Baseline Visual Field Findings in the RUSH2A Study: Associated Factors and Correlation with Other Measures of Disease Severity

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Abstract *[current 249, 250 words max]*

Purpose: Baseline visual fields to monitor the Rate of Progression in *USH2A*-related Retinal Degeneration (RUSH2A) study.

Design: Cross-sectional study within a natural history study.

Setting: Multicenter, international.

Study Population: Usher syndrome type 2 (USH2) (N=80) or autosomal recessive non-syndromic RP (ARRP) (N=47) associated with biallelic disease-causing sequence variants in *USH2A*.

Observation Procedures: Repeatability of full-field static perimetry (SP) and between-eye symmetry of kinetic perimetry (KP) were evaluated with intra-class correlation coefficients (ICC). The association of demographic and clinical characteristics with total hill of vision (V_{TOT}) was assessed with general linear models. Associations between V_{TOT} and other functional and morphologic measures were assessed using Spearman correlation coefficients and t-tests.

Main Outcome Measures: V_{TOT} (SP); III4e isopter area (KP).

Results: USH2 participants had more severe visual field loss than ARRP participants (P=<.001, adjusting for disease duration, age of enrollment). Mean V_{TOT} measures among 3 repeat tests were 32.7±24.1, 31.2±23.4, 31.7±23.9 decibel-steradians (ICC=.96). Better VA, greater photopic ERG 30Hz flicker amplitudes, higher mean microperimetry sensitivity, higher central subfield thickness, absence of macular cysts, and higher III4e seeing area were associated with higher V_{TOT} (all r>.48; P<.05). Mean III4e isopter areas for left (4561±4426 deg^2) and right eyes (4215±4300 deg^2) were concordant (ICC=0.94).

Conclusions: USH2 participants had more visual field loss than participants with *USH2A*-related ARRP, adjusting for duration of disease and age of enrollment. V_{TOT} was repeatable and correlated with other functional and structural metrics, suggesting it may be a good summary measure of disease severity in patients with *USH2A*-related retinal degeneration.

Title *[Current= 126, max=135]*: Baseline Visual Field Findings in the RUSH2A Study: Associated Factors and Correlation with Other Measures of Disease Severity

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Short Title: *[Current=50, max=60]*: Baseline Visual Field Findings in the RUSH2A Study

Meeting Presentation: This material will be presented as a video for the ARVO Learn Platform instead of being presented at the 2020 ARVO Annual Meeting in Baltimore, MD on May 5, 2020

This article contains additional supplemental material available at AJO.com: e-Figure 1 (A-B), e-Figure 2, e-Figure 3 (A-B) and e-Table 1, e-Table 2, e-Table 3, e-Table 4, and e-Table 5.

Introduction *[current 3,930]*

Disease-causing sequence variants in *USH2A* are the most common cause of Usher syndrome type 2 (USH2, a syndromic form of retinitis pigmentosa (RP) with congenital, mild to moderate hearing loss), the commonest cause of combined dual sensory impairment.[1,2] Moreover, *USH2A* variants are also the commonest cause of autosomal recessive non-syndromic RP (ARRP, isolated RP with normal hearing at birth).[1,3-7] Retinal degeneration associated with sequence variants in *USH2A* is characterized by slowly progressive rod, then cone, photoreceptor dysfunction and eventual photoreceptor death, resulting in escalating vision loss. It appears the combination of *USH2A* variants explains whether one has USH2 or non-syndromic RP.[8-10] Retinal degeneration is more severe in patients with USH2 than *USH2A*-related non-syndromic RP.[9] However, the reason is not clearly understood[9] especially since there are many single variants in *USH2A* that have been associated with both Usher syndrome type 2 and non-syndromic RP.[3,8] Therefore, the suggestion that retinal degeneration is more severe in patients with USH2 than *USH2A*-related non-syndromic RP may relate to other genetic modifiers and/or environmental influences.[9] As new treatments for *USH2A*-related retinal degeneration are under development or in early clinical trials,[11,12] a comprehensive understanding of the natural history of disease progression of *USH2A*-related retinal degeneration is essential.

Limited natural history data are available from patients with *USH2A*-related retinal degeneration. In general, the natural history studies to date reporting manual kinetic perimetry (KP) included USH2 patients not genetically characterized.[13-16] None of the prior studies included longitudinal characterization of the retinal phenotype using current standard assessments, such as quantitative static perimetry (SP) employing the volumetric measure of the hill of vision (HOV).[17] Previous studies were mostly retrospective with variable research approaches, and did not use standardized measures such as visual acuity (VA) according to the Early Treatment of Diabetic Retinopathy Study (ETDRS) protocol,[18] either within or across clinical centers. We do not know which structural/functional parameters provide sensitive and reliable outcome measures that reflect change and could be used to monitor progression or treatment effectiveness.

Because *USH2A*-related retinal degeneration is the commonest cause of USH2 and non-syndromic autosomal recessive RP, a multicenter, international, longitudinal natural history study of participants with retinal degeneration associated with *USH2A* sequence variants, the Rate of Progression of *USH2A*-related Retinal Degeneration (RUSH2A) study, was undertaken. The primary objective of the RUSH2A study was to characterize the natural history of retinal degeneration associated with *USH2A* biallelic disease-causing sequence variants over 4 years, using functional, structural, and patient-reported outcome measures, with the goal of identifying outcome measures that can be used to monitor disease progression and treatment response. Secondary study objectives included the evaluation of variability and possible risk factors (genotype, phenotype, environmental, and comorbidities) for progression of these outcome measures.

This report aims to: (1) describe the RUSH2A study design and methods, (2) summarize the baseline characteristics of the enrolled participants, including differences between those with USH2 and those with non-syndromic ARRP, and (3) summarize results of baseline visual fields, including the repeatability of the HOV derived from SP, and the relationships of clinical characteristics and other functional and structural measures with baseline HOV.

Methods

Study Design

This multicenter, longitudinal, international natural history study enrolled participants at 16 clinical sites in Canada, France, Germany, the United Kingdom, and the United States (US). The protocol and informed consent process adhered to the tenets of the Declaration of Helsinki

and were approved by the ethics boards associated with each participating site, including compliance with the associated federal regulations. Informed consent was obtained from all participants prior to enrollment. The RUSH2A protocol is listed on www.clinicaltrials.gov (NCT03146078), with registration completed prior to enrolling the first participant.

Eligibility criteria and genetic screening. The inclusion and exclusion criteria are listed in **e-Table 1** (Supplemental Material at AJO.com). Participants were at least 8 years old with rod-cone degeneration associated with at least 2 disease-causing sequence variants in *USH2A*, based on existing genetic reports from Clinical Laboratory Improvement Amendments (CLIA)-certified laboratories (or equivalent, in non-US countries). Following initial eligibility assessment and enrollment, some participants without a history of hearing loss and presumed non-syndromic ARRP for whom the phase of alleles was unknown underwent additional genetic testing of first-degree relatives to confirm that inheritance of the mutant alleles was *in trans*. Ultimately, participants with (1) USH2 or (2) ARRP with either homozygous or compound heterozygous *USH2A* variants inherited *in trans* were enrolled into this natural history study. After enrollment, an independent audiologist reviewed both the history of hearing loss and the results of baseline audiology exams distinguishing USH2 from ARRP.

Study cohorts and sample size. This study included two cohorts, one with vision of ETDRS letter score of 54 or more and one of ETDRS letter score of 53 or less. Due to the expected high degree of symmetry of retinal disease between eyes,[14,19] most of the testing was performed in one "study eye" designated for each participant. The study eye was the eye with better baseline visual acuity. If both eyes had the same baseline visual acuity, the designation was made at investigator discretion as the eye with more stable fixation or clearer ocular media to permit ophthalmic imaging. The primary cohort included participants with study eye baseline ETDRS letter score of 54 or more (Snellen equivalent 20/80 or better) and stable fixation. Participants in the primary cohort were expected to have further deterioration in vision that could be measured reliably and will be followed in a longitudinal natural history study. A sample size of 100 for the primary cohort was selected to provide a 95% confidence interval half-width of approximately 4% for percentage change over 4 years in visual field area, assuming a mean decrease of 25% with a standard deviation (SD) of 20%.[9,20] The study was also designed to enroll a secondary cohort of 20 participants with study eye baseline ETDRS letter score of 53 or less (Snellen equivalent 20/100 or worse), central visual field of less than 10 degrees diameter, or unstable fixation to complete a baseline visit only. The purpose of the secondary cohort was to obtain cross-sectional data on participants having disease spanning the full range of severity.

Visit schedule and testing procedures. All participants completed a baseline visit. Primary cohort participants will return annually for visits through 4 years. The visit schedule and testing procedures are detailed in **e-Table 2** (Supplemental Material at AJO.com). In brief, in addition to medical history and demographic data, the RUSH2A study collected auditory and olfactory data at baseline to evaluate these as risk factors associated with baseline disease severity and progression of retinal degeneration based on all of the outcome measures, over the 4-year study duration. Visual function testing at baseline and follow up in the primary cohort included best-corrected visual acuity (BCVA), SP, fundus-guided microperimetry (MP), KP, full-field electroretinogram (ERG), and full-field stimulus threshold (FST) measures. Retinal structure was assessed using spectral-domain optical coherence tomography (SD-OCT) in all participants. All testing procedures were performed according to standardized procedures by study-certified technicians as noted in **e-Table 2** (Supplemental Material at AJO.com). Patient-reported outcomes were collected using the Veterans Affairs Low Vision Visual Functioning Questionnaire (VALVVFQ-48) in adults at least 18 years old and the L.V. Prasad-Functional Vision Questionnaire (LVP-FVQ-II) in children <18 years old. Adverse events and medications were collected for the study with the objective to provide historical control data for future clinical trials**.**

Outcome measures. The RUSH2A study aims to evaluate progression of several main outcome measures over 4 years: (1) SP total HOV (V_{TOT}, decibel-steradian (dB-sr)), (**e-Figure 1A** (Supplemental Material at AJO.com)) graded by the Casey Reading Center (CRC; Casey Eye Institute, Oregon Health Sciences University, Portland, OR); (2) seeing area measured by KP using I4e, III4e, and V4e isopter targets, graded by CRC; (3) mean retinal sensitivity measured by MP, graded by the Duke Reading Center (DRC; Duke University, Durham, NC); (4) BCVA using the ETDRS protocol; (5) Ellipsoid Zone (EZ) area measured on SD-OCT, graded by DRC; (6) rod- and cone-mediated retinal sensitivity as measured by FST; and (7) retinal function measured with full-field rod and cone-mediated ERGs. DRC also graded SD-OCTs for central subfield thickness (CST) within the center 1mm and presence of intraretinal cysts defined as round or oval cavities within the retinal layers as additional measures of retinal structure. The primary focus of this paper is to characterize the baseline visual fields in detail; future papers will characterize the remaining outcome measures, (3) through (7) listed above.

Perimetry methods. Full-field automated SP was performed on the Octopus 900 (Haag-Streit, Mason, Ohio) with a custom grid, using the German Adaptive Thresholding Estimation (GATE)[21,22] strategy and a custom "RP 185 point" centrally condensed radial grid extending 65° nasally and superiorly, 67° inferiorly, and 80° temporally with a size V stimulus size (**e-Figure 1A** (Supplemental Material at AJO.com)). Any participants found to have no measurable vision outside of 25 degrees at baseline were intended to be tested with only the central 30-degree grid (V_{30}) at subsequent visits, but V_{30} was analyzed from the full grid for all participants at baseline (**e-Figure 1B** (Supplemental Material at AJO.com)). Historical measures of SP are limited in the number of locations that can be tested in a reasonable time, but the full-threshold testing algorithm employed by the GATE strategy permits testing more locations over a shorter time, and is also better designed to identify and monitor visual field defects due to retinal disease compared to other algorithms, e.g. Swedish Interactive Thresholding Algorithm (SITA).[21] Topographic analysis of SP using an approach called Visual Field Modeling and Analysis (VFMA) produces the three-dimensional, quantitative surface models of V_{TOT}.[22,23] The volume (in unit dB-sr) beneath the surface of the thin-plate spline representation of the HOV and within the external boundary of the grid was quantified (V_{TOT}). The reliability factor (RF) measured subject performance as the sum of false positive and false negative answers divided by the total number of trial questions. False negative responses contribute more to the RF measure in patients with low retinal sensitivity due to RP.[24] For the evaluation of KP, the Octopus perimetry EyeSuite software calculated areas in degree^2 for each isopter automatically. Test vectors originating 10° outside the age-correlated normal isopter were presented every 15° with 4°per second angular velocity. Six reaction-time vectors were presented within seeing areas, with 1 repetition horizontally, vertically, and diagonally, originating from 10°and 30°eccentricity. Scotomas were mapped at 2°per second angular velocity originating from the assumed center and using at least 12 vectors. Blind spots were mapped with the I4e stimulus, or the smallest and least bright stimulus seen, at 2°per second angular velocity with a minimum of 8 vectors originating from the assumed center.

Statistical Methods

The distributions of baseline characteristics and measures of visual function and structure were summarized using means, standard deviations (SDs), medians, quartiles and ranges. SP was performed in the study eye, three times in the primary cohort to characterize within-visit variation in test responses, and only once in the secondary cohort. In the former case, the average of the three V_{TOT} and three V_{30} tests for each of the participants was used for analyses of this measure. Intra-class correlation coefficients (ICC) and the methods of Bland and Altman for assessing agreement between measurements, the repeatability coefficient and Bland-Altman plots, were used to assess variability of SP on tests repeated three times per participant.[25] General linear models adjusted for clinical diagnosis, disease duration, and age of

enrollment were used to assess the association of baseline characteristics with V_{TOT}. In addition, we evaluated the association between baseline V_{TOT} and other functional and structural measures by calculating Spearman correlation coefficients for continuous factors and comparison of means with t-tests for categorical factors.

KP was performed in both eyes for all participants at baseline. Symmetry of left and right eyes areas at baseline was assessed using scatterplots and summarized with ICCs. Bland-Altman plots were used to assess the magnitude of differences and their association with the area size.

Missing data were treated as a separate category for discrete factors, and a missing indicator was created for continuous factors. Continuous covariates were included in all models in continuous form but were categorized for display and ease of interpretation in the tables. All reported *P* values were 2-sided. Statistical analyses were conducted using SAS software version 9.4 (SAS, Inc).

Results

Study Population

One hundred and forty-five participants consented to enroll into the RUSH2A study, of whom 127 were eligible after genetic screening and completed a baseline visit (**e-Figure 2** (Supplemental Material at AJO.com)). Of these 127 participants, 105 (83%) were in the primary cohort, and 22 (17%) were in the secondary cohort. Key baseline characteristics of the participants are provided in **Table 1** and stratified by clinical diagnosis (80 [63%] USH2 and 47 [37%] ARRP). Sixty-eight (54%) of participants were female, 113 (89%) were white. The median (interquartile range ([IQR]) age was 37 (27, 44) years in the USH2 group and 44 (36, 50) years in the ARRP group. The age of onset of disease reported by the participant was younger in the USH2 group than in the ARRP group (median 16 vs 32 years). Although median duration of disease was similar in the USH2 group (16 years) and the ARRP group (12 years), there was a higher percentage with duration ≥20 years in the USH2 group (44% [35 of 80] versus 17% [8 of 47]). Ninety-seven percent (73 of 75) of the USH2 participants had moderate or worse hearing loss, but 9% (4 of 47) of the ARRP participants had moderate hearing loss based on the 4 frequency pure tone average audiology test score (Table 1), and sites reported current hearing aid use in 6 of 47 with ARRP (13%). Hearing loss in subjects in the ARRP group was sensorineural and correlated with age (r^2 = 0.53, P = <0.001), but there was no significant correlation between hearing loss and age in the USH2 group (r^2 = 0.01, P = 0.46). A complete analysis of audiology results for participants in the RUSH2A study will be provided in a separate report. Additional baseline characteristics are summarized in **e-Tables 3 and 4** (Supplemental Material at AJO.com). Pre-existing conditions are summarized in **e-Table 5** (Supplemental Material at AJO.com)**.** 29 (23%) of the 127 participants reported a pre-existing psychiatric disorder. Of these, depression and anxiety were the most commonly reported; 17 (59%) participants reported depression (12 in USH2 and 5 in ARRP), and 15 (52%) participants reported anxiety (7 participants in the USH2 group and 8 in ARRP).

Functional and Structural Measures at Baseline

Functional and structural measures at baseline are summarized in **Table 2.** The median value for V_{TOT} was twice as large in the ARRP participants as in the USH2 participants (32.8 versus 16.0 dB-sr, P<0.001), although both groups were lower than normal subjects (103 dB-sr).[22] However, the median values for V_{30} were similar (9.3 versus 7.5 dB-sr, P=0.13) in both groups, although both groups were also lower than normal participants (27.4 dB-sr).[22] The mean (SD) sensitivity on static perimetry was 9.3 (6.0) dB in USH2 participants, and 11.9 (6.0) dB in ARRP participants). Participants with ARRP had larger seeing areas for all 3 isopters (I4e, III4e, and V4e) compared to participants with USH2. Mean (SD) III4e area for left and right eyes was

4215 (4300) and 4561 (4426) squared degrees, respectively, showing high concordance (ICC=0.94; **e-Figure 3A**(Supplemental Material at AJO.com)), but the seeing area was smaller than the lower limit of normal subjects (12799 squared degrees, data not published), in both groups. Bland-Altman plots (**e-Figure 3B** (Supplemental Material at AJO.com)) show a mean difference (left minus right) between eyes equal to -346 squared degrees with limits of agreement -3340 to 2648 squared degrees. Mean sensitivity of microperimetry was 5.4 (4.9) dB in USH2 participants, and 6.7 (5.1) dB in ARRP participants**.** Detailed microperimetry baseline data, including repeatability and correlation with OCT EZ area will be reported in a future manuscript. The median visual acuity score for all participants was 80 (Snellen equivalent 20/25) and similar in both diagnosis groups. Photopic ERG amplitudes were not measurable in 29% of participants with similar percentages in both diagnosis groups. Cysts were present in OCT scans from 49% of participants with USH2 and 34% of participants with ARRP. The central subfield thickness was similar in both diagnosis groups (overall median 253 microns).

Variability of Static Perimetry Testing

Measures of variability of results within a testing session (reliability factor, false positive rate, and false negative rate) and of variability in V_{TOT} in a participant between testing sessions were examined. Three participants had only two SP tests, and one participant did not have baseline SP; the secondary cohort of participants with more severe disease had only a single baseline. Good reliability was found in both groups with a reliability factor (RF) median (IQR) over all tests of 5.2% (2.1%, 9.1%) in participants with USH2, and 5.1% (3.0%, 7.3%) in participants with ARRP (**Table 3**). The median (IQR) for the false positive rate over all tests was 1% (0%, 4%) and 2% (0%, 3%) and for the false negative rate was 8% (2%,15%) and 8% (5%,11%), respectively for the USH2 and ARRP groups. The overall repeatability for 101 participants with 3 available tests was high and similar when comparing the 3 pairs of test results (ICC overall=0.96, ICC test 1 versus test 2 = 0.98, ICC test 2 versus test 3 = 0.95, test 1 versus test 3= 0.94; repeatability coefficient = 13.7) (**Table 3**). Bland-Altman plots showed mean differences near 0 (<1.5 with 95% limits of agreement of ± 16 dB-sr; **Figure 1)**.

Association of Baseline Characteristics with Total Hill of Vision (V_{TOT})

Mean V_{TOT} values stratified by diagnosis and baseline characteristics are shown in **Table 4**. Among all participants and within each diagnosis group, mean V_{TOT} decreased with increasing duration of disease. After adjustment for duration of disease and age of enrollment, USH2 participants had lower V_{TOT} values compared with ARRP participants (mean difference estimated from linear regression: 13.4 dB-sr with 95% CI [4.2, 22.6], P= <0.001; **Table 5**). After adjustment for clinical diagnosis and age of enrollment, longer disease duration was associated with lower V_{TOT} values (P<0.001), with a mean decrease of 0.45 [95% CI (0.03, 0.88)] dB-sr for each additional year of duration (**Table 5**). Age at enrollment was significantly associated with V_{TOT} when adjusted for clinical diagnosis and disease duration. Older age of enrollment was associated with worse vision (P=0.02). The association of age of enrollment with V_{TOT} remained similar in a sensitivity analysis with USH2 participants only (data not shown). No other baseline characteristic in **Table 4** was found to be significantly associated with V_{TOT} once clinical diagnosis and disease duration were accounted for.

Association of V_{TOT} with Other Measures of Function and Structure

The association of baseline functional and structural measures with V_{TOT} are summarized in **Table 6.** Better BCVA was associated with higher V_{TOT} values (Spearman correlation coefficient r=0.59, P<0.001). Presence of cysts (well-defined round or oval cavities within the retinal layers) in OCT scans was associated with a lower V_{TOT} (mean difference=9.1

dB-sr, P=0.03). Other factors including photopic ERG 30 Hz amplitudes, mean retinal sensitivity on MP, and central subfield thickness within the center 1mm on SD-OCT were all found to be moderately associated with V_{TOT}, with correlation coefficients ranging from 0.48 to 0.55**.** KP III4e area was very strongly associated with V_{TOT} (r=0.92, P <0.001). The correlation coefficients for V_{30} with the measures of function and structure were similar to the corresponding correlation coefficients for V_{TOT} and similar between the two clinical diagnosis groups (r from 0.46 to 0.85; data not shown).

Discussion

The RUSH2A study comprised roughly two-thirds of participants with USH2 and one-third with ARRP and represents a large, diverse population of patients with retinal degeneration due to *USH2A* variants, well-characterized genetically and phenotypically with a broad spectrum of disease severity. The main outcome measure, V_{TOT}, differed between disease groups (USH2 and ARRP) and disease duration. V_{TOT} results were repeatable over 3 repetitions at baseline separated by no more than 10 days in participants in the primary cohort, suggesting the learning effect was minimal and triplicate SP measures at baseline may not be necessary. Similar findings have been shown using V_{TOT} and V_{30} in X-linked RP associated with *RPGR*.[26] Furthermore, many common clinical measures including BCVA, ERG 30Hz flicker amplitudes, mean macular retinal sensitivity on microperimetry, III4e KP area, the presence of intraretinal cysts, and central subfield thickness correlated with the V_{TOT} measured using standard SP protocols and common equipment among the 16 participating centers. The study results suggest that V_{TOT} may provide a reliable outcome measure of disease progression for clinical trials of participants with *USH2A*-related retinal degeneration. V_{30} values were similar between USH2 and ARRP but provided a less sensitive measure of disease severity than V_{TOT}. Greater disease duration significantly correlated with more severe visual field loss as measured by SP, consistent with the progressive nature of *USH2A*-related retinal degeneration.

Participants in the RUSH2A study reported anxiety (11%) and depression (9%) more commonly than other psychiatric disorders. Prior studies of participants with RP have shown significantly greater rates of anxiety and depression compared to controls,[27] with anxiety in 36.5% and depression in 15.5%[28] using a standard questionnaire to measure anxiety and depression. Other studies found significantly increased rates of depressive mood in RP patients (34.8%) compared to controls (17.1%),[29] and depression scores indicative of clinical depression in 25.7% of RP patients.[30] Rates reported in RUSH2A participants were lower than many studies in the literature. The present study relies on patient report of anxiety and depression, and therefore rates in the RUSH2A participants may under-represent the true prevalence of disease. Future studies will report results of quality of life test results using standard instruments at baseline and longitudinally in the RUSH2A study.

It is noteworthy that participants with USH2 had worse visual field sensitivity (V_{TOT} and V_{30}) than participants with ARRP, even after accounting for disease duration and age at enrollment. A previous study comparing participants with USH2 with ARRP due to biallelic *USH2A* sequence variants found that those with USH2 had more severe visual impairment measured by visual field and visual acuity, occurring at least a decade earlier than in those with ARRP.[9] Similarly, in another study ERG 30 Hz flicker amplitudes were lower in participants with USH2 compared to ARRP.[10] More severe truncating sequence variants have been reported in participants with USH2 than ARRP, and hearing loss is also more severe in those with truncating *USH2A* sequence variants compared to missense sequence variants.[31] Genetic characteristics of the RUSH2A population will be reported in a future manuscript, but may provide further insight into the relationship between genotype and phenotype in patients with *USH2A*-related retinal degeneration.

In the RUSH2A study population, older age at enrollment into the study was associated

with lower V_{TOT} as measured by SP, after adjustment for clinical diagnosis and duration of disease. Due to congenital hearing loss, patients with USH2 may be diagnosed at earlier ages than patients with ARRP and similar loss of vision. Thus, the reported duration of vision loss for ARRP patients may be an underestimate of the true duration, so that the estimated mean of 13.4 dB-sr higher V_{TOT} in the ARRP group relative to the USH2 group **(Table 5)** may be an underestimate.

USH2A-related retinal degeneration affects rods, then cones, so rod-mediated measures of retinal function may reflect disease severity earlier and potentially more sensitively than more cone-driven measures such as perimetry or BCVA.[32] Due to the background illumination used in this study, the clinical measures that correlated with V_{TOT} were most likely cone-mediated; but measures of rod function including FST, dark-adapted visual field sensitivity and rod ERGs are included in the RUSH2A study and will be described in future manuscripts. Specifically, dark-adapted perimetry will be performed at 5 sites beginning at the 12-month RUSH2A visit and will be repeated annually for 36 months. Dark-adapted perimetry will be used to determine the proportion of participants with evidence of rod-mediated function. The RUSH2A trial provides an opportunity to determine whether measurement of rod function and rate of loss could provide useful outcome measures for monitoring disease progression during future observational/interventional clinical trials.

In conclusion, V_{TOT} interpolation of SP correlated significantly with diagnosis, disease duration and several clinical measures of retinal structure and function in the RUSH2A study population at baseline. Future work will evaluate genetic risk factors for disease severity, hearing loss, rod-mediated retinal function and the impact of disease on patient quality of life at baseline and during 4 years of longitudinal progression in the RUSH2A study.

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Figure Legend

Figure 1 (a-c). Baseline Static Perimetry Bland-Altman Plots in the RUSH2A Study

Legend: Bland-Altman plot[a] of test 1, 2, and 3 pairwise. Only included participants with 3 fields(N=101). The differences between test 1 and 2, test 2 and 3, test 1 and 3 for V_{TOT} are plotted on the y-axis against their averages on the x-axis.

[a]The Bland-Altman plots only include participants with 3 fields(N=101, not included 22 secondary cohort participants only performed the test once, 1 participant was missing all SP tests, 2 primary cohort participants were missing the second and the third test, and 1 participant was missing the third V_{TOT} value.)

Table 1. Baseline Characteristics by Clinical Diagnosis in the RUSH2A Study

Characteristic	Overall N=127	Clinical Diagnosis USH2 N=80	Clinical Diagnosis ARRP N=47
Gender			
Female	68 (54%)	44 (55%)	24 (51%)
Male	59 (46%)	36 (45%)	23 (49%)
Race/Ethnicity			
White	113 (89%)	70 (88%)	43 (91%)
Hispanic	9 (7%)	7 (8%)	2 (4%)
Asian	5 (4%)	3 (4%)	2 (4%)
Enrollment area			
United States/Canada	83 (65%)	50 (62%)	33 (70%)
Europe/UK	44 (35%)	30 (38%)	14 (30%)
Age at enrollment, yrs [a]			
Median (IQR)	40 (30, 48)	37 (27, 44)	44 (36, 50)
[Min, Max]	[15, 80]	[15, 80]	[24, 75]
<35	44 (35%)	36 (45%)	8 (17%)
35-45	44 (35%)	25 (31%)	19 (40%)
>=45	39 (30%)	19 (24%)	20 (43%)
Age of onset, yrs [b]			
Median (IQR)	19 (14, 30)	16 (13, 22)	32 (20, 41)
[Min, Max]	[5, 65]	[5, 46]	[7, 65]
<16	41 (32%)	36 (45%)	5 (11%)
[16, 25)	40 (32%)	30 (38%)	10 (22%)
>=25	45 (36%)	14 (18%)	31 (67%)
Duration of Disease, yrs [b]			
Median (IQR)	15 (8, 23)	16 (10, 27)	12 (6, 18)
[Min, Max]	[1, 60]	[1, 60]	[1, 36]
<10	37 (29%)	20 (25%)	17 (37%)
[10, 19)	46 (37%)	25 (31%)	21 (46%)
>=20	43 (34%)	35 (44%)	8 (17%)
Severity of hearing loss [c]			
Normal	35 (29%)	0	35 (74%)
Mild	10 (8%)	2 (3%)	8 (17%)
Moderate	58 (48%)	54 (72%)	4 (9%)
Severe	15 (12%)	15 (20%)	0
Profound	4 (3%)	4 (5%)	0
Smoking status			
Yes	33 (26%)	20 (25%)	13 (28%)
No	94 (74%)	60 (75%)	34 (72%)
Current use of dietary supplements			
None	53 (42%)	41 (51%)	12 (26%)
Vitamin A only	11 (9%)	5 (6%)	6 (13%)
DHA only	5 (4%)	3 (4%)	2 (4%)
Lutein only	9 (7%)	5 (6%)	4 (9%)
Combination	49 (38%)	26 (33%)	23 (49%)

[a] 28 participants were not permitted to report date of birth due to regulatory restrictions. Therefore, only year of birth and categorical age was reported. For those participants, July 1st with the reported birth year was imputed as birth date to calculate continuous age

[b] 1 participant in the ARRP group was missing age of onset (a participant-reported field based on their awareness of visual symptoms) and duration of disease (computed based on age of onset and date of enrollment)

[c] Composite score based on 4F-PTA (four frequency air conduction threshold pure-tone average based on 0.5, 1, 2, and 4 kHz). 5 participants in the USH2 group were missing baseline 4F-PTA (3 had cochlear implants in both ears, 2 missed their audiology exam for other reasons)

Table 2. Baseline Functional and Structural Measures in the RUSH2A Study

	Overall N=127	Clinical Diagnosis	
		USH2 N = 80	ARRP N = 47
V_{TOT} (dB-sr) [a]			
Median (IQR)	20.6 (7.7, 46.3)	16.0 (3.6, 35.2)	32.8 (15.1, 54.6)
[Min, Max]	[0.2, 90.5]	[0.2, 81.4]	[2.5, 90.5]
Mean (SD)	27.8 (23.7)	22.5 (21.5)	37.1 (24.7)
V_{30} (dB-sr) [a]			
Median (IQR)	8.3 (3.8, 12.8)	7.5 (2.7, 12.7)	9.3 (5.1, 13.3)
[Min, Max]	[0.2, 22.7]	[0.2, 21.6]	[1.4, 22.7]
Mean (SD)	9.0 (5.9)	8.4 (5.9)	10.0 (5.9)
SP mean sensitivity (dB) [a]			
Median (IQR)	9.3 (5.2, 14.6)	7.8 (4.3, 13.8)	12.1 (7.0, 16.9)
[Min, Max]	[0.4, 24.6]	[0.4, 24.2]	[2.4, 24.6]
Mean (SD)	10.2 (6.1)	9.3 (6.0)	11.9 (6.0)
I4e seeing area (deg^2) [b]			
Median (IQR)	85.8 (22.2, 607.0)	61.4 (12.8, 289.2)	187.7 (27.1, 1770.0)
[Min, Max]	[0.0, 8883.1]	[0.0, 5619.2]	[0.0, 8883.1]
III4e seeing area (deg^2) [b]			
Median (IQR)	2454.6 (431.6, 8064.4)	1362.5 (226.1, 6465.6)	5722.6 (2112.7, 9707.6)
[Min, Max]	[6.7, 13467.0]	[6.7, 13335.0]	[105.9, 13467.0]
V4e seeing area (deg^2) [b]			
Median (IQR)	8798.5(2619, 12344.0)	5912.5 (842.4, 11521.0)	11062.0 (7389.4, 13035.0)
[Min, Max]	[18.6, 15800.0]	[18.6, 15579.0]	[405.5, 15800.0]
VA ETDRS letter score [c]			
Median (IQR)	80.0 (75.0, 85.0)	79.0 (73.5, 85.0)	82.0(77.0, 87.0)
[Min, Max]	[18.0, 94.0]	[18.0, 92.0]	[41.0, 94.0]
Photopic ERG 30 Hz flicker Amplitude (μV) [d]			
N of unmeasurable (0) amplitudes	37 (29%)	25 (32%)	12 (26%)
Median amplitude (IQR)	2.0 (0.0, 7.7)	1.5 (0.0, 5.5)	3.1 (0.0, 20.0)
[Min, Max]	[0.0, 82.2]	[0.0, 82.2]	[0.0, 60.0]
MP mean retinal sensitivity [e]			
Median (IQR)	4.1 (2.5, 8.5)	3.8 (2.2, 8.6)	5.4 (2.7, 8.6)
[Min, Max]	[0.2, 22.8]	[0.2, 22.8]	[0.5, 19.2]
Mean (SD)	6.0 (4.9)	5.5 (4.9)	6.6 (5.3)
Presence of cysts [f]			
Yes	55 (43%)	39 (49%)	16 (34%)
No	70 (55%)	39 (49%)	31 (66%)
Ungradable	2 (2%)	2 (2%)	0
Central subfield thickness (microns) [f]			
Median (IQR)	253.0 (228.0, 285.0)	247.0 (223.0, 280.0)	261.0 (246.0, 288.0)
[Min, Max]	[137.0, 519.0]	[137.0, 519.0]	[175.0, 323.0]

[a] Static perimetry results were graded by a reading center. Results are based on the average of 3 fields when 3 tests were performed (primary cohort); otherwise they based on just the 1 test performed (secondary cohort). Static perimetry data is not included for 1 participant in the ARRP group (participant was not tested).

[b] Kinetic perimetry results were graded by a reading center. Seeing area was calculated as isopter area minus scotoma. Scotoma not tested/measured was treated as 0 in the calculation. 49 participants in the USH2 group and 24 participants in the ARRP group have scotomas not tested/measured and treated as 0. 21 participants in the USH2group and 8 participants in the ARRP group have III4e scotomas not tested/measured and treated as 0 (1 subject was excluded for procedure issues). 28 participants in the USH2group and 14 participants in the ARRP group have V4e scotomas not tested/measured and treated as 0 (2 subjects were excluded for procedure issues).

[c] 5 sites used an ETDRS chart, 10 sites use an electronic visual acuity tester, and 1 site used both

[d] Photopic ERG 30 Hz flicker amplitudes are not included for 1 participant in the USH2 group (participant was not tested)

[e] Microperimetry mean retinal sensitivity results were graded by a reading center. Results are based on the average of first two (out of three) tests. Microperimetry mean retinal sensitivity data are not included for 25 participants in the USH2 and 10 participants in the ARRP group (reasons include: 22 not performed in secondary cohort per protocol; in the primary cohort, 10 were not performed because the site did not have the equipment, 2 were not done, 1 was ungradable).

[f] Presence of any cyst and central subfield thickness on OCT were graded by a reading center. Central subfield thickness data are not included for 1 participant in the USH2 group (due to ungradable image).

Table 3. Baseline Static Perimetry Reliability Measures within Test Session and Variability Among Sessions Data in the RUSH2A Study

		Clinical Diagnosis	
	Overall N =126[a]	**USH2 N=80**	**ARRP N=46**
Reliability Factor (%)[b] Median (IQR)			
Overall	5.1 (2.6, 8.2)	5.2 (2.1, 9.1)	5.1 (3.0, 7.3)
Test 1	4.7 (1.9, 9.0)	4.2 (1.7, 8.8)	5.1 (2.8, 9.8)
Test 2	4.7 (2.5, 9.8)	5.1 (2.4, 11.1)	4.6 (2.6, 7.2)
Test 3	5.0 (2.4, 9.0)	5.4 (2.3, 9.4)	4.5 (2.6, 8.8)
False positives rate (%)[b] Median (IQR)			
Overall	1 (0, 3)	1 (0, 4)	2 (0, 3)
Test 1	0 (0, 4)	0 (0, 4)	1 (0, 3)
Test 2	0 (0, 3)	0 (0, 5)	0 (0, 3)
Test 3	0 (0, 5)	0 (0, 5)	0 (0, 4)
False negatives rate (%)[b] Median (IQR)			
Overall	8 (3, 14)	8 (2, 15)	8 (5, 11)
Test 1	6 (3, 15)	6 (0, 13)	8 (3, 15)
Test 2	7 (3, 16)	8 (3, 19)	7 (3, 13)
Test 3	7 (3, 17)	7 (2, 17)	7 (3, 14)
Intraclass correlation coefficient (95% confidence interval) [c]			
Overall (tests 1,2, and 3)	0.96 (0.94 0.97)		Not applicable
Tests 1 and 2	0.98 (0.97 0.98)		Not applicable
Tests 2 and 3	0.95 (0.93 0.97)		Not applicable
Tests 1 and 3	0.94 (0.92 0.96)		Not applicable
Repeatability coefficient (95% confidence interval) [c]			
Overall (tests 1,2, and 3)	13.7 (9.2, 16.3)		Not applicable

[a]Static perimetry results were graded by a reading center. 1 participant in ARRP group was missing all SP tests and is excluded from this table.

[b]Test 2 and Test 3 data are not included for 24 participants, respectively (22 secondary cohort participants only performed the test once, and 2 primary cohort participants were missing the second and the third test)

[c]Variability analysis data are not included for 25 participants (22 secondary cohort participants only performed the test once, and 2 primary cohort participants were missing the second and the third test, and 1 participant was missing the third V_{TOT} value.)

Table 4. Baseline Mean Full Field Hill of Vision (V_{TOT}) in the RUSH2A Study - Stratified by Clinical Diagnosis and Baseline Characteristics[a]

	Overall		Clinical Diagnosis			
			USH2		ARRP	
Characteristic	N=126	V_{TOT} Mean (SD)	N=80	V_{TOT} Mean (SD)	N=46	V_{TOT} Mean (SD)
Gender						
Female	68	27.4 (22.3)	44	22.9 (20.8)	24	35.5 (23.1)
Male	58	28.4 (25.4)	36	21.9 (22.6)	22	38.9 (26.7)
Race/Ethnicity						
White	112	28.2 (23.7)	70	21.9 (20.8)	42	38.6 (24.8)
Hispanic	9	26.4 (24.4)	7	27.6 (25.6)	2	22.4 (28.1)
Asian	5	21.8 (27.1)	3	23.0 (35.4)	2	20.0 (20.1)
Enrollment area						
United States	83	25.7 (23.0)	50	21.1 (21.2)	33	32.5 (24.1)
Europe/UK	43	32.0 (24.8)	30	24.7 (22.1)	13	48.8 (23.1)
Age at enrollment, yrs[b]						
<35	44	35.7 (23.0)	36	33.1 (20.0)	8	47.6 (32.5)
35-45	43	23.9 (22.4)	25	18.3 (20.1)	18	31.8 (23.6)
>= 45	39	23.1 (24.2)	19	7.8 (15.3)	20	37.8 (22.0)
Duration of Disease, yrs[c]						
<10	36	40.5 (22.6)	20	34.3 (20.0)	16	48.2 (23.9)
[10,20)	46	28.5 (21.9)	25	28.7 (23.5)	21	28.3 (20.4)
>=20	43	15.0 (18.2)	35	11.2 (14.8)	8	31.5 (23.4)
Smoking status						
Yes	33	31.2 (24.7)	20	25.9 (24.2)	13	39.3 (24.2)
No	93	26.6 (23.4)	60	21.3 (20.6)	33	36.3 (25.2)
Current use of dietary supplements						
None	53	32.3 (23.9)	41	25.6 (20.5)	12	55.0 (20.7)
Vitamin A only	11	14.9 (16.0)	5	9.5 (12.7)	6	19.5 (18.1)
DHA only	5	15.8 (13.1)	3	17.0 (15.4)	2	13.9 (14.3)
Lutein only	8	32.2 (23.5)	5	24.4 (21.5)	3	45.3 (24.4)
Combination	49	26.4 (24.9)	26	20.2 (24.6)	23	33.4 (23.7)

[a]Static perimetry results were graded by a reading center. Results are based on the average of 3 fields when 3 tests were performed (primary cohort); otherwise they are based on the 1 test performed (secondary cohort). Static perimetry data is not included for 1 participant in the ARRP group (participant was not tested). Factors are presented categorically to show the data but were analyzed using a continuous version of the factor in the model. None of the other factors in the table were significantly associated with V_{TOT} once disease duration, age of enrollment and clinical diagnosis were accounted for (P-value not shown).

[b]28 participants were not permitted to report date of birth due to regulatory restrictions. Therefore, only year of birth and categorical age was reported. For those participants, July 1st with the reported birth year was imputed as birth date to calculate continuous age

[c]1 participant in the ARRP group was missing age of onset (a participant-reported field based on their awareness of visual symptoms) and duration of disease (computed based on age of onset and date of enrollment)

Table 5. Baseline Mean and Adjusted Mean Full Field Hill of Vision (V_{TOT}) in the RUSH2A Study

Models	N=126[a]	Mean (SD) – decibel steradians	Adjusted Mean (95% CI)- decibel steradians[b]	Difference from Reference Group (95% CI)	P-value[c]
Clinical Diagnosis					<0.001
USH2	80	22.5 (21.5)	22.9 (17.9, 28.0)	Reference	
ARRP	46	37.1 (24.7)	36.3 (29.5, 43.1)	13.4 (4.2, 22.6)	
Duration of disease, yrs[d]					<0.001
<10	36	40.5 (22.6)	39.1 (31.9, 46.3)	Reference	
[10,20)	46	28.5 (21.9)	27.9 (21.7, 34.0)	-11.2 (-20.3, -2.1)	
>=20	43	15.0 (18.2)	21.9 (13.8, 30.1)	-17.2 (-28.9, -5.4)	
Age of enrollment, yrs[e]					0.02
<35	44	35.7 (23.0)	35.4 (27.1, 43.6)	Reference	
35-45	43	23.9 (22.4)	27.7 (21.3, 34.1)	-7.6 (-18.4, 3.1)	
>=45	39	23.1 (24.2)	25.8 (18.8, 32.7)	-9.6 (-21.4, 2.2)	

[a]Static perimetry results were graded by a reading center. Results are based on the average of 3 fields when 3 tests were performed (primary cohort); otherwise they based on just the 1 test performed (secondary cohort). Static perimetry data is not included for 1 participant in the ARRP group (participant was not tested).

[b]Simultaneous adjustment for duration of disease, clinical diagnosis, and age of enrollment

[c]Factors are presented categorically to show the data but were analyzed using continuous version of the factor in the model.

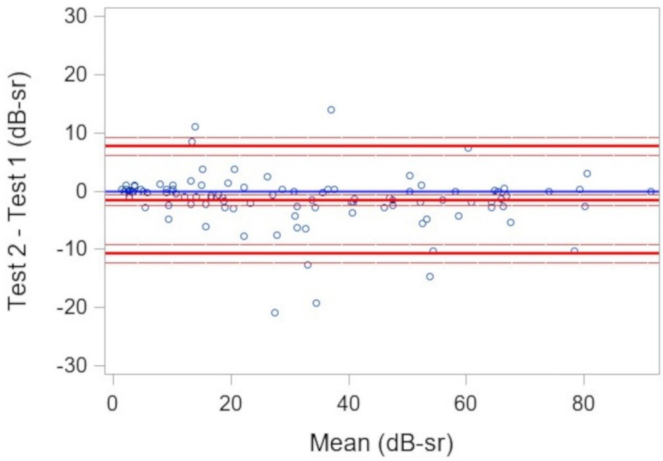
[d]1 participant in the ARRP group was missing age of onset (a participant-reported field based on their awareness of visual symptoms) and duration of disease (computed based on age of onset and date of enrollment)

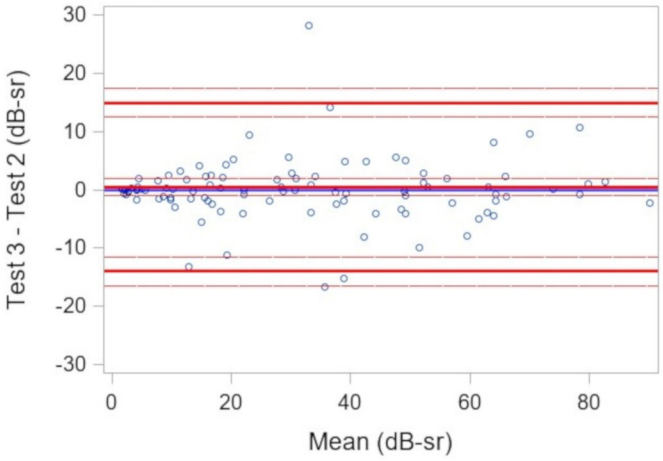
[e]28 participants were not permitted to report date of birth due to regulatory restrictions. Therefore, only year of birth and categorical age was reported. For those participants, July 1st with the reported birth year was imputed as birth date to calculate continuous age

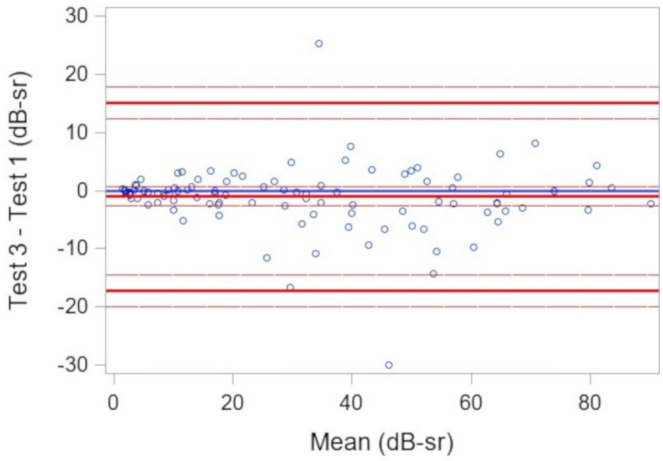
Table 6. Correlation of Baseline V_{TOT} with Other Baseline Functional and Structural Measures in the RUSH2A Study

	Overall		Clinical Diagnosis					
			USH2		ARRP			
	N=126[a]	V_{TOT}[a] Mean (SD)	N=80	V_{TOT}[a] Mean (SD)	N=46	V_{TOT}[a] Mean (SD)	Correlation Coefficient[b]	P Value[b]
III4e seeing area (deg^2)[c]							0.93	<0.001
<710	40	5.8 (6.1)	36	5.8 (6.3)	4	5.4 (4.0)		
[710, 4000)	33	19.0 (8.7)	19	20.8 (9.0)	14	17.0 (8.2)		
[4000, 8000)	25	38.7 (12.4)	13	37.4 (11.4)	12	40.1 (13.9)		
>=8000	27	61.8 (15.5)	12	58.8 (17.4)	15	64.1 (13.9)		
VA ETDRS letter score (approx. Snellen equivalent)[d]							0.59	<0.001
<69 (<20/40)	14	8.6 (12.6)	11	3.3 (3.1)	3	27.9 (16.4)		
69-73 (20/40)	13	15.8 (19.4)	9	12.7 (17.6)	4	22.9 (23.9)		
74-78 (20/32)	24	19.1 (15.8)	17	17.8 (12.5)	7	22.1 (22.8)		
79-83 (20/25)	33	24.1 (20.9)	18	19.2 (18.1)	15	30.0 (23.1)		
>=84 (>=20/20)	42	45.8 (22.6)	25	39.9 (23.1)	17	54.5 (19.5)		
Photopic ERG 30 Hz flicker amplitudes (μV)[e]							0.54	<0.001
0	37	16.9 (16.6)	25	14.5 (16.7)	12	22.1 (15.8)		
(0, 1.8)	20	13.9 (12.4)	15	12.8 (13.0)	5	17.2 (10.8)		
[1.8, 6.8)	34	27.1 (21.2)	24	27.7 (22.9)	10	25.7 (17.6)		
>=6.8	34	48.9 (23.9)	15	37.7 (24.2)	19	57.8 (20.2)		
MP mean retinal sensitivity (dB)[f]							0.55	<0.001
<2	16	20.6 (18.6)	12	14.4 (15.8)	4	39.3 (14.0)		
[2, 4)	28	23.3 (19.2)	17	20.9 (16.3)	11	27.0 (23.4)		
[4, 8)	21	31.0 (23.6)	11	24.4 (21.2)	10	38.3 (25.0)		
>=8	26	53.3 (20.5)	15	47.1 (21.9)	11	61.8 (15.4)		
Presence of cysts[g]							N/A	0.03
Yes	55	23.1 (23.0)	39	20.2 (20.3)	16	30.4 (28.0)		
No	69	32.2 (23.7)	39	25.6 (22.8)	30	40.7 (22.4)		
Ungradable	2	6.4 (6.2)	2	6.4 (6.2)	0	NA		
Central subfield thickness (μm)[g]							0.48	<0.001
<230	32	11.7 (12.9)	28	8.7 (10.4)	4	32.2 (10.6)		
[230, 250)	22	28.8 (23.1)	13	28.7 (24.6)	9	28.9 (22.2)		
[250, 280)	33	30.2 (25.3)	18	24.6 (23.4)	15	36.8 (26.7)		
>=280	38	39.2 (23.1)	20	36.2 (19.6)	18	42.6 (26.5)		

[a]Static perimetry results were graded by a reading center. Results are based on the average of 3 fields when 3 tests were performed (primary cohort); otherwise they based on just the 1 test performed (secondary cohort). Static perimetry data is not included for 1 participant in the ARRP group (participant was not tested).
[b]Correlation coefficients and p-values are based on analyses combining all participants (both USH2and ARRP groups). Factors are presented categorically to show the data but were analyzed using continuous version of the factor in the analysis.
[c]Kinetic perimetry results were graded by a reading group and 8 participants in ARRP group have III4e scotoma not tested/measured and treated as 0 (1 subject was excluded for procedure issues).
[d]5 sites used an ETDRS chart, 10 sites use an electronic visual acuity tester, and 1 site used both
[e]Photopic ERG 30 Hz flicker amplitudes are not included for 1 participant in the USH2group (participant was not tested)
[f]Microperimetry mean retinal sensitivity results were graded by a reading center. Results are based on the average of first two (out of three) tests. Microperimetry mean retinal sensitivity data are not included for 25 participants in the USH2and 10 participants in the ARRP group (reasons include: 22 not performed in secondary cohort per protocol; in the primary cohort, 10 were not performed because the site did not have the equipment, 2 were MP not done, 1 was ungradable).
[g]Presence of any cyst and central subfield thickness on OCT were graded by a reading center. Central subfield thickness data are not included for 1 participant in the USH2group (due to ungradable image). The P-value for presence of any cyst was calculated using T-test.







Highlights

- Disease duration and age are associated with visual field loss in *USH2A*-related RD
- Visual field loss is more severe in USH2 than ARRP
- Total hill of vision is repeatable
- Total hill of vision correlates with function and structure