-B, CSL-O, and CSL-W as a function of sensitivity loss measured at first visit. Thin gray lines depict the reference of no progression, and thick black lines show the mean progression rates. Short lines to the right of each graph indicate the progression rates of nasal (N) and temporal (T) hemifields, and combined (C) results. The letters are placed next to each other in instances in which the progression rates were similar. Data are from selected regions in four quadrants either evaluated pointwise (A) or as regional averages (B). Gray circles = N, unfilled triangles = T.
### Table 2

Coefficients of Repeatability (CR), Progression Rates, and Observation Durations

<table>
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<th>Metric</th>
<th>CR and Progression Rates</th>
<th>Observation Duration†</th>
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<tr>
<td></td>
<td>C</td>
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<tr>
<td>RSL-B</td>
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<td></td>
<td>CR, dB (95% CI)</td>
<td>Rate, dB/y</td>
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<tr>
<td>CNT</td>
<td>9.6 (8.7–12.3)</td>
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</tr>
<tr>
<td></td>
<td>11.7 (9.8–15.7)</td>
<td>2.9 (1.3–4.6)</td>
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<tr>
<td></td>
<td>8.2 (7.1–11.1)</td>
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<td>Pointwise</td>
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<td></td>
<td>CR, dB (95% CI)</td>
<td>Rate, dB/y</td>
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<tr>
<td>CNT</td>
<td>5.1 (4.6–6.8)</td>
<td>0.7 (0.2–1.1)</td>
</tr>
<tr>
<td></td>
<td>6.5 (5.6–11.4)</td>
<td>0.8 (0.4–1.3)</td>
</tr>
<tr>
<td></td>
<td>4.4 (3.9–5.3)</td>
<td>0.6 (0.2–1.1)</td>
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<td></td>
<td>4.6 (4.0–5.6)</td>
<td>0.9 (0.4–1.4)</td>
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</table>

C, combined; N, nasal field; T, temporal field.

* Mean (95% confidence interval).
† In years; calculated using 80% power, \( \alpha = 0.05 \).

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**Figure 3.** Test-retest variability of rod and cone function in ORF15-RPGR/XLRP. (A, B) Bland-Altman plots summarizing pointwise (A) and regional (B) test-retest differences as a function of mean loss for RSL-B (left), CSL-O (middle), and CSL-W (right). Brackets define the CR estimates for nasal (N), temporal (T), and combined (C) visual field locations. Gray circles = N, unfilled triangles = T.
treatment trials. To illustrate the relative performance of the different metrics discussed, Table 2 shows the observation durations required for the simplest case of an average subject being followed up, with 95% chance of false-negative conclusions (α = 0.05) and 80% chance of detecting true-positive results (power = 80%). Using the RSL-B metric on regional averages, the required duration is 4.5 years to detect significant disease progression. The estimate is somewhat shorter in the temporal field (4.0 years) as compared with the nasal field (4.8 years) (Table 2). For the cone sensitivity loss metrics, longer observation periods would be required (Table 2).

Consideration of Retinal Regions With Severe Vision Loss

Our main estimates of progression and variability were limited to retinal loci demonstrating mild or moderate vision loss mediated by rods or cones to minimize floor effects. However, early-phase clinical trials will likely treat, at least initially, retinal regions with severe vision loss until the safety of the intervention is demonstrated. Evaluation and interpretation of severe vision loss is more challenging and requires larger dynamic range to avoid floor effects. Currently, the largest available dynamic range in perimetry is with the use of white stimuli under dark-adapted conditions; this approach has already been used by our group as an outcome measure in a gene therapy clinical trial.6,7,11

In all ORF15-RPGR-XLRP eyes of the current study, we also recorded dark-adapted sensitivity losses with white stimulus (DASL-W). As exemplified by the right eye of P6/F4, mild and moderate RSL-B values were found in the mid- and far-peripheral field (Fig. 4A, left). Vast expanse of the visual field with greater severity of disease showed DASL-W values ranging from 30 to 59 dB at the first visit (Fig. 4A, left). Two years later, DASL-W range had increased from 32 to 64 dB and the average increase in DASL-W was 7.1 dB.

Test-retest variability of the DASL-W evaluated across all patients in the mid- and far-peripheral crescents resulted in a regional CR of 16.9 dB (Fig. 4B), which was larger than the CR values for RSL-B. Next, two regions consisting of six neighboring samples in the perimacular inferonasal and inferotemporal regions were chosen as likely destinations of gene therapy interventions in early-phase trials (Fig. 4A, bold rectangles). The average DASL-W of these two regions across all eyes at the first visit was 40.1 dB, which was consistent with severe disease. Over a 2-year period, average DASL-W progression rate was 3.0 dB per year (Fig. 4C), which was comparable to RSL-B results.

**DISCUSSION**

Dysfunction of rod-photoreceptor–based night vision in many inherited photoreceptor diseases results from the reduction of the number of opsin molecules available to absorb photons.27–33 This reduction in “photon catch” typically occurs due to the degeneration (loss) of a subset of rod photoreceptors and shortening of outer segments in surviving photoreceptors: two processes that tend to co-occur through a mechanism currently not well understood.34–37 Changes in photon catch would predictably correspond to changes in absolute sensitivity to detect dim lights in the dark, assuming all else (e.g., phototransduction and post-phototransduction signaling) being equal. Computerized static perimetry performed under dark-adapted conditions is one of the most practical methods that provides a topographical map of absolute sensitivity across the retina, and thus can provide an

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**FIGURE 4.** Estimation of sensitivity in retinal regions with severe disease stages. (A) In a representative patient P6/F4 at ages 18 and 20, comparison of loci with mild and moderate sensitivity loss estimated with RSL-B (gray numbers) compared with the loci with severe disease probed with DASL-W (black numbers). Bold rectangles illustrate perimacular inferior field regions chosen as likely destinations of subretinal surgery. (B) Test-retest variability of DASL-W obtained in mid-peripheral nasal and far-peripheral temporal field crescents as a function of average value. Brackets define the CR estimates for nasal (N), temporal (T), and combined (C) visual field locations. (C) Estimated progression rates of DASL-W regions as a function of sensitivity loss measured at first visit. Thin gray line depicts the reference of no progression, and thick black line shows the mean progression rate. Progression rates of nasal, temporal, and combined regions are indicated to the right as short black lines. Gray circles = N, unfilled triangles = T.
Rod and Cone Sensitivity in XLRP

Progression of Disease

For each retinal locus sampled with evidence of rod function, the mean progression rate in ORF15-RPGR-XLRP was approximately 2 dB per year. There is very limited literature using comparable methods. USH2A-associated retinal degeneration (USH2A-RD) eyes have previously shown a slower progression rate of 2.1 dB per year in the central visual field, whereas ABCA4-associated STGD eyes have shown a slower progression rate of 1.1 dB per year in the peripheral visual field. In retinitis pigmentosa (RP) patients without a specific molecular diagnosis, rod function measured with a single large stimulus in a cohort of XLRP patients with ORF15 mutations in the RPGR gene to help design clinical treatment trials as well as interpret the results. Importantly, we used a single visit to obtain variability estimates, and two visits separated by a relatively short duration of 2 years to obtain progression estimates for an average patient.

Variability of Sensitivity Loss

Multiple measurements of light sensitivity in the same eye and at the same retinal location will often be unequal even when the time interval between measurements is short enough to avoid the consequences of retinal disease progression. Components of this fluctuation could include technical/physical changes, learning effects (including maturation for young subjects), and short- and long-term variability. In the current report, all data were acquired on the same equipment with the same stimuli and background conditions, and identical thresholding algorithms; thus, contributions from technical/physical changes can be assumed to be minor or insignificant. In addition, all subjects have previously performed other perimetry and were mature enough to perform the testing reliably; thus, it is assumed that a learning effect is also minor or insignificant. Consistent with the second assumption was lack of bias in test-retest differences. Among the remaining components of fluctuation is short-term (also called intratest) variability, which refers to the differences in sensitivity estimates occurring over a short time interval (on the scale of seconds) resulting from the statistics of the frequency-of-seeing functions. Long-term (also called intertest) variability on the other hand refers to fluctuations of attention, changes to yes-no criteria used, level of tiredness, and other random factors over a longer time interval (on the scale of hours to days). The overall CR values estimated in the current work would be expected to have contributions from both short- and long-term variability.

Pointwise CR values for ORF15-RPGR-XLRP rods averaged 9.6 dB in the current study and this was larger than 6.8 dB reported previously in a group of RP patients without a specific molecular diagnosis. The latter work tested a single point in the inferior central visual field, whereas the current study tested dozens of points mostly located in the mid- and far-peripheral visual field, which likely contributed to the greater pointwise variability. The regional CR value for rods of 6.3 dB reported here was similar to the variability of 5.5 dB reported across the average of a wide visual field, but larger than the 3.0 dB reported for the average across a small central region. These results taken together support the hypothesis that averaging across regions reduces the variability, whereas testing of more peripheral loci and greater number of loci increases the variability. For localized interventions, such as gene therapy (typically directed to superior paramacular retina), all else being equal, multiple neighboring retinal locations with RSL-B values between 10 and 20 dB would be expected to provide the ideal conditions for the detection of improvement or toxicity in the short term, and progression of disease in the long term.

For cone function in our ORF15-RPGR-XLRP patients, pointwise CR values averaged 4.7 dB for CSL-W (Table 2). In a comparable study in a group of healthy subjects, CR values were approximately 5 dB in the peripheral visual field. Earlier work in the central visual field of healthy subjects and rare inclusion of patients with pathological changes found CR estimates of 5.0 dB, 6.6 dB, and a range between 3.2 and 12.2 dB. In a study with RP patients, CR values within the central visual field were found to vary with the severity of vision loss and ranged from approximately 5 to 12 dB; in the current study, however, a relationship between CR and severity of vision loss was not apparent (Fig. 3A). Similar to the argument above, for localized interventions, such as gene therapy, multiple neighboring retinal locations with CSL values between 5 and 15 dB would be expected to provide the ideal conditions for the detection of improvement or toxicity in the short term, and progression of disease in the long term.

Outcome Selection for Subretinal Gene Therapy Trials

The progression rates and CRs estimated in the current work based on a prospective 2-year natural history in ORF15-RPGR-
XLRP patients are likely to be useful for the selection of outcome measures for subretinal gene therapy trials, several of which are currently under way. First, our results suggest that averaging of sensitivities sampled across an 80-mm² (900-deg²) retinal region provides important reduction of variability. Second, for the detection of statistically significant vision changes in the short term, outcome measures with the smallest CRs are desirable. Retinal loci in the inferotemporal visual field (superonasal retina) would combine the relatively greater use of inferior visual field for everyday functional vision with the smallest regional CRs recorded for rod and cone measures in the central vision but with slow rod kinetics. Proc Retin Eye Res. 2015;48:62–81.

References
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