RPGRIP1 Landscape Analysis and Clinical Development Report

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Scope of Work: The primary goal of this project is to establish a baseline and foundational understanding for what is known about the clinical manifestation of RPGRIP1 associated retinal dystrophy at the time of this report. This understanding will enable clinical study strategy and design, as well as community engagement.

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Leber congenital amaurosis (LCA) is a group of retinal dystrophies that present in early childhood, with the most severe forms presenting as early as birth. Homozygous and compound heterozygous loss-of-function mutations in RPGRIP1 are commonly reported to cause **LCA type 6** (LCA6), characterized by early infantile onset with a lack of recordable cone ERG by 12 months of age [1–13]. Although LCA6 is the most common RPGRIP1-associated disease, **cone-rod dystrophy 13** (CORD13) and forms of early-onset **retinitis pigmentosa** have also been described [4, 11, 13–16]. Significant symptom overlap exists among the three diagnoses, which we will discuss in detail throughout the report.

RPGRIP1 association with disease

LCA is often diagnosed at birth or within the first months of life. A diagnosis of LCA6 is made after genetic testing indicates mutations in the RPGRIP1 gene. LCA6 is predominately characterized by early and severe vision loss leading to reduced or non-recordable ERG within the first year of life; however, among patients with LCA6, there is variability in the age of onset and severity of ERG findings. Characteristics of LCA can also include infant eye poking, pigmentary retinopathy and maculopathy, disc edema, nystagmus, and retinal vascular attenuation. In most RPGRIP1-mediated LCA cases, cone function is extinguished prior to rod function as seen in ERG recordings. This cone-then-rod function loss leads to reduced visual acuity followed by reduction in peripheral field. Although cone function is lost earlier than rod function, there appears to be a retention of central retina laminar structure and architecture into adolescence and adulthood in some cases [3, 10]. This provides a larger window for treatment with gene therapy and the potential for regaining some photoreceptor function post treatment. Of note, there are also a number of case studies documenting the retention of central visual function in LCA6 patients, further supporting the observation of retained central photoreceptors, but also increasing the heterogeneity of disease progression across patients [1, 3, 10, 13].

CORD13 normally presents later in childhood or adolescence and vision loss is less severe than in LCA6. Cone-rod dystrophy is most commonly associated with an initial loss of color vision, loss of visual acuity, and photophobia by the second decade of life (10-13 years). Symptoms of cone-rod dystrophy also include night blindness, loss of peripheral vision, and significantly reduced ERG recordings.

Retinitis Pigmentosa caused by RPGRIP1 mutations is predominantly diagnosed as early-onset or juvenile RP. RP is characterized by the presence of pale optic discs, night blindness, progressive constriction of visual field, retinal vascular narrowing, retinal pigment changes, and reduced ERG amplitudes. Symptoms of RP, specifically juvenile RP and early-onset, can be similar to those of CORD13, and normally first appear in late childhood or early adolescence.

There is significant overlap in symptoms across all three diagnoses. LCA6 is known as the most severe of the three due to its early age of onset; however, even within the LCA6 diagnosis there

is significant variability in initial disease severity and progression rates. LCA6 and CORD13 are most similar in symptom presentation, but with distinct ages of onset. Across all three diagnoses, there is an overlap in symptoms in presentations that call into question the distinction between the three. This is discussed in more detail in *Symptom Onset* and *Clinical Population Symptom Heterogeneity*. Taken together, this suggests that all three diagnoses could be part of a larger spectrum of retinal dystrophy that cannot easily be defined by one disease grouping.

Disease Overview

Age at onset

LCA6 begins within the first year of life, with most patients displaying symptoms in infancy. CORD13 and early-onset/juvenile RP symptoms can appear any time between 1 year and early adolescence. Most studies discuss early-onset RP as onset before 10 years of age while non-early onset RP generally has an onset after 10 years of age. LCA6 is marked by a severe and swift decline in visual function followed by a relatively non-progressive state after the initial decline. For CORD13, there seems to be a later onset and a similar significant decline in vision around 14-16 years of age [14].

Initial Symptoms and Onset

Age at symptom onset is one of the defining characteristics differentiating the three clinical diagnoses. The vast majority of RPGRIP1 mutations lead to LCA6, with caregivers reporting observed visual impairment beginning in the first year of life, and in some cases even at birth. LCA is one of the most severe retinal dystrophies and can present with either impaired vision or complete blindness. A hallmark of LCA is presentation with Franceschetti's oculo-digital sign, which consists of poking, pressing, and rubbing of the eyes with a knuckle or finger. Infants with LCA may also present with nystagmus or a sluggish pupillary reaction. These symptoms normally lead to extensive eye exams and documentation of severely reduced or extinguished cone and/or rod ERG responses. Fundus exams upon initial assessment can vary—some children present with a normal findings, with deterioration over time, and some children present with signs of fundus deterioration upon initial assessment, including bone spicule formation and pigment deposits.

Initial symptoms of CORD13 present later than those of LCA6, normally in late childhood or early adolescence; however reports of diagnosis as early as 1.5 years old have occurred [15]. Loss of cone-mediated color vision is one of the first symptoms, followed by night blindness, photophobia, and reduced ERG, eventually progressing to light perception only. Fundus granularity and macular degeneration are also seen in CORD13.

RPGRIP1-mediated RP patients generally have good central vision through the first decade of life, at which point visual acuity begins to decline starting with night blindness and gradual

constriction of the visual field. Exams can also show pale optic discs, retinal vascular narrowing, pigmentary changes to the retina, and reduced ERG.

One of the greatest challenges for patients and physicians, is accurate diagnosis, which can create challenges for correct mutation analysis and prompt molecular diagnosis. It is unclear whether the different diagnoses of LCA6, CORD13, or early- onset RP are a result of distinct diseases caused by genetic interactions and distinct pathophysiological events, or if these diagnoses are a part of the same spectrum, primarily influenced by diagnostic bias, age at presentation, and the presence of residual RPGRIP1 activity. A number of studies propose the theory that LCA6, with the earliest age of onset of the three diagnoses, is caused by the most severe mutations (usually PTCs) which leave little to no functional RPGRIP1. Conversely, later onset of retinal dystrophy and a diagnosis of CORD13 and early onset RP or even late onset LCA6 are linked to the same pathology but at a slower rate due to the presence of residual RPGRIP1 function.

List of common clinical phenotypes associated with RPGRIP1 mutations

Symptoms described in clinical reports (including LCA6, CORD13, and RP); most common symptoms are in **bold**

- **Reduced or absent ERG-early**
- **Nystagmus**
- **Vision limited to light perception**
- **Photophobia**
- **Color vision loss**
- **Retinal vascular attenuation**
- **Pigmentary deposits: bone spicule pigmentary deposits, fundus granularity—generally starts in periphery and can vary significantly within and between patients**
- Franceschitti's oculo-digital sign
- Hyperopia/myopia

- Achromatopsia
- Macular degeneration
- Cataracts
- Pale optic discs
- Drussen-like deposits
- Peripheral vision loss
- Waxy- appearing optic nerve head
- Thinning of peripheral or parafoveal retina (OCT)
- Blurred vision
- Absent macular arch
- Chorioretinal atrophy
- Keratoconus/keratoglobus

Clinical diagnoses symptoms, onset, and major decline points

Clinical Diagnosis	Common to all diagnoses	LCA6-specific	CORD13-specific	RP-specific
Symptoms reported in clinical publications specific to RPGRIP1 mutations	• Reduced ERG • Nystagmus • Macular Degeneration/blurred vision • Reduced visual acuity • Fundus pigmentary deposits (bone spicule, granularity)	• Photophobia • Night blindness • Hyperopia • Vascular attenuation • Reduced visual acuity (limited to light perception) • Eye poking in infants • Drusen-like deposits • Peripheral vision loss • Disc pallor • Chorioretinal atrophy • Keratoconus/keratoglobus •	• Central vision loss • Color vision loss • Early Photophobia	• Reduced visual field/reduced peripheral vision • Night blindness • Color vision loss • Waxy discs • Vessel attenuation • Absent peripheral background fluorescence • Tunnel vision • Cataracts
Age at onset	N/A	• Usually in early infancy but before 1 year of age	• Variable—in late childhood/early adolescence	• Variable—anywhere from over 1 year into late adolescence
Age at major decline points	N/A	• Significant and sever visual loss within the first year of life in most cases	• Rapid vision loss between 14-16 years old	• Variable

Specialists seen

Documenting the specialists seen by a patient can provide a clear understanding of the patient journey before a diagnosis as well as the burden of care for the patient and family after diagnosis. Additionally, knowing the key specialists can aid in trial enrollment through early patient identification. Most clinical studies cited in this document are written by experts in the field of IRDs and can be used as a resource for identifying clinical experts. Patients and families should be consulted to develop a more thorough list of specialists. Patients with very early onset vision loss may also have delays or difficulties with speech, social skills, and behavior which may necessitate early involvement of a developmental pediatric specialist [16].

- Pediatric ophthalmologist
- IRD ophthalmologists
- Retinal surgeons
- Genetic counselors
- Developmental pediatric specialist

- Vision therapist
- Occupational therapist

Families of the patient may be referred for genetic testing after a patient's initial diagnosis. Siblings of an affected proband with inherited recessive RPGRIP1 LCA have a 25% chance of also being born with LCA or a 50% chance of being a carrier of one mutation.

Diagnosis

Symptoms that lead to seek diagnosis

Most commonly seen at symptom onset:
- Severe and very early vision loss noticed by lack of eye tracking, lack of eye focus, eye poking
- Night blindness
- Central vision loss
- Photophobia
- Focus towards bright lights in very young children or infants
- Nystagmus

There is significant symptom overlap with all three clinical diagnoses associated with RPGRIP1 mutations; this can lead to misdiagnosis, or a delay in screening with the correct mutation panel. Because RPGRIP1 mutations are most commonly associated with LCA specifically, a patient diagnosed with LCA will likely have genetic testing that includes RPGRIP1. The likelihood of an RP or CORD patient being screened for RPGRIP1 is lower though, as those diseases are not as commonly associated with RPGRIP1 mutations. Diagnosis will likely depend on when the child first sees an ophthalmologist and how quickly that physician can make the diagnosis or their willingness to send the patient to a genetic counselor, as well as insurance coverage for genetic testing.

Symptoms that lead families to seek a diagnosis are similar to those described in the previous section. For LCA6, caregivers sometimes notice a lack of visual response as early as birth or turning only towards very bright lights, and usually within the first 3 months of life. Additionally, caregivers may notice nystagmus and poking or rubbing of the eyes— Franceschetti's oculo-digital sign. It is believed that repeated poking of the eye causes the eye to recede into the eye socket due to the breakdown of the orbital fat cushion overtime, while also causing keratoconus- a bulging of the central cornea. Whether Franceschetti's sign and keratoconus are caused by the rubbing of the eyes or an intrinsic part of the pathology is debated among some researchers. Other symptoms associated with LCA6 that have been observed include hyperopia and cataracts. General LCA symptoms that could be seen with RPGRIP1 mutations include ptosis, strabismus, myopia, microphthalmos, and macular coloboma.

CORD13 and the RP cases normally present later than LCA6. CORD13 has similar initial symptoms as LCA6 in addition to reduced visual acuity, lack of color vision, and photophobia. RP initial symptoms vary, but include photophobia, night blindness, and tunnel vision.

Time to diagnosis

There are no studies that definitively report an average time to diagnosis for RPGRIP1 patients, as diagnosis timelines depend on multiple parameters including clinical presentation, genetic testing, and access to specialists and adequate healthcare. Most patients with RPGRIP1 mutations present with classical LCA in early infancy; however, depending on the physician and healthcare system, genetic testing is not always readily available, so a genetic diagnosis may come at a much older age. Depending on how familiar the physician is with RPGRIP1 mutation symptom presentation, there may also be a delay in genetic diagnosis if a patient presents with a CORD, RP, or other atypical phenotype, which is not as commonly associated with RPGRIP1 mutations.

Early diagnosis with the correct genetic mutation is extremely important for future care and possible enrollment in clinical trials. Multiple groups have designed or are currently working on clinical diagnosis guides or pipelines for IRD identification so the proper diagnosis can be made earlier (see figure 1 that follows). Groups such as Foundation Fighting Blindness have designed inclusive databases where patients and physicians can report clinical findings and disease progression information to assist with further diagnoses and natural history information (see section on *Registries*).

Understanding the patient journey to diagnosis and the burden of disease on both the patient and the family can impact trial design as well as commercial considerations of the value of a gene therapy. The literature on RPGRIP1 associated disease is too sparse to discern details about the path and timelines for diagnosis. To this end, we recommend early engagement with patient communities and KOLs with the end goal of accelerating diagnoses and increasing accessibility.

Figure 1: Diagnosis Flow-Chart that created to help physicians quickly diagnose certain symptoms in LCA [6]
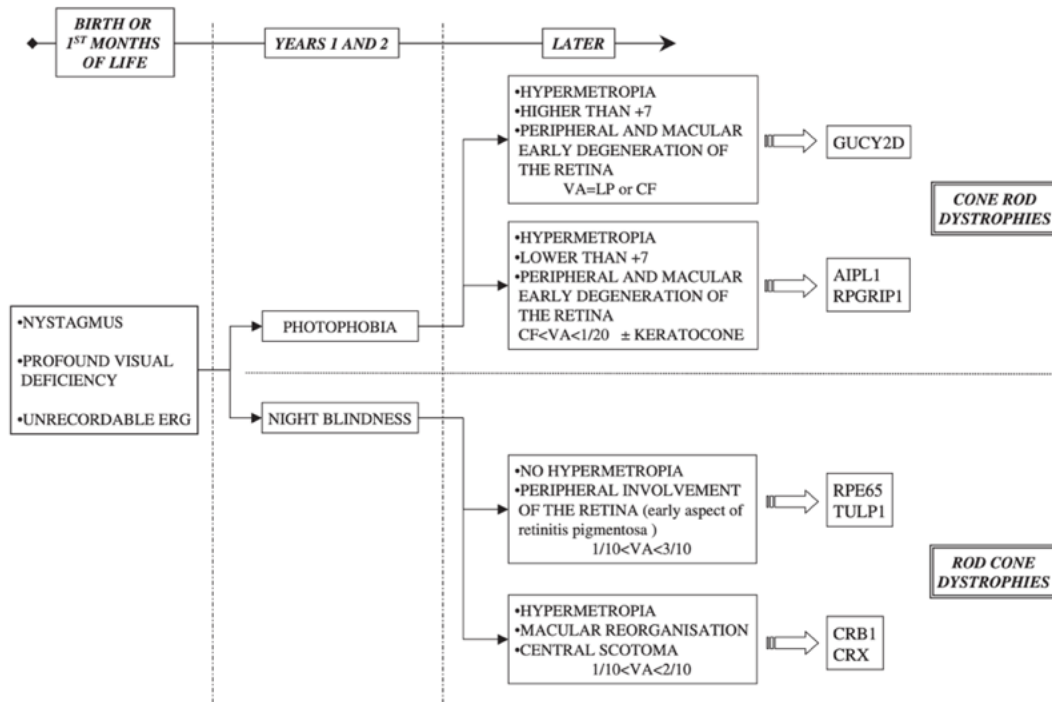
FIGURE 1. Clinical flowchart. LCA and early onset retinitis pigmentosa are divided on the basis of light behavior, the refraction data, the aspect of the retina, and the visual acuity (VA). CF, counting fingers; LP, light perception. This flowchart allows one to direct the molecular analysis of selected LCA genes.

Diagnostic testing- common practices and considerations

- Electroretinogram (ERG) is most commonly used in the diagnosis of LCA. Patients generally present with extinguished or severely reduced ERG from a very early age.
- Fundus exam (fundus fluorescein angiography) is also used, although LCA6 often presents with normal fundus exam at an early age, but a number of abnormalities have been observed in patients.
- Best correlated visual acuity (BCVA) is also commonly used as a diagnostic tool and a measure of visual acuity loss as the patient ages.
- Optical Coherence Tomography (OCT) - Less often but importantly, OCT is used to identify which region of the retina has thinned as the disease progresses—potentially giving insight for gene therapy subretinal injection site.

Prevalence of misdiagnosis in RPGRIP1 diseases has not been published; however, one clinical report noted that a patient was originally misdiagnosed with idiopathic infantile nystagmus [12] Another consideration is the potential for an inconclusive genetic analyses if the mutation screening is only done for coding regions of RPGRIP1. The *Genetic Testing* section discusses this idea in more detail.

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Genetic Testing

Genetic screening for RPGRIP1 is included on genetic panels for a number of different vision phenotypes, as well as intellectual disability. Collectively, panels screen for a range of genetic mutation types including deletions, duplications, mutation scanning of the entire coding region, targeted variant analysis, and sequence analysis of the entire coding region. That being said, a recent study assessed the contribution of non-coding variants to RPGRIP1-mediated IRDs and concluded that non-coding variants in the RPGRIP1 locus associate with disease. This study suggests including genome sequencing or an expanded exon-based sequencing by incorporating a 30 bp flanking region around the sequenced exons as well as copy-number analysis. Exome and genome sequencing revealed potential noncoding pathogenic variants in 7 families that would not have been identified by exome sequencing alone. In six of the seven families, the noncoding pathogenic variants were shown to lead to loss of function in vitro [17]. This becomes critically important when biallelic PVs are crucial for inclusion of patients for gene therapy.

The two figures below are from *Contribution of noncoding pathogenic variants to RPGRIP1-mediated inherited retinal degeneration* (2019) [17] , identifying noncoding pathogenic variants in RPGRIP1-mediated inherited retinal degenerations in patients that had one previously identified coding PV.

Figure 2: RPGRIP1 PVs detected using the GEDi (the Genetic eye disease) diagnostic test which is reported to be 98 percent accurate at detecting spelling variations or mutations in the genetic code of inherited eye disease genes and is highly reproducible between test runs [17]

Table 1 Clinical characteristics and PV in *RPGRIP1*-mediated inherited retinal degeneration patients

ID[a]	Gender	Age at Dx	Variant 1[b]	MAF[c]	Variant 2[b]	MAF[c]	Signs[d]	Method
237–523	F	Early childhood	c.3793ins4(p.V1265Gfs*19)	4×10^{-6}	Exon1-2 dup	N/A	Keratoconus, PSC, asteroid hyalosis, ↓ CV	GEDiGS
281–608	M	Early childhood	c.1615_1624del10(p.E539Qfs*2)	4×10^{-6}	Exon2 dup	N/A	Nystagmus, ON atrophy, ↓ CV	GEDiGS
601–1236	M	Infancy	c.3238+1G>A	0	c.1611+27G>A	0	Nystagmus, scatter hypopigmented spots in retinal periphery	GEDiGS
949–1907	M	Infancy	c.3618-1_3621del5	0	c.1468-263G>C	0	Nystagmus, peripheral atrophy, pigmentary macular changes	GEDiGS
827–1591	M	1.5 yo	c.895_896del2(p.G299Sfs*21)	0	c.2367+23delG	2.3×10^{-4}	Nystagmus, macular atrophy	GEDiES
1797–3128	M	15 yo	c.2302C>T (p.R768*)	2×10^{-5}	Exon19 del	N/A		GEDi
79–194	F	Infancy	c.3793ins4(p.V1265Gfs*19)	4×10^{-6}	c.3793ins4(p.V1265Gfs*19)	4×10^{-6}	Peripheral pigmentary changes, bull's eye macular changes	GEDi
501–336	F	4 months	c.2302C>T(p.R768*)	2×10^{-5}	c.711_711del1(p.P237Pfs*40)	0	Nystagmus	GEDi
690–1378	F	Infancy	c.1084_1087del(p.E362NAfs*12)	0	c.767C>Gp.S256*	0	Nystagmus	GEDi

GEDi is the genetic eye disease (GEDi) diagnostic test[17]
CV color vision, *del* deletion, *Dup* duplication, *MAF* minor allele frequency, *N/A* not available, *ON* optic nerve, *PSC* posterior subcapsular cataract, *ES* exome sequencing, *GS* genome sequencing, *yo* years old
[a] IDs are presented as Ocular Genomics Institute (OGI) family numbers followed by the individual patient number
[b] *RPGRIP1* PV in each of two alleles, with protein alteration in parentheses
[c] Minor allele frequency based on the Genome Aggregation Database (gnomAD)
[d] All patients exhibited diminished visual field, visual acuity, and electroretinography signals in addition to attenuated vessels and bone spicules on fundoscopy. Additional finding are indicated in the table

Figure 3: noncoding pathogenic variants are listed below the RPGRIP1 transcript model [17]
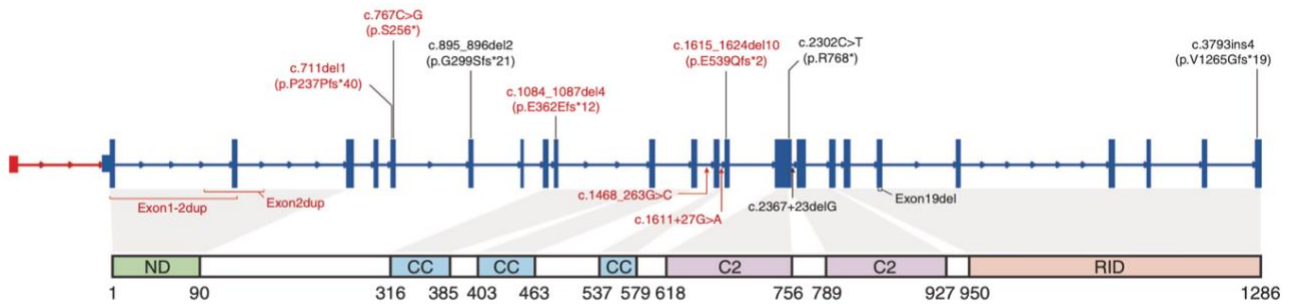
Fig. 4 Summary of *RPGRIP1* PV and gene model. Novel findings are indicated in red with noncoding PV listed below the NM_020366 transcript model and coding PV listed above. The novel exon 1' is shown in red. The RPGRIP1 protein and corresponding domains are shown at the bottom

Genetic Panels Resources
- https://oculargenomics.meei.harvard.edu/services/genetic-diagnostic-testing-service/
- https://www.ncbi.nlm.nih.gov/gtr/all/tests/?term=57096%5Bgeneid%5D

Vision phenotype panels that include RPGRIP1 in screening:
- LCA6
- CORD13
- Intellectual disability
- Inherited Retinal Disorders
- Stargardt Disease & Macular Dystrophies
- Retinitis Pigmentosa
- Autosomal recessive RP
- Hearing and Vision loss
- Nystagmus
- Ciliopathies
- Retinopathy
- Optic Atrophy

Outside of the USA, as of August 7, 2020, at least 9 additional countries have screening capabilities and utilize panels that include RPGRIP1. The following countries have registered RPGRIP1 containing genetic panels in the Genetic Testing Registry:

- Australia
- Canada
- Denmark
- Estonia
- Finland
- Germany
- Portugal
- Spain
- Turkey

This list can help identify potential treatment centers outside of the USA and aid in regulatory planning.

Diagnostic obstacles

Identifying diagnostic obstacles is important for understanding potential bottlenecks to clinical trial enrollment. Anecdotally, it can take a significant amount of time to get the correct diagnosis for rare IRDs. RPGRIP1 patients are likely to face significant delays in diagnosis unless

they are initially seen by a specialist who has experience with RPGRIP1-mediated diseases. Access to genetic testing and correct diagnosis are some of the largest obstacles patients and families encounter. Understanding the average time to diagnosis will impact clinical planning by establishing timelines from diagnosis to dosing of the patient. The later patients are correctly diagnosed and identified, the larger the impact on visual function loss and the fewer intact photoreceptors there will be at the time of dosing. Patient groups and clinicians should be consulted to determine the diagnostic timeline: average time from symptom onset and first clinical visit to genetic confirmation of RPGRIP1 mutations. **Mobilizing direct-to-patient genetic screening options and increasing accessibility of genetic testing for referring physicians will decrease the time to diagnosis and ultimately increase the number of diagnoses.**

Clinical population symptom heterogeneity

Although many patients fall into the classic LCA phenotype—early and severe visual loss followed by relative non-progression—there is significant heterogeneity within the patient population in symptom onset and progression. Within just the LCA6 diagnosis there are extremes in severity: some patients present with completely extinguished ERG and no light perception from the first exam, while some patients retain some visual function into adolescence and even adulthood. Careful consideration should be given to the involvement of all three diagnoses. KOLs should be consulted on the overlap in symptoms and associated pathologies across LCA6, CORD13, RP within different age groups.

Progression

Rate of decline

As of August 7th, 2020 there are no formal natural history studies specifically for RPGRIP1 patients, but disease progression has been discussed in the context of broader LCA studies. Although outliers do exist, the general disease progressions for patients with RPGRIP1 mutations follow a similar pattern. For most of the clinical reports there is a sudden and severe drop in visual function followed by a mostly non-progressive stage. For LCA6, this decline generally occurs before the child is 1 year old. For CORD13 and juvenile or early onset RP, this decline can range from childhood to early/mid adolescence. CORD13 specifically can have a sharp decrease in visual function around 14-16 years old.

Multiple studies report the observation of preserved central photoreceptors in late adolescents and adults with RPGRIP1 mutations, despite the rapid and severe visual decline documented in childhood [3, 10]. This would enable treatment to be delivered through early adulthood or even later if central photoreceptors are present as confirmed by OCT. Photoreceptor retention in the central retina has also anecdotally been confirmed by clinical investigators through personal conversations.

One publication described the retinal architecture of a 19 year old male patient diagnosed with LCA6. The authors used OCT to show that the patient retained normal retinal thickness near the central retina/fovea even with a non-detectable ERG response at 19 years old. There were, however, signs of retinal thinning near the parafoveal region and the peripheral retina [10]. These data suggest a wide therapeutic window that extends into early adulthood, although it is largely believed that early intervention would provide the most meaningful changes to patients. One study investigated retinal structure in the early stages of LCA6 and reported that ONL thickness was well preserved, evaluated by OCT, in patients during early childhood, and that treatment during early childhood would provide the best outcomes for patients with RPGRIP1-mediated LCA [3].

Disease progression for CORD13 and RPGRIP1-mediated RP are not as well defined as LCA6. For CORD13 there is visual loss in late childhood and early adolescence. This normally begins with a loss of color vision followed by night blindness and an eventual rapid loss of visual function around 14-16 years of age [14]. RP progression is similar to CORD13, although RP normally starts with night blindness and visual field constriction in the second decade of life; however, there are clinical reports of presentation in earlier childhood (~1.5years old) for early-onset RP [15]. Longitudinal studies utilizing OCT to characterize anatomical changes in CORD13 and RP are lacking.

Characteristics of decline

Characteristics of decline include further reduction in measurable ERG (if not absent at first exam), restricted peripheral vision, reduction in visual acuity, further vascular attenuation of retina, increased retinal pigment deposits. These all vary among patients and even between eyes of the same patient. In LCA6, cone ERG is normally extinguished early in disease progression (usually before the age of 1 year) while rod ERG is significantly reduced. For CORD13 and RP the age of decline points can vary; however, there is evidence that CORD13 primary decline begins in early adolescence between 14 and 16 years old. For each disease, the quick and severe decline in visual function is normally followed by a more stable, non-progressive disease course where degeneration is much slower.

Therapeutic Window

There are two papers which discuss the potential window for a therapeutic. Both of those papers were described in the previous section. The paper describing the 19 year old with retained central photoreceptors suggests that if the central retina was targeted, there is potentially a larger window for administration of gene therapy. Another study examined retinal architecture using OCT in "early-stage" LCA caused by RPGRIP1 mutations and suggested the best window for therapy would be in early childhood, ideally before 5 years of age because of retained ONL thickness during that stage of the disease [3].

Considerations for therapeutic window (discussed in trial design):
- Safety for pediatric population

- Retention of photoreceptors in the central retina potentially into adulthood
- Efficacy of treatment if already in a "degeneration" portion of the disease progression—after dysfunction but before degeneration
    - RPE65 studies showed necessity of early intervention for prolonged recovery and stabilization in addition to a benefit of intervention early in younger patients with better visual acuity [18, 19].
- Regulatory concerns
- Eye surface volume in younger patients and how this relates to dosing and injection volume
- Differences in onset of various clinical diagnoses

Population and Genetics

Incidence & Prevalence

Prevalence of a rare disease can be hard to determine precisely, and there are likely more cases than the estimates provided here. Early discussions with clinicians are suggested to gain a better understanding of prevalence. LCA is estimated to affect approximately 1 in 33,000-81,000 people in the United States. Most reports of RPGRIP1-LCA6 estimate the United States prevalence at about 4-6% of total LCA patients. This percentage puts a conservative estimate of patients in the United States with LCA6 at 200-600 and 5 new cases per year [1, 2, 5, 6, 11, 15, 20]. One study found RPGRIP1 mutation prevalence to be much higher, around 13.5%, so variation in this percentage is likely [9]. There are no publications estimating prevalence of CORD13 or RP diagnoses in the US; however, some extrapolations can be made from prevalence reports elsewhere. China's prevalence of RPGRIP1-mediated RP is estimated to be ~2% of all RP cases, which would be a significant number of individuals if extrapolated to the United States (2000) [13]. CORD13 is likely to be lower than LCA6 prevalence, as cone-rod dystrophy is estimated to affect fewer people in the United States than LCA6.

Prevalence outside the United states can vary greatly. A worldwide estimate of individuals with RPGRIP1 mutations is likely to be around 20,747 [21]. A clinical report from Spain showed RPGRIP1 mutations were the second most prevalent disease- causing mutation for LCA, after CRB1. They found relative frequencies of mutated RPGRIP1 alleles to be high-- in LCA (20%), early onset RP (15%), and non-early onset RP (15%) [4] (Figure 5). This however was not the same frequency seen in other countries or studies, and likely represents an example of regional incidence differences.

Figure 5: Frequency of RPGRIP1 mutations in LCA and RP are high in a Spanish cohort examined [4]
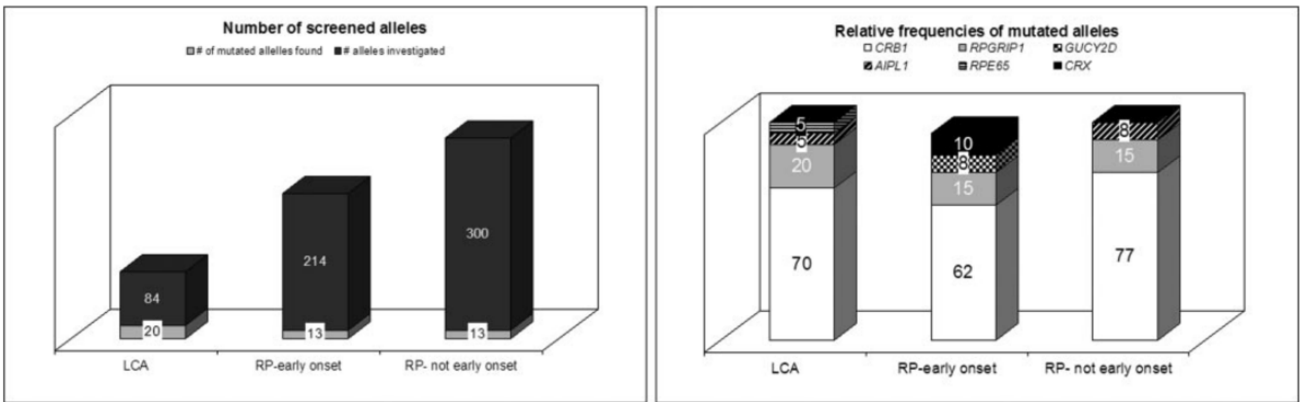
FIGURE 1. *Left*: 84, 214, and 300 alleles were screened in patients with diagnoses of LCA, early-onset RP, and non–early-onset RP, respectively. Within these groups 20 (23.8%), 13 (6.1%), and 13 (4.3%) alleles with disease-causing sequence changes were detected, respectively. *Right*: percentages of alleles with detected sequence changes found in the different clinical groups. *CRB1* is the gene with the most frequent mutations in all three patient groups, followed by *RPGRIP1*. Changes in *RPE65* were found only in the LCA group and changes in *CRX* only in the early-onset RP group.

RPGRIP1 mutation carriers were also examined. Most carriers had 20/20 vision in better eye, corrected with few ERG abnormalities. Some of the carriers also had drusen-like deposits; however, carrier vision was considered normal [22].

Ethnic or geographical enrichment

Patients with RPGRIP1 mutations have been described in many countries, **Appendix Table 1A-C** summarizes published mutation information including the country where the study was conducted.

Common mutations

Nonsense/frameshift, missense, deletions (both large and small), and insertions have all been described for RPGRIP1. There are no reports of significant founder mutations in RPGRIP1 that are concentrated in the LCA6 community. There are many different mutations in RPGRIP1 that cause the three diseases (**Appendix Table 1A-C**).

One of the more prevalent variants, found in multiple studies from multiple countries, is c.3341A>G (p.Asp1114Gly) [2, 4, 6, 9, 11, 15]. Vallespin, 2007 noted that in the population they described (from Spain), for both LCA and RP groups, this was observed at a similar frequency in the control subjects and therefore was likely a benign polymorphism [4].

Mutation characterization & genotype- phenotype correlations

A large majority of published mutations are nonsense or frameshift, leading to a premature termination codon (PTC). These nonsense mutations are considered null mutations because of the likely mRNA degradation through nonsense-mediated decay. Missense mutations can lead to partial protein products and are not considered true null mutations. These partial proteins may retain partial function and are described as hypomorphic mutations. The differences in these two type of mutations is thought to be the underlying reason for differences in severity between LCA phenotypes and CORD phenotypes, where LCA is caused by PTC mutations and

CORD is caused by missense mutations which retain residual RPGRIP1 function [8, 14, 23, 24]. This is the only genotype-phenotype association that has been discussed by multiple groups.

Gene Therapy for RPGRIP1

RPGRIP1 Gene Therapy sequence variant

The RPGRIP1 gene therapy under development at PTC incorporates the benign rs3748361 sequence variant that occurs at a minor allele frequency (MAF) of 0.3260 (range from 0.06131-0.4842) in the general population (Figure 6). The rs3748361 polymorphism results in an amino acid change from a glutamic acid at position 1033 to a glutamine. This variant is documented in multiple databases (Clinvar, dpSNP, gnomAD, [25]) and studies and has no associated clinical consequence.

Figure 6: Allele frequency of rs3748361 across populations studies.

Population Frequencies ❷

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency ▲
▸ African	1482	24172	43	0.06131
▸ Latino	7013	35292	706	0.1987
▸ Ashkenazi Jewish	2576	10332	364	0.2493
▸ Other	2426	7108	441	0.3413
▸ European (non-Finnish)	45173	128016	7982	0.3529
▸ European (Finnish)	9741	24926	1930	0.3908
▸ East Asian	8043	19478	1698	0.4129
▸ South Asian	14794	30556	3651	0.4842
Female	39819	127530	7180	0.3122
Male	51429	152350	9635	0.3376
Total	**91248**	**279880**	**16815**	**0.3260**

RPGRIP1 expression in the human retina

Photoreceptors

Rod and cone photoreceptors are the most relevant cell types for RPGRIP1 gene therapy. RPGRIP1 localization, specifically within photoreceptors, is species specific. In the human retina RPGRIP1 was found to localize to 4 distinct regions: 1) photoreceptor outer segments, 2) amacrine cells of the inner retina, 3) cytoskeleton of photoreceptors, and 4) decorating microtubules [8]. Within the photoreceptor cells, hRPGRIP1 colocalizes with RPGR in restricted

foci in the outer segments [26]. RPGRIP1 was also found to encode several different isoforms that have distinct cellular, subcellular, and biochemical properties. The significance of amacrine cell expression in diseases is unknown and discussed briefly below.

Amacrine cells

There are two studies describing RPGRIP1 expression in amacrine cells of the retina [26, 27]. Data on amacrine cell expression in the human retina shows that the low-weight isoform of RPGRIP1 is found in the amacrine cells of the central retina [27]. It is hypothesized in that publication that amacrine cell expression might explain the extinguished ERG found in LCA6 patients, even when photoreceptors are still maintained and have not yet degenerated.

Considerations for treating pediatric and infantile populations

Ocular volume and surface area

When treating infants or young children it is important to consider the dose of vector per eye. Due to the continuing development of the eye, ocular volume and surface area are reduced in infants. Neonates have 1/6th the volume of the adult eye and 1/3rd by 1 years old. Subretinal surface area is the most relevant consideration for RPGRIP1. That being said, Luxturna used the same volume and dose for children over the age of 12 months old as was used in adult populations, 300 ul at 1.5e11 vg/eye. This may need to be evaluated separately for the RPGRIP1 program.

Retinal Cell Proliferation in infants

While the LCA6 population presents with visual symptoms early in life, often before the age of 1 years old, infantile subretinal injections of a gene therapy is not recommended. Cell proliferation is active in the developing retina and continues until 8-12 months of age, therefore children administered a gene therapy during continued cell proliferation may see initial effects that are diluted over time. For this reason, Luxturna is not recommended in children under 12 months due to concerns of dilution. If a secondary administration is feasible then treating children as young as possible to prevent cellular loss followed by a secondary injection after cell proliferation has ceased could be considered. Developmental specialists and gene therapy researchers working on follow-up administration of a gene therapy should be consulted. This is not an advised strategy for the clinical trial but should be considered after the gene therapy is approved or at least proven safe in younger populations.

Subretinal Delivery

Subretinal injection route of administration is the best method for targeting photoreceptors. This ROA has also been shown to reduce the immune response within the eye compared to intravitreal injection. In RPGRIP1 patients, **photoreceptors within the central retina (surrounding the fovea) are thought survive longer than those of the peripheral retina. For this reason, it is vital to target the central retina with the drug, as this will provide the best opportunity for recovery and/or stabilization** [10, 24]**.**

"Photoreceptors in the central retina appear to persist for long periods of time after visual function becomes immeasurable (Jacobson et al. 2007). Thus LCA6 patients with underlying RPGRIP1 mutations have treatment potential for a gene replacement strategy if targeted to central, but not peripheral, retina."[24]

Subretinal delivery has become the most used ROA because of its vector containment, success in Luxturna, and ability to reduce dose. *Surgical Techniques for Retinal Gene Therapy Delivery* reviews some of the delivery techniques, advantages and disadvantages of each [28]. One consideration for surgical technique is whether or not to create a pre-bleb with a salt solution prior to injection of the drug. The Luxturna administration guide does not include the use of a pre-bleb but other therapies, including a gene therapy choroideremia, have used the pre-bleb technique to increase targeting of retinal cells, prevent the loss of vector material during microdissection of the retina, and increase assurance of correct bleb location.

> *"More recently, individual surgeons and investigators in preclinical trials[13-15] have started raising a subretinal pre-bleb with BSS prior to injection of drug for several reasons. First, the pre-bleb initiates hydrodissection into the subretinal space without wasting any valuable vector and predissection of the correct target treatment location. However, the single-bleb delivery method is also frequently utilized[1, 16] and also has its own advantages (Figure 1). Surgeons utilizing this method believe that the BSS pre-bleb can dilute the subretinal drug content and overstretch the retina, often then requiring multiple dilute subretinal blebs. Additionally, reinjection through the same retinotomy poses a surgical challenge not only in physically locating the retinotomy site (with or without the assistance of intraoperative OCT), but also in ensuring that the retinotomy site is not enlarged upon re-entry or a second adjacent retinotomy is not accidentally made. If this occurs, gene vector may be lost by egress through an enlarged retinotomy wound when injecting."*[28]

Luxturna's recommended area of injection in the final operator's manual is a single bleb with 300uL along the superior vascular arcade, avoiding vascular structures and areas of dense atrophy, at least 2mm away from foveal center [29]. Distinct area for subretinal bleb will need to be discussed with clinicians after retinal structure is assessed. If the retina is thin in the area of injection it is recommended to administer the subretinal injection in multiple small portions to allow the fluid to reabsorb and to reduce the risk of rupture [30–32].

> *"In addition, careful observation of the fovea with real- time intraoperative OCT during the injection of BSS and the vector helped avoid excessive stretching of the foveal tissue and reduced the risk of macular hole. Stretching was also avoided by delivering the subretinal injections in multiple small portions, allowing the subretinal fluid to reabsorb slightly between applications."*[31]

Reviews on best practices and learnings from other clinical trials for subretinal surgery technique are described in detail [30, 33, 34].

It is important, when doing subretinal injections, to assess vector shedding and transgene expression to understand biodistribution. Figure 7 shows data from Luxturna's phase 3 trial.

**Data from the Luxturna Phase 3 trial shows that only 48% of subjects had detectable vector DNA in any of the samples collected (tears serum, whole blood) seen in figure below

Figure 7: Luxturna Phase 3 vector shedding and biodistribution data [39]

Table 8. Summary of Phase 3 Vector Shedding and Biodistribution Data

Category	Total N = 29
Subjects with Any Positive Samples	14 (48%)
Subjects with Only Positive Tear Samples	11 (38%)
Subjects with Only Positive Serum Samples	1 (3%)
Subjects with Both Positive Tear and Serum Samples	2 (7%)

Note: No whole blood samples were positive for AAV2-hRPE65v2 vector DNA.
Source: Module 2.5.5.5.2: Clinical Overview. Study 301 CSR and Study 301 CSR Addendum 2016

Takeaways and important considerations for subretinal injection location: The retina is already thin in most IRD patients, so care will need to be taken in choosing the right delivery area. The fovea is the least likely to detach during delivery but also the most likely to rupture from excessive stretching (thinner than surrounding retinal areas). Consider delivering the injection in one bolus to the macular region adjacent to the fovea or consider splitting the volume and dose into two injections covering a larger region. This would allow for a larger region of coverage; however, necessity of this will need to be determined by region of retina that still contains viable photoreceptors. It is possible that because of the rate of degeneration, multiple injection sites would not be necessary. Two site/bleb injections for delivery of vector creates more regions for backflow so care will need to be taken to ensure the right technique is decided upon for the patients. Multiple salt solution blebs may be considered for creating a larger target zone for vector material, and has been discussed before [30]; however, this is not always recommended and is likely retina specific (consider retinal thickness, integrity, target zone, and readiness of detachment). It is recommended that the exact surgical technique is discussed with the surgical clinicians and clinical KOLs who will be involved with the trial and the area and technique should be mirrored in the Pharmacology-toxicology studies.

Unilateral or bilateral delivery for initial clinical studies: In the phase 3 trial, Luxturna's dosing strategy was one dose in the first eye followed by the contralateral eye no fewer than 6 days post-first eye. A clinical argument can be made that a dramatic difference in vision between the two eyes in children is unnecessary and unethical. In the Luxturna Phase 1 trial, patients waited

a minimum of year to receive the contralateral treatment. The contralateral eye should be treated with the maximal feasible dose so timing will depend upon the timelines for subsequent patient administration and safety review.

Dose and volume

Subretinal gene therapy delivery volumes in humans range from 100 uL in the NightstaRx/Biogen Choroideremia trial up to the highest delivery volume of 450 uL in a gene therapy trial for RP caused by MERTK mutations [35]. The highest used in the Luxturna trials was 300 ul where adverse events were not found to associate with the increased injection volume.

Appendix Table 3 summarizes the volumes and doses used in subretinal gene therapy programs.

Ipsilateral Re-administration

Subretinal injections in the human eye usually cover a quarter to a third of the retinal surface area. While this can have a significant therapeutic impact on visual function, the effects are usually localized to the region around the injection site and the 'bleb.' Researchers are evaluating the safety and efficacy of ipsilateral re-administration approaches to deliver broader therapeutic effects and the ability to increase transgene expression if transgene expression decreases over time. A recent study in NHPs showed that ipsilateral re-administration of the same gene therapy was immunologically well tolerated even in the presence of systemic and local neutralizing antibodies (NAbs) [36]. Changes in the subretinal space were noted in this study for both the initial injection and subsequent injection and included accumulations of suspected inflammatory cells, migrated RPE cells, POS abnormalities, and loss of pigment in the RPE. These observations did not appear to worsen with re-administration and the investigators thought the findings were specific to NHPs, as the observations did not parallel those seen in patients thus far for single injections.

Trial design: Strategic considerations

Clinical Trial Population

For gene therapy, the first-in-human testing will be conducted in patients, rather than healthy volunteers. To this end, many rare disease gene therapy programs incorporate measures of efficacy into their first-in-human trial design. Given what is known about the pathophysiology of RPGRIP1- associated retinal dystrophy and the slow decline in visual function, the most significant challenge to proving an effective therapeutic will be proving an effect within the timeframe of a clinical study, usually 2-3 years. This section outlines critical considerations for framing the clinical study design, which may change based on strategy.

Questions to guide strategy development:
- Is the aim to stabilize function or is there the potential for regain of function?
- Should the FIH focus on a single homogenous population defined by one diagnosis (LCA6, CORD13, etc) or should the population be recruited based on visual function characteristics and retinal photoreceptor disease stage, remaining diagnosis agnostic? What regulatory challenges will this pose later when trying to broaden to the rest of the population?
- Do data exist to support the prediction of a time to therapeutic effect by an intermediate biomarker or measurable outcome? Can these data be generated? What level of risk does this pose to the program overall?
- Is there a subpopulation that has a predictable rate of decline? Will the indication need to be broadened through another trial later?

Discussion points

Consideration of age and measuring efficacy
While mutations in RPGRIP1 are most commonly associated with LCA6, the early age at onset for most patients with LCA6 may present a regulatory and recruitment bottleneck for this program unless the relevant regulatory agency is open to early phase clinical trials in a younger population. Treating a juvenile, adolescent, or early adult population with CORD13, RP, or an older onset of LCA6 would provide an intermediate risk/benefit balance for a first in human study, but the potential for obtaining a measurable effect during the time period of the study is expected to be more limited. It is worth noting that the phase 1 Luxturna trial found the greatest improvement in visual function in children. This group will necessitate more innovative outcome measures dependent upon the degree of visual function, and whether the child can speak or read. If children have some preserved visual function but are not able to read or speak yet, endpoints such as gaze tracking and ERG should be incorporated.

Alternatively, older LCA6 patients with preserved central visual function could be considered for the Phase 1/2 with the hope of pivoting into a Phase 3 if exploratory efficacy readouts yield positive results. Rather than focusing inclusion criteria on a specific diagnosis, inclusion and exclusion criteria should identify adolescent or young-adult patients with RPGRIP1- mediated disease who have preserved central photoreceptors and some preserved visual function. While disease progression in this group may be slow- complicating the measurement of functional stabilization- the regulatory advantage of working with an older group of patients may outweigh the challenges of working with a young pediatric population who present with more severe forms of the disease. Additionally, exploratory outcome measures should focus on identifying a regain of central visual function in these individuals which would provide strategic value for subsequent trial phases.

Subpopulation approach

A study on CORD13 patients suggest that there is often a steep and predictable decline in visual function between the ages of 14 and 16 which may be a good age range to target for trial recruitment initially in a Phase 1/2 design that assesses safety, tolerability, and looks at the potential to stabilize function [14].

Prednisolone Protocols

We would consider use of a prednisolone pretreatment protocol consistent with Luxturna and recent Choroideremia gene therapy trials to prevent reactions to limit the immune reaction to the administration of the gene therapy . Additionally, steroid treatment may be used after subretinal injection in a similar high dose then tapered off.

- Choroideremia: 10-day oral course of prednisolone, starting 2 days before surgery at 1 mg/kg (70–100 mg) for 7 days and then reduced to 40 mg for 1 day, 20 mg for 1 day, and 10 mg for 1 day

- Luxturna: starting 3 days before first injection 1mg/kg per day of prednisone orally for 7 days with a max dose of 40 mg/day regardless of weight. Prednisone tapered until 3 days before injection of second eye, when the steroid regimen was repeated.

Design consideration

Current ocular study design considerations should take advantage of the presence of an untreated contralateral eye in study design development. Contralateral treatments in ongoing gene therapy programs have shown that even in the presence of NAbs, administration in the contralateral eye is both well tolerated and efficacious [36–38]. An FIH study focused on an age group with slower progression could expect to effectively test safety of the product in one eye of each patient with a two or three dose escalation design and then move into the contralateral eye a year or two later at the maximally tolerated dose. Additionally, statistical analyses for efficacy should assess changes in decline between both eyes during the period of the first treatment and then with contralateral treatment. This approach should incorporate a lead-in period whenever possible to establish the rate of decline in each eye, as progression rates may differ.

Bilateral treatment in patients should be considered within a Phase 3 trial and at younger age groups were the potential for stabilization or rescue would be maximized. After the optimal dose for younger clinical populations is established, without safety concerns, the age at treatment should be as close to symptom presentation as feasible within each disease group. With inherited disorders, early sibling genetic testing provides the opportunity to treat a younger age group than the average clinical population because patients are identified earlier, often before the presentation of symptoms. If siblings of a diagnosed patient are confirmed to have inherited the same disease causing RPGRIP1 mutations and identified prior to known visual loss, OCT and ERG should be monitored closely to identify the earliest signs of dysfunction, at which point treatment should be considered.

Safety endpoints

Safety measures for gene therapy clinical trials are fairly standard across different drug products, with additional focus and refinement dependent upon the route of administration and the targeting organ. A list of recommended safety measures for systemic monitoring is listed below. For subretinal injections, safety measures should monitor for AEs seen in other subretinal injections programs as well as complications that may relate to the disease process, corticosteroid regimen, and the age of administration.

Systemic safety monitoring

- CBC measures
- Liver function
- Kidney function
- Coagulation profile
- Urine analysis
- AAV titers
- Peripheral blood PCR
- ASR measurements
- Ophthalmologic AEs
- Systemic AEs

Measures and monitoring for subretinal or ocular adverse events

- Infection and inflammation
- Visual acuity
- Visual function
- Intraocular pressure
- Ophthalmoscopic exam
- Eye and Retinal health and integrity (i.e. detachment, tear, deposits, epiretinal membrane) as measured by OCT, ophthalmoscopy exam, ERG

Most common Adverse Effects (occurred in more than 5% of treated patients) with Luxturna treatment: Conjunctival hyperemia, cataract, increased ocular pressure, retinal tear, dellen, macular hole, subretinal deposits, eye inflammation, eye irritation, eye pain, maculopathy

Outcome measures

Outcome measures should focus on measuring both function and anatomy in the treated and untreated eye for comparison during the course of the study. Functional measures for RPGRIP1-mediated disease should monitor changes in visual acuity, vision assisted mobility, visual field, and threshold sensitivity. ERG should also be used as an early indicator of function, but changes in ERG need to be corroborated by direct functional readouts. Depending on the visual function in the patient at dosing, outcome measures will need to be optimally paired with the targeted treatment population.

Anatomy and pathology related to the disease should also be monitored closely. The use of imaging techniques such as OCT and ophthalmoscopic exams enables the effect of gene therapy on retinal integrity to be measured. Published preclinical studies on RPGRIP1 gene therapy provide early evidence that a gene therapy should stabilize photoreceptor loss especially in the outer nuclear layer of the central retina.

Importantly, patients should be monitored during a lead-in period before dosing to establish measurable timeframes for expected changes in these endpoints and to aid in discriminating between therapy driven changes, surgical intervention-related AEs, and predicted disease decline.

Outcome measures:
- Visual acuity (i.e. BCVA)
- Vision assisted mobility test (i.e. MLMT)
- Visual field assessment (Goldman Perimetry test)
- Light discomfort
- Threshold sensitivity
- Intraocular pressure
- Ophthalmoscopic exam
- OCT
- ERG

Time to follow-up and safety review

During the FIH study, patients should not be dosed less than four weeks apart to allow for adequate time for evaluation of acute immune effects after dosing. Preclinical data from both mice and NHPs suggest that expression driven from genes delivered by the Anc80 capsid begins earlier than genes delivered via other capsids. This difference is estimated to be at least 4 days earlier with Anc80, although peak expression is roughly the same, occurring around 20-22 days after injection. An independent Safety Board should review all data between cohorts before making a recommendation on proceeding to the next higher dose. A review of all accumulated data across the dose groups should be evaluated before making a determination on a maximally tolerated dose, at which point, patients can be treated in the contralateral eye.

During the short-term follow-up (STFU) period all patients should be followed for two years to evaluate the safety, tolerability, efficacy, and pharmacodynamic activity of the gene therapy. Interim analyses present the opportunity to evaluate efficacy before the end of the two years.

In addition to the STFU, regulators expect a long-term follow-up (LTFU) protocol to establish continued efficacy and durability of the gene therapy as well as the safety of the gene therapy product over extended periods of time. LTFU intervals span five to 15 years, often with yearly data collection. The RPGRIP1 gene therapy program is scheduled to be the first in-human testing of the Anc80 capsid. Under these circumstances, LTFU may need to be longer than previously tested capsids such as AAV2 and AAV9.

Given how young the gene therapy field is at a large it is likely to be another 5-10 years before we fully understand long-term durability and safety of gene therapy products.

Patient Data

Patient data is critical for clinical study planning and evaluation of efficacy of a gene therapy. Clinical understanding of RPGRIP1 mutations has been summarized in a number of single patient case studies, family studies, and screening of patients with related diagnoses. The data summarized in this report provides a glimpse of RPGRIP1-driven disease as heterogenous in its presentation and diagnosis, and incomplete in our understanding of progression.
Filling these gaps and generating trial ready comparator datasets will require investment. Additionally, less formal datasets such as patient group-led registries can aid in trail recruitment, mutational understanding, patient education, and patient engagement. There are relatively cheap and quick options for launching a patient registry nowadays which allows for less formal but impactful data collection ahead of a formal natural history study.

Natural History Study

In October 2021, a paper was published which looked at data from 212 previously reported and 16 new patients with RPGRIP1 mutations [41]. Where available, they looked at family history, best corrected visual acuity (BCVA), refraction, comprehensive ocular examination, optical coherence tomography (OCT) imaging, visual fields (VF), and full-field electroretinography (ffERG). They found that biallelic RPGRIP1 mutations were likely to cause early onset, severe retinal degeneration. Null mutations were found to be more damaging than missense mutations which were more likely to be associated with milder vision loss. However, they also found that most patients, regardless of type of mutation, maintained some BCVA for a long period after diagnosis. While this is a relatively small sampling of the RPGRIP1 community, this study is a good start in understanding the progression of vision loss caused by RPGRIP1 mutations.

Additionally, there are all-comer natural history studies and registries for LCA. While not specific to LCA6 and RPGRIP1, these studies may contain data from LCA6 patients alongside other forms of LCA. The following is a genetic decryption study in France for LCA in a large cohort of families.

- https://clinicaltrials.gov/ct2/show/NCT02970266?cond=Leber+Congenital+Amaurosis&draw=2&rank=1

Registries

Foundation Fighting Blindness (FFB) sponsored "Inherited Retinal Degenerative Disease Registry" called My Retina Tracker, with open and continuous self-enrollment. This registry allows patients to self-report, add medical data, family history, and other diagnosis-related data. Patients are also encouraged to maintain their profiles with updated information on their clinical status as a way of tracking disease histories and progression and to provide a longitudinal clinical data set. Clinicians and genetic counselors can also add data to the registry for specific patients. Through the Research Portal, researchers can request access to de-identified data, which can help in identifying patients for clinical trials.

- https://clinicaltrials.gov/ct2/show/NCT02435940?cond=Leber+Congenital+Amaurosis&draw=2&rank=24

Patient Engagement

RPGRIP1 Patient Groups

Unlike other rare disease communities, there is not a centralized RPGRIP1-specific Foundation that represents the community of patients and their families. As of August 7th, 2020, there is a Facebook-based family group with roughly 30-40 members and there are motivated individuals in the community. Having a centralized, unified patient group can help accelerate clinical trial planning, enrollment, execution, and can impact communications with the FDA. In the absence of an active RPGRIP1 patient group we recommend developing a strategy to help build relationships with this community early to avoid future obstacles. This strategy should include direct and indirect engagement through representatives of the community, social media, and development of educational materials.

LCA and RP communities

The wider LCA and RP communities are well organized and can offer an alternative to engagement with an RPGRIP1-specific community. An advantage to partnering with the LCA and RP groups is their experience with clinical trials and collaborations with other drug development programs. This may streamline the process for priority alignment between PTC and the patients and families, but engagement may also be hindered if outreach doesn't involve RPGRIP1 patients and families directly.

LCA and RP patient groups:
- Foundation Fighting Blindness: "the world's leading private funder of retinal disease research." FFB focuses on funding research on inherited retinal disorders such as forms of RP, LCA, Usher Syndrome, and AMD. FFB has significant experience in partnering with industry, patient groups, and academia and would present a strong compliment to patient engagement and trial design alongside direct interactions with the RPGRIP1 community. My Retina Tracker may also be able to help families get genetic testing if insurance will not provide assistance.
    - https://www.fightingblindness.org/
- Sofia Sees Hope—An LCA group with strong patient engagement
    - https://sofiasees.org/

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Appendix

Clinical Report Synopsis: eye phenotypes

Leber Congenital Amaurosis (LCA6) Only

1. Examination of null RPGRIP1 alleles in a multi-family examination of LCA patients [1]
    - RPGRIP1 prevalence estimated in this examined LCA group: 6%
    - Patients with frameshift or nonsense mutations in RPGRIP1 showed clinical characteristics consistent with LCA
        - *Patient 048-044*: 26yo female; nystagmus and vision limited to light perception from early childhood; at 26 there was moderate vascular attenuation and no bone spicule pigment deposits
        - *Patient 048-079*: poor vision since early childhood; consanguineous family
            - at 15yo—nystagmus, vision limited to light perception; hyperopic, with a spherical equivalent of +2.6 averaged between the two eyes; vascular attenuation an bone spicule pigment deposits in midperipheral retina; non-detectable full field ERG response
        - Patient's 2-year-old affected sister: nystagmus and was hyperopic, with a spherical equivalent of +6.9 averaged between the two eyes: able to follow objects with her eyes only in a well-illuminated environment; fundi were close to normal

2. Clinical phenotypes and *genotype-phenotype associations in LCA* showed severe visual impairment from a very early age in RPGRIP1-mediated LCA (LCA6) [9]
    - 5 patients examined had RPGRIP1 mutation variants—mean age of 7 years old
    - Summary findings for all RPGRIP1 patients:
        - had little to know visual acuity upon first exam with only some with fix and follow or slight light perception;
        - no anterior segment findings;
        - posterior segment findings included 4/5 patients with Drussen-like deposits (first report for LCA6);
        - two siblings (age 1yr and 8yr) examined had grossly normal foveae

Table 1. Clinical Features of 37 LCA Patients With Probable Disease-Causing Mutations

Patient No.	Gene	Sequence Variation	Follow-up Age (y)	Follow-up (y)	Initial Better Eye Visual Acuity	Most Recent Better Eye Visual Acuity	Better Eye Rx	Nyctalopia	Photosensitivity
33	*RPGRIP1*	D1114G; heterozygous	5	4	F/F	20/200	+3.25	N	N
34	*RPGRIP1*	3 bp del 1134; heterozygous*	8	7	No F/F	NLP	+2.50	N	N
35	*RPGRIP1*	D1114G; heterozygous	9	3	F/F	10/225	+8.25	Y	N
36	*RPGRIP1*	R890X; homozygous	8	2	LP	NLP	+10.25	Y	N
37	*RPGRIP1*	R890X; homozygous	3	1	No F/F	LP	+10.00	NA	Y

3. Clinical report of 1 patient with LCA who had well preserved central retina architecture at 19yo [10]
    - OCT evaluation conducted on one patient (male) at 19 years old
        - history of reduced visual acuity, night blindness, and peripheral vision loss since childhood.
        - At 19 years old:

- Fundus showed attenuated retinal vessels, waxy-appearing optic nerve heads, and peripheral pigmentary disturbance without clumps or bone spicule changes.
- Visual field was limited to central island only. Standard full-field ERG showed no detectable response
- OCT showed normal thickness of retina near fovea but thinning near para and perifoveal areas; lamination of retina less distinct beyond central retina

4. Genetic analysis and clinical characteristics of LCA families and individual patient in Australia [5]
   o RPGRIP1 mutations found in 2 pedigrees and 4 individuals—3 nonsense, 1 frameshift/deletion
   o Clinical characteristics obtained through ophthalmologic reports
   o RPGRIP1 specific symptoms: nystagmus (all patients), bilateral flat ERG (all patients); photophobia in 2 individuals from 1 pedigree; 2 individuals with night blindness; 1 person with hypermetropia; pigmentary fundus changes
     - This report says "less severe" visual acuity deficits seen in RPGRIP1 pedigrees-- <6/60 visual acuity
     - Franceschetti's oculo-digital sign not reported for the RPGRIP1 patients in this cohort

5. Evaluation of retinal structure in early-stage RPGRIP1-mediated LCA in families and individuals [3]
   o Evaluates retinal structure of "early stage" LCA6 in 4 individuals in 2 families
   o Important clinical takeaway: photoreceptor ONL on OCT is relatively well preserved in the early stages of LCA
     - "The findings for cases 2 and 3 suggest that the ONL is better retained in early childhood than at 5 years of age"---**gene therapy in early childhood would provide the best outcomes**
   - All patients examined had some ONL thinning:
     - Case 1 (EYE20): 13YO girl; visited hospital with congenital nystagmus and visual impairment at 7yo; fundoscope exam showed retinal degeneration with attenuated vessels, chorioretinal atrophy, and peripheral pigmentation; at 7YO fERG showed a reduced rod response and an extinguished cone response; OCT-thinning photoreceptor ONL (ellipsoid zone extinguished; at 11yo lamellar structure not distinct in perifoveal region, but retained in the fovea; at 12yo goldmann perimetry showed constriction of visual field
     - Cases 2 and 3 (EYE64 and 65): 7 yo homozygotic twin boys diagnosed with LCA with congenital nystagmus and visual impairment at 1yr, 3mo; funduscopic examination- both eyes showed retinal degeneration with slightly attenuated vessels, chorioretinal atrophy, and mild peripheral pigmentation. fERG @1 year and 3 months of age showed a subnormal rod response and an extinguished cone response; OCT showed a thinning photoreceptor ONL with a distinct adjacent lamellar architecture at 1 year and 3 months of age- the ellipsoid zone was extinguished; Goldmann perimetry performed at 7 years of age showed concentric constriction of the visual field
     - Case 4 (EYE55): 17-year-old boy with LCA with congenital nystagmus and visual impairment at 7YO; fundoscope exam showed both eyes were normal; fERG @9YO showed extinguished cone response and subnormal rod response; OCT @

9YO showed thinning of photoreceptor ONL with distinct adjacent lamellar architecture; ellipsoid zone was maintained at 9yo but blurred at 17yo; goldmann perimetry showed low sensitivity within each isopter

Retinitis Pigmentosa (early-onset and juvenile) Only

6. Mutation analysis and clinical symptom report of patients with Juvenile Retinitis pigmentosa—some of which were caused by RPGRIP1 mutations [15]
    - 35 unrelated patients with juvenile RP
    - Two patients shown to have identified heterozygous RPGRIP1 mutations (other allele not reported or not stated if homozygous for same allele). Both diagnosed with juvenile isolated RP
        - Patient 25150: age of onset is **1.5yr**; ERG reduction and visual field reduction; visual acuity of 0.1 at the age of 15 and experienced night blindness and color vision impairment; fundoscopy revealed atrophic macular area and ERG severely reduced
    - Patient 25474: age of onset "<20"; poor ocular evaluations

Cone-rod dystrophy (CORD13) Only

7. First clinical report of mutations in RPGRIP1 leading to Cone-Rod dystrophy (CORD) in family analysis [14]
    - Examined 4 consanguineous Pakistani families: therefore very high prevalence of mutations in RPGRIP1, but likely not representative of population due to consanguinity
        - Total individuals screened and numbers of individuals with RPGRIP1 mutations:
            - Family 1: 20 individuals screened, 8 had RPGRIP1 mutations
            - Family 2: 19 individuals screened, 8 had RPGRIP1 mutations
            - 2 additional families: total of 4 affected individuals
        - Total individuals in that group that have RPGRIP 1 mutations:
    - Clinically: all affected individuals had deterioration in central vision and color blindness from an early age and a rapid decline in vision between 14-16 years old; photophobia since childhood; one patient had a macula bull's eye; patients had varying levels of fundus granularity; rod and cone ERG reduced

Multiple clinical diagnoses addressed in same study

8. Correlating visual acuity of patients with LCA and early childhood onset Retinitis Pigmentosa to underlying mutations [11]
    - Multicentered retrospective study
    - 9/157 patients identified had RPGRIP1 mutations (4.6)
        - 8 LCA patients with RPGRRIP1 mutations
            - Seven (77.8%) of the 9 patients had BCVA of worse than 20/400. One patient could not perceive light. Visual acuity ranged from 20/100 to no light perception, with a median of 2.6 (equivalent to vision of counting fingers). None of the patients in this group had keratoconus. Six of the 9 patients (66.7%) in this group had a hyperopic spherical equivalence of >1D.
        - 1 RP patient with an RPGRIP1 mutation
            - The BCVA for this patient was 20/150 at the age of 30 years
    - Overview: in this study patients with RPGRIP1 LCA were found to have a median VA of counting fingers, with 77.8% of patients having VA worse than 20/200. The one patient with

early childhood-onset retinitis pigmentosa had comparably better VA of 20/150 in the better/seeing eye
- o For patient inclusion in the study: the presence of mutations in one of the LCA genes
    - LCA : age limit set at 1 year—onset of visual disturbance was noted by parents before and up to the age of 1 yr
    - Early childhood-onset RP defined as severe visual symptoms commencing after 1 yr of age until 5yo

9. Mutation and clinical analysis of patients with RP, found 3 probands (2 diagnosed with early-onset RP, one diagnosed with [13]
    - o 99 Chinese patients sequenced with targeted gene capture sequencing
    - o Cites prevalence of RP caused by RPGRIP1 mutations in Chinese population at ~2%
    - o Clinical findings:
        - *P065:* 30 yr old male with early onset RP; bilateral blurred vision in childhood; night blindness by age 23; gradual vision and peripheral field loss; @30 YO waxy disc, attenuated retinal vasculature, and some patchy pigments in one eye's peripheral retina; absent peripheral background florescence; macular arch absent; fERG- no detectable rod response and cone response reduced 99%
        - *P024*: 22YO female diagnosed with RP; reported vision weakness for 20+ years; nystagmus noticed at 8 months old followed by gradual loss of visual acuity and night blindness; in last 6 years visual field had losses of peripheral vision and developed tunnel vision by 21 years old; attenuated retinal vasculature with no pigment changes; ERG had extinguished waveform for rods and cones
        - *P030*: 36 YO female with large deletion of exons 1-22 in RPGRIP1; diagnosed with LCA; born with severe visual impairment and nystagmus without other symptoms— "blind at birth"; at age 36 she could detect hand motion; bone corpuscle pigmentary deposits seen in retinal periphery; attenuated vasculature; no rod ERG response; changes to cone ERG (undetermined what exactly)

10. Larger RP study in Germany found 1 patient with an RPGRIP1 mutation [40]
    - o RPGRIP1 patient clinical information:
        - Diagnosed with non-syndromic retinitis pigmentosa at 13 yrs old; reported nyctalopia, dark adaptation problems and glare since childhood; at 37yo he started using visual aids and underwent cataract surgery—visual acuity now was 20/50 in right eye and 20/63 in left eye; visual fields were constricted; fERG showed no detectable response; widespread RPE and photoreceptor atrophy and bone spicule pigmentations
        - Frameshift mutation predicted to cause unstable mRNA or protein truncation
        - Visual acuity was relatively good in early life and remained stable until after 40 years old

Appendix Table 1a: published RPGRIP1 mutations

Table 1a-c key:

*mutations previously descrived in other studies
Common mutation*undetermined pathogenicity
Mutation found in another gene (CRB1)

Table 1a. RPGRIP1 mutation summary from published case studies

Citation	Clinical Diagnosis	Country	Family/design	Exon-allele 1	Allele 1	predicted change1	Exon-allele 2	Allele 2	predicted change2	notes
Hanein, 2004	RPGRIP1 mutation	Multiple countries (20)	4F	15	c.2237G>A	p.Gly746Glu12	15	c.2237G>A	p.Gly746Glu[12]	consanguineous
Hanein, 2004	RPGRIP1 mutation	Multiple countries (20)	9S	intron 8	c.490+3A>G[12]	Aberrant splicing		?	?	
Hanein, 2004	RPGRIP1 mutation	Multiple countries (20)	46S	12	c.1501_1502insTGTC	p.Leu501fsX508[12]		?	?	
Hanein, 2004	RPGRIP1 mutation	Multiple countries (20)	77S	16	c.2534C>T	p.Arg852X	Intron 16	c.2710+1G>A	Aberrant splicing	
Hanein, 2004	RPGRIP1 mutation	Multiple countries (20)	104F	4	c.510delT	p.Tyr170fsX189[12]	4	c.510delT	p.Tyr170fsX189[12]	consanguineous
Hanein, 2004	RPGRIP1 mutation	Multiple countries (20)	126S	21	c.3341A>G	p.Asp1114Gly	16	c.2668C>T	p.Arg890X	
Hanein, 2004	RPGRIP1 mutation	Multiple countries (20)	160S	12	c.1525C>T	p.Gln509X[12]		?	?	
Hanein, 2004	RPGRIP1 mutation	Multiple countries (20)	167S	16	c.2576_2577insTT	p.Ser858fsX865[12]	23	c.3629_3630insG	p.The1210fsX1214[12]	
Dryja, 2001	LCA-RPGRIP1 mutation	N/A (likely USA)	1	23	Asp1176(1-bp del)	causes PTC	3	Trp65Ter	causes PTC	
Dryja, 2001	LCA-RPGRIP1 mutation	N/A (likely USA)	2	18	Gln893(1- bp ins)	causes PTC	18	Gln893(1- bp ins)	causes PTC	consanguineous
Dryja, 2001	LCA-RPGRIP1 mutation	N/A (likely USA)	2	18	Gln893(1- bp ins)	causes PTC	18	Gln893(1- bp ins)	causes PTC	consanguineous
Dryja, 2001	LCA-RPGRIP1 mutation	N/A (likely USA)	3	10	Lys342(1-bp del)	causes PTC	10	Lys342(1-bp del)	causes PTC	
Gerber, 2001	RPGRIP1 mutation	Moroccan origin	SED1	15	G2237A	Gly746Glu	15	G2237A	Gly746Glu	consanguineous
Gerber, 2001	RPGRIP1 mutation	Moroccan origin	SED2	15	G2237A	Gly746Glu	15	G2237A	Gly746Glu	consanguineous
Gerber, 2001	RPGRIP1 mutation	Moroccan origin	NIL1	4	nt 511 deletion	PTC 19aa downstream	4	nt 511 deletion	PTC 19aa downstream	consanguineous
Gerber, 2001	RPGRIP1 mutation	Moroccan origin	NIL2	4	nt 511 deletion	PTC 19aa downstream	4	nt 511 deletion	PTC 19aa downstream	consanguineous
Gerber, 2001	RPGRIP1 mutation	FR, ENGL Ger. and Bulgr.	LOB	16	2566+2 insTT	PTC 3aa downstream	23	ins 3629+1G	frameshift, stop codon, 5aa DS	
Gerber, 2001	RPGRIP1 mutation	Belgian origin	NES	16	2668C>T	R890X	21	A3341G	D1114G	
Gerber, 2001	RPGRIP1 mutation	Moroccan origin	MOU	21	A3341G	D1114G	21	A3341G	D1114G	consanguineous
Gerber, 2001	RPGRIP1 mutation	French	GRA	12	C1525T	Q509X				
Gerber, 2001	RPGRIP1 mutation	French	HER	12	1501+4 insTGTC	PTC 8aa DS				
Gerber, 2001	RPGRIP1 mutation	French	GUY	24	del nt 3835 ± 3837	del E1279				
common mutation*undetermined pathogenicity										

Table 1b. RPGRIP1 mutation summary from published case studies cont.

Citation	Clinical Diagnosis	Country	Family/design	Exon-allele 1	Allele 1	predicted change1	Exon-allele 2	Allele 2	predicted change2	notes
Miyamichi, 2019	RPGRIP1 mutation	Japanese-same family	EYE65	22	c.3565_3571delCGAAGGC	p.(R1189Gfs*7)	22	c.3565_3571delCGAAGGC	p.(R1189Gfs*7)	
Miyamichi, 2019	RPGRIP1 mutation	Japanese	EYE55	11	c.1467+1G>T	splice	17	c.2710+374_2895+78del	deletion	
Booij, 2005	isolated retinites pigmentosa	Dutch and/or Belgian	patient 25140		c.1614_1623 del	p.Glu538Glufs2		?		
Booij, 2005	isolated retinites pigmentosa	Dutch and/or Belgian	Patient 25474		c.3341A>G	p.Asp1114Gly		?		
Abouzeid, 2016	LCA (RPGRIP1 mutation)	Egypt	4yo girl		c.420delG	term 30 codons down		c.420delG	termination 30codons down	consanguineous
Hameed, 2003	CRD (RPGRIP1-mediated)	Pakastan	1CRD--8 patie	16	c. 2480G>T	p.Arg827Leu	16	c. 2480G>T	p.Arg827Leu	8 affected individuals-consanguineous
Hameed, 2003	CRD (RPGRIP1-mediated)	Pakastan	4CRD--8 patie	13	c. 1639G>T	p.Ala547Ser	13	c. 1639G>T	p.Ala547Ser	8 affected individuals-consanguineous
Hameed, 2003	CRD (RPGRIP1-mediated)	Pakastan	5CRD--2 patie	13	c. 1639G>T	p.Ala547Ser	13	c. 1639G>T	p.Ala547Ser	2 affected individuals-consanguineous
Hameed, 2003	CRD (RPGRIP1-mediated)	Pakastan	10CRD--2 pati	13	c. 1639G>T	p.Ala547Ser	13	c. 1639G>T	p.Ala547Ser	2 affected individuals-consanguineous
Jacobson, 2007	LCA	USA	N/A-19YO male		c.1211T>A	Val1211Glu		c.1211T>A	Val1211Glu	
Galvin, 2005	LCA	N/A	pt 33		D1114G					
Galvin, 2005	LCA	N/A	pt 34		3 bp del 1134					
Galvin, 2005	LCA	N/A	pt 35		D1114G					
Galvin, 2005	LCA	N/A	pt 36*		R890X			R890X		siblings
Galvin, 2005	LCA	N/A	pt 37*		R890X			R890X		siblings
Walia, 2010	LCA	USA (varied backgrounds)			D1114G			?		
Walia, 2010	LCA	USA (varied backgrounds)			R890X			R890X		
Walia, 2010	LCA	USA (varied backgrounds)			Q483X			?		
Walia, 2010	LCA	USA (varied backgrounds)			R768X			"Splice"		
Walia, 2010	LCA	USA (varied backgrounds)			1107del A			1107del A		
Walia, 2010	LCA	USA (varied backgrounds)			V1211E			V1211E		
Walia, 2010	LCA	USA (varied backgrounds)			E1033Q			E1033Q		
Walia, 2010	LCA	USA (varied backgrounds)			P882S			?		
Walia, 2010	early onset RP	USA (varied backgrounds)								
common mutation*undetermined pathogenicity										

Appendix Table 1c: published RPGRIP1 mutations continued

Table 1c. RPGRIP1 mutation summary from published case studies cont.

Citation	Clinical Diagnosis	Country	Family/design	Exon-allele 1	Allele 1	predicted change1	Exon-allele 2	Allele 2	predicted change2	notes
Khan, 2013*	LCA*CRD	non-US (Pakistan/Saudi Arabia)	3-prev. descr		c.1107delA;	p.(Glu370Asnfs*5)		c.1107delA;	p.(Glu370Asnfs*5)	sibling of #4
Khan, 2013*	LCA*CRD	non-US (Pakistan/Saudi Arabia)	4-prev. descr		c.1107delA;	p.(Glu370Asnfs*5)		c.1107delA;	p.(Glu370Asnfs*5)	sibling of #3
Khan, 2013*	LCA	non-US (Pakistan/Saudi Arabia)	5		c.2662C>T;	p.(Arg888X)		c.2662C>T;	p.(Arg888X)	
Khan, 2013*	LCA*CRD	non-US (Pakistan/Saudi Arabia)	6-prev. descr		c.1107delA;	p.(Glu370Asnfs*5)		c.1107delA;	p.(Glu370Asnfs*5)	
Khan, 2013*	LCA	non-US (Pakistan/Saudi Arabia)	7-prev. descr		c.3330T>A;	p.(Tyr1110*)		c.3330T>A;	p.(Tyr1110*)	
Khan, 2013*	LCA*CRD	non-US (Pakistan/Saudi Arabia)	8-prev. descr		c.1107delA;	p.(Glu370Asnfs*5)		c.1107delA;	p.(Glu370Asnfs*5)	
Khan, 2013*	LCA*CRD	non-US (Pakistan/Saudi Arabia)	9-prev. descr		c.1107delA;	p.(Glu370Asnfs*5)		c.1107delA;	p.(Glu370Asnfs*5)	
Han, 2017	LCA	Korea	8		c.3565_3571delCGAAGGC	p.Arg1189GlyfsTer7		?		
Han, 2017	LCA	Korea	9		c.2079C>G	p.Tyr693Ter		c.2209_2215+18del		
Huang, 2017	early onset-RP	China	P065	intron 11	c.1468-2A>G	Exon12 del	intron 11	c.1468-2A>G	Exon12 del	
Huang, 2017	RP	China	P024	2	c.154C>T	p.Arg52*	14	c.2020C>T	p.Pro674Ser	previously reported; null
Huang, 2017	LCA	China	P030	Exon1-22	exon 1-22 deletion		Exon1-22	exon 1-22 deletion		
Birtel, 2018	RP	Germany	pt.39	19	c.3100_3238del139	p.Gln1034Thrfs*23	19	c.3100_3238del139	p.Gln1034Thrfs*23	
Vallespin, 2007	LCA	Spain	LCA-0002	21	c.3341A>G	p.Asp1114Gly				disease causing role not proven
Vallespin, 2007	LCA	Spain	LCA-0034	21	c.3341A>G	p.Asp1114Gly				
Vallespin, 2007	LCA	Spain	LCA-0037	14	c. 1767G>T	p.Gln589His				
Vallespin, 2007	LCA	Spain	LCA-0039	16	c.2417C>T	p.Thr806Ile				
Vallespin, 2007	LCA	Spain	LCA-0029	16	c.2435G>A	p.Arg812Gln				
Vallespin, 2007	early onset ARRP	Spain	RP-0310	2	CRB1 c.614C>T	p.Ile205Thr	21	RPGRIP1 c.3341A>G	p.Asp1114Gly*	disease causing role not proven
Vallespin, 2007	early onset ARRP	Spain	RP-0137	8	CRB1 c.2714G>A	Arg905Gln	21	RPGRIP1 c.3341A>G	p.Asp1114Gly*	disease causing role not proven
Vallespin, 2007	early onset ARRP	Spain	RP-0841	16	c.2417C>T	p.Thr806Ile				
Vallespin, 2007	early onset ARRP	Spain	RP-0061	21	c.3341A>G	p.Asp1114Gly*				disease causing role not proven
Vallespin, 2007	early onset ARRP	Spain	RP-0726	21	c.3358A>G	p.Ile1120Leu				
Vallespin, 2007	non-early onset ARRP	Spain	RP-0633	14	c.1767G>T	p.Gln589His				
Vallespin, 2007	non-early onset ARRP	Spain	RP-0621	16	c.2555G>A	p.Arg852Gln				
Vallespin, 2007	non-early onset ARRP	Spain	RP-0647	21	c.3341A>G	p.Asp1114Gly*				disease causing role not proven
Vallespin, 2007	non-early onset ARRP	Spain	RP-0807	21	c.3341A>G	p.Asp1114Gly*				disease causing role not proven
				21	c.3341A>G	p.Asp1114Gly*				disease causing role not proven
*mutations also found in other studies										
common mutation*undetermined pathogenicity										

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Appendix Table 2: RPGRIP1 variants with unknown pathogenicity

Table 2. RPGRIP1 variants with unknown pathogenicity or suspected benign variants					
Citation	**Clinical Diagnosis**	**Country**	**non-pathogenic variant**	**Sequence change**	**notes**
Dryja, 2001	nonpathogenic	N/A (likely USA)	Pro96Gln	CCG vs. CAG	
Dryja, 2001	nonpathogenic	N/A (likely USA)	Lys192Glu	AAA vs. GAA	
Dryja, 2001	nonpathogenic	N/A (likely USA)	Ala520Ser	GCT vs. TCT	
Dryja, 2001	uncertain pathogenicity	N/A (likely USA)	Arg86Trp	CGG vs. TGG	
Dryja, 2001	uncertain pathogenicity	N/A (likely USA)	Asp849Gly	GAT vs. GGT	
Dryja, 2001	uncertain pathogenicity	N/A (likely USA)	Gln562His	CAG vs. CAT	
Booij, 2005	polymorphic sequence change	Dutch and/or Belgian	c.302-16_-15insATA	c.302-16_-15insATA	new discovery
Booij, 2005	polymorphic sequence change	Dutch and/or Belgian	c.907-153_-154delGG	c.907-153_-154delGG	new discovery
Booij, 2005	polymorphic sequence change	Dutch and/or Belgian	p.Pro175Pro	c.525A>G	
Booij, 2005	polymorphic sequence change	Dutch and/or Belgian	p.Lys192Glu	c.574A>G	
Booij, 2005	polymorphic sequence change*	Dutch and/or Belgian	p.Ala547Ser	c.1639G>T	*has been described as pathogenic
Booij, 2005	polymorphic sequence change	Dutch and/or Belgian	p.Pro599Pro	c.1797G>A	
Booij, 2005	polymorphic sequence change	Dutch and/or Belgian	p.Ala764Ala	c.2292G>A	
Booij, 2005	polymorphic sequence change	Dutch and/or Belgian	p.Gln1033Glu	c.3097G>C	
Booij, 2005	polymorphic sequence change	Dutch and/or Belgian	p.Asp1182Asp	c.3546C>T	
Hameed, 2003	Polymorphism	Pakastan	Leu427Leu	CTC to CTT	
Hameed, 2003	Polymorphism	Pakastan	G>A sequence change-intron 9		
Hameed, 2003	Polymorphism	Pakastan	9bp deletion in intronic region of exon 13		
Vallespin, 2007	no proof of pathogenic role*	Spain	p.Asp1114Gly (RPGRIP1),		*previosuly described as pathogenic

Appendix Table 3: dose and volumes for gene therapy clinical trials

Trial	Dose	Volume	Age (years)
Luxturna Phase 1	1.5E+10 vg/ eye	150 ul	8+
	4.80E+10	150 ul	8+
	1.50E+11	300 ul	8+
Luxturna Phase 3	1.50E+11	300 ul	3+
Choroideremia Phase 1/2	1.00E+11	100uL	18+
VEGF Phase 1/2	1.00E+10	100ul	55+
	1.00E+11	100ul	55+
LHON (IVT)	5.00E+09	50 ul	<12
	1.00E+10	50 ul	>12
LHON (IVT) Phase 1	1.08E+09	200 ul	15+
	5.81E+09	200 ul	15+
	2.40E+10	200 ul	15+
	1.00E+11	100 ul	15+
MERTK	5.96E+10	150 ul	14-70
	1.79E+11	450 ul	14-70
CNGA3 ph1/2	1x10e10 vgp		18+
	5x10e10 vgp		18+
	1x10e11 vgp		18+
CNGB3 ph1/2	2.0 x 10^11 vg/mL		18+
	4.0 x 10^10 vg/mL		18+
	1.2 x 10^11 vg/m		18+
	3.6 x 10^11 vg/mL		18+
	3.6 x 10^11 vg/mL		6-17 yr
	1.1 x 10^12 vg/mL		18+
	1.1 x 10^12 vg/mL		6-17 yr
	3.2 x 10^12 vg/mL		18+

Clinical Trial Links:

- ***Voretigene neparvovec*, Luxturna—Spark Therapeutics; AAV2-RPE65**
    - Phase 1:
      https://clinicaltrials.gov/ct2/show/NCT00516477?cond=AAV2-hRPE65v2&phase=01&draw=2&rank=2
    - Phase 1 follow-on:
      https://clinicaltrials.gov/ct2/show/NCT01208389?cond=AAV2-hRPE65v2&draw=2&rank=2
    - Phase 3:
      https://clinicaltrials.gov/ct2/show/NCT00999609?cond=Voretigene+neparvovec&draw=2&rank=3

- **Choroideremia—Biogen/NightstaRx; AAV2-REP1**
    - Phase 1/2:
      https://clinicaltrials.gov/ct2/show/NCT01461213?cond=Choroideremia&draw=2&rank=8
    - Phase 2:
      https://clinicaltrials.gov/ct2/show/NCT02553135?cond=Choroideremia&draw=2&rank=1
    - Phase 2: sponsor, NightStaRx/Biogen
      https://clinicaltrials.gov/ct2/show/NCT03507686?term=biogen&cond=Choroideremia&draw=2&rank=2
      Phase 3: sponsor, NightStaRx/Biogen
      https://clinicaltrials.gov/ct2/show/NCT03496012?term=biogen&cond=Choroideremia&draw=2&rank=3

- Long-term follow up study from the previous NightstaRx studies
  https://clinicaltrials.gov/ct2/show/NCT03584165?term=biogen&cond=Choroideremia&draw=2&rank=4
- Spark also has a phase1/2 for AAV2-hCHM
  https://clinicaltrials.gov/ct2/show/NCT02341807?cond=Choroideremia&draw=2&rank=13

- **Editas Medicine/Allergan CEP290 Clinical Trials**
    - Phase1/2:
      https://clinicaltrials.gov/ct2/show/NCT03872479?term=editas&draw=2&rank=1
- **Other LCA clinical trials**
    - Sanofi GUCY2D phase 1/2, unilateral subretinal injection:
      https://clinicaltrials.gov/ct2/show/NCT03920007?cond=Leber+Congenital+Amaurosis&draw=2&rank=3
    - Meira gtx RPE65 phase1/2 single eye subretinal injection of AAV2/5
      https://clinicaltrials.gov/ct2/show/NCT02781480?cond=Leber+Congenital+Amaurosis&draw=2&rank=8

- Phase 1 LCA RPE65 (not Luxturna from what I can see) https://clinicaltrials.gov/ct2/show/NCT00821340?cond=Leber+Congenital+Amaurosis&draw=2&rank=10