Foundation Fighting Blindness (FFB) Consortium

Rate of Progression in USH2A Related Retinal Degeneration (RUSH2A)

Version 3.0
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Signature Page

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List of Abbreviations

ANCOVA	Analysis of covariance
AOSLO	Adaptive Optics Scanning Laser Ophthalmoscopy
ADRP	Autosomal dominant retinitis pigmentosa
ARRP	Autosomal recessive retinitis pigmentosa
BCVA	Best corrected visual acuity
CFR	Code of Federal Regulations
DAVF	Dark-adapted visual field
DAC	Dark-adapted Chromatic
DHA	Docosahexaenoic acid
DHHS	Department of Health and Human Services
ERG	Electroretinogram
ETDRS	Early Treatment of Diabetic Retinopathy Study
EVA	Electronic visual acuity
EZ	Ellipsoid zone
FDA	Food and Drug Administration
FST	Full-field sensitivity testing
GVF	Goldmann visual field
IOP	Intraocular pressure
IRB	Institutional Review Board
LVP-FVQ-II	L.V. Prasad-Functional Vision Questionnaire
MRDQ	Michigan Retinal Degeneration Questionnaire
MP	Microperimetry
N	Number or sample size
OD	Right Eye
OS	Left Eye
OU	Both eyes
PI	Principal investigator
PRO	Patient-reported outcomes
ROI	Region of interest
RPE	Retinal pigment epithelial cells
RP	Retinitis pigmentosa
RUSH2A	Rate of Progression in USH2A Related Retinal Degeneration
SD	Standard Deviation
SD-OCT	Spectral domain optical coherence tomography
SE	Study eye
VA	Visual acuity
VA LV VFQ-48	48-item Veterans Affairs Low Vision Visual Functioning Questionnaire
VPA	Valproic acid

CHAPTER 1.
1. BACKGROUND AND RATIONALE

1.1 Background

Usher syndrome is a rare disease, affecting 3.0-16.7 (1-3) per 100,000 people in the United States, for a prevalence of 10,285-53,677 (4). However, it represents a leading cause of autosomal recessive deaf-blindness (5). Clinically, Usher syndrome is divided into 3 types, based on the severity and onset of hearing loss (6). Usher syndrome type 1 is associated with profound congenital hearing loss, absent vestibular responses, and retinitis pigmentosa (RP) (or rod greater than cone photoreceptor degeneration) beginning in the first decade of life, and represents 33-44% of all Usher syndrome (6). Usher syndrome type 2 is associated with a sloping audiogram with less severe congenital hearing impairment, normal vestibular responses, and RP beginning in the first or second decade. It represents 56-67% of all Usher syndrome (7, 8). Usher syndrome type 3 is the least common form, accounting for <3% of Usher syndrome, and is associated with progressive hearing loss, variable vestibular responses and variable onset of RP (9-11). Usher syndrome is genetically heterogeneous and at least 11 genes and 4 loci have been associated with autosomal recessive Usher syndrome (6).

The most common gene mutated in patients with Usher syndrome type 2 is *USH2A*, with at least one mutation in 57-79.3% of Usher syndrome type 2 patients (12, 13). Prior studies in patients with Usher syndrome have demonstrated that photoreceptor degeneration is primary with secondary retinal pigment epithelial (RPE) degeneration in patients with mutations in the *MYO7A, PCDH15, USH2A*, and *GPR98* genes, indicating a shared mechanism of photoreceptor degeneration preceding RPE loss (14). However, patients with *USH2A* mutations showed a wider spectrum of disease expression than other causes of Usher syndrome type 2 (15-18) and regions with normal retinal function were observed in some patients (18).

USH2A mutations may also cause RP without congenital hearing loss (RP 39) (12, 19-23) and *USH2A* mutations may represent the most common cause of autosomal recessive RP in the U.S. (12). Retinal degeneration associated with mutations in the *USH2A* gene is characterized by slowly progressive rod, then cone, photoreceptor death, and relentless vision loss over decades. Because the *USH2A* gene is large (790 kb spanning 72 exons with introns varying from 127 base pairs to 78 kilobases in length) (24), it exceeds the carrying capacity of standard adeno-associated or lentiviral vectors used to deliver gene therapy in other autosomal-recessive retinal degenerations, such as *RPE65*-related retinal degeneration, *MERTK*-related retinal degeneration, *USH1B*-related retinal degeneration, and choroideremia (associated with mutations in the *REP1* gene) (25, 26). As new treatments for *USH2A*-related retinal degeneration are developed, a clear understanding of the natural history of disease progression of *USH2A*-related retinal degeneration is necessary.

Limited natural history data are available from patients with Usher syndrome type 2. In general, the natural history studies reported to date provide information that was obtained with manual kinetic perimetry in patients prior to widespread genotyping, and the studies lack adequate robustness to define a population for a treatment trial or the most efficient and relevant endpoints. One study followed 19 patients for 5.58 years and reported that Goldmann visual field (GVF) decline in all Usher syndrome type 2 patients combined is similar to that in RP (27); another study of 58 patients

with Usher syndrome type 2 showed 3 patterns of visual field loss which all shared half-lives between 4.59-6.42 years for the GVF V4e target (28). Two retrospective studies have provided limited natural history data in patients with *USH2A*-related retinal degeneration with and without congenital hearing loss. Further, these studies used older measurement techniques not suitable for multicenter clinical trials (Snellen acuity charts, Goldmann kinetic perimetry), the annual rates of decline were found to be 2.6% for visual acuity, 7.0% for visual field area, and 13.2% for 30 Hz photopic full-field electroretinogram (ERG) amplitudes (29). Earlier this year a retrospective study reported visual acuity, GVF kinetic perimetry, and full-field electroretinography in 225 consecutive European patients with *USH2A*-related retinal degeneration; although many patients in both groups had the same mutations in the *USH2A* gene, patients with Usher syndrome type 2a developed symptoms, were visually impaired and were legally blind at earlier ages than patients with nonsyndromic RP (30).

None of the preceding studies included longitudinal characterization of the retinal phenotype using quantitative standard evaluation modalities, such as spectral-domain optical coherence tomography (SD-OCT) and static perimetry or investigated patient-reported outcomes (PROs). In addition, much of the data was obtained retrospectively without standard research protocols, such as standard measures of visual acuity according to the Early Treatment of Diabetic Retinopathy Study (ETDRS) protocol (31), so it is unclear which parameters provide the most sensitive and robust outcome measures to follow in a longitudinal, multicenter study of disease progression.

SD-OCT scans provide objective, non-invasive measures of outer retinal structure, and have been shown to correlate with visual function in eyes with retinal degeneration (32-34). SD-OCT measures of retinal structure such as the ellipsoid zone (EZ) band width or area may be less variable than functional measures such as visual acuity, visual field, and full-field ERG, and may show significant change during disease progression in shorter times than do standard functional measures (32, 35). However, rates of change in EZ area have not been evaluated and have not been correlated with standard measures of visual function in eyes with *USH2A* mutations. Fundus-guided microperimetry, which evaluates macular function with greater precision and resolution than standard perimetry, and can be used to correlate with SD-OCT measures of retinal structure (36, 37), has also not been examined in patients with *USH2A*-related retinal degeneration. Full-field sensitivity testing (FST) measures the most sensitive rod- and cone-mediated parts of the visual field (38); since *USH2A*-related retinal degeneration affects rods earlier and more severely than cone photoreceptors, and in many patients the rod-mediated full-field ERG is severely reduced below levels that can be reliably measured at diagnosis, FST could provide a sensitive and quantitative measure of rod-mediated function in eyes with *USH2A*-related retinal degeneration. In addition, no studies have reported PRO measures in patients with *USH2A*-mutations; PROs offer an opportunity to develop validated, quantitative outcome measures describing the impact of *USH2A*-related retinal degeneration on patient experience and quality of life, and no studies have reported PROs in patients with USH2A mutations.

Congenital hearing loss is a defining feature of Usher syndrome type 2A, but adult-onset hearing loss has also been reported in non-syndromic RP associated with *USH2A* mutations (16). Standardized audiometric tests could provide outcome measures to characterize the effect of *USH2A* mutations on hearing and provide insight into the extent and severity of dual sensory loss in patients with *USH2A* –related retinal degeneration. Finally, the *USH2A* gene is expressed in

photoreceptor connecting cilia, and normal olfactory function requires normal cilia. Olfactory function has been evaluated in patients with Usher syndrome and results have shown variable degrees of olfactory dysfunction (39-41). The present study will investigate quantitative measures of auditory and olfactory function as a non-invasive, low cost way to characterize the clinical phenotype of patients with *USH2A*-related retinal degeneration.

Newer outcome measures of retinal degeneration such as EZ area, microperimetry, FST thresholds, PROs, audiometry, and olfactory testing have not been validated or examined in patients with *USH2A*-related retinal degeneration, but could provide more sensitive measures of disease progression than traditional measures, such as visual acuity and kinetic visual field sensitivity, that have been described in the past.

1.2 Rationale

This natural history study of patients with *USH2A* mutations will accelerate the development of outcome measures for clinical trials. Sensitive, objective outcome measures of retinal degeneration will greatly facilitate development of treatments for Usher syndrome patients. Together these approaches are expected to have an impact on understanding *USH2A*-related retinal degeneration, developing experimental treatment protocols, and assessing their effectiveness.

The goals and expected impact of this natural history study are to:

1. Report the natural history of retinal degeneration in patients with biallelic mutations in the *USH2A* gene
2. Identify sensitive structural and functional outcome measures to use for future multicenter clinical trials in *USH2A*-related retinal degeneration
3. Identify well-defined subpopulations for future clinical trials of investigative treatments for *USH2A*-related retinal degeneration

1.3 Study Objectives

The primary objectives of the natural history study are to:

1. Characterize the natural history of retinal degeneration associated with biallelic pathogenic mutations in the *USH2A* gene over 4 years, as measured using functional outcome measures (static perimetry, microperimetry, full-field stimulus threshold, electroretinography, and visual acuity; listed in section 1.4.4)
2. Characterize the natural history of retinal degeneration associated with biallelic pathogenic mutations in the *USH2A* gene over 4 years, as measured using structural outcome measures (SD-OCT EZ area; listed in section 1.4.4)
3. Investigate structure-function relationships for insights into the mechanisms of retinal degeneration by relating changes in SD-OCT EZ area to visual field progression in individuals with biallelic pathogenic mutations in the *USH2A* gene
4. Assess for possible genotype, phenotype, and environmental risk factors with progression of the outcome measures at 4 years (listed in section 1.4.4) in individuals with biallelic pathogenic mutations in the *USH2A* gene

Some additional secondary objectives of this study include:

1. Characterize baseline cross-sectional retinal degeneration associated with biallelic pathogenic mutations in the *USH2A* gene (as measured using the main outcome measures listed in section 1.4.4)
2. Investigate comorbidities associated with disease (baseline cross-sectional) and disease progression (longitudinal natural history study) in individuals with biallelic pathogenic mutations in the *USH2A* gene
3. Explore patient reported outcome (PRO) measures associated with disease (baseline cross-sectional) and disease progression (longitudinal natural history study) in individuals with biallelic pathogenic mutations in the *USH2A* gene
4. Evaluate variability and symmetry of left and right eye kinetic perimetry and SD-OCT outcomes at baseline and at 4 years in individuals with biallelic pathogenic mutations in the *USH2A* gene

1.4 Synopsis of Study Design

1.4.1 Study Design

This study is designed as a multicenter, longitudinal, prospective natural history study.

A second cohort of eyes with more severe vision impairment (20/100 or worse or unstable fixation or visual field area <10°) will enroll into a cross-sectional baseline study only.

1.4.2 Major Eligibility Criteria

Key eligibility criteria include:

- Participants with clinical diagnosis of rod-cone degeneration and at least 2 disease-causing mutations in *USH2A* gene from a clinically-certified lab report **will be eligible for the Genetics Screening Phase.** Of these participants, the following additional criteria must be met for the longitudinal natural history or cross-sectional baseline study eligibility:
  - Participants who have a clinical diagnosis of Usher syndrome type 2a (with congenital hearing loss) **will be eligible for the study** with no further segregation analysis
  - Participants who have non-syndromic RP (without congenital hearing loss) must have mutations which are homozygous or heterozygous *in trans* **to be eligible for the study**. This will require additional segregation analyses and genetics evaluation if the phase of the alleles is not already known from the clinically-certified laboratory report

1.4.3 Sample Size

- Participants will be enrolled into the Genetics Screening Phase until the recruitment goals of each cohort (below) are met
- Due to expected symmetry of measurements, one study eye per participant will be enrolled into the study. This will be identified as the eye with better baseline visual acuity. If both eyes have the same baseline visual acuity (within one Snellen line), the determination will

be made at investigator discretion as the eye with better fixation or clearer ocular media to permit ophthalmic imaging

- A **primary cohort** (***study eye baseline*** visual acuity ETDRS letter score of 54 or more [approximate Snellen equivalent 20/80 or better] and stable fixation and clinically determined [on Octopus 900 Pro] kinetic visual field III4e diameter 10° or more in every meridian of the central field) of 100 participants will be enrolled into the longitudinal natural history study
- A **secondary cohort** (***study eye baseline*** visual acuity ETDRS letter score of 53 or less [approximate Snellen equivalent 20/100 or worse] or unstable fixation or clinically determined [on Octopus 900 Pro] kinetic visual field III4e diameter less than 10° in any meridian of the central field) of 20 participants will be enrolled into the baseline cross-sectional study

1.4.4 Main Outcomes

1. Visual field sensitivity measured by static perimetry with topographic analysis (Hill of Vision)
2. Best corrected E-ETDRS visual acuity
3. Mean retinal sensitivity as measured by fundus-guided microperimetry
4. EZ area as measured by SD-OCT
5. Rod- and cone-mediated retinal function as measured by FST
6. Retinal function using full-field ERG amplitudes and timing in response to rod- and cone-specific stimuli

1.4.5 Visit Schedule and Procedures

Table 1. Visit Schedule and Procedures

Procedures	Baseline[c]	12M	24M	36M	48M
Visit Windows	Day 0	Wk 52 ± 4	Wk 104±4	Wk 156± 4	Wk 208± 4
Olfactory Test	X				
Audiology[d]	X				
Patient Reported Outcomes	X		X		X
Demographics/Medical History/Vitals	X				
Concomitant Medications/Medical Conditions	X	X	X	X	X
Complete Ophthalmic Exam[a]	OU	OU	OU	OU	OU
Refraction and E-ETDRS VA Testing (EVA) or ETDRS charts	OU	OU	OU	OU	OU
SD-OCT with measurement of EZ area (Heidelberg Spectralis) [e]	OU	SE	SE	SE	OU
Color Fundus Photos [f]	OU				
Full-field ERG [f, g]	SE				SE[h]
Kinetic visual field area (Octopus 900 Pro) [e]	OU				OU
Static visual field volume (Octopus 900 Pro) [b, e]	SE (x3)	SE	SE	SE	SE
Fundus-guided microperimetry (MAIA, where available) [b, e]	SE (x3)	SE	SE	SE	SE
Full-field Stimulus Threshold (Diagnosys Espion, where available)	SE (x3)	SE (x3)	SE (x3)	SE (x3)	SE (x3)

OU=both eyes SE=study eye

a Ophthalmic exam includes slit-lamp biomicroscopy, indirect ophthalmoscopy and intraocular pressure (IOP). IOP measurements will be taken prior to pupil dilation. Whenever possible the site should make its best effort to ensure that the exam takes place at approximately the same time of the day at each visit and with the same equipment

b Third perimetry may be completed at an additional baseline visit, within 14 days of the designated baseline visit date

c For primary cohort – all tests are required as indicated. For secondary cohort – microperimetry will not be required; static perimetry will only be performed once; all other tests will be completed as noted

d Audiology – to be performed within 30 days of the baseline visit, in primary and secondary cohort participants

e SD-OCT, Kinetic visual field area, static visual field area, and fundus guided microperimetry images will be submitted to a reading center for grading. Details of this process are specified in the RUSH2A Procedures Manual

f Some ERG images and Color Fundus Photos will be collected for quality review/grading as needed. Details of this process are specified in the RUSH2A Procedures Manual

g ERG to be performed within 60 days of the baseline and 48M visits

h If ERG is undetectable at baseline, no need to perform at 48M, at investigator's discretion

1.5 General Considerations

The study is being conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, with the protocol described herein, and with the standards of Good Clinical Practice. The RUSH2A Procedures Manuals provide details of the procedures followed by the clinical sites. Data will be directly collected in electronic case report forms, which will be considered the source data.

The risk level for this protocol is considered to be research not involving greater than minimal risk (45 CFR 46.404). A risk-based monitoring approach will be followed, consistent with the FDA "Guidance for Industry Oversight of Clinical Investigations — A Risk-Based Approach to Monitoring" (August 2013).

CHAPTER 2

2. PARTICIPANT ELIGIBILITY AND ENROLLMENT

2.1 Identifying Eligible Participants and Obtaining Informed Consent

Identifying Eligible Participants

Potential eligibility will be assessed during a routine examination by an investigator prior to obtaining informed consent, as part of usual care. The following will be determined as part of this assessment:

1. Confirm potential participant meets all eligibility criteria for Genetics Screening Phase (section 2.2)
2. If potential participant is already known to have non-syndromic RP with heterozygous mutations inherited *in cis*, then the site should not proceed with enrollment
3. If enrollment for the *anticipated* cohort has closed, then the site should not proceed with enrollment
    a. NOTE: The actual cohort will be determined by baseline testing (section 2.5.2.1)

Obtaining Informed Consent/Assent and Enrollment

Prior to completing any procedures or collecting any data that are not part of usual care, informed consent will be obtained. Depending on ethics board requirements the consent may be written, verbal, or electronic. For adults (18 years or older), the consent will be obtained from the participant. For children (8 to 17 years old), informed consent will be obtained from the parent or guardian and a child assent form will be obtained from minors who are of appropriate age according to ethics board requirements.

The consent process will include the following. For patients who are considered eligible for the study based on a routine-care exam, the study protocol will be discussed with the potential study participant/parent by a study investigator or clinic coordinator. The potential study participant/parent will be given the Informed Consent Form to read. Participants with severe vision/hearing impairment may require a review of the Informed Consent Form by a translator according to ethics board requirements. Potential study participants/parents will be encouraged to discuss the study with family members and personal physician(s) before deciding whether to participate in the study. After the informed consent is completed, enrollment will be accomplished using the study website. Consented participants will initially be enrolled into the Genetics Screening Phase. After completion of this phase, those meeting the additional criteria to enter the study will continue into either the cross-sectional baseline study or the natural history study; those who do not will exit prior to completion of any baseline procedures.

End of Recruitment

Participants *anticipated* to be in the primary cohort (based on routine-care examination) will be enrolled into the Genetics Screening Phase until 100 have been confirmed eligible for the natural history study **and** confirmed in the primary cohort based on study eye visual acuity, fixation, and kinetic visual field area at baseline.

- This means more than 100 primary cohort participants may be screened; the number and reason for screen failures will be tracked

- This also means more than 100 may enroll into the natural history study, if some have already been enrolled into the screening phase when the 100th is confirmed

Participants *anticipated* to be in the secondary cohort (based on routine-care examination) will be enrolled into the Genetics Screening Phase until 20 have been confirmed eligible for the cross-sectional baseline study **and** confirmed in the secondary cohort based on study eye visual acuity, fixation, and kinetic visual field area at baseline.

- This means more than 20 secondary cohort participants may be screened; the number and reason for screen failures will be tracked
- This also means more than 20 may enroll into the cross-sectional baseline study, if some have already been enrolled into the screening phase when the 20th is confirmed

Sites will be notified as the recruitment goals near completion, and efforts will be made to accurately predict the number of participants in queue for screening phase in order to keep the number enrolled beyond the goal at a minimum.

2.2 Eligibility Criteria for Genetics Screening Phase

To be eligible for enrollment into the Genetics Screening Phase, a study participant must meet all of the inclusion criteria and none of the exclusion criteria. All criteria will be reconfirmed prior to entry into the study (either the natural history study or the cross-sectional baseline study) and completion of baseline procedures.

2.2.1 Study Participant Inclusion Criteria

1. Willing and able to complete the informed consent process
2. Ability to return for all study visits over 48 months if in the natural history study
3. Age ≥ 8 years
4. At least 2 disease-causing mutations in *USH2A* gene from a clinically certified lab report

2.2.2 Study Participant Exclusion Criteria

1. Mutations in genes that cause autosomal dominant RP, X-linked RP, or presence of biallelic mutations in autosomal recessive RP/retinal dystrophy genes other than *USH2A*
2. Expected to enter experimental treatment trial at any time during this study
3. History of more than 1 year of cumulative treatment, at any time, with an agent associated with pigmentary retinopathy (including hydroxychloroquine, chloroquine, thioridazine, and deferoxamine)

2.2.3 Ocular Inclusion Criteria

Both eyes must meet all of the following:

1. Clinical diagnosis of a rod-cone degeneration
2. Clear ocular media and adequate pupil dilation to permit good quality photographic imaging
3. Ability to perform kinetic and static perimetry reliably

2.2.4 Ocular Exclusion Criteria

If either eye has any of the following, the patient is not eligible:

1. Current vitreous hemorrhage
2. Current or any history of rhegmatogenous retinal detachment
3. Current or any history of (e.g., prior to cataract or refractive surgery) spherical equivalent of the refractive error worse than -8 Diopters of myopia
4. History of intraocular surgery (e.g., cataract surgery, vitrectomy, penetrating keratoplasty, or LASIK) within the last 3 months
5. Current or any history of confirmed diagnosis of glaucoma (e.g., based on glaucoma visual field, nerve changes, or glaucoma filtering surgery)
6. Current or any history of retinal vascular occlusion or proliferative diabetic retinopathy
7. Expected to have cataract removal surgery during the study
8. History or current evidence of ocular disease that, in the opinion of the investigator, may confound assessment of visual function
9. History of treatment for retinitis pigmentosa that could affect the progression of retinal degeneration (including participation in a clinical trial within the last year or a retained drug delivery device)

2.3 Genetics Screening Phase

1. Participants who are eligible and who provide the appropriate consent (section 2.1 and 2.2) will enroll into the Genetics Screening Phase

2. A Genetics Screening Form will be completed by the site, using the clinically-certified lab report (which was used to identify eligibility criterion #4 under section 2.2.1) to enter clinical diagnosis ("Usher syndrome type 2a," defined as RP with congenital hearing loss, or "non-syndromic RP," defined as RP without congenital hearing loss), and whether mutations were homozygous or heterozygous inherited *in trans* or *in cis*, if known. Depending on the data entered, the following will occur:

   - Participants who have (1) Usher syndrome type 2a or (2) non-syndromic RP with either homozygous *USH2A* mutations or heterozygous *USH2A* mutations inherited *in trans* will be eligible for the study without further genetic screening (section 2.4)

   - Participants who have non-syndromic RP with heterozygous *USH2A* mutations inherited *in cis* will not be eligible for the study and will be exited

   - Participants who have non-syndromic RP and for whom phase of alleles is unknown will be asked to provide a saliva sample, and approach 1-2 first degree relatives to provide a saliva sample for additional genetics review. The first-degree relative(s) will be provided with information on how to provide informed consent and how to complete the saliva kit. The participant's and first-degree relative(s) samples will be shipped to and analyzed by the central lab to determine the phase of the alleles. Details of this process are specified in the RUSH2A Procedures Manuals
     - Those determined to have homozygous *USH2A* mutations or heterozygous *USH2A* mutations inherited *in trans* will reconfirm eligibility (section 2.2) and proceed to the natural history study or cross-sectional baseline study (section 2.4)
     - Those determined to have heterozygous *USH2A* mutations inherited *in cis* will not be eligible for the study and will be exited

2.4 Eligibility Criteria for Natural History Study / Cross-Sectional Baseline Study

To be eligible for the natural history study or the cross-sectional baseline study, the following must be met:

1. Genetics screening criteria (section 2.3) must be met, i.e.:
    a. Participant has (1) Usher syndrome type 2a or (2) non-syndromic RP with either homozygous *USH2A* mutations or heterozygous *USH2A* mutations inherited *in trans*
2. All the eligibility criteria for the Genetics Screening Phase (section 2.2) must be re-confirmed prior to entry into the natural history study or the cross-sectional baseline study

The following additional criteria will be evaluated in the study eye as part of the baseline testing, to determine the cohort and the correct study to enter:

- Participants with baseline visual acuity ETDRS letter score of 54 or more [approximate Snellen equivalent 20/80 or better] and stable fixation and clinically determined [on Octopus 900 Pro] kinetic visual field III4e diameter 10° or more in every meridian of the central field of the study eye ("primary cohort") will be enrolled into the natural history study
- Participants with baseline visual acuity ETDRS letter score of 53 or less [approximate Snellen equivalent 20/100 or worse] or unstable fixation or clinically determined [on Octopus 900 Pro] kinetic visual field III4e diameter less than 10°in any meridian of the central field of the study eye ("secondary cohort") will be enrolled in the cross-sectional baseline study

2.5 Baseline Visit

2.5.1 Historical Information

A history will be elicited from the potential study participant and extracted from available medical records. Data to be collected will include demographic data, medical conditions, and concomitant medications.

2.5.2 Baseline Testing Procedures

The following procedures serve as baseline measures for the study. The testing procedures are detailed in the RUSH2A Procedures Manuals. The visual acuity, kinetic visual field, SD-OCT, static visual field, photos, ERG, and microperimetry testing must be performed by a certified technician. All baseline testing must be completed within 14 days of the designated baseline visit date, except for audiology which must be completed within 30 days, and ERG which must be completed within 60 days.

2.5.2.1 Baseline Testing Performed to Determine Study Eye and Cohort

1. ETDRS visual acuity testing must be performed **in both eyes** (best corrected) on the electronic visual acuity tester (EVA) or ETDRS charts. A protocol refraction is required

2. Kinetic visual field area (using Octopus 900 Pro) must be performed **in both eyes**

Visual acuity testing and kinetic visual field area must be performed prior to static perimetry, microperimetry, full-field stimulus threshold, and full-field ERG to determine the study eye and then cohort, in that specific order.

- The eye with better visual acuity is the study eye. If both eyes have the same baseline visual acuity, the determination will be made at investigator discretion as the eye with better fixation or clearer ocular media to permit ophthalmic imaging
- The baseline study eye visual acuity, fixation, and kinetic visual field area will then determine the cohort (see section 2.4)

2.5.2.2 Remainder of Baseline Testing Performed at Clinical Site

Testing to perform on both eyes:

1. Complete ophthalmic exam. Exam will include slit-lamp biomicroscopy, indirect ophthalmoscopy, and intraocular pressure (IOP). IOP measurements will be taken prior to pupil dilation
2. SD-OCT with measurement of EZ area (Heidelberg Spectralis)
3. Color fundus photos (no equipment requirements)

Testing to perform on study eye only:

1. Static visual field (using Octopus 900 Pro).
    a. For primary cohort, performed three times. Third perimetry may be completed at a subsequent visit (must be completed within 14 days of baseline)
    b. For secondary cohort, performed once
2. Fundus-guided microperimetry (MAIA, where available)
    a. Participants at sites where MAIA is unavailable will not complete this testing
    b. For primary cohort, performed three times. Third microperimetry may be completed at a subsequent visit (must be completed within 14 days of baseline)
    c. For secondary cohort, not performed
3. Full-field stimulus threshold (Diagnosys Espion, where available)
    a. Participants at sites where Diagnosys Espion is unavailable will not complete this testing
    b. Performed three times, within each: blue, red, and white stimulus
4. Full-field ERG (Diagnosys Espion preferred)

Additional testing:

1. Patient Reported Outcomes
    a. Adult patients aged ≥18 and older will be tested using the 48-item Veterans Affairs Low Vision Visual Functioning Questionnaire (VA LV VFQ-48) (42). Additional detail is included in the RUSH2A Procedures Manual
    b. Children aged 8-<18 years will be tested using the second version of the L.V. Prasad-Functional Vision Questionnaire (LVP-FVQ-II) (43). Additional detail is included in the RUSH2A Procedures Manual

2. Olfactory testing

a. Olfactory testing kits will be used. Investigators will be provided with a scoring template to interpret and score the results. Additional detail is included in the RUSH2A Procedures Manual

2.5.2.3 Additional Baseline Testing

The following baseline tests are to be performed **within 30 days** of the baseline visit, at the study site or a non-study site, in primary and secondary cohort participants.

1. **Audiology testing**
   a. Investigators will order audiology tests as specified in the RUSH2A Procedures Manual, to be performed by an audiologist, unless the participant has cochlear implants in both ears. These results will be returned to the clinical site by the patient or audiologist's office, to be entered by the clinical site into the study website. The test results will be reviewed by an audiology expert.

2.5.3 Genetics Committee Review

For participants confirmed eligible and completing the baseline visit, the site will upload the clinically certified lab report (which was used to identify eligibility criterion #4 under section 2.2.1) to the study website for review by a genetics committee. The committee will review this report as well as the data from the Genetics Screening Phase to confirm the mutations as pathogenic or likely pathogenic. Details of the process are described in the RUSH2A Procedures Manuals. Cases that are not confirmed as pathogenic or likely pathogenic will remain in the study and will not be considered ineligible, however their data may be analyzed separately from those with pathogenic mutations.

CHAPTER 3

3. FOLLOW-UP VISIT SCHEDULE AND PROCEDURES

The baseline visit date is considered to be study day 0. Follow up visit schedules and procedures apply only to the primary cohort.

3.1 Follow-up Visit Schedule

Study visits will be conducted at:

- 12 months (52 ± 4 Weeks)
- 24 months (104 ± 4 Weeks)
- 36 months (156 ± 4 Weeks)
- 48 months (208 ± 4 Weeks)

Out-of-window visits may still be completed and used for analysis. Details regarding limits for these windows and when to consider a visit missed are specified in the RUSH2A Procedures Manuals.

Testing procedures at unspecified visits are at investigator discretion. However, it is recommended that procedures that are performed should follow the standard protocol for each procedure.

3.2 Follow-up Visit Testing and Procedures

At each visit, an interval history will be elicited, which will include treatment of the study eye. The following procedures will be performed according to the schedule described below. The testing procedures are detailed in the RUSH2A Procedures Manuals. The visual acuity, kinetic visual field, OCT, static visual field, ERG, and microperimetry testing must be performed by a certified technician.

3.2.1 Testing to Perform at Every Visit

Testing performed on both eyes

1. Complete ophthalmic exam. Exam will include slit-lamp biomicroscopy, indirect ophthalmoscopy, and intraocular pressure (IOP). IOP measurements will be taken prior to pupil dilation
2. ETDRS visual acuity testing in each eye (best corrected) on the EVA or ETDRS charts. A protocol refraction is required

Testing performed on study eye only

1. Static visual field (using Octopus 900 Pro)
2. Fundus-guided microperimetry (MAIA, where available)
    a. Participants at sites where MAIA is unavailable will not complete this testing
3. Full-field stimulus threshold (Diagnosys Espion, where available)
    a. Participants at sites where Diagnosys Espion is unavailable will not complete this testing
    b. Performed three times, within each: blue, red, and white stimulus

Testing performed on study eye only at 12 months, 24 months, and 36 months and on both eyes at 48 months

1. SD-OCT with measurement of EZ area (Heidelberg Spectralis)

3.2.2 Testing to Perform at 24 Months and 48 Months Only

1. Patient Reported Outcomes
    a. Adult patients aged ≥18 will be tested using the 48-item Veterans Affairs Low Vision Visual Functioning Questionnaire (VA LV VFQ-48) (42).
    b. Children aged 8-<18 years will be tested using the second version of the LVP-FVQ-II (43).
    c.

Note: At **48 Months** these PROs may be completed in person or remotely (phone or other remote methods) any time within the 48-month visit window (208 ± 4 weeks). Additional detail is included in the RUSH2A Procedures Manual

3.2.3 Testing to Perform at 48 Months Only

Testing performed on both eyes

1. Kinetic visual field area (using Octopus 900 Pro)

Testing performed on study eye only

1. Full-field ERG (Diagnosys Espion preferred) ***only if responses were recordable at baseline***
    a. If ERG was non-detectable at baseline (defined at investigator discretion), testing is not required
2. Patient Reported Outcome
    a. Adult participants aged ≥18 will be tested using the Michigan Retinal Degeneration Questionnaire (MRDQ) (44)
        i. The MRDQ may be completed in person or remotely (phone or other remote methods) any time within the 48-month visit window (208 ± 4 weeks). Additional detail is included in the RUSH2A Procedures Manual

CHAPTER 4

4. MISCELLANEOUS CONSIDERATIONS

4.1 Treatment for Syndromic and Non-syndromic *USH2A*-Related Retinal Degeneration

Participants with Usher syndrome type 2a and non-syndromic RP should not enroll into experimental treatment trials of underlying conditions related to *USH2A* mutations during the 4-year study duration. Participants who do enroll into such a trial may be exited from RUSH2A upon Executive Committee review.

4.2 Risks and Benefits

4.2.1 Risks and Discomforts

Most examination procedures are considered part of standard care for retinal degenerations. The procedures have been standardized for consistency across centers and are not part of a therapeutic experimental protocol. The only risk for being part of the study over and above standard care is the unlikely chance that sensitive participant information is viewed by someone outside the research team who is not authorized. However, special efforts are being made to ensure that this does not happen. Otherwise, there are no known risks or discomforts beyond those involved in standard clinical care for patients with retinal degeneration involved in participation in this study, which involves systematically collecting information in a prospective fashion. The sections below summarize the risks and discomforts that may be involved in the usual care of the patient during the period of time of prospective data collection.

- Risks associated with testing visual acuity, audiology, olfactory testing, kinetic and static perimetry, and patient reported outcomes include boredom and frustration, but no lasting adverse effects are associated with these noninvasive tests
- Dilating eye drops will be used as part of the examination and before the color fundus photographs, optical coherence tomography scans, full-field ERG test, full-field stimulus threshold, and fundus-guided microperimetry. Dilating eye drops may sting, cause light-sensitivity, or an allergic reaction. There is a small risk of inducing a narrow-angle glaucoma attack from the pupil dilation. However, all participants will have had prior pupil dilation, usually on multiple occasions, and therefore the risk is extremely small. If glaucoma occurs, treatment is available
- IOP Examination and ERG: In rare instances, the cornea may be scratched during measurement of intra-ocular pressure or use of a contact lens electrode. An abrasion like this may be painful, but it heals quickly with no lasting effects. In the event that a participant experiences a corneal abrasion, antibiotic ointment will be administered and an eye patch or gauze may be placed over the eye
- Fundus photographs use bright lights associated with the camera flashes which can be uncomfortable for study participants, but these carry no known risk to the eye or vision
- The risks of genetic testing include emotional and psychological stress when patients may learn they have a genetic disease that could be passed along to their children, if information relating to the family, such as adoption and paternity, could be determined from these tests.

The genetics lab will only be reporting results related to how the mutations are arranged to the coordinating center, the clinical site, and the genetics review committee. All genetic testing information will be kept in confidential laboratory documents and medical records. If data gathered through genetic testing is accidentally released or stolen, it is possible that the information could become available to an insurer, an employer, a relative, or someone else. There are discrimination protections in US Federal Law and many State laws, however there is still a small chance that participants could be harmed if a release occurred

4.2.2 Benefits

Study participants are not expected to benefit directly from participation in this study. Subjects participating in this study may benefit from close attention from the study personnel and PI.

The risks of participating in the study are outweighed by the benefits including increased attention from the study personnel and the ability to contribute to increased understanding of the natural history of USH2A-related retinal degeneration and contribute to future development of treatments.

4.3 Adverse Events Reporting for Safety Monitoring

Information on medical conditions will be collected systematically to provide historical controls for future clinical trials. However, RUSH2A is a natural history study and does not require any specific adverse event reporting to regulatory or oversight bodies. Each Principal Investigator is responsible for abiding by any other reporting requirements specific to his/her IRB or equivalent ethics oversight committee.

4.4 Inclusion of Women and Minorities

We anticipate that study enrollment will be representative of the population of patients with biallelic mutations in the *USH2A* gene. Both males and females are expected to be enrolled in this protocol. All ethnic and racial groups are eligible for participation in this study, with the goal of having appropriate minority representation of those with biallelic mutations in the *USH2A* gene.

4.5 Inclusion of Children

We anticipate that study enrollment will be representative of the population of patients with biallelic mutations in the *USH2A* gene, including those under the age of 18. Children younger than 8 years of age may not be able to perform the study tests reliably so the study will not include children younger than 8 years. Biallelic mutations in the *USH2A* gene can impact individuals in the first or second decade of life, therefore it is imperative that children are included in this natural history study in order to adequately characterize the natural history of retinal degeneration in patients with *USH2A* mutations.

It is the investigators' opinion that the protocol's level of risk falls under Department of Health and Human Services (DHHS) 46.404, which is research not involving greater than minimal risk.

4.6 Study Participant Withdrawal and Losses to Follow-up

A study participant has the right to withdraw from the study at any time. If s/he is considering withdrawal from the study, the Principal Investigator should personally speak to the individual about the reasons, and every effort should be made to accommodate the study participant to allow continued participation if possible.

4.7 Discontinuation of Study

The study may be discontinued by the Executive Committee prior to the pre-planned completion of follow-up for all study participants.

CHAPTER 5

5. STATISTICAL CONSIDERATIONS

The approach to sample size and statistical analyses are summarized below. A detailed statistical analysis plan will be written and finalized prior to the completion of the study. The analysis plan synopsis in this chapter contains the framework of the anticipated final analysis plan.

5.1 Sample Size for Primary Cohort (Natural History Study)

The sample size evaluation focuses on objectives 1 and 2 of the study, to characterize the natural history of retinal degeneration associated with biallelic, pathogenic mutations in the *USH2A* gene on both the structural and functional outcomes of interest (listed in 1.4.4). The approach is summarized in section 5.1.1. Calculations to address objective 4, evaluation of risk factors associated with progression, are summarized in section 5.1.2. A final justification of the selected sample size using the outcome of primary interest, static perimetry volume, is outlined in section 5.1.3, along with an overall synopsis of the impact on the other outcomes of interest.

The sample size for the secondary cohort is a convenience sample, i.e., 20 participants.

5.1.1 Sample Size Considerations for Evaluating Percent Change from Baseline to 4 Years (All Outcomes)

Longitudinal changes on all outcome parameters being collected will be of interest. Change from baseline to 4 years will be evaluated for sample size purposes. **The power/sample size calculations in the following sections may be used to consider <u>percent change</u> on <u>any</u> outcome measure from baseline to 4 years.** Note that only one study eye will be included per participant for the majority of outcome measures, so the calculations below are counting sample size on a participant level.

The primary way sample size was evaluated was by considering the precision around the point estimates for the outcome measures of interest. Figure 1 (including the table of specific values corresponding to the graph) considers various expected standard deviations and the relationship between sample size and the half width of the associated 95% confidence interval (the +/- amount around the estimated mean). The larger this amount, the wider the confidence interval, meaning the range of possible true values grows.

828 **Figure 1. Sample size versus half width of 95% confidence interval for varying true**
829 **population standard deviation values**
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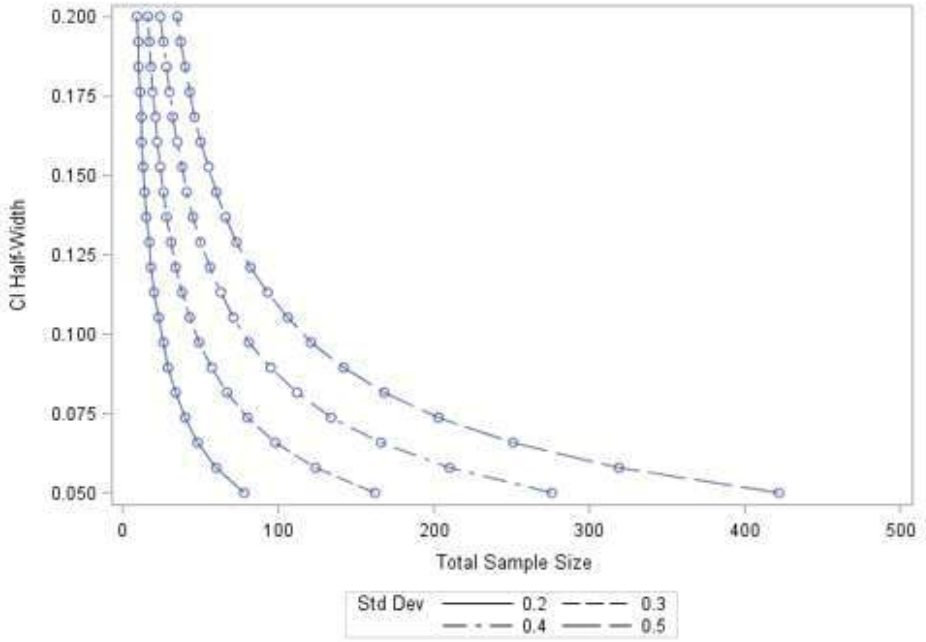

	N=20	N=50	N=70	N=100	N=150
SD=20%	9%	6%	5%	4%	3%
SD=30%	13%	8%	7%	6%	5%
SD=40%	18%	11%	9%	8%	6%
SD=50%	22%	14%	12%	10%	8%

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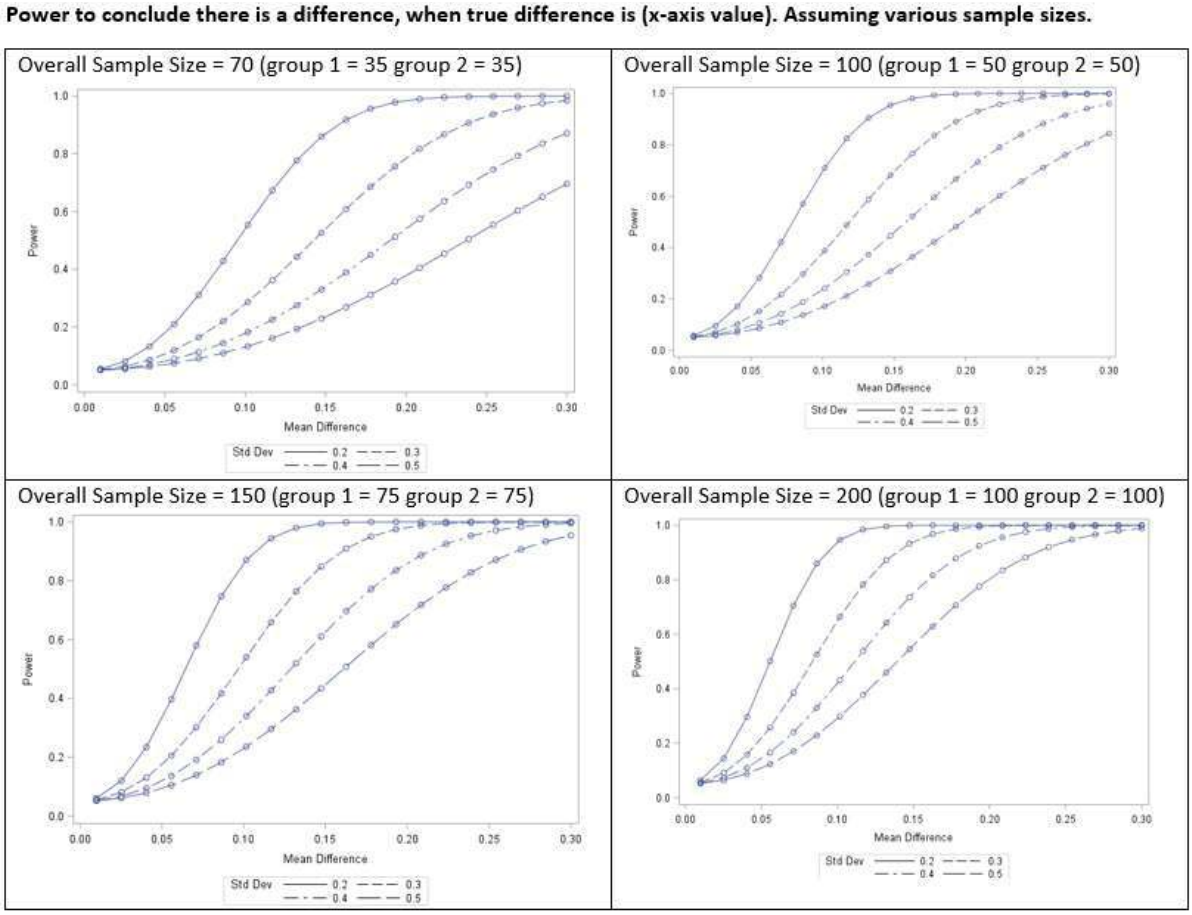
5.1.2 Sample Size Considerations for Comparing Percent Change from Baseline to 4 Years within Subgroups of Interest (All Outcomes)

Another important objective for this natural history study will be to evaluate association of possible risk factors with progression of various functional outcome variables (objective 4). Thus, it will be important to have a large enough total sample size to plan reasonable comparisons between subgroups. Figure 2 considers various expected standard deviations and evaluates the power to detect varying differences in average percent change from baseline to 4 years, comparing subgroups of various equally distributed sizes. If subgroups are not equally sized the detectable difference (with the same power) will be larger.

Note: *within* subgroup point estimates and confidence intervals will also be important. Figure 1 above can be applied to potential subgroup sample sizes as well to consider the precision that would be observed.

Figure 2. Power to conclude there is a difference given varying true difference values, population standard deviation, and sample size



5.1.3 Final Sample Size Justification

5.1.3.1 Static Perimetry Volume

Although longitudinal changes on all outcome parameters being collected will be of interest for objectives 1 and 2, a sample size justification specifically for static perimetry volume (full field Hill of Vision) over 4 years is provided below as an outcome of primary importance.

Data to consider for evaluating sample size:

- Natural history of autosomal recessive retinitis pigmentosa (ARRP) due to USH2A mutations (29)
    - Mean annual Percent Change for visual field area = -7%
- Valproic Acid Protocol (VPA) Data (a phase II multiple site, randomized, placebo-controlled trial of oral valproic acid for autosomal dominant retinitis pigmentosa (ADRP) [data not published]
    - Percent Change from Baseline to 1 year, Mean (Standard Deviation) for OD/OS:
        - Placebo group (N=44) = -0.3% (16%) / -4.9% (17%)

Assumptions made:

- Expect average annual decline in RUSH2A to be greater than that of VPA (patients with ADRP), i.e., 0.3%-4.9% at 1 year. Expect the rate to be more similar to the annual rate of decline reported in patients with ARRP due to USH2A observed on visual field area, i.e. 7% (29). Therefore, assume true decline from baseline is about 6.25% per year or 25% by 4 years
- True SD of percent change at 4 years similar to the VPA 1-year SD of around 20%

Based on these assumptions and the impact as presented in section 5.1.1 and 5.1.2, we have selected a sample size for the primary cohort of 100. It is anticipated that 8 additional patients will enroll into the natural history study due to the lag between closing recruitment into the screening phase and the completion of those pending confirmation in the screening phase. It is also expected that roughly the same number will be discontinued prior to completion of the 48-month visit, thus the final number will be close to the target of 100. With a sample size of 100 participants the half width of a 95% confidence interval around the point estimate would be 4%. A comparison of two equal-sized subgroups (N=50 each), which is the anticipated distribution of syndromic and non-syndromic participants based on a potential recruitment survey completed by clinical sites for the RUSH2A study [data not shown], would have about 80% power to conclude there is a difference if the true difference is 11%.

5.1.3.2 Synopsis of Justification for All Outcomes

The primary objective of the study is to characterize the natural history of retinal degeneration using the main outcome measures listed in section 1.4.4. Therefore, the precision of these estimates (how tight the confidence interval is around the point estimate) for all of the outcomes of interest will be of the greatest importance in the consideration of sample size. With a sample size of 100 participants in the primary cohort, all of these outcomes will have 95% confidence intervals no

wider than +/- 10% (when analyzed in terms of percent change from baseline) if the standard deviation is within 50%. Some outcomes (VA, OCT) will have data on both eyes which will improve the precision further. This was considered acceptable precision to meet our objective for all outcomes of interest.

Furthermore, for the additional objective of evaluating risk factors associated with progression of these outcomes, a sample size of 100 participants will provide enough power to evaluate subgroups, especially those with close to equal distribution. For example, for 2 subgroups of equal size (50 each) there will be at least 70% power to detect differences as small as 10% if standard deviation is within 20%.

5.2 Data Analysis

The analysis plans below are written with respect to the majority of outcomes of interest, which will have data on a single designated study eye for each participant. Two of the outcomes of interest, VA and OCT, will have data on both eyes. Analyses of these outcomes will include data on both eyes, and models and confidence intervals will adjust for correlation between 2 eyes of the same participant.

5.2.1 Primary Objectives Analyses

The primary objectives of the natural history study and brief analysis plan for each are as follows. All primary objectives apply to the **primary cohort only.**

1. Characterize the natural history of retinal degeneration associated with biallelic pathogenic mutations in the *USH2A* gene over 4 years as measured using the main outcome measures, functional and structural (listed in section 1.4.4)
    a. Analysis plan: The distribution of each outcome at each visit will be summarized (including tabulating categorically, as well as means, standard deviations, medians, quartiles, ranges; both the absolute change and percent change will be evaluated). To determine the average annual rate of progression in the population for each outcome, a repeated measures least squares regression model will be fit using all available outcome data at baseline and all annual visits. Multiple imputation will be used to impute the outcome values for all missing time points (including participants who discontinue follow up prior to 48 months). Secondary analyses using binary definitions of outcome measures will also be explored in time to event analyses; Kaplan-Meier estimates with 95% confidence intervals will be calculated

2. Investigate structure-function relationships for insights into the mechanisms of retinal degeneration by relating changes in SD-OCT EZ area to visual field progression in individuals with biallelic pathogenic mutations in the *USH2A* gene
    a. Analysis plan: Scatterplots and Spearman correlation coefficients of changes in SD-OCT EZ area versus visual field progression from baseline to each visit will be evaluated. Repeated measures least squares models will be fit using visual field progression as the dependent variable. Both linearity and the potential for larger variability with increasing EZ area will be evaluated, and transformations and/or higher order polynomial terms will be considered. Multivariate models using

potential risk factors (as assessed below) for visual field progression will be considered

3. Assess for possible genotype, phenotype, and environmental risk factors with progression of the outcome measures (listed in section 1.4.4) in individuals with biallelic pathogenic mutations in the *USH2A* gene
    a. Analysis plan: The distribution of each outcome in terms of both absolute change and percent change from baseline to 4 years will be summarized (including tabulating categorically, as well as means, standard deviations, medians, quartiles), ***stratified by*** categorical levels of each potential risk factor of interest (listed below). The association of factors potentially related to change at 4 years for each outcome measure will be evaluated in univariate and multivariate ANCOVA models (adjusting for baseline). A stepwise selection procedure will be used to build the final model. A threshold of $P<0.10$ will be used to add to the model, and a threshold of $P<0.01$ will be used to remain in the multivariate model. Missing outcome data will be imputed using multiple imputation as noted in the primary analysis. Linearity of continuous factors will be assessed, and possibly quadratic or cubic terms will be considered if non-linear. Secondary analyses using binary definitions of outcome measures will also be explored in time to event analyses; Cox proportional hazard models will be evaluated using a parallel stepwise selection procedure

- Potential factors to evaluate include:
    - Phenotypic factors:
        - Syndromic versus non-syndromic at baseline
        - Severity of hearing loss at baseline
        - Age at onset of vision loss
        - Olfactory status at baseline
    - Genotypic factors:
        - Characterizations of the variants on the USH2A protein
    - Environmental factors:
        - Smoking status at baseline
        - Vitamin A use history at baseline
        - Docosahexaenoic acid (DHA) use history at baseline
        - Light exposure history at baseline

5.2.2 Secondary Objectives Analyses

The secondary objectives of this study and brief analysis plan for each are as follows. These objectives apply to both the **primary and secondary cohorts**.

1. Characterize baseline cross-sectional retinal degeneration associated with biallelic pathogenic mutations in the *USH2A* gene (as measured using the main outcome measures listed in section 1.4.4)
    a. Analysis plan: The distribution of each outcome will be summarized (including tabulating categorically, as well as means, standard deviations, medians, quartiles, ranges)

2. Investigate comorbidities associated with disease (baseline cross-sectional) and disease progression (longitudinal natural history study) in individuals with biallelic pathogenic mutations in the *USH2A* gene
    a. Analysis plan: All medical conditions identified at baseline (in both the primary and secondary cohorts) or at any time during follow up (primary cohort only) will be tabulated. Baseline medical conditions will be cross-tabulated with categorical (severity of disease) versions of the outcome measures of interest at baseline. Development of medical conditions by each annual visit will be cross-tabulated with binary (progression of disease) versions of the outcome measures of interest at each annual visit

3. Explore patient reported outcome (PRO) measures associated with disease (baseline cross-sectional) and disease progression (longitudinal natural history study) in individuals with biallelic pathogenic mutations in the *USH2A* gene
    a. Analysis plan: Rasch analyses will be performed to calibrate both the VA LV VFQ-48 (completed by adults) and the LVP-FVQ-II (completed by children). Equivalence of different language versions will be established by calculating differential item functioning scores as part of the analyses. The scoring of each questionnaire will be completed according to the procedures for each instrument and is detailed further in the separate statistical analysis plan. Baseline scores will be cross-tabulated with categorical (severity of disease) versions of the outcome measures of interest at baseline. Changes in scores will be cross-tabulated with binary (progression of disease) versions of the outcome measures of interest at the 24 and 48 month visits
    b. MRDQ is completed at 48 months in adults only. The cross-sectional analyses described above for baseline will be performed for MRDQ at 48 months.

4. Evaluate variability and symmetry of left and right eye kinetic perimetry and OCT outcomes at baseline and at 4 years in individuals with biallelic mutations in the *USH2A* gene
    a. Analysis plan: Scatterplots and Spearman correlation coefficients of left eye versus right eye will be evaluated for SD-OCT EZ area and kinetic perimetry III4e area at baseline (in both the primary and secondary cohorts) and at 4 years (primary cohort only)

5.2.3 Sensitivity Analyses

Analyses above will be repeated excluding cases that are not confirmed as pathogenic or likely pathogenic by a genetics committee. This will confirm that the results are not influenced by cases that may be ineligible based on genetics expert review but eligible on clinical review. Exclusions or subgroup analyses may be considered as a result of this analysis.

5.2.4 Interim Data Analysis

No formal interim analysis or "stopping guidelines" are planned for determining early stopping according to statistical rules, as no intervention is being studied and thus early efficacy and safety signals will not be applicable.

Interim analyses will be planned for other reasons, including to evaluate data at baseline and annual visits for reporting in preliminary manuscripts, as well as monitoring data for recruitment and retention benchmarks, and quality assurance throughout the duration of the study. The Executive Committee will review and oversee these data and their use in reporting.

CHAPTER 6

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APPENDIX

1. ANCILLARY STUDY: DARK-ADAPTED VISUAL FIELDS (DAVF)

1.1 Background and Rationale

A comprehensive assessment of visual function in patients with *USH2A* mutations involves rod as well as cone-mediated vision. The main RUSH2A protocol is only assessing rod function with the FST, a full-field test that provides no spatial information. The Medmont dark-adapted chromatic (DAC) automated perimeter has been specifically designed to measure rod function across the retina. The RUSH2A trial is an opportunity to determine whether the additional information about rod function and rate of loss is valuable for following participants and a potential outcome measure for treatment trials.

1.2 Objectives

In participants meeting eligibility for the RUSH2A protocol primary cohort, the following questions are of interest:

1) What proportion of participants have evidence of rod function (defined below) at their initial DAVF testing? Is age associated with evidence of rod function?
    a. Participants will be considered to have evidence of rod function if the difference in cyan sensitivity relative to red sensitivity is greater than 5 dB at 3 or more locations (Bennett et al., 2016).
2) In those with evidence of rod function on their initial DAVF test: What is the annual rate of change in sensitivity in rod-mediated regions as measured by cyan mean sensitivity?

1.3 Eligibility and Informed Consent

The DAVF ancillary study will be optional for RUSH2A-certified sites which have the ***Medmont DAC***. Certification requirements for the DAVF ancillary study are detailed in the RUSH2A Procedures Manual.

All RUSH2A-enrolled primary cohort adult participants at sites that are certified to participate in the DAVF ancillary study will be eligible for participation in the ancillary study. Participation in the DAVF ancillary study will be optional. A separate informed consent for the DAVF ancillary study will be obtained according to the same policies as noted in the main RUSH2A protocol. The consent may also include the ability to retrospectively provide images that were captured prior to consenting to the DAVF ancillary study, to be provided and evaluated along with those captured under the RUSH2A ancillary study mechanism.

1.4 Testing Procedures and Schedule

The DAVF testing and data collection procedures are detailed in the RUSH2A Procedures Manual. Testing must be performed by a certified technician, on the Medmont DAC. All DAVF testing will be completed in the **study eye only**.

Participants who consent to participate will complete the initial DAVF test at the 12-month visit. The site will evaluate the DAVF at the 12-month visit to determine if there is evidence of rod function.

- Participants with no rod function on the first test will not have DAVF test performed at subsequent visits.
- Participants with rod function on the first test will complete additional DAVF testing at each subsequent annual visit (24M, 36M, and 48M visits).

1.5 Sample Size and Analysis Considerations

It is anticipated that approximately 75 primary cohort participants will be enrolled into this ancillary study, based on the number of sites expected to participate, and their enrollment volume. Of those, it is expected that approximately 40 will have evidence of rod function at the initial DAVF test and will continue to the follow up testing to measure rate of change. The outcome will have 95% confidence intervals no wider than +/- 12% if the standard deviation is within 40%.

For objective 1, the proportion of ancillary study participants with evidence of rod function at their initial DAVF test, and associated 95% confidence interval, will be calculated. The association of evidence of rod function with age will be evaluated by tabulating the proportion with evidence of rod function stratified by age categories and fitting a logistic regression model.

For objective 2, within participants with evidence of rod function on their initial DAVF test, the annual rate of change in rod function as measured by cyan mean sensitivity will be evaluated in an approach that mirrors that of the primary outcomes for the RUSH2A protocol (Section 5.2.1, item 1).

2. ANCILLARY STUDY: ADAPTIVE OPTICS SCANNING LASER OPHTHALMOSCOPY (AOSLO)

2.1 Background and Rationale

The RUSH2A study is collecting clinical data on the rate of photoreceptor degeneration in patients with *USH2A* mutations. However, the standard clinical tests included in the main RUSH2A protocol do not have the resolution necessary to study cone photoreceptor structure on a cellular level. Adaptive Optics Scanning Laser Ophthalmoscopy (AOSLO) is a non-invasive method of studying macular cone photoreceptors with high resolution. No published studies to date have characterized the longitudinal change in cone structure over time in eyes with USH2A related retinal degeneration.

2.2 Objectives

In patients meeting eligibility for the RUSH2A protocol primary cohort, who also meet criteria to reliably obtain AOSLO, the following questions are of interest:

1) What is the annual rate of change in cone spacing in the macula of patients with retinal degenerations associated with mutations in the *USH2A* gene?
2) Are annual rates of change in cone spacing different between patients with Usher syndrome type 2A and those with non-syndromic RP due to mutations in the *USH2A* gene?

2.3 Eligibility and Informed Consent

The AOSLO ancillary study will be optional for RUSH2A-certified sites which have ***AOSLO systems with split detector capability***. Certification requirements for the AOSLO ancillary study are detailed in the RUSH2A Procedures Manual.

RUSH2A-enrolled <u>adult</u> participants <u>who also meet the following additional screening criteria</u> will be eligible for participation in the ancillary study. Participation in the AOSLO ancillary study will be optional. A separate informed consent for the AOSLO ancillary study will be obtained according to the same policies as noted in the main RUSH2A protocol. The consent may also include the ability to retrospectively provide images that were captured prior to consenting to the AOSLO ancillary study, to be provided and evaluated along with those captured under the RUSH2A ancillary study mechanism.

<u>AOSLO Screening Criteria:</u>

1. Participant must have NO corneal opacification or lack of optical clarity in study eye.
2. Participant must have NO nystagmus or unstable fixation in study eye.
3. Participant must have NO significant dry eye in study eye.

2.4 Testing Procedures and Schedule

The AOSLO testing and data collection procedures are detailed in the RUSH2A Procedures Manual. Testing must be performed by a certified technician, on the AOSLO with split detector capability. All AOSLO testing will be completed in the **<u>study eye only</u>**.

Participants who consent to participate will complete the initial AOSLO testing at the 12-month RUSH2A visit, or within 60 days of the 12-month visit. This initial imaging session will acquire 2 sets of images to create macular montages, to be taken within those 60 days. A designated central AOSLO Principal Investigator will evaluate the initial images to determine if reliability criteria are met (defined below), and to define the regions of interest (ROIs) for grading on initial and future testing.

AOSLO Image Criteria:

- Participant must have reproducible initial AOSLO image montages at 2 initial imaging sessions with quality suitable to identify a minimum of 10 regions of interest, each containing ≥ 50 cones and separated by approximately 1 degree intervals in the central retina, at which reliable cone spacing measures can be made.

Based on the AOSLO PI evaluation based on the above criteria,

- Participants not meeting AOSLO image criteria will not have AOSLO images at subsequent visits
- Participants meeting AOSLO image criteria will have AOSLO images at each subsequent annual visit (24M, 36M and 48M visits), or within 60 days of the associated visit.

For those meeting the AOSLO imaging criteria, initial and follow-up AOSLO images will be graded by at least 3 certified graders trained on grading procedures by the AOSLO PI. These grading procedures are detailed in a separate AOSLO grading manual.

2.5 Sample Size and Analysis Considerations

It is anticipated that approximately 25 primary cohort participants will be enrolled into this ancillary study, based on the number of sites expected to participate, and their enrollment volume. It is expected that all will meet the reliability criteria and will continue to the follow up testing to measure rate of change. The calculation for the estimated percent change in cone spacing will have 95% confidence intervals no wider than +/- 4% if the standard deviation is within 10%.

The cone spacing measure determined by each grader within each ROI will be averaged across all graders. For objective 1, the annual rate of change in cone spacing will be evaluated using a linear mixed effects regression model clustering on region of interest. For objective 2, the association of diagnosis (syndromic versus non-syndromic) on annual rate of change in cone spacing will be evaluated in an approach that mirrors that of the primary outcomes for the RUSH2A protocol (Section 5.2.1, item 3).