Foundation Fighting Blindness (FFB) Consortium

Rate of Progression in *EYS* Related Retinal Degeneration (Pro-EYS)

Version Number: 2.0

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Signature Page

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Version Number: 2.0

18-Dec-2020

FFB Consortium Coordinating Center (CC) Director		
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LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
ACMG	American College of Medical Genetics
ADRP	Autosomal dominant retinitis pigmentosa
AE	Adverse Event
ANCOVA	Analysis of covariance
BCVA	Best corrected visual acuity
BRVT	Berkeley Rudimentary Vision Test
CC	Coordinating Center
CFR	Code of Federal Regulations
CGA	Central Genetics Auditor
CI	Confidence interval
CME	Cystoid macular edema
CSF	Contrast Sensitivity Function
DHA	Docosahexaenoic acid
EC	Ethics Committee
ERG	Electroretinogram
ETDRS	Early Treatment of Diabetic Retinopathy Study
EVA	Electronic Visual Acuity
EZ	Ellipsoid Zone
FAF	Fundus Autofluorescence
FFB	Foundation Fighting Blindness
FST	Full-field stimulus threshold
GCP	Good Clinical Practice
ICF	Informed consent form
ICH	International Committee of Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IOP	Intraocular Pressure
IRB	Institutional Review Board
IS/OS	Inner Segment/ Outer Segment
LLVA	Low Luminance Visual Acuity
MP	Microperimetry
MRDQ	Michigan Retinal Degeneration Questionnaire
N	Number or sample size
OD	Right Eye
OS	Left Eye
OU	Both eyes

ABBREVIATION	DEFINITION
PI	Principal investigator
PRO	Patient reported outcomes
PROMIS®-29	Patient-Reported Outcomes Measurement Information System
QA	Quality Assurance
QC	Quality Control
RBM	Risk-Based Monitoring
RP	Retinitis pigmentosa
SAE	Serious adverse event
SD	Standard deviation
SD-OCT	Spectral domain optical coherence tomography
SP	Static perimetry
TALEN	Transcription activator-like effector nuclease
ULV-VFQ-50	Ultra-Low Vision Visual Functioning Questionnaire
VA	Visual acuity
VA LV VFQ-48	Veterans Affairs Low Vision Visual Functioning Questionnaire
VF	Visual Field
ViSIO-PRO	Visual Symptom and Impact Outcomes Patient Reported Outcome
VPA	Valproic Acid

PROTOCOL SUMMARY

ITEM	DESCRIPTION
Title	Rate of Progression in EYS Related Retinal Degeneration (Pro-EYS)
Précis	This natural history study of patients with *EYS* mutations will accelerate the development of outcome measures for clinical trials. Sensitive, reliable outcome measures of retinal degeneration will greatly facilitate development of treatments for retinitis pigmentosa due to *EYS* mutations. Together these approaches are expected to have an impact on understanding *EYS*-related retinal degeneration, developing experimental treatment protocols, and assessing their effectiveness. The goals and expected impact of this natural history study are to: 1. Describe the natural history of retinal degeneration in patients with biallelic mutations in the *EYS* gene 2. Identify sensitive structural and functional outcome measures to use for future multicenter clinical trials in *EYS*-related retinal degeneration 3. Identify well-defined subpopulations for future clinical trials of investigative treatments for *EYS*-related retinal degeneration
Objectives	1. Characterize the natural history of retinal degeneration associated with biallelic pathogenic mutations in the *EYS* gene over 4 years, as measured using functional, structural, and patient-reported outcome measures 2. Investigate whether structural outcome measures can be validated as surrogates for functional outcomes in individuals with biallelic pathogenic mutations in the *EYS* gene 3. Evaluate possible risk factors (genotype, phenotype, environmental, and comorbidities) for progression of the outcome measures at 4 years in individuals with biallelic pathogenic mutations in the *EYS* gene 4. Evaluate variability and symmetry of left and right eye outcomes over 4 years in individuals with biallelic pathogenic mutations in the *EYS* gene
Study Design	Multicenter, longitudinal, prospective natural history study. Participants will be assigned to one of three "Vision Cohorts" based on visual acuity (VA) and kinetic visual fields (VF).
Number of Clinical Sites	Approximately 30
Endpoint	**Functional Outcomes:** • VF sensitivity as measured by static perimetry with topographic analysis (Hill of Vision) and assessed by a central reading center • Early Treatment of Diabetic Retinopathy Study (ETDRS) Best corrected visual acuity (BCVA) letter score as measured on the Electronic Visual Acuity (EVA) system or ETDRS charts. Berkeley Rudimentary Vision Test (BRVT) will be used for patients unable to see letters • Mean retinal sensitivity as measured by fundus-guided microperimetry (MP) and assessed by a central reading center at selected sites with requisite equipment • Full-field retinal sensitivity as measured by full-field stimulus threshold (FST) testing to blue, white and red stimuli • Best corrected low luminance visual acuity (LLVA) letter score • Contrast sensitivity function (CSF) as measured by the CSV-1000E VectorVision chart • Retinal function using full-field electroretinogram (ERG) amplitudes and timing in response to rod- and cone-specific stimuli **Structural Outcomes:** • Ellipsoid zone (EZ) area as measured by spectral domain optical coherence tomography (SD-OCT) and assessed by a central reading center • Explore qualitative categorization of Fundus Autofluorescence (FAF) pattern as assessed by a central reading center • Explore quantitative measures of FAF as assessed by a central reading center **Patient Reported Outcomes (PRO):** • Vision Cohorts 1 and 2: Veterans Affairs Low Vision Visual Functioning Questionnaire (VA LV VFQ-48), Patient-Reported Outcomes Measurement Information System (PROMIS®-29), Michigan Retinal Degeneration Questionnaire (MRDQ) and Visual Symptom and Impact Outcomes Patient Reported Outcome (ViSIO-PRO) Instrument

<table>
<tr><th>ITEM</th><th>DESCRIPTION</th></tr>
<tr><td></td><td>• Vision Cohort 3: Ultra-Low Vision Visual Functioning Questionnaire (ULV-VFQ-50), and PROMIS®-29, MRDQ and ViSIO-PRO</td></tr>
<tr><td>Population</td><td>Key Eligibility Criteria:
The entire list of eligibility criteria is in section 2.3.1 and must be reviewed at the Screening Visit. All eligibility criteria must be met to enroll into the genetic screening phase. A key subset of those eligibility criteria includes the following.

• Age ≥ 18 years of age
• Clinical diagnosis of retinal dystrophy
• Must meet one of the Genetic Screening Criteria:
➢ <u>Screening Group A:</u> At least 2 disease-causing variants in the EYS gene which are homozygous or heterozygous in trans, based on a report from a clinically-certified lab (or a report from a research lab that has been pre-approved by the study Genetics Committee)
➢ <u>Screening Group B:</u> Only 1 disease-causing variant in the EYS gene, based on a report from a clinically-certified lab (or a report from a research lab which has been pre-approved by the study Genetics Committee)
➢ <u>Screening Group C:</u> At least 2 disease-causing variants in the EYS gene which are unknown phase, based on a report from a clinically-certified lab (or a report from a research lab which has been pre-approved by the study Genetics Committee)

Participants eligible upon initial screening will continue to the genetic screening phase. Following the genetic screening phase, to be eligible to enroll in the study cohort, the following must be documented:

• <u>Final Study Cohort Criteria:</u> At least 2 disease-causing variants in the EYS gene which are homozygous or heterozygous in trans, based on a report from a clinically-certified lab (or a report from a research lab that has been pre-approved by the study Genetics Committee), and confirmed by a Central Genetics Auditor (CGA).</td></tr>
<tr><td>Sample Size</td><td>Sample size rationale is detailed in section 6.1. Recruitment will be based on three Vision Cohorts defined as follows:
• Vision Cohort 1: ~70 participants with the better eye Screening Visit visual acuity ETDRS letter score of 54 or more [approximate Snellen equivalent 20/80 or better] <u>and</u> visual field diameter 10 degrees or more in every meridian of the central field
• Vision Cohort 2: ~20 participants with the better eye Screening Visit visual acuity ETDRS letter score of 19-53 [approximate Snellen equivalent 20/100 - 20/400] <u>or</u> (visual acuity ETDRS letter score of 54 or more [approximate Snellen equivalent 20/80 or better] <u>and</u> visual field diameter less than 10 degrees in any meridian of the central field)
• Vision Cohort 3: ~10 participants with the better eye Screening Visit visual acuity ETDRS letter score of 18 or less [approximate Snellen equivalent 20/500 or worse]

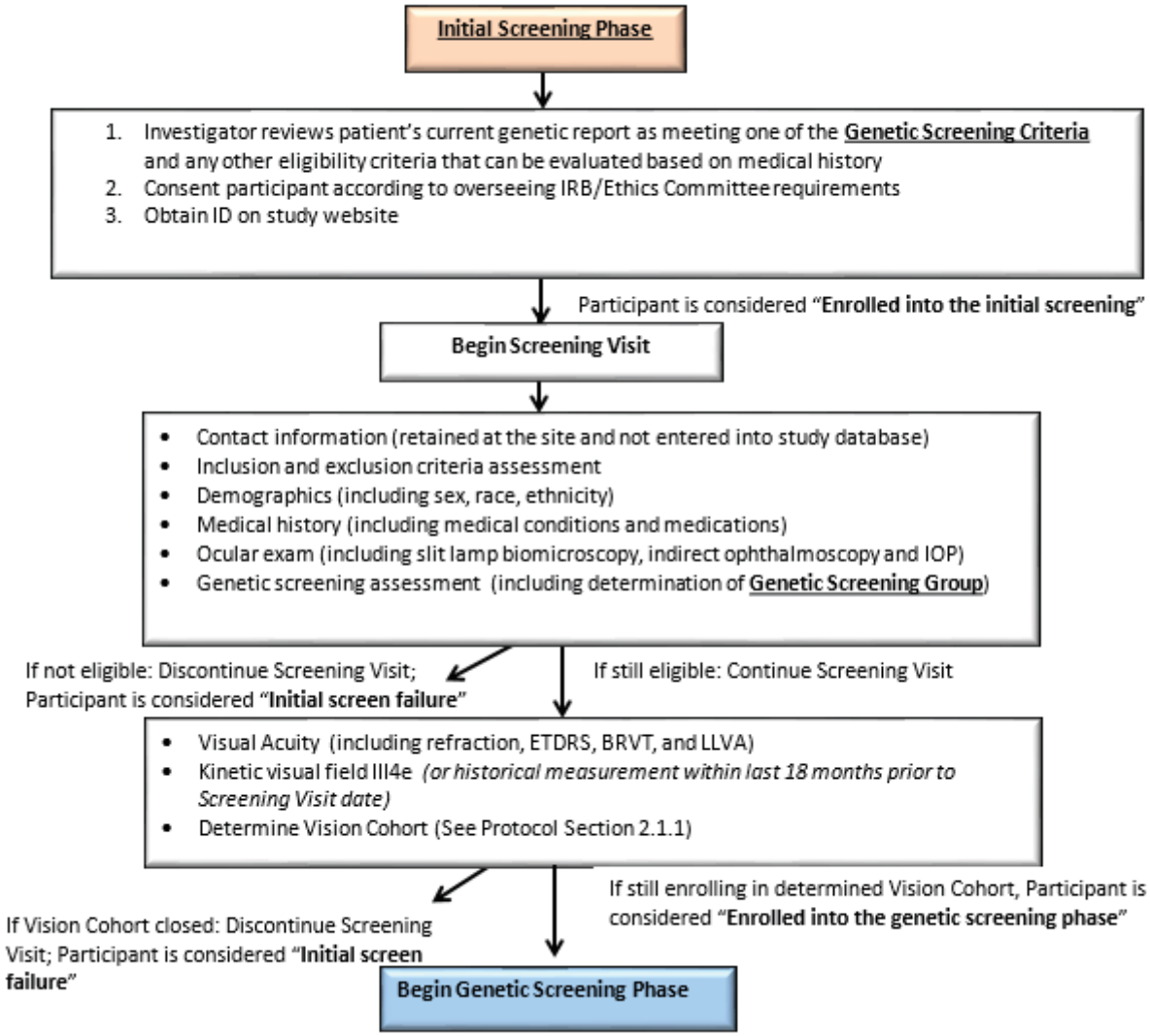
The better eye is defined as the eye with better Screening Visit ETDRS VA. If both eyes have the same VA (defined as the same Snellen equivalent), then the determination will be made at investigator discretion as the eye with better fixation or clear ocular media to permit highest quality retinal imaging.

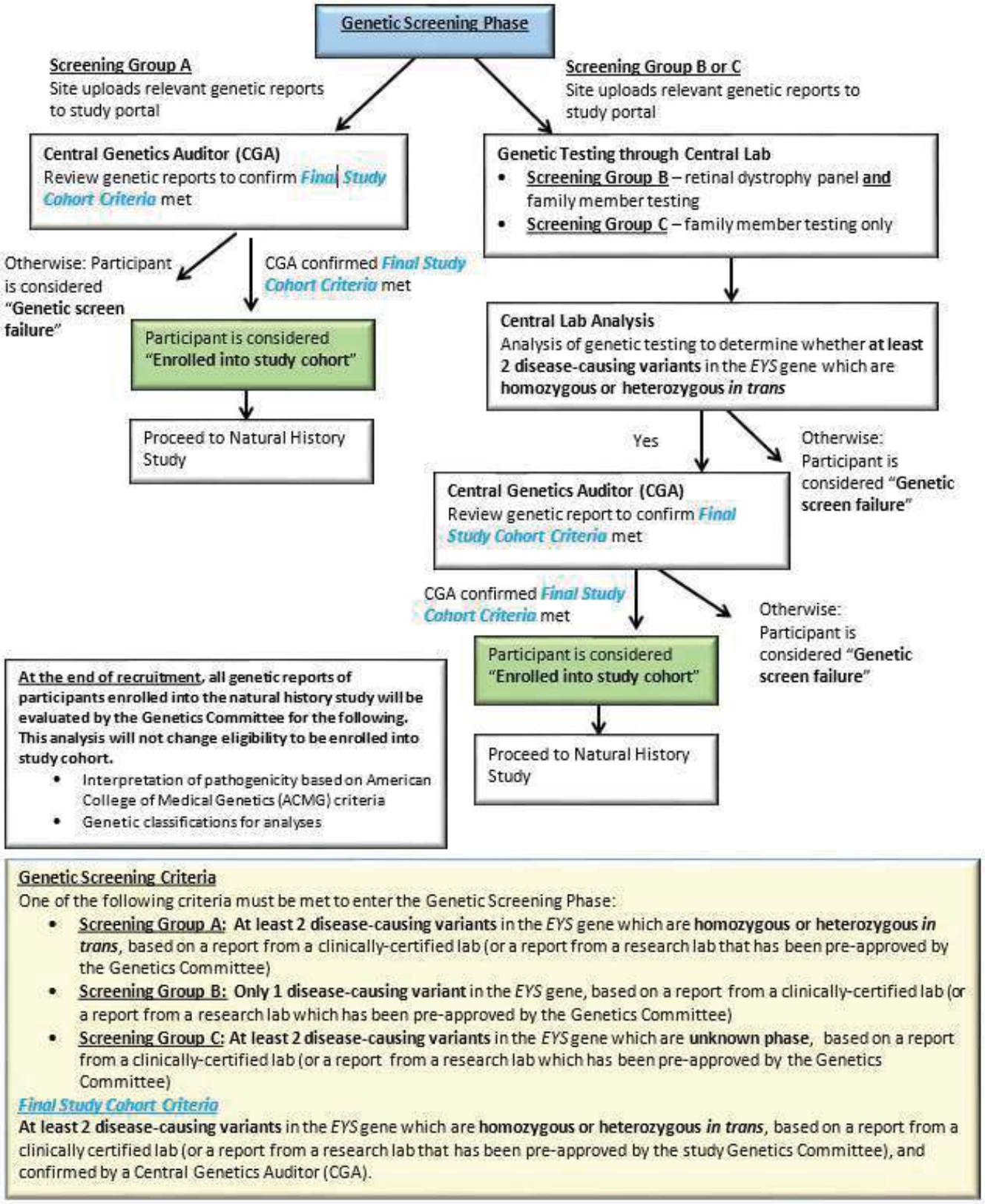
The visual field (VF) is defined as a clinically determined kinetic VF III4e performed within the last 18 months prior to or including the Screening Visit date

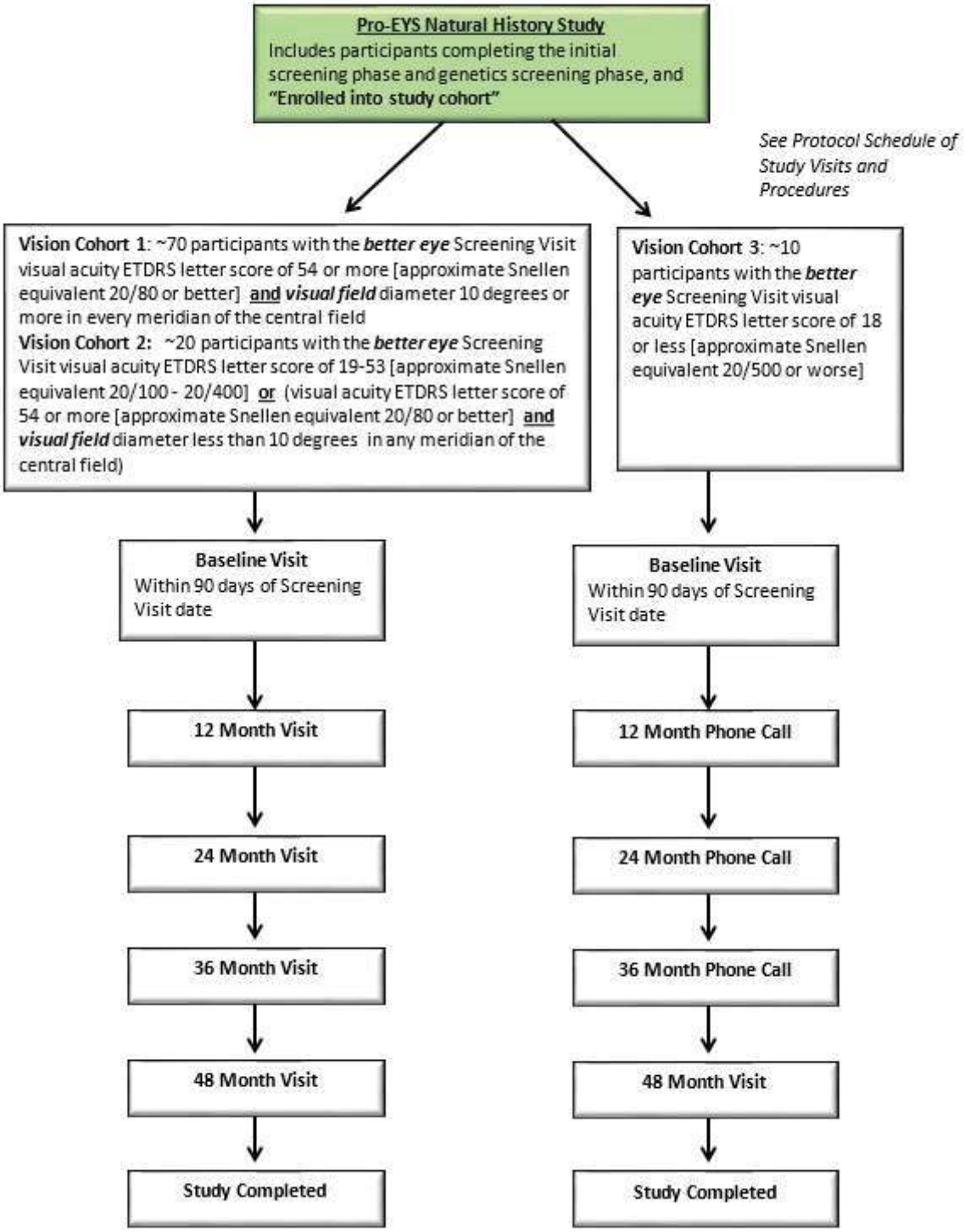
<table>
<tr><th></th><th>VF diameter ≥10° in every meridian</th><th>VF diameter <10° in any meridian</th></tr>
<tr><td>20/80 or better</td><td>Vision Cohort 1</td><td>Vision Cohort 2</td></tr>
<tr><td>20/100-20/400</td><td>Vision Cohort 2</td><td>Vision Cohort 2</td></tr>
<tr><td>20/500 or worse</td><td>Vision Cohort 3</td><td>Vision Cohort 3</td></tr>
</table></td></tr>
<tr><td>Participant Duration</td><td>From the time of screening until the 48-month visit: Approximately 51 Months</td></tr>
</table>

ITEM	DESCRIPTION
	• Screening- Baseline Visit (~ 3 months) • Baseline Visit – 48-month Follow-up Visit (~ 48 months)
Protocol Overview/Synopsis	1. Investigator reviews patient's current genetic report as meeting one of the Genetic Screening Criteria and any other eligibility criteria that can be evaluated based on medical history 2. Consent participant according to overseeing Institutional Review Board (IRB)/Ethics Committee (EC) requirements 3. Obtain ID on study website to ***enroll into initial screening*** 4. Complete a Screening Visit to determine eligibility, Vision Cohort and Genetic Screening Group. Participants meeting criteria to continue will ***enroll into the genetic screening phase***. (See flow chart in next section for details) 5. Complete genetic screening according to the requirements for the given Genetic Screening Group. Participants meeting criteria to continue will ***enroll into the study cohort.*** (See flow chart in next section for details) 6. Participants who ***enroll into the study cohort*** will return to the clinic within 90 days of the Screening Visit date to start baseline testing, and no later than 2 weeks after receiving confirmation of meeting ***final study cohort criteria*** from the CGA 7. Participants in Vision Cohorts 1 and 2 will return to the clinic at 12, 24, 36 and 48 months from the baseline visit start date for follow-up visits. Participants in Vision Cohort 3 will have phone calls with clinical site personnel at 12, 24 and 36 months from the baseline visit date, and a study visit at 48 months 8. After the 48-month follow-up visit, participation in the Pro-EYS study (for all 3 Vision Cohorts) will be completed

SCHEMATIC OF STUDY DESIGN




Genetic Screening Phase
Screening Group A
Site uploads relevant genetic reports to study portal
Screening Group B or C
Site uploads relevant genetic reports to study portal
Central Genetics Auditor (CGA)
Review genetic reports to confirm Final Study Cohort Criteria met
Genetic Testing through Central Lab
Screening Group B – retinal dystrophy panel and family member testing
Screening Group C – family member testing only
Otherwise: Participant is considered "Genetic screen failure"
CGA confirmed Final Study Cohort Criteria met
Participant is considered "Enrolled into study cohort"
Proceed to Natural History Study
Central Lab Analysis
Analysis of genetic testing to determine whether at least 2 disease-causing variants in the EYS gene which are homozygous or heterozygous in trans
Yes
Otherwise: Participant is considered "Genetic screen failure"
Central Genetics Auditor (CGA)
Review genetic report to confirm Final Study Cohort Criteria met
CGA confirmed Final Study Cohort Criteria met
Otherwise: Participant is considered "Genetic screen failure"
Participant is considered "Enrolled into study cohort"
Proceed to Natural History Study
At the end of recruitment, all genetic reports of participants enrolled into the natural history study will be evaluated by the Genetics Committee for the following. This analysis will not change eligibility to be enrolled into study cohort.
Interpretation of pathogenicity based on American College of Medical Genetics (ACMG) criteria
Genetic classifications for analyses
Genetic Screening Criteria
One of the following criteria must be met to enter the Genetic Screening Phase:
Screening Group A: At least 2 disease-causing variants in the EYS gene which are homozygous or heterozygous in trans, based on a report from a clinically-certified lab (or a report from a research lab that has been pre-approved by the Genetics Committee)
Screening Group B: Only 1 disease-causing variant in the EYS gene, based on a report from a clinically-certified lab (or a report from a research lab which has been pre-approved by the Genetics Committee)
Screening Group C: At least 2 disease-causing variants in the EYS gene which are unknown phase, based on a report from a clinically-certified lab (or a report from a research lab which has been pre-approved by the Genetics Committee)
Final Study Cohort Criteria
At least 2 disease-causing variants in the EYS gene which are homozygous or heterozygous in trans, based on a report from a clinically certified lab (or a report from a research lab that has been pre-approved by the study Genetics Committee), and confirmed by a Central Genetics Auditor (CGA).


Pro-EYS Natural History Study
Includes participants completing the initial screening phase and genetics screening phase, and "Enrolled into study cohort"
See Protocol Schedule of Study Visits and Procedures
Vision Cohort 1: ~70 participants with the better eye Screening Visit visual acuity ETDRS letter score of 54 or more [approximate Snellen equivalent 20/80 or better] and visual field diameter 10 degrees or more in every meridian of the central field
Vision Cohort 2: ~20 participants with the better eye Screening Visit visual acuity ETDRS letter score of 19-53 [approximate Snellen equivalent 20/100 - 20/400] or (visual acuity ETDRS letter score of 54 or more [approximate Snellen equivalent 20/80 or better] and visual field diameter less than 10 degrees in any meridian of the central field)
Vision Cohort 3: ~10 participants with the better eye Screening Visit visual acuity ETDRS letter score of 18 or less [approximate Snellen equivalent 20/500 or worse]
Baseline Visit
Within 90 days of Screening Visit date
12 Month Visit
24 Month Visit
36 Month Visit
48 Month Visit
Study Completed
Baseline Visit
Within 90 days of Screening Visit date
12 Month Phone Call
24 Month Phone Call
36 Month Phone Call
48 Month Visit
Study Completed

SCHEDULE OF STUDY VISITS AND PROCEDURES

Visit Schedule for Vision Cohorts 1 and 2

Visit	Screening	Baseline	12M	24M	36M	48M
Visit Target Windows	(up to Day -90) [a]	(Day 0) [b]	Wk 52 ± 4 [c]	Wk 104± 4 [c]	Wk 156± 4 [c]	Wk 208± 4 [c]
Participant-Level Procedures						
Informed Consent	X					
Demographics/Screening Medical History (including pre-existing conditions, patient-reported daily activities and medications)	X					
Physical Exam (including height, weight and blood pressure)		X				
Concomitant Medications/Adverse Events		X	X	X	X	X
Patient Reported Outcomes (VA LV VFQ-48, PROMIS®-29, MRDQ and ViSIO-PRO) [i]		X		X		X
Ocular Procedures - *All testing performed in each eye*						
Complete Ophthalmic Exam [h]	X		X	X	X	X
Visual acuity (including refraction, ETDRS, BRVT if needed, LLVA if needed)	X	X [j]	X	X	X	X
Contrast Sensitivity (VectorVision CSV-1000E)		X	X	X	X	X
SD-OCT with measurement of EZ area (Heidelberg Spectralis)		X	X	X	X	X
Axial Length and Corneal Curvature measurements		X				
Near Infrared Reflectance Photos (Heidelberg Spectralis)		X				
Fundus Autofluorescence (Optos, ***where available***)		X	X	X	X	X
Fundus Autofluorescence (Heidelberg Spectralis, ***where available***)		X	X	X	X	X
Full-field ERG (Diagnosys Espion preferred)		X [d]				X [e]
Full-field Stimulus Threshold (Diagnosys Espion, ***where available***)		X	X	X	X	X
Static perimetry (Octopus 900 Pro)		X [f]	X	X	X	X
Fundus guided microperimetry (MAIA, ***where available***)		X [f]	X	X	X	X
Kinetic VF III4e *for Vision Cohort definition only*	X [g]					

a. All Screening Visit testing must be completed on the same day (with the exception of kinetic VF, as noted below)
b. Baseline Visit date (defined as the start date of all Baseline testing) must be no later than 2 weeks after receiving confirmation of meeting ***final study cohort criteria*** from the CGA (and if possible, within 90 days of the Screening Visit date). All Baseline testing must be completed within 7 days of the Baseline Visit date, except PROs as specified
c. All Follow up visit testing must be completed on the same day, except PROs as specified
d. If ERG has been undetectable in the past, no need to perform at baseline, at investigator's discretion
e. If ERG is undetectable at baseline, no need to perform at 48M, at investigator's discretion
f. For static perimetry and microperimetry, all Vision Cohort 1 and 2 participants will complete two tests at baseline. The results will be compared according to the ***visual field criteria*** (section 3.2) to determine if a third test is needed.
g. Kinetic VF III4e performed within the last 18 months prior to or including the Screening Visit date for Vision Cohort determination only
h. 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
i. PROs will be completed by participants who agree to answer these additional questions; may be completed in person or remotely any time within 6 months after Baseline or +/- 6 weeks of 24 or 48-month follow-up visits
j. If the Baseline visit date is more than 90 days after the Screening Visit date, all Visual acuity procedures must also be completed

Visit Schedule for Vision Cohort 3

Visit	Screening	Baseline	12M	24M	36M	48M
Visit Target Windows	(up to Day -90) [a]	(Day 0)[b]	Wk 52 ± 4 (Phone call only)	Wk 104± 4 (Phone call only)	Wk 156± 4 (Phone call only)	Wk 208± 4[c]
Participant-Level Procedures						
Informed Consent	X					
Demographics/Medical History (including pre-existing conditions, patient-reported daily activities and medications)	X					
Physical Exam (including height, weight and blood pressure)		X				
Concomitant Medications/Adverse Events		X	X	X	X	X
Patient Reported Outcomes (ULV-VFQ-50, PROMIS®-29, MRDQ and ViSIO-PRO))[f]		X				X
Ocular Procedures - *All testing performed in each eye*						
Complete Ophthalmic Exam[e]	X					X
Visual acuity (including refraction, ETDRS, BRVT if needed, LLVA if needed)	X	X[g]				X
SD-OCT with measurement of EZ area (Heidelberg Spectralis)		X				X
Axial Length and Corneal Curvature measurements		X				
Near Infrared Reflectance Photos (Heidelberg Spectralis, ***where available***)		X				
Fundus Autofluorescence (Optos, ***where available***)		X				X
Fundus Autofluorescence (Heidelberg Spectralis, ***where available***)		X				X
Full-field Stimulus Threshold (Diagnosys Espion, ***where available***)		X				X
Kinetic VF III4e *for Vision Cohort definition only*	X[d]					

a. All Screening Visit testing must be completed on the same day (with the exception of kinetic VF, as noted below)
b. Baseline Visit date (defined as the start date of all Baseline testing) must be no later than 2 weeks after receiving confirmation of meeting ***final study cohort criteria*** from the CGA (and if possible, within 90 days of the Screening Visit date). All Baseline testing must be completed within 7 days of the Baseline Visit date, except PROs as specified
c. Follow up visit testing must all be completed on the same day
d. Kinetic VF III4e performed within the last 18 months prior to or including the Screening Visit date, for Vision Cohort determination only
e. 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 the site should make it 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
f. PROs will be completed by participants who agree to answer these additional questions; may be completed in person or remotely any time within 6 months after Baseline or +/- 6 weeks of 48-month follow-up visits
g. If the Baseline visit date is more than 90 days after the Screening Visit date, all Visual acuity procedures must also be completed

Chapter 1: Background Information

1.1 Introduction

Biallelic mutations in *EYS* represent a common cause of retinitis pigmentosa.[1-3] Most commonly individuals present with a rod-cone dystrophy, but cases of cone-rod dystrophy and macular dystrophy have also been reported.[4,5] Mutations in *EYS* account for up to 24% of cases of autosomal recessive retinitis pigmentosa in Japan[9], 16% in Spain[6], 12% in France[7], and 7% in Israel.[8] *EYS* is composed of 44 exons, spans 2 Mb and encodes a 3,165 amino acid protein that has at least 28 EGF domains and 5 C-terminal Laminin G-like domains.[1,2] There are at least four isoforms expressed in the human retina.[10] The exact role of *EYS* is unclear but it has been speculated to be involved in maintenance of the ciliary axoneme in rods and cones.[11-13] Studies of the function of *EYS* have been hampered by the fact that the gene is disrupted in the mouse. Deletion of the gene in zebrafish using transcription activator-like effector nuclease (TALEN) leads to defects in photoreceptor outer segments and results in a cone-rod dystrophy[11] and suggests its importance in photoreceptor function.

In humans, both missense and nonsense mutations have been reported in *EYS*. However, there has not been a strong establishment of genotype-phenotype correlations, and even missense mutations can sometimes cause a severe phenotype. There do not appear to be any hot spots for mutations, but mutations in the carboxyl terminus have been associated with a less common presentation of cone-rod dystrophy.[4] Confusingly, mutations that have been reported to cause cone-rod dystrophy have also been reported to cause rod-cone dystrophy in other patients.[14] Other studies have reported that mutations in the N-terminus of the protein may present with more severe degeneration than mutations in the C-terminus.[15]

The most common phenotype reported in patients is a rod-cone dystrophy with relative central sparing.[7] The average age of onset has been reported to be approximately twenty years with loss of visual acuity starting around age thirty. Most patients have non-recordable electroretinograms (ERGs) at the time of presentation, but patients may demonstrate some preservation on multifocal ERGs.[7] Variation in autofluorescent patterns have been reported to correlate with severity of the disease. Some patients present with a crescent-shaped hyperautofluorescent ring with extension into the nasal/superior retina. This pattern has been associated with more mild disease and recordable ERGs.[15] Posterior subcapsular cataracts are a common manifestation of this mutation and cystoid macular edema (CME) has a reported incidence of 31%.[7]

Retrospective studies have shown the rate of vision loss in *EYS* to be more severe than that of mutations caused by *USH2A*.[16] Loss of visual acuity has been estimated to be 5.7% year with the median age for visual acuity to drop below 20/32 to be 36 years.[16] The average rate of visual field (VF) loss has been estimated to be 23.1% per year and loss of ellipsoid zone (EZ) width to be 3-5% per year.[5,16,17] To date, no large-scale prospective study of visual function has been conducted for patients with biallelic mutations in *EYS*. The Pro-EYS study will conduct a multicenter natural history study in patients with biallelic mutations in *EYS* with the purpose of better understanding disease progression as well as obtaining preliminary data for potential therapeutic trials in the future.

1.2 Scientific Rationale for Study Design

A prospective natural history study is the gold standard for tracking the course of disease. The knowledge of the course of patients with *EYS* mutations will guide the planning of future controlled treatment trials. Identifying the most sensitive and reliable outcome measures of retinal degeneration will greatly facilitate development of treatments with maximum efficiency. Together, these approaches are expected to have an impact on understanding *EYS*-related retinal degeneration, developing investigational treatment protocols, and assessing their effectiveness.

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

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

1.3 Study Objectives

1. Characterize the natural history of retinal degeneration associated with biallelic pathogenic mutations in the *EYS* gene over 4 years, as measured using functional, structural, and patient-reported outcome measures
2. Investigate whether structural outcome measures can be validated as surrogates for functional outcomes in individuals with biallelic pathogenic mutations in the *EYS* gene
3. Evaluate possible risk factors (genotype, phenotype, environmental, and comorbidities) for progression of the outcome measures at 4 years in individuals with biallelic pathogenic mutations in the *EYS* gene
4. Evaluate variability and symmetry of left and right eye outcomes over 4 years in individuals with biallelic pathogenic mutations in the *EYS* gene

1.4 General Considerations

The study is being conducted in compliance 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 (GCP). Employing a prospective longitudinal study design is advantageous because it reflects a systematic method of data collection. This study design incorporates several strategies to minimize bias, detailed below, using considerations from "Rare diseases: Natural History Studies for Drug Development: Guidance for Industry, Draft Guidance."[18] These are considered standard for treatment trials and will enhance the translation of the data from this study to a treatment trial.

- Establishing standardized testing procedures and specific required equipment for all investigators, leading to greater consistency and precision in the information collected
- Training and certification of study staff who will perform the following procedures related to the primary outcomes: Static Perimetry (SP), Optical Coherence Tomography

(OCT), Microperimetry (MP), Fundus autofluorescence (FAF), ERG) by a Reading Center. The Reading Center will grade test results in a uniform manner independently from study sites

- Use of standard, consistent definitions of pre-existing medical conditions, medications and treatments, and adverse events (AEs) across all clinical sites
- A consistent schedule of follow-up visits for all participants with established visit time frames
- A coordinating center (CC) is responsible for monitoring the conduct of the study to ensure adherence to protocol

Chapter 2: Study Enrollment and Screening Visit

2.1 Participant Recruitment and Enrollment

Study participants will be recruited from approximately 30 clinical sites worldwide. All eligible participants will be included without regard to gender, race, or ethnicity. Potential eligibility will be assessed during a routine examination by an investigator prior to obtaining informed consent, as part of usual care, through referrals from other providers or self-referral.

2.1.1 Participant Recruitment Goals and Strategy

Recruitment will be tracked within 3 Vision Cohorts defined as follows. Sample size rationale is detailed in section 6.1.

- **Vision Cohort 1**: ~70 participants with the ***better eye*** Screening Visit visual acuity ETDRS letter score of 54 or more [approximate Snellen equivalent 20/80 or better] **and** ***visual field*** diameter 10 degrees or more in every meridian of the central field
- **Vision Cohort 2:** ~20 participants with the ***better eye*** Screening Visit visual acuity ETDRS letter score of 19-53 [approximate Snellen equivalent 20/100 - 20/400] **or** (visual acuity ETDRS letter score of 54 or more [approximate Snellen equivalent 20/80 or better] **and** ***visual field*** diameter less than 10 degrees in any meridian of the central field)
- **Vision Cohort 3**: ~10 participants with the ***better eye*** Screening Visit visual acuity ETDRS letter score of 18 or less [approximate Snellen equivalent 20/500 or worse]

The ***better eye*** is defined as the eye with better Screening Visit ETDRS VA. If both eyes have the same VA (defined as the same Snellen equivalent), then the determination will be made at investigator discretion as the eye with better fixation or clear ocular media to permit highest quality retinal imaging.

The ***visual field*** (VF) is defined as the clinically determined kinetic VF III4e performed within the last 18 months prior to or including the Screening Visit date.

	VF diameter ≥10° in every meridian	VF diameter <10° in any meridian
20/80 or better	Vision Cohort 1	Vision Cohort 2
20/100-20/400	Vision Cohort 2	Vision Cohort 2
20/500 or worse	Vision Cohort 3	Vision Cohort 3

The Foundation Fighting Blindness (FFB) Consortium Executive Committee will review recruitment progress and feasibility at regular intervals, including an evaluation 5 months after recruitment begins. Initial recruitment goals will be as follows:

- 100 participants ***enrolled into the study cohort*** (Cohorts 1, 2, and 3 combined)
- 10 participants enrolled in Vision Cohort 3

- 90 participants enrolled in Cohort 1 and Vision Cohort 2 combined
  - The FFB Consortium Executive Committee may also recommend individual Vision Cohort maximums based on interim adjusted projections
- If recruitment is not at a rate to meet the initial goals, an interim assessment of feasibility may be made by the FFB Consortium Executive Committee. A minimum of 65 participants enrolled in Vision Cohort 1 and Vision Cohort 2 combined will need to be recruited.

Participants will not be counted as *enrolled into the study cohort* until initial screening and genetic screening have been confirmed as a success (sections 2.3 and 2.4). This means that more participants will be screened than noted above; the number and reasons for screen failures will be tracked. It is also possible that some participants will have completed the Screening Visit and will be awaiting genetic confirmation at the time the enrolled numbers reach the goals above; therefore, the final enrolled numbers may be larger. To limit over-enrollment, clinical sites will be notified as the recruitment goals near completion, efforts will be made to accurately predict numbers in the genetic screening queue, and consent and screening of participants which could contribute to over-enrollment in a given Vision Cohort may be paused.

2.2 Informed Consent and Authorization Procedures

Potential eligibility may be assessed as part of a routine care examination by an investigator prior to obtaining informed consent, as part of usual care, by referral from another physician, or self-referral. Before completing any procedures or collecting any data that are not part of usual care, written informed consent will be obtained, using consent documentation approved by the overseeing IRB/ EC.

The study protocol will be discussed with the potential study participant by study staff. The potential study participant will be given the Informed Consent Form (ICF) to read. Potential study participants with severe vision impairment may be presented with a Short Form, to be read aloud by a clinical staff member if they prefer, following the overseeing IRB/ EC requirements. Potential study participants will be encouraged to discuss the study with family members and their personal physicians(s) before deciding whether to participate in the study.

As part of the informed consent process, each participant will be asked to sign an authorization for release of personal information. The investigator, or his or her designee, will review the study-specific information that will be collected and to whom that information will be disclosed. After speaking with the participant, questions will be answered about the details regarding authorization.

A participant is considered ***enrolled into the initial screening*** when the ICF has been signed and a participant ID has been obtained on the study website.

An immediate family member(s) of study participants may be asked to participate in family member genetic testing as part of the genetic screening phase. In these cases, a family member(s) will be asked to provide a saliva sample (described in section 2.4). An electronic consent form will be reviewed and signed by the family member(s) in order to obtain permission to collect a saliva sample.

2.3 Screening Visit

After the ICF has been signed, a potential participant will be evaluated for study eligibility through the elicitation of a medical history, performance of ophthalmic tests as described below, and genetic testing, if applicable. The Screening Visit date will be the date the Screening Visit testing procedures started. All Screening Visit testing procedures should be completed on this date.

2.3.1 Eligibility Criteria

To be eligible to ***enroll into the genetic screening phase,*** a study participant must meet all of the inclusion criteria and none of the exclusion criteria at the Screening Visit.

2.3.1.1 Participant Criteria

Participant Inclusion Criteria

Participants must meet all of the following inclusion criteria at the Screening Visit in order to be eligible to ***enroll into the genetic screening phase***.

1. Willing to participate in the study and able to communicate consent during the consent process
2. Ability to return for all study visits over 48 months
3. Age ≥ 18 years
4. Must meet one of the Genetic Screening Criteria, defined below:
   - **Screening Group A: At least 2 disease-causing variants** in the *EYS* gene which are **homozygous** or **heterozygous *in trans***, based on a report from a clinically-certified lab (or a report from a research lab that has been pre-approved by the Genetics Committee)
   - **Screening Group B: Only 1 disease-causing variant** in the *EYS* gene, based on a report from a clinically-certified lab (or a report from a research lab which has been pre-approved by the Genetics Committee)
   - **Screening Group C: At least 2 disease-causing variants** in the *EYS* gene which are **unknown phase**, based on a report from a clinically-certified lab (or a report from a research lab which has been pre-approved by the Genetics Committee)

Note pertaining to all Screening Groups: if a participant has a variant(s) of unknown significance, he/she would still qualify as long as there is at least 1 disease-causing variant(s) on the EYS gene.

Participant Exclusion Criteria

Participants must not meet any of the following exclusion criteria at the Screening Visit in order to be eligible to ***enroll into the genetic screening phase***.

1. Mutations in genes that cause autosomal dominant retinitis pigmentosa (ADRP), X-linked retinitis pigmentosa (RP), or presence of biallelic mutations in autosomal recessive RP/retinal dystrophy genes other than *EYS*
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)

2.3.1.2 Ocular Criteria

Ocular Inclusion Criteria

Both eyes must meet all of the following at the Screening Visit in order for a participant to be eligible to ***enroll into the genetic screening phase***.

1. Clinical diagnosis of retinal dystrophy
2. Clear ocular media and adequate pupil dilation to permit good quality photographic imaging

Ocular Exclusion Criteria

If either eye has any of the following at the Screening Visit, the participant is not eligible to ***enroll into the genetic screening phase***.

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 glaucomatous VF changes or nerve changes, or history of glaucoma filtering surgery)
6. Current or any history of retinal vascular occlusion or proliferative diabetic retinopathy
7. History or current evidence of ocular disease that, in the opinion of the investigator, may confound assessment of visual function
8. History or evidence of active treatment for retinitis pigmentosa that could affect the progression of retinal degeneration, including:
    a. Any use of ocular stem cell or gene therapy
    b. Any treatment with ocriplasmin
    c. Treatment with an ophthalmic oligonucleotide within the last 9 months (last treatment date is less than 9 months prior to Screening Visit date)
    d. Treatment with any other product within five times the expected half-life of the product (time from last treatment date to Screening Visit date is at least 5 times the half-life of the given product)

2.3.2 Screening Data Collection and Testing

The study design schematic at the beginning of the protocol shows the flow of the Screening Visit. The following procedures will be performed at the Screening Visit. The testing procedures are detailed in the Pro-EYS Procedures Manuals. An overview of the equipment and technician requirements for all testing is in section 3.4. All ocular testing will be performed in each eye, right eye (OD) first and then left eye (OS).

Participants meeting criteria to continue will be ***enrolled into the genetic screening phase*** (section 2.4***)***. Otherwise, the participant will be an ***initial screen failure*** (section 2.3.3). The below information will be collected at the Screening Visit:

1. Contact information (retained at the clinical site and not entered into study database)
2. Inclusion and exclusion criteria assessment (criteria in sections 2.3.1.1 and 2.3.1.2)
3. Demographics (including sex, race, ethnicity)
4. A medical history will be elicited from the study participant and extracted from available medical records, including patient-reported daily activities, pre-existing medical conditions and medications
5. Complete ophthalmic exam. Exam will include slit-lamp biomicroscopy, indirect ophthalmoscopy, and intraocular pressure (IOP). IOP measurements will be taken prior to pupil dilation
6. Genetic screening assessment (including number and phase of mutations in the *EYS* gene, history of consanguinity, and collection of the source genetic report(s) available at the clinical site)
   - This includes an assessment that the participant meets one of the Genetic Screening Criteria described in section 2.3.1.1. If the participant does not meet the criteria for one of the Genetic Screening Groups (section 2.3.1.1), the remainder of procedures and testing are not required. Participant should be discontinued as an ***initial screen failure*** per section 2.3.3
7. Visual acuity (including refraction, ETDRS, BRVT if needed, LLVA if needed)
   - The VA letter score will determine whether LLVA or BRVT should be performed. The criteria are defined in the Pro-EYS Procedures Manuals.
8. Kinetic VF III4e (or historical measurement performed within the last 18 months prior to or including the Screening Visit date) Vision Cohort determination, based on eye with better visual acuity and kinetic VF above (criteria in section 2.1.1)
   - If the participant's determined Vision Cohort is closed for enrollment, the remainder of procedures and testing are not required. Participant should be discontinued as an ***initial screen failure*** per section 2.3.3

2.3.3 Initial Screen Failures

Participants who do not meet criteria to continue as noted above will be discontinued as an ***initial screen failure***. The Screening Visit Form will still be completed, entering "Not Done" for

testing not finished. A Final Status Form will be completed, and the reason for screen failure will be noted.

2.4 Genetic Screening Phase

Participants passing the initial screening and enrolling into the Genetic Screening Phase will complete the following genetic testing and/or review procedures, according to their Screening Group (defined in section 2.3.1.1). The study design schematic at the beginning of the protocol also summarizes the flow of the Genetic Screening Phase. More detailed procedures are specified in the Pro-EYS Procedures Manual.

- All genetic reports noted below to be uploaded to the study website by the clinical site or Central Lab may be available to and reviewed by the CC, associated clinical site, Central Lab, CGA, Genetics Committee, and investigators involved in oversight of the study (which include the study chair, Operations Committee, and FFB Consortium Executive Committee). All reports will be de-identified prior to uploading

<u>Screening Group A</u>

- The clinical site will upload supporting genetic documentation (including genetic reports) onto the study website
- A CGA will review the genetic documentation provided by the clinical site to verify the genetic screening data entry and appropriate documentation of the ***final study cohort criteria*** (section 2.5***)*** of **at least 2 disease-causing variants** in the *EYS* gene which are **homozygous or heterozygous *in trans***. Additional documentation may be requested as needed to verify the ***final study cohort criteria*** and all genetic screening assessments
    - If ***final study cohort criteria*** are verified, participant will be considered ***enrolled into the study cohort***
    - Otherwise the participant will be a ***genetic screen failure*** (section 2.4.1)

<u>Screening Group B or C</u>

- The clinical site will upload supporting genetic documentation (including genetic reports) onto the study website
- Participants will be asked to provide a saliva sample, and approach at least 1 first-degree relative to provide a saliva sample for additional genetic testing. 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)'s samples will be shipped to and analyzed by the Central Lab to conduct retinal dystrophy panel genetic testing and determine the presence and number of disease-causing variants on the *EYS* gene (Screening Group B only), and the phase of the alleles from the family member testing (Screening Groups B

and C). The Central Lab will provide these assessments and its genetic report(s), which will be uploaded to the study website

- If the Central Lab determines there are at least 2 disease-causing variants in the *EYS* gene which are homozygous or heterozygous *in trans*, the participant's reports will move forward to CGA review
- Otherwise the participant will be a ***genetic screen failure*** (section 2.4.1)

- A CGA will review the reports provided by the clinical site and the Central Lab to verify the genetic screening data entry and appropriate documentation of the ***final study cohort criteria*** (section 2.5) of **at least 2 disease-causing variants** in the *EYS* gene which are **homozygous or heterozygous *in trans*.** Additional documentation may be requested as needed to verify the ***final study cohort criteria*** and all genetic screening assessments
  - If ***final study cohort criteria*** are verified, the participant will be considered ***enrolled into the study cohort***
  - Otherwise the participant will be a ***genetic screen failure*** (section 2.4.1)

2.4.1 Genetic Screen Failures

Participants who do not meet criteria to continue as noted above will be discontinued as a ***genetic screen failure***. A Final Status Form will be completed, and the reason for screen failure will be noted.

2.4.2 Genetics Committee Review

A Genetics Committee will review the genetic documentation of participants with verified ***final study cohort criteria*** and ***enrolled into the study cohort*** for interpretation/evaluation of whether or not the *EYS* mutations are causative of the disease (i.e., pathogenic). Details of the process are described in the Pro-EYS Monitoring Plan. Cases that are not confirmed as disease- causing will remain in the study and will not be considered ineligible, however their data may be analyzed separately from those with pathogenic mutations.

2.5 Participants Enrolled into the Natural History Study

All participants meeting initial screening and eligibility criteria (section 2.3.1) who complete the Genetic Screening Phase (section 2.4) and meet the ***final study cohort criteria*** (defined below) will be considered ***enrolled into the study cohort*** and will complete the natural history study.

- ***Final Study Cohort Criteria***: **At least 2 disease-causing variants** in the *EYS* gene which are **homozygous or heterozygous *in trans***, based on a report from a clinically-certified lab (or a report from a research lab that has been pre-approved by the study genetics committee), and confirmed by a CGA.

Chapter 3: Natural History Study Procedures

3.1 Baseline Visit

Participants meeting criteria to enter the natural history study (section 2.5) will return for a Baseline Visit date within 90 days of the Screening Visit date if possible, and no later than 2 weeks after receiving confirmation of meeting ***final study cohort criteria*** from the CGA. The Baseline Visit date is the date on which the Baseline Visit testing procedures begin. All Baseline Visit testing procedures should be completed within 7 days of the Baseline Visit date, except PROs as specified below.

The testing performed at the Screening Visit will serve as baseline measures for the study and will not be completed again at the Baseline Visit. The only exception is if Baseline Visit date is more than 90 days after Screening Visit date, then visual acuity testing will be repeated (including refraction, ETDRS, LLVA if needed, BRVT if needed).

3.2 Baseline Testing Procedures

The following procedures will be performed at the Baseline Visit. The testing procedures are detailed in the Pro-EYS Procedures Manuals. **An overview of the equipment and certification requirements for all testing is in section 3.4.** All ocular testing will be performed in each eye, OD first and then OS.

1. Medical updates, including new/changed adverse events, ocular procedures, and medications
2. Physical exam (including height, weight, and blood pressure)
3. Patient Reported Outcomes (PROs)- may be completed in person or remotely (phone or other remote methods) any time within 6 months of the Baseline visit)
    a. VA LV VFQ-48 - *Vision Cohorts 1 and 2 only*
    b. ULV-VFQ-50 - *Vision Cohort 3 only*
    c. PROMIS®-29 - *All Vision Cohorts*
    d. MRDQ- *All Vision Cohorts*
    e. ViSIO-PRO- *All Vision Cohorts*
4. Contrast sensitivity - *Vision Cohorts 1 and 2 only*
5. SD-OCT
6. Axial Length and Corneal Curvature measurements
7. Near Infrared Reflectance Photos
8. Fundus Autofluorescence (on Optos, ***where available***)
9. Fundus Autofluorescence (on Heidelberg Spectralis, ***where available***)
10. Full-field ERG -*Vision Cohorts 1 and 2 only*

a. If non-detectable in the past (defined at investigator discretion), testing is not required

11. Full-field Stimulus Threshold

12. Static perimetry - *Vision Cohorts 1 and 2 only*

a. Two tests will be performed. The clinical site will compare the certified technician-determined mean sensitivity from test 1 versus test 2

- If the absolute value of the difference between the two tests is ≤ 2.4 dB then the participant passes static perimetry reliability criteria and a third test is <u>not</u> needed
- If the absolute value of the difference between the two tests is > 2.4 dB then the participant does not pass static perimetry reliability criteria, and a third test <u>is needed</u>

13. Fundus-guided microperimetry- *Vision Cohorts 1 and 2 only*

a. Two tests will be performed. The clinical site will compare the certified technician-determined mean sensitivity from test 1 versus test 2

- If the absolute value of the difference between the two tests divided by the average between them is ≤ 50% OR the absolute value of the difference between the two tests is ≤ 0.5 dB, then the participant passes microperimetry reliability criteria and a third test is <u>not</u> needed
- If the absolute value of the difference between the two tests divided by the average between them is > 50% AND the absolute value of the difference between the two tests is > 0.5 dB then the participant does not pass microperimetry reliability criteria and a third test is needed

3.3 Follow-up Visits

The Baseline Visit date is considered study day 0, from which follow-up visit windows are timed. The Follow-up Visit date will be the date the Follow-up Visit testing procedures started. All Follow-up Visit testing procedures should be completed on the same date, with the exception of PROs, which can be completed ± 6 weeks of the required visits as noted below.

Follow up visits will be conducted at:

Visit	Target	Target Window	Allowable Window
12 Month Visit (Vision Cohorts 1 and 2)	52 Weeks	± 4 Weeks	± 6 Weeks
24 Month Visit (Vision Cohorts 1 and 2)	104 Weeks	± 4 Weeks	± 6 Weeks
36 Month Visit (Vision Cohorts 1 and 2)	156 Weeks	± 4 Weeks	± 6 Weeks
48 Month Visit (All Vision Cohorts)	208 Weeks	± 4 Weeks	± 6Weeks

Visits should be scheduled in the target window whenever possible. If circumstances do not permit this, visits may be scheduled to extend out to the allowable window without being

considered a protocol deviation, but the reason for scheduling outside of the target window will be documented on the visit form. Visits occurring out of the allowable window may still be completed and used for analysis but will be documented as protocol deviations. Details regarding when to consider a visit missed are specified in the Pro-EYS Procedures Manuals.

The goal will be for all participants to complete all scheduled visits. However, participants who (because of unforeseen circumstances) are unable or unwilling to return for all follow-up visits will be permitted to return for key visits only as an alternative to withdrawal from the study. When a participant is placed into this status, missed visits will not be recorded as protocol deviations (since they would not be recorded as protocol deviations if the participant was dropped from the study).

3.3.1 Follow-up Visit Testing Procedures

The following procedures will be performed at the Follow-up Visits as noted below. The testing procedures are detailed in the Pro-EYS Procedures Manuals. An overview of the equipment and certification requirements for all testing is in section 3.4. All ocular testing will be performed in each eye, OD first and then OS.

Vision Cohorts 1 and 2

The following will be performed at the 12 Month, 24 Month, 36 Month, and 48 Month Visits unless otherwise noted.

1. Medical updates, including new/changed AEs, ocular procedures, and medications
2. Patient Reported Outcomes (VA LV VFQ-48, PROMIS®-29, MRDQ, ViSIO-PRO) – *24 Month and 48 Month Visits only*
    a. May be completed in person or remotely (phone or other remote methods) any time within the Allowable Window of the associated visit
3. Complete ophthalmic exam. Exam will include slit-lamp biomicroscopy, indirect ophthalmoscopy, and intraocular pressure (IOP). IOP measurements will be taken prior to pupil dilation
4. Visual acuity (including refraction, ETDRS, BRVT if needed, LLVA if needed)
    a. The visual acuity letter score will determine whether LLVA or BRVT should be performed. The criteria are defined in the Pro-EYS Procedures Manuals.
5. Contrast sensitivity
6. SD-OCT
7. Fundus Autofluorescence (on Optos, ***where available***)
8. Fundus Autofluorescence (on Heidelberg Spectralis, ***where available***)
9. Full-field ERG - *48 Month Visit only*

a. If ERG was non-detectable prior to or at baseline (defined at investigator discretion), testing is not required

10. Full-field Stimulus Threshold

11. Static perimetry

    a. Static perimetry should be completed to the best of the participant's ability. If the investigator feels that the participant will be unable to complete static perimetry reliably at the follow up visit, a waiver may be obtained in advance from the study chair to skip static perimetry

12. Fundus- guided Microperimetry

    a. Fundus- guided microperimetry should be completed to the best of the participant's ability. If the investigator feels that the participant will be unable to complete microperimetry reliably at the follow up visit, a waiver may be obtained in advance from the study chair to skip microperimetry

Vision Cohort 3

The following will be performed at the 48 Month Visit.

1. Medical updates, including new/changed adverse events, ocular procedures, and medications
2. Patient Reported Outcomes (ULV-VFQ-50, PROMIS®-29, MRDQ and ViSIO-PRO)
    a. May be completed in person or remotely (phone or other remote methods) any time within the Allowable Window of the associated visit
3. Complete ophthalmic exam. Exam will include slit-lamp biomicroscopy, indirect ophthalmoscopy, and intraocular pressure (IOP). IOP measurements will be taken prior to pupil dilation
4. Visual acuity (including refraction, ETDRS, BRVT if needed, LLVA if needed)
    a. The visual acuity letter score will determine whether LLVA or BRVT should be performed. The criteria are defined in the Pro-EYS Procedures Manuals.
5. SD-OCT
6. Fundus Autofluorescence (on Optos, ***where available***)
7. Fundus Autofluorescence (on Spectralis, ***where available***)
8. Full-field Stimulus Threshold

Phone contact will be scheduled at 12, 24, and 36 Month intervals. The purpose of the phone contact will be to keep the participants engaged in the study during the interim between the Baseline and 48 Month Follow-up Visits and keep contact information updated. Changes in medications and AEs will also be collected.

3.3.2 Unscheduled Visits

Testing procedures at unscheduled visits are at investigator discretion. However, it is recommended that procedures that are performed should follow the standard protocol for each procedure and by certified personnel whenever possible. Unscheduled visits which occur during the study should be recorded in the FFB Consortium study website. Study images taken during any unscheduled visits are not required to be submitted to the study website.

3.4 Personnel and Equipment Requirements for Study Procedures

The testing procedures are detailed in the Pro-EYS Procedures Manuals. An overview of the equipment and certification requirements for all testing are as follows.

Study Procedures	Equipment Required (if applicable)	Who can Perform
Investigator taking overall responsibility for a visit: oversees that consent process was performed in accordance with IRB/EC requirements, signs off on all eCRFs for a participant, eCRF edits, and protocol deviations	N/A	Certified investigator
Coordinator taking responsibility for the visit: oversees the data entry aspect of the visit, addresses protocol queries and signs off on deviations	N/A	Certified coordinator
Informed consent: explanation/review of study with the potential participant and/or signature of ICF	N/A	Certified investigator/coordinator as permitted by the IRB/EC
Signature of Informed Consent Form	N/A	Certified investigator/ coordinator as permitted by the IRB/EC
Data entry on study website	N/A	Certified coordinator (or certified investigator with additional study website certification)
Sample collection and shipping	Study will provide necessary materials – detailed in Pro-EYS Procedures Manuals	Certified coordinator
Collect information regarding medical history, demographics, physical exam, adverse events, medications	N/A	Does not need to be performed by study certified personnel*
Patient Reported Outcomes	Study will provide necessary materials – detailed in Pro-EYS Procedures Manuals	Certified coordinator
Ocular Exam (including slit-lamp biomicroscopy, indirect ophthalmoscopy and intraocular pressure IOP)	Any equipment is acceptable	Does not need to be performed by study certified personnel*
Visual Acuity - Refraction	N/A	Clinical site personnel certified for refraction
Visual Acuity - ETDRS	EVA system (preferred) otherwise ETDRS charts	Clinical site personnel certified for ETDRS
Visual Acuity - LLVA	EVA system (preferred) otherwise ETDRS charts 2.0 neutral density filter to be provided by study	Site personnel certified for performing ETDRS is also certified to perform LLVA
Visual Acuity - BRVT	BRVT charts provided by study	Clinical site personnel certified for BRVT

Contrast Sensitivity	VectorVision CSV-1000E provided by study	Does not need to be performed by study certified personnel*
SD-OCT	Heidelberg Spectralis	Clinical site personnel certified for SD-OCT
Axial Length and Corneal Curvature	Any equipment is acceptable	Does not need to be performed by study certified personnel*
Near Infrared Reflectance Photos	Heidelberg Spectralis	Clinical site personnel certified for Near Infrared Photos
Fundus Autofluorescence (on Optos)	Optos (where available)	Clinical site personnel certified for Fundus Autofluorescence on Optos
Fundus Autofluorescence (on Heidelberg Spectralis)	Heidelberg Spectralis (where available)	Clinical site personnel certified for Fundus Autofluorescence on Spectralis
Full-field ERG	Diagnosys Espion (preferred)	Clinical site personnel certified for ERG
FST	Diagnosys Espion (where available)	Does not need to be performed by study certified personnel*
Static Perimetry	Octopus 900 Pro (GATE Protocol)	Clinical site personnel certified for SP
Fundus-guided Microperimetry	MAIA (if available)	Clinical site personnel certified for MP
Kinetic Perimetry (historical)	Any equipment is acceptable	(Historical) Does not need to be performed by study certified personnel or recorded in the Pro-EYS Study Staff Delegation Log
** Personnel who will be performing procedure must be documented in the Pro-EYS Study Staff Delegation Log. The Principal Investigator (PI) is responsible for verifying individual qualifications and training specific to performing each type of procedure and ultimate accuracy and integrity of such data*		

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Chapter 4: Unanticipated Problem and Adverse Event Reporting

4.1 Unanticipated Problems

Site investigators will promptly report to the CC all unanticipated problems meeting the criteria below. For this protocol, an unanticipated problem is an incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol related documents, such as the IRB/EC-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places participants or others at a greater risk of harm than was previously known or recognized **(**including physical, psychological, economic, or social harm)

The CC also will report to the IRB all unanticipated problems not directly involving a specific site such as unanticipated problems that occur at the CC or at another participating entity such as a laboratory.

4.2 Adverse Events

4.2.1 Definition

Adverse Event (AE): Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (modified from the definition of AEs in the Integrated Addendum to ICH E6 (R2)).[19]

Serious Adverse Event (SAE): Any untoward medical occurrence that:

- Results in death
- Is life-threatening; (a non-life-threatening event which, had it been more severe, might have become life-threatening, is not necessarily considered a SAE)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions (sight threatening)
- Is a congenital anomaly or birth defect

- Is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the participant or may require medical/surgical intervention to prevent one of the outcomes listed above)

4.2.2 Reportable Adverse Events

For this protocol, a reportable AE includes all events meeting the definition of an AE above.

All AEs—whether volunteered by the participant, discovered by study personnel during questioning, or detected through examination, laboratory test, or other means—will be reported on an AE form online.

The purpose of AE collection for the Pro-EYS study will be to provide historical controls for future clinical trials. As a no greater than minimal risk study, AEs do not require any specific 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.

4.2.3 Relationship of Adverse Event to Study Procedure

The study investigator will assess the relationship of any AE to be related or unrelated to a study procedure by determining if there is a reasonable possibility that the AE may have been caused by the procedure.

To ensure consistency of AE causality assessments, investigators should apply the following general guideline when determining whether an AE is related:

Yes

There is a plausible temporal relationship between the onset of the AE and a study procedure, and the AE cannot be readily explained by the participant's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to a study procedure; and/or the AE abates or resolves upon discontinuation of a study procedure and, if applicable, reappears upon re-challenge.

No

Evidence exists that the AE has an etiology other than a study procedure (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to a study procedure.

4.2.4 Severity (Intensity) of Adverse Event

The severity (intensity) of an AE will be rated on a three-point scale: (1) mild, (2) moderate, or (3) severe. A severity assessment is a clinical determination of the intensity of an event. Thus, a severe AE is not necessarily serious. For example, itching for several days may be rated as severe, but may not be clinically serious.

1. MILD: Usually transient, requires no special treatment, and does not interfere with the participant's daily activities

2. MODERATE: Usually causes a low level of inconvenience, discomfort or concern to the participant and may interfere with daily activities but is usually ameliorated by simple therapeutic measures and participant is able to continue in study
3. SEVERE: Interrupts a participant's usual daily activities, causes severe discomfort, may cause discontinuation of study drug, and generally requires systemic drug therapy or other treatment

Chapter 5: Miscellaneous Considerations

5.1 Treatments During the Study

5.1.1 Treatment for *EYS*-Related Retinal Degeneration

Participants ***enrolled into the final study cohort*** should not plan to enroll into experimental treatment trials of underlying conditions related to *EYS* mutations during the 4-year study duration. Participants who do enroll into such a trial will be evaluated by the FFB Consortium Executive Committee to determine if they may continue participating in the Pro-EYS study.

5.1.2 Treatment for Cystoid Macular Edema

Participants ***enrolled into the final study cohort*** who are receiving treatment for CME throughout the duration of the study may continue doing so without affecting their participation in the Pro-EYS study.

5.1.3 Intraocular Surgical Procedures

Participants ***enrolled into the final study cohort*** who have intraocular surgery during the course of the study should have follow-up visits timed either before the surgery date or at least 3 months after the surgery date. Clinical sites should make reasonable efforts to schedule the participant's follow-up visit as close to the visit target window as possible.

5.2 Risks and Benefits

5.2.1 Risks and Discomforts

Most examination procedures are considered part of standard care for retinal degenerations. The procedures have been standardized for consistency across sites 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 VA, KP, SP, Optos or Spectralis FAF, Near infrared reflectance photos, and PRO may 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 ophthalmic examination and before the SD-OCT, ERG, FST, and MP. 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, a tear ointment may be administered, and an eye patch or gauze may be placed over the eye
- 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. 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

5.2.2 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 *EYS*-related retinal degeneration and contribute to future development of treatments.

5.3 Collection of Pre-Existing Conditions and Medications

Pre-Existing Condition: Any medical condition that is either present at screening, a chronic disease, or a prior condition that could impact the participant's health during the course of the study (e.g., prior myocardial infarction or stroke) should be recorded.

Medications: All medication for the treatment of chronic pre-existing conditions, medical conditions, and/or AEs that the participant is currently taking at screening and during the course of the study should be recorded. Nutraceuticals and preventative treatment also should be recorded.

5.4 Participant Compensation

Participant compensation will be specified in the ICF.

5.5 Participant Withdrawal

Participation in the study is voluntary, and a participant may withdraw at any time. For participants who withdraw, their data will be used up until the time of withdrawal.

5.6 Confidentiality

For security and confidentiality purposes, participants will be assigned an identifier that will be used instead of their name. Protected health information gathered for this study will be shared with the FFB Consortium CC, the Jaeb Center for Health Research in Tampa, Florida, USA. De-identified participant information may also be provided to research sites involved in the study.

Chapter 6: 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.

6.1 Sample Size

The sample size evaluation focuses on objective 1 of the study, to characterize the natural history of retinal degeneration associated with biallelic pathogenic mutations in the *EYS* gene over 4 years on both the structural and functional outcomes of interest. Calculations to address objective 3, evaluation of possible risk factors associated with progression, are summarized. A justification of the selected sample size using percent change for the outcomes of interest is outlined. The precision of the between-eye correlation is also provided.

It should be noted that the sample size for Vision Cohort 3 is a convenience sample, i.e., 10 participants. The objectives of including this population are to:

- Establish baseline FST and PRO measurement data in patients with very low vision to be used in future trials of optogenetics, stem cells and other regenerative technology
- Establish the most extended range of patients with *EYS* mutations in the measures possible
- Obtain cross sectional data in patients with the furthest disease progression
- Establish which tests are the most useful in patients with the furthest disease progression

6.1.1 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 may be used to consider percent change on any outcome measure from baseline to 4 years.

Both eyes of a participant will be assessed for the main outcomes of interest. Thus, if there are N participants, 2N eyes will be available for analysis. However, outcome measures from 2 eyes of a person are typically strongly correlated ($r \geq 0.5$). The contribution of information in this case is $(2/(1+r))$ instead of 2. Values for the multiplier to the number of participants to obtain an effective sample size are given below:

r	Effective N
0.0	2.00
0.1	1.82
0.2	1.67

0.3	1.54
0.4	1.43
0.5	1.33
0.6	1.25
0.7	1.18
0.8	1.11
0.9	1.05
1.0	1.00

One objective is to estimate the correlation between eyes for the outcome measures; therefore, the value of the correlation is not known at the time of study design. We assume here a correlation of 0.8. This assumption is conservative in that it requires a higher number of participants that other plausible values of r.

The primary way sample size is evaluated is by considering the precision around the point estimates for the outcome measures of interest. Table 1 (including the table of specific values corresponding to the graph) provides the half width of the 95% confidence interval (CI) for the estimated mean percent change for combinations of the standard deviation (SD) of the distribution of percent change and sample size. The larger the SD, the wider the CI, meaning the range of possible true values grows.

Table 1. Sample size versus half width of 95% confidence interval for the mean percent change for varying standard deviation values

	Effective Sample Size (N of participants)				
	n=55 (50)	**n= 72 (65)**	**n= 88 (80)**	**n= 99 (90)**	**n=110 (100)**
SD=20%	5%	5%	4%	4%	4%
SD=30%	8%	7%	6%	6%	6%
SD=40%	11%	9%	8%	8%	7%
SD=50%	13%	12%	10%	10%	9%

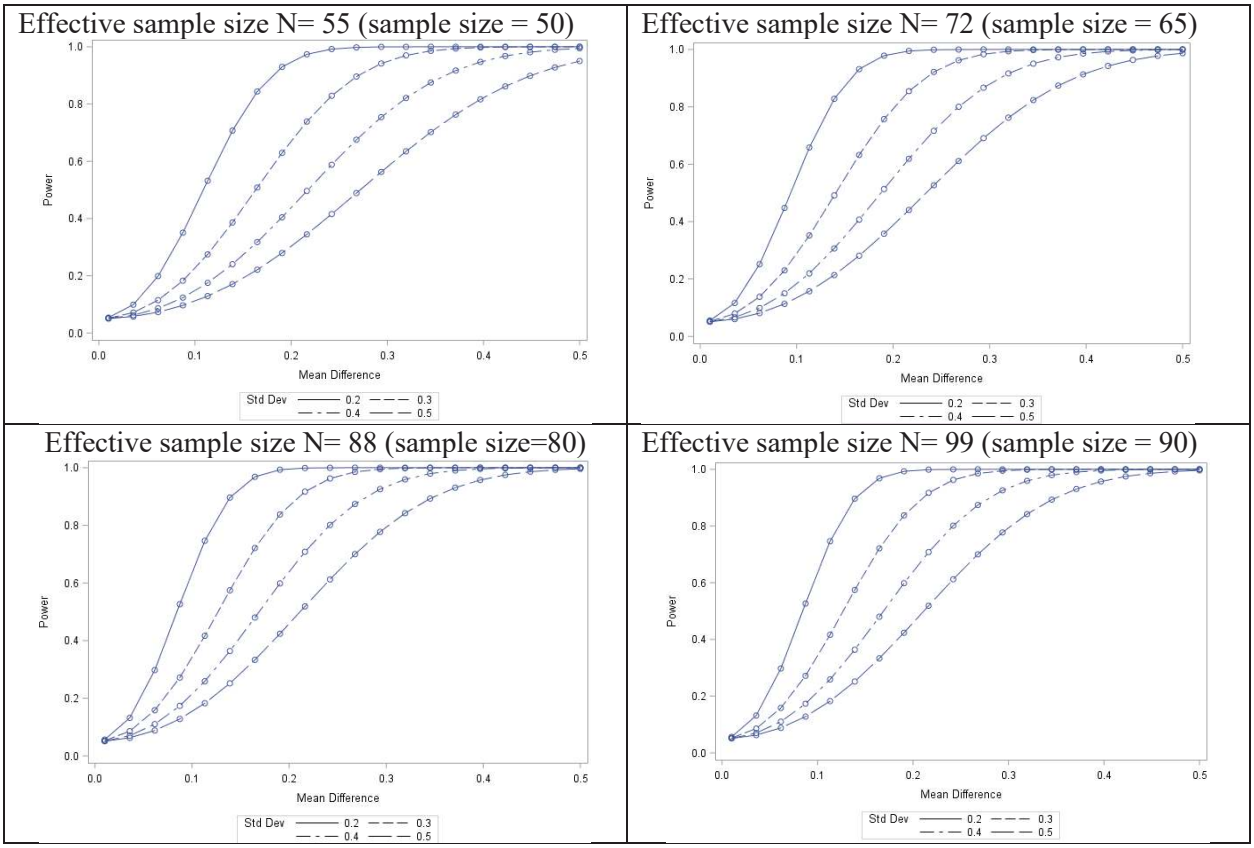
6.1.2 Sample Size Considerations for Comparing Percent Change from Baseline to 4 Years within Subgroups of Interest (All Outcomes)

Another important objective for this natural history study will be to evaluate the association of possible risk factors with progression of various functional outcome variables (objective 3). Thus, it will be important to have a large enough total sample size to plan reasonable comparisons between subgroups. Figure 1 considers various expected SDs 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 CIs will also be important. Table 1 above can be applied to potential subgroup sample sizes as well to consider the precision that would be observed.

Figure 1. 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 in mean percent change is x-axis value. Assuming various sample size (subgroups close to equal distribution).

6.1.3 Sample Size Considerations for Precision of the Estimate of the Correlation between Eyes

The intraclass correlation coefficient is used to assess the strength of correlation between eyes. When both eyes have the same mean for the outcome measure, the intraclass correlation coefficient is equal to the standard Pearson correlation coefficient (r). The distribution of r is not symmetric; therefore, CIs for the estimated correlation coefficient are not symmetric. A transformation of r ($z = 0.5 * \ln((1+r)/1-r)$) is used to create a variable that is asymptotically distributed N (0, 1/(sqrt(N-3))) under the null hypothesis that r=0. The table below provides the 95% CI for different estimates of r from the observed data.

Table 2. 95% Confidence Intervals for an Observed Value of r

	N of patients				
r	**n= 50**	**n= 65**	**n= 80**	**n= 90**	**n= 100**
0.3	(0.02, 0.53)	(0.07, 0.51)	(0.09, 0.49)	(0.10, 0.48)	(0.11, 0.47)
0.4	(0.14, 0.61)	(0.17, 0.59)	(0.20, 0.57)	(0.21, 0.56)	(0.22, 0.55)
0.5	(0.26, 0.68)	(0.29, 0.66)	(0.31, 0.65)	(0.33, 0.64)	(0.34, 0.63)
0.6	(0.39, 0.75)	(0.42, 0.74)	(0.44, 0.72)	(0.45, 0.72)	(0.46, 0.71)
0.7	(0.52, 0.82)	(0.55, 0.81)	(0.57, 0.80)	(0.58, 0.79)	(0.58, 0.79)
0.8	(0.67, 0.88)	(0.69, 0.87)	(0.70, 0.87)	(0.71, 0.86)	(0.72, 0.86)
0.9	(0.83, 0.94)	(0.84, 0.94)	(0.85, 0.93)	(0.85, 0.93)	(0.85, 0.93)

6.1.4 Final Sample Size Justification

Longitudinal changes in all outcome parameters being collected will be of interest for objectives 1 and 3. Information on rates of decline for *EYS*, and for inherited retinal degenerations in general, is very limited.

Data to consider for evaluating sample size:

- Valproic Acid Protocol (VPA) Data (a phase II multiple site, randomized, placebo-controlled trial of oral valproic acid for ADRP) [Placebo group N=44; dataset can be accessed at this link]
  - Percent Change from Baseline to 1 year, Mean (SD):
    - -0.3% (16%) OD
    - -4.9% (17%) OS
- Natural history of 15 participants with *EYS* mutations (McGuigan/Jacobson, 2017)
  - -5.7% per year for VA [8 participants]
  - -5.8% for Inner Segment/Outer Segment (IS/OS) extent
- Natural history of 12 participants with *EYS* mutations (Miyata/Yoshimura, 2016)
  - -5.2 ±3.1% for IS/OS extent

Assumptions made:

- Expect average annual decline in *EYS* to be 6.25% per year or 25% by 4 years
- True SD of percent change at 4 years similar to VPA 1-year SD of around 20%

Based on these assumptions and the impact as presented above, a sample size for the combined Vision Cohorts 1 and 2 of 90 patients or 99 effective eyes has been selected. With an effective sample size of 99, the half width of a 95% CI around the point estimate for percent change would be 4%. A comparison of two equal-sized subgroups would have about 80% power to conclude there is a difference if the true difference is 11%.

Based on the above justification, the total sample size will be 100 participants enrolled. Recruitment is anticipated to take 10 months from the time of study launch.

6.1.4.1 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. Therefore, the precision of these estimates (how tight the CI 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 90 participants (effective sample size N= 99) for Vision Cohorts 1 and 2, all of these outcomes will have 95% CIs no wider than +/- 4% (when analyzed in terms of percent change from baseline) if the SD is within 20%. Based on the available data, we expect the SD to be within 20%, which would yield CIs no wider than +/- 4%. With an even smaller sample size of 65 participants (effective sample size N= 72), all of these outcomes will have 95% CIs no wider than +/- 5% if the SD is within 20%. 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 90 (effective sample size N= 99) participants for Vision Cohorts 1 and 2 will provide enough power to evaluate subgroups, especially those with close to equal distribution. For example, for 2 subgroups of equal size there will be at least 69% power to detect differences as small as 10% if SD is within 20%. With a sample size of 65 (effective sample size N= 72), there will be at least 55% power to detect differences as small as 10% if SD is within 20%.

For objective 4 of evaluating variability and symmetry, a sample size of 90 participants for Vision Cohorts 1 and 2 will have a 95% CI of (0.34, 0.63) when observed r equals to 0.5, and (0.72, 0.86) when observed r equals to 0.8. With a smaller sample size of 65 participants, the 95% CI would be (0.30, 0.66) when observed r equals to 0.5, and (0.70, 0.87) when observed r equals to 0.8. This is considered acceptable precision to meet our objective.

6.2 Data Analysis

The analysis plans below are written with respect to the majority of outcomes of interest. Analyses will include data on both eyes for each participant, and models and confidence intervals will adjust for correlation between 2 eyes of the same participant.

6.2.1 Primary Objectives Analyses

The primary objectives of the natural history study and brief analysis plan for each are as follows.

1. Characterize the natural history of retinal degeneration associated with biallelic pathogenic mutations in the *EYS* gene over 4 years, as measured using functional, structural, and PRO measures

    a. Analysis plan for functional and structural measures: The distribution of each outcome at each visit will be summarized (including tabulating categorically, as well as means, SDs, medians, quartiles, ranges; both the absolute change and percent change will be evaluated, tests performed multiple times will be analyzed using average of all available tests). 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. Mixed effects linear models for the continuous outcome measures and for the time-to-event analyses will also be applied and the fit of the models compared.

b. Analysis plan for PRO measures: Rasch analyses will be performed to calibrate both the VA LV VFQ-48 (completed by Vision Cohorts 1 and 2), PROMIS®-29, MRDQ (completed by all Vision Cohorts), ViSIO-PRO (completed by all Vision Cohorts), and the ULV-VFQ-50 (completed by Vision Cohort 3). 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 a 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.

2. Investigate whether structural outcome measures can be validated as surrogates for functional outcomes in individuals with biallelic pathogenic mutations in the *EYS* gene

   a. Analysis plan: Scatterplots and Spearman correlation coefficients of changes in SD-OCT EZ area versus VF progression from baseline to each visit will be evaluated. Repeated measures least squares models will be fit using VF 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 VF progression will be considered.

3. Evaluate possible risk factors (genotype, phenotype, environmental, and comorbidities) for progression of the outcome measures at 4 years in individuals with biallelic pathogenic mutations in the *EYS* 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 analysis of covariance (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 risk factors to evaluate include:
    - Phenotypic:
        - Clinical diagnosis
        - Age of onset of initial vision symptoms
        - Gender
        - Race/ethnicity
        - Visual acuity
        - Lens Status (phakic/pseudophakic/aphakic)
        - ERG 30 Hz flicker cone amplitudes b-wave (continuous)
        - FAF pattern as measured qualitatively
        - SD-OCT (as factors related to SP Hill Of Vision (HOV))
            - Presence of cysts
            - Central subfield thickness
        - MP
            - Mean retinal sensitivity
        - SP (as factors related to SD-OCT EZ area)
            - Volume of 30 degrees HOV
            - Mean sensitivity
            - Full field HOV
    - Genotypic:
        - Characterizations of the variants on the *EYS* protein
    - Environmental factors
        - Smoking status at baseline
        - Vitamin A use at baseline
        - Docosahexaenoic acid (DHA) use at baseline
        - Lutein use at baseline

4. Evaluate variability of repeat perimetry testing and symmetry of left and right eye outcomes over 4 years in individuals with biallelic pathogenic mutations in the *EYS* gene

    a. <u>Analysis plan for variability of repeat perimetry testing at baseline:</u> Scatterplots and Spearman correlation coefficients for pairs (first versus second) of testing values for each repeated perimetry test. Bland-Altman plots of the inter-eye difference versus the mean value will be inspected and a linear regression model for the differences will be used to test whether the slope is 0 and whether variability changes with greater mean values. The intraclass correlation coefficient of the values and the within-person variance will be estimated.

b. Analysis plan for the symmetry of left eye versus right eye: At baseline and each subsequent testing time, the symmetry of the test result values from the left and right eyes will be assessed and the symmetry of the change from baseline from the left and right eyes will be assessed for each follow-up visit. Bland-Altman plots of the inter-eye difference versus the mean value will be inspected and a linear regression model for the differences will be used to test whether the intercept is 0 and the slope is 0. The intraclass correlation coefficient of the values will be estimated.

6.2.2 Sensitivity Analyses

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

6.2.3 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 are not 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 FFB Consortium Executive Committee will review and oversee these data and their use in reporting.

Chapter 7: Data Collection and Monitoring

7.1 Case Report Forms and Other Data Collection

The main study data are collected on electronic case report forms (eCRFs). When data are directly collected in eCRFs, this will be considered the source data. For any data points for which the eCRF is not considered source (e.g., lab results which are transcribed from a printed report into the eCRF), the original source documentation must be maintained in the participant's study chart or medical record. This source must be readily verifiable against the values entered into eCRF. Even where all study data are directly entered into the eCRFs at office visits, evidence of interaction with a live subject must be recorded (e.g., office note, visit record, etc.).

The Central Lab will generate genetic reports from the retinal dystrophy genetic panel testing and/or family member testing analysis as applicable. These reports will be uploaded to the FFB Consortium study website and made available to the clinical site.

The CGA will review the genetic lab report(s) submitted by the clinical site during genetic screening against the genetic eCRF data to ensure that the data entered by the clinical site are consistent with the source(s) provided prior to the Baseline visit. The CGA will document his/her verification of these genetic data on the FFB Consortium study website and the clinical site will be notified of the results of the review.

In addition to providing interpretation/evaluation of whether or not the *EYS* mutations are causative of the disease on the FFB Consortium study website (see section 2.4.2), the Genetics Committee will review and provide approval for the use of genetic reports from research labs to be used for determining participant eligibility.

Reading Centers will conduct grading of the study data collected for MP, SD-OCT, SP and FAF using the FFB Consortium study website. A Reading Center will conduct quality review only of the first ERG obtained from each clinical site using the FFB Consortium study website. These data will remain in the study database and will not be provided to the clinical site.

Each participating site will maintain appropriate medical and research records for this trial, in compliance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E6 and regulatory and institutional requirements for the protection of confidentiality of participants.

7.2 Study Records Retention

Study documents should be retained for a minimum of six years from the date on which the CC receives IRB approval to close the study. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the CC, if applicable. It is the responsibility of the CC to inform the investigator when study documents no longer need to be retained.

7.3 Quality Assurance and Monitoring

Designated personnel from the CC will be responsible for maintaining quality assurance (QA) and quality control (QC) systems to ensure that the clinical portion of the trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the

applicable regulatory requirements, as well as to ensure that the rights and wellbeing of trial participants are protected and that the reported trial data are accurate, complete, and verifiable.

Consistent with the Integrated Addendum to ICH E6 (R2)[19], a risk-based monitoring (RBM) plan will be developed and revised as needed during the course of the study. This plan describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

As much as possible, remote monitoring will be performed in real-time with on-site monitoring performed to evaluate the verity and completeness of the key site data. Elements of the RBM plan may include:

- Qualification assessment, training, and certification for sites and site personnel
- Oversight of IRB/EC coverage and informed consent procedures
- Central (remote) data monitoring: validation of data entry, data edits/audit trail, protocol review of entered data and edits, statistical monitoring, study closeout
- On-site monitoring (site visits): source data verification, site visit report
- Communications with site staff
- Participant retention and visit completion
- Quality control reports
- Management of noncompliance
- Documenting monitoring activities
- AE reporting

CC representatives or their designees may visit the study facilities at any time in order to maintain current and personal knowledge of the study through review of the records, comparison with source documents, observation and discussion of the conduct and progress of the study. The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

7.4 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or procedure requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

The site PI and study staff delegated to study responsibilities are responsible for knowing and adhering to their IRB/EC requirements. Further details about the handling of protocol deviations will be included in the monitoring plan.

Chapter 8: Ethics/Protection of Human Participants

8.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Participants of Research codified in 45 Code of Federal Regulations (CFR) Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH E6.

8.2 Institutional Review Boards and Ethics Committees

The protocol, ICF(s), recruitment materials, and all participant materials will be submitted to the IRB or EC for review and approval. Approval of both the protocol and the ICF(s) must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB or EC before the changes are implemented to the study. All changes to the consent form will be IRB or EC approved; a determination will be made regarding whether previously consented participants need to be re-consented.

8.3 Informed Consent Process

8.3.1 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. All consent forms will be IRB-or EC- approved and in the case of written consent, the participant will be given the opportunity to carefully read and review the document. For any form of consent presented (written or verbal), the investigator or his/her designee (as approved by the IRB/EC) will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Participants will be asked to carefully consider the consent form presented to them and have any questions answered prior to signing.

Participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. Participants must sign the ICF prior to any procedures being done specifically for the study. Participants may withdraw consent at any time throughout the course of the trial. A copy of the ICF will be given to participants for their records. The rights and welfare of participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

8.3.2 Participant and Data Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, the funder(s) and their agents. This confidentiality is extended to cover genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The CC, other authorized vendors or representatives of the funder, representatives of the IRBs/ECs, or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to medical records (office, clinic, or hospital) for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB/EC, institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the FFB Consortium CC, located at the Jaeb Center for Health Research in Tampa, Florida. This will not include the participant's contact or identifying information, unless otherwise specified in the informed consent form. Rather, a unique study identification number will identify individual participants and their research data. The study data entry and study management systems used by clinical sites and by the FFB Consortium CC research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the FFB Consortium CC.

8.4 Stored Specimens

With the participant's approval and as approved by the IRB/ECs, de-identified biological samples collected for genetic testing will be stored at the Central Lab, until 12 months after the study is completed, after which they will be destroyed.

Chapter 9: References

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