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

Alejandro J. Roman,¹ Artur V. Cideciyan,¹ Sharon B. Schwartz,¹ Melani B. Olivares,¹ Elise Heon,² and Samuel G. Jacobson¹

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 on at least two visits separated by fewer than 120 days using Goldmann visual field (GVF) and ETDRS visual acuity (VA) in a retrospective review. GVFs 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 normal 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 (log₁₀nsu), 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 retinis pigmentosa showed that the *RPE65*-LCA patients had higher variability in kinetic field extent. VA variability in *RPE65*-LCA fell within reported results for retinis 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 retinis 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 than 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

From the ¹Scheie Eye Institute, Department of Ophthalmology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania; and ²Department of Ophthalmology and Vision Sciences, The Hospital for Sick Children, University of Toronto, Toronto, Canada.

Supported by grants from the Macula Vision Research Foundation, Foundation Fighting Blindness, and National Institutes of Health/National Eye Institute (EY017280). AVC is a Research to Prevent Blindness Senior Scientific Investigator.

Submitted for publication November 20, 2012; revised January 9, 2013; accepted January 14, 2013.

Disclosure: A.J. Roman, None; A.V. Cideciyan, None; S.B. Schwartz, None; M.B. Olivares, None; E. Heon, None; S.G. Jacobson, None

Corresponding author: Samuel G. Jacobson, Scheie Eye Institute, University of Pennsylvania, 51 N. 39th Street, Philadelphia, PA 19104; jacobsons@mail.med.upenn.edu.

quantified as reported previously.¹⁴ 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.^{14–16} 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.¹⁴ As in our previous studies,^{13,17–22} 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.^{7,9} 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,^{23,24} scored as the number of letters correctly read after adjusting for distance, and expressed as $\log_{10}$ 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 20/1600.²⁴ 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 $1.96 \sqrt{2s_w}$, where s_w is the within-person SD obtained by ANOVA²⁵ using $\log_{10}$ -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 $\log_{10}$ -converted GVF values. Intervisit fluctuation is shown (Fig. 1B)

FIGURE 1. Intervisit variability of kinetic visual fields in *RPE65*-LCA. (A) Visual field extent in one eye of patients with *RPE65* mutations, measured by the same examiner and perimeter in two or three visits within a 120-day interval. Ordinate is in nsu; mean normal extent corresponds to 100 nsu. *Bottom:* Examples of Goldmann perimeter charts (shown as left eyes) recorded on different visits for four representative patients. (B) Variability shown as intervisit differences (referenced to median or first visit when $n = 2$) against mean visual field extent. Differences do not depend strongly on mean level of extent. *Dashed lines* depict 95% confidence limits for determining significant change between a pair of visits.

as a function of mean extent among visits. The repeatability coefficient was 0.248 ($\log_{10}$ nsu) (Fig. 1B, dashed lines). This is equivalent to a $\times/\div 1.77$ factor (-44% , $+77\%$) in GVF extent in nsu.²⁶ The 95% confidence interval for the repeatability coefficient was 0.19 to 0.31 ($\log_{10}$ nsu).

VA measures for all visits are ranked by mean acuity (Fig. 2A). Our cohort of *RPE65*-LCA patients had a mean VA ranging from $\log_{10}$ MAR = 0.14 to 1.96 (20/40–20/1250). The individual measures fluctuated within a range of approximately $\log_{10}$ MAR = 0.2 (10 letters) around the measurement at the middlemost visit (Fig. 2B). The repeatability coefficient was $\log_{10}$ MAR = 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 ($\log_{10}$ MAR).

FIGURE 2. Intervisit variability of best-corrected VAs in patients with RPE65-LCA. (A) Multiple visits in different days within an interval of fewer than 85 days are shown ranked by mean VA across visits. *Right:* Scale indicates equivalent Snellen fractions. (B) Fluctuation around the mean for individual VA data (subtraction of visit data minus mean of all visits for each patient) illustrating the range of variation. (C) Variability shown as intervisit differences (referenced to median or first visit when $n = 2$) against mean acuity, showing no strong dependence of differences with acuity level. *Dashed lines* depict 95% confidence limits for determining significant change between two visits. *Right:* Scale is a correspondence to letters read on an ETDRS chart.

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,⁶ 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.,⁶ 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,

examiner 2), averaging ± 0.183 ($\log_{10}$ nsu). In another study that analyzed GVF,⁷ 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 ($\log_{10}$). In a more recent study,⁹ a different estimate of repeatability was used and limits of $\pm 36.3\%$ 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 $+53\%$ for one study,⁶ -32% to $+47\%$ for another study,⁷ and -44% to $+77\%$ for the present study. We estimated the relative number of participants with different levels of normalized VF extent among the different studies (Table) and found dissimilar cohort compositions (bar diagrams in Table). Normal data referenced or contained in the respective studies were used for normalization.

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 ($r = 0.138$, $P = 0.59$). Next, we postulated that fixation instability (nystagmus) may contribute to the measured variability of GVF. 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 withstanding). Another study of a retinal degeneration patient cohort ($n = 29$) that included patients with RP or CRD (conerod 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

DISCUSSION

Knowing the variability of functional outcomes is critical for studies designed to determine safety and efficacy in clinical trials of ocular therapy or to monitor the natural history of disease.^{7,8,29-31} The goal of the present study was to fill in a gap of missing data pertaining to intervisit variability of commonly used visual function outcomes in a population of LCA patients with a disease that has recently been treated with genetic as well as pharmacological interventions. Although there was use of purpose-developed methods to assess outcome in such

TABLE. Summary and Comparison of Visual Field and VA Variability Studies

Goldmann Visual Field					
Study (n, age, repetitions)	Diagnosis	Composition by VF extent*	Coef. of Repeatability† (log ₁₀ nsu) (nsu)		Method, Target
Ross et al., 1984 (17, 13-68, 2)	RP		± 0.183	-34% to +53%	2D area (chart), II-4e
Berson et al., 1985 (28, 6-49, 2)‡	RP		± 0.167	-32% to +47%	2D area (chart), V-4e
Bittner et al., 2011 (28, 19-76, 2) (reduced set, n=23)	RP		N/A §	±36.3% ±17.7%	3D area (retina), V-4e
This study (18, 11-40, 2-3 #)	RPE65-LCA		± 0.248	-44% to +77%	3D area (visual field), V-4e
Visual Acuity					
Study (n, age, repetitions)	Diagnosis	VA range	Coef. of Repeatability† (logMAR) ETDRS letters		Method
Berson et al., 1985 (32, 6-49, 2)‡	RP	20/20 to 20/200	± 0.078	± 3.9	Snellen chart
Birch et al., 1999 (29, 5-61, 2)	RP, CRD	20/40 to 20/125	± 0.212	± 10.6	ETDRS chart
This study (18, 11-40, 2-16**)	RPE65-LCA	20/25 to 20/1800	± 0.170	± 8.5	ETDRS chart
Rosser et al., 2003 (50, <50, 2)	Normal subjects	better than 20/30	± 0.110	± 5.5	ETDRS chart

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).

‡ Subjects randomly selected from larger cohort.

§ Study used a different repeatability measure.

|| Excludes patients with higher variability

number of repetitions=3 for 15 patients, and 2 for the remaining three.

** number of repetitions: avg=12, range 2 to 16.

studies, there was also reliance on the time-honored methods of kinetic perimetry and VA³²⁻³⁴ (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 ungenotyped 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.^{7,8,28} 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^{6,7} used planar areas on the GVF chart, while another⁹ 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

in terms of extent of visual field is also heterogeneous (Table). For example, the cohort in one study⁹ has most subjects with extents less than or equal to 17% of normal (using normal mean GVF extent of 741 mm²)³⁷; in another study,⁶ most patients have extents ranging from 50% to 83% of normal; and a further study⁷ 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 interpolation³⁸ and may result in lower variability estimates than when using a direct assignment of the line logMAR value.^{39,40}

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^{41,42} (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-LCA⁴¹ 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.⁴² 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,^{43,44} 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,^{20,27,45} also deserve specific attention but may not be appropriate for monitoring by traditional GVF and VA outcomes.

References

- Jacobson SG, Cideciyan AV. Treatment possibilities for retinitis pigmentosa. *N Engl J Med*. 2010;363:1669–1671.
- Cideciyan AV. Leber congenital amaurosis due to RPE65 mutations and its treatment with gene therapy. *Prog Retin Eye Res*. 2010;29:398–427.
- Fishman GA, Jacobson SG, Alexander KR, et al. Outcome measures and their application in clinical trials for retinal degenerative diseases: outline, review, and perspective. *Retina*. 2005;25:772–777.
- Bramall AN, Wright AF, Jacobson SG, McInnes RR. The genomic, biochemical, and cellular responses of the retina in inherited photoreceptor degenerations and prospects for the treatment of these disorders. *Annu Rev Neurosci*. 2010;33:441–472.
- Wright AF, Chakarova CF, Abd El-Aziz MM, Bhattacharya SS. Photoreceptor degeneration: genetic and mechanistic dissection of a complex trait. *Nat Rev Genet*. 2010;11:273–284.
- Ross DF, Fishman GA, Gilbert LD, Anderson RJ. Variability of visual field measurements in normal subjects and patients with retinitis pigmentosa. *Arch Ophthalmol*. 1984;102:1004–1010.
- Berson EL, Sandberg MA, Rosner B, Birch DG, Hanson AH. Natural course of retinitis pigmentosa over a three-year interval. *Am J Ophthalmol*. 1985;99:240–251.
- Birch DG, Anderson JL, Fish GE. Yearly rates of rod and cone functional loss in retinitis pigmentosa and cone-rod dystrophy. *Ophthalmology*. 1999;106:258–268.
- Bittner AK, Iftikhar MH, Dagnelie G. Test-retest, within-visit variability of Goldmann visual fields in retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. 2011;52:8042–8046.
- Jacobson SG, Aleman TS, Cideciyan AV, et al. Identifying photoreceptors in blind eyes caused by RPE65 mutations: prerequisite for human gene therapy success. *Proc Natl Acad Sci U S A*. 2005;102:6177–6182.
- Jacobson SG, Aleman TS, Cideciyan AV, et al. Human cone photoreceptor dependence on RPE65 isomerase. *Proc Natl Acad Sci U S A*. 2007;104:15123–15128.
- Jacobson SG, Cideciyan AV, Aleman TS, et al. Photoreceptor layer topography in children with leber congenital amaurosis caused by RPE65 mutations. *Invest Ophthalmol Vis Sci*. 2008;49:4573–4577.
- Jacobson SG, Aleman TS, Cideciyan AV, et al. Defining the residual vision in leber congenital amaurosis caused by RPE65 mutations. *Invest Ophthalmol Vis Sci*. 2009;50:2368–2375.
- Jacobson SG, Yagasaki K, Feuer WJ, Román AJ. Interocular asymmetry of visual function in heterozygotes of X-linked retinitis pigmentosa. *Exp Eye Res*. 1989;48:679–691.
- Weleber RG, Tobler WR. Computerized quantitative analysis of kinetic visual fields. *Am J Ophthalmol*. 1986;101:461–468.
- Dagnelie G. Conversion of planimetric visual field data into solid angles and retinal areas. *Clin Vision Sci*. 1990;5:95–100.
- Schwartz SB, Aleman TS, Cideciyan AV, et al. Disease expression in Usher syndrome caused by VLGR1 gene mutation (USH2C) and comparison with USH2A phenotype. *Invest Ophthalmol Vis Sci*. 2005;46:734–743.
- Herrera W, Aleman TS, Cideciyan AV, et al. Retinal disease in Usher syndrome III caused by mutations in the clarin-1 gene. *Invest Ophthalmol Vis Sci*. 2008;49:2651–2660.
- Stone EM, Luo X, Heon E, et al. Autosomal recessive retinitis pigmentosa caused by mutations in the MAK gene. *Invest Ophthalmol Vis Sci*. 2011;52:9665–9673.
- Jacobson SG, Cideciyan AV, Aleman TS, et al. Human retinal disease from AIPL1 gene mutations: foveal cone loss with minimal macular photoreceptors and rod function remaining. *Invest Ophthalmol Vis Sci*. 2011;52:70–79.
- Jacobson SG, Cideciyan AV, Gibbs D, et al. Retinal disease course in Usher syndrome 1B due to MYO7A mutations. *Invest Ophthalmol Vis Sci*. 2011;52:7924–7936.
- Huang WC, Wright AF, Roman AJ, et al. RPGR-associated retinal degeneration in human X-linked RP and a murine model. *Invest Ophthalmol Vis Sci*. 2012;53:5594–5608.
- Early Treatment Diabetic Retinopathy Study Research Group. Photocoagulation for diabetic macular edema. Report number 1. *Arch Ophthalmol*. 1985;103:1796–1806.
- Bressler NM, Bressler SB, Hawkins BS, et al. Submacular surgery trials randomized pilot trial of laser photocoagulation versus surgery for recurrent choroidal neovascularization secondary to age-related macular degeneration: I. Ophthalmic outcomes submacular surgery trials pilot study report number 1. *Am J Ophthalmol*. 2000;130:387–407.
- Bland JM, Altman DG. Measurement error. *BMJ*. 1996;313:744.

26. Nevill AM, Atkinson G. Assessing agreement between measurements recorded on a ratio scale in sports medicine and sports science. *Br J Sports Med.* 1997;31:314-318.
27. Jacobson SG, Cideciyan AV, Peshenko IV, et al. Determining consequences of retinal membrane guanylyl cyclase (RetGC1) deficiency in human Leber congenital amaurosis en route to therapy: residual cone-photoreceptor vision correlates with biochemical properties of the mutants. *Hum Mol Genet.* 2013; 22:168-183.
28. Rosser DA, Cousens SN, Murdoch IE, Fitzke FW, Laidlaw DAH. How sensitive to clinical change are ETDRS logMAR visual acuity measurements? *Invest Ophthalmol Vis Sci.* 2003;44: 3278-3281.
29. Berson EL, Rosner B, Sandberg MA, et al. A randomized trial of vitamin A and vitamin E supplementation for retinitis pigmentosa. *Arch Ophthalmol.* 1993;111:761-772.
30. Berson EL, Rosner B, Sandberg MA, et al. Clinical trial of lutein in patients with retinitis pigmentosa receiving vitamin A. *Arch Ophthalmol.* 2010;128:403-411.
31. Fishman GA, Bozbeyoglu S, Massof RW, Kimberling W. Natural course of visual field loss in patients with Type 2 Usher syndrome. *Retina.* 2007;27:601-608.
32. Aleman TS, Jacobson SG, Chico JD, et al. Impairment of the transient pupillary light reflex in Rpe65(-/-) mice and humans with leber congenital amaurosis. *Invest Ophthalmol Vis Sci.* 2004;45:1259-1271.
33. Roman AJ, Schwartz SB, Aleman TS, et al. Quantifying rod photoreceptor-mediated vision in retinal degenerations: dark-adapted thresholds as outcome measures. *Exp Eye Res.* 2005; 80:259-272.
34. Roman AJ, Cideciyan AV, Aleman TS, Jacobson SG. Full-field stimulus testing (FST) to quantify visual perception in severely blind candidates for treatment trials. *Physiol Meas.* 2007;28: N51-N56.
35. Brown B, Lovie-Kitchin J. Repeated visual acuity measurement: establishing the patient's own criterion for change. *Optom Vis Sci.* 1993;70:45-53.
36. Bittner AK, Ibrahim MA, Haythornthwaite JA, Diener-West M, Dagnelie G. Vision test variability in retinitis pigmentosa and psychosocial factors. *Optom Vis Sci.* 2011;88:1496-1506.
37. Massof R, Dagnelie G, Benzsawel T, Palmer R, Finkelstein D. First order dynamics of visual field loss in retinitis pigmentosa. *Clin Vision Sci.* 1990;5:1-26.
38. Ferris FL III, Kassoff A, Bresnick GH, Bailey I. New visual acuity charts for clinical research. *Am J Ophthalmol.* 1982;94: 91-96.
39. Bailey IL, Bullimore MA, Raasch TW, Taylor HR. Clinical grading and the effects of scaling. *Invest Ophthalmol Vis Sci.* 1991;32:422-432.
40. Vanden Bosch ME, Wall M. Visual acuity scored by the letter-by-letter or probit methods has lower retest variability than the line assignment method. *Eye (Lond).* 1997;11:411-417.
41. Maguire AM, High KA, Auricchio A, et al. Age-dependent effects of RPE65 gene therapy for Leber's congenital amaurosis: a phase 1 dose-escalation trial. *Lancet.* 2009;374:1597-1605.
42. Bennett J, Ashtari M, Wellman J, et al. AAV2 gene therapy readministration in three adults with congenital blindness. *Sci Transl Med.* 2012;4:120ra15.
43. Gao S-Q, Maeda T, Okano K, Palczewski K. A microparticle/hydrogel combination drug-delivery system for sustained release of retinoids. *Invest Ophthalmol Vis Sci.* 2012;53: 6314-6323.
44. Kaylor JJ, Yuan Q, Cook J, et al. Identification of DES1 as a vitamin A isomerase in Müller glial cells of the retina. *Nat Chem Biol.* 2013;9:30-36.
45. Cideciyan AV, Rachel RA, Aleman TS, et al. Cone photoreceptors are the main targets for gene therapy of NPHP5 (IQCB1) or NPHP6 (CEP290) blindness: generation of an all-cone Nphp6 hypomorph mouse that mimics the human retinal ciliopathy. *Hum Mol Genet.* 2011;20:1411-1423.