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Purpose: The purpose of this study was to evaluate baseline best corrected visual acuity (BCVA), full-field electroretinography (ERG), full-field stimulus thresholds (FST), and their relationship with baseline demographic and clinical characteristics in the Rate of Progression in Usher syndrome type 2 (*USH2A*)-related Retinal Degeneration (RUSH2A) multicenter study.

Methods: Participants had Usher syndrome type 2 (USH2, $N = 80$) or autosomal recessive nonsyndromic retinitis pigmentosa (ARRP, $N = 47$) associated with biallelic variants in the *USH2A* gene. Associations of demographic and clinical characteristics with BCVA, ERG, and FST were assessed with regression models.

Results: In comparison to ARRP, USH2 had worse BCVA (median 79 vs. 82 letters; $P < 0.001$ adjusted for age), lower rod-mediated ERG b-wave amplitudes (median 0.0 vs. 6.6 µV; $P < 0.001$) and 30 Hz flicker cone-mediated ERG amplitudes (median 1.5 vs. 3.1 µV; $P = 0.001$), and higher (white, blue, and red) FST thresholds (means [−26, −31, −23 dB] vs. [−39, −45, −28 dB]; $P < 0.001$ for all stimuli). After adjusting for age, gender, and duration of vision loss, the difference in BCVA between diagnosis groups was attenuated ($P = 0.09$). Only diagnosis was associated with rod- and cone-mediated ERG parameters, whereas both genders ($P = 0.04$) and duration of visual loss ($P < 0.001$) also were associated with FST white stimulus.

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Conclusions: USH2 participants had worse BCVA, ERG, and FST than ARRP participants. FST was strongly associated with duration of disease; it remains to be determined whether it will be a sensitive measure of progression.

Translational Relevance: Using standardized research protocols in RUSH2A, measures have been identified to monitor disease progression and treatment response and differentiate features of prognostic relevance between USH2 and ARRP participants with *USH2A* mutations.

Introduction

Variants in the Usher syndrome type 2 (*USH2A*) gene are among the most common causes of inherited retinal degenerations (IRDs).[1] Biallelic variants can result in early partial sensorineural hearing loss combined with retinitis pigmentosa (RP) - namely Usher syndrome type 2 (USH2), the most common form of Usher syndrome. In addition, *USH2A* variants account for 12% to 25% of individuals with nonsyndromic autosomal recessive RP (ARRP), thereby also representing the most common cause of ARRP.[2,3] Given that treatment trials for *USH2A*-related retinal degeneration are ongoing, and that additional trials are planned, it is imperative to learn more about the natural history of *USH2A*-mediated disease in order to select the best outcome measures of change in visual function. *The Rate of Progression of USH2A-related Retinal Degeneration (RUSH2A)* study was initiated in 2017. The goal of this multicenter, international, longitudinal natural history study is to evaluate the role of state-of-the-art testing modalities to determine the most effective and time-sensitive methods for monitoring these individuals in future trials. Secondary goals are to identify risk factors that influence the rate of progression, to evaluate relationships among different functional and structural measures, and to identify a pool of well-characterized potential participants for anticipated treatment trials.

Best-corrected visual acuity (BCVA), full-field electroretinography (ERG) and the full-field stimulus threshold (FST) test are three of the outcome measures evaluated in the study herein. BCVA was measured with the Early Treatment of Diabetic Retinopathy Study (ETDRS) eye charts, and the electronic visual acuity (EVA) equivalent, which are in widespread use in clinical trials.[4,5] The ERG is among the most widely used measures for diagnosing and following individuals with IRDs and, in the present study, were used to assess rod- and cone-mediated responses at baseline. The ERG was complemented by the FST test, because individuals with moderate to severe visual loss often have an ERG that is unmeasurable.[6,7] The objectives of the current report are to describe BCVA, ERG, and FST measures at baseline in the RUSH2A study, to evaluate correlations between these functional measures, and to evaluate their associations with clinical characteristics.

Methods

Study Design

Details of the design of this multicenter, longitudinal natural history study (NCT03146078) have been described previously.[8] Briefly, 127 participants were enrolled at 16 clinical sites in North America and Europe. The study adhered to the tenets of the Declaration of Helsinki and was approved by the institutional review boards (IRBs) or ethics boards associated with each participating site.

Participants were at least 8 years old with a clinical diagnosis of rod-cone degeneration associated with at least 2 disease-causing *USH2A* sequence variants in trans. A committee reviewed all genetic reports to confirm the variants as pathogenic or likely pathogenic. The majority of testing was performed in the "study" eye, defined as the eye with better baseline BCVA. The primary cohort included 105 participants with a baseline ETDRS letter score of 54 or greater (20/80 or better) in the better eye, central visual field at least 10 degrees diameter, and stable fixation. A secondary cohort of 22 participants with ETDRS letter score of 53 or worse (20/100 or worse), central visual field less than 10 degrees diameter, or unstable fixation, was enrolled to complete a baseline visit only. Both cohorts are combined in this baseline cross-sectional report.

Outcome Measures

The visit schedule and testing procedure for this prospective study have been documented previously.[8] The primary focus of the current report is baseline BCVA, ERG, and FST measures. All measurements were performed by study certified technicians following standardized protocols. Following subjective

refraction, BCVA was measured as the ETDRS letter score on the EVA tester or ETDRS charts.[4,5] Only BCVA from study eyes was used for analyses.

Full-field ERG was performed following the International Society for Clinical Electrophysiology of Vision (ISCEV) protocol.[9] The full-field ERG measures in the current analyses included the amplitude of the b-wave from the dark adapted dim-flash 0.01 cd.s/m2 ERG response (DA 0.01 ERG), which reflects a rod-driven bipolar cell response; the amplitude of the b-wave of the dark adapted standard flash 3.0 cd.s/m2 ERG (DA 3.0 ERG), which arises from a combined response of both rod and cone systems; and the trough-to-peak amplitude of the light-adapted 30 Hz flicker (LA 3.0 flicker ERG), which is cone-driven.

FST was performed on the Espion E^3 (Diagnosys LCC, Lowell, MA). White, blue, and red stimuli were used for FST testing, with a two parameter Weibull function determining the actual threshold, while considering false positives and false negatives.[7] In the FST protocol for all three colors, the baseline of 0 dB was defined as 0.1 cd/m^2. Thresholds were measured in triplicates for each color and the averaged result from the three tests were used for each color in order to determine receptor type mediating threshold.

Statistical Methods

The distributions of BCVA, ERG, and FST measures were summarized using means and standard deviations (SDs) or medians and interquartile ranges (IQRs), depending on the distribution of the data. The percentage of eyes with no response on ERG testing was also reported. Correlations among BCVA, ERG, and FST measures were assessed with Spearman correlation coefficients.

Associations between participant characteristics and BCVA, ERG, and FST outcomes were assessed. Linear regression models were used to assess the association between each participant characteristic and BCVA score. A stepwise selection method was used to determine a multivariable model for BCVA score. Clinical diagnosis was forced into the model and other factors with P value < 0.05 were considered as statistically significant and remained in the final model. Because BCVA scores have a skewed distribution, the ranked normal score transformation of the BCVA score was used as an outcome variable in the regression model. Similar regression models were used for the FST outcomes. All three ERG outcomes had a large proportion of zero responses and the b-wave amplitude had a skewed distribution; analyses for these outcomes were performed with generalized linear regression models for the Tweedie distribution and a log link function.[10] All analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC) and reported P values are two-sided.

Results

Study Population

A detailed description of the RUSH2A participant enrollment flow and baseline characteristics was previously published.[8] A total of 127 participants were enrolled into the study, with 105 in the primary cohort and 22 in the secondary cohort. The clinical diagnosis based on the self-reported hearing loss history was USH2 for 80 (63%) participants and ARRP for 47 (37%) participants. The median age was 37 years (IQR = 27 to 44 years) for the USH2 group and 44 years (IQR = 36 to 50 years) for the ARRP group. Overall, 68 of the participants (54%) were women, 113 (89%) were white, and 83 (65%) were enrolled in the US or Canadian sites. The median age at onset of first noticed vision loss was 16 years (IQR = 13 to 22 years) for the USH2 group and 32 years (IQR = 20 to 41 years) in the ARRP group. Among the 122 participants with audiology testing data, 97% (73 of 75) of the USH2 participants had moderate or worse hearing loss, whereas only 9% (4 of 47) of the ARRP participants had moderate adult-onset hearing loss.

Summary of BCVA, ERG, and FST Findings at Baseline

Summary of the BCVA, ERG, and FST findings at baseline are provided in Table 1 for the entire study group and stratified by clinical diagnosis. Overall, 75 (59%) of the participants had a baseline BCVA of > 79 letters ($\geq$ 20/25). Fifty-four percent of the USH2 group and 68% of the ARRP group had BCVA > 79 letters. Those with USH2 had significantly worse BCVA scores (median, 79 letters), than those with ARRP (median, 82 letters; $P < 0.001$ after adjusting for age).

All but one RUSH2A participant had ERG testing. Scotopic responses to a dim-flash (DA 0.01) were unmeasurable in 40 (51%) of participants with USH2 compared with 19 (40%) of those with ARRP. The median rod-driven b-wave amplitude was significantly larger in ARRP than in USH2 (6.6 vs. 0.0 µV, $P < 0.001$ after adjusting for age; see Table 1). A lower percentage of the overall cohort had unmeasurable mixed rod-cone DA 3.0 ERG responses (35%) than DA 0.01 ERG responses (47%; see Table 1). The amplitudes for the DA 3.0 ERG b-wave responses were also significantly larger in ARRP than USH2 (median 11.6 vs. 5.0 µV,

Table 1. Best Corrected Visual Acuity (BCVA), Electroretinography (ERG), and Full-Field Stimulus Threshold (FST) Measures by Clinical Diagnosis

	Overall ($N = 127$)	USH2 ($N = 80$)	ARRP ($N = 47$)	*P* Value
BCVA Letter Score				
<69 (< 20/40)	14 (11%)	11 (14%)	3 (6%)	
69–73 (20/40)	14 (11%)	9 (11%)	5 (11%)	
74–78 (20/32)	24 (19%)	17 (21%)	7 (15%)	
79–83 (20/25)	33 (26%)	18 (23%)	15 (32%)	
≥84 (≥20/20)	42 (33%)	25 (31%)	17 (36%)	
Median (IQR)	80 (75, 85)	79 (74, 85)	82 (77, 87)	<0.001[a]
ERG Responses				
No. with test results	126	79	47	
DA 0.01 ERG amplitude (μV)				
Unmeasurable, n	59 (47%)	40 (51%)	19 (40%)	
Median (IQR)	0.7 (0.0, 7.4)	0.0 (0.0, 5.0)	6.6 (0.0, 19.0)	<0.001[b]
DA 3.0 ERG b-wave amplitude (μV)				
Unmeasurable, n (%)	44 (35%)	30 (38%)	14 (30%)	
Median (IQR)	6.2 (0.0, 15.5)	5.0 (0.0, 11.8)	11.6 (0.0, 64.0)	<0.001[b]
LA 3.0 flicker ERG amplitude (μV)				
Unmeasurable, n	37 (29%)	25 (32%)	12 (26%)	
Median (IQR)	2.0 (0.0, 7.7)	1.5 (0.0, 5.5)	3.1 (0.0, 20.0)	0.001[b]
FST (dB)				
No. with test results	93	56	37	
White stimulus, *mean ± SD*	−32 ± 13	−26 ± 10	−39 ± 13	<0.001[a]
Blue stimulus, *mean ± SD*	−36 ± 14	−31 ± 11	−45 ± 14	<0.001[a]
Red stimulus, *mean ± SD*	−25 ± 7	−23 ± 6	−28 ± 8	<0.001[a]

[a] *P* value calculated using linear regression model, adjusting for age.
[b] *P* value calculated using generalized linear regression model with Tweedie distribution, adjusting for age.

$P < 0.001$ after adjusting for age). The percentage of unmeasurable LA 3.0 flicker ERG responses was even lower in the entire cohort (29%), and median amplitudes were significantly higher in the ARRP group than the USH2 group (3.1 vs. 1.5 μV, $P = 0.001$ after adjusting for age; Fig. 1A, Table 1). Compared with normal individuals (typical mean ± SD: 28 ± 2.5 ms),[11] participants with ARRP had a delay in LA 3.0 flicker ERG implicit time (mean ± SD: 38 ± 6 ms) that was significantly ($P = 0.005$) greater than implicit time delay observed in USH2 (mean ± SD: 34 ± 6 ms; Fig. 1B). FST was not available at all sites, with 93 participants (73%) undergoing testing. There were significant differences for all three stimuli, white, blue, and red, between clinical diagnosis groups ($P < 0.001$), with the ARRP group showing lower thresholds (less severely impaired retinal function) than the USH2 group (see Table 1). Figure 2 shows representative FST threshold results from three different participants and a normal subject. Each graph shows the probability of a positive response against the stimulus intensity. Panel A, (USH2, age 55 years old), with a white threshold of −20 dB, represents an example of a participant with primarily cone mediated thresholds, because the thresholds to the photopically matched blue and red were similar. The participant shown in panel B (USH2, age 19 years old) has a threshold to white of −35 dB. The difference in blue and red thresholds was 10 dB, suggesting that rods are mediating the response to the blue (and white) stimulus. The participant shown in panel C (USH2, age 61 years old) had a mean white threshold of −55 dB. Thresholds for the blue stimulus were 25 dB lower than thresholds to red, consistent with rod mediation and similar to the blue-red threshold difference seen in a normal observer (panel D, age 25 years old).[6]

The relationship between FST white stimulus threshold and blue-red threshold difference by duration of vision loss is shown in Figure 3 for all participants. A difference between blue and red FST thresholds of ≤ 10 dB appears to indicate cone-mediated dark-adapted thresholds. The upper right region of the

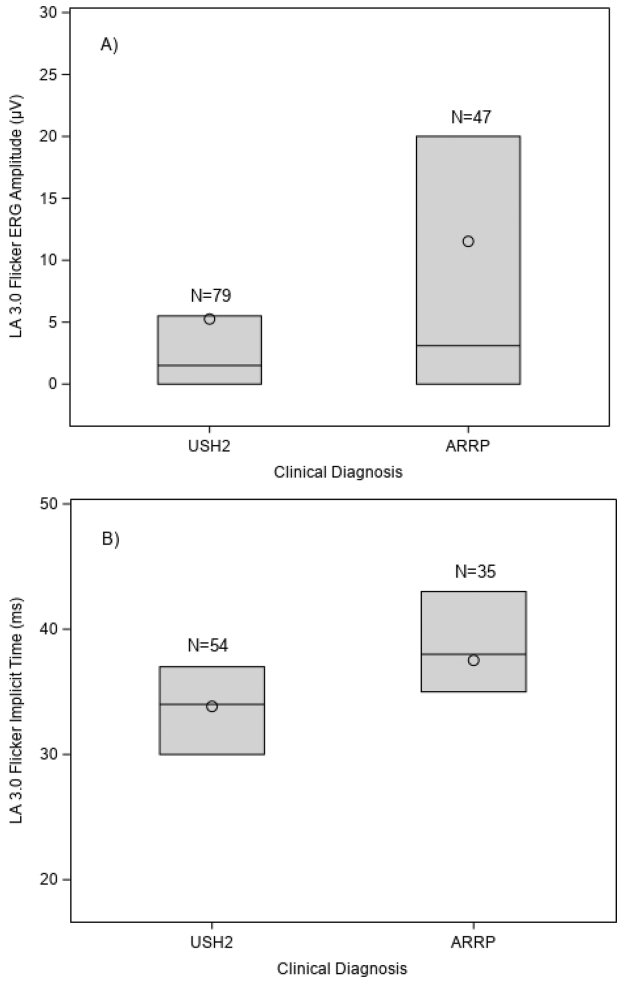

Figure 1. Light-adapted 3.0 flicker Electroretinography (ERG) response by clinical diagnosis. (**A**) Amplitude; (**B**) implicit time. The bottom and top of each box denote the 25th and 75th percentiles, the line inside the box denotes the median and the circle is the mean.

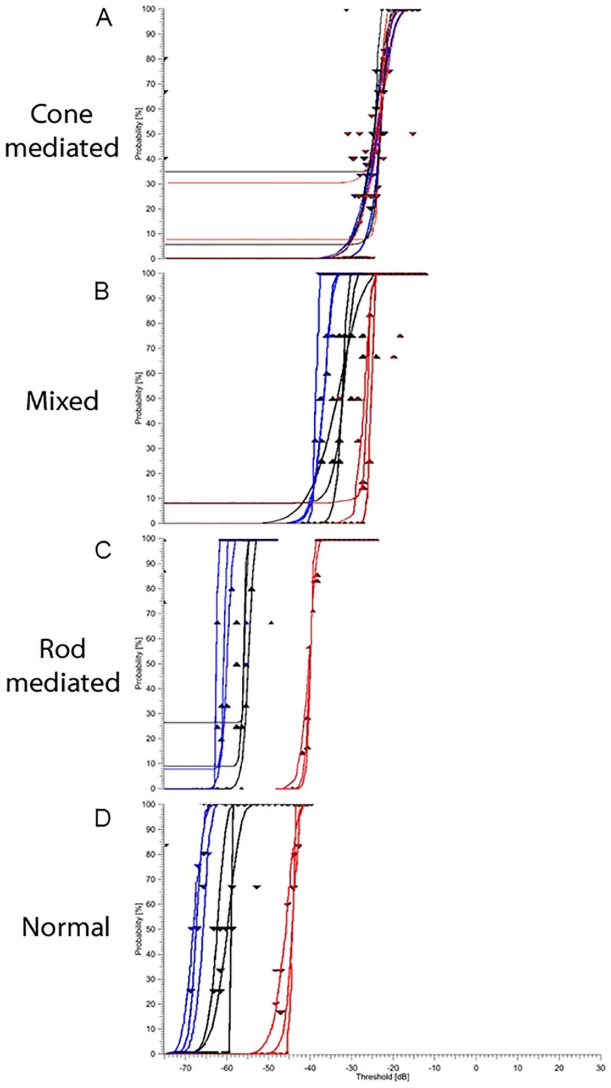

Figure 2. Full-field stimulus thresholds (FST) threshold results from three different participants and one normal subject. Black, blue, and red colors represent responses from white, blue, and red stimuli, respectively. (**A**) Cone-mediated (USH2, age 55 years old); (**B**) mixed (USH2, age 19 years old); (**C**) rod-mediated (USH2, age 61 years old); (**D**) normal (age 25 years old).[7]

graph shows that approximately 40 of 93 participants (43%) with FST data showed no definite evidence of rod function. For these participants, white thresholds were above −30 dB and blue-red differences around zero, indicating that cones might be primarily mediating FST thresholds. The lower left corner shows participants with blue-red differences of ≥20 dB indicating rod mediation. Based on these results, the lower limit for cone mediation of the white stimulus appears to be −30 dB. Thus, when a participant has a white threshold below −30 dB, we can assume that the patient has rods mediating the threshold. Those with remaining rod function are primarily those with < 20 years of reported vision loss (39 of 44), whereas participants with cone-mediated thresholds tended to have > 20 years of reported vision loss (28 of 49).

Correlations Among BCVA, ERG, and FST Measures

Correlations among BCVA, ERG, and FST measures are shown in Table 2. Not surprisingly, most measures are at least moderately correlated, because all reflect the severity of disease. As expected, there is a low correlation (0.17) of the BCVA with the DA 0.01 ERG, but the correlation with LA 3.0 flicker ERG though stronger, is still a limited correlation (0.30). The FST white threshold was moderately correlated with BCVA (−0.60; Supplementary Figure S2) and with LA 3.0 flicker (−0.55) and DA 3.0 (−0.64) ERG (see Table 2).

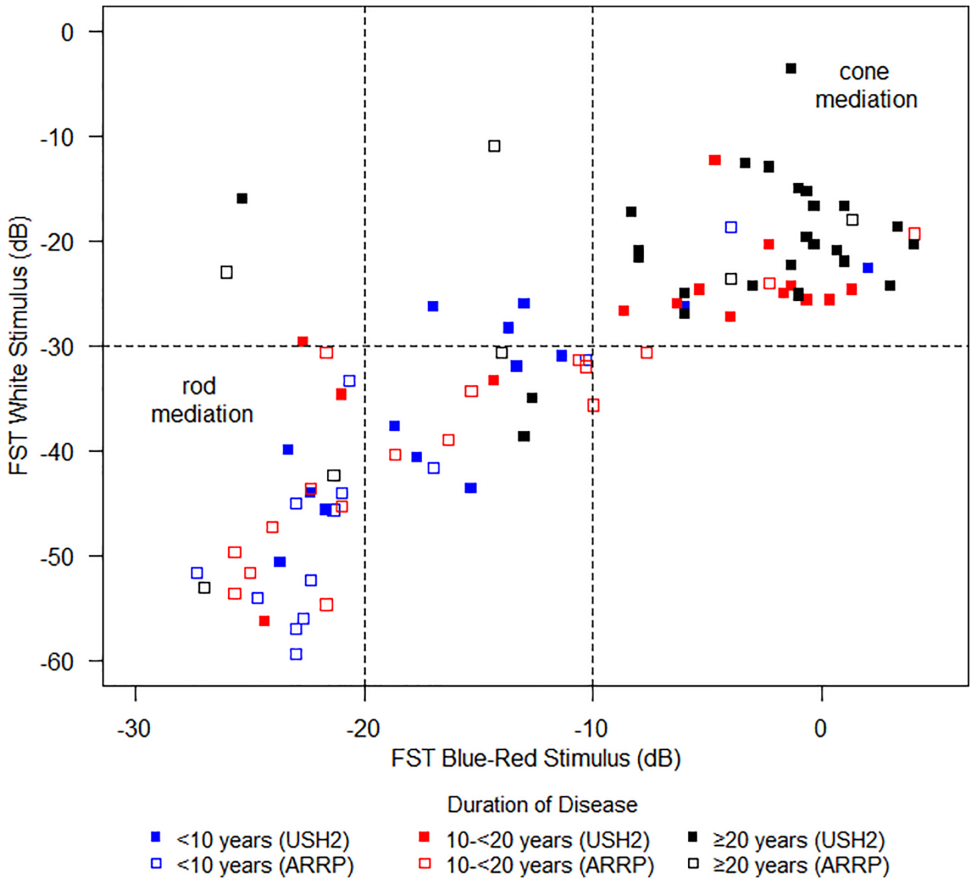

Figure 3. Full-field stimulus thresholds (FST) white versus blue-red by duration of disease and clinical diagnosis. Filled symbols represent USH2 participants and open symbols represent ARRP participants. Blue, red, and black symbols represent duration of disease at < 10 years, 10 to < 20 years, and ≥20 years, respectively.

Association of Baseline Characteristics with BCVA

Median (IQR) of BCVA letter score by participant characteristics are shown in Table 3. Several participant characteristics were significantly associated with lower BCVA letter score in univariable analyses: USH2 phenotype ($P = 0.03$), older age at enrollment ($P < 0.001$), and longer duration of vision loss ($P < 0.001$). From the multivariable analysis, age at enrollment, duration of vision loss, and gender were retained in the model, with women having significantly lower VA scores than men. Clinical diagnosis was marginally associated with BCVA letter score after taking other factors into account. Race/ethnicity, smoking status, and dietary supplement use were not associated with baseline BCVA letter score.

Association of Baseline Characteristics with ERG

The variables associated with LA 3.0 flicker ERG trough-to-peak amplitude are shown in Table 4. Clinical diagnosis is the only factor that was significantly associated with LA 3.0 flicker ERG response, with the median amplitude in the ARRP group being roughly twice the amplitude in the USH2 group ($P = 0.004$). As shown in Supplementary Figure S1, age was not associated with DA 0.01 ERG amplitude (panel A), DA 3.0 DA amplitude (panel B), or LA 3.0 flicker amplitude (panel C). We also analyzed DA 0.01 ERG amplitude and LA 3.0 flicker ERG implicit times (data not shown); the only characteristic associated with either of these parameters was clinical diagnosis.

Association of Baseline Characteristics with FST

The participant characteristics associated with the white FST thresholds are shown in Table 5. Clinical diagnosis was a strong determinant, with mean threshold to white stimulus being 13 dB lower (i.e. better sensitivity) in the ARRP group than in the USH2 group. Duration of vision loss was also strongly associated with white thresholds; participants with ≥20 years

Table 2. Spearman Correlation Coefficients Among Best Corrected Visual Acuity (BCVA), Electroretinography (ERG), and Full-Field Stimulus Threshold (FST) Measures

	Best Corrected Visual Acuity	Electroretinography			Full-Field Stimulus Threshold (FST)		
	(BCVA) ($N = 127$)[a]	DA 0.01 ERG ($N = 126$)	LA 3.0 Flicker ($N = 126$)	DA 3.0 ERG ($N = 126$)	White ($N = 93$)	Blue ($N = 93$)	Red ($N = 93$)
BCVA							
Correlation	1.0	+0.17	+0.30	+0.30	−0.60	−0.56	−0.58
P value		0.06	<0.001	<0.001	<0.001	<0.001	<0.001
DA 0.01 ERG							
Correlation	+0.17	1.0	+0.61	+0.69	−0.40	−0.40	−0.45
P value	0.06		<0.001	<0.001	<0.001	<0.001	<0.001
LA 3.0 flicker ERG							
Correlation	+0.30	+0.61	1.0	+0.82	−0.55	−0.52	−0.42
P value	<0.001	<0.001		<0.001	<0.001	<0.001	<0.001
DA 3.0 ERG							
Correlation	+0.30	+0.69	+0.82	1.0	−0.64	−0.62	−0.59
P value	<0.001	<0.001	<0.001		<0.001	<0.001	<0.001
FST White							
Correlation	−0.60	−0.40	−0.55	−0.64	1.0	+0.96	+0.83
P value	<0.001	<0.001	<0.001	<0.001		<0.001	<0.001
FST Blue							
Correlation	−0.56	−0.40	−0.52	−0.62	+0.96	1.0	+0.76
P value	<0.001	<0.001	<0.001	<0.001	<0.001		<0.001
FST Red							
Correlation	−0.58	−0.45	−0.42	−0.59	+0.83	+0.76	1.0
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	

[a]Among 127 participants with VA scores, 126 had ERG results and 93 had FST results; all participants with ERG results had FST results.

disease duration having a mean threshold 18 dB higher than those with < 10 years disease duration. Gender was only marginally associated with white thresholds ($P = 0.04$). Mean unadjusted gender difference (men and women) in FST white thresholds was only 1.7 dB (95% confidence interval [CI] = −3.7 to 7.0), however, in the final multivariable regression model, mean adjusted gender difference was 4.3 dB (95% CI = 0.2 to 8.5).

Disease Asymmetry

Disease asymmetry between the right eye and left eye was assessed on BCVA letter scores. The mean difference between the right eye and left eye (OD–OS) was −1.0 letters (95% CI = −2.3 to 0.3) and the intraclass correlation coefficient was 0.85. The difference was similar in both the USH2 group (mean = −0.9) and the ARRP group (mean = −1.2). No significant correlation was found between the difference in BCVA letter scores and gender ($P = 0.21$) or duration of disease ($P = 0.15$).

Discussion

The majority of participants enrolled in the RUSH2A study were middle-aged (median age 40 years old),[8] with USH2 participants being slightly younger (median 37 years) than the ARRP group (median 44 years). Most participants in the USH2 group (median 79 letters; 20/25) and the ARRP group (median 82 letters; 20/25) retained good BCVA, consistent with results from previous studies.[12] Sandberg et al.[2] found that the majority of 125 patients with *USH2A* sequence variants had one to two lines of acuity loss at 30 years of age and the median age of legal blindness due to decreased BCVA was 65 years. Calzetti et al.[13] reported acuity better than 20/50 at age 50 in all 14 patients with USH2. Despite the gener-

Table 3. Participant Characteristics Associated With Best Corrected Visual Acuity (BCVA)

	N	BCVA Letter Score Median (Q1, Q3)	Univariable Analysis *P* Value	Multivariable Analysis *P* Value
All	127	80 (75, 85)		
Clinical diagnosis			0.03	0.09
USH2	80	79 (74, 85)		
ARRP	47	82 (77, 87)		
Age at enrollment, y			<0.001[a]	0.04
<30 y	30	82 (77, 89)		
30 to < 40 y	34	80 (76, 84)		
40 to < 50 y	37	82 (77, 85)		
≥50 y	26	72 (64, 79)		
Gender			0.09	0.01
Female	68	80 (73, 84)		
Male	59	80 (75, 86)		
Duration of disease, y[b]			<0.001[a]	0.004[a]
<10	37	83 (77, 87)		
10 to < 20	46	81 (76, 86)		
≥20	43	75 (66, 82)		
Race/ethnicity			0.44	NA[c]
Non-Hispanic white	113	80 (75, 85)		
Other	14	80 (75, 81)		
Smoking status			0.33	NA[c]
Ever smoked daily	33	82 (77, 84)		
Never smoked daily	94	79 (74, 85)		
Dietary supplement use			0.59	NA[c]
None	53	82 (75, 86)		
Vitamin A only	11	78 (74, 82)		
DHA only	5	77 (76, 80)		
Lutein only	9	79 (76, 84)		
Multiple supplements	49	79 (72, 85)		

[a]Variable was analyzed as continuous.

[b]One 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]Factors with *P* values > 0.05 in the stepwise selection process were not included in the final multivariable model.

ally good acuity, we nevertheless found that BCVA in USH2 was significantly worse than in patients with ARRP, despite a slightly younger median age. Others have also reported that acuity is better in nonsyndromic patients than syndromic patients at the same age.[14] Among the participant characteristics evaluated, only age of enrollment, disease duration as measured from reported age of visual loss, and gender were associated with BCVA. The association with gender has not been found previously and has no obvious explanation.

Unlike BCVA, the full-field ERG was severely reduced in our participants. The majority had unmeasurable DA 0.01 ERG responses (mean age 40 years, range = 14–80), severely limiting the value of this as a prospective measure in clinical trials. Similarly, others have reported unmeasurable DA 0.01 ERGs by age 26 years[15] and in 17 of 18 patients in another cohort.[13] As anticipated in a rod-cone dystrophy, cone responses to LA 3.0 flicker ERG were less affected and remained measurable in the majority of participants at all ages. Similar to other studies,[16] the ARRP group had significantly larger LA 3.0 flicker ERG amplitudes than the USH2 group despite being older on average. In a multivariate analysis, no participant characteristic other than clinical diagnosis was associated with LA 3.0 flicker ERG amplitude. Unmeasurable responses were found at all ages and there was only a weak corre-

Table 4. Participant Characteristics Associated With Light-Adapted 3.0 Flicker Electroretinography (ERG) Amplitude

	N	LA 3.0 Flicker ERG Amplitude (μV) Median (Q1, Q3)	Unmeasurable %	Univariable Analysis *P* Value[a]	Multivariable Analysis *P* value[a]
All	126	2.0 (0.0, 7.7)	29%		
Clinical diagnosis				0.004	0.004
USH2	79	1.5 (0.0, 5.5)	32%		
ARRP	47	3.1 (0.0, 20.0)	26%		
Age at enrollment, y				0.69[b]	NA[c]
<30 y	30	3.1 (0.2, 6.8)	23%		
30 to < 40 y	34	2.4 (0.0, 6.7)	32%		
40 to < 50 y	36	1.9 (0.0, 12.9)	31%		
≥50 y	26	1.8 (0.0, 7.2)	31%		
Gender				0.49	NA[c]
Female	68	2.0 (0.0, 6.6)	34%		
Male	58	2.2 (0.1, 11.7)	24%		
Duration of disease, y[d]				0.06[b]	NA[c]
<10	37	5.9 (1.8, 12.8)	16%		
10 to < 20	46	1.7 (0.0, 6.2)	33%		
≥20	42	0.8 (0.0, 4.5)	38%		
Race/ethnicity				0.28	NA[c]
Non-Hispanic white	112	2.0 (0.0, 7.6)	29%		
Other	14	3.0 (0.0, 7.7)	36%		
Smoking status				0.52	NA[c]
Ever smoked daily	33	1.5 (0.0, 7.2)	33%		
Never smoked daily	93	2.3 (0.0, 7.7)	28%		
Dietary supplement use				0.13	NA[c]
None	52	2.1 (0.0, 7.9)	35%		
Vitamin A only	11	2.0 (0.0, 7.2)	27%		
DHA only	5	0.0 (0.0, 1.3)	60%		
Lutein only	9	8.8 (1.8, 10.3)	22%		
Multiple supplements	49	2.2 (0.1, 6.7)	22%		

[a]Continuous variable for DA 3.0 flicker amplitude was used as dependent variable.
[b]Variable was analyzed as continuous.
[c]Factors with *P* values > 0.05 in the stepwise selection process were not included in the final multivariable model.
[d]One 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).

lation with BCVA, presumably because most of the cones that contribute to the cone ERG are extrafoveal. Delayed LA 3.0 flicker ERG implicit times are characteristic of RP[17] and were present in virtually all patients in the RUSH2A cohort. The USH2 group had significantly less delays than the ARRP group, presumably because flicker responses in patients with small fields are dominated by foveal cones, which are faster than parafoveal cones.[18]

FST was originally developed to provide a metric in extremely low vision patients who could not perform other tests reliably.[6,7] FST does not require stable fixation and reflects the global light-sensitivity of the remaining photoreceptors. Although it is a less spatially specific measure compared to, for example, microperimetry or static perimetry, it has been used to provide a key reproducible outcome measure for a registration trial of the gene therapy voretigene neparvovec-rzyl (Luxturna, Spark Therapeutics) for patients with *RPE65*-related retinal dystrophy.[19] Here, for the first time, it is being used as a functional measure in a large prospective study. Using blue and

Table 5. Participant Characteristics Associated With Full-Field Stimulus Thresholds (FST) White Stimulus

	N	FST White Stimulus (dB) Mean ± SD	Univariable Analysis *P* Value	Multivariable Analysis *P* Value
All	93	−32 ± 13		
Clinical diagnosis			<0.001	<0.001
USH2	56	−26 ± 10		
ARRP	37	−39 ± 13		
Age at enrollment, y			0.61[a]	NA[b]
<30 y	20	−33 ± 9		
30 to < 40 y	26	−31 ± 12		
40 to < 50 y	27	−32 ± 15		
≥50 y	20	−30 ± 15		
Gender			0.53	0.04
Female	51	−31 ± 12		
Male	42	−32 ± 14		
Duration of disease, y[c]			<0.001[a]	<0.001[a]
<10	27	−40 ± 11		
10 to < 20	33	−33 ± 11		
≥20	33	−22 ± 9		
Race/ethnicity			0.53	NA[b]
Non-Hispanic white	84	−32 ± 13		
Other	9	−29 ± 10		
Smoking status				
Ever smoked daily	26	−31 ± 13	0.94	NA[b]
Never smoked daily	67	−32 ± 13		
Dietary supplement use				
None	35	−32 ± 12	0.55	NA[b]
Vitamin A only	8	−33 ± 13		
DHA only	5	−24 ± 6		
Lutein only	5	−37 ± 9		
Multiple supplements	40	−31 ± 15		

[a]Variable was analyzed as continuous.
[b]Factors with *P* values > 0.05 in the stepwise selection process were not included in the final multivariable model.
[c]Computed from age of onset (a participant-reported field based on their awareness of visual symptoms) and date of enrollment.

red stimuli, it was possible to identify participants with primarily rod-mediated thresholds to the white stimulus. White thresholds were strongly associated with clinical diagnosis and duration of vision loss, and weakly associated with gender. It remains to be determined whether unexpected gender differences in BCVA and FST will be present on future visits. The moderate correlation between FST threshold and BCVA was unexpected but may be consistent with the more general finding that cone loss (and reduced BCVA) occurs after substantial loss of rod function. It remains to be determined whether FST will be a sensitive method for following progression in this population.

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References

1. Pontikos N, Arno G, Jurkute N, et al. Genetic basis of inherited retinal disease in a molecularly characterized cohort of more than 3000 families from the United Kingdom [In Press]. *Ophthalmology*, https://doi.org/10.1016/j.ophtha.2020.1004.1008.
2. Sandberg MA, Rosner B, Weigel-DiFranco C, McGee TL, Dryja TP, Berson EL. Disease course in patients with autosomal recessive retinitis pigmentosa due to the USH2A gene. *Invest Ophthalmol Vis Sci*. 2008;49(12):5532–5539.
3. García-García G, Aller E, Jaijo T, et al. Novel deletions involving the USH2A gene in patients with Usher syndrome and retinitis pigmentosa. *Mol Vis*. 2014;20:1398–1410.
4. Beck RW, Moke PS, Turpin AH, et al. A computerized method of visual acuity testing: adaptation of the early treatment of diabetic retinopathy study testing protocol. *Am J Ophthalmol*. 2003;135(2):194–205.
5. Ferris FL, 3rd Kassoff A, Bresnick GH, Bailey I. New visual acuity charts for clinical research. *Am J Ophthalmol*. 1982;94(1):91–96.
6. 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(8):N51–N56.
7. Klein M, Birch DG. Psychophysical assessment of low visual function in patients with retinal degenerative diseases (RDDs) with the Diagnosys full-field stimulus threshold (D-FST). *Doc Ophthalmol*. 2009;119(3):217–224.
8. Duncan JL, Liang W, Maguire MG, et al. Baseline visual field findings in the RUSH2A study:

associated factors and correlation with other measures of disease severity. *Am J Ophthalmol*. 2020;219:87–100.
9. McCulloch DL, Marmor MF, Brigell MG, et al. ISCEV Standard for full-field clinical electroretinography (2015 update). *Doc Ophthalmol*. 2015;130(1):1–12.
10. Kurz CF. Tweedie distributions for fitting semicontinuous health care utilization cost data. *BMC Med Res Methodol*. 2017;17(1):171.
11. Birch DG, Anderson JL. Standardized full-field electroretinography. Normal values and their variation with age. *Arch Ophthalmol*. 1992;110(11):1571–1576.
12. Stingl K, Kurtenbach A, Hahn G, et al. Full-field electroretinography, visual acuity and visual fields in Usher syndrome: a multicentre European study. *Doc Ophthalmol*. 2019;139(2):151–160.
13. Calzetti G, Levy RA, Cideciyan AV, et al. Efficacy outcome measures for clinical trials of USH2A caused by the common c.2299delG mutation. *Am J Ophthalmol*. 2018;193:114–129.
14. Hendriks M, Verhoeven VJM, Buitendijk GHS, et al. Development of refractive errors-What can we learn from inherited retinal dystrophies? *Am J Ophthalmol*. 2017;182:81–89.
15. 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(2):734–743.
16. Sengillo JD, Cabral T, Schuerch K, et al. Electroretinography reveals difference in cone function between syndromic and nonsyndromic USH2A patients. *Sci Rep*. 2017;7(1):11170.
17. Berson EL. Retinitis pigmentosa and allied diseases: applications of electroretinographic testing. *Int Ophthalmol*. 1981;4(1-2):7–22.
18. Birch DG, Fish GE. Focal cone electroretinograms: aging and macular disease. *Doc Ophthalmol*. 1988;69(3):211–220.
19. Russell S, Bennett J, Wellman JA, et al. Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial. *Lancet*. 2017;390(10097):849–860.