Foundation Fighting Blindness (FFB) Consortium

Gyrate Atrophy Ocular and Systemic Study

Protocol Identifying Number: GYROS

Funded by: Foundation Fighting Blindness and Conquering Gyrate Atrophy Foundation

Version Number: 1.2

28-Nov-2022

Key Roles and Signature Page
Gyrate Atrophy Ocular and Systemic Study

Protocol Identifying Number: GYROS

Version Number: v1.2
28-Nov-2022

GYROS Protocol Co-Chair	
Name, degree	Mandeep Singh, M.D.
Signature/Date	
GYROS Protocol Co-Chair	
Name, degree	David Valle, M.D.
Signature/Date	
JCHR GYROS Protocol Director (Sponsor)	
Name, degree	Allison Ayala, MS
Signature/Date	
FFB Representative (Funder)	
Name, degree	Todd Durham, PhD.
Signature/Date	
Statistician	
Name, degree	Maureen Maguire, PhD, FARVO
Signature/Date	

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
AE	Adverse Event
ARD	Arginine Restricted Diet
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
EC	Ethics Committee
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
GA	Gyrate Atrophy
ICF	Informed consent form
ICH	International Committee of Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IOP	Intraocular Pressure
IRB	Institutional Review Board
KKI	Kennedy Krieger Institute
LLVA	Low Luminance Visual Acuity
LPV-FVQ II	L.V. Prasad-Functional Vision Questionnaire
MRDQ	Michigan Retinal Degeneration Questionnaire
MP	Microperimetry
N	Number or sample size
OAT	ornithine-δ-aminotransferase
OCTA	Optical coherence tomography angiography
OD	Right Eye
OS	Left Eye
OU	Both eyes
PI	Principal investigator
PRO	Patient reported outcomes

ABBREVIATION	DEFINITION
PROMIS®-29	Patient-Reported Outcomes Measurement Information System
PSC	Posterior sub-capsular
QA	Quality Assurance
QC	Quality Control
RBM	Risk-Based Monitoring
RP	Retinitis pigmentosa
RPE	Retinal pigment epithelium
SAE	Serious adverse event
SD	Standard deviation
SD-OCT	Spectral domain optical coherence tomography
SP	Static perimetry
VA	Visual acuity
ViSIO-PRO	Visual Symptom and Impact Outcomes Patient Reported Outcome
VF	Visual Field
VPA	Valproic Acid

PROTOCOL SUMMARY

ITEM	DESCRIPTION
Title	GYROS: Gyrate Atrophy Ocular and Systemic Study
Précis	Gyrate atrophy (GA) is a rare inherited chorioretinal degeneration that is associated with hyperornithinemia, an inborn error of metabolism caused by autosomal recessive mutations in the ornithine aminotransferase (*OAT*) gene. GA is characterized by childhood-onset nyctalopia and sharply demarcated areas of chorioretinal atrophy that initially involve the midperipheral fundus. The atrophic areas typically coalesce and enlarge towards the posterior pole in the second and third decades of life, leading to severe visual field constriction and vision loss if left untreated. The current standard care treatment of GA is an arginine restricted diet that is implemented in practice using dietary protein restriction with essential amino acid supplementation. However, dietary treatment is highly burdensome on patients and negatively impacts quality of life such that only about ~20% of patients can comply. Strict adherence to dietary protein restriction (particularly through adolescence), essential amino acid supplementation, and nutritional management of body weight especially during intercurrent illness and pregnancy are among the challenges of treatment. Periods of suboptimal dietary control led to plasma ornithine elevation and progressive chorioretinal degeneration. To facilitate a future interventional gene therapy clinical trial, there is a need to evaluate natural history of and the relationship between potential clinical trial outcome measures.
Objectives	The objectives of the *OAT* gene natural history study are as follows: **1. Natural History** **a.** Characterize the natural history of retinal degeneration associated with disease-causing *OAT* variants in the presence of standard care dietary treatment regimens over four (4) years, using functional, structural, and patient-reported outcome measures. **b.** Characterize the natural history of ornithine levels associated with disease-causing *OAT* variants in the presence of standard care dietary treatment regimens over four (4) years. **c.** Determine within-patient variability of ornithine levels associated with disease-causing *OAT* variants in the presence of standard care dietary treatment regimens over four (4) years. **d.** Evaluate inter-eye correlation of ocular measures. **2. Metabolic-Structure-Function Relationships** **a.** Explore relationship of structural outcomes with functional outcomes in individuals with disease-causing *OAT* variants. **b.** Explore relationship of plasma ornithine levels with structural and functional outcomes in individuals with disease-causing *OAT* variants. **3. Identify Rapid Progressors** **a.** Explore possible risk factors (genotype, phenotype, environmental, comorbidities, and dietary therapy/supplements) for progression of the functional, structural, and patient-reported outcome measures over four (4) years in individuals with disease-causing *OAT* variants. **b.** Explore possible risk factors (genotype, phenotype, environmental, comorbidities, and dietary therapy/supplements)

<table>
<tr><th>ITEM</th><th>DESCRIPTION</th></tr>
<tr><td></td><td>for ornithine levels over four (4) years in individuals with disease-causing OAT variants.</td></tr>
<tr><td>Impact</td><td>The expected impact of the OAT gene natural history study is to inform a future interventional clinical trial design and implementation, including the following:
1. Determine within-patient variability of ornithine levels.
2. Develop quantitative measures of progression of the area of preserved retina and establish its reproducibility, sensitivity to change, and relationship with other measures.
3. Establish rates of progression of retinal degeneration on all functional, structural, and patient-reported outcome measures, and determine which measures are most sensitive to change.
4. Determine primary time points and duration for a planned future treatment trial.
5. Use variability and inter-eye correlation of outcomes for trial sample size calculations.
6. Identify candidates for the future trial, including eligibility criteria based on risk factors and cut points for severity of disease most likely to benefit from treatment.
7. Establish study procedures and workflows for practical implementation of the same testing procedures in a future trial.</td></tr>
<tr><td>Study Design</td><td>Multicenter, longitudinal, prospective natural history study. Participants will be assigned to one (1) of three (3) Vision Cohorts based on visual acuity (VA) and kinetic visual fields (VF).</td></tr>
<tr><td>Number of Sites</td><td>Approximately 12</td></tr>
<tr><td>Outcomes</td><td>Structural Outcomes:
<table>
<tr><th>Key Assessments</th><th>Test</th><th>Equipment</th><th>Reading Center</th></tr>
<tr><td>Primary: Quantitative measures of progression of the area of preserved retina and of the area of lesions in standardized regions*</td><td>Fundus autofluorescence (FAF)</td><td>Optos</td><td>Yes</td></tr>
<tr><td>Primary: Use with FAF for assessments noted above</td><td>Ultra-Widefield Color Photography</td><td>Optos</td><td>Yes</td></tr>
<tr><td>Ellipsoid zone (EZ) area</td><td>Spectral domain optical coherence tomography (SD-OCT)</td><td>Heidelberg Spectralis</td><td>Yes</td></tr>
<tr><td>Presence and type of cataract (posterior subcapsular and cortical cataract) **</td><td>Red reflex photography</td><td>Digital camera capable of capturing external eye photos
Megapixels of >=3MP
Magnification of 30-40 degrees
Digital camera export in PNG, TIF, DICOM or BMP</td><td>Yes</td></tr>
</table></td></tr>
</table>

ITEM	DESCRIPTION

Foveal avascular zone (FAZ) area and macular vessel density	OCTA *Ancillary test at subset of sites*	Pre-Approved list	Yes

*Other approaches to grading methods may be explored
**Ocular exam by investigator will include clinical grading of presence of nuclear sclerosis cataract.

Functional Outcomes:

Key Assessments	Test	Equipment	Reading Center
Visual field sensitivity measured with quantitative topographic analysis (Hill of Vision)	Static Perimetry	Octopus 900 Pro	Yes
Early Treatment of Diabetic Retinopathy Study (ETDRS) best corrected visual acuity (BCVA) letter score	Visual Acuity	Electronic Visual Acuity (EVA) system or ETDRS charts	N/A
Low visual acuity test - for participants unable to see ETDRS letters	Low Visual Acuity	Berkeley Rudimentary Vision Test (BRVT)	N/A
ETDRS best corrected low luminance visual acuity letter score	Low Luminance Visual Acuity (LLVA)	Electronic Visual Acuity (EVA) system or ETDRS charts	N/A
Mean macular sensitivity	Fundus-Guided Microperimetry	MAIA	Yes
Full-field retinal sensitivity	Full-field stimulus threshold (FST) testing to blue, white, and red stimuli	Diagnosys Espion	No

Metabolic Outcomes:

- **Primary:** Plasma ornithine level; obtained by fasting plasma amino acids panel and evaluated by a central lab
- **Primary:** Blood spot ornithine level; obtained by fasting blood spot test amino acids panel and evaluated by a central lab
- **Secondary**: Proline, lysine, glutamine, glutamate, arginine, and related metabolite: creatine and its precursor guanidinoacetate; obtained by fasting plasma amino acids panel and evaluated by a central lab

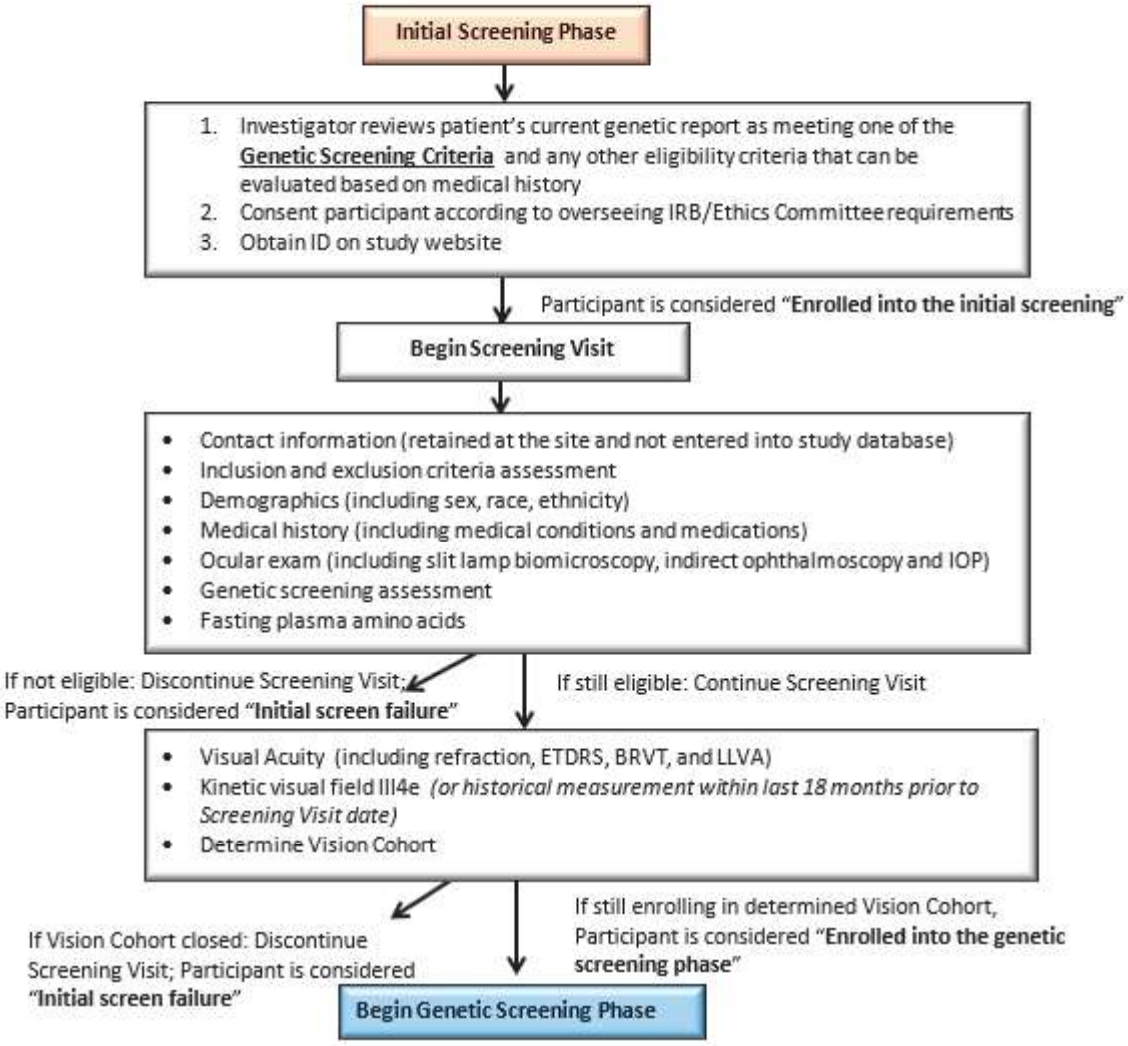
Patient Reported Outcomes (PRO):

- **Adults 18 years or older:**

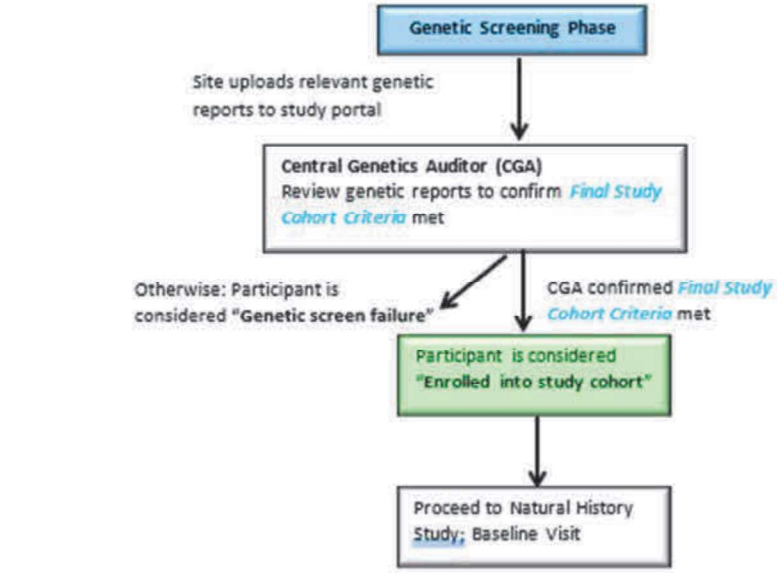
ITEM	DESCRIPTION
	○ Michigan Retinal Degeneration Questionnaire (MRDQ) ○ Visual Symptom and Impact Outcomes Patient Reported Outcome (ViSIO-PRO) ○ Patient-Reported Outcomes Measurement Information System (PROMIS®-29) • **<u>Adolescents 12-17 years</u>:** ○ Visual Symptom and Impact Outcomes Patient Reported Outcome (ViSIO-PRO) ○ L. V. Prasad-Functional Vision Questionnaire (LVP-FVQ II)
Risk Factors of Interest	• Body weight and height • Assessment of B6 supplements and daily arginine and protein intake, based on patient-reported food record sheet evaluated by central expert dietician review. • Biochemical validation of new and rare variants in *OAT* gene, based on skin biopsy evaluated by a central lab.
Population	**Key Eligibility Criteria:** The complete list of eligibility criteria is in **Chapter 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. **1.** Age ≥ 12 years of age **2.** Clinical diagnosis of retinal dystrophy **3.** **<u>Must meet one of the Genetic Screening Criteria</u>:** • **At least 2 disease-causing variants** in the *OAT* 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), ***OR*** • **At least 2 disease-causing variants** in the *OAT* gene with **unknown phase**, 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 must meet both of the following phenotype criteria:** **(1)** Classic fundus appearance of gyrate atrophy (based on investigator discretion), **AND** **(2)** Elevated ornithine levels >300 µmol/L (documented on any prior lab report). 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>:** Must meet one of the **Genetic Screening Criteria** and have the genetic criteria confirmed by a Central Genetics Auditor (CGA).
Sample Size and Recruitment	Sample size rationale is detailed in **Chapter 8.2**. Recruitment will be tracked with approximate distribution in three (3) Vision Cohorts. A goal of at least forty-five (45) participants will be enrolled, with approximately twenty-five (25) in Vision Cohort 1, fifteen (15) in Vision Cohort 2, and five (5) in Vision Cohort 3.

<table>
<tr><th>ITEM</th><th>DESCRIPTION</th></tr>
<tr><td>Phase</td><td>Natural History Study</td></tr>
<tr><td>Vision Cohort Groups</td><td>Vision Cohort Definitions
All participants will be designated into one of the following Vision Cohorts based on data entered at the Screening Visit. See Section 2.1.1.1 Target Study Sample Size for the detailed definitions.
<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 forty-eight (48) month visit: Approximately fifty-one (51) Months.
Screening – Baseline Visit, sixty (60) days
Baseline Visit – forty-eight (48) month Follow-up Visit, forty-eight (48) months.</td></tr>
<tr><td>Study Duration (planned)</td><td>Sixty-one (61) months from first enrollment until last participant visit</td></tr>
<tr><td>Protocol Overview/ Synopsis</td><td>1. Investigator reviews patient’s current genetic report as meeting 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.
a. See flow chart in next section for details.
5. Complete genetic screening, including review of genetic reports by CGA. Participants meeting criteria to continue will enroll into the study cohort.
a. See flow chart in next section for details.
6. Participants who enroll into the study cohort will return to the clinic within sixty (60) days of the Screening Visit date to start baseline testing, and no later than two (2) weeks after receiving confirmation of meeting final study cohort criteria from the CGA.
7. Participants will return to the clinic at 12, 24, 36 and 48 months from the baseline visit start date for follow-up visits.
8. After the 48-month follow-up visit, participation in the study, for all three (3) Vision Cohorts, will be completed.</td></tr>
</table>

SCHEMATIC OF STUDY ENROLLMENT



SCHEMATIC OF GENETIC SCREENING PHASE



Final Study Cohort Criteria

- **At least 2 disease-causing variants** in the *OAT* 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)
  OR
- **At least 2 disease-causing variants** in the *OAT* gene with unknown **phase**, 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 must meet both of the following phenotype criteria**
  (1) Classic fundus appearance of gyrate atrophy (based on investigator discretion) **AND**
  (2) Elevated ornithine levels >300 µmol/L (documented on any prior lab report)

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

SCHEDULE OF STUDY VISITS AND PROCEDURES: VISION COHORT 1 & VISION COHORT 2

Visit	Screening	Baseline	12M	24M	36M	48M
Visit Target Windows	(Up to Day -60) [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 / Eligibility Assessment	X					
Demographics/Screening Medical History (including pre-existing conditions, symptomology history, patient-reported daily activities, and medications)	X					
Determination of Vision Cohort	X					
3mm punch skin biopsy[i]		X				
Concomitant Medications	X	X	X	X	X	X
Adverse Events		X	X	X	X	X
Patient Reported Outcomes (PROMIS-29, MRDQ, ViSIO-PRO, LVP-FVQ II)[h]		X		X		X
Ocular Procedures - *All testing performed in each eye*						
Complete Ophthalmic Exam[f]	X		X	X	X	X
Intraocular Pressure[g]	X		X	X	X	X
Visual acuity (including refraction, ETDRS, BRVT if needed, LLVA if needed)	X		X	X	X	X
SD-OCT volume scans (Heidelberg Spectralis)		X	X	X	X	X
SD-OCT vertical and horizontal scans (Heidelberg Spectralis)		X	X	X	X	X
Axial Length and Corneal Curvature measurements		X		X		X
Fundus Autofluorescence (FAF) (Optos)		X	X	X	X	X
Ultra-Widefield Color Photo (Optos)		X	X	X	X	X
Optical coherence tomography angiography (OCTA)[j]		X	X	X	X	X
Red reflex photo [k]		X	X	X	X	X
Full-field Stimulus Threshold (Diagnosys Espion)		X	X	X	X	X
Static perimetry (Octopus 900 Pro)		X[d]	X	X	X	X
Fundus guided microperimetry (MAIA)		X[d]	X	X	X	X
Kinetic VF III4e *for Vision Cohort definition only*	X[e]					

a. All Screening Visit testing **must** be completed on the same day (except for kinetic VF, as noted below).

b. Baseline Visit date (defined as the start date of all Baseline testing) must be no later than two (2) weeks after receiving confirmation of meeting ***final study cohort criteria*** from the CGA. All Baseline testing must be completed within **ten (10)** 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. For static perimetry and microperimetry, all Vision Cohort 1 and 2 participants will complete two (2) tests at baseline. The results will be compared according to the ***visual field criteria*** to determine if a third test is needed.

e. Historical measurement performed within the last eighteen (18) months prior to the Screening Visit. If a historical measurement is not available, then perform the Kinetic VF III4e at the Screening Visit.

f. Ophthalmic exam includes slit-lamp biomicroscopy and indirect ophthalmoscopy. 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.

g. IOP measurements will be taken prior to pupil dilation.

h. PROs may be completed in person or remotely any time within six (6) months of the Baseline visit or any time within the allowable window of the associated visit window (not required to be the same day as the rest of the visit). Required PROs depend on age, see outcomes grid.

i. Collected by certified investigator; sent to JHU/KKI Biochemical Genetics Lab.

j. If site does not have the equipment that would be required for the ancillary study, then participation in this test will be waived, with approval from the Coordinating Center. Site must use a pre-approved equipment for this test.

k. Digital camera capable of capturing external eye photos, Megapixels of >=3MP, Magnification of 30-40 degrees, Digital camera export in PNG, TIF, DICOM or BMP.

SCHEDULE OF STUDY VISITS AND PROCEDURES: VISION COHORT 3

Visit	Screening	Baseline	12M	24M	36M	48M
Visit Target Windows	(Up to Day -60) [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 / Eligibility Assessment	X					
Demographics/Screening Medical History (including pre-existing conditions, symptomology history, patient-reported daily activities, and medications)	X					
Determination of Vision Cohort	X					
3mm punch skin biopsy [h]		X				
Concomitant Medications	X	X	X	X	X	X
Adverse Events		X	X	X	X	X
Patient Reported Outcomes (PROMIS-29, MRDQ, ViSIO-PRO, LVP-FVQ II) [g]		X		X		X
Ocular Procedures - *All testing performed in each eye*						
Complete Ophthalmic Exam [e]	X		X	X	X	X
Intraocular Pressure [f]	X		X	X	X	X
Visual acuity (including refraction, ETDRS, BRVT if needed, LLVA if needed)	X		X	X	X	X
SD-OCT volume scans (Heidelberg Spectralis)		X	X	X	X	X
SD-OCT vertical and horizontal scans (Heidelberg Spectralis)		X	X	X	X	X
Axial Length and Corneal Curvature measurements		X		X		X
Fundus Autofluorescence (FAF) (Optos)		X	X	X	X	X
Ultra-Widefield Color Photo (Optos)		X	X	X	X	X
Optical coherence tomography angiography (OCTA) [i]		X	X	X	X	X
Red reflex photo [j]		X	X	X	X	X
Full-field Stimulus Threshold (Diagnosys Espion)		X	X	X	X	X
Kinetic VF III4e *for Vision Cohort definition only*	X [d]					

a. All Screening Visit testing must be completed on the same day (except for kinetic VF, as noted below).

b. Baseline Visit date (defined as the start date of all Baseline testing) must be no later than two (2) weeks after receiving confirmation of meeting ***final study cohort criteria*** from the CGA). All Baseline testing must be completed within **ten (10)** 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. Historical Kinetic VF III4e if available. Kinetic VF III4e ***does not*** need to be performed at screening if a historical measurement is not available.

e. Ophthalmic exam includes slit-lamp biomicroscopy and indirect ophthalmoscopy. 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.

f. IOP measurements will be taken prior to pupil dilation.

g. PROs may be completed in person or remotely any time within six (6) months of the Baseline visit or any time within the allowable window of the associated visit window (not required to be the same day as the rest of the visit). Required PROs depend on age, see outcomes grid.

h. Collected by certified investigator; sent to JHU/KKI Biochemical Genetics Lab.

i. If site does not have the equipment that would be required for the ancillary study, then participation in this test will be waived, with approval from the Coordinating Center. Site must use a pre-approved equipment for this test.

j. Digital camera capable of capturing external eye photos, Megapixels of >=3MP, Magnification of 30-40 degrees, Digital camera export in PNG, TIF, DICOM or BMP.

FOOD RECORD AND SAMPLE COLLECTION SCHEDULE FOR ALL VISION COHORTS

	Screening	Baseline	4M	8M	12M	16M	20M	24M	28M	32M	36M	40M	44M	48M
Clinical site coordinator contacts participant [a]		X	X	X	X	X	X	X	X	X	X	X	X	X
Three-day food record [a]		X	X [b]	X [b]	X [b]	X [b]	X [b]	X [b]	X [b]	X [b]	X [b]	X [b]	X [b]	X
Target Windows for Sample Collection			Wk 17 ± 4	Wk 34 ± 4	Wk 52 ± 4	Wk 69 ± 4	Wk 86 ± 4	Wk 104 ± 4	Wk 121 ± 4	Wk 138 ± 4	Wk 156 ± 4	Wk 173 ± 4	Wk 190 ± 4	Wk 208 ± 4
Fasting plasma amino acids[c]	X	X (2)			X			X			X			X
Fasting blood spot amino acids[d]		X	X[d]	X[d]	X	X[d]	X[d]	X	X[d]	X[d]	X	X[d]	X[d]	X
Request Albumin Lab Records[e]		X			X			X			X			X
Body Weight		X			X			X			X			X
Height [f]		X			X			X			X			X

a. The Pre-Sample Participant Telephone Contact should occur four (4) – ten (10) days prior to scheduled sample collection date. Food record should occur for three (3) days leading up to the sample collection date. For at-home samples, the Post-Sample Collection Telephone Call should be completed within one (1) day after the scheduled sample collection date.

b. The Food Record is only collected if the participant is on an arginine-restricted diet. If the participant is not following an arginine-restricted diet, then the food record will not be recorded and collected. See **Section 4.3 Food Record and Blood Sample Collection** for more details.

c. **Baseline only:** two (2) fasting plasma samples collected on two (2) different days, within ten (10) days of Baseline Visit date.

d. Participant and/or caretaker administered remotely. Samples will be collected on all participants.

e. Request the most recent albumin records from the participant's overseeing physician. The albumin labs will be requested only if the participant is on an arginine-restricted diet.

f. Height will be measured annually in participants less than twenty-one (21) years of age at enrollment, otherwise height will only be collected at the Baseline Visit.

Chapter 1: Background Information

1.1 Introduction

Gyrate atrophy (GA) of the choroid and retina (GA, OMIM 258870) is a rare and slowly progressive chorioretinal degeneration that leads to severe loss of vision. The prevalence of GA is thought to be approximately 1 in 1,500,000 live births worldwide, but in certain populations (e.g., Finns) is as high as 1:50,000 live births. Inherited as an autosomal recessive trait, the primary defect in GA is deficiency of the mitochondrial matrix enzyme, ornithine-δ-aminotransferase (*OAT*) resulting from biallelic loss of function mutations in the *OAT* gene located at 10q26. *OAT* deficiency accounts for the hyperornithinemia (plasma levels are ~15-fold increased over normal) present in all GA children and adults ingesting an unrestricted diet.

1.1.1 Clinical

The early symptoms of GA include myopia and reduced night vision in late childhood. Disease progression is characterized by progressive visual field constriction, visual acuity loss, and reduced daytime vision that invariably culminate in functional and legal blindness by mid-adulthood. Visual disability is typically severe and is associated with difficulties in educational, vocational, mental, and personal aspects of life. Ophthalmologic exam reveals a pathognomonic pattern of chorioretinal degeneration starting as punctate lesions in the mid-periphery which gradually enlarge forming sharply demarcated circular areas of atrophy often with hyperpigmented margins. Within the circular areas, the atrophy involves the photoreceptor cell layer, retinal pigment epithelium (RPE), and choroid. The full field electroretinogram (ERG) amplitudes are typically reduced by the second decade of life. Over the ensuing decades, the atrophic lesions increase in number and size, coalescing to form scalloped margins and progressing toward the posterior pole of the eye. Patients can also develop cystoid macular edema (CME) usually in the third decade or beyond. In all patients, posterior sub-capsular (PSC) cataracts develop toward the end of the second decade of life. Aside from their ocular phenotype, GA patients are healthy. Although, they can have abnormal electroencephalograms (EEGs). However, the frequency of seizures is not increased. Some have mild proximal muscle weakness that is not of clinical consequence.

1.1.2 Metabolism

OAT is a pyridoxal phosphate (vitamin B6) dependent homohexameric transaminase, which catalyzes the reversible conversion of ornithine and α-ketoglutarate to Δ1-pyrroline-5-carboxylate (P5C) and glutamate. Each *OAT* monomer has a pyridoxal phosphate covalently bound to lysine 292. P5C is in non-enzymatic equilibrium with glutamate semialdehyde (GSA) and is an intermediate in the synthesis of glutamate and proline. When arginine levels (derived from the breakdown of dietary or endogenous protein) are adequate, the net flux of the *OAT* reaction is in the direction of P5C synthesis[1]. When dietary arginine is inadequate, the net flux reverses to favor ornithine synthesis. Thus, the *OAT* reaction serves an anapleurotic function for the urea cycle and serves a necessary function for de novo arginine synthesis. Loss of this anapleurotic function explains why a few infants with GA present with arginine deficiency and neonatal hyperammonemia[1]. *OAT* activity is expressed in many cell types including high levels in the RPE but is low to undetectable in photoreceptors[2]. Deficiency of *OAT* was shown to be the

primary defect in GA by our group in 1977.[3] *OAT* activity in GA patient fibroblasts is < 5% of control values[2].

1.1.3 Molecular

The *OAT* gene and cDNA were cloned in 1988 and shortly thereafter the first disease producing mutation was identified[4-6]. Since then, more than seventy-five (75) disease causing loss of function variants have been identified[2,7]. As with other rare autosomal recessive conditions, the frequency of certain disease producing variants is enriched in certain populations. For example, in Finland where GA is 5- to 10-fold more frequent than in other populations, most patients are homozygous for the *OAT*- L402P allele reflecting a founder effect in the ancestors of modern-day Finns[6]. A few *OAT* disease alleles encode a Vitamin B6 mutant enzyme, most of these are missense variants near the B6 binding lysine 292, e.g. A226V[2,8].

Interestingly, ornithine levels are increased in a second, rare, autosomal recessive inborn error, the Hyperornithinemia- Hyperammonemia-Homocitrullinuria syndrome (HHHS, OMIM 238970). The plasma ornithine values in these patients range from 200-1020 μM but are generally lower than those observed in GA. HHHS patients have episodic hyperammonemia often with a degree of intellectual disability and spasticity. More than 100 patients have been reported and only one is known to have retinal abnormalities but without the funduscopic change characteristic of GA[9]. It has been shown that the primary defect in HHHS is loss of function mutations in the SLC25A15 gene which encodes ORC1 (aka ORNT1), the inner mitochondrial membrane carrier protein that transports ornithine from the cytosol into the mitochondrial matrix in exchange for matrix citrulline plus H+, an essential step for normal functioning of the urea cycle[10,11].

1.2 Treatment

Development of treatment of GA has been hampered by several factors:

- The disorder is quite rare so that almost no centers have adequate numbers of patients to compare the course of treated versus non-treated individuals.
- The patients come to medical attention at different ages and given the high allelic heterogeneity, often have different *OAT* genotypes.
- There is currently no pathophysiologic model explaining the unique sensitivity of the retina to this inborn error of ornithine metabolism.
- The progress of the untreated disease is slow and there are no systemic manifestations of hyperornithinemia. As an example, patients on diet therapy did not notice any acute negative consequences of going off-diet.
- There is no well-studied cohort of untreated patients available for comparison to a treated group.

Despite these challenges, several approaches have been recently reviewed[12].

1.2.1 Arginine Restricted Diet (ARD)

When the basic defect in GA was identified, it was determined that since ornithine is derived from arginine released by the breakdown of dietary or endogenous protein, a diet which

maintains adequate nutrition but in which natural protein was restricted should reduce ornithine accumulation. Due to the severity of the required protein restriction, essential amino acids (EAA) were added to the diet to avoid deficiencies of these amino acids and care was taken to provide adequate calories, minerals, and vitamins[13-16]. This approach was shown to reduce ornithine levels to near normal (< 200 μM) and with supervision can be maintained by some patients for years with no ill effects. Ultimately, > 25 patients started this diet; most found compliance difficult and only a few were able to maintain reduce levels of ornithine for greater than five (5) years[17]. Evaluation of the consequences of systemically reduce ornithine levels on the progress of the retinal degeneration was challenging for the reasons described above. However, based on fundus photos, visual acuity measures, and other tests, in patients followed over five (5) years or more it was concluded that reduction of plasma ornithine slowed or even stopped the retinal degeneration[17].

This clinical impression was supported by two additional lines of evidence, one in patients and the other in a mouse model of GA. First, as described above, there is substantial heterogeneity in the rate of progression of GA, which is explained in part by allelic heterogeneity in the *OAT* mutations causing GA. To confirm this, intra- vs inter-familial variation was compared in the ocular phenotype in six (6) GA sibling pairs and found that the ocular phenotypes of siblings were more similar than those of unrelated GA patients of a similar age. From this observation, it was reasoned that in families with multiple affected siblings, the older could serve as the best control for the younger. In two (2) families, both siblings were placed, one older and one younger, on an ARD at the same time. The severity of the younger sibling to that of the older was compared when the younger reached the age of the older at the time of institution of an ARD. In both families, the retinal phenotype of the younger, who had been on an ARD for 5-7 years by the time they reached the age of diagnosis of the older (untreated at that point) was far less severe. This observation strongly supported the hypothesis that reduction of ornithine accumulation is beneficial[18]. Second, using gene targeting, a knockout mouse model of GA was produced.[1] Post-weaning, homozygous *OAT* null mice had hyperornithinemia to an extent like human GA patients. Their ERG, retinal histology and ultrastructure were normal at two (2) months of age but by age six (6) months these parameters were clearly abnormal and by twelve (12) months of age they had unrecordable ERGs and a severe generalized retinal degeneration[19]. By contrast, GA mice placed on an ARD at two (2) months of age maintained near normal plasma ornithine levels and at twelve (12) months of age had normal ERGs and completely normal retinal histology and ultrastructure[20]. These unambiguous results strongly indicate that systemic correction of ornithine accumulation prevents retinal degeneration in GA. As described above, however, an ARD poses an adherence challenge and only ~20% of patients can comply, and all require careful and repeated evaluation to ensure adequate nutrition.

1.2.2 Pyridoxine Supplementation

For many inherited enzyme deficiencies involving vitamin B6-dependent enzymes (all transaminases, many decarboxylases), a fraction of the patients exhibited some improvement when treated with pyridoxine, vitamin B6, a precursor of pyridoxal phosphate, the active form of the vitamin. Normal pyridoxine intake is ~ 1mg/day while the therapeutic dose ranges from 200 – 500 mg/day. This approach is completed initially on all new GA patients, instructing them to maintain their usual diet and defining a response as a > 30% reduction in ornithine levels. Using these criteria, < 10% of patients respond. In those patients for whom skin fibroblasts are available, B6 responsivity in the *OAT* activity assays was also evaluated. The B6 responsive

patients identified have not exhibited a complete correction of ornithine levels, and for those whose ornithine concentrations on B6 >200 μM, a modified ARD was recommended[8].

1.2.3 Creatine Supplementation

The first step in the creatine biosynthetic pathway is catalyzed by arginine: glycine amidinotransferase (AGAT), a cytosolic enzyme that is inhibited by ornithine, one of the reaction products. For this reason, hyperornithinemic GA patients have less than 10% of normal levels of plasma creatine and its degradation product, creatinine[21]. However, trials with creatine therapy have not prevented progression of the retinal degeneration[22-24] and patients with inborn errors of creatine biosynthesis and similar reductions in creatine do not have retinal degeneration[24]. These observations have reduced enthusiasm for creatine treatment.

1.2.4 Proline Supplementation

Plasma proline levels are normal in GA, but some studies have indicated that proline is an important energy source for RPE cells and largely is derived from ornithine in a pathway that depends on *OAT* activity[2,25]. Proline crosses the blood brain barrier poorly[2] and dietary supplementation with proline has been tried in GA with no beneficial effect. However, this approach may be worth additional studies, especially if advances in understanding the pathophysiology of GA support a role for reduced proline consumption by the RPE.

1.2.5 Lysine Supplementation

Plasma lysine levels are reduced in GA patients with a mean near the lower limit of normal.[2] This is because ornithine and lysine share a common dibasic amino acid transporter in the kidney and the high levels of ornithine compete with lysine to increase renal losses of lysine. If lysine levels could be maintained at high, supra-normal levels it should be possible to increase urinary losses of ornithine. Evidence from GA mice and GA patients indicates that this approach works but requires high doses of oral lysine which many (most) patients do not tolerate. Moreover, to be effective, lysine levels must be maintained at supra-normal levels which could have detrimental consequences over the long-term.

1.3 Rationale

The treatment for GA that has produced clearly positive results is systemic reduction of ornithine with an ARD. This diet is challenging and only a small fraction of patients can maintain the diet. The results, to date, support the hypothesis that ornithine is directly involved in pathophysiology and that systemic reduction is sufficient for a therapeutic benefit. Pyridoxine supplementation treatment should be beneficial in those patients who have B6-responsive *OAT* mutations regardless of the pathophysiological mechanism and all patients merit a trial with clear criteria for a beneficial effect (>30% decrease in plasma ornithine on an unrestricted diet). To date, depending on *OAT* genotype, only a few GA patients respond. There is little or no evidence supporting the other therapeutic approaches currently. A well-controlled natural history study would be extremely valuable for future therapeutic trials.

There is limited information from previous natural history studies of patients with GA[12,26]. The available information is based on cross-sectional data from small numbers of patients, or from longitudinal studies that are either short term treatment studies or natural history studies with outcomes based on outdated imaging and visual function methodology[17].

1.4 Study Objective

Natural History - Characterize the natural history of ornithine levels and retinal degeneration (RD) associated with disease-causing *OAT* variants in the presence of standard care dietary treatment regimens over 4 years.

Metabolic-Structure-Function Relationships - Explore the relationships between structural and functional RD outcome measures and plasma ornithine levels.

Identify Rapid Progressors – Explore the relationship of possible risk factors (genotype, phenotype, environmental, comorbidities, and standard care dietary regimen) with severity and progression of metabolic, functional, structural, and patient-reported outcomes.

1.5 Potential Risks and Benefits

1.5.1 Known Potential Risks

Most examination procedures are considered part of standard care for retinal degenerations. This study will be capturing information about participants that includes identifiable, personal information, like date of birth (if permitted by site's regulatory bodies). The study has procedures in place to protect that information. However, there is a chance that a loss of that protection could occur. This would be a loss of confidentiality. There are special efforts being made to ensure that this does not happen.

The sections below summarize the risks and discomforts that may occur during the period of prospective data collection.

- Risks associated with testing visual acuity (VA), kinetic perimetry (KP), microperimetry (MP), static perimetry (SP), Optos fundus autofluorescence (FAF), Optos Color Photos, red reflectance photos, Optical coherence tomography angiography (OCT-A), and patient reported outcome (PRO) questionnaires may include boredom and frustration. However, 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 spectral domain optical coherence tomography (SD-OCT), full-field stimulus threshold (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. Therefore, the risk is extremely small. If glaucoma occurs, treatment is available.
- **Intraocular Pressure (IOP) Examination:** In rare instances, the cornea may be scratched during measurement of IOP. An abrasion like this may be painful, but it heals quickly with no lasting effects. If a participant experiences a corneal abrasion, ophthalmic ointment may be administered, and an eye patch or gauze may be placed over the eye.
- **Venipuncture**: Blood draws can cause common mild reactions including pain, bruising, or redness at the sampling site. Less common reactions include bleeding from the sampling site, formation of a small blood clot or swelling of the vein and surrounding tissues, and fainting. The risk of local infection is less than 1 in 1000.

- **Fingerstick Risks:** About two (2) drops of blood will be removed by fingerstick for measuring ornithine, amino acid, and arginine. Pain is common at the time of lancing. In about 1 in 10 cases, a small amount of bleeding under the skin will produce a bruise. A small scar may persist for several weeks. The risk of local infection is less than 1 in 1000.
- **Skin biopsy:** Complications associated with a three (3) mm punch biopsy of the skin include a brief sting when the local anesthetic (numbing medicine) is injected at the biopsy site, the possibility of local bleeding and bruising, pain, infection, allergic reaction to the numbing medicine used in the procedure, or damage to the structures beneath the skin site (such as an artery or a nerve). A small scar may persist for several years.

1.5.2 Known Potential Benefits

Study participants are not expected to benefit directly from participation in this study.

1.5.3 Risk Assessment

The known potential risks above, except for venipuncture, fingerstick collection and skin biopsy, are standard-of-care procedures that do not pose additional risks to participate in the study.

- The venipuncture is needed to collect plasma samples to monitor the ornithine levels in relation to progression of Gyrate Atrophy.
- A fingerstick blood spot collection is needed to obtain a blood spot sample. This will determine if this procedure is comparable to the plasma sample collection in evaluating the ornithine levels over time.
- A skin biopsy is an incisional procedure of the skin. The potential complications include localized bleeding, scarring, bruising, pain, redness, swelling, and damage to nerves and blood vessels. These complications are rare, and when they occur, are mild and self-limiting. This procedure will be used to collect the participant's cells, which are fibroblasts. These cells will be used to measure the activity of the participant's *OAT* enzyme and the response to vitamin B6. The research goal is to understand the impact of *OAT* mutations on plasma ornithine levels and retinal degeneration. The skin biopsy is necessary to quantify *OAT* enzyme activity in each participant as it is not possible to derive this information from the blood sample, thus providing direct confirmation on the pathogenicity and functional severity of the mutation in each subject. This information will inform the analysis of the plasma ornithine and retinal degeneration outcome measures for the duration of this trial and will complement the development of dietary guidelines for treatment trials in slowing the progression of Gyrate Atrophy. The cells can be stored frozen indefinitely with participant consent. These cells can be re-cultured to perform additional studies as needed to provide a better understanding of Gyrate Atrophy.

1.6 General Considerations

The study is being conducted in compliance with the policies described in the study policies document, 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.[27] 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 primary outcome procedures 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.
- When feasible, data will be directly collected in electronic case report forms, which will be considered the source data.

Chapter 2: Study Enrollment and Screening

2.1 Participant Recruitment and Enrollment

Enrollment will proceed with the goal of at least forty-five (45) participants completing the trial. Approximately fifty (50) individuals may be enrolled in the study to achieve this goal. Participants who have signed consent and started the Screening process may be permitted to continue into the trial, if eligible, even if the enrollment goal has been reached.

Study participants will be recruited from approximately twelve (12) clinical sites worldwide. All eligible participants will be included without regard to gender, race, or ethnicity. The primary recruitment strategy will be from the patients referred from the Investigators. However, recruitment materials may be made available at retinal disorder websites upon IRB approval.

The target population for GYROS will be patients with GA associated with disease-causing variants in the *OAT* gene. The male: female distribution of patients with *OAT*-related retinal degeneration is expected to be approximately 1:1, therefore both genders will be eligible to enroll. Additionally, *OAT*-related retinal degeneration affects patients of all racial and ethnic groups, thus enrollment will be conducted without regard to race or ethnicity.

It is expected that GYROS enrollment will be representative of the population of patients with gyrate atrophy associated with disease-causing variants in the *OAT* gene, including those under the age of eighteen (18). GA can impact individuals in the first or second decade of life, therefore it is important that children are included in this natural history study to characterize the natural history of retinal degeneration in patients with gyrate atrophy.

2.1.1 Participant Recruitment Goals and Strategy

2.1.1.1 Target Study Sample Size

Recruitment will be tracked by three (3) vision cohorts defined below. Reference **Chapter 6.1 Sample size** for the rationale.

Vision Cohort 1 Approximately Twenty-five (25) participants	Criteria that must be met in the **better eye*** at the 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 Approximately Fifteen (15) participants	Criteria that must be met in the **better eye*** at the 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 Approximately Five (5) participants	Criteria that must be met in the **better eye*** at the Screening Visit: ○ **visual acuity** ETDRS letter score of 18 or less (approximate Snellen equivalent 20/500 or worse).

***Better eye definition**	The **better eye** is defined as the eye with the better Screening Visit ETDRS visual acuity. However, if both eyes have the same visual acuity, which is defined as the same Snellen equivalent, then the determination will be made at investigator discretion. In this scenario, the investigator will consider the eye with better fixation or clearer ocular media to permit highest quality retinal imaging.
****Visual Field**	The **visual field** (VF) is defined as the clinically determined **kinetic VF III4e** performed within the last 18 months prior to the Screening Visit or performed on the day of the Screening Visit.

	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

2.1.1.2 Participant Recruitment

The Foundation Fighting Blindness (FFB) Consortium Executive Committee will review recruitment progress and feasibility at regular intervals.

Initial recruitment goals will include forty-five (45) participants **enrolled into the study vision cohorts** 1, 2, and 3 combined. 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 forty (40) participants enrolled in Vision Cohorts 1 and 2 combined will be targeted.

2.1.1.3 Participant Enrollment

Participants will not be counted as enrolled into the study cohort until Initial Screening and Genetic Screening have been confirmed. This means that potentially more participants will be screened. The number and reasons for screen failures will be tracked. It is possible that 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 and efforts will be made to accurately predict numbers in the genetic screening queue.

2.2 Informed Consent and Authorization Procedures

Study participants must be age of majority to consent for themselves, e.g., at least eighteen (18) years of age in the US. For potential participants who are minors, e.g., under eighteen (18) years of age in the US, a parent/legal guardian (referred to subsequently as “parent”) will be provided with the Informed Consent Form to read and will be given the opportunity to ask questions.

Potential participants meeting the IRB’s minimum age of assent will be given an Assent Form to read and discuss with their parents and study personnel. If the parents and child agree

to participate, the Informed Consent Form and Child Assent Form will be signed. A copy of the consent form will be provided to the participant and their parents, and another copy will be added to the participant's study record.

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 to read. Potential study participants will be encouraged to discuss the study with family members and their personal physician(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.

Participants who become age of majority, e.g., eighteen (18) years of age in the US, during the study will need to re-consent with an adult Informed Consent Form.

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 will 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 the inclusion criteria and none of the exclusion criteria at the Screening Visit.

2.3.1.1 Participant Inclusion Criteria

Participants must meet all the following inclusion criteria at the Screening Visit 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. Willing and able to complete all study visit assessments at each visit over the forty-eight (48) month study period.
3. Age ≥ 12 years
4. Adult participants must have the capacity to consent on their own behalf.
5. Must meet one (1) of the **Genetic Screening Criteria** below:
    - **At least 2 disease-causing variants** in the *OAT* 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.

    OR

- **At least 2 disease-causing variants** in the *OAT* gene with **unknown phase**, 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 **must meet both of the following phenotype criteria:**
    1. Classic fundus appearance of gyrate atrophy (based on investigator discretion) **AND**
    2. Elevated ornithine levels >300 µmol/L (documented on any prior lab report).

2.3.1.2 Ocular Inclusion Criteria

Participant must meet the following criteria at the Screening Visit to **enroll into the genetic screening phase**.

6. Both eyes must have a clinical diagnosis of retinal dystrophy
7. Both eyes must permit good quality photographic imaging (for example, but not limited to, clear ocular media, adequate pupil dilation, stable fixation).

2.3.1.3 Participant Exclusion Criteria

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

1. Single pathogenic or likely pathogenic genetic variants known to be associated with autosomal dominant retinitis pigmentosa/retinal dystrophy (AD, heterozygous), X-linked retinitis pigmentosa/retinal dystrophy (XL, hemizygous), or mitochondrial inheritance.
2. Expected to enter experimental treatment trial at any time during this study.
3. History of more than one (1) year of cumulative treatment, at any time, with an agent associated with pigmentary retinopathy, including amiodarone, chloroquine, deferoxamine, hydroxychloroquine, pentosan polysulfate, tamoxifen, and deferoxamine.

Note: Since this is a Natural History Study collecting data on the progression of GA, pregnant women will not be specifically excluded from participation.

2.3.1.4 Ocular Exclusion Criteria

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

4. Current vitreous hemorrhage.
5. Current complications of pathological myopia (for example, but not limited to, myopic maculopathy including atrophy, scar, choroidal neovascularization, schisis) that could inhibit imaging studies of gyrate atrophy-related chorioretinal degeneration or assessment of visual function.
6. History of intraocular surgery (for example, but not limited to cataract surgery, vitrectomy, penetrating keratoplasty, or LASIK) within the last 3 months.

7. Current or any history of confirmed diagnosis of glaucoma (for example, but not limited to glaucomatous VF changes or nerve changes, or history of glaucoma filtering surgery).
8. Current or any history of retinal vascular occlusion or proliferative diabetic retinopathy.
9. Any clinically significant cystoid macular edema, as determined by the Investigator, that is not stable three (3) months before screening; use of chronic, prophylactic treatment with topical or oral agents (for example, but not limited to, dorzolamide) is allowed.
10. History or current evidence of ocular disease that, in the opinion of the investigator, may confound assessment of visual function (for example, but not limited to, tractional or rhegmatogenous retinal detachment, any vitreoretinal surgery, retinal vascular occlusion, proliferative diabetic retinopathy).
11. The following medications and treatments are prohibited as they can affect progression of retinitis pigmentosa or confound the assessments. The participant must not have received or plan to receive the following treatments.
    i. Any use of ocular stem cell or gene therapy:
    ii. Any treatment with ocriplasmin.
    iii. Treatment with Ozurdex (dexamethasone), Iluvien or Yutiq (fluocinolone acetonide) intravitreal implant.
12. The following medications and treatments are excluded within the specified timeframe:
    i. Treatment with an ophthalmic oligonucleotide within the last 9 months (last treatment date is less than 9 months prior to Screening Visit date).
    ii. Treatment with any other product for retinal dystrophy 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).
    iii. Treatment that can alter VA between Screening and Baseline (e.g., periorbital injections.

2.3.2 Screening Data Collection and Testing

Please reference the schematic of study design at the beginning of the protocol for the flow of the study visit procedures. The following procedures will be performed at the Screening Visit. The testing procedures are detailed in the **GYROS Clinical Site Manual of Procedures**. An overview of the equipment and technician requirements for all testing is in **Chapter 5.1 Study Procedure Requirements**. All ocular testing will be performed in each eye, right eye (OD) first and then left eye (OS). Screening procedures will last approximately four (4) hours.

Participants meeting criteria to continue will be **enrolled into the genetic screening phase**. Otherwise, the participant will be an **initial screen failure**.

The following procedures will be performed, data collected, eligibility criteria checked, and documented at the Screening Visit.

1. Inclusion and exclusion criteria assessed
2. Demographics: date of birth, sex, race, and ethnicity

3. Contact information: this will be retained at the site and not entered on the study database
4. 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.
    - Participant will sign a release of medical information form to collect medical records and laboratory records.
5. Concomitant medications
6. Genetic screening assessment, including number and phase of mutations in the *OAT* 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. If the participant does not meet the criteria for one of the **Genetic Screening Criteria**, then the remainder of procedures and testing are not required. The participant will be discontinued as an **initial screen failure**.
7. Complete ophthalmic examination to include:
    - Slit lamp biomicroscopy
    - Indirect ophthalmoscopy
8. IOP
    - IOP measurements will be taken prior to pupil dilation.
9. VA (including refraction, ETDRS, BRVT if needed, LLVA if needed).
    - The VA letter score will determine whether LLVA or BRVT will be performed. The criteria are defined in the GYROS Clinical Site Manual of Procedures.
10. Kinetic VF III4e
    - **Vision Cohort 1 and 2:** Historical measurement performed within the last eighteen (18) months prior to the Screening Visit. If a historical measurement is not available, then perform the Kinetic VF III4e at the Screening Visit.
    - **Vision Cohort 3:** Collect the most recent historical measurement prior to the Screening Visit, if available. Kinetic VF III4e does not need to be performed at screening if a historical measurement is not available.
11. Determination of Vision Cohort
    - If the participant's determined Vision Cohort is closed for enrollment, the remainder of procedures and testing are not required. Participant will be discontinued as an **initial screen failure**.
12. Sample Collection
    - The Site Personnel will collect one (1) fasting plasma sample.

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 submit their genetic lab reports for CGA review. The schematic of study design at the beginning of the protocol summarizes the flow of the **Genetic Screening Phase**. Reference the **GYROS Clinical Site Manual of Procedures** for detailed procedures.

All genetic reports will be uploaded to the FFB study website by the clinical site. These reports may be reviewed by the CC, associated clinical site, Central Genetics Auditor (CGA), Genetics Committee, and investigators involved in the oversight of the study (which includes the study chair, Operations Committee, and Executive Committee). All genetic reports will be de-identified and redacted of all personal data prior to uploading on the FFB study website.

The clinical site will upload supporting genetic documentation, including the genetic reports, on the FFB study website. The CGA will review the genetic documentation provided by the clinical site to verify the genetic screening data entry and appropriate documentation of the **Genetic Screening Criteria.** Additional documentation may be requested as needed to verify the **Genetic Screening Criteria** and all genetic screening assessments.

- If **Genetic Screening Criteria** are verified, then the participant will be considered **enrolled into the study cohort**.
- If the final study cohort is not verified, then the participant will be a **genetic screen failure**.

2.4.1.1 Genetic Screen Failures

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

2.4.1.2 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 and evaluation of whether the *OAT* mutations are causative of the disease (i.e., pathogenic, likely pathogenic). 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 Final Study Cohort Criteria

The Final Study Cohort Criteria is participants that **meet one of the Genetic Screening Criteria, and have the genetic criteria confirmed by a Central Genetics Auditor (CGA).**

Participants that meet the initial screening and eligibility criteria, who complete the Genetic Screening Phase, and who meet the **final study cohort criteria** will be considered **enrolled into the study cohort**. These participants should complete the natural history study.

Chapter 3: Baseline Visit

3.1 Baseline Visit

Participants meeting criteria to enter the natural history study will return for a Baseline Visit within sixty (60) days after receiving confirmation of meeting **final study cohort criteria** from the CGA.

The Baseline Visit date will be documented as the date testing procedures began. All Baseline Visit testing procedures will be completed within **ten (10) days** of the Baseline Visit date, unless specified below.

3.2 Food Record and Blood Sample Collection

All participants will complete the food record and blood sample collection as described below.

(1) Participant Contact

- The Clinical Site Coordinator will contact the participants (via phone or other remote interface) four (4) to ten (10) days prior to the first of the two (2) scheduled sample collection date. The **Pre-Sample Collection Telephone Call** will include instructions and reminders of:
  - The two (2) sample collection dates.
  - Fasting instructions.
  - Food record collection instructions.

(2) Food Record

- The participant will complete a food record to document their daily consumption during the following period:
  - **Begin** three (3) days prior to the first sample collection date
  - **End** one (1) day prior to the last sample collection date
- The participant will digitally send (e-mail or text) a picture of the food record documentation and either mail the original food tracking record to the site or bring it to the site at their Baseline Visit.
- The Clinical Site Coordinator will enter the data from the food record on the FFB Study Website.
- A Central Dietitian Reviewer (CDR) will review food record on FFB Study Website to determine nutritional content. This information will be documented on the FFB Study Website.

(3) Sample Collection

- Site Personnel will collect the following:
  - Two (2) **fasting plasma samples** on two (2) different days, within ten (10) days of the Baseline Visit date.

- One (1) **fasting blood spot fingerstick** on the same day as one of the fasting plasma samples.
  - All samples will be shipped to the Kennedy Krieger Institute (KKI).
- The Clinical Site Coordinator would request the most recent lab records with an albumin result if the participant indicated they are currently on an arginine-restricted diet.

3.3 Baseline Procedures

The following procedures will be performed at the Baseline Visit. The testing procedures are detailed in the **GYROS Clinical Site Manual of Procedures**. An overview of the equipment and certification requirements for all testing is in **Chapter 5.1: Study Procedure Requirements**. All ocular testing will be performed in each eye, OD first and then OS. Baseline procedures will last approximately seven (7) – eight (8) hours. Baseline procedures will span over ten (10) days.

3.3.1 Baseline Data Collection and Testing Cohorts 1 & 2

1. Medical updates to include:
   - New or changed adverse events (AEs)
   - New ocular procedures
   - New or changed medications
     - Request medical records and laboratory records if applicable.
2. Patient Reported Outcomes
   - Adults: PROMIS®-29, MRDQ, ViSIO-PRO
   - Adolescents: LPV-FVQ II, ViSIO-PRO
   - Children: LPV-FVQ II
     - The PROs may be completed in person or remotely (phone or other remote methods) any time within six (6) months of the Baseline Visit (not required to be the same day as the rest of the Baseline Visit).
3. Skin biopsy (3mm punch skin biopsy)
   - Ship to Johns Hopkins University Department of Genetic Medicine Research Lab
4. Spectral Domain Optical Coherence Tomography (SD-OCT)
   - Volume Scan
   - Vertical and Horizontal Scan
5. Axial Length and Corneal Curvature Measurements
6. Fundus Autofluorescence
7. Widefield color photo

8. Optical coherence tomography angiography (OCTA)

- Ancillary test; may be waived with approval from the Coordinating Center

9. Red reflex photo

10. Full field Stimulus Threshold (FST)

11. Static perimetry

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

  (a) If the absolute value of the difference between the two tests is ≤ 2.4 dB, then the participant passes static perimetry reliability criteria. A third test is not needed.

  (b) 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. A third test will be required.

12. Fundus guided microperimetry

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

  (a) 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 the microperimetry reliability criteria. A third test is not needed.

  (b) 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. A third test will be required.

13. Measurements to include:

- Weight
- Height

3.3.2 Baseline Data Collection and Testing Cohort 3

1. Medical updates to include

- New or changed adverse events (AEs)
- New ocular procedures
- New or changed medications
  - Request medical records and laboratory records if applicable.

2. Patient Reported Outcomes

- Adults: PROMIS®-29, MRDQ, ViSIO-PRO

- Adolescents: LPV-FVQ II, ViSIO-PRO
- Children: LPV-FVQ II
  - The PROs may be completed in person or remotely (phone or other remote methods) any time within six (6) months of the Baseline Visit (not required to be the same day as the rest of the Baseline Visit).

3. Skin biopsy (3mm punch skin biopsy)

- Ship to Johns Hopkins University Department of Genetic Medicine Research Lab

4. Spectral Domain Optical Coherence Tomography (SD-OCT)

- Volume Scan
- Vertical and Horizontal Scan

5. Axial Length and Corneal Curvature Measurements

6. Fundus Autofluorescence

7. Widefield color photo

8. Optical coherence tomography angiography (OCTA)

- Ancillary test; may be waived with approval from the Coordinating Center

9. Red reflex photo

10. Full field Stimulus Threshold (FST)

11. Measurements to include:

- Weight
- Height

Chapter 4: Follow-up Visits

4.1 Follow-up Visits

Follow-up Visits for all vision cohorts will occur annually. The Baseline Visit date is considered study day zero (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 will be completed on the same date other than the PROs as noted in **Section 4.4 Follow-up Procedures**.

4.2 Target Timelines

Target dates and windows for each study Follow-up Visit for all vision cohorts are shown below. Dates and windows are timed from Baseline Visit Date. Visits will be scheduled in the target window.

Table 4-1. Schedule for Follow-up Visits

VISIT SCHEDULE	TARGET DATE	TARGET WINDOW (AROUND TARGET DATE)
12 Month Visit	52 Weeks	± 4 Weeks
24 Month Visit	104 Weeks	± 4 Weeks
36 Month Visit	156 Weeks	± 4 Weeks
48 Month Visit	208 Weeks	± 4 Weeks

Table 4-2. Schedule for Remote Blood Spot Collection

BLOOD SPOT SCHEDULE	TARGET DATE	TARGET WINDOW (AROUND TARGET DATE)
4 Months	17 Weeks	± 4 Weeks
8 Months	34 Weeks	± 4 Weeks
16 Months	69 Weeks	± 4 Weeks
20 Months	86 Weeks	± 4 Weeks
28 Months	121 Weeks	± 4 Weeks
32 Months	138 Weeks	± 4 Weeks
40 Months	173 Weeks	± 4 Weeks
44 Months	190 Weeks	± 4 Weeks

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.

Additional office visits may occur as needed.

4.3 Food Record and Blood Sample Collection

All participants will be asked to complete the blood sample collection as described below. Clinical Site Coordinators will complete a **Pre-Sample Collection Telephone Call** four (4) to ten (10) days prior to the scheduled sample collection date. For at-home samples, the **Post-Sample Collection Telephone Call** should be completed within one (1) day after the scheduled sample collection date. Reference the **GYROS Clinical Site Manual of Procedures** for more details.

(1) Participant Contact

- The Clinical Site Coordinator will contact the participants (via phone or other remote interface) four (4) to ten (10) days prior to the scheduled sample collection date. The **Pre-Sample Collection Telephone Call** will include instructions and reminders of:
  - The next sample collection date.
  - Fasting instructions.
  - Food record collection instructions *(if applicable; see Food Record instructions below)*.
- For at-home sample collections the Clinical Site Coordinator will contact the participants (via phone or other remote interface) after the scheduled sample collection date, within one (1) day preferred. The **Post-Sample Collection Telephone Call** will include the collection of:
  - Information on the participant's last meal.
  - Completion of the blood spot sample.
  - Completion of the Food Record.

(2) Food Record

- **<u>Participants who indicated following an arginine-restricted diet at Screening</u> will be asked to complete a food record every four (4) months between Baseline and the 48M visit.**
  - If a participant initiates an arginine-restricted diet after Screening, the participant will complete a food record every four (4) months going forward.
  - If a participant following an arginine-restricted diet at screening stops following an arginine-restricted diet at any point in the study, the participant will continue to complete the food record every four (4) months.
- **ALL participants will complete the food record for the 48M visit.**
- The participants will complete the food record every day for three (3) days prior to the fasting sample collection.

- The participant will digitally send (e-mail or text) a picture of the food record to the site and either mail the original food record document to the site or bring it to the site at their next visit.
- The Clinical Site Coordinator will transcribe the data from the food record on the FFB Study Website.
- A Central Dietitian Reviewer (CDR) will review food record on FFB Study Website to determine nutritional content. This information will be entered into the FFB Study Website.

(3) Sample Collection

- **At the annual Follow-up Visits**, the Clinical Site Personnel will collect one (1) **fasting plasma sample** on all participants and ship to KKI. The sample collection procedures are detailed in the GYROS Clinical Site Manual of Procedures.
- **At the annual Follow-up Visits**, the Clinical Site Personnel will collect one (1) **fasting blood spot fingerstick** on all participants and ship to KKI. The sample collection procedures are detailed in the GYROS Clinical Site Manual of Procedures.
- **At the annual Follow-up Visits**, the Clinical Site Coordinator would request the most recent lab records with an albumin result if the participant indicated they are ***currently*** on an arginine-restricted diet.
- **Every four (4) months between annual Follow-up Visits**, one (1) **fasting blood spot fingerstick** will be collected on all participants. The participant or caretaker will collect the sample and ship to the central laboratory, KKI. The blood spot collection kits and instructions will be provided. The sample collection procedures are detailed in the GYROS Clinical Site Manual of Procedures.

4.4 Follow-up Procedures

The following procedures will be performed at the Follow-Up Visits, unless otherwise specified. The testing procedures are detailed in the GYROS Clinical Site Manual of Procedures. An overview of the equipment and certification requirements for all testing is in **Chapter 5.1: Study Procedure Requirements**. All ocular testing will be performed in each eye, OD first and then OS. Follow-up visit procedures will last approximately seven (7) – eight (8) hours.

4.4.1 Follow-up Data Collection and Testing Cohorts 1 & 2

1. Medical updates to include:
   - New or changed adverse events (AEs)
   - New ocular procedures
   - New or changed medications
     - Request medical records and laboratory records if applicable.
2. Patient Reported Outcomes

- Complete at 24-Month and 48-Month Follow-up Visits Only
- Adults: PROMIS®-29, MRDQ, ViSIO-PRO
- Adolescents: LPV-FVQ II, ViSIO-PRO
- Children: LPV-FVQ II
    - The PROs may be completed in person or remotely (phone or other remote methods) any time within the Allowable Window of the associated visit, (not required to be the same day as the rest of the Follow up Visit).

3. Complete ophthalmic examination to include:

- Slit lamp biomicroscopy
- Indirect ophthalmoscopy

4. IOP

- IOP measurements will be taken prior to pupil dilation.

5. VA (including refraction, ETDRS, BRVT if needed, LLVA if needed)

- The visual acuity letter score will determine whether LLVA or BRVT will be performed. The criteria are defined in the GYROS Clinical Site Manual of Procedures.

6. SD-OCT

- Volume Scan
- Vertical and Horizontal Scan

7. Axial Length and Corneal Curvature Measurements

- Complete at 24-Month and 48-Month Follow-up Visits Only

8. Fundus Autofluorescence

9. Widefield color photo

10. OCTA

- Ancillary test; may be waived with approval from the Coordinating Center

11. Red reflex photo

12. Full field Stimulus Threshold test (FST)

13. Static perimetry

14. Fundus guided microperimetry

15. Measurements to include:

- Weight
- Height
    - Measured annually in participants <21 years of age at enrollment.

4.4.2 Follow-up Data Collection and Testing Cohort 3

1. Medical updates to include
   - New or changed adverse events (AEs)
   - New ocular procedures
   - New or changed medications
     - Request medical records and laboratory records if applicable
2. Patient Reported Outcomes
   - Complete at 24-Month and 48-Month Follow-up Visits Only
   - Adults: PROMIS®-29, MRDQ, ViSIO-PRO
   - Adolescents: LPV-FVQ II, ViSIO-PRO
   - Children: LPV-FVQ II
     - The PROs may be completed in person or remotely (phone or other remote methods) any time within the Allowable Window of the associated visit, (not required to be the same day as the rest of the Follow up Visit).
3. Complete ophthalmic examination to include:
   - Slit lamp biomicroscopy
   - Indirect ophthalmoscopy
4. IOP
   - IOP measurements will be taken prior to pupil dilation.
5. VA (including refraction, ETDRS, BRVT if needed, LLVA if needed)
   - The visual acuity letter score will determine whether LLVA or BRVT will be performed. The criteria are defined in the GYROS Clinical Site Manual of Procedures.
6. SD-OCT
   - Volume Scan
   - Vertical and Horizontal Scan
7. Axial Length and Corneal Curvature Measurements
   - Complete at 24-Month and 48-Month Follow-up Visits Only
8. Fundus Autofluorescence
9. Widefield color photo
10. OCTA
    - Ancillary test; may be waived with approval from the Coordinating Center
11. Red reflex photo
12. FST

13. Measurements to include:

- Weight
- Height
  - Measured annually in participants <21 years of age at enrollment.

4.4.3 Unscheduled Visits

Testing procedures at unscheduled visits are at the investigator's discretion. However, it is recommended that procedures performed during these visits follow the standard protocol for each procedure and be performed by certified personnel. Unscheduled visits will be recorded on the FFB Consortium study website. Study images taken during unscheduled visits do not require submission to the study website.

Chapter 5: Testing Procedures and Questionnaires

5.1 Study Procedure Requirements

The study procedure instructions are detailed in the **GYROS Clinical Site Manual of Procedures**. An overview of the equipment and certification requirements for all testing are provided in the table below.

Study Procedures	Description	Equipment Required (If applicable)	Site Personnel Delegation
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 Form (ICF) Process	Explanation/review of study with the potential participant and/or signature of ICF.	N/A	Certified investigator as permitted by the IRB/EC
Signature of Informed Consent Form	The participant and/or LAR sign the ICF. The person obtaining the ICF will also sign.	N/A	Certified investigator as permitted by the IRB/EC
Data entry on study website	Data collected from the study participant will be directly entered on the FFB Study Website (strongly encouraged) or written on the paper CRF and transcribed on the FFB Study Website within seven (7) days.	Computer and internet connection	Certified coordinator or certified investigator with additional study website certification
Collect information regarding medical history, demographics, adverse events, medications	Sites will collect medical history, demographic information, adverse events, and medications from the participant during each visit. This information can be confirmed by requesting medical records if needed.	N/A	Certified investigator or coordinator
Patient Reported Outcomes	There will be a total of four (4) questionnaires. Three (3) for adults and one (1) – two (2) for minors depending on their age.	Study will provide	Certified investigator or coordinator
Ocular Exam	Including slit lamp biomicroscopy and indirect ophthalmoscopy	Any equipment is acceptable	Certified investigator
IOP	Measurement of the fluid pressure inside the eye.	Any equipment is acceptable	Clinical site personnel performing this test does not require a study

Study Procedures	Description	Equipment Required (If applicable)	Site Personnel Delegation
			specific certification. However, they must be trained and delegated by the PI per the SSDL.
Visual Acuity – Refraction	Refraction is done as a routine part of an eye exam to achieve best corrected visual acuity measures	N/A	Clinical site personnel certified for refraction
Visual Acuity – ETDRS	Traditional measure of central visual function that represents foveal cone function,	EVA system, ETDRS charts	Clinical site personnel certified for ETDRS
Visual Acuity – LLVA	LLVA tests vision function in low luminance conditions.	EVA system, or ETDRS charts 2.0 neutral density filter to be provided by study	Clinical site personnel certified for performing ETDRS is also certified to perform LLVA
Visual Acuity – BRVT	BRVT incorporates a three-level hierarchy for visual acuity testing better suited for low vision participants.	BRVT charts provided by study	Clinical site personnel certified for BRVT
Spectral Domain Optical Coherence Tomography - Volume Scan	Scans provide objective, non-invasive measures of retinal structure, including loss of inner/outer segment junction and have been shown to correlate with visual function in eyes with retinal degeneration	Heidelberg Spectralis	Clinical site personnel certified for SD-OCT
Spectral Domain Optical Coherence Tomography - Vertical and Horizontal Scan	Scans provide objective, non-invasive measures of retinal structure, including loss of inner/outer segment junction and have been shown to correlate with visual function in eyes with retinal degeneration	Heidelberg Spectralis	Clinical site personnel certified for SD-OCT and vertical and horizontal scan
OCTA	Provides information on foveal avascular zone enlargement and decreased macular vascular density	Site must use pre-approved device.	Clinical site personnel certified for OCTA
Axial Length and Corneal Curvature	Axial length measures the distance between the anterior and posterior poles of the eye. Corneal curvature determines the power of the cornea.	Any equipment is acceptable	Clinical site personnel performing this test does not require a study

Study Procedures	Description	Equipment Required (If applicable)	Site Personnel Delegation
			specific certification. However, they must be trained and delegated by the PI per the SSDL.
Fundus Autofluorescence	Captures areas of absent autofluorescence which corresponds with areas of chorioretinal atrophy seen on clinical exam	Optos	Clinical site personnel certified for widefield fundus autofluorescence
Widefield color photo (UWF color)	Complementary imaging modality that delineates the borders of the atrophic lesions classically associated with GA	Optos	Clinical site personnel certified for widefield color photo
Red reflex photo	Captures the area and severity of posterior subcapsular cataracts commonly associated with GA	Digital camera capable of capturing external eye photos, Megapixels of >=3MP, Magnification of 30-40 degrees, Digital camera export in PNG, TIF, DICOM or BMP	Clinical site personnel certified for red reflex photo
Full field Stimulus Threshold test (FST)	Measures rod- and cone-mediated parts of the visual field	Diagnosys Espion	Clinical site personnel performing this test does not require a study specific certification. However, they must be trained and delegated by the PI per the SSDL.
Static perimetry (SP)	A static field of vision test involves illuminating an object that does not move in the patient's field of vision to evaluate the vision in this manner.	Octopus 900 Pro (GATE Protocol)	Clinical site personnel certified for SP

Study Procedures	Description	Equipment Required (If applicable)	Site Personnel Delegation
Fundus guided microperimetry (MP)	This test will map out how bright a spot needs to be for the participant to be see it at specific locations inside the eye.	MAIA	Clinical site personnel certified for MP
Kinetic Perimetry (KP)	A kinetic visual field test involves moving an object in and out of a patient's peripheral vision to find the edge of the patient's field of vision.	Any equipment is acceptable	Does not need to be performed by study certified personnel or recorded on the GYROS Study Staff Delegation Log as this is a historical test.
Three (3) day food record	A three-day food record is designed to get an accurate description of a participant's typical daily diet.	Source provided by the study	Certified investigator or coordinator
Blood sample collection: • **Fasting plasma amino acids**	The Amino Acids Analysis, Plasma is a blood test that measures 36 amino acids and intermediary metabolites.	N/A	Does not need to be performed by study certified personnel or recorded on the GYROS Study Staff Delegation Log.
Blood sample processing: • **Fasting plasma amino acids**	During centrifugation, the barrier gel rises to separate the heavier cell mass from the lighter liquid portion.	Calibrated centrifuge	Certified investigator, coordinator, or appropriately trained laboratory staff
Measurements: • **Weight** • **Height**	Collect the participants height and weight using a calibrated scale in the office. BMI is automatically calculated on the FFB Study Website.	Calibrated scale	Certified investigator or coordinator
Skin Biopsy (3mm punch skin biopsy)	The skin is performed by stabilizing the area with the thumb and forefinger and stretched slightly perpendicular to the skin tension lines. A punch is held perpendicular to the skin, and is rotated into the skin with a firm, constant pressure.	Kit provided by the study	Certified investigator
Participant Contact Reminders to complete the three (3) day food record	The Clinical Site Coordinator will complete a **Pre-Sample Collection Telephone Call** to the participants (via phone or other remote interface) four (4) to ten (10) days prior to the scheduled sample collection date.	N/A	Certified investigator or coordinator

Study Procedures	Description	Equipment Required (If applicable)	Site Personnel Delegation
	The Clinical Site Coordinator will complete a **Post-Sample Collection Telephone Call** for at-home samples within one (1) day after the scheduled sample collection date.		
Food Record Collection and transcription of data on the FFB Study Website.	The Clinical Site Coordinator will collect and transcribe the data from the food record on the FFB Study Website	N/A	Certified investigator or coordinator

5.2 Questionnaires

The following questionnaires will be administered in the study by a certified investigator or coordinator. Each questionnaire takes about fifteen (15) minutes to administer.

Questionnaire	Type	Description	Vision Cohort	Age
Michigan Retinal Degeneration Questionnaire (MRDQ)	Vision Function	The MRDQ is a psychometrically validated patient-reported outcome measure for inherited retinal degenerations questionnaire which consists of fifty-nine (59) questions measuring seven (7) unidimensional domains: central vision, color vision, contrast sensitivity, scotopic function, photopic peripheral vision, mesopic peripheral vision, and photosensitivity.	All	Adults >= 18 years
PROMIS-29® (Patient-Reported Outcomes Measurement Information System®)	Global physical, mental, and social health	The PROMIS®-29 contains items from seven PROMIS domains: depression; anxiety; physical function; pain interference; fatigue; sleep disturbance; and ability to participate in social roles and activities. The seven domains cover the most relevant areas of self-reported health for most people with chronic illness. There is also one 11-point rating scale for pain intensity.	All	Adults >= 18 years
Visual Symptom and Impact Outcomes Patient Reported Outcome (ViSIO-PRO)	Vision Function and HRQoL	The ViSIO-PRO is an instrument designed to assess visual function symptoms, impacts on functional vision, and impacts on wider health-related quality of life (HRQoL). It has been designed for completion by adolescents (12-17) and adults (18 +) with retinitis pigmentosa (RP).	All	Adolescents (12-17) and Adults (18+)
L. V. Prasad-Functional Vision	Vision Function	The LVP-FVQ-II questionnaire consists of twenty-three (23)	All	Children (<18 years)

Questionnaire (LVP-FVQ II)		questions. The questionnaire is used to assess self-reported difficulties in performing daily tasks in children with visual impairment.		

823

Chapter 6: Unanticipated Problem and Adverse Event Reporting

6.1 Unanticipated Problems

Site investigators will promptly report all unanticipated problems meeting the criteria below on an eCRF. Sites overseen by the JCHR IRB must report Unanticipated Problems to the IRB within seven (7) calendar days of recognition. For this protocol, an unanticipated problem is an incident, experience, or outcome that meets all 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-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 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.

These instances must be reported to the JCHR IRB within seven (7) calendar days of recognition. The Director of the Human Research Protection Program (HRPP) will report to the appropriate regulatory authorities and applicable relying IRBs if the JCHR IRB determines that the event indeed meets the criteria of an Unanticipated Problem that requires further reporting to fulfill the reporting obligations of the HRPP.

6.2 Adverse Events

6.2.1 Definitions

Adverse Event (AE): Any untoward medical occurrence, including laboratory findings, associated with study procedures whether the event is considered related. Progression of GA retinal dystrophy including new or worsening of anticipated clinical signs or symptoms, which are collected and assessed as unequivocally associated with the disease progression should NOT be reported as AEs unless the disease progression is greater than anticipated in the natural course of the disease.

Serious Adverse Event (SAE): Any untoward medical occurrence that results in any of the following outcomes:

- Death.
- A life-threatening adverse event: (a non-life-threatening event which, had it been more severe, might have become life-threatening, is not necessarily considered a serious adverse event).

- Inpatient hospitalization or prolongation of existing hospitalization.
- A persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly or birth defect.

An important medical event that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical and surgical intervention to prevent one of the outcomes listed in this definition.

Note: As this is a Natural History Study, the Investigator(s) will make the categorical determinations of Adverse Events, as described above, and will report each determination to the Coordinating Center, as per data collection.

6.2.2 Reportable Adverse Events

For this protocol, a reportable adverse event includes all events meeting the definition of an adverse event.

All reportable Adverse Events whether volunteered by the participant, discovered by study personnel during questioning, or detected through ophthalmological examination, laboratory test, or other means will be reported on an adverse event form online.

The purpose of AE collection for the GYROS 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. However, each Principal Investigator is responsible for abiding by any other reporting requirements specific to their IRB or equivalent ethics oversight committee

6.2.3 Relationship of Adverse Event to Study Procedure

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

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

Yes

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

No

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

6.2.4 Severity (Intensity) of Adverse Event

A severity assessment is a clinical determination of the intensity of an event. Thus, a severe adverse event is not necessarily serious. For example, itching for several days may be rated as severe, but may not be clinically serious.

The severity (intensity) of an adverse event will be rated on a three-point scale: (1) mild, (2) moderate, or (3) severe.

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 and causes severe discomfort.

6.2.5 Expectedness

As this is a Natural History Study, the expectedness for a serious adverse event will not be assessed.

6.2.6 Coding of Adverse Events

Adverse events will be coded using the MedDRA dictionary. To facilitate coding, the site will enter a preliminary MedDRA code

6.2.7 Outcome of Adverse Event

The outcome of each reportable adverse event will be classified by the investigator as follows:

1. **RECOVERED/RESOLVED (COMPLETE RECOVERY) –** The participant recovered from the AE/SAE without sequelae. Record the AE/SAE stop date.
2. **RECOVERED/RESOLVED WITH SEQUELAE –** AE/SAE where the subject recuperated but retained pathological conditions resulting from the prior disease or injury. Record the AE/SAE stop date.
3. **FATAL –** A fatal outcome is defined as the SAE that resulted in death. Only the event that was the cause of death should be reported as fatal. AEs/SAEs that were ongoing at the time of death; however, were not the cause of death, will be recorded as "resolved" at the time of death.
4. **ONGOING NOT RECOVERED/NOT RESOLVED –** An ongoing AE/SAE is defined as an ongoing event with an undetermined outcome.
    - An ongoing outcome will require follow-up by the site to determine the outcome of the AE/SAE.
    - The outcome of an ongoing event at the time of death that was not the cause of death, will be updated and recorded as "resolved" with the date of death recorded as the stop date.

5. **ONGOING (MEDICALLY STABLE) –** AE/SAE is ongoing, but medically stable. For example, a chronic condition where no further change is expected.

If any reported adverse events are ongoing when a participant completes the study (or withdraws), they will be followed until they are either resolved, or have no prospect of improvement or change, even after the participant has completed all applicable study visits/contacts. For all other adverse events, data collection will end at the time the participant completes the study.

Note: participants should continue to receive appropriate medical care for an adverse event after their participation in the study ends.

If a participant is lost to follow up and participant outcome cannot be determined, outcome classification will be the last known outcome.

6.3 Timing of Event Reporting

Investigator(s) are responsible for reporting Adverse Events on the electronic case report form (eCRF) through the study website in a timely manner.

Each Principal Investigator (PI) is responsible for reporting serious study-related adverse events and abiding by any other reporting requirements specific to his/her Institutional Review Board (IRB) or Ethics Committee (EC). Where the JCHR IRB is the overseeing IRB, sites must report all serious, related adverse events regardless of whether they are expected/anticipated and regardless of whether they are fatal or life-threatening within seven (7) calendar days.

Chapter 7: Miscellaneous Considerations

7.1 New or ongoing Medical Conditions and Medications

7.1.1 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 study (e.g., prior myocardial infarction or stroke).

7.1.2 Medical Conditions during the study

In addition to conditions meeting the reporting requirements for an adverse event or as described above, the following medical conditions should also be reported: (1) new diagnosis of a chronic disease (i.e., not present at the time of enrollment), and (2) any medical condition that could affect the participant's ability to carry out any aspect of the protocol or could affect an outcome assessment. These will be reported as adverse events. See **Chapter 6.2 Adverse Events** for more detail.

7.1.3 Medications

All medication for the treatment of chronic pre-existing conditions, medical conditions, and/or adverse events that the participant is currently taking at screening and during the study should be recorded. Nutraceuticals and preventative treatments that are of interest to the study will be recorded.

7.2 Prohibited Medications, Treatments, and Procedures

7.2.1 Prohibited Medications and Treatment for *OAT*-Related Retinal Degeneration

Participants who are **enrolled into the final study cohort** should not receive IRD treatments during the study. This includes enrolling into an experimental treatment trial of underlying conditions related to *OAT* mutations during the 4-year study duration. However, if the participants enroll in a treatment trial the Executive Committee will be consulted and will determine if the participant may continue in the study.

Examples of prohibited medications and treatments include, but are not limited to the following:

- use of ocular stem cell or gene therapy
- ocriplasmin
- ophthalmic oligonucleotide
- Ozurdex (dexamethasone)
- Iluvien or
- Yutiq (fluocinolone acetonide) intravitreal implant
- Amiodarone, chloroquine, deferoxamine, hydroxychloroquine, pentosan polysulfate, tamoxifen, thioridazine

7.2.2 Intraocular Surgical Procedures

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

7.2.3 Treatment for Cystoid Macular Edema (CME)

Participants **enrolled into the final study cohort**, who need to receive treatment for CME during the study, may do so with an oral or topical dorzolamide, topical nonsteroidal or injectable corticosteroid, but not a steroid implant.

7.3 Pregnancy Reporting

If a pregnancy occurs, the participant will remain in the study. The occurrence of pregnancy will be reported to the Coordinating Center within seven (7) days of the site's discovery of the pregnancy (including at screening) and the Pregnancy Notification form will be completed within seven (7) calendar days. Sites will collect concomitant medications throughout the pregnancy. If an Adverse Event occurs because of the pregnancy, then the site will record the Adverse Event on the Adverse Event form.

7.4 Participant Compensation

Participant compensation will be specified in the informed consent form.

7.5 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.

7.6 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 coordinating center, the Jaeb Center for Health Research in Tampa, FL. De-identified participant information may also be provided to research sites involved in the study.

Chapter 8: Statistical Considerations

8.1 Statistical and Analytical

The approach to sample size and statistical analyses are summarized below. The analysis plan synopsis in this chapter contains the framework of the anticipated final analysis plan.

8.2 Sample Size

The sample size evaluation focuses on objective 1a of the study, to characterize the natural history of retinal degeneration associated with disease-causing *OAT* variants in the presence of standard care dietary treatment regimens over four (4) years, using functional, structural, and patient-reported outcome measures. A justification of the selected sample size using percent change for the outcomes of interest is outlined. The precision of estimates and of the correlation between structure-based, function-based, and metabolic-based outcomes involved in objective two (2) and of the correlation between eyes in the outcomes is also provided. Calculations to address objective three (3), explore risk factors associated with progression (i.e., compare subgroups for dichotomous risk factors), are summarized.

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

Longitudinal changes on all outcome parameters being collected will be examined with the change in preserved retina being of particular interest. Change from baseline to four (4) years will be evaluated for sample size purposes, although statistical analyses will include the data from each annual visit. The power and sample size calculations below may be used to consider percent change on any outcome measure from baseline to four (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 two (2) eyes of a person are typically strongly correlated ($r \geq 0.5$). The contribution of information from the two (2) eyes in this case is (2/(1+r)) instead of two (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

The correlation between eyes (inter-eye correlation) for each outcome measure will be estimated during the study and 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 than lower plausible values of r.

The primary way sample size is evaluated is by considering the precision around the point estimates for the percent change in the outcome measures over four (4) years. **Table 6.1** provides the half width of the 95% confidence interval (CI) for the estimated mean percent change per year 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 6.1. 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=11 (10)	**n= 22 (20)**	**n= 33 (30)**	**n= 44 (40)**	**n=55 (50)**
SD=20%	12%	8%	7%	6%	5%
SD=30%	18%	13%	10%	9%	8%
SD=40%	24%	17%	14%	12%	11%
SD=50%	30%	21%	17%	15%	13%

Note: The shaded column corresponds to the sample size deemed feasible based on preliminary patient counts and financial constraints. Forty (40) participants from Vision Cohorts 1 and 2 will be followed for progression.

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

Another objective for this natural history study will be to explore the association of risk factors with progression of various functional outcome variables (objective 3). **Figure 6.1** considers various expected SDs of the distribution of percent change from baseline to four (4) years per eye and evaluates the power to detect varying differences in average percent change from baseline to four (4) years, comparing subgroups of various equally distributed sizes. If subgroups are not equally sized the smallest detectable difference (with the same power) will be larger. Testing will be performed with a Type I (α) error rate of 0.05.

Note: *within* subgroup point estimates and Cis will also be important. **Table 6.1** above can be applied to potential subgroup sample sizes as well to consider the precision that would be observed.

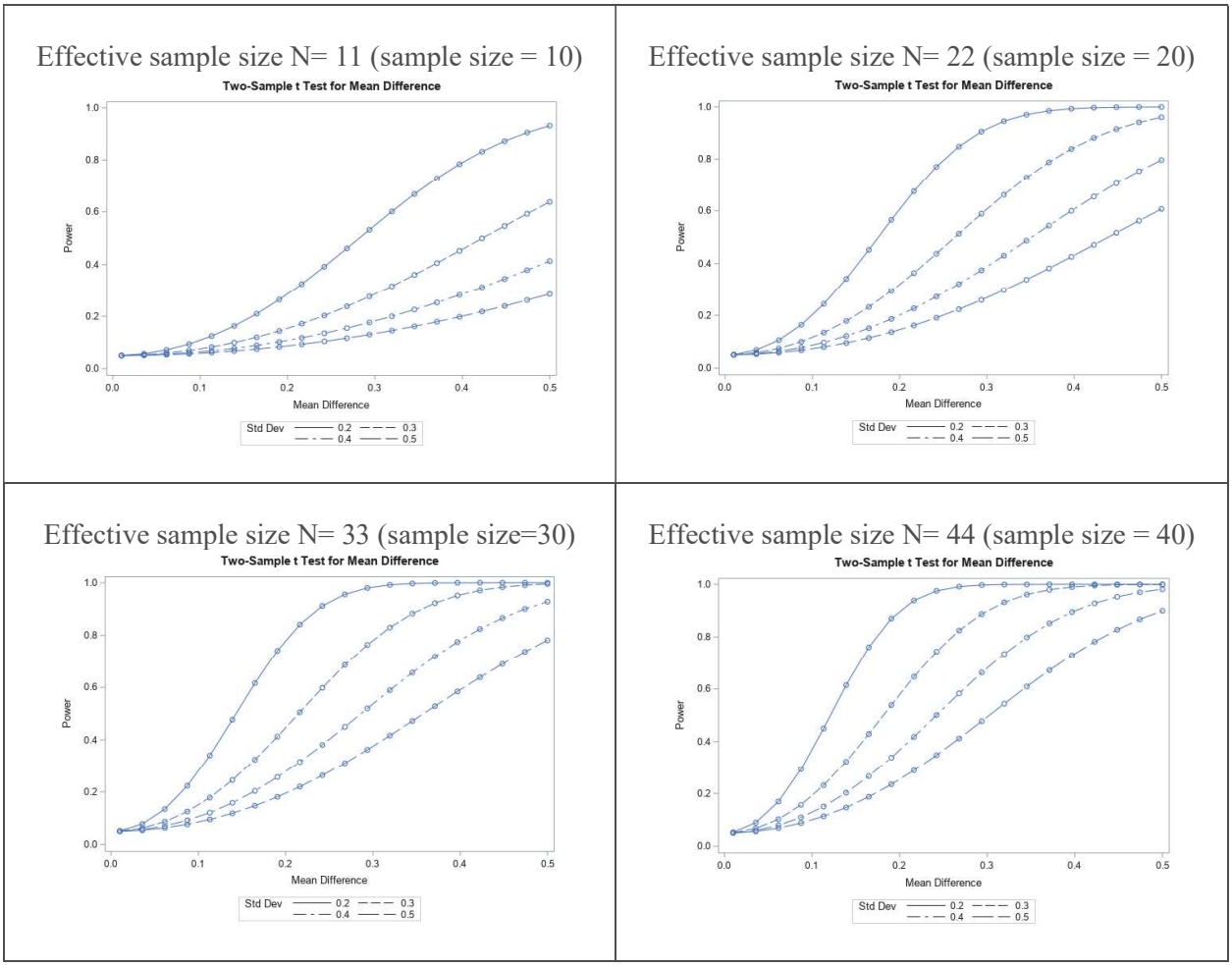


Figure 6.1. Power to conclude there is a difference given varying true difference values, population standard deviation, and sample size.

For the graphs is Figure 6.1, the y-axis if for the power to conclude that there is a difference when the true difference in mean percent change is the x-axis value. Each of the four graphs is for a different total sample size with the number of eyes in each of the two subgroups being approximately fifty percent (50%). Forty (40) participants from Vision Cohorts 1 and 2 will be followed.

For example, with an effective sample size of twenty-two (22) and an effective sample size of eleven (11) in each subgroup, to have power of 80% or more, the true difference needs to be approximately 1.25 SDs (a mean difference of 25% if the SD of the percent change over four (4) years 20%). If the effective sample size is thirty-three (33), the true difference needs to be approximately 1.0 SD (a mean difference 20% if the SD of the percent change over four (4) years is 20%). If the effective sample size is forty-four (44), the true difference needs to be approximately 0.86 SDs (a mean difference 17% if the SD of the percent change over four (4) years is 20%). Such mean differences are considered large.

8.2.3 Sample Size Considerations for Precision of the Estimate of the Correlation Between Structure-based, Function-based, and Metabolic-based Outcomes and of the Correlation Between Eyes

The Pearson correlation coefficient (r) can be used to assess the correlation between two different outcome measures. 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 intraclass correlation coefficient is used to assess the strength of agreement 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 table below provides the 95% CI for different estimates of r from the observed data.

Table 6.2A. 95% Confidence Intervals for an Observed Value of the Correlation between Outcome Measures.

	Effective sample size				
r	**n= 11**	**n= 22**	**n= 33**	**n= 44**	**n=55**
0.3	(-0.37,0.76)	(-0.14,0.64)	(-0.05,0.58)	(0.00,0.55)	(0.04,0.52)
0.4	(-0.26,0.81)	(-0.03,0.70)	(0.07,0.65)	(0.12,0.62)	(0.15,0.60)
0.5	(-0.14,0.85)	(0.10,0.76)	(0.19,0.72)	(0.24,0.69)	(0.27,0.68)
0.6	(0.00,0.88)	(0.24,0.82)	(0.32,0.78)	(0.37,0.76)	(0.40,0.75)
0.7	(0.17,0.92)	(0.39,0.87)	(0.47,0.84)	(0.51,0.83)	(0.53,0.81)
0.8	(0.38,0.95)	(0.57,0.91)	(0.63,0.90)	(0.66,0.89)	(0.68,0.88)
0.9	(0.65,0.97)	(0.77,0.96)	(0.81,0.95)	(0.82,0.94)	(0.83,0.94)

Table 6.2B. 95% Confidence Intervals for an Observed Value of the Inter-eye Correlation.

	N of patients				
r	**n= 10**	**n= 20**	**n= 30**	**n= 40**	**n=50**
0.3	(-0.41,0.78)	(-0.16,0.66)	(-0.07,0.60)	(-0.01,0.56)	(0.02,0.53)
0.4	(-0.31,0.82)	(-0.05,0.72)	(0.05,0.66)	(0.10,0.63)	(0.14,0.61)
0.5	(-0.19,0.86)	(0.07,0.77)	(0.17,0.73)	(0.22,0.70)	(0.26,0.68)
0.6	(-0.05,0.89)	(0.21,0.82)	(0.31,0.79)	(0.35,0.77)	(0.39,0.75)
0.7	(0.13,0.92)	(0.37,0.87)	(0.45,0.85)	(0.50,0.83)	(0.52,0.82)
0.8	(0.34,0.95)	(0.55,0.92)	(0.62,0.90)	(0.65,0.89)	(0.67,0.88)
0.9	(0.62,0.98)	(0.76,0.96)	(0.80,0.95)	(0.82,0.95)	(0.83,0.94)

Note: The shaded column corresponds to the sample size deemed feasible based on preliminary patient counts and financial constraints. Forty (40) participants from Vision Cohorts 1 and 2 will be followed and used for assessing inter-eye correlation of change in outcome measures over time.

8.2.4 Final Sample Size Justification

Longitudinal changes in all outcome parameters being collected will be of interest for objectives one (1) and three (3). Information on rates of decline using the proposed outcome measures for *OAT* variants does not exist.[12,26] Information on rates of decline for inherited retinal degenerations in general is limited.

Data to consider for evaluating sample size:

- Valproic Acid Protocol (VPA) Data (phase II multiple sites, randomized, placebo-controlled trial of oral valproic acid for ADRP) [Placebo group N=44; dataset can be accessed at https://public.jaeb.org/ffb/stdy]
    - Percent Change from Baseline to 1 year, Mean (SD):
        - -0.3% (16%) OD
        - -4.9% (17%) OS

Assumptions made:

- Expect average annual decline due to *OAT* will be 5% per year or 20% by four (4) years.
- True SD of percent change at four (4) years is like VPA 1-year SD of approximately 20%.

If thirty (30) study participants or thirty-three (33) effective eyes are recruited:

- The half width of a 95% CI around the point estimate for mean percent change would be seven (7) %.
- A comparison of two equal-sized subgroups would have about 80% power to conclude there is a difference if the true difference is 20%.
- For the objective of evaluating the agreement in outcome between eyes, a sample size of thirty (30) participants for Vision Cohorts 1 and 2 will yield a 95% CI of (0.17,0.73) when the observed r equals 0.5, and (0.62,0.90) when the observed r equals 0.8.
- For the objective of evaluating the correlation between outcomes, an effective sample size of thirty-three (33) participants for Vision Cohorts 1 and 2 will have a 95% CI of (0.19,0.72) when the observed r equals 0.5, and (0.63,0.90) when the observed r equals 0.8.

If forty (40) study participants or forty-four (44) effective eyes are recruited:

- The half width of a 95% CI around the point estimate for mean percent change would be six (6) %.
- A comparison of two equal-sized subgroups would have about 80% power to conclude there is a difference if the true difference is 17%.
- For the objective of evaluating the agreement in outcome between eyes, a sample size of forty (40) participants for Vision Cohorts 1 and 2 will yield a 95% CI of (0.22,0.70) when the observed r equals 0.5, and (0.65,0.89) when the observed r equals 0.8.

- For the objective of evaluating the correlation between outcomes, an effective sample size of forty-four (44) participants for Vision Cohorts 1 and 2 will yield a 95% CI of (0.24,0.69) when the observed r equals 0.5, and (0.66,0.89) when the observed r equals 0.8.

8.3 Data Analysis

The analysis plans below are written with respect to most outcomes of interest. Analyses will include data on both eyes for each participant, and confidence intervals will be adjusted for correlation between two (2) eyes of the same participant.

8.3.1 Primary Objective Analysis

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

1. **Natural History**
   - **(a)** Characterize the natural history of retinal degeneration associated with disease-causing *OAT* variants over four (4) years, as measured using functional, structural, and patient-reported outcome measures.

     i. **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 per visit will be analyzed using the average of all available tests). To determine the average annual rate of progression in the population for each outcome, a mixed effects model will be fit using all available outcome data at baseline and all annual visits. Both the correlation in outcomes over time and the correlation in outcomes between eyes will be accommodated by the model.[28] Mixed models adequately accommodate missing values when missing values are missing at random (MAR). A second method of accommodating missing values will be through multiple imputation for the outcome values for all missing time points (including participants who discontinue follow up prior to forty-eight (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.

     ii. **PRO measures:** The scoring of each questionnaire will be completed according to the procedures recommended by the developers of the questionnaire for each instrument. 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. A generalized linear model adjusted for baseline differences will be explored.

Partially completed questionnaires will be managed using methods recommended by the developers of the specific questionnaire. When no questionnaire is completed, we will use statistical methods that mitigate the impact of missing data, specifically, mixed effects regression models and multiple imputation for the outcome values for all missing time points.

(b) Characterize the natural history of ornithine levels associated with disease-causing *OAT* variants in the presence of standard care dietary treatment regimens over four (4) years.

i. Longitudinal annual measures of ornithine will be explored by graphing the values for everyone ("spaghetti" plots) and analyzed to detect any linear trend in time with mixed effects models.

(c) Determine within-patient variability of ornithine levels associated with disease-causing *OAT* variants in the presence of standard care dietary treatment regimens over four (4) years.

i. The within-person standard deviation of ornithine levels will be estimated for the group of patients using a mixed effects model. Models will be based on levels from blood dot tests and from plasma testing over the four (4) years to determined long -term variability and on levels from blood dot tests over periods of six (6) months and of twelve (12) months to determine short-term variability. The coefficient of repeatability will be calculated to estimate the difference from baseline that would need to be exceeded to indicate that a treatment was causing an effect beyond random variability.

2. Metabolic, Structure, and Function Relationships

(a) Evaluate cross-sectional associations at baseline and each annual visit with scatterplots and Spearman correlation coefficients. Evaluate the association of changes in each outcome measure with changes the other outcome measures at each visit with scatter plots and Spearman correlation coefficients. Use mixed effects regression models to evaluate the association of change in each outcome measure with changes in each of the other outcome measures. With so many outcomes and only forty (40) patients, results will be interpreted cautiously because of both low powers to detect clinically meaningful associations and elevated Type I error rates due to multiple comparisons.

3. Identify Rapid Progressors

(a) The distribution of each outcome in terms of both absolute change and percent change from baseline to four (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). Potential risk factors to evaluate include phenotypic factors including the baseline level of each of the structural and functional

measures and ornithine level, genotype, and adherence to the arginine-restricted diet as noted below. Univariable regression models of each outcome measure will be fit for each of the potential risk factors. With so many potential risk factors and outcomes and only forty (40) patients, results will be interpreted cautiously because of both low powers to detect clinically meaningful differences and elevated Type I error rates due to multiple comparisons.

i. Phenotypic:
- Age of onset of initial vision symptoms
- Gender
- Race/ethnicity
- Body weight/BMI
- Lens Status (phakic/pseudophakic/aphakic) and area of subcapsular cataract on red reflex photos
- Baseline foveal avascular zone (FAZ) area and macular vessel density on OCTA
- Baseline visual acuity
- Baseline low luminance visual acuity
- Baseline area of preserved retina on fundus autofluorescence and color photos
- Baseline full Hill of Vision (HOV) on static perimetry
- Baseline mean sensitivity on static perimetry
- Baseline mean retinal sensitivity on microperimetry
- Baseline full-field stimulus (FST) levels
- Baseline ERG amplitude and timing
- Baseline ellipsoid zone (EZ) area on OCT
- Baseline ornithine level

ii. Genotypic
- Characterizations of the *OAT* variants

iii. Environmental Factors
- Adherence to arginine-restricted diet
- Smoking

Chapter 9: Data Collection and Monitoring

9.1 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 electronic case report forms, this will be considered the source data. However, it is recommended that a progress note is completed to document the events of the visit. For any data points for which the eCRF is not considered source (e.g., lab results that 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.) and provided to the coordinating center for review.

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.

9.1.1 Central Lab

The Kennedy Krieger Institute, Central Lab, will complete an analysis from the fasting amino acid panel, and blood spot collection. The lab will directly enter the laboratory results on the FFB Study Website.

Results for ornithine and arginine will be provided for the site.

9.1.2 Research Lab

The Johns Hopkins University Department of Genetic Medicine Research Lab will receive the skin biopsy samples to grow fibroblasts. The tissue sample analysis will be entered directly in the FFB Study Website.

9.1.3 Central Genetics Auditor (CGA)

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.

9.1.4 Genetics Committee

In addition to providing evaluation of whether the *OAT* mutations are causative of the disease on the FFB Consortium study website, the Genetics Committee will review and provide approval for the use of genetic reports from research labs to be used for determining participant eligibility.

9.1.5 The Johns Hopkins Institute for Clinical and Translational Research (ICTR) Research Nutrition

The site will enter the food record documentation on the FFB Study Website. ICTR will review the three (3) day food records and analyze for arginine, vitamin A, and vitamin B6 intake. Total protein and caloric intake will be documented as well. ICTR will enter the nutrition analysis assessment data directly on the FFB Study Website.

9.1.6 Reading Centers

Reading Centers will conduct grading of the study data collected using the FFB Consortium study website. The Reading Centers will provide the graded data through a data transfer or by entering the graded data on the study website. These data will remain in the study database and will not be provided to the clinical site.

9.2 Study Records Retention

Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH E6 and regulatory and institutional requirements for the protection of confidentiality of participants.

Study documents will be retained for a minimum of six (6) 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 sponsor. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

9.3 Quality Assurance and Monitoring

Designated personnel from the Coordinating Center 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, Good Clinical Practice (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.

A risk-based monitoring (RBM) plan will be developed and revised as needed during the study, consistent with the FDA "Guidance for Industry Oversight of Clinical Investigations — A Risk-Based Approach to Monitoring" (August 2013).[29] Study conduct and monitoring will conform with 21 Code of Federal Regulations (CFR) 312.[30] 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.

The data of most importance for monitoring at the site are participant eligibility and adverse events. Therefore, the RBM plan will focus on these areas. 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 may include:

- Qualification assessment, training, and certification for sites and site personnel
- Oversight of Institutional Review Board (IRB) 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
- Patient retention and visit completion
- Quality control reports
- Management of noncompliance
- Documenting monitoring activities
- Adverse event reporting and monitoring

Coordinating Center representatives or their designees may visit the study facilities at any time to maintain current and personal knowledge of the study through review of 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.

9.4 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.

A significant (or major) deviation is any deviation that departs from the established materials in such a way that it poses an increase in the risk to the study participants, adversely affects the welfare, rights, or safety of the research study participants, or negatively influences the scientific study integrity. As a result of significant deviations, corrective and preventive actions are to be developed by the site and implemented promptly.

The site PI and study staff are responsible for knowing and adhering to their IRB/EC requirements.

Chapter 10: Ethics/Protection of Human Participants

10.1 Ethical Standard

The Principal Investigator (PI) will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Participants of Research in accordance with ICF E6/GCP, EC requirements, and local laws and regulations, as applicable.

10.2 Institutional Review Boards

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

10.3 Informed Consent Process

10.3.1 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. Extensive discussion of risks and benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved, and the participant will be asked to read and review the document. The investigator 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. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing.

The participants will have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the 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.

10.3.2 Participant and Data Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and 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 study monitor, other authorized representatives of the sponsor, representatives of the IRB, regulatory agencies or company supplying study product 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, 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 Coordinating Center, located at the Jaeb Center for Health Research in Tampa, FL. This will not include the participant's contact or identifying information, unless otherwise specified in the informed consent form. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by the FFB Consortium Coordinating Center 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 Coordinating Center.

10.3.3 Future Use of Stored Specimens and Data

With the participant's approval and as approved by the IRB/ECs, de-identified biological skin fibroblasts and blood samples collected for analysis will be stored at The Kennedy Krieger Institute until the FFB Study Team notifies them in writing to destroy the samples. The blood samples will be destroyed one (1) year after the end of the study. The skin biopsy samples will be stored indefinitely with the participant's consent.

10.3.4 Future Use of Ocular Images

Data collected for this study will be analyzed and stored at the FFB Coordinating Center and the Reading Centers. After the study is completed, the archived data will be transmitted to and stored at the FFB Consortium Coordinating Center, under the supervision of Allison Ayala, for use by other researchers including those outside of the study. Permission to transmit data to the FFB Consortium Coordinating Center will be included in the informed consent. This data will contain images of the retina, which are considered identifiable, but no directly identifying information will be share, such as name, as described in the consent forms.

During the conduct of the study, an individual participant can choose to withdraw consent to have their ocular images stored for future research. However, withdrawal of consent will not be possible after the study is completed.

Chapter 11: References

1. Wang T, Lawler AM, Steel G, Sipila I, Milam AH, Valle D. Mice lacking ornithine-delta-aminotransferase have paradoxical neonatal hypoornithinaemia and retinal degeneration. *Nat Genet.* 1995;11(2):185-190.
2. Valle D, Simell O. The Hyperornithinemias. In: Valle DL, Antonarakis S, Ballabio A, Beaudet AL, Mitchell GA, eds. *The Online Metabolic and Molecular Bases of Inherited Disease.* New York, NY: McGraw-Hill Education; 2019.
3. Valle D, Kaiser-Kupfer MI, Del Valle LA. Gyrate atrophy of the choroid and retina: deficiency of ornithine aminotransferase in transformed lymphocytes. *Proceedings of the National Academy of Sciences of the United States of America.* 1977;74(11):5159-5161.
4. Mitchell GA, Brody LC, Looney J, et al. An initiator codon mutation in ornithine-delta-aminotransferase causing gyrate atrophy of the choroid and retina. *The Journal of clinical investigation.* 1988;81(2):630-633.
5. Mitchell GA, Looney JE, Brody LC, et al. Human ornithine-delta-aminotransferase. cDNA cloning and analysis of the structural gene. *The Journal of biological chemistry.* 1988;263(28):14288-14295.
6. Mitchell GA, Brody LC, Sipila I, et al. At least two mutant alleles of ornithine delta-aminotransferase cause gyrate atrophy of the choroid and retina in Finns. *Proceedings of the National Academy of Sciences of the United States of America.* 1989;86(1):197-201.
7. Brody LC, Mitchell GA, Obie C, et al. Ornithine delta-aminotransferase mutations in gyrate atrophy. Allelic heterogeneity and functional consequences. *J Biol Chem.* 1992;267(5):3302-3307.
8. Michaud J, Thompson GN, Brody LC, et al. Pyridoxine-responsive gyrate atrophy of the choroid and retina: clinical and biochemical correlates of the mutation A226V. *Am J Hum Genet.* 1995;56(3):616-622.
9. Lemay JF, Lambert MA, Mitchell GA, et al. Hyperammonemia-hyperornithinemia-homocitrullinuria syndrome: neurologic, ophthalmologic, and neuropsychologic examination of six patients. *J Pediatr.* 1992;121(5 Pt 1):725-730.
10. Camacho JA, Obie C, Biery B, et al. Hyperornithinaemia-hyperammonaemia-homocitrullinuria syndrome is caused by mutations in a gene encoding a mitochondrial ornithine transporter. *Nat Genet.* 1999;22(2):151-158.
11. Palmieri F, Scarcia P, Monné M. Diseases Caused by Mutations in Mitochondrial Carrier Genes SLC25: A Review. *Biomolecules.* 2020;10(4).
12. Balfoort BM, Buijs MJN, Ten Asbroek A, et al. A review of treatment modalities in gyrate atrophy of the choroid and retina (GACR). *Mol Genet Metab.* 2021;134(1-2):96-116.
13. Valle D, Walser M, Brusilow S, Kaiser-Kupfer MI, Takki K. Gyrate atrophy of the choroid and retina. Biochemical considerations and experience with an arginine-restricted diet. *Ophthalmology.* 1981;88(4):325-330.
14. Kaiser-Kupfer MI, de Monasterio F, Valle D, Walser M, Brusilow S. Visual results of a long-term trial of a low-arginine diet in gyrate atrophy of choroid and retina. *Ophthalmology.* 1981;88(4):307-310.

15. Valle D, Walser M, Brusilow SW, Kaiser-Kupfer M. Gyrate atrophy of the choroid and retina: amino acid metabolism and correction of hyperornithinemia with an arginine-deficient diet. *J Clin Invest.* 1980;65(2):371-378.
16. Kaiser-Kupfer MI, de Monasterio FM, Valle D, Walser M, Brusilow S. Gyrate atrophy of the choroid and retina: improved visual function following reduction of plasma ornithine by diet. *Science.* 1980;210(4474):1128-1131.
17. Kaiser-Kupfer MI, Caruso RC, Valle D, Reed GF. Use of an arginine-restricted diet to slow progression of visual loss in patients with gyrate atrophy. *Arch Ophthalmol.* 2004;122(7):982-984.
18. Kaiser-Kupfer MI, Caruso RC, Valle D. Gyrate atrophy of the choroid and retina. Long-term reduction of ornithine slows retinal degeneration. *Arch Ophthalmol.* 1991;109(11):1539-1548.
19. Wang T, Milam AH, Steel G, Valle D. A mouse model of gyrate atrophy of the choroid and retina. Early retinal pigment epithelium damage and progressive retinal degeneration. *J Clin Invest.* 1996;97(12):2753-2762.
20. Wang T, Steel G, Milam AH, Valle D. Correction of ornithine accumulation prevents retinal degeneration in a mouse model of gyrate atrophy of the choroid and retina. *Proceedings of the National Academy of Sciences of the United States of America.* 2000;97(3):1224-1229.
21. Sipilä I, Simell O, Arjomaa P. Gyrate atrophy of the choroid and retina with hyperornithinemia. Deficient formation of guanidinoacetic acid from arginine. *J Clin Invest.* 1980;66(4):684-687.
22. Vannas-Sulonen K, Sipilä I, Vannas A, Simell O, Rapola J. Gyrate atrophy of the choroid and retina. A five-year follow-up of creatine supplementation. *Ophthalmology.* 1985;92(12):1719-1727.
23. Heinänen K, Näntö-Salonen K, Komu M, et al. Creatine corrects muscle 31P spectrum in gyrate atrophy with hyperornithinaemia. *Eur J Clin Invest.* 1999;29(12):1060-1065.
24. Hanna-El-Daher L, Braissant O. Creatine synthesis and exchanges between brain cells: What can be learned from human creatine deficiencies and various experimental models? *Amino Acids.* 2016;48(8):1877-1895.
25. Du J, Zhu S, Lim RR, Chao JR. Proline metabolism and transport in retinal health and disease. *Amino Acids.* 2021;53(12):1789-1806.
26. Elnahry AG, Elnahry GA. Gyrate Atrophy of the Choroid and Retina: A Review. *European Journal of Ophthalmology.* 2021:11206721211067333.
27. Food and Drug Administration. Rare Diseases: Natural History Studies for Drug Development. In. *Draft Guidance for Industry,*2019.
28. Ying GS, Maguire MG, Glynn RJ, Rosner B. Tutorial on Biostatistics: Longitudinal Analysis of Correlated Continuous Eye Data. *Ophthalmic epidemiology.* 2021;28(1):3-20.
29. Food and Drug Administration. Guidance for Industry Oversight of Clinical Investigations - A Risk-Based Approach to Monitoring. In:2013.
30. Food and Drug Administration. 21 Code of Federal Regulations (CFR) 312. In:2022.