Foundation Fighting Blindness (FFB) Consortium

Rate of Progression in USH2A Related Retinal Degeneration (RUSH2A)

Version 3.0
17 December, 2020

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### Signature Page

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**Version Number: 3.0**

**17-Dec-2020**

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Reason: I am approving this document
Location:  
Date: 2020-12-18 12:10-05:00
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<thead>
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<th>Description</th>
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<tbody>
<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
</tr>
<tr>
<td>AOSLO</td>
<td>Adaptive Optics Scanning Laser Ophthalmoscopy</td>
</tr>
<tr>
<td>ADRP</td>
<td>Autosomal dominant retinitis pigmentosa</td>
</tr>
<tr>
<td>ARRP</td>
<td>Autosomal recessive retinitis pigmentosa</td>
</tr>
<tr>
<td>BCVA</td>
<td>Best corrected visual acuity</td>
</tr>
<tr>
<td>CFR</td>
<td>Code of Federal Regulations</td>
</tr>
<tr>
<td>DAVF</td>
<td>Dark-adapted visual field</td>
</tr>
<tr>
<td>DAC</td>
<td>Dark-adapted Chromatic</td>
</tr>
<tr>
<td>DHA</td>
<td>Docosahexaenoic acid</td>
</tr>
<tr>
<td>DHHS</td>
<td>Department of Health and Human Services</td>
</tr>
<tr>
<td>ERG</td>
<td>Electroretinogram</td>
</tr>
<tr>
<td>ETDRS</td>
<td>Early Treatment of Diabetic Retinopathy Study</td>
</tr>
<tr>
<td>EVA</td>
<td>Electronic visual acuity</td>
</tr>
<tr>
<td>EZ</td>
<td>Ellipsoid zone</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FST</td>
<td>Full-field sensitivity testing</td>
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<tr>
<td>GVF</td>
<td>Goldmann visual field</td>
</tr>
<tr>
<td>IOP</td>
<td>Intraocular pressure</td>
</tr>
<tr>
<td>IRB</td>
<td>Institutional Review Board</td>
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<tr>
<td>LVP-FVQ-II</td>
<td>L.V. Prasad-Functional Vision Questionnaire</td>
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<tr>
<td>MRDQ</td>
<td>Michigan Retinal Degeneration Questionnaire</td>
</tr>
<tr>
<td>MP</td>
<td>Microperimetry</td>
</tr>
<tr>
<td>N</td>
<td>Number or sample size</td>
</tr>
<tr>
<td>OD</td>
<td>Right Eye</td>
</tr>
<tr>
<td>OS</td>
<td>Left Eye</td>
</tr>
<tr>
<td>OU</td>
<td>Both eyes</td>
</tr>
<tr>
<td>PI</td>
<td>Principal investigator</td>
</tr>
<tr>
<td>PRO</td>
<td>Patient-reported outcomes</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>RPE</td>
<td>Retinal pigment epithelial cells</td>
</tr>
<tr>
<td>RP</td>
<td>Retinitis pigmentosa</td>
</tr>
<tr>
<td>RUSH2A</td>
<td>Rate of Progression in USH2A Related Retinal Degeneration</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SD-OCT</td>
<td>Spectral domain optical coherence tomography</td>
</tr>
<tr>
<td>SE</td>
<td>Study eye</td>
</tr>
<tr>
<td>VA</td>
<td>Visual acuity</td>
</tr>
<tr>
<td>VA LV VFQ-48</td>
<td>48-item Veterans Affairs Low Vision Visual Functioning Questionnaire</td>
</tr>
<tr>
<td>VPA</td>
<td>Valproic acid</td>
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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 REPI 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
degenereation 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

<table>
<thead>
<tr>
<th>Procedures</th>
<th>Baseline&lt;sup&gt;e&lt;/sup&gt;</th>
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<th>24M</th>
<th>36M</th>
<th>48M</th>
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<td>Visit Windows</td>
<td>Day 0</td>
<td>Wk</td>
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<tr>
<td></td>
<td>52 ± 4</td>
<td>104±4</td>
<td>156±4</td>
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<td>Olfactory Test</td>
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<td>Patient Reported Outcomes</td>
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<td>Concomitant Medications/Medical Conditions</td>
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<td>Refraction and E-ETDRS VA Testing (EVA) or ETDRS charts</td>
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<td>SD-OCT with measurement of EZ area (Heidelberg Spectralis)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>OU</td>
<td>SE</td>
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<tr>
<td>Full-field ERG&lt;sup&gt;k,e&lt;/sup&gt;</td>
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<td>Kinetic visual field area (Octopus 900 Pro)&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Static visual field volume (Octopus 900 Pro)&lt;sup&gt;b,e&lt;/sup&gt;</td>
<td>SE (x3)</td>
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<td>SE</td>
<td>SE</td>
<td>SE</td>
</tr>
<tr>
<td>Fundus-guided microperimetry (MAIA, where available)&lt;sup&gt;b,e&lt;/sup&gt;</td>
<td>SE (x3)</td>
<td>SE</td>
<td>SE</td>
<td>SE</td>
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</tr>
<tr>
<td>Full-field Stimulus Threshold (Diagnosys Espion, where available)</td>
<td>SE (x3)</td>
<td>SE (x3)</td>
<td>SE (x3)</td>
<td>SE (x3)</td>
<td>SE (x3)</td>
</tr>
</tbody>
</table>

<sup>a</sup> 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.<br><br><sup>b</sup> Third perimeter may be completed at an additional baseline visit, within 14 days of the designated baseline visit date.<br><br><sup>c</sup> 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.<br><br><sup>d</sup> Audiology – to be performed within 30 days of the baseline visit, in primary and secondary cohort participants<br><br><sup>e</sup> 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.<br><br><sup>f</sup> 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.<br><br><sup>g</sup> ERG to be performed within 60 days of the baseline and 48M visits<br><br><sup>h</sup> 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 deferroxamine)

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 Dipters 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
  o 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)
  o 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 \textit{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 (\textit{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 (\textit{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 \textbf{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 percent change on any 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.
Figure 1. Sample size versus half width of 95% confidence interval for varying true population standard deviation values.
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**

*Power to conclude there is a difference, when true difference is (x-axis value). Assuming various sample sizes.*

- **Overall Sample Size = 70 (group 1 = 35 group 2 = 35)**
- **Overall Sample Size = 100 (group 1 = 50 group 2 = 50)**
- **Overall Sample Size = 150 (group 1 = 75 group 2 = 75)**
- **Overall Sample Size = 200 (group 1 = 100 group 2 = 100)**
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
development 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<0.01 will be used to add to the model, and a threshold of P<0.05 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:
  o Phenotypic factors:
    ▪ Syndromic versus non-syndromic at baseline
    ▪ Severity of hearing loss at baseline
    ▪ Age at onset of vision loss
    ▪ Olfactory status at baseline
  o Genotypic factors:
    ▪ Characterizations of the variants on the USH2A protein
  o 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

6. REFERENCES


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 adult participants who also meet the following additional screening criteria
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.

AOSLO Screening Criteria:
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 study eye only.
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).