Intervisit Variability of Visual Parameters in Leber Congenital Amaurosis Caused by RPE65 Mutations

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Purpose. To determine the intervisit variability of kinetic visual fields and visual acuity in patients with Leber congenital amaurosis (LCA) caused by mutations in the RPE65 (Retinal Pigment Epithelium–specific protein 65kDa) gene.

Methods. RPE65-LCA patients (n = 20; ages 11–40 years) were studied at least two visits separated by fewer than 120 days using Goldmann visual field (GVF) and ETDRS visual acuity (VA) in a retrospective review. GVF were quantified by computing the spherical coordinates of their vertices and calculating the solid angle subtended, and reported in normalized solid-angle units (nsu) as a percentage of average normalized field extent. Repeatability coefficients were calculated using 95% confidence intervals on log10-converted variables.

Results. Visual field extents in RPE65-LCA spanned a wide range from 4 to 95 nsu. The repeatability coefficient was 0.248 (log10(nsu)), suggesting cutoffs for significant change (in nsu) of +77% for improvement and −44% for worsening. VA in RPE65-LCA ranged from logMAR = 0.14 to 1.96 (20/40 to 20/1250). The repeatability coefficient was 0.170 (logMAR) (≥8.5 ETDRS letters). Comparisons with published studies of ungenotyped retinitis pigmentosa showed that the RPE65-LCA patients had higher variability in kinetic field extent. VA variability in RPE65-LCA fell within reported results for retinitis pigmentosa.

Conclusions. Variability data for GVF and VA are provided to permit interpretation of the significance of increases and decreases of these functional outcomes in ongoing and planned clinical trials of therapy for LCA caused by RPE65 mutations. (Invest Ophthalmol Vis Sci. 2013;54:1378–1383) DOI:10.1167/iovs.12-11341

Inherited retinal degenerations have recently become targets for novel treatment strategies using various methods.1 Leber congenital amaurosis (LCA) caused by mutations in the RPE65 (Retinal Pigment Epithelium–specific protein 65kDa) gene is one of the first retinal disorders to be approached with novel therapies, and patients with RPE65-LCA are taking part in several ongoing clinical trials. Gene augmentation therapy (reviewed by Cideciyan2) and the use of an oral retinoid (ClinicalTrials.gov Identifier: NCT01014052) are two therapeutic strategies currently being evaluated.

Traditional outcome measures to determine safety and efficacy in ocular clinical trials are visual acuity and visual fields.3 Knowledge of the intervisit variability of these outcome measures is key to evaluating the safety and efficacy of treatment. Intervisit variability of these parameters has been reported in retinitis pigmentosa (RP), a molecularly and phenotypically heterogeneous group of retinal degenerations,4,5 and there are differences in approach and in results.6–9 Studies have been reported in LCA, or specifically RPE65-LCA, even though visual acuity (VA) and kinetic visual fields are included as outcome measures in many of the ongoing clinical trials of RPE65-LCA.

We studied a cohort of patients with RPE65-LCA to quantify intervisit variability of kinetic visual fields and VAs. The results should enable trials that use these parameters as outcomes to better interpret their results and to determine the significance of changes measured.

Subjects and Methods

Subjects

All procedures followed the Declaration of Helsinki and the study was approved by the University of Pennsylvania’s institutional review board. Informed consent, assent, and parental permission were obtained. There were 20 patients (ages 11–40, mean 22.1 years; 13 female, 7 male) with a clinical diagnosis of LCA and with RPE65 mutations.10–13 The patients underwent clinical eye examinations. All patients had nystagmus but to different degrees. Two patients had visual field extents smaller that 2% of normal mean and were excluded from the visual field analyses. Repeat visual acuity data were not available in one patient and another had only light perception (LP) vision; these two patients were not included in the visual acuity analysis study. The study was a retrospective review, and all RPE65-LCA patients were included who had repeated measurements within a 120-day interval (i.e., no greater interval than 120 days for Goldmann kinetic visual field [GVF] and 85 days for VA). Inclusion was not based on motivation or experience. Although the rate of progression in RPE65-LCA is believed to be slow, there are currently no published data on natural history of the disease by these metrics. This study assumes the lack of detectable effect of disease progression over a 120-day interval.

Kinetic Perimetry

GVFs were performed by one of the authors (SGJ) using the V-4e target under standard conditions on the same calibrated perimeter, and
quantified as reported previously. The extent of visual islands and scotomas were obtained by manually tracing the isopter polygons on scanned images of the perimeter charts, computing the spherical coordinates of their vertices and calculating the solid angle subtended. This spherical mapping removes the area distortion due to the perimeter projection on a flat surface. Extents for visual field islands were added, and those for scotomas were subtracted. In a previous study, we obtained a mean (SD) solid angle of 3.23 (0.17) steradians in a group of 22 normal subjects; the measurements were made by the same investigator as in the present study. As in our previous studies, GVF extent in patients is reported as a percentage of the mean normal visual field. These GVF extent units are called normalized solid-angle units (nsu) for brevity and convenience, and log-converted for consistency and comparability with the previous literature.

In RPE65-LCA patients, two or three measurements of GVF extent were performed on different days, with intervisit intervals ranging from 1 to 118 days (mean, 43 days; n = 5 patients with two visits, n = 13 patients with three visits).

VA
Best-corrected VA was measured using Early Treatment Diabetic Retinopathy Study (ETDRS) methodology, scored as the number of letters correctly read after adjusting for distance, and expressed as logMAR of the minimum angle of resolution (logMAR). In two of the patients, a chart distance of 0.5 m was used to extend the measurable acuity range to 20/1600. Acuity was measured on several visits (range 2 to 16; mean 12 visits), done on different days with intervisit intervals ranging from 3 to 85 days (mean 63 days). Ambient and chart luminance were checked routinely per manufacturer requirements.

Data Analysis
The 95% repeatability coefficients for GVF extent and VA were calculated as 1.96 \( \sqrt{\frac{\text{SD}^2}{n}} \), where \( \text{SD} \) is the within-person SD obtained by ANOVA using log10-converted data from multiple visits. Data from one eye per patient were used for the analysis, selecting the eye with better VA at the earliest visit or randomly if acuities were the same. It is to be noted that the term, repeatability coefficient, in this study refers to inter- and not intravisit data.

RESULTS
Visual Field and VA Variability in RPE65-LCA
Visual field extents for our cohort of RPE65-LCA patients spanned a wide range (4 to 95 nsu). These GVF extents are displayed graphically and ranked by the average of their visits (Fig. 1A, top). Pairs of GVF charts are shown for four representative patients whose ages were (left to right, Fig. 1A, middle) 27, 23, 18, and 27 years, respectively. The patterns of the field abnormalities were different among patients: there are examples of central and peripheral islands separated by midperipheral scotomas, central island only, altitudinal and midperipheral defects, and a full field. There was no obvious relation between age and visual field extent. When measured at repeated visits, different changes in polygon shape for the isopters were observed. Islands could appear to expand or contract, scotomas could merge, and residual bridges of function between visual islands appeared or disappeared. There was no uniform pattern of change within the cohort studied. Patients with greater differences between visits could have either increases or decreases in apparent extent. There are examples of both directions of change at different levels of overall dysfunction and at different ages.

The coefficient of repeatability was determined from log10-converted GVF values. Intervisit fluctuation is shown (Fig. 1B) as a function of mean extent among visits. The repeatability coefficient was 0.248 (log 10nsu) (Fig. 1B, dashed lines). This is equivalent to a 1/\( \sqrt{1 - 0.248} \) factor (−44%, +77%) in GVF extent in nsu. The 95% confidence interval for the repeatability coefficient was 0.19 to 0.31 (log10nsu).

VA measures for all visits are ranked by mean acuity (Fig. 2A). Our cohort of RPE65-LCA patients had a mean VA ranging from logMAR = 0.14 to 1.96 (20/40–20/1250). The individual measures fluctuated within a range of approximately logMAR = 0.2 (10 letters) around the measurement at the middlemost visit (Fig. 2B). The repeatability coefficient was logMAR = 0.170, which is equivalent to approximately ±8.5 letters using ETDRS methodology (Fig. 2C). The 95% confidence interval for the repeatability coefficient was 0.15 to 0.19 (logMAR).
examiner 2), averaging ±0.183 (log10 nsu). In another study that analyzed GVE, there was a 0.252 change limit using a 99% confidence interval on visual field equivalent circular diameter obtained from areas quantified with a planimeter after conversion to natural logarithms (n = 28 RP patients, V-4e target). For comparison with the present work, this number can be converted to a 95% interval, to areas, and to common logarithms by successively applying factors of 0.7609, 2, and \ln(10)\(^{-1}\) respectively (with normality assumption). This results in limits for significant change of ±0.167 (log10). In a more recent study, a different estimate of repeatability was used and limits of ±56.3% on the original variable (n = 28 RP patients, V-4e target) were reported. For comparison with this study’s results, the log-converted variable in the other reports can be expressed in percentage change on the original variable, resulting in −34% to +55% for one study, and −32% to +47% for another study. There was no strong dependence on mean VA (r = 0.138, P = 0.59). Next, we postulated that fixation instability (nystagmus) may contribute to the measured variability of GVE. Fixation instability was quantified under visualization of the fundus.27

Were there any obvious reasons for the higher variability in our RPE65-LCA patients compared to the reports of variability in RP patients? We first postulated that VA may have been more impaired in those with greater kinetic field variability, possibly due to instability of fixation during the studies. The relationship between GVF variability and average VA was plotted and there was no strong dependence on mean VA (r = 0.59). Next, we postulated that fixation instability (nystagmus) may contribute to the measured variability of GVE. Fixation instability was quantified under visualization of the fundus.27

The magnitude of fixation instability was inversely related to VA as previously published.11 There was a weak tendency for the eyes with greater fixation instability to show greater GVF variability (r = 0.26).

Comparison with Intervisit Variability of VA in Studies of RP

Variability of VA in RPE65-LCA can also be compared with published work that studied VA in patients with RP (Table). As a reference, there is a report that lists the limits in 50 normal subjects with better than 20/30 VA as ±0.110 (logMAR) or ±5.5 letters.28 For an RP cohort (n = 32), one study7 calculated ±0.235 limits using natural log conversion and a 99% interval. This corresponds to ±0.078 (logMAR) (±3.9 letters) after conversion to common logs and a 95% interval (normality withstand). Another study of a retinal degeneration patient cohort (n = 29) that included patients with RP or CRD (cone-rod dystrophy) reported ±0.212 (logMAR) limits or ±10.6 ETDRS letters.8 The interval of ±0.170 (logMAR) or ±8.5 letters obtained in the present study for RPE65-LCA patients falls between those obtained for these two studies and is greater than reported variability for the normal subjects.28

Relationship of Kinetic Perimetry Results in RPE65-LCA to Those in Studies of RP

The intervisit variability for GVF and VA estimated for RPE65-LCA can be viewed in the context of those values previously reported in populations of RP that used similar methodology (Table). In one study of GVF, individual test-retest data were reported such that the repeatability coefficient can be directly calculated. Data in a cohort of 17 patients with RP (Ross et al., Table 6, II-4e target) for OD and OS tested by two examiners result in limits of ±0.193 (OD, examiner 1), ±0.155 (OS, examiner 1), ±0.173 (OD, examiner 2), and ±0.212 (OS,
studies, there was also reliance on the time-honored methods of kinetic perimetry and VA 32–34 (Koenekoop RK, et al. IOVS 2012;53:ARVO E-Abstract 4642). Intervisit variability data may not have been defined specifically for RPE65-LCA because there was an assumption that a number of previous studies in unengotyped RP would serve the purpose.6–9 Within the RP groups studied previously, there were some complexities of assessing variability across patients. For example, in a recent study that aimed to quantify GVF variability in RP,9 patients were separated by high and low levels of variability using a classification limit. There has even been a recommendation that individual assessments (for VA) of variability be done to address across-patient heterogeneity.35 Attempts have been made to explain the sources of these patient-specific differences, including mean value of the variable, operator training, and psychosocial factors.36 Such predictive factors could eventually be used to preclassify the patients and obtain more specific variability limits. The need of a preclassification step, however, introduces another uncertainty factor: the classification criteria must be relevant and consistent across the whole interval of interest, which can span years for slowly progressing diseases.

The present method to assess variability has been frequently used for several visual function metrics, including VA and GVF extent.7,8 In the present study, as in others, we calculated a repeatability coefficient to obtain limits for significant change. This analysis predicts that the difference of the measures taken in two different sessions has a 95% probability of being within the limits for significant change, if the difference was only due to chance. The degree of variability in VA for our cohort of patients with RPE65-LCA (0.170 logMAR) falls between the results of the two RP studies in the Table.7,8 For GVF, however, coefficients in the RP cohorts were smaller and outside the confidence interval of the one calculated for the RPE65-LCA patients in the present study (Table; 0.248 log10nsu), suggesting that claims of positive change in GVF from interventions must be made with caution in this disease.

Caveats about Comparisons with Previous Studies
It has been valuable to try to relate the results from the present study to those of earlier work, even if RPE65-LCA is a different disorder than what has been referred to as typical RP, which is itself not a single disease entity.4,5 We acknowledge that there are factors in the earlier studies and in the present work that make them dissimilar and our attempted comparisons are thus imperfect. For example, two studies of kinetic fields used planar areas on the GVF chart, while another estimated spherical retinal areas using a model eye, and the present study uses visual field solid angles. Even after normalizing to an average normal field extent, there are distortions associated with each method causing central and peripheral areas to be weighted differently.16 The composition of RP patient cohorts

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**Table. Summary and Comparison of Visual Field and VA Variability Studies**

<table>
<thead>
<tr>
<th>Goldmann Visual Field Study</th>
<th>Diagnosis</th>
<th>Computation by VF extent*</th>
<th>Coef. of Repeatability† (log10 nsu)</th>
<th>Method, Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ross et al., 1984 (17, 13-68, 2)</td>
<td>RP</td>
<td>17 33 50 67 83</td>
<td>± 0.183 -34% to +53%</td>
<td>2D area (chart), II-4e</td>
</tr>
<tr>
<td>Berson et al., 1985 (28, 6-49, 2)</td>
<td>RP</td>
<td>17 33 50 67 83</td>
<td>± 0.167 -32% to +47%</td>
<td>2D area (chart), V-4e</td>
</tr>
<tr>
<td>Biltner et al., 2011 (28, 19-76, 2)</td>
<td>RP</td>
<td>N/A §</td>
<td>±36.3% +17.7%</td>
<td>3D area (retina), V-4e</td>
</tr>
<tr>
<td>This study (18, 11-40, 2-3 #)</td>
<td>RPE65-LCA</td>
<td>17 33 50 67 83</td>
<td>± 0.248 -44% to +77%</td>
<td>3D area (visual field), V-4e</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Visual Acuity Study</th>
<th>Diagnosis</th>
<th>VA range</th>
<th>Coef. of Repeatability† (logMAR)</th>
<th>ETDRS letters</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berson et al., 1985 (32, 6-49, 2)</td>
<td>RP</td>
<td>20/20 to 20/200</td>
<td>± 0.078 ± 3.9</td>
<td>Snellen chart</td>
<td></td>
</tr>
<tr>
<td>Birch et al., 1999 (29, 5-61, 2)</td>
<td>RP, CRD</td>
<td>20/40 to 20/125</td>
<td>± 0.212 ± 10.6</td>
<td>ETDRS chart</td>
<td></td>
</tr>
<tr>
<td>This study (18, 11-40, 2-16**)</td>
<td>RPE65-LCA</td>
<td>20/25 to 20/1800</td>
<td>± 0.170 ± 8.5</td>
<td>ETDRS chart</td>
<td></td>
</tr>
<tr>
<td>Roser et al., 2003 (50, &lt;50, 2)</td>
<td>Normal subjects</td>
<td>better than 20/30</td>
<td>±0.110 ± 5.5</td>
<td>ETDRS chart</td>
<td></td>
</tr>
</tbody>
</table>

N/A, not available; RP, retinitis pigmentosa; CRD, cone-rod dystrophy
* Extent units normalized to mean normal, using normative data as included in each study.
† Coefficients of repeatability (95% confidence limits for determining significant change, using log-converted variables).
‡ Includes patients with higher variability
§ Number of patients excluded from the comparison:
# Number of patients = 13 for 15 patients, 2 for the remaining three.
** Number of repetitions: avg=12, range 2 to 16.
in terms of extent of visual field is also heterogeneous (Table). For example, the cohort in one study has most subjects with extents less than or equal to 17% of normal (using normal mean GVF extent of 741 mm²); in another study, most patients have extents ranging from 50% to 83% of normal; and a further study and the present work include patients with a wider range of GVF extents (Table, bar diagrams). Dissimilar methodologies in studies of VA should also be considered. For example, the ETDRS scoring method of counting letters implies some interpolation and may result in lower variability estimates than when using a direct assignment of the line logMAR value.

**Implications of the Results in Recent and Future Clinical Trials of RPE65-LCA**

Some of the early reports after gene therapy and oral cis-retinoid trials in RPE65-LCA have stated that there were improvements, or data trends indicating improvement, after treatment (Koenekoop RK, et al. *IOVS* 2012;53:ARVO E-Abstract 4642). Such reported results can now be evaluated from the perspective of the intervisit variability limits established in the current study. For example, the GVF improvements reported in many patients taking part in a gene therapy trial in RPE65-LCA would fall within the intervisit variability reported in the current study. This conclusion would also be true for most GVF results after a readministration trial in previously untreated eyes of three patients. Further, some of the percentage GVF improvements cited in a trial of oral cis-retinoid that included patients with RPE65 mutations (Koenekoop RK, et al. *IOVS* 2012;53:ARVO E-Abstract 4642) would fall within the intervisit variability that we measured in this study.

Do we need to establish variability limits for every population of retinal degeneration patients before embarking on a therapeutic initiative or can we rely on results from previously studied retinopathies? At this early stage of attempting to improve vision by treatment of previously incurable monogenic retinal degenerations, it seems prudent to determine variability, rather than assume it or extrapolate from other disorders. With new and refined approaches being developed specifically for treatment of RPE65-LCA, there is sufficient justification to establish variability limits for such traditional outcomes as GVF and VA. Among the early-onset retinal degenerations or LCA, the phenotype of RPE65-LCA may be one of only a few LCA groups that can be monitored with VA and GVF. Hereditary retinopathies with more severe visual dysfunction than RPE65-LCA and some evidence of therapeutic proof-of-concept in animal studies, such as other forms of LCA, also deserve specific attention but may not be appropriate for monitoring by traditional GVF and VA outcomes.

**References**


