Clinical characteristics and management of retinitis pigmentosa

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CLINICAL CHARACTERISTICS AND MANAGEMENT OF RETINITIS PIGMENTOSA

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"When you have eliminated all which is impossible, then whatever remains, however improbable, must be the truth."

Arthur Conan Doyle, *The Case-Book of Sherlock Holmes, 1927*

Voor mijn familie

LIST OF ABBREVIATIONS

AAV	Adeno-associated virus
ADRP	Autosomal dominant retinitis pigmentosa
AON	Antisense oligonucleotide
ARRP	Autosomal recessive retinitis pigmentosa
BCVA	Best-corrected visual acuity
CAI	Carbonic anhydrase inhibitor
CI	Confidence interval
CME	Cystoid macular edema
CNTF	Ciliary neurotrophic factor
CRB1	Crumbs homologue 1
CRD	Cone-rod dystrophy
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CRT	Central retinal thickness
DA	Dark-adapted
dB	Decibel
DHA	Docosahexaenoic acid
ELM	External limiting membrane
EOG	Electro-oculogram
ERM	Epiretinal membrane
ESC	Embryonic stem cell
ETDRS	Early Treatment Diabetic Retinopathy Study
EZ	Ellipsoid zone
FAF	Fundus autofluorescence
FDA	Food and Drug Administration
ffERG	Full-field electroretinography
FST	Full-field stimulus
GFAP	Glial fibrillary acid protein
HDR	Homology directed repair
ICSI	Intracytoplasmic sperm injection
INL	Inner nuclear layer
IOL	Intraocular lens
IPSC	Induced pluripotent stem cells
IRD	Inherited retinal dystrophy
ISCEV	International Society for Clinical Electrophysiology of Vision
IVF	In vitro fertilization
LA	Light-adapted
LCA	Leber congenital amaurosis
LogMAR	Logarithm of the minimal angle of resolution
LRAT	Lecithin retinol acyl transferase

LVRS	Low vision rehabilitation services
MD	Macular dystrophy
MFERG	Multifocal electroretinogram
MP	Microperimetry
MRNA	Messenger RNA
MSC	Mesenchymal stem cell
NAC	N-acetylcysteine
NACA	N-acetylcysteine amide
NGS	Next-generation sequencing
NHEJ	Non-homologous end joining
NIPT	Non-invasive prenatal test
ONL	Outer nuclear layer
ORF15	Open reading frame 15
PACG	Primary angle closure glaucoma
PGT	Preimplantation genetic testing
RD	Retinal detachment
RP	Retinitis pigmentosa
RPC	Retinal progenitor cell
RPE	Retinal pigment epithelium
RPE65	RPE specific protein 65
SD	Standard deviation
SD-OCT	Spectral-domain optical coherence tomography
SE	Standard error
VEGF	Vascular endothelium growth factor
VF	Visual field
WES	Whole-exome sequencing
WGS	Whole-genome sequencing
XLRP	X-linked retinitis pigmentosa

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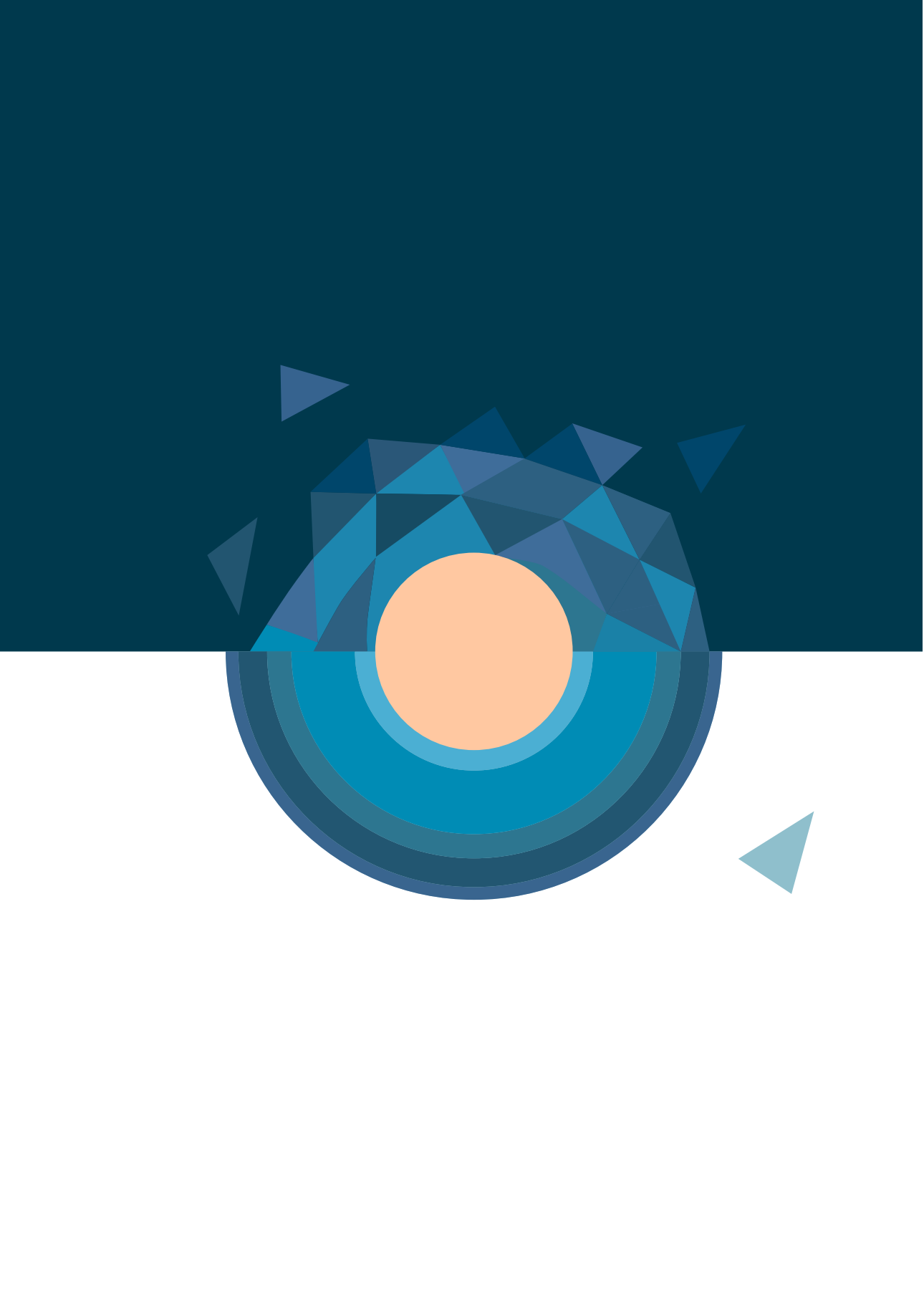

CHAPTER 1

GENERAL INTRODUCTION

Partly adapted from:

Retinitis Pigmentosa: Current Clinical Management and Emerging Therapies

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INTRODUCTION

The eye is a complex organ that is responsible for creating our sense of vision. It consists of several parts that together focus incoming light onto the light-sensitive photoreceptor cells located in the retina. In a sense, the visual process of the eye can be explained by using a camera as an analogy, as the eye and a camera share many similarities in their structure and function (Figure 1).[1]

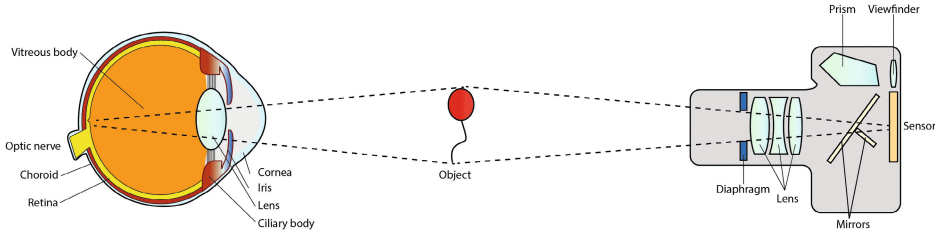


Figure 1. The shared similarities in structures between a human eye and a digital camera. An eye and a camera both have lenses and a light-sensitive region at the posterior segment. Also, an aperture is present in the eye (the pupil created by the iris) and the camera (created by the diaphragm) that control the amount of light that enters both structures.

Vision starts when a particle of light reaches the eye. Light is then refracted by the cornea and the lens, and focused on the retina. Likewise, the lens in a camera focuses incoming light onto a film or digital sensor. This ability to focus light is important, as it allows for clear images to be formed. The pupil of the eye, which is formed by the iris, acts as an aperture to control the amount of light that enters the eye. In a camera, a diaphragm is present that can manipulate the aperture. In low-light conditions, the pupil dilates to allow more light to enter, whereas in a camera, the diaphragm can be adjusted to reach a similar purpose. This way, the eye and camera are able to function under a range of lighting conditions. In the eye, light eventually reaches the retina and the photoreceptors, which converts incoming light into electrical signals which are then transmitted through the optic nerve to the brain. Similarly, in a camera, light is captured on a digital sensor and this information is then processed by the software and hardware of the camera, subsequently converted into digital images.

It is conceivable that any defects in any component of the eye or a camera will impact their functionality. For example, damage to the lens of the eye or camera may cause incorrect focusing of light, resulting in blurry vision or poor image quality. Also, severe damage to the photoreceptors in the eye or the film sensor impedes the transmission of electrical signals and thus visual information, leading to incomplete and unclear images, or no information at all.

The camera and eye share many similarities in terms of the importance of light, the ability to manipulate light, and processing of visual information. They also share

a fundamental purpose: to allow us to view and perceive the world around us. To preserve the quality of visual information in the eye, it is crucial that the components that form the visual pathway remain intact.

The retina

The retina is a complex structure that contains specialized cells that convert light into electrical signals that are then transmitted to the brain via the optic nerve. The retina contains several distinct layers that can be divided between the inner and outer retina (Figure 2).

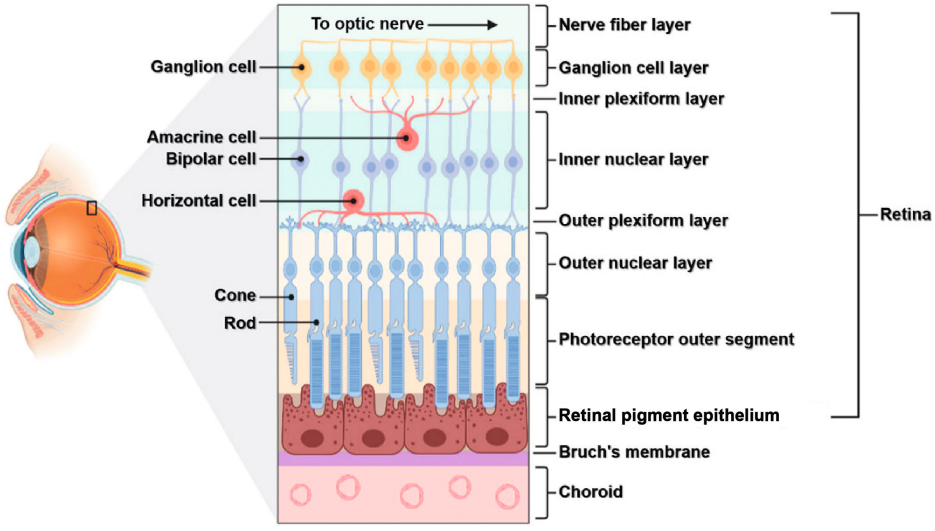


Figure 2. The layers of the retina (adapted from Yang et al., 2021[3]).

The inner retina

The inner retina contains the inner limiting membrane, nerve fiber layer, ganglion cell, inner plexiform and inner nuclear layer.[2] The nerve fiber layer contains the axons of the ganglion cells, whose cells bodies are found in the ganglion cell layer. The ganglion cells synapse with axons of the amacrine and bipolar cells, found in the inner plexiform and inner nuclear layer, and transmit electrical signals to the brain via the optic nerve.

The outer retina

The outer retina contains the outer plexiform layer, the outer nuclear layer, the photoreceptor layer and the retinal pigment epithelium.[2] The outer plexiform and nuclear layers house the inner segment and cell bodies of photoreceptors, and their synapses with bipolar and amacrine cells. There are two main types of photoreceptor cells: rods, which are responsible for contrast viewing and peripheral vision; and cones, which are mainly used for high-acuity detail and color vision. The density of

photoreceptors differs between parts of the retina.[4,5] Rods are scarcely available in the macula and absent at the fovea, and are increasingly available towards the peripheral retina. Conversely, density of cones increases towards the macula and the fovea, and is less so towards the peripheral retina.

The outer segments are primary cilia that contain hundreds stacks of membrane discs, tightly packed together. In these photoreceptor outer segments, particles of light (photons) are captured and transformed into electrical signals using a process known as **phototransduction**.

Phototransduction
In brief, phototransduction is initiated when photons are absorbed by the G-coupled protein opsin found in the outer segments of photoreceptors.[4] Opsin is bound by the light-sensitive chromophore 11-*cis*-retinal, holding opsin in its inactive state. Absorption of light causes the transformation of 11-*cis* retinal to its all-*trans* configuration, and opsin undergoes a conformational change that activates the receptor protein. In turn, the activated opsin stimulates the G-protein transducin, that causes the breakdown of cyclic guanosine monophosphate into its inactive form via the enzyme phosphodiesterase, leading to a decreased concentration of cyclic guanosine monophosphate in the cells.[6] The falling concentration of cyclic guanosine monophosphate leads to the closing ion channels in the photoreceptor cell membrane, resulting in membrane hyperpolarization. The hyperpolarization of the photoreceptor cell causes a decreased release of the neurotransmitter glutamate at the synapse with bipolar cells.[6] Bipolar cells send this graded change in membrane potential to the retinal ganglion cells. The retinal ganglion cells, whose axons become the optic nerve, generate electrical signals that are subsequently sent to the brain. The electrical signals are received and processed by the brain, resulting in the perception of light and formation of images. Following phototransduction, opsins require recharging with 11-*cis* retinal to restore light sensitivity. A crucial part of this recharging process takes place in the retinal pigment epithelium (RPE).

Retinal pigment epithelium
The RPE layer contains a single layer of cells that lies between the photoreceptor layer and the choroid. The main function of the RPE cells is to provide support and nourishment to the photoreceptor layer through several methods such as:

1. The melanin found in RPE cells absorb excess light to prevent it to cause damage to the photoreceptor cells.[7]
2. RPE cells transport nutrients such as glucose and fatty acids from the choroid to the photoreceptor cells.
3. RPE cells move waste products produced by the photoreceptors to the choroid for disposal.

4. RPE cells are responsible for the regeneration of opsin that are depleted during phototransduction via a chemical reaction known as the **visual cycle**.

Visual cycle

The visual cycle is responsible for the regeneration of visual pigment following phototransduction (Figure 3). When the chromophore 11-*cis* retinal is bound to opsin, they are known as visual pigment, as this bonding allows for the detection of photons. By themselves, opsins are not light-sensitive. When activated by a photon, 11-*cis* retinal undergoes photoisomerization to all-*trans* retinal, which induces a change in conformation of opsin, thus starting phototransduction as mentioned previously. The newly formed all-*trans* retinal is not light-sensitive, and opsin must release all-*trans* retinal and bind to new 11-*cis* retinal to continue detecting photons.

In the classical visual cycle, all-*trans* retinal is reduced to all-*trans* retinol in the outer segments of photoreceptors by RDH8[8]. All-*trans* retinol is then transported to the RPE, where it is used to generate all-trans retinyl esters together with lecithin retinol acyl transferase (LRAT). Subsequently, all-*trans* retinyl ester is then isomerized and hydrolyzed *into* 11-*cis*-retinol using RPE65. Retinol dehydrogenase-5 then oxidizes 11-*cis* retinol into 11-*cis* retinal, which is then transported back to the photoreceptors to regenerate visual pigment.

Cones are believed to rely mainly on the classical visual cycle, but are also known to have access to an alternative recycling cycle.[9] Many of the necessary enzymes are found in the cones themselves, making them less dependent of the RPE. Instead of the RPE, this pathway relies on the Müller cells to recycle chromophores and supply it selectively to cones. This alternative, and possibly more rapid, intraretinal supply of chromophores is believed to contribute to the ability of cones to adapt to a dynamic range of bright light, and to adapt to the dark at a faster rate than rods.[9]

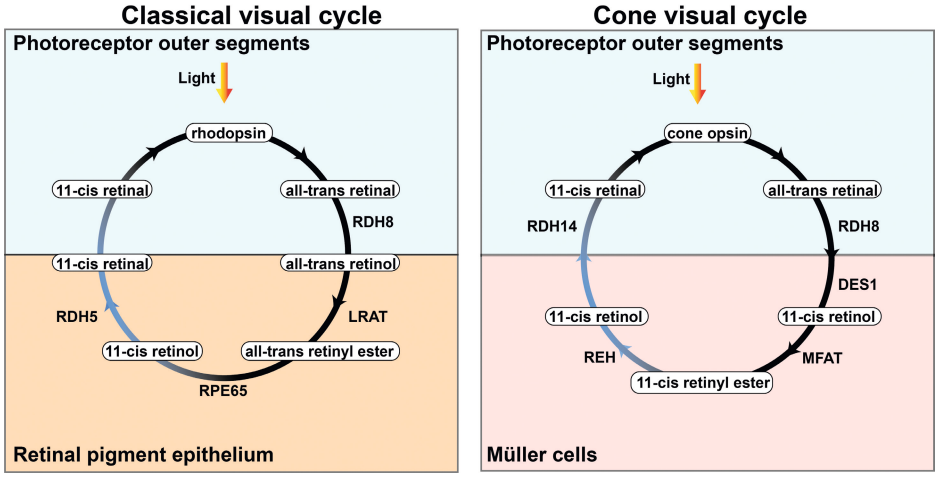


Figure 3. Overview of the classical and cone visual cycle (adapted from Tsin et al., 2018).[8] These visual cycles are needed to regenerate visual pigment, which is needed to detect photons. Cone visual pigment has access to an alternative visual cycle via Müller cells, making them less dependent on the retinal pigment epithelium for regeneration. DES = dihydroceramide desaturase. LRAT = lecithin retinol acyltransferase. MFAT = multifunction O-acyltransferase. RDH = retinol dehydrogenase. REH = retinyl ester hydrolase. RPE65 = retinoid isomerohydrolase.

Overall, the retina is a highly complex structure that contains many steps that are essential for the correct functioning of the visual system as a whole. The importance of each individual step is underscored, as an interruption of these steps has been identified as a source of visual impairment in humans.[8] A common cause of these interruptions are underlying genetic defects, which cause a spectrum of diseases known as inherited retinal dystrophies. The most commonly known variant of inherited retinal dystrophy is **retinitis pigmentosa**, which will be the focus of this thesis.

RETINITIS PIGMENTOSA

Retinitis pigmentosa (RP) is a collective term used to describe a heterogeneous group of inherited retinal dystrophies (IRDs) that are characterized by primary loss of rod photoreceptors, followed by secondary loss of cone photoreceptors.[10] This degenerative process leads to a gradual loss in visual function in affected individuals and may ultimately lead to loss of visual functions in more advanced stages.[10, 11] RP has a variable prevalence of 1 in 750-9000 individuals, depending on the geographic location of the reported study.[10-17] Higher incidences of RP are typically found in regions with high rates of consanguinity and in (semi-)isolated populations.[16, 18] In the Western population, the global prevalence of RP has been estimated to be around 1 in 3000-5000 individuals.[10, 11]

The term 'retinitis pigmentosa' was first introduced by Dutch physician F.C. Donders in 1857, after a few previous reports of possible RP, including potentially the first fundus drawing of a patient with RP (or choroideremia) by Dutch physician Van Trigt, a PhD student of Donders, soon after the introduction of the ophthalmoscope.[19-22] While the term 'retinitis pigmentosa' is considered a misnomer, it is still widely used in clinical and academic settings.[10] The term 'rod—cone dystrophy' is often used interchangeably with RP, as it denotes the order of photoreceptor degeneration occurring in this retinal disease.[23-26]

While RP was previously considered to be untreatable, promising medical advances, particularly the development of genetic therapies, have paved the way for potential therapies that may slow down or halt photoreceptor degeneration, or even restore some degree of visual function.[27] Our improved understanding of the cellular mechanisms and genetic background underlying IRDs, along with the immune-privileged characteristics of the eye, has heralded gene therapy as one of the most promising therapies for RP.[28] Proof-of-concept studies in murine and canine models have shown the potential of gene therapy for *RPE65*-associated retinopathy, which has led to the initiation of human clinical gene therapy trials.[29-33] The positive results in both safety profiles and clinical endpoints in these trials have resulted in the approval of voretigene neparvovec as the first FDA-approved gene-therapy for patients with *RPE65*-associated retinopathy, which is now commercially available as Luxturna®.[29, 34-36] This major milestone has sparked a surge in interest for other IRDs and their candidate genes, and several gene-therapy clinical trials have already commenced and terminated.[28, 36-40]

Despite rapid developments in genomic medicine, some important considerations remain for the implementation of these therapeutic strategies. For instance, while gene therapy holds promise for patients with IRDs, not every patient will be eligible for this treatment. Generally, genetic therapies require identification of the causative

gene and photoreceptor viability in order to be effectively applied.[37] Many IRD patients do not meet both of these criteria for gene therapy. As such, patients require therapy tailored to their genetic condition and disease stage, or a therapy that can be universally applied regardless of the underlying genetic cause.[27, 34, 37] Furthermore, when designing a clinical trial, chosen outcome measures need to be relevant and meaningful for the intended retinal disease and patient.[41] As RP is mostly a progressive, degenerative disease, timely intervention would provide the most benefit. [42]

Additionally, RP is associated with an increased risk of other ocular complications, such as cataract and cystoid macular edema (CME), which may cause additional visual disturbances.[43, 44] The combination of RP with other potentially vision-impairing complications often causes significant visual impairment at an early age, which also impacts a patient's physical and mental health.[45] Currently, several management options for RP exist, ranging from genetic and psychological counseling to the treatment of RP-associated complications. Although these management options are considered supportive, they certainly provide some relief of the physical, mental and social-emotional burden that patients may experience.[46]

We aim to update and familiarize readers with the current tools for the clinical management of RP, as new management modalities have become available over the years. This information can be used by clinicians to provide patients with updated insights into current management options, to weigh their benefits and drawbacks, and in turn, advise patients in the management of their disease.

Pathophysiology of RP

RP is mostly a monogenic disease, in which most disease-associated genetic variants are expressed in photoreceptor or RPE cells, although digenic inheritance has also been described.[10, 47] As each gene has its own function, genetic variants lead to different biochemical changes within the retina. Eventually, these changes result in the degeneration of photoreceptors and RPE cells. To date, more than 90 genes have been linked to RP, and it is likely that this number will increase over the years due to ongoing improvements in diagnostic testing techniques (RetNet, https://sph.uth.edu/RetNet/; accessed on 01 November 2022).[10, 48, 49] RP is a highly heterogeneous disease, both clinically and genetically, and shows considerable overlap with other IRDs. Identical disease-associated genetic variants may manifest in different clinical entities, whereas different variants in different genes may also result in similar phenotypes.[49] An overview of the different causative genes in RP and their overlap with other IRDs is shown in the Figure 4.

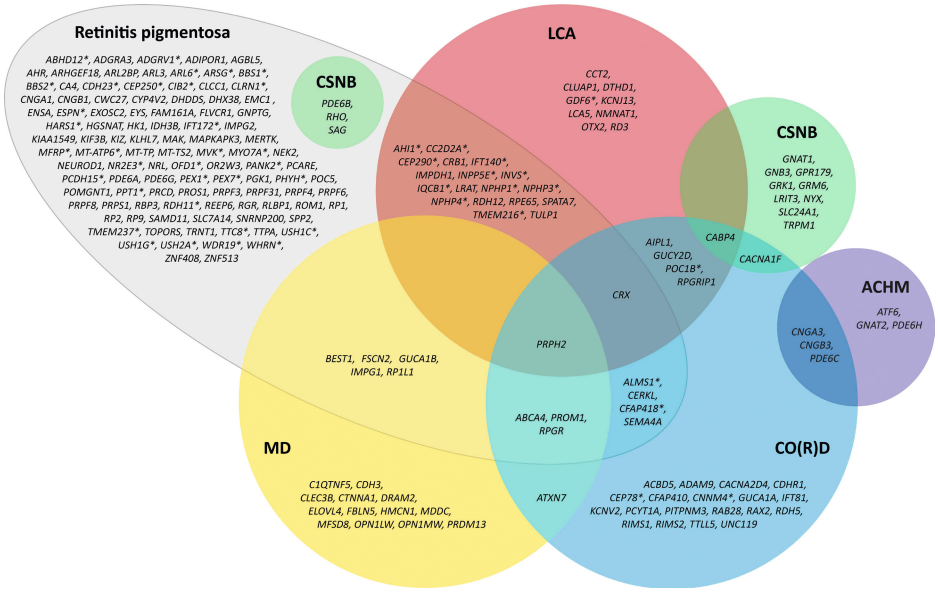


Figure 4. Venn diagram of currently identified genes associated with retinitis pigmentosa (RP) and their genetic overlap with other inherited retinal dystrophies. For example, variants in the *RHO* gene can manifest in either RP or congenital stationary night blindness phenotypes. All genes included are registered in the Online Mendelian Inheritance in Man (OMIM) database and follow the up-to-date symbols of the HUGO Gene Nomenclature Committee (HGNC). Genes that are associated with syndromic forms of RP are marked with an asterisk (*). ACHM = achromatopsia; CO(R)D = cone(-rod) dystrophy; CSNB = congenital stationary night blindness; LCA = Leber Congenital Amaurosis; MD = macular dystrophy.

Classification of RP

Mode of Inheritance

RP comprises a spectrum of retinal phenotypes, some of which may exhibit unique clinical characteristics. Several classification systems have been proposed. The most common method to classify patients with RP is by their Mendelian mode of inheritance. RP can be inherited as autosomal dominant (adRP; 15-25%), autosomal recessive (arRP; 5-20%) or X-linked recessive (XLRP; 5-15%).[10, 50] Other inheritance patterns for RP, albeit very rare, also exist, namely in X-linked dominant, mitochondrial, and digenic forms.[23] Patients with no positive family history or definitive molecular diagnosis are termed isolated or simplex cases. These simplex cases are assumed to be primarily autosomal recessive, although other inheritance forms are also conceivable.[51]

Non-Syndromic and Syndromic forms of RP

RP can also manifest with extra-ocular symptoms, which occurs in 20-30% of all cases.[10, 23] The most common extra-ocular symptom in combination with RP is hearing loss, in the context of Usher syndrome.[52] Patients are classified into 'syndromic RP' or 'non-

syndromic RP' categories, based on the distinction of whether extra-ocular features are present or absent, respectively. Additionally, most patients with syndromic RP can be further classified into either 'inborn errors of metabolism (IEM)' or 'ciliopathies'.[53]

IEM includes a large group of genetic disorders in which the function of a crucial enzyme in one of the metabolic pathways is lost (e.g. carbohydrate, protein, or glycogen storage pathways).[53] IEM has a predilection for the brain, and in turn can also affect the retina as it is part of the central nervous system.[53] Examples include adult Refsum disease (RP, neurodegeneration, ataxia, hearing loss, anosmia, and cardiac/skeletal/skin involvement), Bassen-Kornzweig syndrome (RP, fat malabsorption, acanthocytosis, low blood cholesterol, neurodegeneration) and PHARC syndrome (polyneuropathy, hearing loss, ataxia, RP and cataract).[54-58]

Ciliopathies are a group of disorders that affect the assembly or function of primary cilia. Cilia are microtubular extensions of the plasma membrane and are a component of nearly every cell type. As a consequence, genetic defects in the cilia are typically pleiotropic, affecting more than one system.[53] In the retina, the proximal end of the photoreceptors' outer segments is connected to their inner segments via the connecting cilium. Other organs that are often affected in ciliopathies are the inner ear, kidney, liver, and central nervous system.[53] Known ciliopathies that can manifest with retinal degeneration include Usher syndrome, Joubert syndrome (retinal degeneration, intellectual disability, polydactyly, ataxia), Senior-Loken syndrome (retinal degeneration and nephronophthisis), and Bardet-Biedl syndrome (RP, intellectual disability, polydactyly, obesity, and hypogonadism), among others.[53, 55, 56, 59-61]

Clinical Symptoms

RP involves the primary degeneration of rods, followed by the secondary degeneration of cones.[11] As each photoreceptor type plays a specific role in the establishment of vision, there is a classic order in which the clinical symptoms of RP manifest. Due to the initial loss of rod photoreceptors, which are primarily used for vision in dim light conditions and peripheral visual functions, patients experience difficulty or inability to see in dark or dimly lit environments, which is commonly known as 'night blindness' or nyctalopia.[24] The second symptom found in RP is a progressive loss of peripheral visual fields, although this may be unnoticed in the initial stages of disease due to compensating mechanisms.[62] When the degeneration of photoreceptors further expands, so do the visual field defects. Constriction of visual fields progresses over time, eventually reaching the central part of the visual field. In advanced stages of RP, only a small residual central island of visual field may remain - with or without peripheral remnants - which results in severely constricted vision known clinically as 'tunnel vision' (Figure 5).[63, 64] As a result of visual field loss, one of the major perceived

difficulties in patients with RP is mobility, which requires input from both central and peripheral vision.[65]

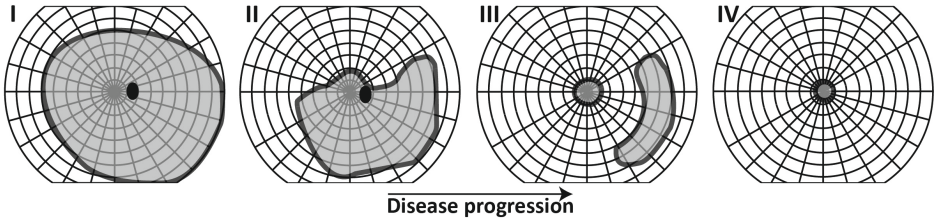


Figure 5. Illustrative example of typical visual field progression in a patient with retinitis pigmentosa using kinetic perimetry. Visual fields can be within normal limits in early stages of disease (I), although visual field defects may already be present but not detectable within the used target stimulus. With time, constriction of the visual fields occurs, with defects typically being symmetric and expanding more rapidly outwards and slower inwards (II,III). Ultimately, a small central remnant of visual field may remain in end-stage retinitis pigmentosa, which is commonly experienced and known as 'tunnel vision' (IV). Note that the clinical course of visual field loss varies between individuals and may follow a progression pattern that is different from this illustration.

Cone photoreceptors, which are densely packed in the macula, are responsible for visual acuity and color vision.[66] Gene variants that target specifically rods but not cones (e.g., disease-associated variants in the *RHO* gene affecting rhodopsin, a rod-specific protein) can still cause death of cone photoreceptors. It remains unclear how cone degeneration in these specific circumstances occurs. Several theoretical concepts have been suggested for the secondary degeneration of cones, including the lack of trophic factors, such as rod-derived cone viability factor, nutrient shortages, oxidative stress and microglial activation, which are induced following rod photoreceptor apoptosis.[66-69] Loss of cone photoreceptors leads to a gradual loss of central vision once sufficient cones in the macula are compromised. This process can ultimately lead to severe visual impairment or even functional blindness based on criteria established by the World Health Organization.[70] Importantly, most patients with RP in advanced stages of their disease will likely retain some degree of residual vision, and total blindness, i.e., no light perception, is uncommon.[71] Previous studies reported that 7-8% of patients with generalized RP end up with a vision of counting fingers or worse in their fourth or fifth decade of life, while less than 1% of RP patients progress to no light perception.[71, 72] In addition to central vision loss, patients may lose color vision, and they may have increased sensitivity to light (i.e., photophobia).[24, 73] Photopsia, i.e., seeing light flashes or static noise when no light enters the eye, is very common in RP, possibly due to reduced afferent nerve impulses or spontaneous signaling from the inner retina.[10, 74]

Disease Onset and Prognosis

The onset, severity and progression of symptoms in RP are highly variable, even in affected individuals from the same family. (Epi)genetic and possibly environmental modifiers are believed to contribute to phenotypic variability, which complicates the establishment of potential genotype-phenotype correlations.[75] It remains difficult to establish a visual prognosis for RP as a group of conditions, although a rough estimate of disease progression can be determined based on the mode of inheritance and the underlying genetic defect, as well as previous information on the clinical course.[76] More severe phenotypes with early-onset disease and the rapid decline in visual function are typically observed in patients with arRP or XLRP, as these variants generally result in loss of function of a crucial protein in the visual pathway.[23, 77-80] High myopia (refractive error of -6 diopters or more) may be associated with a more rapid disease progression, for instance in *RPGR*-associated X-linked RP.[78, 80, 81] In contrast, patients with adRP (e.g., due to *RHO* mutations) mostly demonstrate a relatively mild disease course compared to arRP or XLRP, and they may even retain considerable central and peripheral visual function up until the eighth decade of life.[82-84] The disease course of RP is best understood in the most prevalent genes associated with RP (e.g., *RHO* and *RPGR*) as more extensive retrospective and prospective studies have been performed in these genes; thus, their visual prognosis can be more accurately estimated.[32, 77, 80, 82, 83, 85-87]

Diagnostic Testing in RP

The management of RP starts by establishing the diagnosis through extensive clinical and genetic testing. Early diagnosis of RP enables early prevention and management of complications, disease monitoring and genetic counselling (e.g., family planning). Clinical examination, including the assessment of visual functions, provides relevant information for visual rehabilitation services and helps affected individuals make informed choices about their professional life. Genetic testing is important for visual prognosis, family planning, and for potential inclusion into clinical trials and gene therapy when available. In this chapter, we discuss the principles of clinical and genetic testing methods used for the diagnosis of RP.

Clinical Testing and Evaluation

Clinical evaluation of patients with presumed RP consists of a comprehensive ophthalmic examination that includes best-corrected visual acuity (BCVA), intraocular pressure, slit-lamp, fundus, perimetric, retinal imaging, and electrophysiological evaluation.

Fundus Findings

The classical clinical hallmarks of RP seen in fundus examinations include a pale optic disc, retinal vessel attenuation and intraretinal hyperpigmentation. While intraretinal hyperpigmentation typically has a bone-spicule-like appearance, it may also present

as nummular, salt and pepper-like, or with granular pigmentation. A non-pigmented form of RP also exists ('RP sine pigmenti'), instead of the typical bone-spicule-like hyperpigmentation.[10, 88, 89] These retinal changes typically occur bilaterally and show a high degree of symmetry, although cases of unilateral RP have also been described.[90, 91] Other fundus findings, albeit less common, include optic nerve drusen, CME, epiretinal membrane formation, and Coats-like disease, a (mid)peripheral exudative vasculopathy characterized by telangiectatic vessels, focal serous retinal detachment and lipid exudate deposition.[75] The onset and presentation of the aforementioned fundus findings differ highly between individuals and may even present in atypical forms. Sector RP is considered an atypical, mild form of RP, which is more common in patients with adRP.[78, 82, 92-94] Degeneration in sector RP has a predilection for the inferior nasal hemisphere of the retina with corresponding superior visual field defects.[67] A widespread, generalized disease similar to classic RP may develop with time, although this is not necessarily the case for all patients with sector RP.[82]

Differential Diagnosis

A complete medical history, review of other body systems, and sometimes laboratory testing is necessary to distinguish between RP and other conditions that can masquerade as RP. The list of differential diagnoses in RP is extensive and includes infectious (e.g., syphilis or congenital rubella), drug-induced (e.g., chloroquine or thioridazine), iatrogenic (e.g., laser photocoagulation), metabolic (e.g., gyrate atrophy due to hyperornithinemia) and nutritional etiologies (e.g., vitamin A and zinc deficiencies), as well as a range of non-RP-inherited retinal dystrophies (e.g., choroideremia, congenital stationary night blindness and Oguchi disease).[24, 95] In addition, it is important to rule out several metabolic diseases that may present with fundus findings mimicking RP including abetalipoproteinemia (Bassen-Kornzweig disease), ataxia with vitamin E deficiency, and adult Refsum disease, among others.[60, 96, 97] This distinction from RP is crucial as disease progression in some metabolic diseases can be combated. For instance, in the case of Abetalipoproteinemia and ataxia with vitamin E deficiency, disease progression can be slowed with specific vitamin supplements, while disease progression in adult Refsum disease can be slowed by limiting the intake of food high in phytanic acid.[98, 99] A delayed diagnosis and, consequentially, delayed treatment, may have significant and irreversible consequences for patients with these diseases.[97]

Electrophysiological Testing

Electrophysiological testing plays a major role in the diagnosis and follow-up of RP, as well as the differentiation of RP from other diagnoses. Among all electrophysiological tools, full-field electroretinography (ffERG) is the most common technique used for diagnosing RP, which follows the guidelines established by the International Society for Clinical Electrophysiology of Vision (ISCEV).[100] In brief, the ffERG evaluates the retinal function in response to light stimulus. A dim white single flash in a dark-adapted

eye (i.e., scotopic test conditions) invokes a rod response, whereas a flickering white light (30-Hz) in a light-adapted eye elicits a cone response.[100] When RP becomes detectable in ffERG, i.e. when the retina is sufficiently affected, scotopic responses demonstrate a significant reduction in amplitudes of both a- and b-waves, which are responses mostly derived from photoreceptor and bipolar cells, respectively (Figure 6). Ultimately, both scotopic and photopic responses can be fully extinguished and are non-recordable in end-stage disease.[24] Other diagnostic tools that measure retinal function include multifocal ERG (mfERG), which assesses macular function, and dark adaptometry, which measures the time it takes for photoreceptors to retain maximal sensitivity following photoreceptor bleaching.[101-103] These other electrophysiological testing tools play a smaller role in the initial diagnosis of RP, and are instead sometimes used to complement ffERG/clinical findings and to rule out other potential diagnoses.

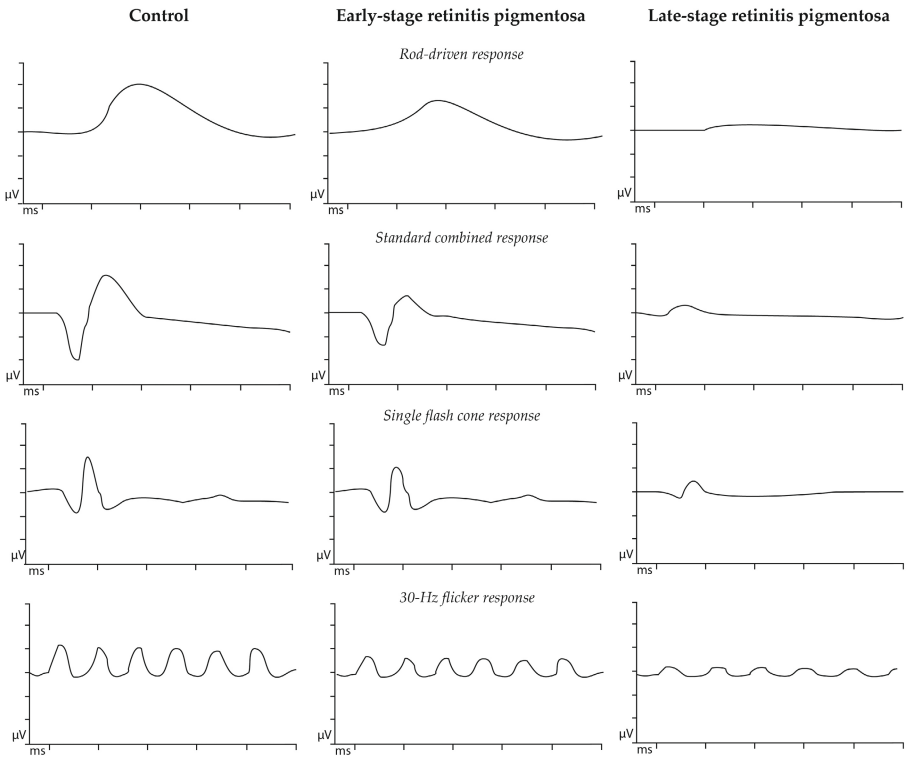


Figure 6. Example full-field electroretinography recordings in a healthy patient and in patients with different disease stages of retinitis pigmentosa. Different stimuli are used to establish the diagnosis of retinitis pigmentosa, which is based on the guidelines of the International Society for Clinical Electrophysiology of Vision (ISCEV). In patients with advanced stages of diseases, rod-driven responses are severely diminished or even absent, whereas residual cone-driven responses may still remain.

Perimetry Testing

As ffERG responses eventually become non-recordable in patients with advanced forms of RP, ffERG is not useful for monitoring disease progression.[104] Instead, kinetic visual fields and multimodal imaging techniques are used to further monitor progression, as these can be utilized even in advanced stages of disease.

Visual field testing is a key in the functional evaluation of RP. When performed in early phases of disease, visual field testing demonstrates progressive, midperipheral visual field loss. With time, a midperipheral ring scotoma develops, which typically expands more rapidly towards the periphery than centrally.[75] Goldmann perimetry is often considered the standard for the detection of visual field progression in RP. In Goldmann perimetry, a light stimulus is presented outwards and is slowly moved inwards by an operator until the stimuli are visibly seen by the patient.[105] This process is then repeated multiple times while using different stimuli, in order to map the extent of a patient's visual field. Limitations of Goldmann kinetic perimetry include significant variability in patients with low vision/unstable fixation, and inter-operator variability.[106-108] While Goldmann kinetic perimetry is still commonly used in clinical settings, it is gradually being replaced by other visual field testing methods, such as computerized (semi-) automated perimetry devices, in clinical practice, research and clinical trials.[104, 109]

Microperimetry (MP) is a semi-automated perimetry device that correlates stimuli presented to the central retina using fundus tracking.[109] The test is performed by having the patient fixate on a central point while different stimuli are presented at various locations on the retina. The patient's ability to perceive the stimulus at each location is recorded and used to create a 'retinal sensitivity map'. This yields a more precise point-by-point correlation and follow-up.[109] MP is often employed in clinical trials for IRDs in combination with traditional outcome measures (i.e., visual acuity and visual fields).[110-112] Recent studies have shown that changes in retinal sensitivity can be detected within relatively short time frames, preceding changes in BCVA.[104, 113, 114] As BCVA is affected in later stages of RP, it is difficult to assess disease progression based on BCVA in short follow-up periods, such as in the context of clinical trials.[112] Therefore, MP can prove beneficial in clinical trials as a complementary outcome measure to detect disease progression and to assess treatment outcome. It is important to note that MP is not a replacement for traditional visual acuity testing, as it is not appropriate for all patients with RP. Measuring disease progression with MP becomes more difficult in patients with poor fixation (e.g., patients with low vision or nystagmus), which in turn causes variability in measurements. Another limitation is that MP only allows for sensitivity mapping of the central retina.

Dark-adapted (DA) static perimetry was developed to measure rod-and-cone function across larger extents of the retina.[115, 116] In contrast to light-adapted perimetry, DA can

be used to discriminate between rod and cone functions by testing each loci with different stimuli.[117] Each testing loci is exposed to a cyan (505 nm) and red (626 nm) stimuli. As rods are less sensitive to red stimuli, a large threshold difference between stimuli indicates rod mediation.[118] DA static perimeters are commercially available but can also be performed on current standard perimeters by modifications.[117]

Full-Field Stimulus Threshold Testing

Another psychophysical tool is the full-field-stimulus threshold (FST), which has become a key outcome measure in gene-therapy trials.[40, 77, 119] The FST was developed as a tool to quantify retinal sensitivity in patients with end-stage IRD as these patients commonly lacked the vision and fixation needed for other outcome measurements tools.[120] In brief, the purpose of the FST is to measure the retinal threshold, which is defined as the stimulus intensity and is seen 50% of the time by a patient. Different stimuli (red, blue and white) yield differentiation between rod, cone or mixed rod-cone responses, and stimuli are typically presented multiple times to account for test-retest reliability. As the FST measures the thresholds of the entire retina, a limitation of this measurement tool is the lack of spatial information. Still, the FST has been able to demonstrate treatment efficacy across multiple gene-therapy trials.[29, 30, 32, 120, 121]

Multimodal Imaging

Multimodal imaging, including widefield fundus imaging, spectral-domain optical coherence tomography (SD-OCT), and fundus autofluorescence (FAF) imaging, is used to visualize the extent of retinal degeneration in patients with RP. Widefield fundus imaging yields for a comprehensive overview of the retina, which can be used to monitor progression in RP. Multiple studies have used structural markers on SD-OCT, such as the central retinal thickness and/or ellipsoid zone (EZ) band width, as another means of tracking disease progression.[122-128] In addition, SD-OCT yields the detection of secondary complications associated with RP, such as the presence of CME and epiretinal membrane. FAF is a non-invasive imaging technique that measures the level of autofluorescent lipofuscin components in the photoreceptors and RPE. A hyperautofluorescent macular ring can typically be observed in earlier disease stages of RP and indicates the transition zones between healthy and degenerating retina, which are often accompanied by progressive thinning of the EZ, external limiting membrane (ELM) and outer nuclear layer (ONL) on SD-OCT (Figure 7).[87] It is important to note that hyperfluorescent rings are not specific to RP and can also be seen in other retinal diseases such as cone-rod dystrophies. Gradual constriction of hyperautofluorescent rings towards the central retina occurs in RP, whereas gradual expansion of the ring is observed in cone-rod dystrophies due to differences in order of photoreceptor degeneration. In advanced stages of RP, when extensive photoreceptor and RPE degeneration has occurred, resulting in the depletion of lipofuscin levels in the retina and RPE, extensive hypo-autofluorescent areas are seen on FAF (Figure 7).

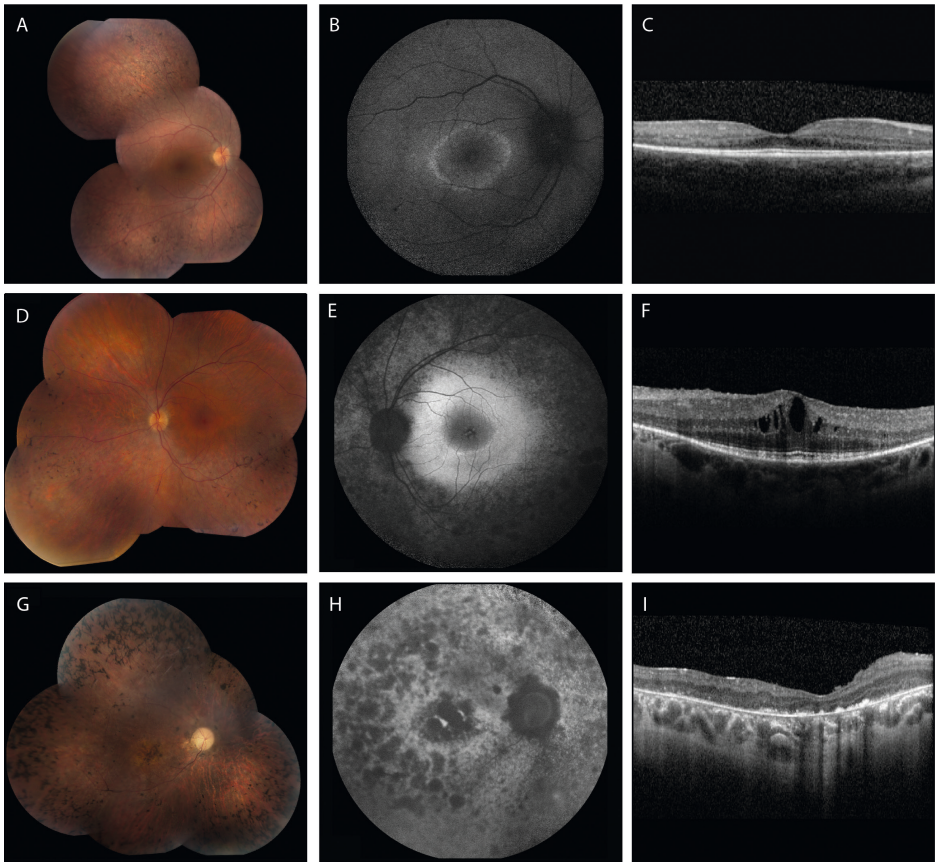


Figure 7. Multimodal imaging in three patients with retinitis pigmentosa (RP). (**A-C**): Multimodal imaging in a patient with RP caused by a variant in the *RHO* gene showing the clinical hallmarks of RP, including attenuated vessels and bone-spicule-like hyperpigmentation in the (mid)peripheral retina (**A**). On autofluorescence imaging, a small hyperfluorescent ring is observed in the macula (**B**). (**C**), Spectral-domain optical coherence imaging shows a relatively intact central retina with loss of the outer retinal layers (i.e. ellipsoid zone and external limiting membrane) outside this area. (**D-F**): Multimodal imaging in a different patient with *RHO*-associated RP reveals hypo-autofluorescent areas in the midperipheral retina and around the vascular arcades, with a broad hyperautofluorescent ring-like region in the macula (**E**). The foveal area shows hypo-autofluorescence some petaloid, likely due to the presence of cystoid macular edema that masks underlying autofluorescence. (**F**), SD-OCT confirms the presence of CME along with the perifoveal loss of the outer retinal layers. (**G-I**): More extensive bone-spicule-like hyperpigmentation is observed in this patient with advanced *RPGR*-associated RP, showing not only hyperpigmentation in the midperipheral retina, but also in the fovea (**G**). Autofluorescence imaging (**H**) shows some residual regions of normal or increased autofluorescence together with regions of mottled hypo-autofluorescence that also include the fovea. As expected, there is clear outer retinal and retinal pigment epithelium loss on optical coherence tomography (**I**).

Genetic Testing

Due to the clinical variability of RP and its phenotypic overlap with other IRDs, a diagnosis based on clinical findings alone is not sufficient. Therefore, genetic testing has become indispensable in the diagnosis and management of RP. With the approval of gene therapy for *RPE65*-associated IRD, and several first-in-human trials on other genetic therapies for a range of IRD-associated genes, it is pivotal to offer genetic testing to patients when available and affordable. Genetic testing allows for the assessment of a patient's potential eligibility for these ongoing and upcoming trials, and facilitates genetic counseling and provides a more accurate clinical prognosis.[129] There are several genetic diagnostic techniques available, and we briefly discuss the advantages and disadvantages of these modalities.

Sanger Sequencing

Sanger sequencing, a first-generation sequencing technique, has been the gold standard for DNA sequencing for several decades and is still considered by many to be the gold standard for single-gene or low-throughput sequencing.[130] Sanger sequencing starts with polymerase chain reaction amplification of the region of interest, followed by targeted sequencing of up to 800 base pairs.[131-133] While Sanger sequencing is fast and cost effective for single genes, it is outperformed by newer techniques when the sequencing of multiple targets is needed.[134]

Next-Generation Sequencing

Next-generation sequencing (NGS), also called second-generation sequencing, is currently the primary approach for molecular analysis in IRDs. NGS distinguishes itself from Sanger sequencing by allowing for parallel sequencing of multiple parts of DNA from multiple samples (i.e., multiplexing). Because large amounts of DNA and RNA snippets can be sequenced in a short time using this method, it is also called high-throughput sequencing.[135, 136] Currently, NGS can genetically solve up to 60–80% of all sequenced RP/IRD patients.[137-140] In the remaining unsolved patients, periodic re-examination of genomic data could prove valuable as new disease-causing variants are discovered and new bioinformatic and data analytical tools are developed over time. Within NGS, three main techniques exist that are used for the identification of genomic variants: targeted gene sequencing, whole-exome sequencing (WES) and whole-genome sequencing (WGS).

Targeted Gene Sequencing

Targeted gene sequencing allows for the sequencing of specific regions that are clinically relevant to the disease of interest. For RP, a custom gene panel is created that sequences all exonic and intronic regions associated with RP and related IRDs.[131] Targeted sequencing is an effective approach for initial screening of RP for several reasons as follows: it allows for greater read depth of targeted regions; regions are predefined and therefore more likely to be clinically relevant; and samples are

screened at reduced costs and computational burden when compared to WES and WGS techniques.[129] Targeted gene sequencing is not useful for the detection of novel genes, as these new regions are not sequenced until they are specifically added to the existing gene panel. If a novel gene is found for RP, previously used gene panels need to be redesigned and revalidated.[129]

Whole- Exome Sequencing

WES exclusively targets protein-coding exons, also known as the exome, which makes up to approximately 1-2% of a patient's entire genome.[129, 141] WES provides coverage of more than 95% of the entire exome, in which 85% of all pathogenic variants are expected to reside.[141] Furthermore, WES can screen intronic variants close to target exons, e.g., splice-site variants.[140] As such, WES is a reliable tool to detect novel, mostly monogenic, variants in patients with genetically unsolved RP. A major limitation of WES is its inability to comprehensively detect structural variants, copy-number variants and chromosomal rearrangements.[140]

Whole-Genome Sequencing

WGS targets the entire genome, which consists of over three billion nucleotides, and thus exceeds the coverage of previously mentioned NGS techniques.[129] This allows WGS to uncover variants not detected using WES, including copy number variants, intergenic variants and deep intronic variants.[129] Despite the better coverage of WGS, there are several drawbacks that should be considered. Due to its wider coverage, WGS generates large clusters of information, more so than any other NGS technique, which includes an increase in secondary, accidental findings.[142] These large datasets obtained from WGS require greater levels of processing and analyzing, not to mention larger amounts of data storage and increased financial costs, compared to other NGS techniques.[139, 140]

Recommendations for Genetic Testing

In summary, considering the sheer number of genes involved in the pathogenesis of RP, NGS is often preferred over conventional Sanger sequencing. Out of all NGS techniques, targeted gene sequencing is typically the primary approach for genetic screening. Using broad, IRD-based gene panels allows for maximum coverage of relevant regions using a single test and provides the best balance between sensitivity, cost efficiency and computational burden compared to other NGS techniques.[140] When the underlying cause remains unresolved following targeted gene panel testing, other higher-targeting sequencing techniques (WES or WGS) can be employed to elucidate the exact genetic basis of the disease. Newer third-generation sequencing techniques also exist, which employ real-time DNA molecular sequencing, and allow for longer reads.[143, 144] However, these methods are still under development and are not commonly used in clinical practice.

Genetic Counseling

Because RP is a heritable disease, genetic counseling plays an important role in the management of RP. The aim of genetic counseling is to advise and inform patients of the physical, psychosocial, and familial implications of genetic findings on RP.[129, 145] Genetic counseling takes place prior to and after genetic testing and can be provided by a subspecialized ophthalmologist, clinical geneticist or by another specialized genetic counselor.[49, 145-148] The organization of genetic counseling services differs between centers and across different countries, depending on the availability of genetic counseling professionals.[129] A recent study in the US demonstrated that most ophthalmologists (and/or optometrists) performed some degree of genetic counseling during patient visits, but these practices were often limited to taking a family history or explaining the inheritance pattern due to time constraints and/or due to limited knowledge in genetics.[149] Therefore, in most cases, patients should be referred to a clinical geneticist or genetic counselor for more comprehensive counseling. While both professions provide genetic counseling, clinical geneticists are physicians subspecializing in genetic testing, counseling and establishing the diagnosis, whereas genetic counselors primarily focus on providing counseling services.[147]

Genetic counseling starts prior to genetic testing (i.e., pre-test counseling), in which patients are informed of the potential importance and implications of genetic testing for their disease, the limitations of genetic testing and potential ethical concerns.[148, 150] Genetic counseling needs to be tailored to the needs and profile of the patient. Genetic counseling involves informing patients of the hereditary nature of their disease, the prognosis and management and the risk of the disease expressing itself in other family members.[151] Obtaining family data is important to determine the causality of newly discovered variants, for example through pedigree mapping, linkage analysis and segregation analysis.[152] Recurrence risks are best estimated if the disease follows Mendelian inheritance laws and if the underlying genetic defect is known; thus it is best discussed following genetic testing (i.e., post-test counseling). The diagnostic rates of genetic testing have improved due to the advent of NGS testing techniques, which have led to more personalized counseling and more accurate estimates of recurrence risks. However, these increased diagnostic rates have also led to an increase in incidental findings of variants of unknown significance. Genetic findings need to be correctly interpreted, placed into clinical contexts and appropriately conveyed to patients, which requires a high level of expertise on ophthalmogenetics.[149]

With regard to genetic testing techniques, the likelihood of finding genetic mutations unrelated to the retinal disorder increases when techniques are able to detect more genetic variations.[153, 154] These findings are known as secondary findings and are mostly found with WES and WGS.[130] This is an important aspect of counseling because patients also have the right "to not know", which should be disclosed in the consent form for genetic testing.[145, 155, 156] Once a secondary finding is found, it may be

ethically problematic to uphold this right to not know, because a secondary finding can have implications for patient health or reproduction.[146, 157] Each secondary finding should be assessed for their causality, clinical significance and actionability.[158] A list of recommended genes and variants has been published by the American College of Genetics and Genomics, which includes clinical significant genes, such as *BRCA1* and *BRCA2*.[159] Additionally, due to the lower read depth of WES and WGS (compared to more narrow techniques), there is a higher chance to miss a variant.[133] Another important aspect of genetic counseling is to psychologically guide patients, who consider presymptomatic testing and to assess the social impact for the patient. For patients with RP, this may have an impact on informed choices about education, professional life and lifestyle. In some cases, diagnosis also has consequences for insurance, such as disability income insurance. If there is a higher risk of having affected offspring, then the option for preconception and pre-implantation counseling can and should be discussed.

Preconception Counseling

Once the mode of inheritance is established, genetic counselors are able to estimate the risk of recurrence and to counsel on reproductive choices. Several reproductive choices are as follows: (1) to conceive naturally - if the risk of inheritance is relatively low, the disease impact is judged acceptable, or if other options are in contrast with their personal beliefs; (2) to receive gamete or embryo donation - which allows for one parent to keep a genetic link with the child (via gamete donation), while also decreasing the risk of passing genetic conditions to their offspring; (3) to adopt - so that the genetic trait is not inherited, although the possibility for the adoptee to carry other medical health problems still remains; (4) or to decide to remain childless.[129, 160]

If patients decide to conceive naturally, it is also possible to screen whether the fetus is affected with an inherited eye condition, using prenatal testing if the causative genetic variants are known. Invasive prenatal genetic tests, such as chorionic villus sampling or amniocentesis, carry a small chance of miscarriage, which may deter patients from taking these tests, although this risk has been significantly reduced over recent decades.[161] Non-invasive prenatal testing (NIPT) also exists, which yields the detection of genetic conditions based on cell-free DNA in maternal blood, but this is not available yet for RP. A genetic counselor will be able to guide patients in selecting the right option for prenatal screening if required.[162]

Pre-Implantation Genetic Testing

Another option for family planning is conceiving via assisted means, such as *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI). Pre-implantation genetic testing (PGT) is then employed prior to IVF or ICSI, which is formerly known as pre-implantation genetic diagnosis.[163, 164] PGT is a technique that screens the genetic material of an embryo after in vitro fertilization and before implantation.[163-166] In many

ways, PGT resembles other forms of prenatal diagnostics. PGT can be subcategorized into six categories as follows: PGT-A (focused on aneuploidies screening); PGT-M (focused on monogenic disorders and diagnosing); PGT-SR (focused on structural rearrangements in a chromosome); combined PGT (combining PGT-A and PGT-M); extended PGT (focused on polygenic disorders); and non-invasive PGT (using blastocentesis or analysis of exhausted culture media as an alternative for embryo biopsy).[163, 165] PGT-M and combined PGT are mainly used to detect underlying gene variants linked to RP, while PGT-A and PGT-SR are subcategories describing screening focused on chromosome abnormalities. The subcategory also determines what kind of genetic screening method is used, with PGT-M mainly using NGS techniques.[163] The amount of DNA extracted for PGT-M testing is very low, thus pre-screening of the variants of interest is usually performed in order to increase the accuracy of the testing. This can be carried out by genetically testing both parents and possibly other family members, increasing the accuracy of detecting a single gene mutation.[163] The main advantage of PGT is the avoidance of selective abortion, as PGT makes it unlikely for the fetus to carry the screened genetic defect. Genetic counseling must always precede PGT, as patients must be informed of the advantages and limitations of this technique, and patients must understand that the possibility of misdiagnosis due to allele dropout, contamination or mosaicism is still present, although small.[167, 168]

AIMS AND OUTLINE

The aim of this thesis is to expand the knowledge on IRDs, with a particular focus on RP. This thesis details an extensive characterization and progression analysis of several genetic subtypes of RP, in preparation for human clinical trials. The second part of this thesis evaluates the current clinical management of RP, analysing several treatment options on their impact on both objective and subjective outcome measures.

Chapter 1 introduces the anatomy of the eye and the retina, along with an overview of RP and IRDs. The chapter also covers various clinical and genetic testing tools used to diagnose these conditions.

Chapter 2 provides an in-depth characterization and natural history studies of several common and uncommon forms of RP**. Chapter 2.1.** describes the natural history of *RHO*-associated RP, in one of the largest cohorts analysed to date. Clinical characteristics of common *RHO*-associated subtypes (generalized or sector RP) are given, and their respective natural disease course are compared. A correlation with several markers on imaging are investigated, in order to establish potential surrogate endpoints for future trials. **Chapter 2.2.** reveals the clinical and genetic characteristics of *RPGR*-associated IRDs, which is the most common X-linked form of RP. We investigate the use of more recent psychophysical tools, such as microperimetry and FST, in this cohort.

Furthermore, we describe the histopathological features of a post-mortem retina obtained from a patient carrying a variant in *RPGR*. **Chapter 2.3.** contains the findings of a rare syndromic form of RP known as PHARC syndrome. Neurological, audiological and in particular ophthalmic findings are described. **Chapter 2.4.** describes the first prospective, longitudinal natural history study in patients with *CRB1*-associated IRDs. The results include the 2-year progression analysis of the most common clinical outcome measures used, including BCVA, visual fields, microperimetry, ffERG and FST.

Chapter 3 evaluates the quality of life and the efficacy of current clinical management options for patients with RP. **Chapter 3.1.** investigates the visual outcome of cataract surgery in patients with RP. The study explores the potential benefit and risks of this treatment in this specific patient group, and it determines risk factors that influence visual outcome. **Chapter 3.2.** studies the impact of a low vision aid called the OrCam MyEye 2.0 on the daily activities and vision-related quality of life of patients with RP. The OrCam MyEye 2.0 is a portable camera that can be mounted to a pair of glasses, which converts visual stimuli (text, products, people) into audio for transmission. **Chapter 3.3.** investigates the quality of life in patients with *CRB1*-associated IRDs using a validated questionnaire, and we determine which quality of life aspects are most affected over the natural disease course of 4 years in untreated patients.

Chapter 4 discusses the findings of the previous chapters, and provides an overview of the current clinical management of RP, and emerging therapies.

The **Appendix** contains the English and Dutch summary of this thesis, acknowledgements, information about the author and a list of publications.

REFERENCES

1. Ferland M. Comparison of the human eye to a camera. science.com. 2023. https://sciencing.com/comparison-human-eye-camera-6305474.html
2. Hoon M, Okawa H, Della Santina L, Wong RO. Functional architecture of the retina: development and disease. *Prog Retin Eye Res*. Sep 2014;42:44-84. doi:10.1016/j.preteyeres.2014.06.003
3. Yang S, Zhou J, Li D. Functions and Diseases of the Retinal Pigment Epithelium. Mini Review. *Frontiers in Pharmacology*. 2021-July-28 2021;12doi:10.3389/fphar.2021.727870
4. Molday RS, Moritz OL. Photoreceptors at a glance. *J Cell Sci*. Nov 15 2015;128(22):4039-45. doi:10.1242/jcs.175687
5. Jonas JB, Schneider U, Naumann GOH. Count and density of human retinal photoreceptors. *Graefe's Archive for Clinical and Experimental Ophthalmology*. 1992/10/01 1992;230(6):505-510. doi:10.1007/BF00181769
6. Yau KW, Hardie RC. Phototransduction motifs and variations. *Cell*. Oct 16 2009;139(2):246-64. doi:10.1016/j.cell.2009.09.029
7. Sparrow JR, Hicks D, Hamel CP. The retinal pigment epithelium in health and disease. *Curr Mol Med*. Dec 2010;10(9):802-23. doi:10.2174/156652410793937813
8. Tsin A, Betts-Obregon B, Grigsby J. Visual cycle proteins: Structure, function, and roles in human retinal disease. *J Biol Chem*. Aug 24 2018;293(34):13016-13021. doi:10.1074/jbc.AW118.003228
9. Wang JS, Kefalov VJ. The cone-specific visual cycle. *Prog Retin Eye Res*. Mar 2011;30(2):115-28. doi:10.1016/j.preteyeres.2010.11.001
10. Verbakel SK, van Huet RAC, Boon CJF, et al. Non-syndromic retinitis pigmentosa. *Progress in Retinal and Eye Research*. 2018/03/27/ 2018;66:157-186. doi:https://doi.org/10.1016/j.preteyeres.2018.03.005
11. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. *The Lancet*. 2006/11/18/ 2006;368(9549):1795-1809. doi:10.1016/S0140-6736(06)69740-7
12. Haim M. Epidemiology of retinitis pigmentosa in Denmark. *Acta Ophthalmol Scand Suppl*. 2002;(233):1-34. doi:10.1046/j.1395-3907.2002.00001.x
13. Na K-H, Kim HJ, Kim KH, et al. Prevalence, Age at Diagnosis, Mortality, and Cause of Death in Retinitis Pigmentosa in Korea—A Nationwide Population-based Study. *American Journal of Ophthalmology*. 2017/04/01/ 2017;176:157-165. doi:https://doi.org/10.1016/j.ajo.2017.01.014
14. Bunker CH, Berson EL, Bromley WC, Hayes RP, Roderick TH. Prevalence of Retinitis Pigmentosa in Maine. *American Journal of Ophthalmology*. 1984/03/01/ 1984;97(3):357-365. doi:https://doi.org/10.1016/0002-9394(84)90636-6
15. Xu L, Hu L, Ma K, Li J, Jonas JB. Prevalence of Retinitis Pigmentosa in Urban and Rural Adult Chinese: The Beijing Eye Study. *European Journal of Ophthalmology*. 2006/11/01 2006;16(6):865-866. doi:10.1177/112067210601600614
16. Sen P, Bhargava A, George R, et al. Prevalence of Retinitis Pigmentosa in South Indian Population Aged Above 40 Years. *Ophthalmic Epidemiology*. 2008/01/01 2008;15(4):279-281. doi:10.1080/09286580802105814
17. Nangia V, Jonas JB, Khare A, Sinha A. Prevalence of retinitis pigmentosa in India: the Central India Eye and Medical Study. https://doi.org/10.1111/j.1755-3768.2012.02396.x. *Acta Ophthalmologica*. 2012/12/01 2012;90(8):e649-e650. doi:https://doi.org/10.1111/j.1755-3768.2012.02396.x
18. Sharon D, Banin E. Nonsyndromic retinitis pigmentosa is highly prevalent in the Jerusalem region with a high frequency of founder mutations. *Mol Vis*. 2015;21:783-792.

19. Von Ammon FA. *Klinische Darstellungen der Krankheiten und Bildungsfehler des Menschlichen Auges, der Augenlider, und der Thränewerkzeug*. G. Reimer; 1838.
20. van Trigt AC. De Speculo Oculi. *Nederlandsch Lancet*. 1853;
21. Langenbeck BCR. *Observations anatomico-pathologica.* 1836.
22. Donders FC. Beiträge zur pathologischen Anatomie des Auges. *Archiv für Ophthalmologie*. 1857/03/01 1857;3(1):139-165. doi:10.1007/BF02720685
23. Musarella MA, MacDonald IM. Current Concepts in the Treatment of Retinitis Pigmentosa. *Journal of Ophthalmology*. 2010/10/11 2011;2011:753547. doi:10.1155/2011/753547
24. Hamel CP. Retinitis pigmentosa. *Orphanet J Rare Dis*. 2006;1:40-40. doi:10.1186/1750-1172-1-40
25. Marmor MF. The electroretinogram in retinitis pigmentosa. *Archives of Ophthalmology*. 1979;97(7):1300-1304. doi:10.1001/archopht.1979.01020020042009
26. Marmor MF, Aguirre G, Arden G, et al. Retinitis Pigmentosa: A Symposium on Terminology and Methods of Examination. *Ophthalmology*. 1983/02/01/ 1983;90(2):126-131. doi:https://doi.org/10.1016/S0161-6420(83)34587-5
27. Aguirre GD. Concepts and Strategies in Retinal Gene Therapy. *Invest Ophthalmol Vis Sci*. 2017;58(12):5399-5411. doi:10.1167/iovs.17-22978
28. Chiu W, Lin T-Y, Chang Y-C, et al. An Update on Gene Therapy for Inherited Retinal Dystrophy: Experience in Leber Congenital Amaurosis Clinical Trials. *Int J Mol Sci*. 2021;22(9):4534. doi:10.3390/ijms22094534
29. Maguire AM, High KA, Auricchio A, et al. Age-dependent effects of RPE65 gene therapy for Leber's congenital amaurosis: a phase 1 dose-escalation trial. *Lancet (London, England)*. 10/23 2009;374(9701):1597-1605. doi:10.1016/S0140-6736(09)61836-5
30. Bainbridge JWB, Smith AJ, Barker SS, et al. Effect of Gene Therapy on Visual Function in Leber's Congenital Amaurosis. *New England Journal of Medicine*. 2008/05/22 2008;358(21):2231-2239. doi:10.1056/NEJMoa0802268
31. Sodi A, Banfi S, Testa F, et al. RPE65-associated inherited retinal diseases: consensus recommendations for eligibility to gene therapy. *Orphanet J Rare Dis*. 2021/06/04 2021;16(1):257. doi:10.1186/s13023-021-01868-4
32. Acland GM, Aguirre GD, Bennett J, et al. Long-term restoration of rod and cone vision by single dose rAAV-mediated gene transfer to the retina in a canine model of childhood blindness. *Mol Ther*. Dec 2005;12(6):1072-82. doi:10.1016/j.ymthe.2005.08.008
33. Acland GM, Aguirre GD, Ray J, et al. Gene therapy restores vision in a canine model of childhood blindness. *Nat Genet*. May 2001;28(1):92-5. doi:10.1038/ng0501-92
34. Ameri H. Prospect of retinal gene therapy following commercialization of voretigene neparvovec-rzyl for retinal dystrophy mediated by RPE65 mutation. *Journal of Current Ophthalmology*. 02/1601/30/ received 01/31/accepted 2018;30(1):1-2. doi:10.1016/j.joco.2018.01.006
35. Maguire AM, Russell S, Wellman JA, et al. Efficacy, Safety, and Durability of Voretigene Neparvovec-rzyl in RPE65 Mutation–Associated Inherited Retinal Dystrophy: Results of Phase 1 and 3 Trials. *Ophthalmology*. 2019/09/01/ 2019;126(9):1273-1285. doi:https://doi.org/10.1016/j.ophtha.2019.06.017
36. Pierce EA, Bennett J. The Status of RPE65 Gene Therapy Trials: Safety and Efficacy. *Cold Spring Harb Perspect Med*. 2015;5(9):a017285-a017285. doi:10.1101/cshperspect.a017285
37. Cehajic Kapetanovic J, McClements ME, Martinez-Fernandez de la Camara C, MacLaren RE. Molecular Strategies for RPGR Gene Therapy. *Genes (Basel)*. 2019;10(9):674. doi:10.3390/genes10090674

38. Fischer MD, McClements ME, Martinez-Fernandez de la Camara C, et al. Codon-Optimized RPGR Improves Stability and Efficacy of AAV8 Gene Therapy in Two Mouse Models of X-Linked Retinitis Pigmentosa. *Molecular therapy : the journal of the American Society of Gene Therapy*. 2017;25(8):1854-1865. doi:10.1016/j.ymthe.2017.05.005

39. Bennett J, Ashtari M, Wellman J, et al. AAV2 gene therapy readministration in three adults with congenital blindness. *Sci Transl Med*. 2012;4(120):120ra15-120ra15. doi:10.1126/scitranslmed.3002865

40. Jacobson SG, Cideciyan AV, Roman AJ, et al. Improvement and decline in vision with gene therapy in childhood blindness. *N Engl J Med*. 2015;372(20):1920-1926. doi:10.1056/NEJMoa1412965

41. Ku CA, Pennesi ME. The new landscape of retinal gene therapy. https://doi.org/10.1002/ajmg.c.31842. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics*. 2020/09/01 2020;184(3):846-859. doi:https://doi.org/10.1002/ajmg.c.31842

42. Chivers M, Li N, Pan F, Wieffer H, Slowik R, Leartsakulpanitch J. The Burden of X-Linked Retinitis Pigmentosa on Patients and Society: A Narrative Literature Review. *Clinicoecon Outcomes Res*. 2021;13:565-572. doi:10.2147/CEOR.S297287

43. Antonio-Aguirre B, Swenor B, Canner JK, Singh MS. Risk of cystoid macular edema following cataract surgery in retinitis pigmentosa: an analysis of United States claims from 2010 to 2018. *Ophthalmology Retina*. 2022;doi:10.1016/j.oret.2022.04.018

44. Bastek JV, Heckenlively JR, Straatsma BR. Cataract Surgery in Retinitis Pigmentosa Patients. *Ophthalmology*. 1982;89(8):880-884. doi:10.1016/S0161-6420(82)34700-4

45. Chaumet-Riffaud AE, Chaumet-Riffaud P, Cariou A, et al. Impact of Retinitis Pigmentosa on Quality of Life, Mental Health, and Employment Among Young Adults. *American Journal of Ophthalmology*. 2017/05/01/ 2017;177:169-174. doi:https://doi.org/10.1016/j.ajo.2017.02.016

46. Parmeggiani F, Sato G, De Nadai K, Romano MR, Binotto A, Costagliola C. Clinical and Rehabilitative Management of Retinitis Pigmentosa: Up-to-Date. *Curr Genomics*. 2011;12(4):250-259. doi:10.2174/138920211795860125

47. Dryja TP, Hahn LB, Kajiwara K, Berson EL. Dominant and digenic mutations in the peripherin/RDS and ROM1 genes in retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. 1997;38(10):1972-1982.

48. Daiger SP, B.J.F. R, Greenberg J, Christoffels A, W. H. Data services and software for identifying genes and mutations causing retinal degeneration. *Invest Ophthalmol Vis Sci*. 1998;39:S295doi:https://sph.uth.edu/RetNet/

49. Branham K, Schlegel D, Fahim AT, Jayasundera KT. Genetic testing for inherited retinal degenerations: Triumphs and tribulations. https://doi.org/10.1002/ajmg.c.31835. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics*. 2020/09/01 2020;184(3):571-577. doi:https://doi.org/10.1002/ajmg.c.31835

50. Dias MF, Joo K, Kemp JA, et al. Molecular genetics and emerging therapies for retinitis pigmentosa: Basic research and clinical perspectives. *Prog Retin Eye Res*. Mar 2018;63:107-131. doi:10.1016/j.preteyeres.2017.10.004

51. Jay M. On the heredity of retinitis pigmentosa. *Br J Ophthalmol*. Jul 1982;66(7):405-16. doi:10.1136/bjo.66.7.405

52. Mathur P, Yang J. Usher syndrome: Hearing loss, retinal degeneration and associated abnormalities. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. 2015/03/01/ 2015;1852(3):406-420. doi:https://doi.org/10.1016/j.bbadis.2014.11.020

53. Tatour Y, Ben-Yosef T. Syndromic Inherited Retinal Diseases: Genetic, Clinical and Diagnostic Aspects. *Diagnostics (Basel)*. 2020;10(10):779. doi:10.3390/diagnostics10100779

54. Blankman JL, Long JZ, Trauger SA, Siuzdak G, Cravatt BF. ABHD12 controls brain lysophosphatidylserine pathways that are deregulated in a murine model of the neurodegenerative disease PHARC. *Proceedings of the National Academy of Sciences*. 2013;110(4):1500. doi:10.1073/pnas.1217121110

55. Nguyen X-T-A, Almushattat H, Strubbe I, et al. The Phenotypic Spectrum of Patients with PHARC Syndrome Due to Variants in ABHD12: An Ophthalmic Perspective. *Genes (Basel)*. 2021;12(9)doi:10.3390/genes12091404

56. Yoshimura H, Hashimoto T, Murata T, et al. Novel ABHD12 Mutations in PHARC Patients: The Differential Diagnosis of Deaf-Blindness. *Annals of Otology, Rhinology & Laryngology*. 2015/05/01 2015;124(1_suppl):77S-83S. doi:10.1177/0003489415574513

57. Jansen GA, Oftnan R, Ferdinandusse S, et al. Refsum disease is caused by mutations in the phytanoyl–CoA hydroxylase gene. *Nature Genetics*. 1997/10/01 1997;17(2):190-193. doi:10.1038/ng1097-190

58. Schwartz JF, Rowland LP, Eder H, et al. Bassen-Kornzweig Syndrome: Deficiency of Serum β-Lipoprotein: A Neuromuscular Disorder Resembling Friedreich's Ataxia, Associated with Steatorrhea, Acanthocytosis, Retinitis Pigmentosa, and a Disorder of Lipid Metabolism. *Archives of Neurology*. 1963;8(4):438-454. doi:10.1001/archneur.1963.00460040108010

59. Fiskerstrand T, H'Mida-Ben Brahim D, Johansson S, et al. Mutations in ABHD12 Cause the Neurodegenerative Disease PHARC: An Inborn Error of Endocannabinoid Metabolism. *The American Journal of Human Genetics*. 2010/09/10/ 2010;87(3):410-417. doi:https://doi.org/10.1016/j.ajhg.2010.08.002

60. Waterham HR, Wanders RJA, Leroy BP. Adult Refsum Disease. In: Adam MP, Ardinger HH, Pagon RA, et al, eds. *GeneReviews(®)*. University of Washington, Seattle

61. Chen HY, Welby E, Li T, Swaroop A. Retinal disease in ciliopathies: Recent advances with a focus on stem cell-based therapies. *Transl Sci Rare Dis*. Jul 4 2019;4(1-2):97-115. doi:10.3233/trd-190038

62. Weleber RG, Gregory-Evans K. Chapter 17 - Retinitis Pigmentosa and Allied Disorders. In: Ryan SJ, Hinton DR, Schachat AP, Wilkinson CP, eds. *Retina (Fourth Edition)*. Mosby; 2006:395-498.

63. Grover S, Fishman GA, Brown J. Patterns of visual field progression in patients with retinitis pigmentosa. *Ophthalmology*. 1998/06/01/ 1998;105(6):1069-1075. doi:https://doi.org/10.1016/S0161-6420(98)96009-2

64. Xu M, Zhai Y, MacDonald IM. Visual Field Progression in Retinitis Pigmentosa. *Invest Ophthalmol Vis Sci*. 2020;61(6):56-56. doi:10.1167/iovs.61.6.56

65. Timmis MA, Allsop J, Baranian M, et al. Visual Search Behavior in Individuals With Retinitis Pigmentosa During Level Walking and Obstacle Crossing. *Invest Ophthalmol Vis Sci*. 2017;58(11):4737-4746. doi:10.1167/iovs.17-21573

66. Campochiaro PA, Mir TA. The mechanism of cone cell death in Retinitis Pigmentosa. *Progress in Retinal and Eye Research*. 2018/01/01/ 2018;62:24-37. doi:https://doi.org/10.1016/j.preteyeres.2017.08.004

67. Georgiou M, Grewal PS, Narayan A, et al. Sector Retinitis Pigmentosa: Extending the Molecular Genetics Basis and Elucidating the Natural History. *American Journal of Ophthalmology*. 2021/01/01/ 2021;221:299-310. doi:https://doi.org/10.1016/j.ajo.2020.08.004

68. Aït-Ali N, Fridlich R, Millet-Puel G, et al. Rod-derived cone viability factor promotes cone survival by stimulating aerobic glycolysis. *Cell*. May 7 2015;161(4):817-32. doi:10.1016/j.cell.2015.03.023

69. Léveillard T, Sahel JA. Rod-derived cone viability factor for treating blinding diseases: from clinic to redox signaling. *Sci Transl Med*. Apr 7 2010;2(26):26ps16. doi:10.1126/scitranslmed.3000866

70. World Health Organization. International Classification of Diseases 11th Revision. Accessed December 25th, 2020, https://icd.who.int/en

71. Vezinaw CM, Fishman GA, McAnany JJ. VISUAL IMPAIRMENT IN RETINITIS PIGMENTOSA. *RETINA*. 2020;40(8)

72. Fishman GA. Retinitis Pigmentosa: Visual Loss. *Archives of Ophthalmology*. 1978;96(7):1185-1188. doi:10.1001/archopht.1978.03910060019004

73. Fishman GA, Young RS, Vasquez V, Lourenço P. Color vision defects in retinitis pigmentosa. *Ann Ophthalmol*. May 1981;13(5):609-18.

74. Bittner AK, Diener-West M, Dagnelie G. A survey of photopsias in self-reported retinitis pigmentosa: location of photopsias is related to disease severity. *Retina (Philadelphia, Pa)*. Nov-Dec 2009;29(10):1513-1521. doi:10.1097/IAE.0b013e3181af0d57

75. Chang S, Vaccarella L, Olatunji S, Cebulla C, Christoforidis J. Diagnostic challenges in retinitis pigmentosa: genotypic multiplicity and phenotypic variability. *Curr Genomics*. 2011;12(4):267-275. doi:10.2174/138920211795860116

76. Fishman GA, Fishman M, Maggiano J. Macular lesions associated with retinitis pigmentosa. *Arch Ophthalmol*. May 1977;95(5):798-803. doi:10.1001/archopht.1977.04450050076006

77. Cideciyan AV, Charng J, Roman AJ, et al. Progression in X-linked retinitis pigmentosa due to ORF15-RPGR mutations: assessment of localized vision changes over 2 years rod and cone sensitivity in XLRP. *Invest Ophthalmol Vis Sci*. 2018;59(11):4558-4566. doi:10.1167/iovs.18-24931

78. Nguyen X-T-A, Talib M, van Schooneveld MJ, et al. RPGR-Associated Dystrophies: Clinical, Genetic, and Histopathological Features. *Int J Mol Sci*. 2020;21(3):835. doi:10.3390/ijms21030835

79. Sandberg MA, Rosner B, Weigel-DiFranco C, Dryja TP, Berson EL. Disease course of patients with X-linked retinitis pigmentosa due to RPGR gene mutations. *Invest Ophthalmol Vis Sci*. 2007;48(3):1298-1304. doi:10.1167/iovs.06-0971

80. Talib M, van Schooneveld MJ, Thiadens AA, et al. Clinical and Genetic characteristics of Male patients with RPGR-associated Retinal Dystrophies: A long-Term Follow-up Study. *RETINA*. 2019;39(6):1186-1199. doi:10.1097/iae.0000000000002125

81. Talib M, van Schooneveld MJ, Van Cauwenbergh C, et al. The Spectrum of Structural and Functional Abnormalities in Female Carriers of Pathogenic Variants in the RPGR Gene. *Invest Ophthalmol Vis Sci*. Aug 1 2018;59(10):4123-4133. doi:10.1167/iovs.17-23453

82. Nguyen X-T-A, Talib M, van Cauwenbergh C, et al. CLINICAL CHARACTERISTICS AND NATURAL HISTORY OF RHO-ASSOCIATED RETINITIS PIGMENTOSA: A Long-Term Follow-Up Study. *RETINA*. 2021;41(1)

83. Berson EL, Rosner B, Weigel-DiFranco C, Dryja TP, Sandberg MA. Disease progression in patients with dominant retinitis pigmentosa and rhodopsin mutations. *Invest Ophthalmol Vis Sci*. 2002;43(9):3027-3036.

84. Berson EL, Sandberg MA, Rosner B, Birch DG, Hanson AH. Natural Course of Retinitis Pigmentosa Over a Three-Year Interval. *American Journal of Ophthalmology*. 1985;99(3):240-251. doi:10.1016/0002-9394(85)90351-4

85. Talib M, van Schooneveld MJ, van Genderen MM, et al. Genotypic and phenotypic characteristics of *CRB1*-associated retinal dystrophies. *Ophthalmology*. 2017;124(6):884-895. doi:10.1016/j.ophtha.2017.01.047

86. Talib M, Van Cauwenbergh C, De Zaeytijd J, et al. CRB1-associated retinal dystrophies in a Belgian cohort: genetic characteristics and long-term clinical follow-up. *British Journal of Ophthalmology*. 2021:bjophthalmol-2020-316781. doi:10.1136/bjophthalmol-2020-316781

87. Tee JJL, Yang Y, Kalitzeos A, Webster A, Bainbridge J, Michaelides M. Natural History Study of Retinal Structure, Progression, and Symmetry Using Ellipzoid Zone Metrics in RPGR-Associated Retinopathy. *American Journal of Ophthalmology*. 2019/02/01/ 2019;198:111-123. doi:10.1016/j.ajo.2018.10.003

88. Pearlman JT, Flood TP, Seiff SR. Retinitis pigmentosa without pigment. *Am J Ophthalmol*. Apr 1976;81(4):417-9. doi:10.1016/0002-9394(76)90296-8

89. Pearlman JT. Letter: Nonpigmented retinitis pigmentosa and the neurologist. *Arch Neurol*. Aug 1976;33(8):590. doi:10.1001/archneur.1976.00500080068015

90. Weller JM, Michelson G, Juenemann AG. Unilateral retinitis pigmentosa: 30 years follow-up. *BMJ Case Rep*. 2014;2014:bcr2013202236. doi:10.1136/bcr-2013-202236

91. Marsiglia M, Duncker T, Peiretti E, Brodie SE, Tsang SH. Unilateral retinitis pigmentosa: a proposal of genetic pathogenic mechanisms. *European journal of ophthalmology*. Jul-Aug 2012;22(4):654-660. doi:10.5301/ejo.5000086

92. Kranich H, Bartkowski S, Denton MJ, et al. Autosomal dominant 'sector' retinitis pigmentosa due to a point mutation predicting an Asn-15-Ser substitution of rhodopsin. *Human Molecular Genetics*. 1993;2(6):813-814. doi:10.1093/hmg/2.6.813

93. Ramon E, Cordomí A, Aguilà M, et al. Differential light-induced responses in sectorial inherited retinal degeneration. *J Biol Chem*. 2014;289(52):35918-35928. doi:10.1074/jbc.M114.609958

94. Shah SP, Wong F, Sharp DM, Vincent AL. A Novel Rhodopsin Point Mutation, Proline-170-histidine, Associated with Sectoral Retinitis Pigmentosa. *Ophthalmic Genetics*. 2014/12/01 2014;35(4):241-247. doi:10.3109/13816810.2014.924014

95. Balfoort BM, Buijs MJN, ten Asbroek ALMA, et al. A review of treatment modalities in gyrate atrophy of the choroid and retina (GACR). *Molecular Genetics and Metabolism*. 2021/09/01/ 2021;134(1):96-116. doi:https://doi.org/10.1016/j.ymgme.2021.07.010

96. Benomar A, Yahyaoui M, Meggouh F, et al. Clinical comparison between AVED patients with 744 del A mutation and Friedreich ataxia with GAA expansion in 15 Moroccan families. *Journal of the Neurological Sciences*. 2002/06/15/ 2002;198(1):25-29. doi:https://doi.org/10.1016/S0022-510X(02)00057-6

97. Jayaram H, Downes SM. Midlife diagnosis of Refsum Disease in siblings with Retinitis Pigmentosa – the footprint is the clue: a case report. *Journal of Medical Case Reports*. 2008/03/12 2008;2(1):80. doi:10.1186/1752-1947-2-80

98. Gouras P, Carr RE, Gunkel RD. Retinitis Pigmentosa in Abetalipoproteinemia: Effects of Vitamin A. *Invest Ophthalmol Vis Sci*. 1971;10(10):784-793.

99. Iwasa K, Shima K, Komai K, Nishida Y, Yokota T, Yamada M. Retinitis pigmentosa and macular degeneration in a patient with ataxia with isolated vitamin E deficiency with a novel c.717 del C mutation in the TTPA gene. *J Neurol Sci*. Oct 15 2014;345(1-2):228-30. doi:10.1016/j.jns.2014.07.001

100. McCulloch DL, Marmor MF, Brigell MG, et al. ISCEV Standard for full-field clinical electroretinography (2015 update). *Documenta Ophthalmologica*. 2015/02/01 2015;130(1):1-12. doi:10.1007/s10633-014-9473-7

101. Hood DC, Odel JG, Chen CS, Winn BJ. The Multifocal Electroretinogram. *Journal of Neuro-Ophthalmology*. 2003;23(3)

102. Alexander KR, Fishman GA. Prolonged rod dark adaptation in retinitis pigmentosa. *Br J Ophthalmol*. 1984;68(8):561-569. doi:10.1136/bjo.68.8.561

103. Stavrou P, Good PA, Broadhurst EJ, Bundey S, Fielder AR, Crews SJ. ERG and EOG abnormalities in carriers of X-linked retinitis pigmentosa. *Eye (Lond)*. 1996;10 (Pt 5):581-9. doi:10.1038/eye.1996.134

104. Menghini M, Cehajic-Kapetanovic J, MacLaren RE. Monitoring progression of retinitis pigmentosa: current recommendations and recent advances. *Expert Opin Orphan Drugs*. 2020;8(2-3):67-78. doi:10.1080/21678707.2020.1735352

105. Talib M, Dagnelie G, Boon CJF. Recording and Analysis of Goldmann Kinetic Visual Fields. *Methods Mol Biol*. 2018;1715:327-338. doi:10.1007/978-1-4939-7522-8_24

106. Barnes CS, Schuchard RA, Birch DG, et al. Reliability of Semiautomated Kinetic Perimetry (SKP) and Goldmann Kinetic Perimetry in Children and Adults With Retinal Dystrophies. *Transl Vis Sci Technol*. 2019;8(3):36-36. doi:10.1167/tvst.8.3.36

107. Barry MP, Bittner AK, Yang L, Marcus R, Iftikhar MH, Dagnelie G. Variability and Errors of Manually Digitized Goldmann Visual Fields. *Optometry and vision science : official publication of the American Academy of Optometry*. 2016;93(7):720-730. doi:10.1097/OPX.0000000000000869

108. Bittner AK, Iftikhar MH, Dagnelie G. Test-Retest, Within-Visit Variability of Goldmann Visual Fields in Retinitis Pigmentosa. *Invest Ophthalmol Vis Sci*. 2011;52(11):8042-8046. doi:10.1167/iovs.11-8321

109. Pfau M, Jolly JK, Wu Z, et al. Fundus-controlled perimetry (microperimetry): Application as outcome measure in clinical trials. *Prog Retin Eye Res*. May 2021;82:100907. doi:10.1016/j.preteyeres.2020.100907

110. Dimopoulos IS, Tseng C, MacDonald IM. Microperimetry as an Outcome Measure in Choroideremia Trials: Reproducibility and Beyond. *Invest Ophthalmol Vis Sci*. 2016;57(10):4151-4161. doi:10.1167/iovs.16-19338

111. Schönbach EM, Wolfson Y, Strauss RW, et al. Macular Sensitivity Measured With Microperimetry in Stargardt Disease in the Progression of Atrophy Secondary to Stargardt Disease (ProgStar) Study: Report No. 7. *JAMA Ophthalmology*. 2017;135(7):696-703. doi:10.1001/jamaophthalmol.2017.1162

112. Iftikhar M, Kherani S, Kaur R, et al. Progression of Retinitis Pigmentosa as Measured on Microperimetry: The PREP-1 Study. *Ophthalmology Retina*. 2018/05/01/ 2018;2(5):502-507. doi:https://doi.org/10.1016/j.oret.2017.09.008

113. Nguyen XT, Talib M, van Schooneveld MJ, et al. CRB1-Associated Retinal Dystrophies: A Prospective Natural History Study in Anticipation of Future Clinical Trials. *Am J Ophthalmol*. Feb 2022;234:37-48. doi:10.1016/j.ajo.2021.07.021

114. Miura G, Baba T, Tatsumi T, Yokouchi H, Yamamoto S. The Impact of Cataract Surgery on Contrast Visual Acuity and Retinal Sensitivity in Patients with Retinitis Pigmentosa. *J Ophthalmol*. 2021;2021:2281834. doi:10.1155/2021/2281834

115. Bennett LD, Klein M, Locke KG, Kiser K, Birch DG. Dark-Adapted Chromatic Perimetry for Measuring Rod Visual Fields in Patients with Retinitis Pigmentosa. *Transl Vis Sci Technol*. Jul 2017;6(4):15. doi:10.1167/tvst.6.4.15

116. Jacobson SG, Voigt WJ, Parel J-M, et al. Automated Light- and Dark- Adapted Perimetry for Evaluating Retinitis Pigmentosa. *Ophthalmology*. 1986/12/01/ 1986;93(12):1604-1611. doi:https://doi.org/10.1016/S0161-6420(86)33522-X

117. McGuigan DB, III, Roman AJ, Cideciyan AV, et al. Automated Light- and Dark-Adapted Perimetry for Evaluating Retinitis Pigmentosa: Filling a Need to Accommodate Multicenter Clinical Trials. *Invest Ophthalmol Vis Sci*. 2016;57(7):3118-3128. doi:10.1167/iovs.16-19302

118. Grewal MK, Chandra S, Bird A, Jeffery G, Sivaprasad S. Scotopic thresholds on dark-adapted chromatic perimetry in healthy aging and age-related macular degeneration. *Scientific Reports*. 2021/05/14 2021;11(1):10349. doi:10.1038/s41598-021-89677-4

119. Cideciyan AV, Jacobson SG, Beltran WA, et al. Human retinal gene therapy for Leber congenital amaurosis shows advancing retinal degeneration despite enduring visual improvement. *Proc Natl Acad Sci U S A*. Feb 5 2013;110(6):E517-25. doi:10.1073/pnas.1218933110

120. Roman AJ, Cideciyan AV, Wu V, Garafalo AV, Jacobson SG. Full-field stimulus testing: Role in the clinic and as an outcome measure in clinical trials of severe childhood retinal disease. *Progress in Retinal and Eye Research*. 2022/03/01/ 2022;87:101000. doi:https://doi.org/10.1016/j.preteyeres.2021.101000

121. Wang X, Yu C, Tzekov RT, Zhu Y, Li W. The effect of human gene therapy for RPE65-associated Leber's congenital amaurosis on visual function: a systematic review and meta-analysis. *Orphanet J Rare Dis*. Feb 14 2020;15(1):49. doi:10.1186/s13023-020-1304-1

122. Cabral T, Sengillo JD, Duong JK, et al. Retrospective analysis of structural disease progression in retinitis pigmentosa utilizing multimodal imaging. *Scientific Reports*. 2017/09/04 2017;7(1):10347. doi:10.1038/s41598-017-10473-0

123. Cai CX, Locke KG, Ramachandran R, Birch DG, Hood DC. A comparison of progressive loss of the ellipsoid zone (EZ) band in autosomal dominant and X-linked retinitis pigmentosa. Invest Ophthalmol Vis Sci. 2014;55(11):7417-7422.

124. Hariri AH, Zhang HY, Ho A, et al. Quantification of ellipsoid zone changes in retinitis pigmentosa using en face spectral domain–optical coherence tomography. *JAMA Ophthalmology*. 2016;134(6):628-635. doi:10.1001/jamaophthalmol.2016.0502

125. Liu G, Li H, Liu X, Xu D, Wang F. Structural analysis of retinal photoreceptor ellipsoid zone and postreceptor retinal layer associated with visual acuity in patients with retinitis pigmentosa by ganglion cell analysis combined with OCT imaging. *Medicine*. 2016;95(52):e5785-e5785. doi:10.1097/MD.0000000000005785

126. Phadikar P, Saxena S, Ruia S, Lai TYY, Meyer CH, Eliott D. The potential of spectral domain optical coherence tomography imaging based retinal biomarkers. *International Journal of Retina and Vitreous*. 01/09; 12/07/accepted 2017;3:1. doi:10.1186/s40942-016-0054-7

127. Schuerch K, Marsiglia M, Lee W, Tsang SH, Sparrow JR. Multimodal Imaging of Disease-Associated Pigmentary Changes in Retinitis Pigmentosa. *Retina (Philadelphia, Pa).* 2016;36(Suppl 1):S147-S158. doi:10.1097/IAE.0000000000001256

128. Xue K, Oldani M, Jolly JK, et al. Correlation of Optical Coherence Tomography and Autofluorescence in the Outer Retina and Choroid of Patients With Choroideremia. *Invest Ophthalmol Vis Sci*. 07/12; 05/22/accepted 2016;57(8):3674-3684. doi:10.1167/iovs.15-18364

129. Méjécase C, Malka S, Guan Z, Slater A, Arno G, Moosajee M. Practical guide to genetic screening for inherited eye diseases. *Therapeutic Advances in Ophthalmology*. 2020/01/01 2020;12:2515841420954592. doi:10.1177/2515841420954592

130. Salmaninejad A, Motaee J, Farjami M, Alimardani M, Esmaeilie A, Pasdar A. Next-generation sequencing and its application in diagnosis of retinitis pigmentosa. *Ophthalmic Genet*. Oct 2019;40(5):393-402. doi:10.1080/13816810.2019.1675178

131. Kumar KR, Cowley MJ, Davis RL. Next-Generation Sequencing and Emerging Technologies. *Semin Thromb Hemost*. Oct 2019;45(7):661-673. doi:10.1055/s-0039-1688446

132. Crossley BM, Bai J, Glaser A, et al. Guidelines for Sanger sequencing and molecular assay monitoring. *J Vet Diagn Invest*. Nov 2020;32(6):767-775. doi:10.1177/1040638720905833

133. Ng PC, Kirkness EF. Whole genome sequencing. *Methods Mol Biol*. 2010;628:215-26. doi:10.1007/978-1-60327-367-1_12

134. Fahim AT, Daiger SP, Weleber RG. Nonsyndromic Retinitis Pigmentosa Overview. In: Adam MP, Ardinger HH, Pagon RA, et al, eds. *GeneReviews(®)*. University of Washington, Seattle

135. Poptsova MS, Il'icheva IA, Nechipurenko DY, et al. Non-random DNA fragmentation in next-generation sequencing. *Sci Rep*. Mar 31 2014;4:4532. doi:10.1038/srep04532

136. França LT, Carrilho E, Kist TB. A review of DNA sequencing techniques. *Q Rev Biophys*. May 2002;35(2):169-200. doi:10.1017/s0033583502003797

137. Ge Z, Bowles K, Goetz K, et al. NGS-based Molecular diagnosis of 105 eyeGENE® probands with Retinitis Pigmentosa. *Scientific Reports*. 2015/12/15 2015;5(1):18287. doi:10.1038/srep18287

138. Glöckle N, Kohl S, Mohr J, et al. Panel-based next generation sequencing as a reliable and efficient technique to detect mutations in unselected patients with retinal dystrophies. *European Journal of Human Genetics*. 2014/01/01 2014;22(1):99-104. doi:10.1038/ejhg.2013.72

139. Farrar GJ, Carrigan M, Dockery A, et al. Toward an elucidation of the molecular genetics of inherited retinal degenerations. *Hum Mol Genet*. Aug 1 2017;26(R1):R2-r11. doi:10.1093/hmg/ddx185

140. Dockery A, Whelan L, Humphries P, Farrar GJ. Next-Generation Sequencing Applications for Inherited Retinal Diseases. *Int J Mol Sci*. 2021;22(11)doi:10.3390/ijms22115684

141. Rabbani B, Tekin M, Mahdieh N. The promise of whole-exome sequencing in medical genetics. *Journal of Human Genetics*. 2014/01/01 2014;59(1):5-15. doi:10.1038/jhg.2013.114

142. Hart MR, Biesecker BB, Blout CL, et al. Secondary findings from clinical genomic sequencing: prevalence, patient perspectives, family history assessment, and health-care costs from a multisite study. *Genet Med*. 2019;21(5):1100-1110. doi:10.1038/s41436-018-0308-x

143. Ozsolak F. Third-generation sequencing techniques and applications to drug discovery. *Expert Opin Drug Discov*. 2012;7(3):231-243. doi:10.1517/17460441.2012.660145

144. Xiao T, Zhou W. The third generation sequencing: the advanced approach to genetic diseases. *Transl Pediatr*. 2020;9(2):163-173. doi:10.21037/tp.2020.03.06

145. Resta R, Biesecker BB, Bennett RL, et al. A new definition of Genetic Counseling: National Society of Genetic Counselors' Task Force report. *J Genet Couns*. Apr 2006;15(2):77-83. doi:10.1007/s10897-005-9014-3

146. Ciarleglio LJ, Bennett RL, Williamson J, Mandell JB, Marks JH. Genetic counseling throughout the life cycle. *J Clin Invest*. Nov 2003;112(9):1280-6. doi:10.1172/jci20170

147. Middleton A, Hall G, Patch C. Genetic counselors and Genomic Counseling in the United Kingdom. https://doi.org/10.1002/mgg3.123. *Molecular Genetics & Genomic Medicine*. 2015/03/01 2015;3(2):79-83. doi:https://doi.org/10.1002/mgg3.123

148. Patch C, Middleton A. Genetic counselling in the era of genomic medicine. *Br Med Bull*. Jun 1 2018;126(1):27-36. doi:10.1093/bmb/ldy008

149. Strait S, Loman R, Erickson L, DeBenedictis M. Inherited retinal degeneration current genetics practices – a needs assessment. *Ophthalmic Genetics*. 2020/11/01 2020;41(6):533-538. doi:10.1080/13816810.2020.1804943

150. McGuire AL, Caulfield T, Cho MK. Research ethics and the challenge of whole-genome sequencing. *Nat Rev Genet*. Feb 2008;9(2):152-6. doi:10.1038/nrg2302

151. Shintani K, Shechtman DL, Gurwood AS. Review and update: current treatment trends for patients with retinitis pigmentosa. *Optometry*. Jul 2009;80(7):384-401. doi:10.1016/j.optm.2008.01.026

152. MacArthur DG, Manolio TA, Dimmock DP, et al. Guidelines for investigating causality of sequence variants in human disease. *Nature*. Apr 24 2014;508(7497):469-76. doi:10.1038/nature13127

153. Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med*. Jul 2013;15(7):565-74. doi:10.1038/gim.2013.73

154. Kalia SS, Adelman K, Bale SJ, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genet Med*. Feb 2017;19(2):249-255. doi:10.1038/gim.2016.190

155. Yang M, Kim JW. Principles of Genetic Counseling in the Era of Next-Generation Sequencing. *Ann Lab Med*. Jul 2018;38(4):291-295. doi:10.3343/alm.2018.38.4.291

156. Berkman BE, Hull SC. The "right not to know" in the genomic era: time to break from tradition? *Am J Bioeth*. 2014;14(3):28-31. doi:10.1080/15265161.2014.880313

157. Bennett RL, Hampel HL, Mandell JB, Marks JH. Genetic counselors: translating genomic science into clinical practice. *J Clin Invest*. Nov 2003;112(9):1274-9. doi:10.1172/jci20113

158. Nilsson MP, Emmertz M, Kristoffersson U, et al. Germline mutations in BRCA1 and BRCA2 incidentally revealed in a biobank research study: experiences from re-contacting mutation carriers and relatives. *J Community Genet*. Jul 2018;9(3):201-208. doi:10.1007/s12687-017-0341-5

159. Miller DT, Lee K, Chung WK, et al. ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genetics in Medicine*. 2021/08/01 2021;23(8):1381-1390. doi:10.1038/s41436-021-01172-3

160. Severijns Y, de Die-Smulders CEM, Gültzow T, de Vries H, van Osch LADM. Hereditary diseases and child wish: exploring motives, considerations, and the (joint) decision-making process of genetically at-risk couples. *J Community Genet*. 2021;12(3):325-335. doi:10.1007/s12687-021-00510-x

161. Liehr T, Lauten A, Schneider U, Schleussner E, Weise A. Noninvasive Prenatal Testing - When Is It Advantageous to Apply. *Biomedicine Hub*. 2017;2(1):1-11. doi:10.1159/000458432

162. Ahmed K, Ahmed M, Potrata B, et al. Patient attitudes towards prenatal diagnostic testing for inherited retinal disease. https://doi.org/10.1002/pd.4644. *Prenatal Diagnosis*. 2015/09/01 2015;35(9):913-918. doi:https://doi.org/10.1002/pd.4644

163. Fesahat F, Montazeri F, Hoseini SM. Preimplantation genetic testing in assisted reproduction technology. *J Gynecol Obstet Hum Reprod*. May 2020;49(5):101723. doi:10.1016/j.jogoh.2020.101723

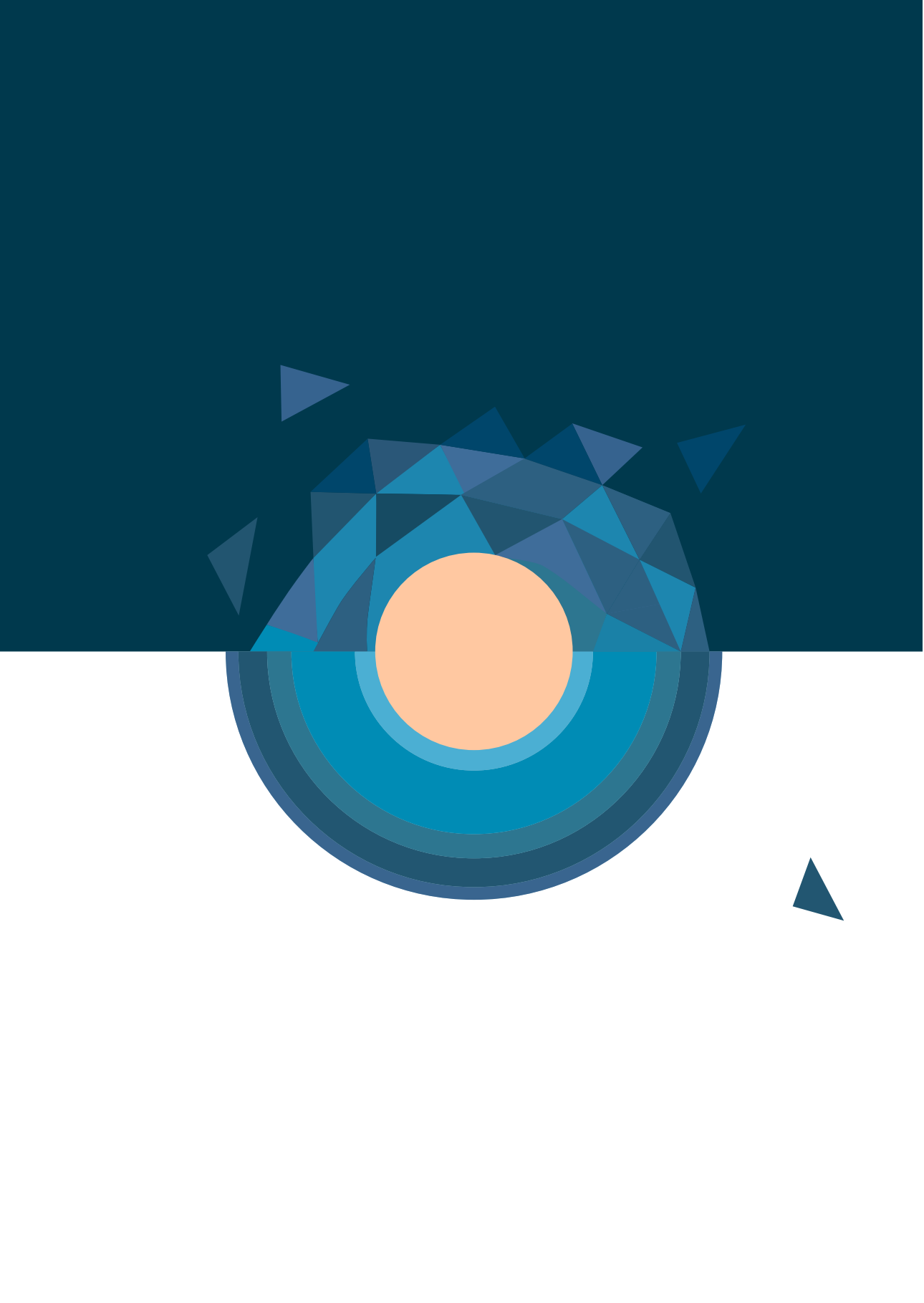
164. Huang X, Liu Y, Yu X, et al. The clinical application of preimplantation genetic diagnosis for X-linked retinitis pigmentosa. *J Assist Reprod Genet*. May 2019;36(5):989-994. doi:10.1007/s10815-019-01434-9

165. Greco E, Litwicka K, Minasi MG, Cursio E, Greco PF, Barillari P. Preimplantation Genetic Testing: Where We Are Today. *Int J Mol Sci*. Jun 19 2020;21(12)doi:10.3390/ijms21124381

166. Murphy NM, Samarasekera TS, Macaskill L, Mullen J, Rombauts LJF. Genome sequencing of human in vitro fertilisation embryos for pathogenic variation screening. *Sci Rep*. Mar 2 2020;10(1):3795. doi:10.1038/s41598-020-60704-0

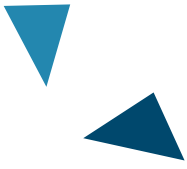
167. Hlavatá L, Ďuďáková Ľ, Trková M, et al. [Preimplantation genetic diagnosis and monogenic inherited eye diseases]. *Cesk Slov Oftalmol*. Fall 2016;72(5):167-171. Preimplantační genetická diagnostika a dědičná onemocnění oka.

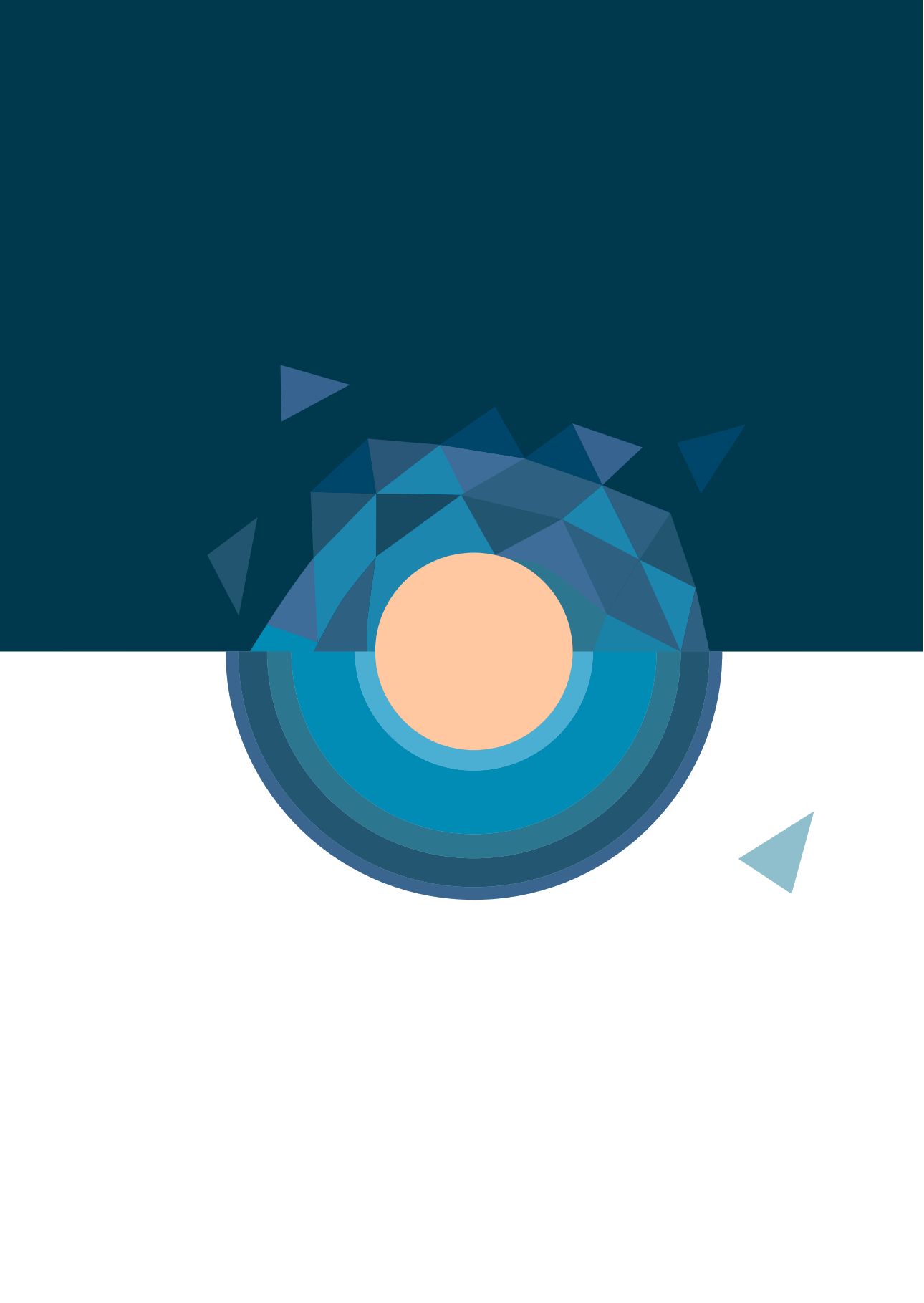
168. Cooper AR, Jungheim ES. Preimplantation genetic testing: indications and controversies. *Clin Lab Med*. 2010;30(3):519-531. doi:10.1016/j.cll.2010.04.008



PART I

CLINICAL CHARACTERISTICS AND NATURAL HISTORY STUDIES





CHAPTER 2.1

CLINICAL CHARACTERISTICS AND NATURAL HISTORY OF *RHO*-ASSOCIATED RETINITIS PIGMENTOSA: A LONG-TERM FOLLOW-UP STUDY

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ABSTRACT

Purpose
To investigate the natural history of *RHO*-associated retinitis pigmentosa (RP).

Methods
A multicenter, medical chart review of 100 patients with autosomal-dominant *RHO*-associated RP.

Results
Based on visual fields, time-to-event analysis revealed median ages of 52 and 79 years to reach low vision (central visual field <20°) and blindness (central visual field <10°), respectively. For best-corrected visual acuity (BCVA), the median age to reach mild impairment (20/67≤ BCVA <20/40) was 72 years, whereas this could not be computed for lower acuities. Disease progression was significantly faster in patients with a generalized RP phenotype (n = 75; 75%) compared to patients with a sector RP phenotype (n = 25; 25%), in terms of decline rates of BCVA ($p < 0.001$), and V4e retinal seeing areas ($p < 0.005$). The foveal thickness of the photoreceptor-retinal pigment epithelium (PR+RPE) complex correlated significantly with BCVA (Spearman's $\rho=0.733$; $p < 0.001$).

Conclusions
Based on central visual fields, the optimal window of intervention for *RHO*-associated RP is before the 5th decade of life. Significant differences in disease progression are present between generalized and sector RP phenotypes. Our findings suggest that the PR+RPE complex is a potential surrogate endpoint for BCVA in future studies.

INTRODUCTION

Mutations in the *RHO* gene are associated with the autosomal-dominant form of retinitis pigmentosa (RP).[1] Initial symptoms of RP include night blindness or peripheral visual field loss, which can be followed by loss of central vision in advanced stages of disease. To date, over 150 mutations in the *RHO* gene have been described, which are responsible for 25-30% of all autosomal-dominant retinitis pigmentosa (adRP) cases.[2] *RHO* mutations have also been described in congenital stationary night blindness,[3] and even more rarely, in forms of autosomal-recessive RP.[4] Another phenotype commonly described in *RHO* mutations is sector RP, which is characterized by regional photoreceptor degeneration, typically confined to the inferior quadrant of the retina.[5, 6] Sector RP is considered a stationary to slowly progressive disease, but may eventually lead to a more severe, diffuse RP phenotype.[7]

The *RHO* gene encodes the protein rhodopsin, located in the outer segment of rod photoreceptor cells, containing extracellular, transmembrane, and cytoplasmic domains.[1] Previous studies have shown that the clinical expression of *RHO*-associated RP correlates with the protein domain affected by the mutation.[8] Mutations affecting the cytoplasmic domain of rhodopsin are more likely to cause a severe RP phenotype, with early loss of rod and cone function. In contrast, patients with mutations affecting the extracellular domain generally have a milder phenotype, with relatively preserved rod and cone function, and slower disease progression.[9, 10]

No curative treatment for *RHO*-associated RP is currently available, but promising results have been achieved by knockdown-and-replacement gene therapy in animal models.[11, 12] To guide the design of upcoming clinical trials, more insight into the natural disease progression in *RHO*-associated RP is necessary. A more detailed clinical disease profile will aid in the selection of eligible candidates, and the establishment of appropriate clinical endpoints for future trials. The purpose of this longitudinal study was to provide a description of the clinical variability and the natural disease course in patients with *RHO*-associated RP in a large cohort.

MATERIALS AND METHODS

Study population

Patients with *RHO*-associated RP were collected from the patient database for hereditary eye diseases (Delleman Archive) at the Amsterdam University Medical Centers (The Netherlands), from various other Dutch tertiary referral centers within the RD5000 consortium,[13] and from Ghent University Hospital in Belgium. Inclusion criteria were: a molecular confirmation of a (likely) pathogenic variant in the *RHO* gene, or a first-degree relative with similar clinical findings and molecular

confirmation of a *RHO* mutation. In total, 100 patients with *RHO*-associated RP were included for analysis in this study. Approval from the Ethics Committee was obtained prior to the study, as well as local Institutional Review Board approval in all participating centers. Dutch participants provided informed consent for the use of their patient data for research purposes. For Belgian subjects, the local Ethics Committee waivered the need for informed consent on the condition of pseudonymization.

Data collection

A standardized review of medical records was performed for data on the initial symptoms, best-corrected visual acuity (BCVA), findings on slit-lamp examination and fundoscopy, Goldmann visual fields (GVF), full-field electroretinogram (ERG), spectral-domain optical coherence tomography (SD-OCT), and fundus autofluorescence (FAF) imaging, where available. GVFs were digitized and converted to retinal seeing areas using methods previously described by Dagnelie.[14]

In patients with available OCT and FAF imaging in Heidelberg (Heidelberg Engineering, Heidelberg, Germany), automatic and manual measurement of retinal layers were performed using the inbuilt software of Heidelberg. The thickness of the photoreceptor-retinal pigment epithelium complex (PR+RPE), was defined as the foveal distance between the external limiting membrane and the basal border of the retinal pigment epithelium, as described previously (see Figure, Supplemental Digital Content 1).[15] All measurements were performed by two authors (XN and MT), and reviewed by CJFB in case of inconsistency between the aforementioned two authors.

Statistical analysis

Data were analyzed using SPSS version 23.0 (IBM Corp, Armonk, NY, USA) and the R-software environment.[16] Findings with a p-value of <0.05 were considered statistically significant. Normally and non-normally distributed data were displayed as means with standard deviations (SD), and medians with interquartile ranges (IQR), respectively. To measure the time to visual impairment, a time-to-event analysis was performed using the nonparametric maximum likelihood estimator (NPMLE) method to account for left, interval and right censored data. Visual impairment endpoints were based on the criteria of the World Health Organization: No visual impairment (BCVA $\geq$ 20/40), mild visual impairment (20/67 $\leq$ BCVA $<$ 20/40), low vision (20/200 $\leq$ BCVA $<$ 20/67), severe visual impairment (20/400 $\leq$ BCVA $<$ 20/200) or blindness (BCVA $<$ 20/400). For visual fields, the following endpoints were used: mild impairment (20° $\leq$ central visual field $<$ 70°), low vision (central visual field $<$ 20°) and blindness (central visual field $<$ 10°). Due to the presence of repeated measurements, a linear mixed model analysis was performed to measure disease progression. For hand movement vision, light perception vision and no light perception, logMAR values of 2.7, 2.8 and 2.9 were used, respectively.[17] Structure-function correlations were analyzed using Spearman correlation coefficients. To analyze

genotype-phenotype associations, patients were stratified into 3 groups, based the affected protein domain: cytoplasmic, transmembrane or extracellular.

RESULTS

Clinical and genetic characteristics

One-hundred patients from 47 families, with autosomal dominant *RHO* mutations, were included from the Dutch (n=63; 63%) and Belgian (n=37; 37%) population. No differences in baseline characteristics between these two populations were present (see Table, Supplemental Digital Content S2). Patients with available longitudinal data (n=72; 72%) had a median follow-up time of 6.9 years (IQR 11.9; range: 0.2-41.0) and a median of 5.0 visits (IQR 6.0, range: 2.0 – 31.0). Seventy-five patients (75%) had a generalized form of RP on fundus examination (Figure 1), whereas 25 patients (25%) showed a sector RP phenotype, with pigmentary changes confined to the inferior hemisphere in all cases. The clinical characteristics of the entire cohort are summarized in Table 1.

In total, 23 different missense mutations, 1 in-frame deletion, and 1 novel splice site mutation were found in the *RHO* gene (see Table, Supplemental Digital Content S3). Patients were stratified, based on the affected protein domain, as carrying extracellular (n=64; 64%), transmembrane (n=20; 20%), or cytoplasmic (n=15; 16%) mutations, excluding the splice site mutation. We found a high proportion of extracellular mutations (24/25; 96%) in the sector RP group. The most common pathogenic variant in this study, p.(Glu181Lys), was found in 4 families, comprising 23 out of the 63 Dutch patients (37%). This mutation was not found in the Belgian cohort. Common mutations exclusively found in the Belgian cohort were p.(Ile255del) (n=6) and p.(Tyr178Asp) (n=6), each belonging to a single family, accounting for 12 out of the 37 Belgian patients (32%).

Table 1. Characteristics of patients with RHO-associated (sector) retinitis pigmentosa at last examination.

Characteristics	Total (n=100)	Generalized RP (n=75)	Sector RP (n=25)	P-value
Male (%)	44 (44)	34 (46)	10 (39)	0.642
Age at last examination (n=100)				
Mean ± SD	43.5 ± 18.5	42.5 ± 19.3	45.5 ± 22.6	0.204
Initial symptoms (n=55)				
Nyctalopia, n (%)	41 (75)	28 (74)	2 (40)	
Visual field loss, n (%)	5 (9)	3 (8)	1 (20)	
Visual acuity loss, n (%)	3 (5)	2 (5)	1 (20)	
Multiple symptoms, n (%)	6 (11)	5 (13)	1 (20)	0.890‡
Age at onset in years (n=55)				
Early childhood, n (%)	21 (38)	16 (29)	5 (9)	
Median age (IQR)	13.5 (12.5)	11.0 (11.8)	15.7 (12.5)	0.829

Table 1. Characteristics of patients with RHO-associated (sector) retinitis pigmentosa at last examination. (continued)

Characteristics	Total (n=100)	Generalized RP (n=75)	Sector RP (n=25)	P-value
Mean refractive error, in D (n=66)				
Mean ± SD	0.8 ±2.85	1.0 ± 3.27	0.2 ± 1.4	0.298
BCVA in the better seeing eye (n=95)				
Median BCVA, in Snellen (IQR)	20/25 (20/30)	20/30 (20/30)	20/20 (20/200)	**<0.001**
BCVA in the worst seeing eye (n=95)				
Median BCVA, in Snellen (IQR)	20/30 (20/25)	20/33 (20/30)	20/22 (20/60)	**0.002**
Electroretinography patterns (n=52)				
Normal responses, n (%)	2 (4)	-	2 (11)	
Reduced responses, n (%) †	5 (10)	1 (3)	4 (22)	
Rod-cone patterns, n (%)	28 (54)	16 (47)	12 (67)	
Minimal responses, n (%)	6 (11)	6 (18)	-	
Non-detectable, n (%)	11 (21)	11 (32)	-	**<0.001**‡
Retinal seeing areas, V4e (n=57)*				
Median seeing areas, in mm^2 (IQR)	256.4 (545.6)	128.0 (552.4)	487.2 (248.3)	0.067
Retinal seeing areas, I4e (n=55)*				
Median seeing areas, in mm^2 (IQR)	17.4 (101.4)	14.5 (66.5)	130.6 (194.36)	**0.011**
Visual field patterns (n=75)				
Normal, n (%)	1 (1)	1 (2)	-	
Peripheral constriction, n (%)	24 (32)	19 (32)	5 (31)	
Midperipheral scotoma, n(%)	6 (8)	5 (9)	1 (6)	
Central island with peripheral remnants, n (%)	18 (24)	15 (25)	3 (19)	
Central preservation, n (%)	19 (25)	18 (30)	1 (6)	
Superior hemisphere, n (%)	7 (10)	1 (2)	6 (38)	**0.002**‡
Cystoid macular edema, n (%)	16/32 (50)	14/25 (56)	2/7 (29)	0.225‡
Central retinal thickness (n=32)*				
Median thickness in μm (IQR)	253.0 (94.0)	254.0 (122.0)	248.5 (92.5)	0.858
Outer nuclear layer thickness (n=32)*				
Median thickness in μm (IQR)	97.5 (47.25)	89.5 (52.5)	110.5 (41.9)	0.121
PR+RPE thickness (n=32)*				
Median thickness in μm (IQR)	90.5 (18.3)	89.5 (20.0)	95.3 (18.1)	0.147
Ellipsoid zone band width (n= 26)*				
Mean width in μm ± SD	2704.5 ± 1881.9	2125.0 ± 1544.8	4277.5 ± 1909.6	**0.007**
Hyper-AF ring diameter (n=15)*				
Horizontal border in μm ± SD	3541.6 ± 1930.6	3484.9 ± 2058.5	3910.3 ± 1009.4	0.571
Vertical border in μm ± SD	2652.7 ± 1567.7	2594.9 ± 1628.0	3201.5 ± 883.2	0.286

*Significant p values (p<0.05) are indicated in bold. The last available examination was used for documentation. RP, retinitis pigmentosa; SD, standard deviation; D, diopters; IQR, interquartile range; PR+RPE, photoreceptor-retinal pigment epithelium; hyper-AF, hyperautofluorescent. *Averaged between eyes. † No clear rod or cone response was documented. ‡ Fisher's exact test was performed.*

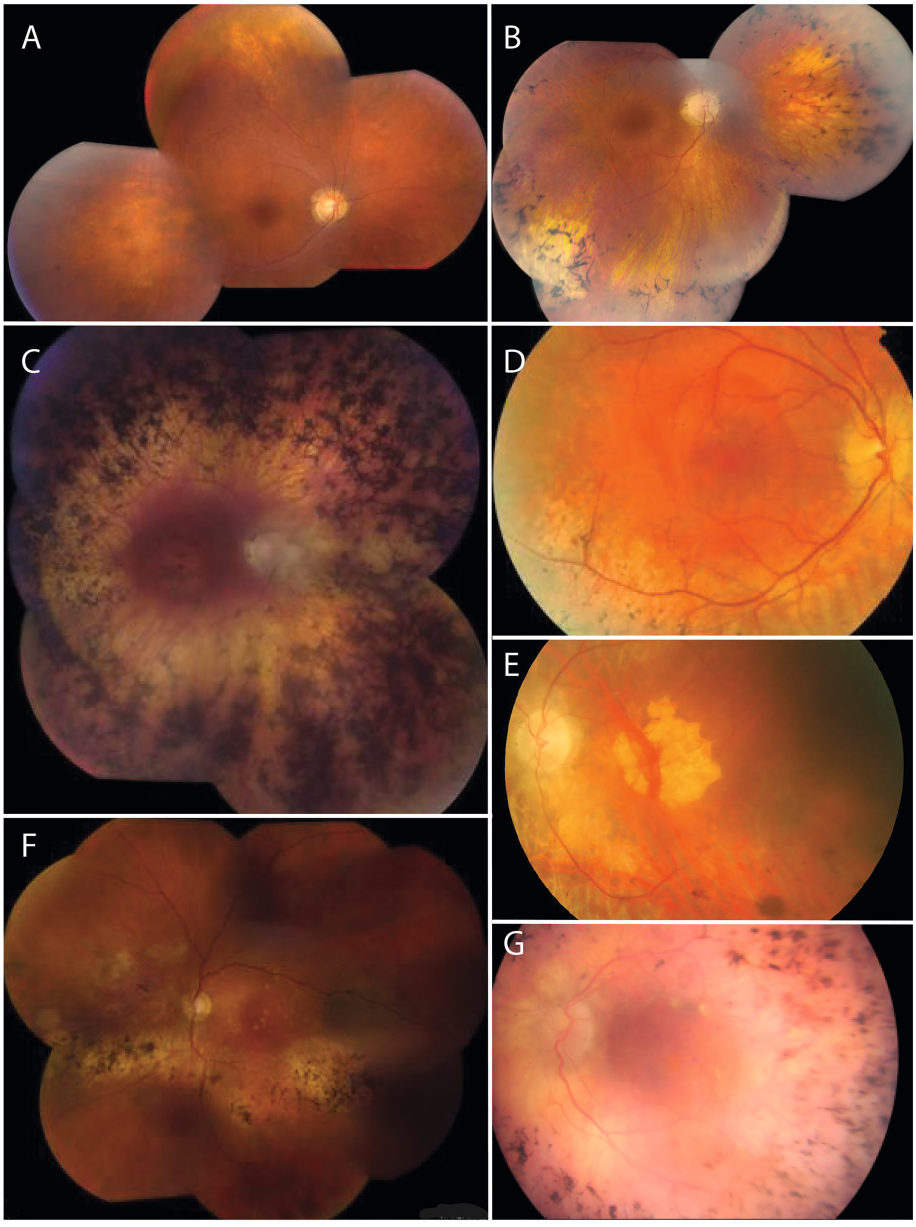

Figure 1. Colour fundus photography in this cohort of *RHO*-associated retinitis pigmentosa (RP). A-C, Illustrations of interfamilial and intrafamilial variability fundus in a family with p.(Tyr178Asn) mutations in the *RHO* gene. A, Patient-ID 65, aged 38, with mutation p.(Tyr178Asn). Peripapillary atrophy and moderate peripheral chorioretinal atrophy was present on fundus photography. No intraretinal hyperpigmentation was seen (best-corrected visual acuity [BCVA]: 20/20 in both eyes). B, Patient-ID 66, aged 45 years, with *RHO* mutation p.(Tyr178Asn). Fundus photography showed chorioretinal atrophy and bone-spicule hyperpigmentation in the midperiphery (BCVA right eye: 20/25; BCVA left eye: 20/67). C, Patient-ID 63, aged 50

years, carrying a p.(Tyr178Asn) in *RHO*. Fundus photography revealed optic disc pallor, attenuated vessels, ring-shaped atrophy in the macula and diffuse intraretinal bone-spicule hyperpigmentations (BCVA was 20/400 in both eyes). D, Patient-ID 52, aged 45 years, with *RHO* mutation p.(Glu28His). A sectorial RP phenotype is seen on colour fundus photography of the right eye, with peripapillary atrophy, and atrophic areas and bone spicule hyperpigmentation around the inferior vascular arcade (BCVA: 20/20 in both eyes). E, Patient-ID 51, aged 87 years, with *RHO* mutation p.(Leu40Pro). Fundus photography showed mild optic disc pallor with retinal atrophy following the superior and inferior vascular arcade. Geographic atrophy is visible at the macula of the left eye, resulting in a BCVA of only light perception. F, Patient-ID 80, aged 74 years, carrying a p.(Asn15Ser) mutation in *RHO*. Fundus photography of the left eye revealed paravascular atrophy mainly in the inferior quadrant with pigment clumping around these atrophic areas. Drusen can be seen around the macula (BCVA right eye: 20/22; BCVA left eye: 20/67). G, Patient-ID 48, aged 74 years, with the p.(Asp190Gly) *RHO* mutation, showing advanced RP. A pale fundus with a waxy pale optic disc, severely attenuated vessels, cystoid macular edema and bone-spicule hyperpigmentation in the midperipheral retina (BCVA right eye: 20/667; BCVA left eye: 20/1000).

Visual function

BCVA data were available for 95 patients, with a high degree of symmetry between eyes (Spearman's $\rho = 0.888$; $p < 0.001$). In 25 patients, a degree of BCVA-based visual impairment was present during the last examination (see Figure, Supplemental Digital Content 4). Time-to-event analysis of the entire cohort revealed a median age of 72 years to reach mild visual impairment, while the median ages for low vision, severe and blindness could not be computed (Figure 2). First occurrences of low vision were seen from the 3th decade of life onwards, whereas severe visual impairment and blindness were seen from the 5th decade of life onwards. In the sector RP cohort, the first occurrence of blindness was seen after the 8th decade of life in a patient with age-related macular degeneration. Linear mixed-model analysis revealed an age effect on mean BCVA, which was 0.012 logMAR (-2.9%; $p < 0.001$) per year for the entire cohort. BCVA decline was significantly faster in patients with a generalized RP phenotype than in patients with a sector RP phenotype ($p = 0.002$), with BCVA progression rates of 0.012 logMAR (-3.8%; $p < 0.001$) and -0.002 logMAR (+0.4%; $p = 0.671$) per year, respectively. For generalized RP patients, we found no differences in baseline BCVA values ($p=0.360$) or in progression slopes ($p = 0.168$) between affected protein domains.

Initial ERG findings were documented in 52 patients (Table 1). Minimal and non-detectable ERG responses were only found in the generalized RP group. The mean age at which non-detectable ERG responses were first observed was 35.3 years (SD 16.1; range 17.1-63.7). Longitudinal ERG data were available for 10 patients, with a mean follow-up time of 6.7 years (SD 4.0; range 0.5-13.6). Eight patients showed no clear changes in ERG-patterns during follow-up. Two patients, aged 29 and 26, displayed rod-cone patterns at the initial visit, which progressed to minimal and absent responses over a time span of 8.6 and 13.6 years, respectively.

Original GVF records were available for 59 patients, with high degree of symmetry between eyes for the V4e (Spearman's $\rho = 0.957$; $p < 0.001$), and the I4e (Spearman's $\rho = 0.935$; $p < 0.001$) retinal seeing areas. Various patterns of visual field defects were

observed, ranging from mild concentric constriction to central islands (Figure 3). Intrafamilial variability was present, as patients carrying the p.(Glu181Lys) mutation could demonstrate different visual field defects (Figure 3A-B). Time-to-event analysis of the visual fields revealed median ages of 44, 52 and 79 years for mild visual impairment, low vision and blindness, respectively (Figure 1B). For the V4e seeing areas, a faster decline of retinal seeing areas was observed in generalized RP patients compared to sector RP patients ($p = 0.005$), with a significant decline rate of -5.6% per year ($p < 0.001$) for generalized RP patients, but not in sector RP (+1.7% per year, $p = 0.477$). No differences in V4e seeing areas were seen between domains at baseline ($p = 0.240$), nor with increasing age ($p = 0.085$) in generalized RP patients. For the I4e seeing areas, we found the age effect to be -5.5% per year ($p < 0.001$) in generalized RP patients, and +0.2% per year ($p = 0.930$) in sector RP patients. For generalized RP patients, we found differences at baseline ($p = 0.013$), with larger I4e seeing areas in patients with extracellular mutations than patients with transmembrane ($p = 0.005$) or cytoplasmic ($p = 0.026$) mutations. No differences in progression slopes of I4e seeing areas were observed between affected domains ($p = 0.233$).

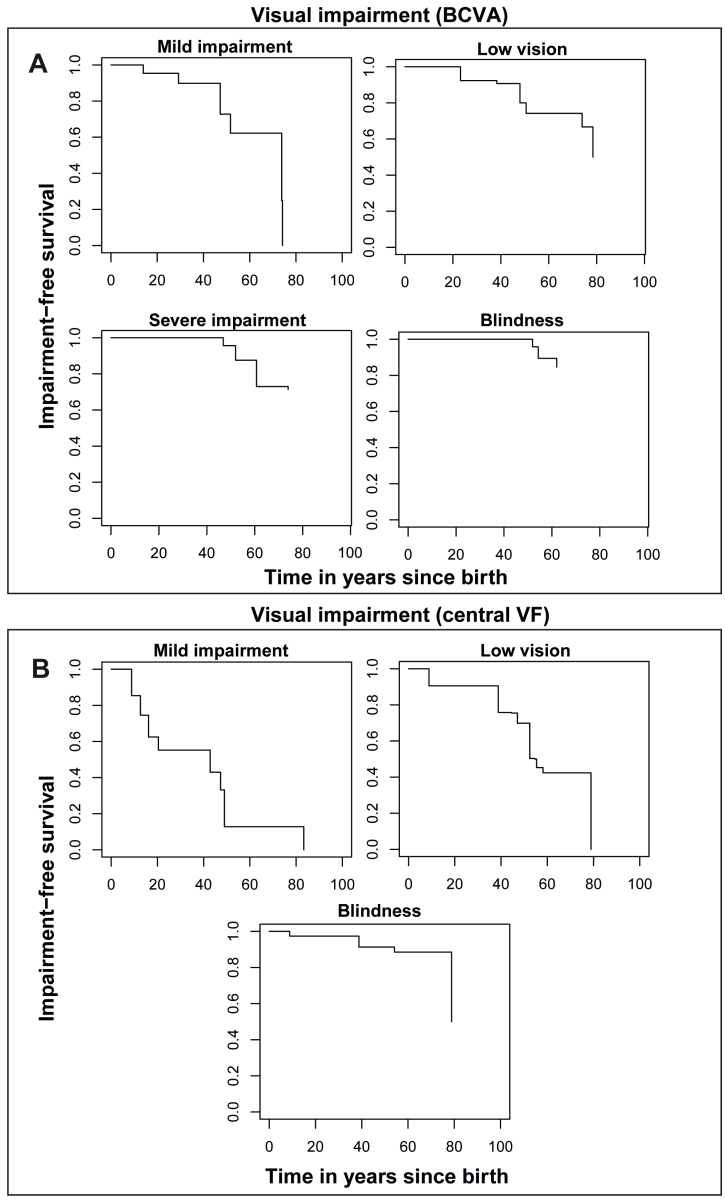

Figure 2. Time-to-event analysis illustrating the time to reaching visual impairment based on the definition set by the World Health Organization. Due to the presence of left, interval and right censored data to estimate the time to low vision, severe impairment and blindness, the nonparametric maximum likelihood estimator (NPMLE) was employed. **A**. Time-to-event analysis of the best-corrected visual acuity (BCVA) in the better-seeing eye demonstrating the time to reaching mild visual impairment ((20/67 ≤ BCVA < 20/40), low vision (20/200 ≤ BCVA < 20/67), severe visual impairment (20/400 ≤ BCVA < 20/200) or blindness (BCVA < 20/400). **B**. For central visual fields (central VF), the following endpoints were used: mild impairment (20° ≤ central VF < 70°), low vision (central VF < 20°) and blindness (central VF < 10°).

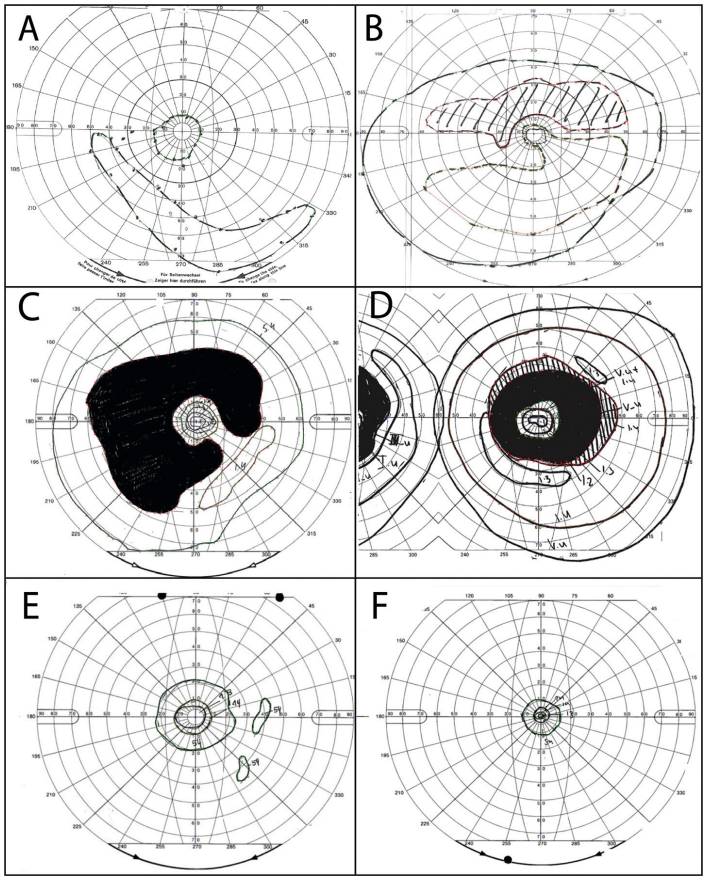

Figure 3. Representative patterns of visual field loss in patients with *RHO*-associated retinitis pigmentosa. **A-B**, intrafamilial variability in a family carrying the p.(Glu181Lys) mutation. **A**, Patient ID-30, aged 54 years, showed a central island with peripheral remnants (best-corrected visual acuity [BCVA] right eye: 20/100; BCVA left eye: 20/125) on kinetic perimetry, while the younger brother (**B**), aged 46 years, had an absolute scotoma in the superior hemifield (BCVA: 20/16 in both eyes). **C**, Patient-ID 52, aged 43 years, with the *RHO* missense mutation p.(Glu28His). An incomplete midperipheral annular scotoma was visible in the left eye (BCVA right eye: 20/25; BCVA left eye: 20/20). **D**, Patient-ID 39, aged 52 years, with a mutation c.937-2A>C (p.(?)) splice site mutation in *RHO*, showing a complete ring scotoma in the right eye (BCVA right eye: 20/67; BCVA left eye: 20/400). **E**, Patient-ID 59, aged 22 years, with *RHO* mutation p.Arg135Trp, showed severe constriction of the V4e and I4e isopters with central preservation of the visual field and small midperipheral visual field remnants (BCVA right eye: 20/67; BCVA left eye: 20/40). **F**, Patient-ID 44 (38 years of age), carrying the p.(Asp190Tyr) *RHO* mutation, had marked peripheral visual field loss with only a residual central island remaining (BCVA right eye: 20/50; BCVA left eye: 20/32).

Multimodal imaging

SD-OCT imaging was performed in 32 patients, with thickness measurements of retinal layers specified in Table 1**.** We found cystoid macular edema (CME) in 16 out of 32 patients (50%), which was located in the fovea for 12/16 (75%) patients.

No significant age effect was found on central retinal thickness (CRT) (p = 0.371) or ONL thickness (p = 0.502), after exclusion of patients with foveal CME. For PR+RPE measurements, advancing age was associated with the loss of PR+RPE thickness (-0.6%/year; p = 0.030), which was not affected by a sector RP phenotype (p = 0.611). The PR+RPE thickness was the only parameter, after exclusion of patients with foveal CME and correction for multiple testing, that correlated with BCVA (Table 2). The macular ellipsoid zone (EZ) band width, measurable in 26/32 (81%) patients, decreased with advancing age (-3.8%; p < 0.001), which was not significantly different in sector RP patients (p = 0.589). However, a larger EZ band width at baseline was seen in patients with a sector RP phenotype (p = 0.018). For generalized RP patients, no differences in the EZ band width at baseline (p = 0.185) or for the decline rates (p=0.886) were observed between affected protein domains. In 6/26 patients (23%), the EZ band width went beyond the scanning range on SD-OCT (Figure 4A). A granular interrupted aspect of the EZ band was seen in 9/26 (35%) patients.

FAF imaging, available for 38 patients, revealed hyper-autofluorescent (hyper-AF) and hypo-autofluorescent (hypo-AF) patterns in variable degrees, including a hyper-AF macular ring in 26/38 (68%) patients (Figure 4). In patients with sector RP, a common pattern seen on FAF was a hypo-AF distribution along the inferior vascular arcade, which corresponded with areas of degeneration seen on fundus photography (Figure 4D). A high degree of correlation was found between EZ band width and the horizontal (ρ = 0.923; p < 0.001), and vertical borders (ρ = 0.937; p < 0.001) of the hyper-AF macular ring.

Table 2. Structure and function correlations in *RHO*-associated retinitis pigmentosa.

	BCVA (logMAR)		Seeing retinal area V4e		Seeing retinal area I4e	
Visual function parameter	Spearman's rho	P-value	Spearman's rho	P-value	Spearman's rho	P-value
SD-OCT imaging						
CRT*	-0.370	0.095	0.291	0.274	0.086	0.770
ONL thickness*	-0.234	0.366	-0.029	0.923	-0.162	0.596
PR+RPE thickness*	-0.733	**<0.001**	0.528	0.053	0.556	0.049
EZ band width	-0.506	0.054	0.198	0.517	0.280	0.379
FAF imaging						
Horizontal ring diameter	-0.339	0.235	0.632	0.024	0.643	0.021
Vertical ring diameter	-0.245	0.419	0.615	0.037	0.566	0.059

The most recent mean values between right and left eyes were used for analysis. Imaging and measurements were performed using Heidelberg inbuilt software (Spectralis SD-OCT + HRA, Heidelberg Engineering, Heidelberg, Germany). Significance level was set at 0.003 following Bonferroni correction. Significant values are in bold.
*BCVA = best-corrected visual acuity. SD-OCT = spectral domain optical coherence tomography. EZ = ellipsoid zone. CRT = central retinal thickness. ONL = outer nuclear layer. PR+RPE = photoreceptor + retinal pigment epithelium complex. FAF = short-wave autofluorescence. * Patients with cystoid macular edema located in the fovea were excluded from analysis.*

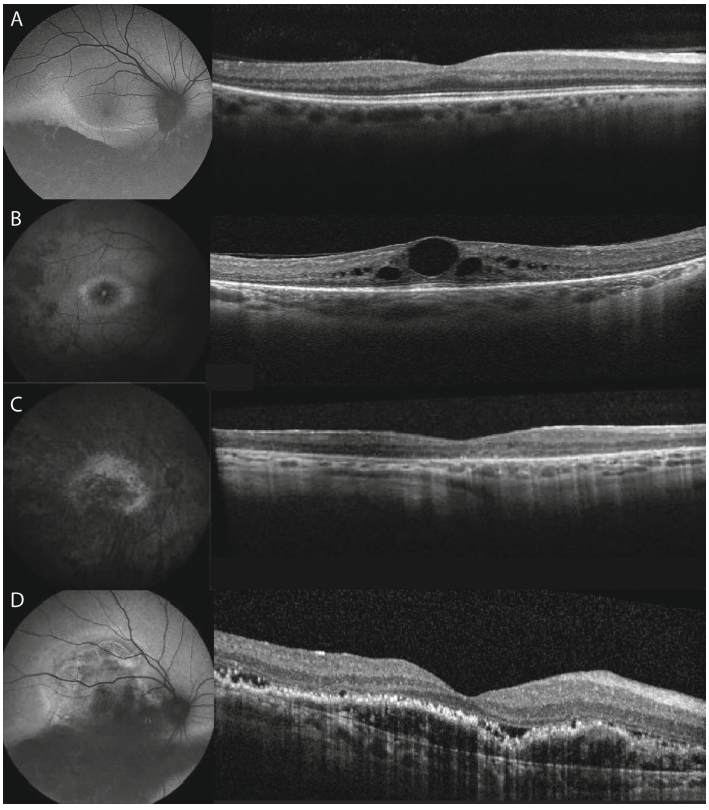

Figure 4. Fundus autofluorescence (FAF) and corresponding spectral-domain optical coherence tomography (SD-OCT) imaging in patients with *RHO*-associated retinitis pigmentosa. **A**, Patient-ID 81, aged 47 years, carrying the missense mutation p.(Asn15Ser). FAF imaging showed an inferior sectoral hypo-autofluorescence (hypo-AF), and ERG responses were reduced in a rod-cone pattern. SD-OCT imaging in this patient revealed central preservation of outer retinal layers, but with thinning of these layers in the peripheral macula. The ellipsoid zone (EZ) band width went beyond the scanning range nasally (best-corrected visual acuity [BCVA] right eye: 20/20; BCVA left eye: 20/22). **B**, Patient-ID 70, aged 26 years, with a p.(Glu181Lys) missense mutation. A well-demarcated hyper-autofluorescent (hyper-AF) ring was visible on FAF, with hypo-AF regions inside the hyper-AF ring. A small hyper-AF spot was seen at the foveal site, corresponding to the site of cystoid macular edema on SD-OCT. The midperipheral retina displayed normal AF regions surrounded by granular hypo-AF in variable intensities. Cystoid fluid collections were present in the inner and outer nuclear layer (BCVA right eye: 20/28; BCVA left eye, 20/33). **C**, Patient-ID 88, aged 60 years, carrying a p.(Pro347Leu) missense mutation in the *RHO* gene. FAF imaging reveals the near-absence of AF in the fovea and midperipheral retina, with residual AF remaining in the posterior pole. No evident hyper-AF ring is seen. Profound atrophy of all retinal layers is present on SD-OCT, with increased visibility of the underlying choroidal vessels. Granular remnants of the EZ are seen at the central fovea, but the EZ is completely absent in the peripheral macula (BCVA was 20/400 in both eyes). **D**, Patient-ID 80, aged 74 years, carrying the *RHO* missense mutation p.(Asn15Ser). FAF imaging showed sectorial degeneration hypo-AF along the inferior vascular arcade, corresponding with the RPE atrophy seen on fundus photography (Figure 1F). On SD-OCT, a pigment epithelial detachment was observed. The partly hyperreflective structures underlying the RPE detachment suggest presence of a neovascular membrane. The different layers of the neuroretina were still discernible, with a BCVA of 20/33 and 20/22 in the right and left eye, respectively.

DISCUSSION

This multicenter study provides a detailed description of the natural history of *RHO*-associated adRP, using cross-sectional and longitudinal data. We found a high prevalence of the p.(Gly181Lys) mutation (n=23), in 4 different families, accounting for 37% of the Dutch cohort. This mutations has rarely been described in other populations, which is suggestive of a founder effect of p.(Gly181Lys) in the Dutch *RHO*-associated adRP patients. Common mutations exclusively found in the Belgian patients of our cohort were p.(Ile255del) and p.(Tyr178Asp). The p.(Tyr178Asp) mutation has never been described outside the Belgian population,[18] whereas the p.(Ile255del) mutation has previously been found in a single Irish family.[19]

Several mutations, such as the p.(Glu181Lys), found in this cohort could present as either generalized or sector RP, which underlines the potential influence of genetic and/or other modifiers on the phenotype. As suggested previously, it is possible that sector RP will eventually transition into a generalized RP phenotype in later stages of the disease.[7] However, we were not able to observe this transition in any of our patients. In addition, we found patients that retained a sector RP phenotype up to the 8th decade of life.

Our reported progression rates of BCVA (-3.8%/year) and V4e seeing areas (-5.6%/year) in patients with generalized RP were faster than those reported by a previous natural history study on *RHO*-associated RP, which reported rates of -1.8% per year for BCVA and -2.6% per year for V4e seeing areas.[9] One possible explanation for this discrepancy is the difference in statistical methods. In contrast to the study of Berson and collaegues,[9] we analyzed patients with a sector RP separately, as these patients demonstrated minimal disease progression and may contribute to a ceiling effect. Additionally, there are notable genetic differences between our population and the one in the American study by Berson and colleagues. The p.(Pro23His) mutation, which is the most common *RHO* mutation in the United States (36% in their cohort), is known to express a particularly mild phenotype, and is also described in patients with sector RP.[20] To the best of our knowledge, this founder mutation has never been reported in European studies, including the present one.[21, 22]

Patients with a generalized form of RP were stratified based on the domains affected by *RHO* mutations. At baseline, we found larger EZ band widths on OCT and I4 seeing areas on GVF in patients carrying extracellular mutations, compared to patients with transmembrane or cytoplasmic mutations. No differences in annual decline rates of EZ band widths and I4e retinal seeing areas were found between mutated protein domains. In addition, mutations causing sector RP, showing minimal disease progression, were predominantly found in the extracellular domains. These findings support previous research in suggesting that extracellular mutations causes

milder phenotypes in *RHO*-associated RP.[23-25] The differences in disease expression between affected domains can be attributed to the biochemical defects caused by the mutations within these domains, although the role of external modifiers such as increased light exposure, especially in the development of sector RP, may also play a role.[2, 7]

A limitation of this study is its retrospective nature, which limited a complete ascertainment of clinical data. ERG data were not available for all patients, and were mainly performed at baseline for diagnostic purposes. For this reason, a previously used classification system could not be applied to this cohort.[10, 23, 24] A prospective standardized natural history study on *RHO*-associated RP, which is currently unavailable to the best of our knowledge, should be able to address such limitations.

Pre-clinical studies on several gene knockdown and replacement strategies for *RHO*-associated RP have shown promising results, paving the way for human gene therapy trials.[11, 12] Our current clinical findings can have significant implications for future clinical treatment trials. We found a high degree of between-eye symmetry for all visual parameters (BCVA, V4e and I4e seeing retinal areas), supporting the use of the fellow eye as a control in intervention studies. On SD-OCT imaging, we found a high prevalence of CME, which may be a concern for future gene therapy trials, as the presence of CME may alter the retinal morphology, challenging correct injection of viral vectors into the subretinal space, and posing additional risks for per- and postoperative complications.[26] The borders of the hyper-AF ring on FAF imaging, which demarcates the transition zone between affected and unaffected retina,[27] correlated strongly with the EZ band width. FAF imaging should be used in conjunction with SD-OCT, in order to capture disease progression in less advanced stages of *RHO*-associated RP, in which the EZ band width can go beyond the 30° scanning range of SD-OCT (occurring in 23% of our cases).

The optimal intervention window for *RHO*-associated RP is before the 5th decade of life, as time-to-event analysis of visual fields revealed median ages of 52 and 79 for low vision and blindness, respectively. The use of visual acuity as an endpoint may be impractical for *RHO*-associated RP, due to the relatively late onset of BCVA-based impairment. Therefore, the use of surrogate endpoints for visual acuity could accelerate the measurement of disease progression and treatment response. We and others have previously found that the PR+RPE complex has been suggested to be a good predictor of visual acuity.[15, 28-30] Similar results were found in this study, as the PR+RPE complex correlated strongly with BCVA, and was the only parameter that remained significant after correction for multiple testing. The PR+RPE complex can potentially be used to identify early structural changes before visual acuity loss may be noticeable, which can be particularly useful in diseases with relatively slow disease progression such as *RHO*-associated RP. In anticipation of future clinical trials for *RHO*,

the establishment of potential clinical endpoints are necessary steps for an optimal study design. In this regard, this study highlights the potential use of the PR+RPE complex as a surrogate endpoint for BCVA in future clinical trials.

ACKNOWLEDGEMENTS

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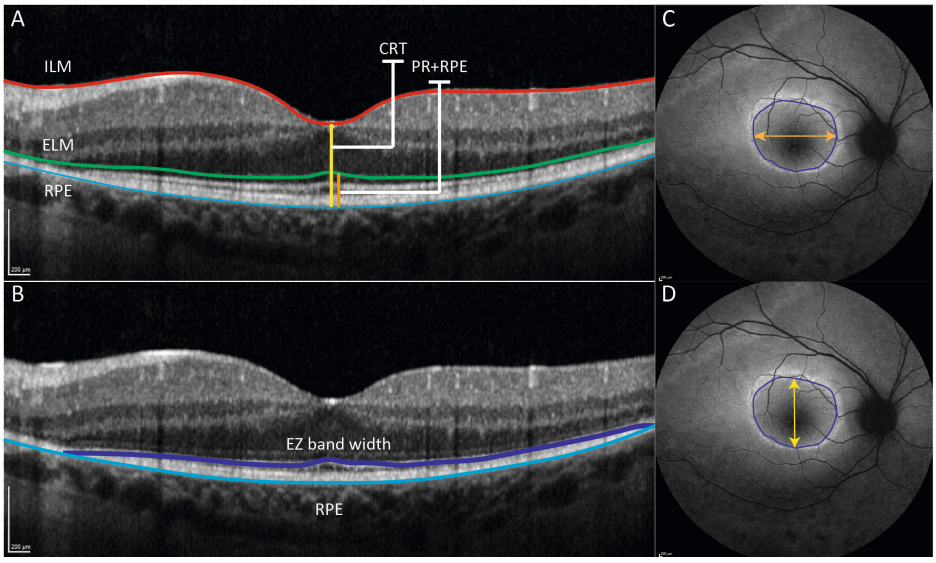
REFERENCES

1. Dryja TP, Hahn LB, Cowley GS, et al. Mutation spectrum of the rhodopsin gene among patients with autosomal dominant retinitis pigmentosa. Proceedings of the National Academy of Sciences of the United States of America. 1991;88(20):9370-9374.
2. Verbakel SK, van Huet RAC, Boon CJF, et al. Non-syndromic retinitis pigmentosa. Progress in Retinal and Eye Research. 2018;66:157-186.
3. Zeitz C, Gross AK, Leifert D, et al. Identification and functional characterization of a novel rhodopsin mutation associated with autosomal dominant CSNB. Investigative Ophthalmology & Visual Science. 2008;49(9):4105-4114.
4. Comitato A, Di Salvo MT, Turchiano G, et al. Dominant and recessive mutations in rhodopsin activate different cell death pathways. Hum Mol Genet. 2016;25(13):2801-2812.
5. Kranich H, Bartkowski S, Denton MJ, et al. Autosomal dominant 'sector' retinitis pigmentosa due to a point mutation predicting an Asn-15-Ser substitution of rhodopsin. Human Molecular Genetics. 1993;2(6):813-814.
6. Berson EL, Rosner B, Sandberg MA, et al. Ocular findings in patients with autosomal dominant retinitis pigmentosa and a rhodopsin gene defect (pro-23-his). Archives of Ophthalmology. 1991;109(1):92-101.
7. Ramon E, Cordomí A, Aguilà M, et al. Differential light-induced responses in sectorial inherited retinal degeneration. J Biol Chem. 2014;289(52):35918-35928.
8. Iannaccone A, Man D, Waseem N, et al. Retinitis pigmentosa associated with rhodopsin mutations: Correlation between phenotypic variability and molecular effects. Vision Research. 2006;46(27):4556-4567.
9. Berson EL, Rosner B, Weigel-DiFranco C, et al. Disease progression in patients with dominant retinitis pigmentosa and rhodopsin mutations. Investigative Ophthalmology & Visual Science. 2002;43(9):3027-3036.
10. Mendes HF, van der Spuy J, Chapple JP, et al. Mechanisms of cell death in rhodopsin retinitis pigmentosa: implications for therapy. Trends in Molecular Medicine.11(4):177-185.
11. Tsai Y-T, Wu W-H, Lee T-T, et al. Clustered regularly interspaced short palindromic repeats-based genome surgery for the treatment of autosomal dominant retinitis pigmentosa. Ophthalmology. 2018.
12. Cideciyan AV, Sudharsan R, Dufour VL, et al. Mutation-independent rhodopsin gene therapy by knockdown and replacement with a single AAV vector. Proceedings of the National Academy of Sciences. 2018;115(36):E8547.
13. van Huet RAC, Oomen CJ, Plomp AS, et al. The RD5000 Database: facilitating clinical, genetic, and therapeutic studies on inherited retinal diseases. Investigative Ophthalmology & Visual Science. 2014;55(11):7355-7360.
14. Dagnelie G. Technical note. Conversion of planimetric visual field data into solid angles and retinal areas. Clinical Vision Science. 1990;5(1):95-100.
15. Spaide RF, Curcio CA. Anatomical correlates to the bands seen in the outer retina by optical coherence tomography: literature review and model. Retina. 2011;31(8):1609-1619.
16. R: A language and environment for statistical computing [computer program]. Vienna, Austria: R Foundation for Statistical Computing; 2010.
17. Talib M, van Schooneveld MJ, Thiadens AA, et al. CLINICAL AND GENETIC CHARACTERISTICS OF MALE PATIENTS WITH RPGR-ASSOCIATED RETINAL DYSTROPHIES: A Long-Term Follow-up Study. RETINA. 2019;39(6):1186-1199.

18. Van Cauwenbergh C, Coppieters F, Roels D, et al. Mutations in splicing factor genes are a major cause of autosomal dominant retinitis pigmentosa in Belgian families. PLoS ONE. 2017;12(1):e0170038.
19. Inglehearn CF, Bashir R, Lester DH, et al. A 3-bp deletion in the rhodopsin gene in a family with autosomal dominant retinitis pigmentosa. American Journal of Human Genetics. 1991;48(1):26-30.
20. Oh KT, Weleber RG, Lotery A, et al. Description of a new mutation in rhodopsin, pro23ala, and comparison with electroretinographic and clinical characteristics of the pro23his mutation. Archives of Ophthalmology. 2000;118(9):1269-1276.
21. Blanco-Kelly F, García-Hoyos M, Cortón M, et al. Genotyping microarray: Mutation screening in Spanish families with autosomal dominant retinitis pigmentosa. Molecular Vision. 2012;18:1478-1483.
22. Audo I, Manes G, Mohand-Saïd S, et al. Spectrum of rhodopsin mutations in French autosomal dominant rod-cone dystrophy patients. Investigative Ophthalmology & Visual Science. 2010;51(7):3687-3700.
23. Cideciyan AV, Hood DC, Huang Y, et al. Disease sequence from mutant rhodopsin allele to rod and cone photoreceptor degeneration in man. Proceedings of the National Academy of Sciences of the United States of America. 1998;95(12):7103-7108.
24. Jacobson SG, McGuigan DB, Sumaroka A, et al. Complexity of the class B phenotype in autosomal dominant retinitis pigmentosa due to rhodopsin mutations. Investigative Ophthalmology & Visual Science. 2016;57(11):4847-4858.
25. Schuster A, Weisschuh N, Jägle H, et al. Novel rhodopsin mutations and genotype-phenotype correlation in patients with autosomal dominant retinitis pigmentosa. British Journal of Ophthalmology. 2005;89(10):1258.
26. Xue K, Groppe M, Salvetti AP, et al. Technique of retinal gene therapy: delivery of viral vector into the subretinal space. Eye. 2017;31(9):1308-1316.
27. Aizawa S, Mitamura Y, Hagiwara A, et al. Changes of fundus autofluorescence, photoreceptor inner and outer segment junction line, and visual function in patients with retinitis pigmentosa. Clinical & Experimental Ophthalmology. 2010;38(6):597-604.
28. Eliwa TF, Hussein MA, Zaki MA, et al. Outer retinal layer thickness as good visual predictor in patients with diabetic macular edema. RETINA. 2018;38(4).
29. Lim JI, Tan O, Fawzi AA, et al. A pilot study of Fourier-domain optical coherence tomography of retinal dystrophy patients. American Journal of Ophthalmology. 2008;146(3):417-426.
30. Talib M, van Schooneveld MJ, Van Cauwenbergh C, et al. The Spectrum of Structural and Functional Abnormalities in Female Carriers of Pathogenic Variants in the RPGR Gene. Investigative Ophthalmology & Visual Science. 2018;59(10):4123-4133.

SUPPLEMENTAL CONTENT



Supplementary Figure S1. Measurement of structural layers and hyperautofluorescent foveal rings using the Heidelberg System (Spectralis SD-OCT + HRA, Heidelberg Engineering, Heidelberg, Germany). Errors in segmentation were corrected manually. (**A**) Measurement of the central retinal thickness (CRT), which was defined as the foveal thickness between the internal limiting membrane (ILM) and the retinal pigment epithelium (RPE) layer. Measurement of the photoreceptor-retinal pigment epithelium complex (PR+RPE), was defined as the foveal thickness between the external limiting membrane (ELM) and the basal border of the RPE layer. (**B**) The ellipsoid zone band width (dark blue) was followed nasally and temporally until it was indistinguishable from the retinal pigment epithelium (light blue). (**C-D**) On fundus autofluorescence imaging, the inner borders of the hyperautofluorescent ring were measured in the horizontal (C) and vertical axis (D) as illustrated.

Supplemental Digital Content 2. Cohort characteristics of Dutch and Belgian patients with *RHO*-associated dystrophies.

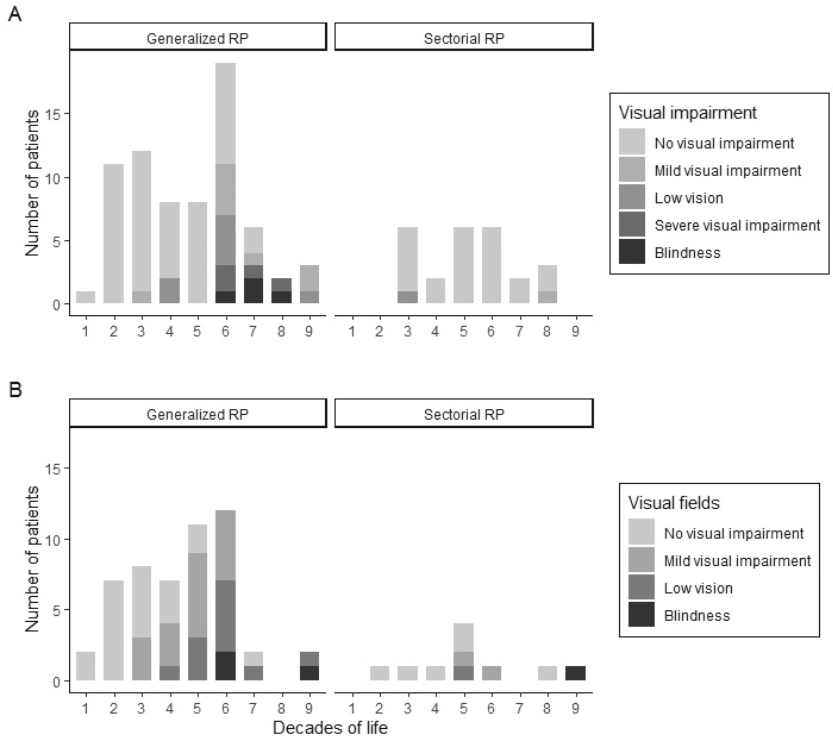
Characteristics	Total	Dutch patients	Belgian patients	P-value
Age at last examination (years), n	100	63	37	
Mean ± SD	43.5±18.5	44.6±18.3	41.6±18.7	0.426
Range	10.0-87.7	14.3-87.7	10.0-82.4	
Follow-up time (years), no.	72	44	28	
Median FU time (IQR)	6.9 (11.9)	6.8 (13.3)	6.9 (7.5)	0.238
Range	0.2-41.0	0.2-36.4	0.2-41.0	
BCVA at the last visit (decimals), n	95	61	34	
Median BCVA, Snellen (IQR)	20/25 (20/30)	20/25 (20/30)	20/27 (20/36)	0.368
Range	LP-20/12	20/1000-20/12	LP-20/20	
Seeing areas at last visit (I4e, mm^2), n	55	25	30	
Median isopter size ((IQR)	17.4 (101.4)	29.1	15.0	0.106
Range	0.8-667.8	1.2-667.8	0.8-292.5	
Seeing areas at last visit (V4e, mm^2), n	57	24	33	
Median isopter size (IQR)	256.4 (545.6)	322.1 (547.5)	205.6 (545.6)	0.559
Range	4.0-764.9	14.5-763.9	3.7-764.9	

SD = standard deviation. FU = follow-up. BCVA = best-corrected visual acuity. IQR = interquartile range. LP = light perception.

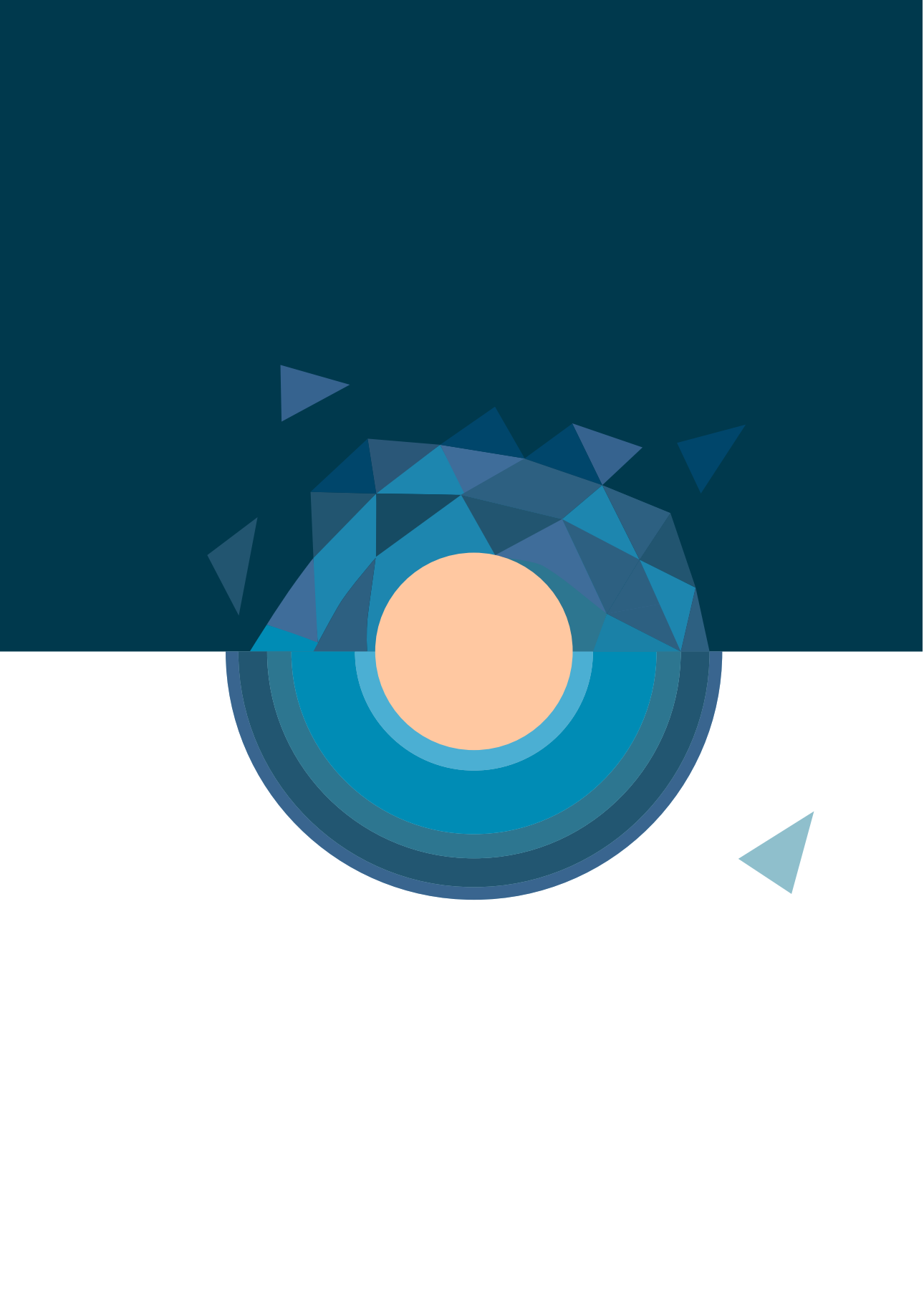
Supplemental Digital Content 3. Overview of mutations included in this study of *RHO*-associated retinitis pigmentosa.

Families affected	#	Nucleotide change	Amino acid change	Domain*	References
1	2	c.11C>A	p.(Thr4Lys)†	Extracellullar	Van den Born et al. 1994
3	4	c.44A>G	p.(Asn15Ser)†	Extracellullar	Yoshi et al. 1998
2	2	c.50C>T	p.(Thr17Met)†	Extracellullar	Dryja et al. 1991
2	2	c.68C>T	p.(Pro23Leu)†	Extracellullar	Dryja et al. 1991
3	8	c.84G>T	p.(Gln28His)†	Extracellullar	Fernandez al. 2014
1	1	c.119T>C	p.(Leu40Pro)	Transmembrane	De Sousa Dias et al. 2015
1	1	c.133T>C	p.(Phe45Leu)	Transmembrane	Dryja et al. 2000
1	3	c.265G>C	p.(Gly89Arg)	Transmembrane	Van Cauwenbergh et al. 2017
3	7	c.403C>T	p.(Arg135Trp)	Cytoplasmic	Jacobson et al 1991
1	4	c.512C>A	p.(Pro171Gln)	Transmembrane	Antiolo et al. 1994
1	6	c.532T>G	p.(Tyr178Asp)	Extracellullar	Van Cauwenbergh et al. 2017
1	1	c.538C>T	p.(Pro180Ser)	Extracellullar	Neveling et al. 2012
4	23	c.541G>A	p.(Glu181Lys)†	Extracellullar	Blanco-Kelly et al. 2012 Coussa et al. 2015
2	3	c.563G>A	p.(Gly188Glu)	Extracellullar	Macke et al. 1993
1	1	c.568G>A	p.(Asp190Asn)†	Extracellullar	Tsui et al. 2008
3	5	c.569A>G	p.(Asp190Gly)†	Extracellullar	Dryja et al. 1991
5	7	c.568G>T	p.(Asp190Tyr)	Extracellullar	Blanco-Kelly et al. 2012
1	1	c.641T>A	p.(Ile214Asn)†	Transmembrane	Neveling et al. 2012
1	1	c.759G>T	p.(Met253Ile)	Transmembrane	Van Huet et al. 2015
1	6	c.763_765del	p.(Ile256del)	Transmembrane	Inglehearn et al. 1991
1	3	c.911T>A	p.(Val304Asp)	Transmembrane	Van Cauwenbergh et al. 2017
1	1	c.937-2A>C	Splice site	-	Novel
2	3	c.1028G>A	p.(Ser343Asn)	Cytoplasmic	Van Cauwenbergh et al. 2017
2	2	c.1033G>A	p.(Val345Met)	Cytoplasmic	Grøndahl al. 2006
3	3	c.1040C>T	p.(Pro347Leu)	Cytoplasmic	Blanco-Kelly et al. 2012 Dryja et al. 1990

*# = Frequency of affected individuals. RP = generalized retinitis pigmentosa. *Protein domain predicted to be affected by RHO mutations. †Mutations associated with a sector RP phenotype.*



Supplemental Digital Content 4. Visual impairment in patients with *RHO*-associated retinitis pigmentosa (RP) by decades of life, classified as either generalized RP or sector RP. **A,** Visual impairment based on last available best-corrected-visual acuity (BCVA), based on the criteria of the World Health Organization (WHO): No visual impairment (BCVA ≥ 20/40), mild visual impairment (20/67 ≤ BCVA < 20/40), low vision (20/200 ≤ BCVA <20/67), severe visual impairment (20/400 ≤ BCVA < 20/200) or blindness (BCVA < 20/400). **B,** For visual impairment based on last available central visual fields (central VF; V4e), the following endpoints were used: mild visual impairment (20° ≤ central VF < 70°), low vision (central VF < 20°) and blindness (central VF < 10°).



CHAPTER 2.2

RPGR-ASSOCIATED DYSTROPHIES: CLINICAL, GENETIC, AND HISTOPATHOLOGICAL FEATURES

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ABSTRACT

This study describes the clinical, genetic, and histopathological features in patients with *RPGR*-associated retinal dystrophies. Nine male patients from 8 unrelated families underwent comprehensive ophthalmic examination. Additionally, the histopathology of the right eye from a patient with an end-stage cone-rod-dystrophy (CRD)/sector retinitis pigmentosa phenotype was examined. All *RPGR* mutations causing a CRD phenotype were situated in exon ORF15. The mean best-corrected visual acuity (BCVA, decimals) was 0.58 (standard deviation [SD]: 0.34; range: 0.05 – 1.13), and the mean spherical refractive error was -4.1D (SD: 2.11; range: -1.38 to -8.19). Hyperautofluorescent rings were observed in 6 patients. Full-field electroretinography responses were absent in all patients. Visual field defects ranged from peripheral constriction to central islands. The mean macular sensitivity on microperimetry was 11.6 dB (SD: 7.8; range: 1.6 - 24.4), and correlated significantly with BCVA ($r = 0.907$; $p = 0.001$). Histological examination showed disruption of retinal topology and stratification, with more severe loss found in the peripheral regions. Reactive gliosis was seen in the inner layers of all regions. Our study demonstrates the highly variable phenotype found in *RPGR*-associated retinal dystrophies. Therapies should be applied at the earliest signs of photoreceptor degeneration, prior to remodeling of the inner retina.

INTRODUCTION

Retinitis pigmentosa (RP) is the most common inherited retinal dystrophy, affecting approximately 1 in 3000 individuals.[1] Its predominant feature is the irreversible loss of rod photoreceptors, with secondary loss of cone photoreceptors. Patients therefore typically present with symptoms of nyctalopia and peripheral visual field constriction, prior to symptoms of central vision loss. X-linked RP (XLRP) accounts for 5-15% of all RP cases, and is recognized as one of the most severe forms of RP.[2, 3] Mutations in *RPGR* gene are responsible for 70-90% of all XLRP cases.[4-6] Symptom onset in affected males starts in childhood years, and is described to reach blindness within the 4th decade of life.[2, 7, 8] Despite the X-linked inheritance of *RPGR,* female carriers may also be affected by XLRP.[9, 10]

Other phenotypes caused by mutations in the *RPGR* gene include X-linked cone-rod dystrophies (CRD) and cone dystrophies (CD), in which degeneration predominantly affects cones, with or without later involvement of rod.[11] Compared to XLRP, the age at onset is later in XL-CRD/CD, typically starting around the 4th decade of life, with initial symptoms being visual acuity loss, color vision defects and variable photophobia.[2, 12]

The *RPGR* gene encodes the retinitis pigmentosa GTPase regulator (RPGR) protein, and is able to express multiple isoforms through alternative splicing. The most common protein isoforms found in the retina are RPGR^{ORF15}, and to a lesser extent, the constitutive protein RPGR$^{1-19}$.[13, 14] The RPGR^{ORF15} transcript, which is believed to play a key role in the intraflagellar transport processes, contains exon 1-14 and exon ORF15, which is formed by alternatively spliced exon 15 and intron 15.[15] Exon ORF15 consists of multiple acidic glutamate-glycine repeats, which promotes polymerase arrest and replication slippage, thus making it a mutational hotspot for XLRP.[16] CRD/CD-disease causing variants in *RPGR* are also located in the ORF15 region, and are typically located at the 3' end of exon ORF15.[17]

At present, no approved treatments for *RPGR*-associated IRDs are available. Recent advances have been made in animal models using subretinal *RPGR*-mediated gene therapy, which has shown an increase in structural and functional survival of photoreceptors.[18,19] These findings have paved the way for human *RPGR*-targeted gene therapy, and phase I/II/III clinical trials are currently ongoing (NCT03252847, NCT03116113 and NCT03316560).[20] To facilitate a better understanding of *RPGR*-associated retinal dystrophies and in view of future therapies, this study provides an extensive prospective phenotypic evaluation of patients harboring mutations in the *RPGR* gene. In addition, we report the histological changes in the retina of a donor *RPGR* patient who had a clinical diagnosis of advanced CRD.

RESULTS

Clinical examination

In total, 9 male patients from 8 different families underwent clinical evaluation, with a mean age of 30.2 years (standard deviation [SD]: 11.54; range: 17.8 – 48.9) at most recent examination. Patients had a diagnosis of RP (n = 8) or CRD (n = 1). Eight different mutations were found, which were located in exon 1-14 (n = 3) or exon ORF15 (n = 5, including the CRD patient), and were generally frameshift mutations (n = 6), followed by nonsense (n = 1) and missense (n = 1) mutations (Table S1). An overview of the clinical findings is provided in Table 1.

For RP patients, the mean age at onset was 6.1 years (SD: 2.8; range: 5.0 - 13.0), with initial symptoms being nyctalopia (n = 6; 75%) or visual field loss (n = 2; 25%). The mean best-corrected-visual-acuity (BCVA; decimals) was 0.57 (SD: 0.36; range: 0.05 - 1.13), and the mean spherical refractive error (SER) ranged from -1.88 D to -8.19 D (mean: -4.32 D; SD: 2.15). Besides the extensive prospective phenotypic evaluation, we were able to retrieve longitudinal BCVA data from medical records of all patients with a mean follow-up of 11.5 years. In patient A-1, visual acuity remained relatively stable until the 4th decade of life, and declined afterwards. BCVA loss correlated significantly with increasing age ($r = -0.857$; $p = 0.008$) in patients with RP. In the CRD patient (H-9), loss of visual acuity was the first symptom, which presented after the 4th decade of life. After initial presentation, BCVA loss (from 0.85 to 0.76) was already seen in a short time span of 0.9 years. The course of BCVA decline of the entire cohort is presented in Figure 1**.**

Table 1. Clinical characteristics of patients with *RPGR*-associated retinal dystrophies at last examination.

					BCVA		SER		Lens status		Fundus features				Goldmann perimetry (V4e)		
Family-ID	Age	Age at onset	Initial symptom	DD	OD	OS	OD	OS	OD	OS	Optic pallor	Attenuated vessels	Bone-spicules	Other relevant findings	Visual field patterns	Retinal seeing areas (mm2) OD|OS	
A-1	45	5	VF loss	RP	0.05	0.05	-1,00	-2,75	Mild PSC	Mild PSC	Yes	Yes	Yes	Bull's eye appearance of macula	Central island with peripheral remnant	95.3	120.4
B-2*	20	5	Night blindness	RP	0.80	1.00	-5,00	-5,13	Clear	Clear	Yes	No	Yes	Optic disc drusen	Peripheral constriction	532.7	481.0
B-3*	20	6	Night blindness	RP	1.25	1.00	-1,88	-0,88	Clear	Clear	Yes	No	Yes	Optic disc drusen	Central island with peripheral remnant	148.8	158.7
C-4	30	5	Night blindness	RP	0.36	0.38	-5,63	-4,88	Clear	Clear	Yes	Yes	Yes	Macular atrophy	Central islands	84.6	73.8
D-5	33	13	VF loss	RP	0.66	0.52	-5,75	-4,38	Clear	Clear	Yes	Yes	Yes	Patches of preserved RPE	Central island with peripheral remnant	279.5	247.6
E-6	22	5	Night blindness	RP	0.50	0.40	-4,13	-4,50	Clear	Clear	Yes	Yes	Yes	Epiretinal membrane	Central island with peripheral remnant	115.2	101.83
F-7	18	5	Night blindness	RP	0.70	1.00	-8,88	-7,50	Clear	Clear	Yes	Yes	Yes	RPE alterations	Midperipheral scotoma	632.2	669.5
G-8	36	5	Night blindness	RP	0.10	0.10	-3,63	-3,25	Clear	Clear	Yes	Yes	Yes	Macular atrophy	Central island	24.8	39.2
H-9	49	48	VA loss	CRD	0.80	0.72	-3,25	-1,50	Clear	Clear	Yes	No	No	Bull's eye appearance of macula	Central scotoma	1213.32	782.04

*DD = diagnosis; BCVA = best-corrected visual acuity; OD = right eye; OS = left eye; SER = spherical refractive error; VF = visual field; RP = retinitis pigmentosa; PSC = posterior subcapsular cataract; BS = bone-spicule-like pigmentation; NR = non-recordable responses; RPE = retinal pigment epithelium; VA = visual acuity; CRD = cone-rod dystrophy; HM = hand movements; LP = light perception vision. BCVA is shown in Snellen decimals. Visual fields were digitized into retinal seeing areas. *Patient B-2 and B-3 are 2nd degree relatives.*

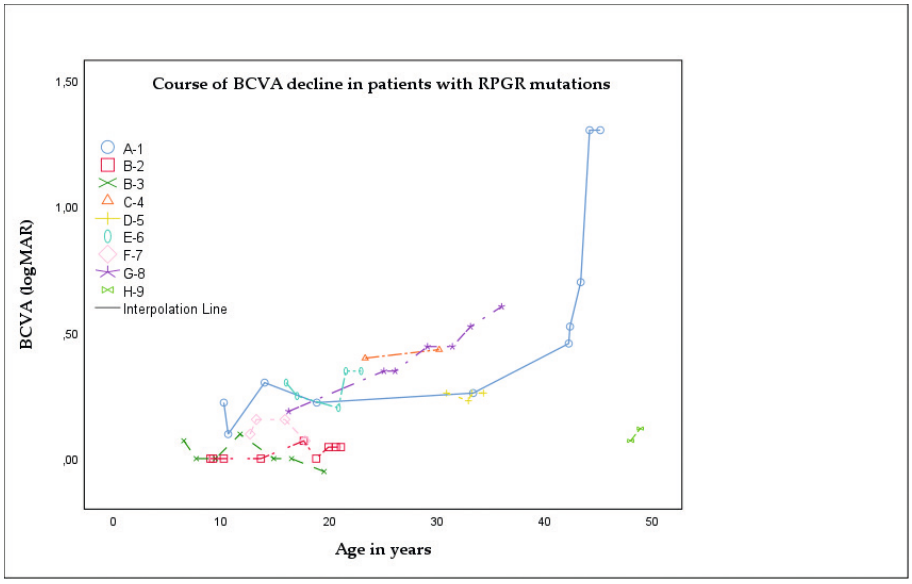


Figure 1. Graph demonstrating the change in mean best-corrected visual acuity (BCVA) in relation to the age in years in this cohort. Snellen BCVA data were transformed into logMAR values. Data from the same subject are shown using interpolation lines connecting the points. All patients had a retinitis pigmentosa phenotype, except for patient H-9, who exhibited a cone-rod dystrophy phenotype.

On fundoscopy, clinical hallmarks of RP, including optic disc pallor, vascular attenuation and bone-spicule-like hyperpigmentation, were observed to various degrees (Figure 2). Optic nerve head drusen were seen in 2 patients. All patients showed macular abnormalities, ranging from moderate retinal pigment epithelium (RPE) changes to profound atrophy. Regions of RPE atrophy on fundus examination corresponded with hypo-autofluorescent (hypo-AF) lesions seen on fundus autofluorescence (FAF) imaging. A macular hyperautofluorescent (hyper-AF) ring was seen in 5 out of 8 RP patients. On spectral-domain optical coherence (SD-OCT) imaging, a common feature in RP patients was the loss of the outer retinal bands (external limiting membrane, ellipsoid zone and the inner/outer segments) in the retina peripheral of the central macula, with relative structural and functional sparing of the central macula. In the CRD patient, central macular atrophy was seen, with FAF showing a central hypo-AF area surrounded by a hyper-AF ring (Figure 2D). No bone-spicules were seen in this patient. SD-OCT imaging showed loss of outer retinal bands focused at the (para)fovea. None of the patients had cystoid macular edema during examination.

Full-field electroretinography (ffERG) responses were absent in all RP patients at the time of examination. Based on previous ERG data (available for 5 RP patients), the mean age at which ERG amplitudes were found to be non-detectable was 11.6

years (SD: 6.7; range: 6.0 – 23.0). In the CRD patient, photopic responses were more severely reduced than scotopic responses, consistent with a CRD phenotype. We performed dark-adapted full-field stimulus thresholds (FST) testing in 6 patients using white and chromatic stimuli (Table S2). The mean general FST sensitivity for the white stimulus, averaged between eyes, was -31.1 dB (SD: 21.6; range: -61.0 to -8.9). The mean blue-red difference was 11.2 dB (SD: 10.9; range: -1.0 to 21.9), with most patients demonstrating a mixed rod-cone mediated response (n = 4, including the CRD patient), and 2 patients showing a cone-mediated response.

The size of visual fields on kinetic perimetry (V4e stimuli), averaged between eyes, ranged between 42.0 mm^2 and 997.7 mm^2 (median: 153.8; interquartile range [IQR]: 185.3), which correlated with age in RP patients (Spearman's rho = -0.714; p = 0.047). Visual field abnormalities ranged from peripheral constriction to central islands in patients with RP, and a central scotoma in the CRD patient (Table 1). In addition, we successfully performed microperimetry testing in 16 eyes from 9 patients (Table S2). Fixation stability was reported as stable (n = 12), relatively unstable (n = 2), or unstable (n = 2); the latter being the case in both eyes from patient A-1 (Snellen BCVA of 0.05 in both eyes). The stability of fixation, as measured using the 95% bivariate contour ellipse area (BCEA) values, correlated with BCVA (r = -0.926; p = 0.003). The mean retinal sensitivity was 11.6 dB (SD: 7.8; range: 1.6 - 24.4), which significantly correlated with BCVA (r = 0.907; p = 0.001), and not with the size of V4e retinal seeing fields (r = 0.553; p = 0.123). In RP patients with macular atrophy, some residual sensitivity at the central macula could still be observed (Figure 3).

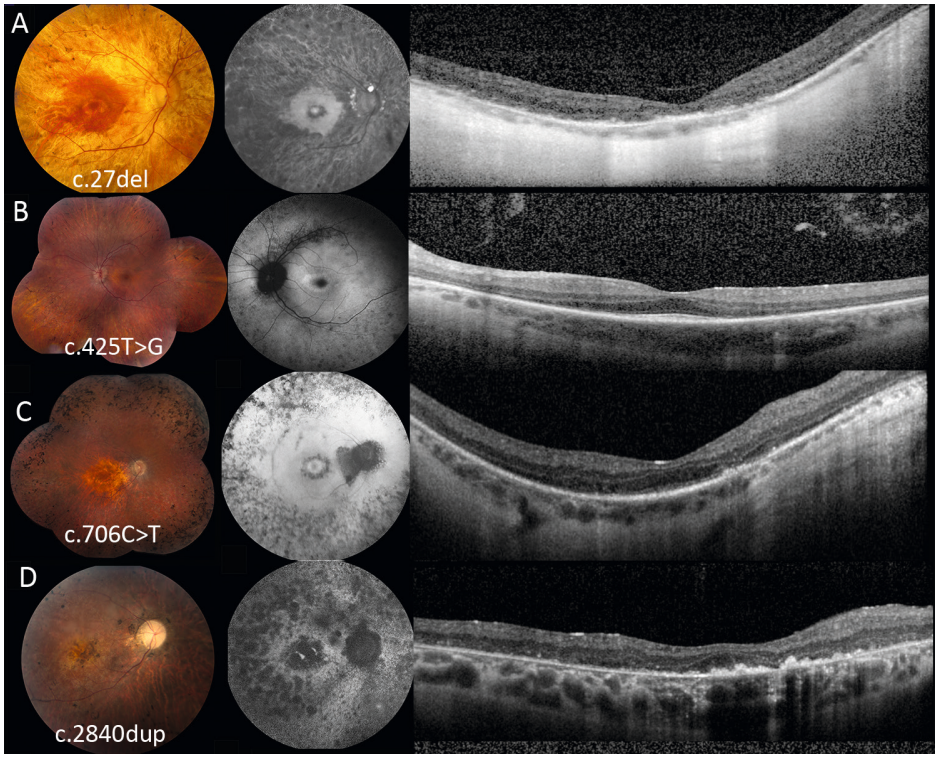


Figure 2. Color fundus photographs and corresponding fundus autofluorescence (FAF) and spectral-domain optical coherence tomography (SD-OCT) images of patients carrying mutations in the *RPGR* gene. (**A**) Right eye of patient A-1, with a best-corrected visual acuity (BCVA) of 0.05, showing a tilted optic disc, vascular attenuation and bone-spicule hyperpigmentation in the midperiphery. Degenerative changes are seen across the entire retina, with sparing of the central macula. An atrophic perifoveal ring is present, resembling a bull's eye maculopathy. Correspondingly, FAF imaging in this patient showed hypo-autofluorescent (hypo-AF) lesions throughout the posterior pole, with sparing of the central macula, and some optic disc drusen. On SD-OCT imaging, atrophy of outer retinal layers is observed, with some relative preservation of the ellipsoid zone (EZ) at the fovea. (**B**) Composite fundus photograph of the left eye of patient B-2 (BCVA of 0.9), showing optic nerve head drusen, normal vessels, and bone-spicule hyperpigmentation in the periphery. FAF imaging showed hypo-AF regions along the vascular arcades and far periphery. A macular hyperautofluorescent (hyper-AF) ring is observed, which matches the extent of EZ loss seen on SD-OCT. (**C**) Right eye of patient G-8, with a BCVA of 0.25. Accumulation of hyperpigmented clumps in the macular region is observed, as well as outside the retinal vascular arcades. On FAF imaging, granular hypo-AF lesions are seen in the posterior pole, and a large central hypo-AF area is seen. SD-OCT imaging shows atrophy of retinal layers, with increased choroidal visibility. Remnants of the outer retinal bands are present in the (para)fovea, together with hyperreflective elevations at the level of the retinal pigment epithelium (RPE) that seem to correspond to the hyperpigmented deposits on fundus photography. (**D**) The left eye of patient H-9 (BCVA of 0.76), with a cone-rod dystrophy phenotype. A bull's eye appearance is seen in the macula on fundus examination, without the presence of bone-spicule-like deposits. Similar findings are seen on FAF imaging, with hyper-AF spot in the fovea, where the outer retinal layers are relatively preserved on SD-OCT, surrounded by a hypo-AF ring of outer retinal and RPE atrophy, which itself is surrounded by a hyper-AF ring.

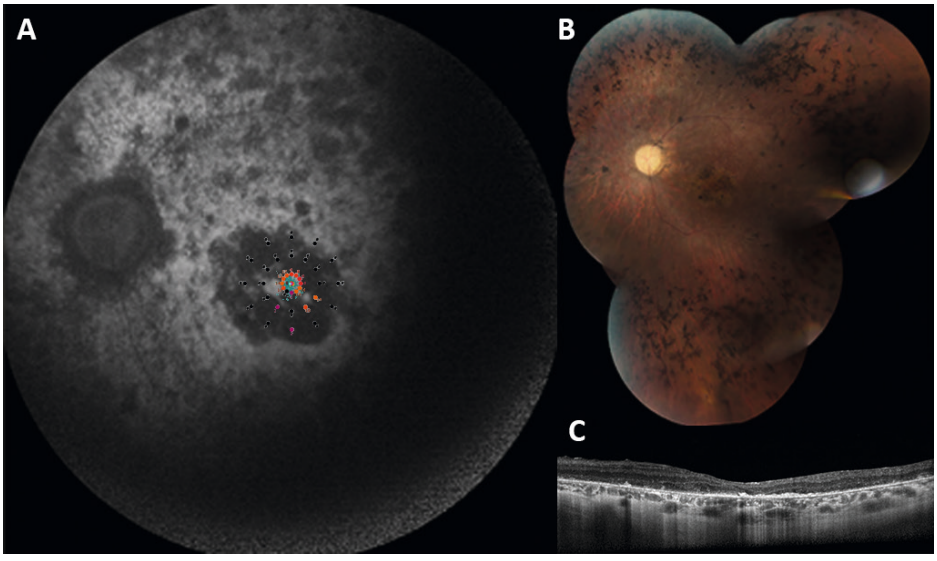


Figure 3. Multimodal imaging and microperimetry in the left eye of patient G-8 (best-corrected visual acuity of the left eye: 0.10). (**A**) Microperimetry revealed reduced, but measurable sensitivity (red and orange sensitivity points) at the central 2° of the fovea, with nearly complete absence of sensitivity outside this region (black sensitivity points). Superimposed microperimetry data on the fundus autofluorescence (FAF) image demonstrated that loss of retinal sensitivity on microperimetry aligned with hypo-autofluorescent lesions on FAF. (**B**) The corresponding fundus image showed clinical hallmarks of retinitis pigmentosa and macular atrophy. (**C**) Spectral-domain optical coherence tomography (SD-OCT) showed remnants of the outer retina at the (para)fovea.

Retinal histology

Medical records of the donor patient (I-10), carrying the p.Glu1031Glyfs*58 (c.3092del) *RPGR* mutation, were obtained. First available records of this patient were found at the age of 32, with BCVA loss as initial symptom (BCVA OD: 0.5; BCVA OS: 0.63). ERG examination revealed non-detectable photopic responses and reduced scotopic amplitudes, suggesting a CRD phenotype. Goldmann kinetic perimetry (V4e stimulus) showed a superior hemifield defect (Figure 4C). BCVA remained relatively stable, until the patient revisited the clinic at the age of 58 (BCVA OD: 0.3; BCVA OS: 0.5). Cataract surgery due to posterior subcapsular cataract in the left eye resulted in improvement of BCVA in the left eye, but no BCVA improvement was seen in the right eye after uncomplicated cataract surgery. The course of BCVA regression/improvement of this patient is shown in Figure 4A. The last ophthalmic examination was available at the age of 89. At last examination, only hand movements and light perception were observed in the right and left eye, respectively. On fundus examination, peripapillary atrophy extending to the macular region was seen. Bone-spicules were present mostly in the inferior and nasal quadrants, suggesting a mixed CRD /sector RP phenotype (Figure 4B). The right eye of patient I-10 was retrieved postmortem at the age of 94, and was prepared for histologic examination after removal of the cornea for donation purposes.

Sections of the macular and peripheral regions were processed for microscopic examination. Gross examination of the right eye showed bone-spicule-like pigmentation predominantly in the inferonasal quadrant (Figure 4E). Due to profound atrophy in the macular region, it was difficult to accurately distinguish and pinpoint the fovea. In all sections, degenerative changes and disorganization of the retinal laminae were evident. In the macular sections, the loss of photoreceptor outer segments was observed, which was accompanied by reduction of photoreceptor cells and closing of the subretinal space. While the retinal pigment RPE layer was atrophic, it was not entirely lost in the macular region.

Peripheral regions showed more extensive retinal remodeling than the macular region, with severe disruption of the normal topology and stratification. Complete loss of the photoreceptor layer and major reduction of the RPE layer was observed. Some remaining patches of the RPE layer were still visible (Figure 4H, red arrowhead). In sections devoid of a pigmented RPE layer, RPE cells migrated and accumulated around the retinal vessels in the inner retina (Figure 4G, yellow arrowhead). Immunohistochemical staining showed positivity for GFAP across the entire retina, in both macular and peripheral sections (Figure 4I-K**)**, signifying reactive gliosis.

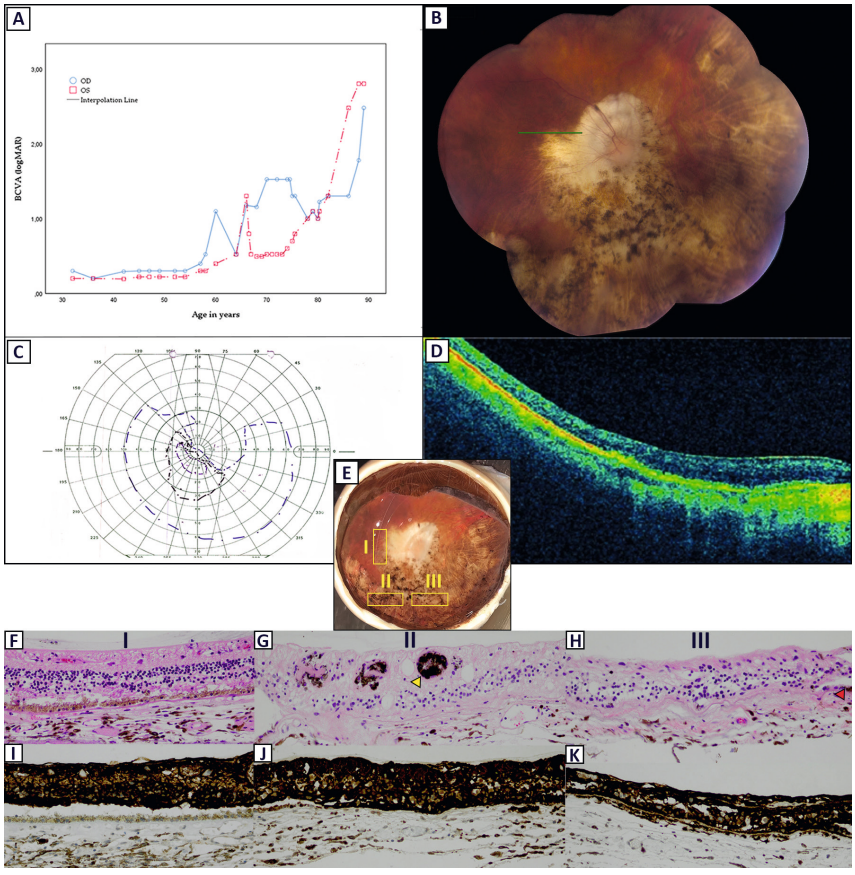


Figure 4. Clinical characteristics and postmortem histopathological examination in patient I-10 at the age of 94, who carried the p.Glu1031Glyfs*58 (c.3092del) mutation in *RPGR*. (**A**) Line graph detailing the best-corrected visual acuity (logMAR) course regression in this patient. Cataract extraction was performed at the age of 66 (left eye; OS) and 74 (right eye; OD). For hand movement vision, light perception vision and no light perception, logMAR values of 2.7, 2.8 and 2.9 were used, respectively. (**B**) Fundus examination of the right eye at age 89 showed a pale optic disc with extensive peripapillary and macular atrophy. Retinal vessels were attenuated or obliterated. Bone-spicule like pigmentation is mainly seen in the inferior-nasal quadrant. The green line illustrates the spectral-domain optical coherence tomography (SD-OCT) section line. (**C**) Goldmann kinetic perimetry at the age of 67 showed a superior hemifield defect. (**D**) SD-OCT imaging revealed profound degeneration of all retinal layers. (**E**) Macroscopic examination of the right eye. A schematic drawing indicating the approximate macular (I) and peripheral (II and III) regions sectioned and processed for further examination. (**F**) Section of the macular region (I) showing loss of photoreceptor segments and closing of the subretinal space. The retinal pigment epithelium (RPE) layer is atrophic, but still visible in this section (hematoxylin-eosin [H&E], 200x). (**G**) Complete absence of photoreceptor outer segments and RPE cells is observed in this peripheral section (II). Inner layers are highly atrophic and disorganized. Migration of RPE cells into retinal vessels is shown (yellow arrowhead; H&E, 200x). (**H**) Clear disruption of the normal topology and stratification is also seen in this section (III), although there are still some RPE cells remaining (red arrowhead [H&E, 200x]). (**I-K**) A positive immunoreactivity for glial fibrillary acid protein (GFAP) is seen across all retinal layers in both macular and peripheral regions (GFAP, 200x).

DISCUSSION

In this study, we describe the clinical and genetic characteristics of *RPGR* patients at different stages of disease based on extensive prospective structural and functional phenotyping. The patients in this cohort demonstrated typical features of RP or CRD. An early onset of symptoms was present in patients with RP, with initial symptoms being night blindness or peripheral visual field loss. As such, we found moderate to severe concentric visual field defects in all patients. In contrast, the first symptoms occurred at the age of 48 in the CRD patient. After onset of disease, BCVA gradually declined in the majority of patients. The fastest BCVA decline was seen in patient A-1, who demonstrated a rapid decline of BCVA after the 4th decade of life. Fundus examination revealed profound atrophy in the macula of this patient. While macular involvement occurs early in CRD, it typically is not seen in RP patients until end-stage disease.[12, 21] Retinal imaging in this study showed RPE atrophy on FAF in various patterns, and loss of outer retinal bands on SD-OCT, which were all consistent with either an RP or CRD phenotype. A hyper-AF ring was found in 6 out of 9 patients, including the CRD patient. Previous studies have shown that the hyper-AF ring correlates with presence of the EZ band, and demarcates the transition between healthy and affected retina.[2, 22, 23] Constriction of this hyper-AF ring in RP indicates disease progression, whereas – conversely - expansion of the hyper-AF ring in CRD patients suggests disease progression.[2, 24]

Defining disease severity is essential for gene therapy trials, as preserved rods and cones are required for successful subretinal delivery.[25] For the assessment of rod and cone function, several functional and psychophysical measurement tools exist, and these methods were explored in this study. Microperimetry measures the retinal sensitivity of the macular region, and is able to detect changes within a short time span.[26] In our cohort, we were able to detect retinal sensitivity loss in all patients, which correlated with the loss of visual acuity. Patients with macular atrophy still retained some residual function at the fovea, although severely reduced, which may be targeted for treatment. The benefit of microperimetry in gene therapy trials is the ability to measure the individual retinal points exposed to treatment, and to correlate these with fundus locations. However, possible challenges with microperimetry may arise when patients with end-stage disease are included in clinical trials. Our study and others have found that fixation stability is correlated with visual acuity.[27, 28] Fixation instability causes greater variation in measurements, which may impact the repeatability and reliability of testing.[27, 28] Therefore, inherent test-retest variation should be taken into account when assessing retinal function with microperimetry. FST is a psychophysical test that measures the sensitivity of the entire retina, even in those without adequate fixation capabilities, and in patients who do not have measurable rod and cone responses on ffERG.[29, 30] Using FST, we found mixed rod-cone mediated responses in 4 patients (including the CRD patient), and cone-mediated responses in

2 RP patients. Patients with cone-mediated responses in our cohort showed macular atrophy and widespread RPE loss, suggesting that cone-mediated responses are indicators for more advanced disease stages of RP.[29] Other studies have shown a strong correlation between FST and residual ffERG amplitudes, suggesting that FST can be a potential replacement for ERG.[31, 32] In patients with *RPGR*-associated RP, ERG responses are often severely reduced or absent from an early age, as was the case in our cohort, and may not be an optimal parameter for future trials. FST could potentially replace ERG as a psychophysical outcome for future *RPGR*-related studies, and is especially useful in patients with severe disease and fixation instability. However, as FST measures the entire retina, it is unable to localize the individual retinal areas mediating the responses. Therefore, caution must be exercised when interpreting FST results in clinical trials, as they may not co-localize with the area of treatment.

Some possible genotype-phenotype correlations could be identified within this cohort. Nine different variants in *RPGR* were detected, including the mutation found in the donor patient (I-10). Frameshift mutations were the most common mutation type, and were mainly located in exon ORF15. It is worth noting that the mutations causing CRD phenotypes, p.(Glu1031Glyfs*58), the eye of patient I-10 in whom histopathological studies were performed and p.(Glu1071Alafs*16) (patient H-9), were found at the 3' of ORF15. Our data support previous studies that suggested that mutations at the 3' terminal of ORF15 cause predominant CRD phenotypes.[4, 17] It is believed that frameshift mutations in the exon encoding the N-terminal RCC1-like domain of RPGR are more prone to nonsense-mediated decay (NMD), leading to lower levels of the transcript and may therefore be more likely to cause severe RP phenotypes.[4] In contrast, mutations in ORF15 are located at the terminal exon and are less likely to result in NMD, resulting in milder RP (towards the 5' end) and CRD (towards the 3' end) phenotypes.[4, 17] This is not always the case, as mutations located close to the downstream of ORF15 have also been reported to result in RP phenotypes.[2, 33] In some cases, ORF15 mutations even resulted in both RP and CRD phenotypes, suggesting the potential influence of genetic and/or environmental modifiers on the phenotype.[16]

We also evaluated the retinal histopathology of an affected 94-year old patient with an end-stage CRD/sector RP phenotype. There have been only a few histopathologic studies of eyes from patients with known *RPGR* mutations.[34, 35] Similar to CRD patient H-9, initial symptoms presented at a much later time, at the age of 32, than the RP patients in this cohort. The longitudinal BCVA follow-up of 57 years in this patient showed that visual acuity gradually deteriorated. Strikingly, fundus examination in this patient showed extensive peripapillary atrophy and bone-spicule hyperpigmentation mainly in the nasal and inferior quadrants, corresponding with a superior hemifield defects on kinetic perimetry. Predilection for superior visual field loss have been described in patients with sector RP caused by mutations in the *RHO* gene, and rarely in patients with *RPGR* mutations.[25, 36, 37] Sectoral changes are believed to reflect a

milder phenotype, and can develop into a widespread disease when followed over decades.[36] On histologic examination, we found a severely atrophic and disorganized retina in all sections. There was a widespread loss of photoreceptors, more so in the peripheral retina than in the macula, which may explain the remaining visual acuity of only hand movements in this patient's right eye. Immunohistochemical analysis showed an intense staining for GFAP across the entire inner retina in the peripheral region, indicating the process of reactive gliosis by Müller cells.[38] Inner remodeling is detrimental for the application of bionic (retinal chip) and biological (gene, optogenetic or stem cell) rescue strategies. These therapies rely on remaining target neurons and may have limited success when glial seal formation has taken place.[39, 40] Therapies should therefore preferably be applied at the earlier signs of degeneration, prior to inner retinal remodeling. Interestingly, GFAP staining was also seen in macular sections, which revealed less degeneration compared to peripheral sections, and showed no bone-spicule-like deposits on fundus examination. This suggests that remodeling of the inner retina may already take place before clinical signs are observable on fundoscopy and retinal imaging.[40] No clinical imaging techniques exist that can accurately track the earliest stages of remodeling, which implicates the optimal timing for therapy.[40] The exact impact of retinal remodeling and gliosis on the long-term efficacy of therapeutic strategies is still unclear, and needs to be further elucidated.

In conclusion, we present a comprehensive clinical, genetic and histopathologic overview of patients with *RPGR*-associated retinal dystrophies. The clinical presentation of *RPGR*-related retinal dystrophies is highly variable, but genotype-phenotype correlations can be discerned. We found that microperimetry and FST can be useful functional parameters for evaluation in future trials. In end-stage disease, remodeling takes place also in the inner retina, which may precede clinically observable signs, complicating future therapeutic strategies. Therefore, treatments should preferably be applied in an early disease stage.

MATERIALS AND METHODS

Clinical examination

Male participants were ascertained from the RD5000 registry, which is a national registry for inherited retinal dystrophies.[41] Comprehensive ophthalmologic assessment was performed at the Leiden University Medical Center (LUMC), including measurement of BCVA using the Early Treatment of Diabetic Retinopathy Study (ETDRS) chart, slit-lamp examination, color vision testing (Hardy-Rand-Rittler [HRR]), and fundus photography (Topcon TRC-50DX, Topcon Medical Systems, Inc. Oakland, NJ, USA). SD-OCT and FAF imaging were performed using the Spectralis HRA+OCT system (Heidelberg Engineering, Heidelberg, Germany).

ffERG was performed according to International Society for Clinical Electrophysiology of Vision (ISCEV) standards. ffERG was not conducted in patients that were previously reported to have non-recordable responses (n = 5). FST testing was performed in 6 patients using the Espion Colordome™ LED full-field stimulator (Diagnosys LCC, Lowell, MA, USA). Both eyes were tested, first using the white stimulus, followed by red and blue stimuli. Thresholds were tested three times for each color, and were averaged to determine the final thresholds. The difference in thresholds between blue and red stimuli determined whether responses were either: rod-mediated (difference of > 22dB), cone-mediated (difference of < 3dB) or mixed (difference between 3 and 22 dB) [29]. Goldmann kinetic perimetry was performed using V4e stimuli, and visual fields were digitized using a method previously used by Dagnelie.[42] In addition, microperimetry (MAIA, Centervue, Padova, Italy) was performed in all patients under mesopic conditions, first by performing the "4-2 fixed protocol" to minimize a learning effect, afterwards followed by the "4-2 strategy" protocol for formal testing.

The present study was approved by the Medical Ethics Committee of the Erasmus Medical Center (MEC-2010-359), as well by the Local Review Board of the LUMC (P11.100). Informed consent was retrieved for all participants, and the study adhered to the tenets of the Declarations of Helsinki. When available, clinical data from medical records were retrieved for longitudinal evaluation.

Retinal histology

The right eye was obtained 15 hours postmortem from a 94-year old male patient (I-10) with a confirmed mutation in the *RPGR* gene. Prior written informed consent for organ and tissue use in research was given by the donor. Relevant tissues were fixed in 4% paraformaldehyde for 24 hours, and embedded in paraffin. The embedded blocks were cut at 4 µm sections and stained with hematoxylin-eosin. For immunohistochemical staining, following deparaffinization, rehydration and heat-induced epitope retrieval, the tissue sections were incubated with primary glial fibrillary acid protein (GFAP; monoclonal mouse antibody, clone 6f2; DakoCytomation, Glostrup, Denmark; dilution 1:400) for 30 minutes and counterstained with hematoxylin.[43] Slides were externally validated using appropriate control tissues, and were reviewed by an ophthalmic pathologist (R.M.V).

Genetic analysis

Genomic DNA was extracted from peripheral blood samples according to standard protocols. Genetic analysis was performed at the Department of Clinical Genetics of the Amsterdam University Medical Centers (Amsterdam UMC), the Netherlands, and methods used have been published previously.[2, 10, 44] Primers used in this study can be accessed upon reasonable request.

Statistical Analysis

Data were analyzed using SPSS version 25.0 (IBM Corp, Armonk, NY, USA). Findings with a p-value of < 0.05 were considered statistically significant. Normally and non-normally distributed data were displayed as means with SD, and medians with IQR, respectively. Depending on the distribution, either Pearson's or Spearman's test was performed for correlation testing.

REFERENCES

1. Hartong, D.T.; Berson, E.L.; Dryja, T.P. Retinitis pigmentosa. *The Lancet* 2006, *368*, 1795-1809, doi:10.1016/S0140-6736(06)69740-7.
2. Talib, M.; van Schooneveld, M.J.; Thiadens, A.A.; Fiocco, M.; Wijnholds, J.; Florijn, R.J.; Schalij-Delfos, N.E.; van Genderen, M.M.; Putter, H.; Cremers, F.P.M., et al. Clinical and Genetic characteristics of Male patients with RPGR-associated Retinal Dystrophies: A long-Term Follow-up Study. *RETINA* 2019, *39*, 1186-1199, doi:10.1097/iae.0000000000002125.
3. Tee, J.J.L.; Yang, Y.; Kalitzeos, A.; Webster, A.; Bainbridge, J.; Weleber, R.G.; Michaelides, M. Characterization of Visual Function, Interocular Variability and Progression Using Static Perimetry–Derived Metrics in RPGR-Associated Retinopathy. *Investigative Ophthalmology & Visual Science* 2018, *59*, 2422-2436, doi:10.1167/iovs.17-23739.
4. Sharon, D.; Sandberg, M.A.; Rabe, V.W.; Stillberger, M.; Dryja, T.P.; Berson, E.L. RP2 and RPGR Mutations and Clinical Correlations in Patients with X-Linked Retinitis Pigmentosa. *American Journal of Human Genetics* 2003, *73*, 1131-1146.
5. Bader, I.; Brandau, O.; Achatz, H.; Apfelstedt-Sylla, E.; Hergersberg, M.; Lorenz, B.; Wissinger, B.; Wittwer, B.r.; Rudolph, G.n.; Meindl, A., et al. X-linked Retinitis Pigmentosa: RPGR Mutations in Most Families with Definite X Linkage and Clustering of Mutations in a Short Sequence Stretch of Exon ORF15. *Investigative Ophthalmology & Visual Science* 2003, *44*, 1458-1463, doi:10.1167/iovs.02-0605.
6. Pelletier, V.; Jambou, M.; Delphin, N.; Zinovieva, E.; Stum, M.; Gigarel, N.; Dollfus, H.; Hamel, C.; Toutain, A.; Dufier, J.-L., et al. Comprehensive survey of mutations in RP2 and RPGR in patients affected with distinct retinal dystrophies: genotype–phenotype correlations and impact on genetic counseling. *Human Mutation* 2007, *28*, 81-91, doi:10.1002/humu.20417.
7. Bird, A.C. X-linked retinitis pigmentosa. *British Journal of Ophthalmology* 1975, *59*, 177, doi:10.1136/bjo.59.4.177.
8. Sandberg, M.A.; Rosner, B.; Weigel-DiFranco, C.; Dryja, T.P.; Berson, E.L. Disease course of patients with X-linked retinitis pigmentosa due to RPGR gene mutations. *Investigative Ophthalmology & Visual Science* 2007, *48*, 1298-1304, doi:10.1167/iovs.06-0971.
9. Nanda, A.; Salvetti, A.P.; Clouston, P.; Downes, S.M.; MacLaren, R.E. Exploring the Variable Phenotypes of RPGR Carrier Females in Assessing their Potential for Retinal Gene Therapy. *Genes* 2018, *9*, 643, doi:10.3390/genes9120643.
10. Talib, M.; van Schooneveld, M.J.; Van Cauwenbergh, C.; Wijnholds, J.; ten Brink, J.B.; Florijn, R.J.; Schalij-Delfos, N.E.; Dagnelie, G.; van Genderen, M.M.; De Baere, E., et al. The Spectrum of Structural and Functional Abnormalities in Female Carriers of Pathogenic Variants in the RPGR Gene. *Investigative Ophthalmology & Visual Science* 2018, *59*, 4123-4133, doi:10.1167/iovs.17-23453.
11. Ebenezer, N.D.; Michaelides, M.; Jenkins, S.A.; Audo, I.; Webster, A.R.; Cheetham, M.E.; Stockman, A.; Maher, E.R.; Ainsworth, J.R.; Yates, J.R., et al. Identification of Novel RPGR ORF15 Mutations in X-linked Progressive Cone-Rod Dystrophy (XLCORD) Families. *Investigative Ophthalmology & Visual Science* 2005, *46*, 1891-1898, doi:10.1167/iovs.04-1482.
12. Thiadens, A.A.H.J.; Soerjoesing, G.G.; Florijn, R.J.; Tjiam, A.G.; den Hollander, A.I.; van den Born, L.I.; Riemslag, F.C.; Bergen, A.A.B.; Klaver, C.C.W. Clinical course of cone dystrophy caused by mutations in the RPGR gene. *Graefes Arch Clin Exp Ophthalmol* 2011, *249*, 1527-1535, doi:10.1007/s00417-011-1789-3.
13. Megaw, R.D.; Soares, D.C.; Wright, A.F. RPGR: Its role in photoreceptor physiology, human disease, and future therapies. *Experimental Eye Research* 2015, *138*, 32-41, doi:10.1016/j.exer.2015.06.007.
14. Kirschner, R.; Rosenberg, T.; Schultz-Heienbrok, R.; Lenzner, S.; Feil, S.; Roepman, R.; Cremers, F.P.M.; Ropers, H.-H.; Berger, W. RPGR Transcription Studies in Mouse and Human Tissues Reveal a Retina-Specific Isoform That Is Disrupted in a Patient With X-Linked Retinitis Pigmentosa. *Human Molecular Genetics* 1999, *8*, 1571-1578, doi:10.1093/hmg/8.8.1571.

15. Hosch, J.; Lorenz, B.; Stieger, K. RPGR: Role in the photoreceptor cilium, human retinal disease, and gene therapy. *Ophthalmic Genetics* 2011, *32*, 1-11, doi:10.3109/13816810.2010.535889.

16. Yang, L.; Yin, X.; Feng, L.; You, D.; Wu, L.; Chen, N.; Li, A.; Li, G.; Ma, Z. Novel Mutations of RPGR in Chinese Retinitis Pigmentosa Patients and the Genotype-Phenotype Correlation. *PLOS ONE* 2014, *9*, e85752, doi:10.1371/journal.pone.0085752.

17. Demirci, F.Y.K.; Rigatti, B.W.; Wen, G.; Radak, A.L.; Mah, T.S.; Baic, C.L.; Traboulsi, E.I.; Alitalo, T.; Ramser, J.; Gorin, M.B. X-Linked Cone-Rod Dystrophy (Locus COD1): Identification of Mutations in RPGR Exon ORF15. *The American Journal of Human Genetics* 2002, *70*, 1049-1053, doi:10.1086/339620.

18. Beltran, W.A.; Cideciyan, A.V.; Lewin, A.S.; Iwabe, S.; Khanna, H.; Sumaroka, A.; Chiodo, V.A.; Fajardo, D.S.; Román, A.J.; Deng, W.-T., et al. Gene therapy rescues photoreceptor blindness in dogs and paves the way for treating human X-linked retinitis pigmentosa. *Proceedings of the National Academy of Sciences of the United States of America* 2012, *109*, 2132-2137, doi:10.1073/pnas.1118847109.

19. Fischer, M.D.; McClements, M.E.; Martinez-Fernandez de la Camara, C.; Bellingrath, J.-S.; Dauletbekov, D.; Ramsden, S.C.; Hickey, D.G.; Barnard, A.R.; MacLaren, R.E. Codon-Optimized RPGR Improves Stability and Efficacy of AAV8 Gene Therapy in Two Mouse Models of X-Linked Retinitis Pigmentosa. *Mol Ther* 2017, *25*, 1854-1865, doi:10.1016/j.ymthe.2017.05.005.

20. Cehajic Kapetanovic, J.; McClements, M.E.; Martinez-Fernandez de la Camara, C.; MacLaren, R.E. Molecular Strategies for RPGR Gene Therapy. *Genes* 2019, *10*, 674, doi:10.3390/genes10090674.

21. van Huet, R.A.C.; Estrada-Cuzcano, A.; Banin, E.; Rotenstreich, Y.; Hipp, S.; Kohl, S.; Hoyng, C.B.; den Hollander, A.I.; Collin, R.W.J.; Klevering, B.J. Clinical Characteristics of Rod and Cone Photoreceptor Dystrophies in Patients With Mutations in the C8orf37 Gene. *Investigative Ophthalmology & Visual Science* 2013, *54*, 4683-4690, doi:10.1167/iovs.12-11439.

22. Tee, J.J.L.; Yang, Y.; Kalitzeos, A.; Webster, A.; Bainbridge, J.; Michaelides, M. Natural History Study of Retinal Structure, Progression, and Symmetry Using Ellipzoid Zone Metrics in RPGR-Associated Retinopathy. *American Journal of Ophthalmology* 2019, *198*, 111-123, doi:10.1016/j.ajo.2018.10.003.

23. Lima, L.H.; Burke, T.; Greenstein, V.C.; Chou, C.L.; Cella, W.; Yannuzzi, L.A.; Tsang, S.H. Progressive Constriction of the Hyperautofluorescent Ring in Retinitis Pigmentosa. *American journal of ophthalmology* 2012, *153*, 10.1016/j.ajo.2011.1008.1043, doi:10.1016/j.ajo.2011.08.043.

24. Barnes, C.S.; Schuchard, R.A.; Birch, D.G.; Dagnelie, G.; Wood, L.; Koenekoop, R.K.; Bittner, A.K. Reliability of Semiautomated Kinetic Perimetry (SKP) and Goldmann Kinetic Perimetry in Children and Adults With Retinal Dystrophies. *Translational vision science & technology* 2019, *8*, 36-36, doi:10.1167/tvst.8.3.36.

25. Charng, J.; Cideciyan, A.V.; Jacobson, S.G.; Sumaroka, A.; Schwartz, S.B.; Swider, M.; Roman, A.J.; Sheplock, R.; Anand, M.; Peden, M.C., et al. Variegated yet non-random rod and cone photoreceptor disease patterns in RPGR-ORF15-associated retinal degeneration. *Hum Mol Genet* 2016, *25*, 5444-5459, doi:10.1093/hmg/ddw361.

26. Bagdonaite-Bejarano, L.; Hansen, R.M.; Fulton, A.B. Microperimetry in Three Inherited Retinal Disorders. *Seminars in Ophthalmology* 2019, *34*, 334-339, doi:10.1080/08820538.2019.1622025.

27. Jolly, J.K.; Xue, K.; Edwards, T.L.; Groppe, M.; MacLaren, R.E. Characterizing the Natural History of Visual Function in Choroideremia Using Microperimetry and Multimodal Retinal Imaging. *Investigative Ophthalmology & Visual Science* 2017, *58*, 5575-5583, doi:10.1167/iovs.17-22486.

28. Dimopoulos, I.S.; Tseng, C.; MacDonald, I.M. Microperimetry as an Outcome Measure in Choroideremia Trials: Reproducibility and Beyond. *Investigative Ophthalmology & Visual Science* 2016, *57*, 4151-4161, doi:10.1167/iovs.16-19338.

29. Jacobson, S.G.; Aleman, T.S.; Cideciyan, A.V.; Roman, A.J.; Sumaroka, A.; Windsor, E.A.M.; Schwartz, S.B.; Heon, E.; Stone, E.M. Defining the Residual Vision in Leber Congenital Amaurosis Caused by RPE65 Mutations. *Investigative Ophthalmology & Visual Science* 2009, *50*, 2368-2375, doi:10.1167/iovs.08-2696.

30. Russell, S.; Bennett, J.; Wellman, J.A.; Chung, D.C.; Yu, Z.-F.; Tillman, A.; Wittes, J.; Pappas, J.; Elci, O.; McCague, S., et al. Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial. *Lancet (London, England)* 2017, *390*, 849-860, doi:10.1016/S0140-6736(17)31868-8.

31. Roman, A.J.; Cideciyan, A.V.; Aleman, T.S.; Jacobson, S.G. Full-field stimulus testing (FST) to quantify visual perception in severely blind candidates for treatment trials. *Physiological Measurement* 2007, *28*, N51-N56, doi:10.1088/0967-3334/28/8/n02.

32. Messias, K.; Jägle, H.; Saran, R.; Ruppert, A.D.P.; Siqueira, R.; Jorge, R.; Messias, A. Psychophysically determined full-field stimulus thresholds (FST) in retinitis pigmentosa: relationships with electroretinography and visual field outcomes. *Documenta Ophthalmologica* 2013, *127*, 123-129, doi:10.1007/s10633-013-9393-y.

33. Vervoort, R.; Lennon, A.; Bird, A.C.; Tulloch, B.; Axton, R.; Miano, M.G.; Meindl, A.; Meitinger, T.; Ciccodicola, A.; Wright, A.F. Mutational hot spot within a new RPGR exon in X-linked retinitis pigmentosa. *Nature Genetics* 2000, *25*, 462-466, doi:10.1038/78182.

34. Aguirre, G.D.; Yashar, B.M.; John, S.K.; Smith, J.E.; Breuer, D.K.; Hiriyanna, S.; Swaroop, A.; Milam, A.H. Retinal Histopathology of an XLRP Carrier with a Mutation in the RPGR Exon ORF15. *Experimental Eye Research* 2002, *75*, 431-443, doi:10.1006/exer.2002.2037.

35. Huang, W.C.; Wright, A.F.; Roman, A.J.; Cideciyan, A.V.; Manson, F.D.; Gewaily, D.Y.; Schwartz, S.B.; Sadigh, S.; Limberis, M.P.; Bell, P., et al. RPGR-associated retinal degeneration in human X-linked RP and a murine model. *Investigative ophthalmology & visual science* 2012, *53*, 5594-5608, doi:10.1167/iovs.12-10070.

36. Ramon, E.; Cordomí, A.; Aguilà, M.; Srinivasan, S.; Dong, X.; Moore, A.T.; Webster, A.R.; Cheetham, M.E.; Garriga, P. Differential light-induced responses in sectorial inherited retinal degeneration. *J Biol Chem* 2014, *289*, 35918-35928, doi:10.1074/jbc.M114.609958.

37. Kranich, H.; Bartkowski, S.; Denton, M.J.; Krey, S.; Dickinson, P.; Duvigneau, C.; Gal, A. Autosomal dominant 'sector' retinitis pigmentosa due to a point mutation predicting an Asn-15-Ser substitution of rhodopsin. *Human Molecular Genetics* 1993, *2*, 813-814, doi:10.1093/hmg/2.6.813.

38. Henriksen, B.S.; Marc, R.E.; Bernstein, P.S. Optogenetics for retinal disorders. *J Ophthalmic Vis Res* 2014, *9*, 374-382, doi:10.4103/2008-322X.143379.

39. Marc, R.E.; Jones, B.W.; Watt, C.B.; Strettoi, E. Neural remodeling in retinal degeneration. *Progress in Retinal and Eye Research* 2003, *22*, 607-655, doi:10.1016/S1350-9462(03)00039-9.

40. Jones, B.W.; Pfeiffer, R.L.; Ferrell, W.D.; Watt, C.B.; Marmor, M.; Marc, R.E. Retinal remodeling in human retinitis pigmentosa. *Experimental eye research* 2016, *150*, 149-165, doi:10.1016/j.exer.2016.03.018.

41. van Huet, R.A.C.; Oomen, C.J.; Plomp, A.S.; van Genderen, M.M.; Klevering, B.J.; Schlingemann, R.O.; Klaver, C.C.W.; van den Born, L.I.; Cremers, F.P.M. The RD5000 Database: facilitating clinical, genetic, and therapeutic studies on inherited retinal diseases. *Investigative Ophthalmology & Visual Science* 2014, *55*, 7355-7360, doi:10.1167/iovs.14-15317.

42. Dagnelie, G. Technical note. Conversion of planimetric visual field data into solid angles and retinal areas. *Clinical Vision Science* 1990, *5*, 95-100.

43. Bais, B.; Kubat, B.; Motazedi, E.; Verdijk, R.M. Amyloid Precursor Protein and Ubiquitin Immunohistochemistry Aid in the Evaluation of Infant Autopsy Eyes With Abusive Head Trauma. *American Journal of Ophthalmology* 2015, *160*, 1285-1295.e1286, doi:10.1016/j.ajo.2015.09.005.

44. Roepman, R.; Bernoud-Hubac, N.; Schick, D.E.; Maugeri, A.; Berger, W.; Ropers, H.-H.; Cremers, F.P.M.; Ferreira, P.A. The retinitis pigmentosa GTPase regulator (RPGR) interacts with novel transport-like proteins in the outer segments of rod photoreceptors. *Human Molecular Genetics* 2000, *9*, 2095-2105, doi:10.1093/hmg/9.14.2095.

SUPPLEMENTAL CONTENT

Supplemental Table 1. Mutations in the *RPGR* gene found in the patients included in this study.

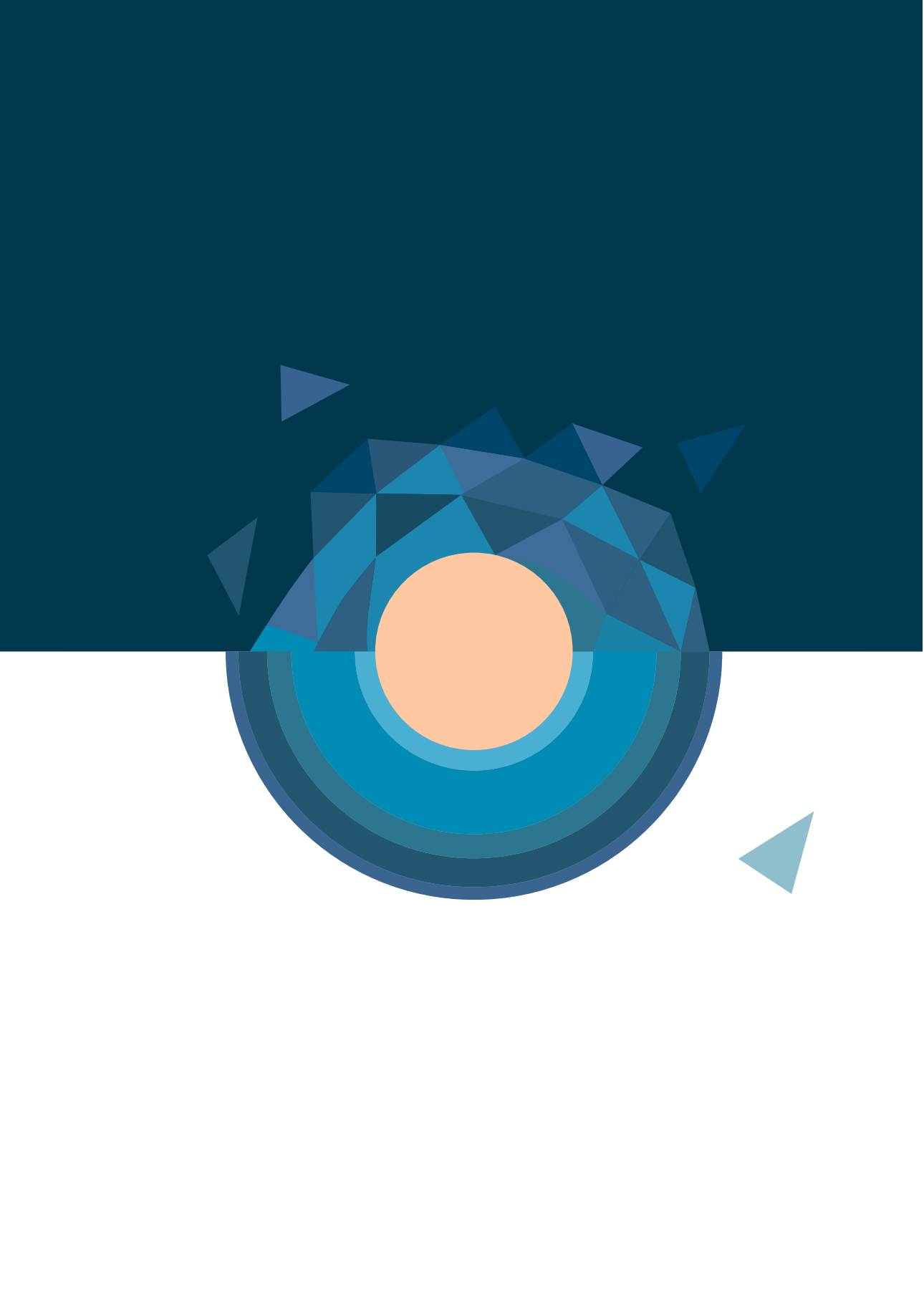
Family-ID	cDNA change	Protein effect	Exon	Diagnosis	Reference
A-1	c.27del	p.(Asp10Ilefs*58)	1	RP	Talib et al. 2018
B-2	c.425T>G	p.(Ile142Ser)	5	RP	Talib et al. 2018
B-3	c.425T>G	p.(Ile142Ser)	5	RP	Talib et al. 2018
C-4	c.706C>T	p.(Gln236*)	7	RP	Buraczynska et al. 1997
D-5	c.2236_2237del	p.(Glu746Argfs*23)	ORF15	RP	Vervoort et al. 2000
E-6	c.2323_2324del	p.(Arg775Glufs*59)	ORF15	RP	Breuer et al. 2002
F-7	c.2838_2839del	p.(Glu947Glyfs*131)	ORF15	RP	Pelletier et al. 2007
G-8	c.2840dup	p.(Glu949Glyfs*130)	ORF15	RP	Neidhardt et al. 2008
H-9	c.3212-3218del	p.(Glu1071Alafs*16)	ORF15	CRD	This study
Donor	c.3092del	p.(Glu1031Glyfs*58)	ORF15	CRD	Demirci et al. 2002

Nucleotide changes in cDNA are noted with transcript NM_001034853.1 as reference. RP = retinitis pigmentosa; CRD = cone-rod dystrophy.

Supplemental Table 2. Retinal sensitivity values in patients with *RPGR*-associated dystrophies.

	Microperimetry		Full-stimulus threshold		
Family-ID	**Mean Retinal sensitivity (dB)**	**Fixation stability OD/OS**	**Mean white (dB)**	**Mean blue-red difference**	**Mediated response**
A-1	3.20	Unstable/Unstable	NP	NP	-
B-2	15.45	Stable/Stable	NP	NP	-
B-3	24.35	Stable/Stable	-43.77	21.86	Mixed
C-4	5.45	RU/RU	-10.72	-1.00	Cone
D-5	14.55	Stable/Stable	-45.57	20.62	Mixed
E-6	8.00	Stable/NP	-16.87	6.31	Mixed
F-7	11.50	Stable/Stable	NP	NP	-
G-8	3.10	NP/Stable	-8.89	-.71	Cone
H-9	20.50	Stable/Stable	-60.98	20.23	Mixed

RU = relatively unstable fixation; NP = not performed. Due to technical reasons, not all tests were performed in all patients. The difference in thresholds between blue and red stimuli determined whether responses were either: rod-mediated (difference of > 22dB), cone-mediated (difference of < 3dB) or mixed (between 3- 22 dB).



CHAPTER 2.3

THE PHENOTYPIC SPECTRUM OF PATIENTS WITH PHARC SYNDROME DUE TO VARIANTS IN *ABHD12:* AN OPHTHALMIC PERSPECTIVE

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ABSTRACT

This study investigated the phenotypic spectrum of PHARC (polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and early-onset cataract) syndrome caused by biallelic variants in the *ABHD12* gene. Fifteen patients from 12 different families were included, with a mean age of 36.7 years (SD ± 11.0; range 17.5 to 53.9) at most recent examination. The presence and onset of neurological, audiological and ophthalmic symptoms were variable, with no evident order of symptom appearance. The mean best-corrected visual acuity was 1.1 logMAR (SD ± 0.9; range 0.1 to 2.8; equivalent to 20/250 Snellen) and showed a trend of progressive decline. Different types of cataract were observed in 13 out of 15 patients (87%), which also included congenital forms of cataract. Fundus examination revealed macular involvement in all patients, ranging from alterations of the retinal pigment epithelium to macular atrophy. Intraretinal spicular hyperpigmentation was observed in 7 out of 15 patients (47%). From an ophthalmic perspective, clinical manifestations in patients with PHARC demonstrate variability with regard to their onset and severity. Given the variable nature of PHARC, an early multidisciplinary assessment is recommended to assess disease severity.

INTRODUCTION

PHARC (polyneuropathy, hearing loss, ataxia, retinitis pigmentosa [RP], and early-onset cataract) is an acronym for a rare, neurodegenerative disease caused by biallelic variants in the *ABHD12* gene.[1, 2] *ABHD12* is located on chromosome 20 and encodes the α/β-hydrolase domain-containing protein 12 (ABHD12), which is highly expressed in the central nervous system (CNS), and plays a vital role in lipid metabolism. *In vitro*, ABHD12 inactivates the main endocannabinoid lipid transmitter 2-arachidonyl glycerol (2-AG), which acts on the cannabinoid receptor 1 and 2 (CB1 and CB2) by converting the 2-AG into the metabolites arachidonate and glycerol.[3,4] *In vivo*, ABHD12 serves as a lyso-phosphatidylserine (lyso-PS) lipase, which degrades lyso-PS that is biosynthesized by *ABHD16A*.[5] Disruption of ABHD12 in mice leads to i) accumulation of lyso-PS in the cerebellum breaching the homeostatic threshold, inducing continuous stimulation of the Purkinje neurons, leading to deregulated cerebellar activity; ii) increased levels of microglial activation and inflammation.[5-7] Accompanying this inflammatory response in mice are behavioral deficits, including sensorimotor defects and hearing loss, which resembles the phenotype described in patients with PHARC syndrome.[5-7]

Patients with PHARC syndrome demonstrate clinical variability with regard to disease onset, severity and progression.[1, 8-10] Polyneuropathy is typically one of the first findings in patients with PHARC syndrome, which usually manifests in childhood. Early signs of polyneuropathy include distal muscle weakness, sensory disturbances, pes cavus, and Achilles tendon contractures.[1, 9, 11] Sensorineural hearing loss is present in most patients with PHARC, with severity varying from moderate hearing loss to profound deafness.[1, 8] RP is reported in the second or third decade of life, with fundoscopy showing optic disc pallor, retinal vessel attenuation and intraretinal specular hyperpigmentation.[1, 8] As a result of RP, patients experience night blindness, constricted visual fields and, ultimately, central vision loss when retinal degeneration reaches the fovea.[12] While PHARC syndrome encompasses neurological, auditory and ophthalmic findings, not all of these findings are necessarily present at initial presentation.[1, 2] Depending on the presenting symptoms, patients may first be misdiagnosed with other neurodegenerative diseases that give rise to roughly similar phenotypes, such as Charcot-Marie-Tooth, Usher type 3, and adult Refsum disease.[1,11]

Because of the considerable challenges of diagnosing PHARC syndrome in patients, it is pivotal to gain more insight into the clinical and genetic characteristics of this neurodegenerative disease. From an ophthalmic perspective, little is known about the retinal phenotype in PHARC syndrome. Expanding our clinical and genetic knowledge on PHARC syndrome may provide insights into its onset, its natural history and the existence of genotype-phenotype correlations. In turn, this will ameliorate our understanding of the function of ABHD12 in humans, which may aid in opening avenues for potential future treatment strategies in the future. To this end, we describe the ophthalmic and associated clinical findings in patients with PHARC syndrome with biallelic *ABHD12* variants.

MATERIALS AND METHODS

Patient population

Clinical data were retrospectively obtained from the Amsterdam University Medical Centers (The Netherlands), Radboud University Medical Center (The Netherlands), Ghent University Hospital (Belgium), and Moorfields Eye Hospital (United Kingdom). Patients with biallelic (likely) pathogenic variants in *ABHD12* and a clinical diagnosis of PHARC, were included in the study. Five patients (D-6, F-8, G-9, H-10 and I-11) have been described previously [10, 13]. This study was approved by the Medical Ethics Committee of the Erasmus University Medical Center (MEC-2010-359, approval date: 10/10/2013) and by the local review board of the Amsterdam University Medical Centers (approval date: 18/11/2013). The study adhered to the Tenets of the Declaration of Helsinki and most patients provided informed consent for the use of their clinical data for research purposes. For Belgian patients, the local ethics committee waivered the need for informed consent on the condition of pseudonymization.

Data collection

Data were obtained through standardized review of medical records and included: sociodemographic information, medical history, age at onset, (previous) clinical diagnosis, best-corrected visual acuity (BCVA), slit-lamp examination, fundus findings, full-field electroretinogram (ffERG), spectral-domain optical coherence tomography (SD-OCT) imaging, and fundus autofluorescence (FAF) imaging. Neurological and audiological data, if available, comprised of a complete physical examination, nerve conduction studies, magnetic resonance brain imaging (MRI), and audiometric testing.

Genetic Analysis

Genomic DNA was obtained from peripheral blood sample using standard protocols. Genetic analysis was performed at each respective center and was performed using a combination of Sanger sequencing and next-generation sequencing, which included targeted gene panel testing, whole exome sequencing and whole genome sequencing. Nucleotide numbering was based on the coding reference NM_001042472.3. For missense variants, pathogenicity predictions from SIFT, Align GVGD, and Polyphen-2 were compared (Supplemental Table 1). The pathogenicity of each variant was assessed and classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines.[14]

Statistical Analysis

Data analysis was performed using R version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria). The normality of data was analyzed using the Shapiro-Wilk test and was also visually plotted. Continuous data were either presented as mean, standard deviations (SD), and range, whereas categorical data were presented as frequencies and percentages. BCVA data was converted to logarithm of the minimum

angle of resolution (logMAR) values for statistical analysis. For vision categories of counting fingers, hand movements, light perception and no light perception, logMAR values of 2.6, 2.7, 2.8 and 2.9 were used, respectively [15].

RESULTS

Patient characteristics

Fifteen patients from 12 different families were included in this study. An overview of the clinical and genetic characteristics of included patients is provided in Table 1. Most patients were male (n = 12; 80%), and the mean age at most recent examination was 36.7 years (SD ± 11.0; range 17.5 to 53.9). Previous (mis)diagnoses, available for 13 patients (87%), included forms of retinal degeneration (e.g. non-syndromic RP or Usher, n = 9; 69%), Charcot–Marie–Tooth (n = 2; 17%), spinocerebellar ataxia (n = 1; 8%), and optic neuropathy (n = 1; 8%). Phytanic acid levels were also assessed in 5 patients (A-1, B-2, C-3, C-4 and C-5) to rule out adult Refsum disease.

In total, 13 different *ABHD12* variants were found in this cohort, 3 of which were missense variants, 3 splice site variants, 4 nonsense variants, and 3 frameshift variants (Table 1 and Supplemental Table 1). The most common variant in this cohort was the frameshift variant c.337_338delGAinsTTT, which was present in more than half of the cohort in either homozygous or compound heterozygous form. This variant in exon 3 was predicted to result in a substitution of asparagine with phenylalanine at codon 113, introducing a premature termination codon (p.[Asp113Phefs*15]).

Table 1. Genetic and clinical characteristics at last examination of patients with biallelic *ABHD12* variants.

		Genetic analysis		Presence of PHARC syndrome symptoms and age at symptom onset/diagnosis (years)				
Family-ID	**Sex, age**	**Allele 1/ Allele 2**	**Protein change**	**Polyneuropathy**	**Hearing loss**	**Ataxia**	**Retinitis pigmentosa**	**Cataract**
A-1	M, 47	c.337_338delGAinsTTT / c.1075del	p.(Asp113Phefs*15) / p.(Val359Phefs*27)	Pes cavus, hammertoes, distal sensory loss, and absent tendon reflexes; age 8	Yes; age 28	Yes; age 8	Asymptomatic, detected during electrophysiological testing at age 45	Yes; age 36
B-2	F, 32	c.337_338delGAinsTTT / c.337_338delGAlinsTTT	p.(Asp113Phefs*15) / p.(Asp113Phefs*15)	Yes; childhood	Yes; age 17	Yes; age 45	Reduced visual acuity; age 32	Posterior subcapsular cataract; age 32
C-3[*]	M, 33	c.337_338delGAinsTTT / c.423-1_425del	p.(Asp113Phefs*15) / p.(?)	Asymptomatic; but detected during examination at age 27	No¶	Yes; age 27	Night blindness; age 14	Sutural cataract; age 3
C-4[*]	M, 33	c.337_338delGAinsTTT / c.423-1_425del	p.(Asp113Phefs*15) / p.(?)	Distal muscle weakness and sensory loss; childhood	Yes; NA	Yes; age 27	Night blindness; age 21	Sutural cataract; age 3
C-5*	M, 38	c.337_338delGAinsTTT / c.423-1_425del	p.(Asp113Phefs*15) / p.(?)	Abnormal gait pattern; childhood	Yes, 20	Yes; age 31	Night blindness	Star-shaped cataract; age 4
D-6	M, 42	c.477G>A / c.557G>C	p.(Trp159*) / p.(Arg186Pro)	Distal sensory loss and reduced tendon reflexes; age 35	Yes; age 36	Yes; NA	Reduced visual acuity; age 29	Cortical cataract; age 29
E-7[†]	F, 36	c.337_338delGAinsTTT / c.337_338delGAlinsTTT	p.(Asp113Phefs*15) / p.(Asp113Phefs*15)	NA[‡]	Yes; age 12	Yes; NA	Visual field loss; age 31	Posterior subcapsular cataract; age 32
F-8	M, 53	c.784C>T / c.867+5G >A	p.(Arg262*) / p.(?)	Distal sensory loss; age 53[‡]	Yes; age 20	NA	Reduced visual acuity; age 18	No

Table 1. Genetic and clinical characteristics at last examination of patients with biallelic *ABHD12* variants. (continued)

		Genetic analysis		Presence of PHARC syndrome symptoms and age at symptom onset/diagnosis (years)				
Family-ID	**Sex, age**	**Allele 1/ Allele 2**	**Protein change**	**Polyneuropathy**	**Hearing loss**	**Ataxia**	**Retinitis pigmentosa**	**Cataract**
G-9	M, 34	c.620-2A>G / c.620-2A>G	p.(?) / p.(?)	Lower limb muscle weakness; age 31‡	Yes; age 20	NA	Reduced visual acuity and night blindness; age 22	Yes; age 26
H-10†	M, 22	c.193C>T / c.193 C>T	p.(Arg65*) / p.(Arg65*)	Lack of coordination; age 7‡	No¶	NA	Reduced visual acuity; age 16	No
I-11†	M, 53	c.374C>T / c.1154T>C	p.(Thr125Met) / p.(Leu385Pro)	NA, but epilepsy and learning difficulties‡	Yes; age 44	NA	Reduced visual acuity and night blindness; age 30	Posterior polar cataract; age 41
J-12*	M, 20	c.337_338delGAinsTTT/ c.337_338delGAinsTTT	p.(Asp113Phefs*15) / p.(Asp113Phefs*15)	Yes; age 20	Yes; age 16	No	Night blindness; age 16	Star-shaped cataract; age 17
J-13*	M, 17	c.337_338delGAinsTTT/ c.337_338delGAinsTTT	p.(Asp113Phefs*15) / p.(Asp113Phefs*15)	Yes; age 18	Yes; age 10	No	Reduced visual acuity; age 10	Star-shaped cataract; age 10
K-14	F, 46	c.1063C>T/ c.1063C>T	p.(Arg355*) / p.(Arg355*)	Yes; age 47	Yes; NA	Yes; NA	Yes; NA	Cerulean cataract, NA
L-15	M, 39	c.337_338delGAinsTTT/ c.341dup	p.(Asp113Phefs*15) / p.(Leu114Phefs*14)	NA‡	Yes; age 33	NA	Night blindness; age 23	Star-shaped cataract; age 29

*Nucleotide numbering is based on reference sequence NM_001042472.3. * Patients C-3, C-4 and C-5 are siblings and patients J-12 and J-13 are siblings. † Consanguineous parents. ‡ Neurological evaluation/electrophysiological testing was not performed or available in these patients. ¶ Patients reported no subjective hearing loss, although audiometric testing results were not available. F = female; M = male; NA = data not available.*

Clinical examination

The onset of neurological, auditory and ophthalmic symptoms was variable, with no apparent order of symptom occurence (Table 1). Results from nerve conduction studies were available for 9 patients (60%), which revealed various degrees of demyelinating polyneuropathy, even in an asymptomatic patient. Patient C-3 had no subjective complaints of sensory or motor deficits, despite both of his siblings (patients C-4 and C-5) being diagnosed with severe demyelinating polyneuropathy in childhood years. Still, upon neurological evaluation, a subtle foot drop and absent Achilles tendon reflexes were detected, with nerve conduction studies revealing a demyelinating polyneuropathy. Hearing loss was not subjectively present in 2 patients (patients C-3 and H-10), although formal audiometric testing results were not available in these patients. Similarly, the presence of ataxia was observed in less than half of the cohort, although the absence of ataxia could not be excluded in 5 patients as neurological examination was not performed or data were not available. MRI was performed in 6 patients (40%; patients A-1, C-3, C-4, C-5, E-7, J-12 and J-13), with signs of cerebellar atrophy in 1 patient with ataxia (patient C-5) and 2 patients without ataxia (J-12 and J-13).

The ophthalmic findings in this cohort at last visit are described in Table 2. Loss of BCVA was observed in all patients (100%), with a mean BCVA of 1.1 logMAR (SD ± 0.9; range 0.1 to 2.8), which is equivalent to approximately 20/250 Snellen acuity. Four patients (A-1, B-2, E-7 and J-13), who carried the variant c.337_338delGAinsTTT in either homozygous or compound heterozygous form, had relatively preserved BCVA (BCVA ≥ 20/40 Snellen in the better-seeing eye). In contrast, the remaining patients, despite being in a similar age range, had visual acuities that could be classified as low vision (BCVA < 20/70 Snellen in the better-seeing eye) or worse. Patients with preserved BCVA were not significantly younger than those with low vision (-4.6 years, $p = 0.496$; independent t-test).

Slit-lamp examination revealed cataracts in 13 patients (87%), of whom 10 patients (77%) underwent uncomplicated cataract extraction. Various types of cataract were observed in this cohort, which also included congenital forms of cataract (Table 1). In 4 patients (patients C-5, J-12, J-13, and L-15), lens opacities were located in the posterior surface of the lens and followed a star-shaped distribution (Figure 1). Patients underwent their first cataract extraction and intraocular lens implementation at a mean age of 30.3 (SD ± 8.8; range 19.0 to 44.0).

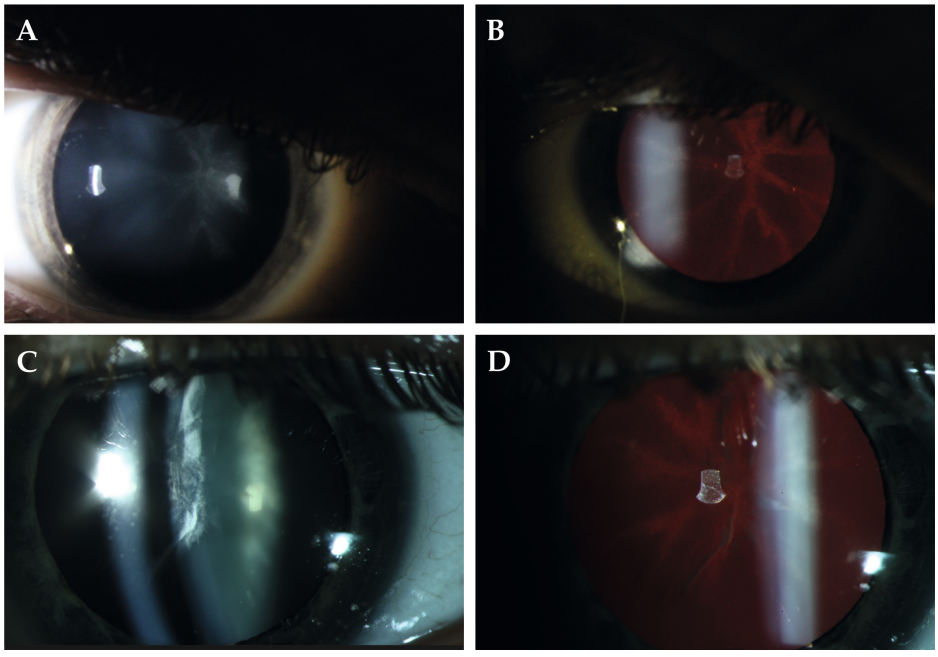

Figure 1. Slit-lamp findings in 2 patients with PHARC syndrome. **A-B**. Slit-lamp photographs of the right eye of patient J-13 at the age of 17. Best-corrected visual acuity was 20/50 Snellen in this eye. Direct illumination demonstrated the presence of cataract in the posterior surface of the lens. Retroillumination revealed that the observed opacity followed a star-shaped distribution, which seemed to delineate the crystalline lens sutures of the posterior cortex. Similar findings were found in the contralateral eye and in both eyes of his sibling (patient J-12, not shown). **C-D**. The right eye of patient L-15 (age 37) showed opacities in both the anterior and posterior cortex. Best-corrected visual acuity was 20/100 during this visit. Retroillumination showed anterior cortical cataract and a star-shaped opacity in the posterior surface.

Table 2. Summary of ophthalmic findings at most recent examination in this cohort of patients with biallelic *ABHD12* variants.

Family-ID	Sex, age	BCVA (OD; OS)	Lens status; age at first surgery	ffERG	Fundus findings		Spectral-domain optical coherence tomography	Fundus autofluorescence
					Macular changes	Bone spicules		
A-1	M, 47	20/22; 20/22	Pseudophakic; surgery at age 36	RCD	RPE alterations	No	Epiretinal membrane, degeneration of the outer retina with preservation of ELM and EZ at the (para)fovea	Hypo-AF regions in midperiphery with a macular hyper-AF ring
B-2	F, 32	20/25; 20/25	Pseudophakic; surgery at age 32	RCD	RPE alterations	No	Degeneration of the outer retina with preservation of ELM and EZ at the (para)fovea	Central hypo-AF surrounded by a hyper-AF ring
C-3	M, 33	20/200; 20/200	Pseudophakic; surgery at age 26	NA	Atrophy	Yes	Degeneration of the outer retina	NA
C-4	M, 33	20/125; 20/100	Pseudophakic; surgery at age 21	MR	Atrophy	Yes	Epiretinal membrane, degeneration of the outer retina, CME ODS at age 29, resolved at age 31	Hypo-AF lesions in the midperiphery with hyper-AF changes in the central macula
C-5	M, 38	20/134; 20/134	Pseudophakic; surgery at age 19	MR	Atrophy	Yes	Epiretinal membrane, degeneration of the outer retina, CME ODS at age 30, resolved at age 32	NA
D-6	M, 42	20/400; 20/400	Cortical cataract	RCD	Atrophy	No	Degeneration of the outer retina	Central hypo-AF with a hyper-AF foveal spot
E-7	F, 36	20/29; 20/29	Pseudophakic; surgery at age 29	RCD	RPE alterations	No	Degeneration of the outer retina with preservation of ELM and EZ at the (para)fovea	Hypo-AF regions in midperiphery with a macular hyper-AF ring
F-8	M, 53	LP; LP	Clear lens	NA	Atrophy	Yes	Extensive atrophy of all retinal layers	Generalized hypo-AF

Table 2. Summary of ophthalmic findings at most recent examination in this cohort of patients with biallelic *ABHD12* variants. (continued)

Family-ID	Sex, age	BCVA (OD; OS)	Lens status; age at first surgery	ffERG	Fundus findings		Spectral-domain optical coherence tomography	Fundus autofluorescence
					Macular changes	Bone spicules		
G-9	M, 34	20/400; 20/400	Pseudophakic; surgery at age 34	RCD	Atrophy	No	Extensive atrophy of all retinal layers at the fovea, with relatively preserved layers in the perifovea	Central hypo-AF
H-10	M, 22	20/240; 20/240	Clear lens	RCD	Bull's eye	No	Degeneration of the outer retina	Central hypo-AF
I-11	M, 53	HM; HM	Pseudophakic; surgery at age 44	NA	Atrophy & Macular hole OS	Yes	Degeneration of the outer retina. Macular hole OS.	Mottled patches of hypo-AF in nasal region with hypo-AF in the central macula
J-12	M, 20	20/200; 20/200	Pseudophakic; surgery at age 20	NA	Atrophy	No	Degeneration of the outer retina.	Central hypo-AF with hyper-AF borders
J- 13	M, 17	20/50; 20/40	Star-shaped cataract	NA	Atrophy	No	Degeneration of the outer retina with preservation of ELM and EZ at the (para)fovea	Hyper-AF ring surrounded by a larger hyper-AF ring
K-14	F, 46	HM; 20/400	Cerulean cataract	RCD	Atrophy	Yes	Degeneration of the outer retina.	Central hypo-AF with a hyper-AF foveal spot. Several hypo-AF lesions along the superior vascular arcade.
L-15	M, 39	20/134; 20/200	Pseudophakic; surgery at age 39	RCD	Atrophy	Yes	Degeneration of the outer retina. CME ODS at age 33, resolved at age 39	Generalized hypo-AF with preserved AF in the central macula.

Findings were similar between eyes unless specified otherwise. AF = autofluorescence; BCVA = best-corrected visual acuity; CME = cystoid macular edema; EZ = ellipsoid zone; ELM = external limiting membrane; F = female; HM = hand movements; LP = light perception; M = male; MR = minimal responses; NA = not available; RCD = rod-cone dystrophy.

In Figure 2**,** we present representative fundus and multimodal imaging findings of this cohort. Fundus examination revealed signs of retinal degeneration in all patients, although a clinical hallmark of RP -intraretinal spicular hyperpigmentation- was only observed in 7 out of 15 patients (47%; Table 2). Patients with intraretinal spicular hyperpigmentation had worse logMAR BCVA than those without pigmentation (+0.9 logMAR BCVA, p = 0.019; independent t-test). Macular involvement was present in all patients (100%), ranging from retinal pigment epithelium alterations to macular atrophy. Full-field electroretinography data were available for 10 patients (67%), showing a rod-cone dystrophy pattern (n = 8; 80%) or minimal scotopic and photopic responses (n = 2; 20%).

FAF imaging was available for 13 out of 15 patients (87%). A common finding was the presence of a hypo-autofluorescent area in the central macula in 6 out 13 patients (46%; Figure 2-B). Other FAF patterns included: a macular hyperautofluorescent ring in 4 patients with relatively preserved BCVA (patients A-1, B-2, E-7 and J-13; Figure 2-E); and generalized, mottled hypo-autofluorescence in patient F-8 who had light perception vision (Figure 2-H). A description of the other FAF patterns observed is provided in Table 2. SD-OCT imaging showed degeneration of the outer retinal layers, the external limiting membrane and the ellipsoid zone, in all patients outside the central macula (100%), and, to various extents, within the central macula. Preservation of the outer retinal layers in the central macula was observed in the four patients with preserved BCVA (patients A-1, B-2, E-7 and J-13; Figure 2-F). Cystoid macular edema was observed in 3 patients (20%), which resolved several years later without treatment.

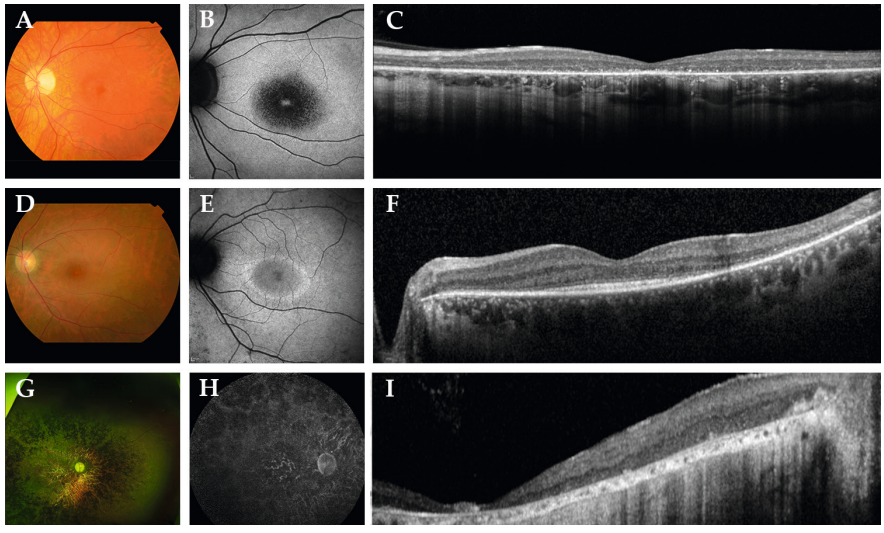

Figure 2. Representative color fundus photographs with corresponding fundus autofluorescence (FAF) and spectral-domain optical coherence tomography (SD-OCT) images in this cohort of patients with

biallelic *ABHD12* variants. (**A-C**) The left eye of patient D-6, a 42-year-old man with Snellen best-corrected visual acuity (BCVA) of 20/400. Fundus photography revealed a slightly pale optic disc, atrophic macular changes, and retinal pigment epithelium (RPE) changes in the midperipheral retina, in the absence of spicular hyperpigmentation. FAF imaging showed a region of hypo-autofluorescence (hypo-AF) in the central macula with a hyperautofluorescent (hyper-AF) spot in the fovea. On SD-OCT, loss of the external limiting membrane and ellipsoid zone was observed. (**D-F**) The left eye of a 36-year old woman, patient E-7, with Snellen BCVA of 20/29. Fundus imaging showed macular and midperipheral alterations, with no evident spicular hyperpigmentation. On FAF imaging, hypo-AF zones of RPE degeneration were present outside the macula, and a macular hyper-AF ring was observed. SD-OCT showed preservation of the outer retinal layers in the (para)fovea. (**G-I**) Patient F-8, a 53-year old man with light perception visual acuity, showed extensive degeneration across the entire retina with dense spicular hyperpigmentation reaching the posterior pole. FAF imaging demonstrated generalized hypo-AF due to the extensive RPE atrophy. SD-OCT showed marked chorioretinal atrophy.

DISCUSSION

In this retrospective study, we report the genetic and clinical characteristics of 15 patients with PHARC syndrome with variable severity, caused by variants in the *ABHD12* gene. This study aimed to expand our clinical knowledge on PHARC syndrome, as it is a rare neurodegenerative disease with less than 50 cases currently described in the literature.[11, 16] The advent of next-generation sequencing has made it possible to identify disease-causing variants on novel genes at a rapid pace, and the ongoing improvements in this technology may lead to an increased detection of patients with biallelic *ABHD12* variants/PHARC syndrome in the future.[17] In our study, we found 13 different variants in *ABHD12*, with the most common variant being c.337_338delGAinsTTT. This is a relatively well-known variant, as it was the first described variant in the Norwegian cohort of Fiskerstrand *et al*., which may suggest a common European ancestry.[1, 2] No evident genotype-phenotype correlation could be established, although 4 patients with this variant on 1 or 2 allele(s) had relatively preserved BCVA and intact ELM/EZ compared to the other patients in this cohort, which suggests a relatively milder ocular phenotype. However, we were unable to correlate ophthalmic findings with neurological or audiological assessments (e.g. nerve conduction studies, MRI and audiograms), as these evaluations were either i) not (routinely) performed; ii) performed in a much earlier or later stage of disease than ophthalmic examinations; iii) or not available due to the retrospective nature of this study. In order to establish potential genotype-phenotype correlations, quantitative data from all involved clinical disciplines need to be obtained within similar time frames, which requires a coordinated interdisciplinary approach. This remains challenging as patients show variability in the manifestation of neurological, audiological, and ophthalmic symptoms, which may result in misdiagnosis, delayed diagnosis and delayed referral for assessment of these symptoms. This was also

the case for our cohort, as the presence and onset of neurological, audiological and ophthalmic symptoms were variable, even in those from within the same family. No evident order of appearance of symptoms was observed, as neurological symptoms could precede ophthalmic symptoms and vice versa. This variable onset of the different symptoms resulted in different diagnoses at initial visit, including (non-) syndromic forms of RP, Charcot–Marie–Tooth and adult Refsum disease, consequently delaying an accurate diagnosis of PHARC syndrome. A patient's self-reported onset of symptoms may not be a particularly reliable indicator for the presence of disease, as self-reported data are susceptible to recall bias, and patients may be asymptomatic in early disease stages [18]. The latter is illustrated by patient C-3, who was asymptomatic for neurological deficits, but was revealed to have demyelinating sensorimotor polyneuropathy on nerve conduction studies. Similarly, the 47-year-old patient A-1 reported no subjective symptoms of RP, although a rod-cone degeneration pattern of the retina was established during full-field ERG. Most likely, disease progression must reach a certain threshold before noticeable symptoms are reported by patients. Establishing the onset and severity of *ABHD12*-associated clinical findings objectively is crucial to determine the natural history of PHARC syndrome, as well as to establish genotype-phenotype correlations. Ideally, patients should undergo objective testing from all involving clinical disciplines at time of diagnosis, and they should also be monitored over a prolonged period of time.

In all patients with available neurological data, demyelinating polyneuropathy was detected in all, with variable presence of ataxia and cerebellar atrophy. These findings are consistent with previous studies on PHARC syndrome.[1, 11] The exact etiology of neurologic deficits in patients with PHARC remains unclear, but previous studies have shown that accumulation of lyso-PS in the cerebellum due to disruptive ABHD12 leads to increased levels of microglial activation and neuroinflammation, which is the presumed cause for neurological deficits in *abhd12* knockout mice.[5-7] Ataxia without cerebellar atrophy might be explained based on the cellular localization of ABHD12 in the Purkinje neurons. When ABHD12 activity is absent, the Purkinje neurons are constantly stimulated by the accumulated lyso-PS, resulting in deregulated cerebellar function.[19] Consistent with previous studies, we found that hearing loss was present in the majority of patients in childhood/early adolescence.[1, 10, 20, 21] To date, it remains unclear where ABHD12 localizes and interacts in the inner ear, which is crucial for determining the cause of hearing loss in patients with PHARC syndrome. Previous studies have shown that microglial cells are in abundance in the inner ear, and can be modulated by distant inflammation by the central nervous system.[22] It could be that microglial cells in the inner ear undergo similar inflammation processes as in the central nervous system due to disruptive ABHD12, with consequent hearing loss, although this hypothesis requires further testing.

From an ophthalmic perspective, we found that the majority of patients (57%) did not exhibit typical intraretinal spicular hyperpigmentation as a characteristic sign of RP on fundus examination, despite patients experiencing subjective symptoms of RP. This phenotype resembles an atypical variant of RP without pigmentation.[23] These patients may demonstrate an early remodeling stage of RP, in which pigmented RPE cells still needs to migrate to the inner retina, before their demise leads to the classic sign of spicular hyperpigmentation as seen on fundoscopy.[23-25] Longer follow-up in patients with PHARC syndrome is required to confirm this hypothesis. Despite the absence of spicular pigment migration in patients, the diagnosis rod-cone dystrophy could still be established using ffERG, which highlights the importance of electrophysiological testing in this disease. FAF patterns were variable, although a common finding was the early presence of a hypo-AF region in the macula, indicative of early macular involvement and early loss of visual acuity. The extent of macular damage was not assessed in this study, but could potentially be measured using multifocal/pattern ERG or microperimetry, the latter being commonly used as an outcome measure in gene therapy trials.[26, 27] Consistent with FAF findings, SD-OCT showed degeneration of the outer retinal layers in all patients, with preservation of the ELM/EZ at (para)fovea in only 4 patients who had relatively preserved BCVA values despite being in similar age ranges as those with no preservation. Therefore, multimodal imaging techniques - in conjunction with more sensitive outcome measures of the macula - are valuable tools to assess the disease severity in the retina, and can likely be used to monitor disease progression in patients with PHARC syndrome.

Visually significant cataract was observed between the second and fourth decade of life in the majority of patients, which is within the reported age range of cataract found in patients with non-syndromic forms of RP.[28-30] The exact pathogenesis of cataract formation in patients with RP is still unknown, although it has been hypothesized that the degenerative retina of patients with RP increases the levels of proinflammatory cytokines and chemokines in the vitreous, which may reach and change the homeostatic state of the natural lens, resulting in formation of cataract.[30-31] It is unclear whether this hypothesis on cataract morphogenesis is also applicable to patients with *ABHD12* variants, as several patients in this cohort were diagnosed with cataract types that suggest a congenital origin.[32] A single case of posterior polar cataract was also reported in the study of Fiskerstrand and colleagues, although a description of cataract morphology was not provided for the other patients of this cohort.[1] In addition, we reported a star-shaped cataract in the posterior cortex of the lens in several related and unrelated patients, that presumably delineated the crystalline lens sutures of the posterior cortex, which is also suggestive for a congenital origin.[32, 33]

The molecular and cellular basis for degeneration in the retina and cataract formation due to *ABHD12* variants remains to be elucidated. To our knowledge, while the expression of *ABHD12* in the brain has been established, limited studies have investigated the expression of *ABHD12* in the neurosensory retina or in the natural lens of the eye.[1] Previous studies in *abhd12* knockout mice showed no degenerative changes in the retina and no lens opacities, despite exhibiting neurological and auditory deficits, which suggests limited expression of *ABHD12* in the eye.[19] Naturally, these findings should be interpreted with caution, as recapitulating human diseases with murine models remains challenging. By analyzing the Human Protein Atlas, we observed some degree of *ABHD12* expression in photoreceptor, bipolar and horizontal cells (available from http://www.proteinatlas.org).[34] A high expression of *ABHD12* is found in microglial cells, which are the resident immune cells of the brain and are also present in the plexiform layers of the retina.[1, 35] Microglial cells possibly play an initiating role in the degeneration of the retina in patients with *ABHD12* variants, which aligns with the hypotheses of the microglial cell possibly being one of the driving forces behind neurodegeneration in the central nervous system and inner ear.[1, 8] Establishing the causative agent for neurodegeneration in patients with ABHD12 variants will be pivotal for the development of treatment modalities for this neurodegenerative disease. For inherited retinal dystrophies, promising results have been achieved with gene therapy, resulting in improvements in visual function.[36, 37] Given the size of the primary transcript of *ABHD12* (1.1 kb, NM_001042472.3), *ABHD12* will likely fit into AAV vectors and is therefore a potential candidate for gene augmentation therapy. If the underlying cause of PHARC syndrome has a metabolic and/or immunological basis, suppressing or inhibiting targets in relevant pathways, such as the previously mentioned lyso-PS pathway, could prove an attractive alternative approach [19]. Further research into the molecular and biochemical basis of *ABHD12* will likely determine the most optimal path for treatment.

In conclusion, we report the phenotype of patients with PHARC syndrome due to biallelic *ABHD12* variants. Rod-cone dystrophy is present in all patients with PHARC syndrome with early macular involvement, although this finding may vary widely in its onset and severity. Given the variability in symptoms and clinical findings in patients with PHARC syndrome, patients should be evaluated in a multidisciplinary setting, involving ophthalmologists, neurologists, audiologists/otologists, and geneticists, when PHARC syndrome is either suspected or genetically confirmed.

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REFERENCES

1. Fiskerstrand, T., et al., *Mutations in ABHD12 Cause the Neurodegenerative Disease PHARC: An Inborn Error of Endocannabinoid Metabolism.* The American Journal of Human Genetics, 2010. 87(3): p. 410-417.
2. Fiskerstrand, T., et al., *A novel Refsum-like disorder that maps to chromosome 20.* Neurology, 2009. 72(1): p. 20.
3. Savinainen, J.R., S.M. Saario, and J.T. Laitinen, *The serine hydrolases MAGL, ABHD6 and ABHD12 as guardians of 2-arachidonoylglycerol signalling through cannabinoid receptors.* Acta physiologica (Oxford, England), 2012. 204(2): p. 267-276.
4. Blankman, J.L., G.M. Simon, and B.F. Cravatt, *A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol.* Chemistry & biology, 2007. 14(12): p. 1347-1356.
5. Shin, M., et al., *Lipid-metabolizing serine hydrolases in the mammalian central nervous system: endocannabinoids and beyond.* Biochimica et biophysica acta. Molecular and cell biology of lipids, 2019. 1864(6): p. 907-921.
6. Joshi, A., et al., *Biochemical characterization of the PHARC-associated serine hydrolase ABHD12 reveals its preference for very-long-chain lipids.* J Biol Chem, 2018. 293(44): p. 16953-16963.
7. Kamat, S.S., et al., *Immunomodulatory lysophosphatidylserines are regulated by ABHD16A and ABHD12 interplay.* Nature Chemical Biology, 2015. 11(2): p. 164-171.
8. Chen, D.-H., et al., *Two novel mutations in ABHD12: expansion of the mutation spectrum in PHARC and assessment of their functional effects.* Human mutation, 2013. 34(12): p. 1672-1678.
9. Eisenberger, T., et al., *Targeted next-generation sequencing identifies a homozygous nonsense mutation in ABHD12, the gene underlying PHARC, in a family clinically diagnosed with Usher syndrome type 3.* Orphanet journal of rare diseases, 2012. 7: p. 59-59.
10. Nishiguchi, K.M., et al., *Exome Sequencing Extends the Phenotypic Spectrum for ABHD12 Mutations: From Syndromic to Nonsyndromic Retinal Degeneration.* Ophthalmology, 2014. 121(8): p. 1620-1627.
11. Thimm, A., et al., *Genotype-phenotype correlation in a novel ABHD12 mutation underlying PHARC syndrome.* Journal of the Peripheral Nervous System, 2020. 25(2): p. 112-116.
12. Verbakel, S.K., et al., *Non-syndromic retinitis pigmentosa.* Progress in Retinal and Eye Research, 2018. 66: p. 157-186.
13. Igelman, A.D., et al., *Expanding the clinical phenotype in patients with disease causing variants associated with atypical Usher syndrome.* Ophthalmic Genetics, 2021: p. 1-10.
14. Richards, S., et al., *Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.* Genetics in Medicine, 2015. 17(5): p. 405-423.
15. Grover, S., et al., *Visual Acuity Impairment in Patients with Retinitis Pigmentosa.* Ophthalmology, 1996. 103(10): p. 1593-1600.
16. Ellingford, J.M., et al., *Functional and in-silico interrogation of rare genomic variants impacting RNA splicing for the diagnosis of genomic disorders.* bioRxiv, 2019: p. 781088.
17. Mardis, E.R., *The impact of next-generation sequencing technology on genetics.* Trends in Genetics, 2008. 24(3): p. 133-141.
18. Althubaiti, A., *Information bias in health research: definition, pitfalls, and adjustment methods.* Journal of Multidisciplinary Healthcare, 2016. 9: p. 211-217.

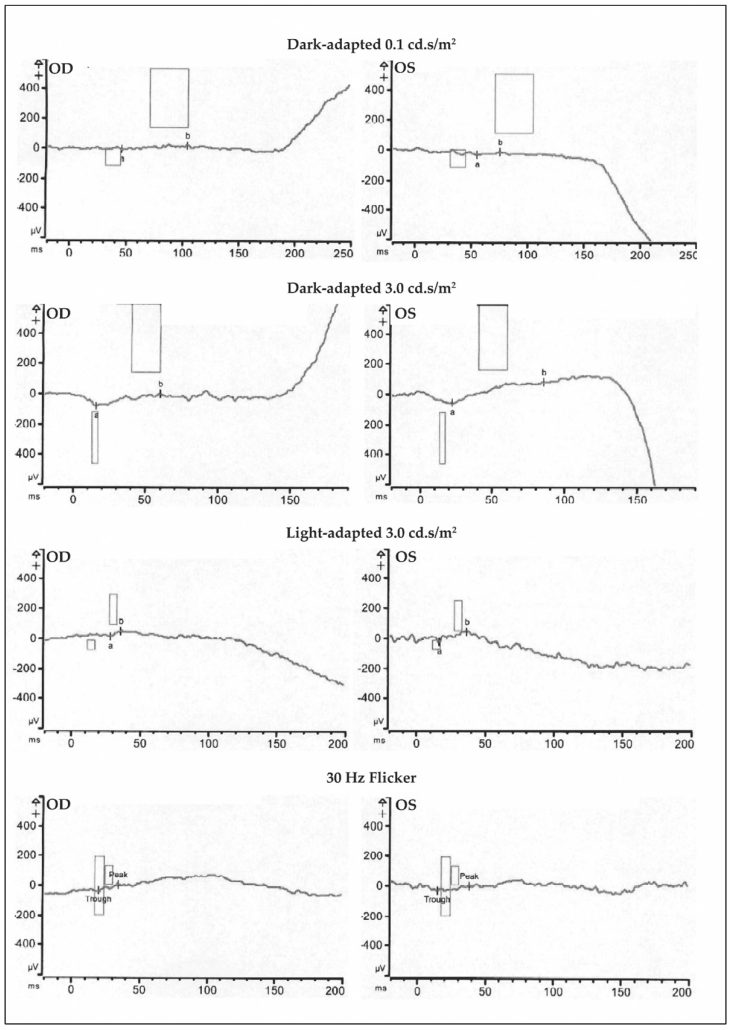
19. Blankman, J.L., et al., *ABHD12 controls brain lysophosphatidylserine pathways that are deregulated in a murine model of the neurodegenerative disease PHARC*. Proceedings of the National Academy of Sciences, 2013. 110(4): p. 1500.
20. Yoshimura, H., et al., *Novel ABHD12 Mutations in PHARC Patients: The Differential Diagnosis of Deaf-Blindness*. Annals of Otology, Rhinology & Laryngology, 2015. 124(1_suppl): p. 77S-83S.
21. Frasquet, M., et al., *Phenotypical features of two patients diagnosed with PHARC syndrome and carriers of a new homozygous mutation in the ABHD12 gene.* Journal of the Neurological Sciences, 2018. 387: p. 134-138.
22. O'Malley, J.T., J.B. Nadol, Jr., and M.J. McKenna, *Anti CD163+, Iba1+, and CD68+ Cells in the Adult Human Inner Ear: Normal Distribution of an Unappreciated Class of Macrophages/Microglia and Implications for Inflammatory Otopathology in Humans*. Otology & neurotology : official publication of the American Otological Society, American Neurotology Society [and] European Academy of Otology and Neurotology, 2016. 37(1): p. 99-108.
23. Takahashi, V.K.L., et al., *Rates of Bone Spicule Pigment Appearance in Patients With Retinitis Pigmentosa Sine Pigmento.* American Journal of Ophthalmology, 2018. 195: p. 176-180.
24. Li, Z.-Y., D.E. Possin, and A.H. Milam, *Histopathology of Bone Spicule Pigmentation in Retinitis Pigmentosa.* Ophthalmology, 1995. 102(5): p. 805-816.
25. Nguyen, X.-T.-A., et al., *RPGR-Associated Dystrophies: Clinical, Genetic, and Histopathological Features.* International journal of molecular sciences, 2020. 21(3): p. 835.
26. Pfau, M., et al., *Fundus-controlled perimetry (microperimetry): Application as outcome measure in clinical trials*. Progress in Retinal and Eye Research, 2020: p. 100907.
27. Hood, D.C., et al., *ISCEV standard for clinical multifocal electroretinography (mfERG) (2011 edition).* Documenta ophthalmologica. Advances in ophthalmology, 2012. 124(1): p. 1-13.
28. Jackson, H., et al., *Outcome of cataract surgery in patients with retinitis pigmentosa.* British Journal of Ophthalmology, 2001. 85(8): p. 936.
29. Fishman, G.A., R.J. Anderson, and P. Lourenco, *Prevalence of posterior subcapsular lens opacities in patients with retinitis pigmentosa.* British Journal of Ophthalmology, 1985. 69(4): p. 263.
30. Yoshida, N., et al., *Factors Affecting Visual Acuity after Cataract Surgery in Patients with Retinitis Pigmentosa.* Ophthalmology, 2015. 122(5): p. 903-908.
31. Hong, Y., et al., *A Review of Complicated Cataract in Retinitis Pigmentosa: Pathogenesis and Cataract Surgery*. Journal of Ophthalmology, 2020. 2020: p. 6699103.
32. Bell, S.J., et al., *Congenital cataract: a guide to genetic and clinical management*. Therapeutic Advances in Rare Disease, 2020. 1: p. 2633004020938061.
33. Gupta, A., et al., *In vivo SS-OCT imaging of crystalline lens sutures.* Biomedical Optics Express, 2020. 11(10): p. 5388-5400.
34. Uhlén, M., et al., *Tissue-based map of the human proteome.* Science, 2015. 347(6220): p. 1260419.
35. Rashid, K., I. Akhtar-Schaefer, and T. Langmann, *Microglia in Retinal Degeneration*. Frontiers in immunology, 2019. 10: p. 1975-1975.
36. Maguire, A.M., et al., *Efficacy, Safety, and Durability of Voretigene Neparvovec-rzyl in RPE65 Mutation–Associated Inherited Retinal Dystrophy: Results of Phase 1 and 3 Trials*. Ophthalmology, 2019. 126(9): p. 1273-1285.
37. Gardiner, K.L., et al., *Long-Term Structural Outcomes of Late-Stage RPE65 Gene Therapy.* Molecular Therapy, 2020. 28(1): p. 266-278.

SUPPLEMENTAL CONTENT

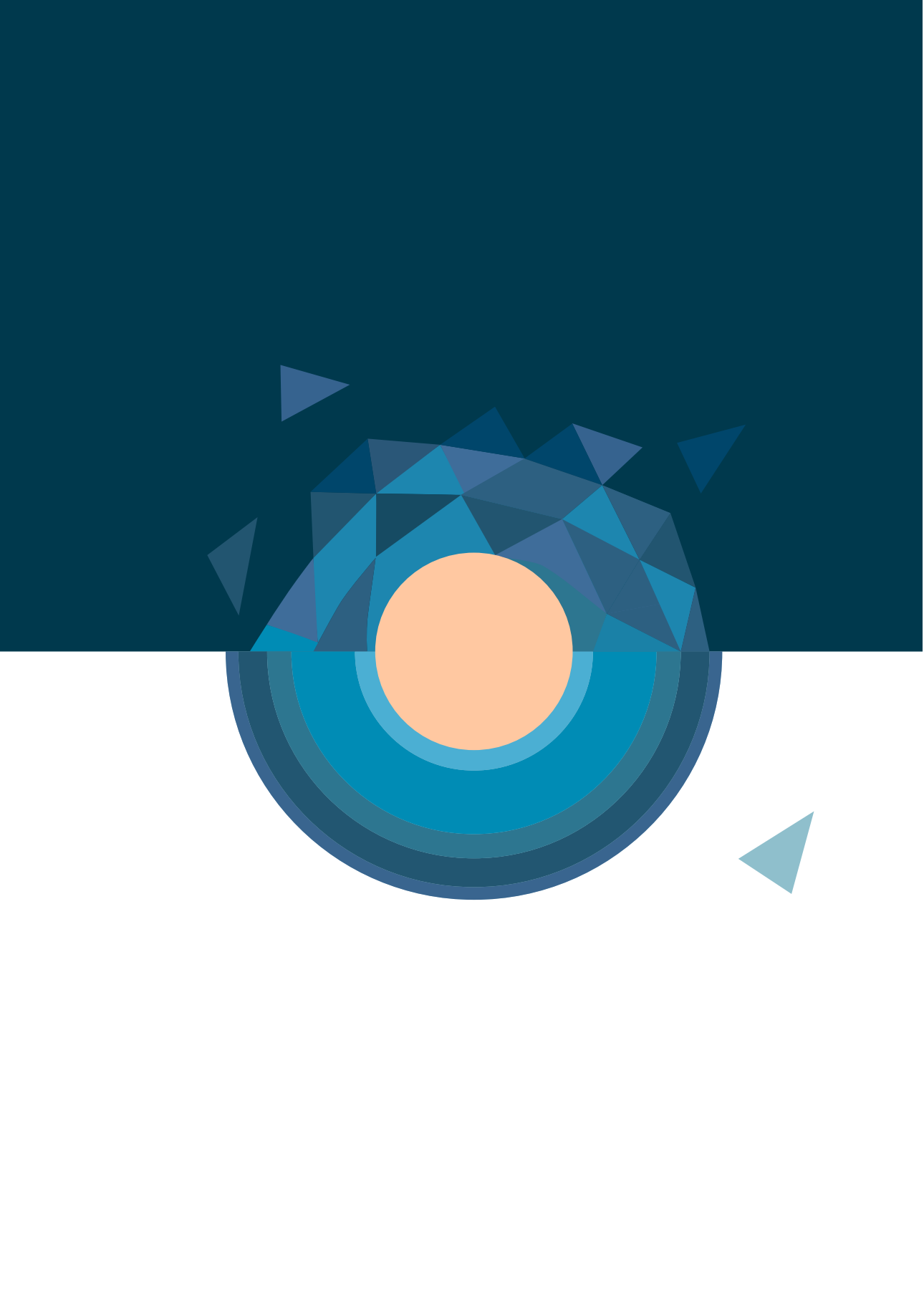
Supplemental Table 1. Overview of *ABHD12* variants found in this study.

Nucleotide change	Protein effect	Mutation type	Frequency (n =)	SIFT	Align GVGD	Polyphen-2	References
c.193C>T	p.(Arg65*)	Nonsense	2	-	-	-	Eisenberger *et al.* 2012, Igelman *et al.* 2021
c.337_338delinsTTT	p.(Asp113Phefs*15)	Frameshift	13	-	-	-	Fiskerstrand *et al.* 2010
c.341dup	p.(Leu114Phefs*14)	Frameshift	1	-		-	**This study**
c.374C>T	p.(Thr125Met)	Missense	1	Affects protein function	C65	Probably damaging	Igelman *et al.* 2021
c.423-1_425del	p.(?)	Splice site	3	-	-	-	**This study**
c.477G>A	p.(Trp159*)	Nonsense	1	-	-	-	Nishiguchi *et al.* 2014
c.557G>C	p.(Arg186Pro)	Missense	1	Affects protein function	C65	Probably damaging	Nishiguchi *et al.* 2014
c.620-2A>G	p.(?)	Splice site	2	-	-	-	Igelman *et al.* 2021
c.784C>T	p.(Arg262*)	Nonsense	1	-	-	-	Thimm *et al.* 2020, Igelman *et al.* 2021
c.867+5G >A	p.(?)	Splice site	1	-	-	-	Ellingford *et al.* 2019
c.1063C>T	p.(Arg355*)	Nonsense	2	-	-	-	**This study**
c.1075del	p.(Val359Phefs*27)	Frameshift	1	-	-	-	**This study**
c.1154T>C	p.(Leu385Pro)	Missense	1	Affects protein function	C65	Probably damaging	Igelman *et al.* 2021

Nucleotide numbering is based on the reference sequence NM_001042472.3. The Human Gene Mutation Database was accessed on July 5th, 2021.



Supplemental Figure S1. Full-field electroretinography (ERG) results of a patient with PHARC (polyneuropathy, hearing loss, ataxia, retinitis pigmentosa and early-onset cataract) syndrome. Full-field ERG responses in both eyes of patient A-1 at the age of 47. Full-field ERGs were performed according to the 'International Society for Clinical Electrophysiology of Vision' standards. Rod responses are (nearly) absent in both eyes, with minimal cone responses measured on light-adapted ERGs. cd.s./m2 = candela seconds per square meter; Hz = hertz; μV=microvolts; ms = milliseconds; OD = right eye; OS = left eye.



CHAPTER 2.4

CRB1-ASSOCIATED RETINAL DYSTROPHIES: A PROSPECTIVE NATURAL HISTORY STUDY IN ANTICIPATION OF FUTURE CLINICAL TRIALS

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ABSTRACT

Purpose
To describe the natural disease course of *CRB1*-associated retinal dystrophies, and to identify clinical endpoints for future clinical trials.

Design
Single center, prospective cohort study.

Methods
An investigator-initiated nationwide collaborative study that included 22 patients with *CRB1*-associated retinal dystrophies. Patients underwent ophthalmic assessment at baseline and 2 years after baseline. Clinical examination included best-corrected visual acuity (BCVA) using ETDRS charts, Goldmann kinetic perimetry (V4e isopter seeing retinal areas), microperimetry, full-field electroretinography (ERG), full-field stimulus threshold (FST), fundus photography, spectral-domain optical coherence tomography and fundus autofluorescence imaging.

Results
Based on genetic, clinical and electrophysiological data, patients were diagnosed with retinitis pigmentosa (n = 19; 86%), cone-rod dystrophy (n = 2; 9%) or isolated macular dystrophy (n = 1; 5%). Two-year analysis of the entire cohort showed no significant changes in BCVA ($p = 0.069$) or V4e isopter seeing retinal areas ($p = 0.616$), although signs of clinical progression were present in individual patients. Macular sensitivity measured on microperimetry revealed a significant reduction at 2-year follow-up ($p < 0.001$). FST responses were measurable in patients with non-recordable ERGs. On average, FST responses remained stable during follow-up.

Conclusion
In *CRB1*-associated retinal dystrophies, visual acuity and visual field measures remain relatively stable over the course of 2 years. Microperimetry showed a significant decrease in retinal sensitivity during follow-up, and may be a more sensitive progression marker. Retinal sensitivity on microperimetry may serve as a functional clinical endpoint in future human treatment trials for *CRB1*-associated retinal dystrophies.

INTRODUCTION

A wide range of related retinal dystrophies (RDs), including Leber congenital amaurosis (LCA), retinitis pigmentosa (RP) and cone(-rod) dystrophies, can be caused by variants in the *CRB1* gene.[1-4] LCA is considered the most severe retinal dystrophy, presenting at birth or early infancy, and is characterized by severe visual impairment, nystagmus, poor pupillary responses, and absent responses on electroretinography.[4] RP is characterized by primary degeneration of rod photoreceptors, with secondary cone degeneration. Initial symptoms in RP typically include night blindness due to degeneration of the rods, followed by concentric visual field loss, and eventually central vision loss later in life due to cone dysfunction.[5] RP comprises a broad spectrum of phenotypic presentations, and can become symptomatic at different ages, ranging from early childhood (i.e. juvenile RP) to middle age, caused by a broad spectrum of genes.[6]

The *CRB1* gene encodes the transmembrane protein Crumbs homologue 1 (CRB1) which, in mammals, localizes to the subapical region of Müller and photoreceptor cells.[7-10] The canonical isoform of CRB1 consists of 19 epidermal growth factor domains and 3 laminin A globular-like domains, and a short cytoplasmic tail that contains FERM/PDZ binding motifs.[11] Recently, a novel isoform of CRB1, CRB1-B, was also discovered, which is presumed to be more abundant in the human retina than its canonical form.[12] While the function of CRB1 in the human retina has not been fully elucidated, it has been suggested to play a key role in cell polarity, cell-to-cell adhesion, photoreceptor morphogenesis and retinal maturation.[8,13-16] The role of CRB1 in retinal development is supported by the abnormal thickening and coarse lamination of the inner retinal layers that has been described in the majority of cases of *CRB1*-associated RDs, which strikes a resemblance to an immature retina.[17] Other clinical features described in *CRB1*-associated RDs include hyperopia, optic nerve drusen, preservation of para-arteriolar retinal pigment epithelium, cystoid macular edema, nummular pigmentation and Coats-like exudates.[18]

Currently, no treatment exists for patients with *CRB1*-associated RDs, but proof-of-concept of adeno-associated virus-mediated gene transfer was achieved using murine models.[15,19] As *CRB1* gene therapy is being developed, it is crucial to determine adequate clinical endpoints ahead of these upcoming trials.[10] This requires an optimal understanding of the disease; its variability and its progression, based on retrospective and prospective natural history studies.[20] A retrospective study previously performed by our study group provided insights into the progressive decline in visual acuity and visual fields in patients with *CRB1* variants, showing that the optimal window for treatment is likely within the first 2 to 3 decades of life based on these outcome measures.[18] However, our knowledge on the feasibility of other psychophysical

outcome measures, such as microperimetry and full-field-stimulus thresholds, remains limited.[18,21]

Herein, we report the first prospective natural history study performed in 22 patients with biallelic *CRB1* variants. The objective of this study was to describe the disease progression in *CRB1*-associated retinal dystrophies, and to determine potential clinical endpoints in anticipation of future therapeutic trials. Based on these findings, we provide the first recommendations and considerations for the study design of upcoming *CRB1* clinical trials.

METHODS

Patient recruitment

This nationwide collaborative study recruited patients from 2 different registries: the RD5000 database, which is a national registry for inherited retinal diseases; and the Delleman archive for genetic eye diseases at Amsterdam University Medical Center.[22] Inclusion criteria for this study were the presence of biallelic *CRB1* variants with a RD phenotype, and a best-corrected visual acuity (BCVA) of ≥20/400 Snellen acuity. In total, 22 patients with biallelic *CRB1* variants were included in the study, of which 10 patients (45%) originated from a previously described genetically isolated population.[21,23] Patients were examined at baseline and 2 years after baseline at Leiden University Medical Center.

The study protocol, genetic findings, and baseline characteristics of the included patients have been described in detail elsewhere, and are briefly described herein.[24] The current study presents the 2-year follow-up data, and describes the longitudinal findings in this cohort.

The study protocol was approved by the Medical Ethics Committee of the Erasmus Medical Center, as it was performed within the framework of the RD5000, and from the local review board of Leiden University Medical Center. Informed consent was obtained from individuals and/or legal guardians, and the study adhered to the tenets of the Declaration of Helsinki.

Clinical examination

Refraction and BCVA were measured monocularly, using the Early Treatment Diabetic Retinopathy Study (ETDRS) letter chart. ETDRS letters were converted to Logarithm of the Minimum Angle of Resolution (logMAR) values for statistical analysis. Visual fields (V4e isopter) were obtained using Goldmann perimetry, which were subsequently converted to digital seeing retinal areas (in mm^2) using a method described by Dagnelie.[25] A change ≥20% in retinal seeing area was considered clinically significant

based on previous test-retest reliability studies in RP patients.[26] Macular sensitivity was assessed by MAIA microperimetry (Centervue, Padova, Italy) using the standard 37-stimuli grid pattern under mesopic conditions. Fixation stability was quantified using the 95% bivariate contour ellipsoid areas (BCEA), which encompasses 95% of all the fixation points during examination. To minimize a learning effect, subjects first underwent a practice session (fixed strategy), prior to formal testing using the 4-2 threshold strategy.[27,28] For follow-up measurements, the inbuilt follow-up software of the MAIA microperimetry was used, enabling accurate reassessment of the same test loci evaluated at baseline. If automatic alignment failed, manual alignment was performed using characteristic retinal landmarks.

After 30 minutes of dark-adaption, full-field electroretinography (ERG) responses were recorded on the Diagnosys (Cambridge, UK) using Dawson Trick Litzkow electrodes, which incorporated the International Society for Clinical Electrophysiology Standards (ISCEV). ERGs were only repeated at follow-up in patients with residual ERG function. Full-field stimulus threshold (FST; Diagnosys LLC, Lowell, MA, USA) testing was performed in a subset of patients using white and chromatic stimuli with the reference luminance (0 dB) set to 0.01 cds/m^2 (25 cd/m^2 presented for 4 ms). Based on FST data by previous studies, the normal threshold for white stimuli, while accounting for differences in reference luminance, should be set at -53 dB.[29-31] Thresholds were measured in triplicate for each stimulus and were averaged per eye. Differences between averaged chromatic sensitivities were used to determine whether responses were rod-mediated (blue-red difference of >22 dB), cone-mediated (blue-red difference of <3 dB), or mixed rod-and-cone-mediated (blue-red difference between 3-22 dB).[29,32]

Retinal imaging included fundus photography (Topcon TRC-50DX, Topcon Medical Systems, Inc. Oakland, NJ, USA), spectral-domain coherence tomography (SD-OCT; Spectralis, Heidelberg Engineering, Germany), and 488 nm wavelength fundus autofluorescence (FAF; Heidelberg Engineering, Germany). On SD-OCT, the laminar organization of the inner retinal layers (inner limiting membrane through external limiting membrane) was categorized into 3 different grades: [1] normal organization without coarse lamination; [2] normal organization with coarse lamination; and [3] relative disorganization with coarse lamination. In addition, the integrity of two hyperreflective bands of the outer retina were evaluated at the (para)fovea (within 2.5 mm of the foveal center) and perifovea (outside 2.5 mm of the foveal center): the external limiting membrane (ELM), and the ellipsoid zone (EZ). The retinal bands were either defined as: continuous, discontinuous or indiscernible. Overall definitions and example gradings of the inner and outer retina are provided in Supplementary Figure 1**.** SD-OCT images were assessed by two authors (XN and MT) and reviewed by CJFB in case of discrepancy between the aforementioned two authors.

Statistical Analysis

Data analysis was performed using R version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria). The normality of data was analyzed using the Shapiro-Wilk test, and was also visually plotted. Continuous data were either presented as mean, standard deviations (SD), and range, in the case of normal distribution; and as median, interquartile ranges (IQR), and range, in the case of non-normal distribution. Categorical data were presented as frequencies and percentages. Changes in parameters between baseline and follow-up were assessed using a linear mixed effect model while accounting for paired eye data within patients. Correlation testing was performed using Spearman's correlation test, using data of the right eye only. The level of significance was set at 0.05. Bonferroni adjustments were applied for multiple testing where appropriate.

RESULTS

Clinical and genetic characteristics

Twenty-two patients, of which 10 (45%) originated from a previously described genetically isolated population, were assessed at baseline and at 2-year follow-up.[24] A summary of the clinical findings in this cohort is provided in Table 1, and is also described for each individual patient in Supplemental Table S1. Patients had a median age of 25.7 years (IQR 19.4; range 6.2 – 74.8) at baseline, and a mean follow-up time of 2.04 years (SD ± 0.05; range 1.97 – 2.19). Based on ERG patterns and clinical examination, a clinical diagnosis of RP (n = 19; 86%), cone-rod dystrophy (CRD; n = 2; 9%), or macular dystrophy (n = 1; 5%) was made. The median self-reported age at onset was 3.0 years (IQR 7.8; range 0.8 – 49.0) and the median disease duration (age at onset subtracted from current age) was 18.7 years (IQR 16.7; range 4.7 – 39.3). An adult onset of symptoms was reported by 2 patients.

Table 1. Summary of the clinical characteristics of patients with *CRB1*-associated retinal dystrophies at last examination.

Characteristic	Total (n = 22)
Age in years	
Mean ± SD	29.3 ± 16.0
Median (IQR)	27.8 (19.4)
Range	8.3 to 76.9
Gender, n (%)	
Female	13 (59%)
Male	9 (41%)
Clinical diagnosis, n (%)	
Retinitis pigmentosa	19 (86%)
Cone-rod dystrophy	2 (9%)

Table 1. Summary of the clinical characteristics of patients with *CRB1*-associated retinal dystrophies at last examination. (continued)

Characteristic	Total (n = 22)
Macular dystrophy	1 (5%)
Age at onset in years	
Mean ± SD	8.2 ± 11.9
Median (IQR)	3.0 (7.8)
Range	0.8 to 49.0
Initial symptoms, n (%)	
Nyctalopia	5 (23%)
Visual field loss	8 (36%)
Visual acuity loss	7 (32%)
Nystagmus	2 (9%)
Disease duration in years	
Mean ± SD	19.0 ± 10.7
Median (IQR)	18.7 (16.7)
Range	4.7 to 39.3
Best-corrected visual acuity in ETDRS	
Mean ± SD	38.6 ± 19.7
Median (IQR)	35.8 (27.1)
Range	8.5 to 76.5
SER in diopters	
Mean ± SD	2.2 ± 2.9
Median (IQR)	2.4 (3.9)
Range	-5.9 to 6.7
Axial length in mm^2	
Mean ± SD	21.1 ± 1.7
Median (IQR)	20.8 (1.7)
Range	19.0 to 26.3
V4e isopter seeing retinal areas in mm^2	
Mean ± SD	258.6 ± 230.9
Median (IQR)	189.4 (261.7)
Range	14.5 to 744.4
Electroretinography patterns, n (%)	
Normal responses	1 (5%)
Cone-rod pattern	2 (9%)
Minimal responses	1 (5%)
Non-detectable	18 (81%)

Findings were averaged between eyes. ETDRS = Early Treatment Diabetic Retinopathy Study; IQR = interquartile range; SD = standard deviation; SER = spherical equivalent of the refractive error.

Fifteen different *CRB1* variants were present in this cohort, of which 12 were missense variants, 1 splice-site variant, 1 in-frame deletion, and 1 nonsense variant (Figure 1 and Supplemental Table S1). The most common variant found in this cohort was the founder variant c.3122T>C (p.Met1041Thr), which was present in a homozygous manner

in all 10 patients from the genetic isolate, and in a compound heterozygous manner in 1 patient from outside the isolate. In 10 out 11 patients (91%), including the patient from outside the isolate, this variant caused an early-onset RP phenotype. Patient P10 exhibited a late-onset CRD phenotype, despite originating from the genetic isolate. No other variants were found in patient P10 using targeted next generation sequencing. Additionally, 2 patients with the variant p.(Thr631Cys) in a compound heterozygous manner, showed relative preservation of visual function and retinal structure at later ages compared to other RP patients in this cohort, which was suggestive for a milder form of RP.

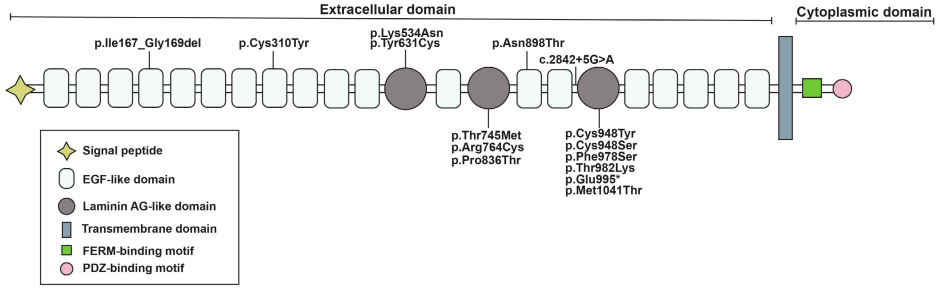

Figure 1. A schematic drawing of the CRB1 protein structure and the variants found in our cohort. The canonical protein CRB1 isoform (NM_201253.2) is comprised of EGF-like domains, laminin AG-like domains, and a short cytoplasmic tail containing FERM and PDF-binding domains. In total, fifteen different variants were found in this cohort, which have all been described previously.[24] The variant p.(Met1041Thr) was the most common variant found in this cohort, which was found in all 10 patients that originated from a Dutch genetic isolate, and in 1 patient from outside the genetic isolate.

Visual acuity and refraction

The mean BCVA of the study eyes was 41.1 ETDRS letters (SD ± 18.3; range 18.0 to 78.0; equivalent to 0.88 logMAR or 20/150 Snellen) at baseline and 38.6 ETDRS letters (SD ± 19.7; range 8.5 – 76.5; equivalent to 0.93 logMAR or 20/170 Snellen) at 2-year follow-up. While a trend for a lower ETDRS score at follow-up was observed, this finding was not statistically significant (-2.5 ETDRS letters, 95% CI: -5.2 to 0.2; p = 0.069). A loss of ≥15 ETDRS letters (i.e. a loss of 3 ETDRS lines) was measured in 5 eyes of 5 patients (11%) at the 2-year follow-up, whose initial ages ranged from 22 to 31 years (Figure 2A). In 2 out of 5 eyes (40%) with a BCVA loss of ≥15 ETDRS letters, clinical examination showed significant posterior subcapsular cataract at both visits. Patient P1 underwent cataract surgery in both eyes between visits, with no improvement in BCVA. No new cases of cataract were seen at follow-up. Spherical equivalent of the refractive error, excluding pseudophakic patients, did not significantly change between visits (-0.09 D, 95% CI: -0.34 to 0.15; p = 0.455).

Kinetic perimetry and microperimetry

The median size of V4e isopter seeing retinal areas, averaged between both eyes of each individual patient, was 176.0 mm^2 (IQR 241.9; range 17.7 – 739.2) at baseline. Overall, there was no significant change in V4e seeing retinal areas at the 2-year follow-up visit (-3.5 mm^2, 95% CI: -17.4 to +10.3; p = 0.616). A loss of ≥20% in V4e seeing retinal areas was seen in 7 eyes of 6 patients (16%), of whom 2 patients had BCVA-based severe visual impairment (BCVA ≤35 ETDRS letters). Moreover, 7 eyes of 4 patients showed an increase of ≥20% in V4e retinal seeing areas from baseline (Figure 2B). These patients all had severe visual impairment based on visual acuity or visual fields (P20, central visual field <10° from point of fixation).

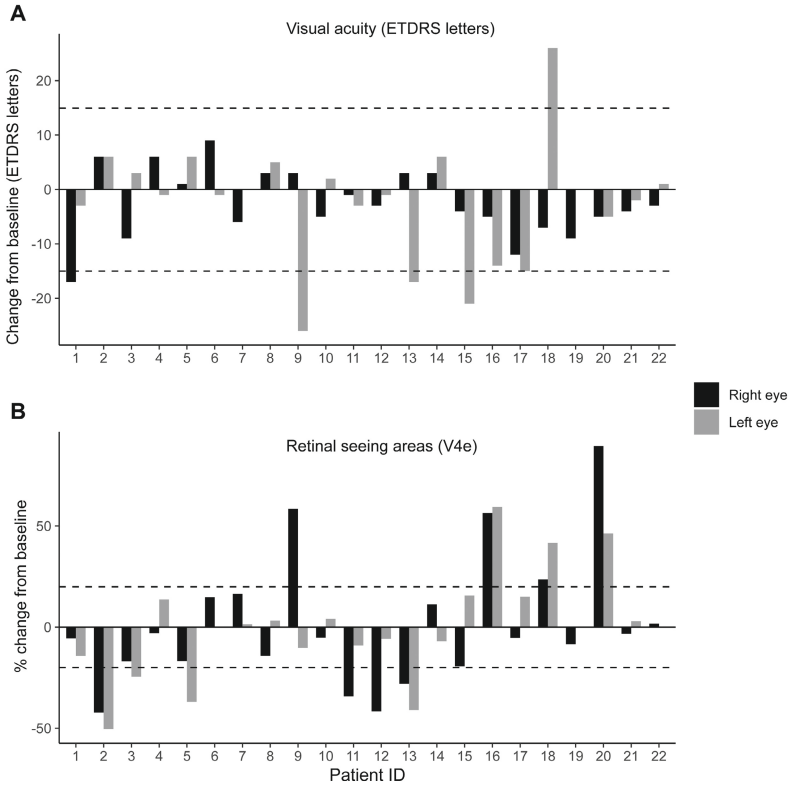

Figure 2. Changes in best-corrected visual acuity (BCVA) using Early Treatment Diabetic Retinopathy Study (ETDRS) letters and visual fields (retinal seeing areas, V4e stimuli) between baseline and 2-year follow-up for each patient. Positive values reflect an improvement from baseline, whereas negative values signify a decrease from baseline. **A**. The absolute change in ETDRS letters was used to illustrate differences from baseline. The threshold for clinical significant BCVA changes was defined as a change of ≥15 ETDRS letters (dashed lines). **B**. For visual fields, the percentage change in retinal seeing areas was used, which was considered clinically significant if it exceeded a 20% change (dashed lines). Patients showing ≥20% improvement in visual field size had severe visual impairment (<35 ETDRS) or severely constricted visual fields (central diameter <20°).

Microperimetry data were available for 36 out of 44 eyes (82%). Microperimetry testing could not be reliably performed in a subset of patients due to age (patients P7, P16) or severe visual impairment (P19, left eye; P20, both eyes). The left eye of patient P2 was also excluded, as this eye was erroneously tested using different threshold settings at follow-up. Figure 3 shows representative microperimetry measurements performed in this cohort. Median BCEA 95% values were 32.9°(SD ± 49.6; range 1.3 to 187.4) and 44.0° (SD ± 49.7; range 1.0 to 178.2) for the right and left eyes, respectively. Higher BCEA values, indicating a more unstable fixation, were seen in patients with worse logMAR BCVA (Spearman's ρ = 0.615; p = 0.004). The mean macular sensitivity was 8.5 dB (SD ± 7.6; range 0.0 – 24.3) and 7.6 dB (SD ± 7.5; range 0.0 to 26.3) for right and left eyes, accordingly. Macular sensitivity correlated with logMAR BCVA (Spearman's ρ = -0.734; p <0.001). In 9 out of 36 eyes (25%), macular sensitivity was ≤1 dB (Figure 3, patient P9). Analysis of the microperimetry testing grid (37 testing loci) showed that patients had a mean of with no measurable sensitivity (+3.5 loci, 95% CI: 0.4 to 6.5; p = 0.027) at follow-up.

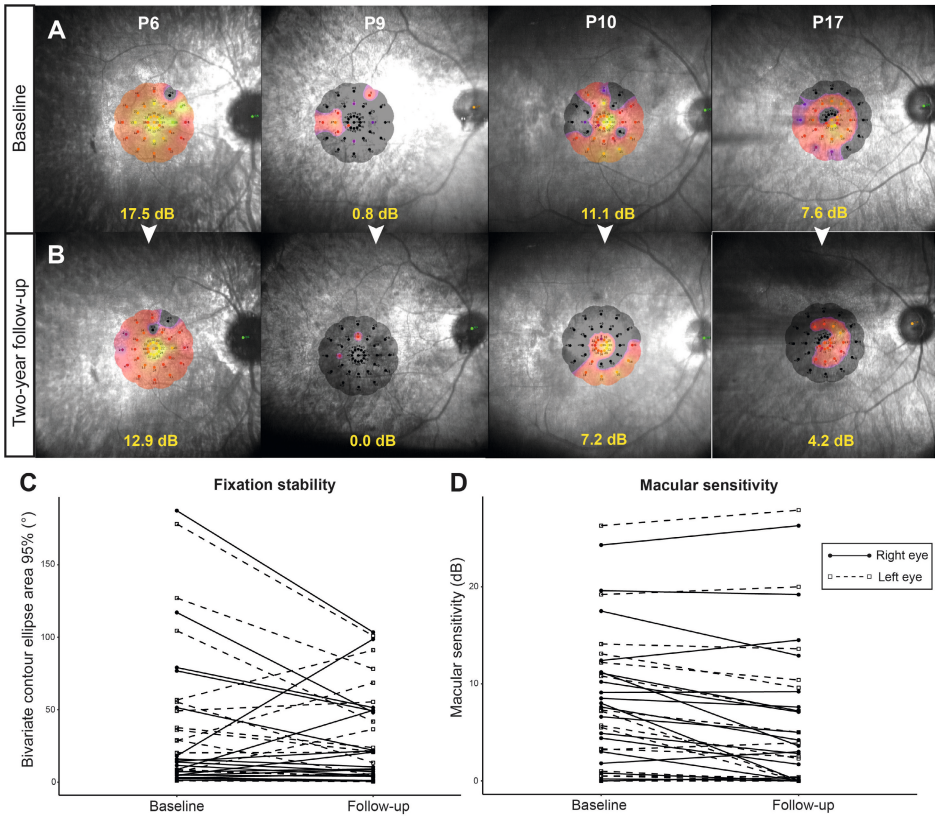

Figure 3. Macular sensitivity (MS) measurements on MAIA microperimetry in patients with *CRB1*-associated retinal dystrophies. Color-coded heat maps are used to demonstrate sensitivity values at each individual loci. Gray regions reflect areas where no sensitivity was measured (absolute scotomas). Mean MS values are

shown in yellow. **A**. MS measurements at baseline in 4 patients with *CRB1*-associated retinal dystrophies. **B**. At 2-year follow-up, MS loss was present in all 4 patients. Note that patient P17 was diagnosed with mild posterior subcapsular cataract, which may contributed to MS loss measured on follow-up. **C**. A spaghetti plot showing longitudinal changes in fixation stability, using bivariate contour ellipse areas (BCEA), in all included study eyes (n = 36). Higher BCEA values signify a more unstable fixation. **D**. A spaghetti plot was also used to illustrate changes in MS for all included study eyes. A significant decline in MS was observed at 2-year follow-up (p <0.001).

Electroretinography and full-field stimulus testing

Scotopic and photopic responses were minimal or non-recordable at baseline in all patients with RP (Table 1). ERGs in P10 and P21 followed a cone-rod dystrophy pattern, whereas P22 (the patient with a macular dystrophy phenotype) demonstrated full-field scotopic and photopic responses within normal limits. Patients with residual responses showed no significant changes in ERG patterns over follow-up. FST measurements were available for 14 patients (64%) at baseline and available for 20 patients (91%) at follow-up, as FST was not available at the start of this study. Two patients (P2 and P16) were not able to reliably perform FST, most likely due to young age. Therefore, to provide a more accurate and complete overview of FST measurements in this cohort, FST responses from the final visit were used for analysis. The mean thresholds for the white, blue and red stimuli at last visit were -38.6 dB (SD ± 12.5; range -57.0 to -11.9), -42.7 dB (SD ± 13.2; range -61.1 to -13.4) and -26.3 dB (SD ± 8.9; range -40.0 to -10.4), respectively. Sensitivity thresholds for the white stimuli were best preserved in patients with mild RP and cone(-rod) dystrophies (Figure 4A). Based on the difference in thresholds between blue and red stimuli, FST responses in the 40 included eyes were rod-mediated (n = 15; 38%), mixed rod-cone mediated (n = 23; 57%), or cone-mediated (n = 2; 5%) (Figure 4B). Cone-mediated responses could still be detected in both eyes of patient P4 with early-onset RP who had severe visual impairment and severely restricted visual fields. We were also able to determine FST responses in the left eye of patient P19 (light perception BCVA), who still had mixed FST responses, but was nearing cone-mediated vision. In patients with longitudinal FST data (14/20; 67%), we found no significant changes in white (-1.7 dB; 95% CI:-3.6 to 0.3; p = 0.098), blue (-0.5 dB, 95% CI: -2.6 to 1.5; p = 0.610) or red (-1.0 dB; 95% CI:-2.3 to 0.3; p = 0.132) FST responses.

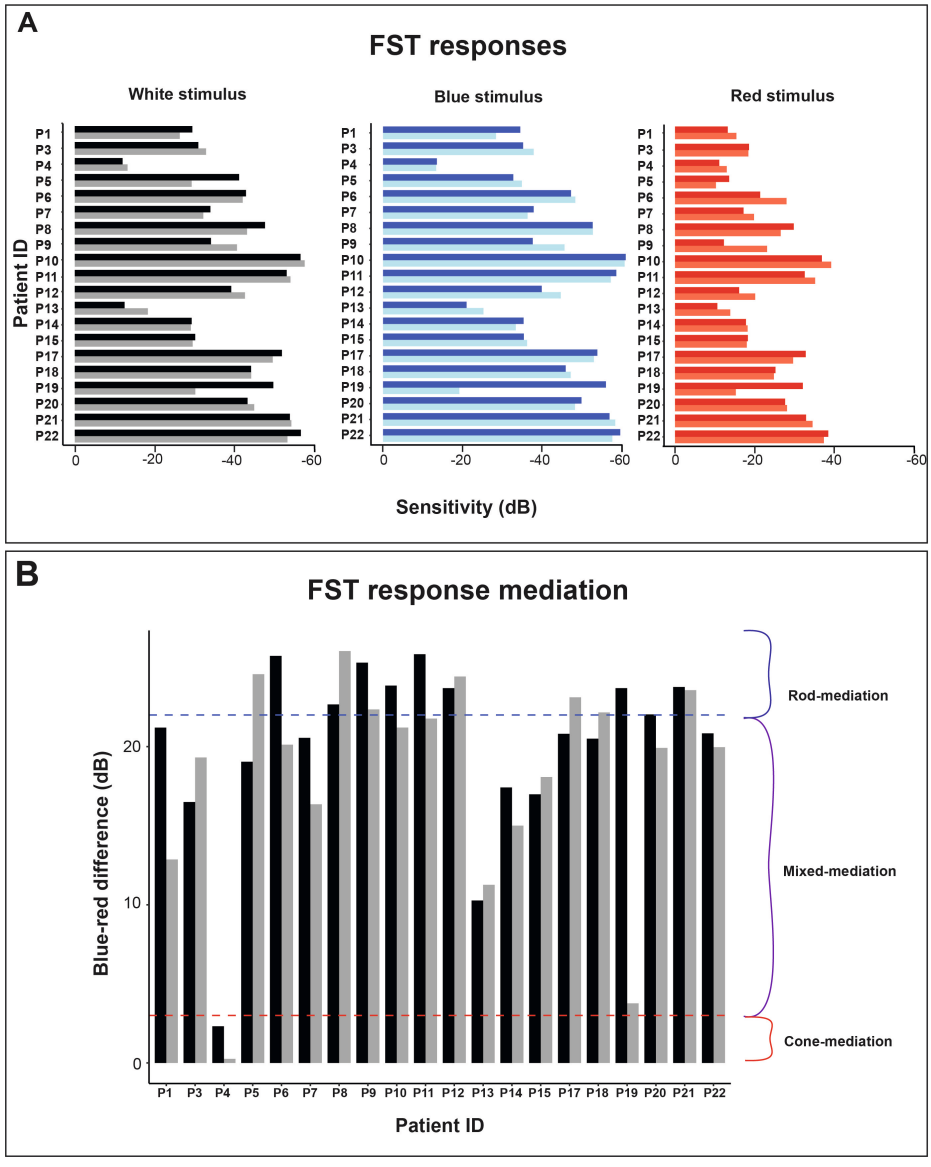

Figure 4. Full-field sensitivity thresholds (FST) responses obtained in 20 patients with *CRB1*-associated retinal dystrophies at 2-year follow-up. Two patients (P2 and P16) were unable to reliably perform FST testing due to young age. **A**. FST responses were obtained using white and chromatic stimuli (blue and red). The grouped bars represent the right eye (darker shaded bars) and left eyes (lighter shaded bars) of a single patient. **B**. Differences between blue and red responses were calculated for each patient. The blue-red difference determined whether FST responses were rod-mediated (difference of >22 dB), cone-mediated (difference <3 dB), or mixed rod-and-cone-mediated (difference between 3 and 22 dB).

Retinal imaging

SD-OCT and FAF data were available in 21 of 22 patients (95%). Retinal imaging could not be performed in patient P16 with early-onset RP due to limited cooperation (aged 6) and nystagmus. A common observation seen on SD-OCT was retinal thickening, which was observed in 20 of 21 patients (95%). Cystoid macular edema and/or cystoid spaces were present in 14 eyes of 8 patients at baseline (38%), which resolved completely in 4 eyes (28%) at follow-up without treatment. The mean central retinal thickness at baseline, after exclusion of patients with cystoid macular edema or cysts, was 133.0 µm (SD ± 50.9, range 59.5 to 236.0), which did not significantly change at follow-up (-10.31 µm, 95% CI: -34.5 to 13.8; p = 0.371). The structure of the inner retina of patients was categorized into 3 different grades: [1] normal organization without coarse lamination (n = 5; 24%); [2] normal organization with coarse lamination (n = 8; 38%); and [3] relative disorganization with coarse lamination (n = 8; 38%) (Supplemental Figure 1 and Supplemental Table. S2). The hyperreflective outer retinal bands, ELM and EZ, were discontinuous or absent at the (para)fovea and perifovea in 18 of 21 patients (86%, Supplemental Table S2). A degree of preservation of the ELM and EZ integrity was observed in patients with mild RP (P11 and P20), and in the patient with a macular dystrophy phenotype (P22). These 3 patients had better baseline logMAR BCVA (-0.6 logMAR, 95% CI: -1.1 to -0.1; p = 0.015) and macular sensitivity (+13.7 dB, 95% CI: 7.6 to 20.2; p <0.001) values compared to the other patients in this cohort. Qualitatively, there were no clear changes in ELM and EZ band integrity on SD-OCT imaging at 2-year follow-up examinations, despite a decline in visual acuity in several patients (Figure 5). As the integrity of ELM and EZ layers were severely affected in the majority of patients (e.g. Figure 5A and 5B), quantitative analysis of the retinal bands could not be reliable performed. On FAF imaging, the predominant pattern observed was generalized hypo-autofluorescence in the (mid)peripheral retina, with residual autofluorescence at the central macula, albeit to varying degrees (Figure 5A). FAF imaging was also able to confirm our fundoscopic findings of preserved RPE regions adjacent to retinal arterioles (Figure 5B). Consistent with SD-OCT findings, autofluorescence signals were best preserved in patients with mild RP and macular dystrophy (Figure 5C). The FAF patterns of each patient are described in Supplemental Table S2, which remained unchanged at follow-up.

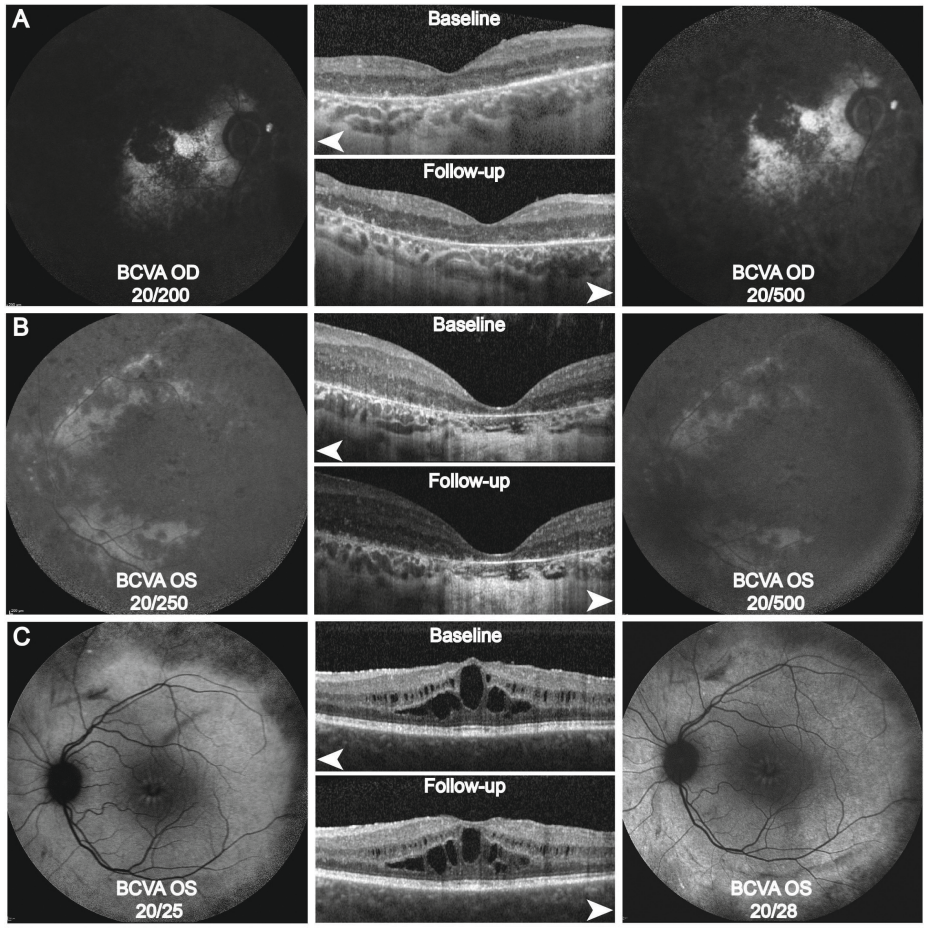

Figure 5. Representative spectral-domain optical coherence tomography (SD-OCT) scans and corresponding fundus autofluorescence (FAF, white arrowheads) images at baseline and at 2-year follow-up in 3 patients with *CRB1*-associated retinitis pigmentosa. Best-corrected visual acuity (BCVA) is shown for each eye in Snellen notation. **A**. Patient P1, aged 30, showed characteristic features of a *CRB1* retina, including inner retinal thickening and coarse lamination of individual retinal layers. Outer retinal bands were nearly absent. FAF imaging in this patient showed overall absence of autofluorescence (AF) in the posterior pole, with some residual AF between the central macula and optic disc. **B**. Patient P13, aged 21, also showed retinal thickening and coarse lamination, in addition to severe foveal atrophy. AF signals were nearly absent, with some preservation of RPE alongside the vascular arterioles. **C**. Patient P11, aged 31, exhibited a mild form of RP. Unlike other RP patients in this cohort, the retinal structure of the inner and outer retina was retained, aside from the presence of cystoid macular edema. Consistent with SD-OCT findings, FAF imaging showed relative preservation of AF in the posterior pole, with signs of degeneration in the (mid)periphery.

DISCUSSION

In this prospective natural history study, we evaluated the functional and structural changes in patients with biallelic *CRB1* variants causing a spectrum of retinal dystrophies, as we anticipate the start of gene therapeutic trials for *CRB1*-associated RDs in the near future. Our two-year analysis of the cohort showed that visual acuity and visual fields did not significantly change during follow-up. BCVA and visual fields are parameters with relatively low sensitivity for early disease changes, and may not be suitable as primary outcome measures in clinical trials on diseases that have relatively slow progression rates, such as RP.[33] Still, in 5 eyes of 5 different patients, aged between 22 and 31 years, we found a BCVA loss of more than 15 ETDRS letters (equivalent to +0.3 logMAR), which is considered a clinically significant change in clinical trials and by regulatory agencies.[34,35] This finding suggests a faster decline around the 3rd decade of life, although the contribution of significant cataract, which was the case for 2 out of 5 eyes, should not be disregarded. This is in line with our retrospective natural history study, which reported median ages of 18, 32, and 44 years to reach moderate visual impairment, severe visual impairment, and blindness, respectively.[18] Based on BCVA data, we suggest that the optimal window for treatment is before the 3rd decade of life. Ideally, patients should be treated at the earliest and safest opportunity to gain the most benefit from gene therapy.

Regarding visual fields, we found that 7 eyes of 6 patients showed progression within the 2-year follow-up period, defined as a loss of ≥20% of the seeing retinal area, which is the test-retest limit in RP patients as found by Bittner and collagues.[26] However, these changes should be interpreted with caution, as greater variability in visual field measurements is predicted in patients with more advanced stages of BCVA- or visual field-based impairment.[36-38] This is evidently demonstrated in our cohort as 4 severe visually impaired patients showed improvements up to 90% in visual fields areas at follow-up, in absence of intervention. Goldmann kinetic perimetry assumes stable and foveal fixation, which is not always the case in patients with severe RP such as in the current study.[26] Instead, other perimetry modalities, such as semi-automated kinetic perimetry or wide-field static perimetry, could be used in future studies for peripheral visual field assessment, as they take fixation stability into consideration and limit operator-dependent variability.[39]

Fundus-tracking perimetry, also known as microperimetry, is a commonly used tool for monitoring disease progression and for assessing treatment efficacy in trials involving other RDs, such as Stargardt disease, choroideremia and X-linked RP.[32, 40, 41, 42] In these RDs, subtle changes in the retina over short periods of time were detectable on microperimetry, preceding detection on conventional parameters.[27, 43] Similar results were found in our cohort, in which we detected a significant decline in macular sensitivity between visits, while no significant decline in BCVA was detected

in the 2-year period. Thus, microperimetry is a sensitive progression marker, and has the potential to serve as a clinical endpoint in treatment trials for *CRB1*-associated RD. However, due to the subjective nature of psychophysical metrics, measurements on microperimetry are inherently susceptible to variability, which is affected by factors including age, the type of retinal disease, disease severity, learning effects, and natural variance.[27, 44] While our study accounted for potential learning effects, formal intrasession and intersession reliability testing was not performed. As the main goal of phase III gene therapy trials is treatment efficacy, a patient's ability to reliably perform microperimetry testing could potentially be an inclusion criterion. Analysis of microperimetry was also impeded by the increasing amount of absolute scotoma points, which resulted in nearly undetectable sensitivity thresholds (macular sensitivity ≤1 dB) in 25% of the study eyes. Reporting the macular sensitivity, which is calculated using the average sensitivity of all testing loci, may not be an ideal approach, as this underestimates the change occurring in individual loci with detectable sensitivity.[27, 40, 45] Other methods that investigate regional sensitivity changes, along with test-retest reliability testing, should be explored in future studies.[44]

On electrophysiological testing, ERG responses were non-recordable in the majority of RP patients, implying that ffERG has no value in monitoring disease progression in patients with *CRB1*-associated RP. An alternative approach to assess residual photoreceptor function is the measurement of sensitivity thresholds using FST, which can be performed regardless of fixation capabilities or ERG function.[46] FST testing showed rod or mixed responses in this cohort, which shows that functional photoreceptors are still present despite this severe early-onset disease. Cone-mediated responses were found in patient P4, which is suggestive for end-stage disease as these responses are typically found in patients with a LCA phenotype.[13,47] As such, FST can provide valuable knowledge on remaining photoreceptor function, and in turn, disease severity, which can guide the selection of eligible candidates for therapeutic intervention.[32] However, FST responses do not appear to be sensitive markers for disease progression over a relatively short period, as we found no significant changes in FST responses over the course of 2 years. Small, localized changes occurring over several years possibly go unnoticed, as FST measures the sensitivity of the entire retina, without revealing spatial information.[30] Nevertheless, FST is potentially capable of measuring a treatment effect, as shown in previous gene therapy trials, and should be considered as a clinical endpoint.[48-50]

In keeping with previous studies, SD-OCT imaging in patients with *CRB1*-associated RP revealed an abnormally thickened inner retina (95%), which could be accompanied by coarse lamination of inner retinal layers and/or cystoid macular edema.[11, 17, 18] It has been postulated that the loss of CRB1 function stimulates proliferation of retinal progenitor cells, and also disrupts naturally occurring apoptosis during retinal development.[9, 17] This phenomenon is in direct contrast with other molecular forms of RP/LCA,

where progressive thinning of the inner retinal layers typically occurs.[51] Regardless of inner retinal thickening, most patients (62%) showed a relatively preserved laminar organization, which may be amenable for gene therapy treatment. The hyperreflective retinal bands, ELM and EZ, were typically discontinuous or indiscernible, consistent with FAF findings, owing to the rapid disease progression at an early age in *CRB1*-associated RP, which impeded quantitative analysis.[51] Despite the state of the inner and outer retina, retinal sensitivity could still be measured using psychophysical metrics, indicating that SD-OCT findings do not necessarily reflect remaining photoreceptor function in patients with *CRB1*-associated RDs. There is an urgent need for reliable methods for accurate quantification and localization of remaining photoreceptors, as viable photoreceptors are a prerequisite for effective treatment with gene therapy.[53, 54, 55] A potential method is the use of adaptive optics, as it allows for the assessment of photoreceptor viability on a cellular level, which, in turn, can shed light on their amenability for gene therapy treatment.[55 ,56, 57] It would be of great interest to assess in future studies whether photoreceptors can be adequately identified using adaptive optics considering the severe, early-onset degeneration and the characteristic retinal phenotype seen in *CRB1* patients.

Our study has several limitations. We included a relatively small cohort of 22 patients with *CRB1*-associated retinal dystrophies, which limited the possibility of a more in-depth (subgroup) analysis. Furthermore, as patients were observed over a period of 2 years, it is possible that parameters with low sensitivity for disease progression in our current study, such as visual acuity and FST, will be able to demonstrate progression over a longer observation period. Novel outcome measurements used in the assessment of gene therapy, such as multi-luminance mobility tests, dark-adapted chromatic perimetry and pupil campimetry, were also not assessed in this study.[58, 59] Future studies that follow a large group of patients with *CRB1* variants over a longer period of time, while also assessing the feasibility of more recent outcome measures, would be invaluable to extend our current findings.

In conclusion, this is the first prospective natural history study performed in patients with RDs associated with biallelic *CRB1* variants. Our study discusses the feasibility of commonly used outcome measures as clinical endpoints in clinical trials, and their potential caveats. BCVA and visual fields measures show stability over 2 years and need to be complemented with more sensitive progression markers. Microperimetry and FST show most potential as clinical endpoints, but further investigation into their reliability, validity and feasibility is required. The findings in this study can be used to aid the design of interventional studies, paving the way for *CRB1* gene therapy trials in the near future.

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Financial disclosure
The Leiden University Medical Center (LUMC) is the holder of patent application PCT/NL2014/050549, which describes the potential clinical use of CRB2; JW is listed as inventor on this patent, and JW is an employee of the LUMC.

REFERENCES

1. Khan KN, Robson A, Mahroo OAR, et al. A clinical and molecular characterisation of CRB1-associated maculopathy. *Eur J Hum Genet.* 2018;26(5):687-694.
2. Vincent A, Ng J, Gerth-Kahlert C, et al. Biallelic Mutations in CRB1 Underlie Autosomal Recessive Familial Foveal Retinoschisis. *Investigative Ophthalmology & Visual Science.* 2016;57(6):2637-2646.
3. Lotery AJ, Jacobson SG, Fishman GA, et al. Mutations in the CRB1 Gene Cause Leber Congenital Amaurosis. *Archives of Ophthalmology.* 2001;119(3):415-420.
4. den Hollander AI, Heckenlively JR, van den Born LI, et al. Leber congenital amaurosis and retinitis pigmentosa with Coats-like exudative vasculopathy are associated with mutations in the crumbs homologue 1 (CRB1) gene. *American journal of human genetics.* 2001;69(1):198-203.
5. Verbakel SK, van Huet RAC, Boon CJF, et al. Non-syndromic retinitis pigmentosa. *Progress in Retinal and Eye Research.* 2018;66:157-186.
6. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. *The Lancet.* 2006;368(9549):1795-1809.
7. Quinn PM, Buck TM, Mulder AA, et al. Human iPSC-Derived Retinas Recapitulate the Fetal CRB1 CRB2 Complex Formation and Demonstrate that Photoreceptors and Müller Glia Are Targets of AAV5. *Stem Cell Reports.* 2019;12(5):906-919.
8. Quinn PM, Pellissier LP, Wijnholds J. The CRB1 Complex: Following the Trail of Crumbs to a Feasible Gene Therapy Strategy. *Front Neurosci.* 2017;11:175.
9. Alves CH, Pellissier LP, Wijnholds J. The CRB1 and adherens junction complex proteins in retinal development and maintenance. *Progress in Retinal and Eye Research.* 2014;40:35-52.
10. Boon N, Wijnholds J, Pellissier LP. Research Models and Gene Augmentation Therapy for CRB1 Retinal Dystrophies. *Front Neurosci.* 2020;14:860.
11. Bujakowska K, Audo I, Mohand-Saïd S, et al. CRB1 mutations in inherited retinal dystrophies. *Hum Mutat.* 2012;33(2):306-315.
12. Ray TA, Cochran K, Kozlowski C, et al. Comprehensive identification of mRNA isoforms reveals the diversity of neural cell-surface molecules with roles in retinal development and disease. *Nat Commun.* 2020;11(1):3328-3328.
13. Stingl KT, Kuehlewein L, Weisschuh N, et al. Chromatic Full-Field Stimulus Threshold and Pupillography as Functional Markers for Late-Stage, Early-Onset Retinitis Pigmentosa Caused by CRB1 Mutations. *Transl Vis Sci Technol.* 2019;8(6):45-45.
14. Alves CH, Boon N, Mulder AA, Koster AJ, Jost CR, Wijnholds J. CRB2 Loss in Rod Photoreceptors Is Associated with Progressive Loss of Retinal Contrast Sensitivity. *Int J Mol Sci.* 2019;20(17).
15. Pellissier LP, Quinn PM, Alves CH, et al. Gene therapy into photoreceptors and Müller glial cells restores retinal structure and function in CRB1 retinitis pigmentosa mouse models. *Human Molecular Genetics.* 2015;24(11):3104-3118.
16. Mehalow AK, Kameya S, Smith RS, et al. CRB1 is essential for external limiting membrane integrity and photoreceptor morphogenesis in the mammalian retina. *Human Molecular Genetics.* 2003;12(17):2179-2189.
17. Jacobson SG, Cideciyan AV, Aleman TS, et al. Crumbs homolog 1 (CRB1) mutations result in a thick human retina with abnormal lamination. *Human Molecular Genetics.* 2003;12(9):1073-1078.
18. Talib M, van Schooneveld MJ, van Genderen MM, et al. Genotypic and phenotypic characteristics of *CRB1*-associated retinal dystrophies. *Ophthalmology.* 2017;124(6):884-895.

19. Buck TM, Vos RM, Alves CH, Wijnholds J. AAV-CRB2 protects against vision loss in an inducible CRB1 retinitis pigmentosa mouse model. *Molecular Therapy - Methods & Clinical Development*. 2021;20:423-441.

20. Talib M, Boon CJF. Retinal Dystrophies and the Road to Treatment: Clinical Requirements and Considerations. *The Asia-Pacific Journal of Ophthalmology*. 2020;9(3).

21. Mathijssen IB, Florijn RJ, van den Born LI, et al. LONG-TERM FOLLOW-UP OF PATIENTS WITH RETINITIS PIGMENTOSA TYPE 12 CAUSED BY CRB1 MUTATIONS: A Severe Phenotype With Considerable Interindividual Variability. *Retina*. 2017;37(1):161-172.

22. van Huet RAC, Oomen CJ, Plomp AS, et al. The RD5000 Database: facilitating clinical, genetic, and therapeutic studies on inherited retinal diseases. *Investigative Ophthalmology & Visual Science*. 2014;55(11):7355-7360.

23. van den Born LI, van Soest S, van Schooneveld MJ, Riemslag FCC, de Jong PTVM, Bleeker-Wagemakers EM. Autosomal Recessive Retinitis Pigmentosa With Preserved Para-arteriolar Retinal Pigment Epithelium. *American Journal of Ophthalmology*. 1994;118(4):430-439.

24. Talib M, van Schooneveld MJ, Wijnholds J, et al. Defining inclusion criteria and endpoints for clinical trials: a prospective cross-sectional study in CRB1-associated retinal dystrophies. *Acta Ophthalmologica*. 2021;*Epub ahead of print*.

25. Dagnelie G. Technical note. Conversion of planimetric visual field data into solid angles and retinal areas. *Clinical Vision Science*. 1990;5(1):95-100.

26. Bittner AK, Iftikhar MH, Dagnelie G. Test-Retest, Within-Visit Variability of Goldmann Visual Fields in Retinitis Pigmentosa. *Investigative Ophthalmology & Visual Science*. 2011;52(11):8042-8046.

27. Wu Z, Ayton LN, Guymer RH, Luu CD. Intrasession Test–Retest Variability of Microperimetry in Age-Related Macular Degeneration. *Investigative Ophthalmology & Visual Science*. 2013;54(12):7378-7385.

28. Talib M, Jolly JK, Boon CJF. Measuring Central Retinal Sensitivity Using Microperimetry. In: Boon CJF, Wijnholds J, eds. *Retinal Gene Therapy: Methods and Protocols*. New York, NY: Springer New York; 2018:339-349.

29. Roman AJ, Cideciyan AV, Aleman TS, Jacobson SG. Full-field stimulus testing (FST) to quantify visual perception in severely blind candidates for treatment trials. *Physiological Measurement*. 2007;28(8):N51-N56.

30. Roman AJ, Schwartz SB, Aleman TS, et al. Quantifying rod photoreceptor-mediated vision in retinal degenerations: dark-adapted thresholds as outcome measures. *Experimental Eye Research*. 2005;80(2):259-272.

31. Klein M, Birch DG. Psychophysical assessment of low visual function in patients with retinal degenerative diseases (RDDs) with the Diagnosys full-field stimulus threshold (D-FST). *Documenta Ophthalmologica*. 2009;119(3):217.

32. Dimopoulos IS, Tseng C, MacDonald IM. Microperimetry as an Outcome Measure in Choroideremia Trials: Reproducibility and Beyond. *Investigative Ophthalmology & Visual Science*. 2016;57(10):4151-4161.

33. Fishman GA, Jacobson SG, Alexander KR, et al. Outcome measures and their aplication in clinical trials for retinal degenerative diseases: Outline, Review, and Perspective. *RETINA*. 2005;25(6).

34. Lam BL, Feuer WJ, Schiffman JC, et al. Trial end points and natural history in patients with G11778A Leber hereditary optic neuropathy : preparation for gene therapy clinical trial. *JAMA ophthalmology*. 2014;132(4):428-436.

35. Kiser AK, Mladenovich D, Eshraghi F, Bourdeau D, Dagnelie G. Reliability and Consistency of Visual Acuity and Contrast Sensitivity Measures in Advanced Eye Disease. *Optometry and Vision Science*. 2005;82(11).

36. Barnes CS, Schuchard RA, Birch DG, et al. Reliability of Semiautomated Kinetic Perimetry (SKP) and Goldmann Kinetic Perimetry in Children and Adults With Retinal Dystrophies. *Transl Vis Sci Technol.* 2019;8(3):36-36.
37. Barry MP, Bittner AK, Yang L, Marcus R, Iftikhar MH, Dagnelie G. Variability and Errors of Manually Digitized Goldmann Visual Fields. *Optometry and vision science : official publication of the American Academy of Optometry.* 2016;93(7):720-730.
38. Roman AJ, Cideciyan AV, Schwartz SB, Olivares MB, Heon E, Jacobson SG. Intervisit Variability of Visual Parameters in Leber Congenital Amaurosis Caused by RPE65 Mutations. *Investigative Ophthalmology & Visual Science.* 2013;54(2):1378-1383.
39. Kumaran N, Rubin GS, Kalitzeos A, et al. A Cross-Sectional and Longitudinal Study of Retinal Sensitivity in RPE65-Associated Leber Congenital Amaurosis. *Investigative ophthalmology & visual science.* 2018;59(8):3330-3339.
40. Schönbach EM, Strauss RW, Muñoz B, et al. Longitudinal Microperimetric Changes of Macular Sensitivity in Stargardt Disease After 12 Months: ProgStar Report No. 13. *JAMA Ophthalmology.* 2020;138(7):772-779.
41. Georgiou M, Singh N, Kane T, et al. Long-Term Investigation of Retinal Function in Patients with Achromatopsia. *Investigative ophthalmology & visual science.* 2020;61(11):38-38.
42. Buckley TMW, Jolly JK, Menghini M, Wood LJ, Nanda A, MacLaren RE. Test-retest repeatability of microperimetry in patients with retinitis pigmentosa caused by mutations in RPGR. *Clinical & Experimental Ophthalmology.* 2020;48(5):714-715.
43. Jolly JK, Xue K, Edwards TL, Groppe M, MacLaren RE. Characterizing the Natural History of Visual Function in Choroideremia Using Microperimetry and Multimodal Retinal Imaging. *Investigative Ophthalmology & Visual Science.* 2017;58(12):5575-5583.
44. Pfau M, Jolly JK, Wu Z, et al. Fundus-controlled perimetry (microperimetry): Application as outcome measure in clinical trials. *Progress in Retinal and Eye Research.* 2020:100907.
45. Iftikhar M, Kherani S, Kaur R, et al. Progression of Retinitis Pigmentosa as Measured on Microperimetry: The PREP-1 Study. *Ophthalmology Retina.* 2018;2(5):502-507.
46. Maguire AM, High KA, Auricchio A, et al. Age-dependent effects of RPE65 gene therapy for Leber's congenital amaurosis: a phase 1 dose-escalation trial. *Lancet.* 2009;374(9701):1597-1605.
47. Collison FT, Park JC, Fishman GA, McAnany JJ, Stone EM. Full-Field Pupillary Light Responses, Luminance Thresholds, and Light Discomfort Thresholds in CEP290 Leber Congenital Amaurosis Patients. *Investigative Ophthalmology & Visual Science.* 2015;56(12):7130-7136.
48. Russell S, Bennett J, Maguire AM, High KA. Voretigene neparvovec-rzyl for the treatment of biallelic RPE65 mutation–associated retinal dystrophy. *Expert Opinion on Orphan Drugs.* 2018;6(8):457-464.
49. Russell S, Bennett J, Wellman JA, et al. Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial. *Lancet.* 2017;390(10097):849-860.
50. Klein M, Mejia P, Galles D, Birch DG. Full-Field Stimulus Thresholds (FSTs) in Subjects with Inherited Retinal Degenerations (IRDs) – a 10 Years Review. *Investigative Ophthalmology & Visual Science.* 2018;59(9):54-54.
51. Tee JJL, Yang Y, Kalitzeos A, Webster A, Bainbridge J, Michaelides M. Natural History Study of Retinal Structure, Progression, and Symmetry Using Ellipzoid Zone Metrics in RPGR-Associated Retinopathy. *American Journal of Ophthalmology.* 2019;198:111-123.
52. Bouzia Z, Georgiou M, Hull S, et al. GUCY2D-Associated Leber Congenital Amaurosis: A Retrospective Natural History Study in Preparation for Trials of Novel Therapies. *American journal of ophthalmology.* 2020;210:59-70.

53. Gardiner KL, Cideciyan AV, Swider M, et al. Long-Term Structural Outcomes of Late-Stage RPE65 Gene Therapy. *Molecular Therapy.* 2020;28(1):266-278.

54. Aguirre GD. Concepts and Strategies in Retinal Gene Therapy. *Investigative ophthalmology & visual science.* 2017;58(12):5399-5411.

55. Georgiou M, Kalitzeos A, Patterson EJ, Dubra A, Carroll J, Michaelides M. Adaptive optics imaging of inherited retinal diseases. *The British journal of ophthalmology.* 2018;102(8):1028-1035.

56. Genead MA, Fishman GA, Rha J, et al. Photoreceptor structure and function in patients with congenital achromatopsia. *Investigative ophthalmology & visual science.* 2011;52(10):7298-7308.

57. Georgiou M, Fujinami K, Michaelides M. Inherited retinal diseases: Therapeutics, clinical trials and end points—A review. *Clinical & Experimental Ophthalmology.* 2021;49(3):270-288.

58. Stingl K, Kempf M, Bartz-Schmidt KU, et al. Spatial and temporal resolution of the photoreceptors rescue dynamics after treatment with voretigene neparvovec. *British Journal of Ophthalmology.* 2021:bjophthalmol-2020-318286.

59. Maguire AM, Russell S, Wellman JA, et al. Efficacy, Safety, and Durability of Voretigene Neparvovec-rzyl in RPE65 Mutation–Associated Inherited Retinal Dystrophy: Results of Phase 1 and 3 Trials. *Ophthalmology.* 2019;126(9):1273-1285.

SUPPLEMENTAL CONTENT

Table S1. Patient's clinical and genetic characteristics at baseline in the *CRB1* cohort

ID	Age, Sex	Allele 1 ; Allele 2	Dx	Age at onset (y)	First symptom	ETDRS BCVA	SER (D)	Axial length (mm)	Lens status (OD/OS)	ERG pattern	V4e (mm^2)	Fundus features			
												Vitreous	ONH drusen	Bone-spicules	PPRPE
P1	M, 29	p.(Met1041Thr); p.(Met1041Thr)	RP	6	VF loss	47.5	+0.6	21.4	PSC	ND	156.3	Vitreous cells	✓	✓	×
P2	F, 13	p.(Met1041Thr); p.(Met1041Thr)	RP	1-2	Nyctalopia	39.5	+3.1	19.8	Clear	ND	35.4	Vitreous cells	✓	✓	✓
P3	F, 16	p.(Met1041Thr); p.(Met1041Thr)	RP	1	VF loss	54.0	+0.6	20.8	Clear	ND	140.2	Vitreous cells	×	✓	✓
P4	F, 38	p.(Met1041Thr); p.(Met1041Thr)	RP	<1	Nyctalopia	31.5	+1.0	20.7	PSC	ND	36.4	Clear	×	✓	✓
P5	M,41	p.(Met1041Thr); p.(Met1041Thr)	RP	2	VF loss	26.5	+3.9	21.3	PSC	ND	82.7	Vitreous cells	✓	✓	×
P6	F, 11	p.(Met1041Thr); p.(Met1041Thr)	RP	3	VF loss	60.0	+3.2	19.5	Clear	ND	278.5	Vitreous cells	✓	✓	✓
P7	F, 9	p.(Met1041Thr); p.(Met1041Thr)	RP	2	Nystagmus	19.0	+6.7	19.1	Clear	ND	275.7	Clear	×	✓	×
P8	F, 10	p.(Met1041Thr); p.(Met1041Thr)	RP	3	VF loss	26.5	+7.1	19.5	Clear	MR	537.4	PRH	×	✓	✓
P9	F, 28	p.(Met1041Thr); p.(Met1041Thr)	RP	8	VF loss	20.0	+5.8	20.6	Clear	ND	174.5	Asteroid hyalosis	✓	✓	✓
P10	M, 39	p.(Met1041Thr); p.(Met1041Thr)	CRD	34-35	VA loss	78.0	+2.5	21.4	Clear	CRD	729.4	Vitreous veils	✓	✓	×
P11	M, 31	p.(Tyr631Cys); p.(Glu995*)	MildRP	7-8	VA loss	75.5	+1.0	21.7	Clear	ND	324.6	Vitreous cells	×	✓	×
P12	F, 26	p.(Asn898Thr); p.(Asn898Thr)	RP	9	VA loss	57.0	+1.4	20.9	Clear	ND	312.5	Vitreous cells	✓	✓	✓

Table S1. Patient's clinical and genetic characteristics at baseline in the *CRB1* cohort (continued)

ID	Age, Sex	Allele 1 ; Allele 2	Dx	Age at onset (y)	First symptom	ETDRS BCVA	SER (D)	Axial length (mm)	Lens status (OD/OS)	ERG pattern	V4e (mm^2)	Fundus features			
												Vitreous	ONH drusen	Bone-spicules	PPRPE
P13	M, 21	p.(Cys948Tyr); p.(Met1041Thr)	RP	2	Nyctalopia	21.0	+2.6	19.9	PSC	ND	202.5	Vitreous cells	×	✓	✓
P14	F, 24	p.(Arg764Cys); p.(Glu995*)	RP	2	Nyctalopia	36.0	+5.3	19.4	PSC	ND	82.3	Clear	×	✓	×
P15	F, 31	p.(Arg764Cys); p.(Glu995*)	RP	1	Nyctalopia	41.5	+4.8	19.2	Clear	ND	160.7	Vitreous cells	×	✓	✓
P16	M, 6	p.(Cys310Tyr); p.(Phe978Ser)	RP	1-2	Nystagmus	18.0	+3.4	20.6	Clear	ND	177.6	Clear	×	✓	×
P17	M, 23	p.(Tyr631Cys); p.(Cys948Tyr)	RP	12	VA loss	45.0	-0.6[b]	22.8	PF / PSC	ND	450.9	No vitreous[c]	×	✓	×
P18	F, 12	p.(Thr745Met); c.2842+5G>A	RP	3	VF loss	29.5	+5.0	20.3	Clear	ND	125.9	Vitreous cells	×	✓	✓
P19	M, 53	p.(Lys534Asn); p.(Thr745Met)	RP	17	VA loss	27.5	+0.3[b]	21.5	PF / PF	ND	16.7	No vitreous[c]	×	✓	×
P20	M, 74	p.(Tyr631Cys); p.(Thr982Lys)	MildRP	49	VF loss	66.5	-0.8[b]	24.3	PF / PF	ND	19.1	Vitreous cells	×	✓	×
P21	F, 31	p.(Pro836Thr); p.(Cys948Ser)	CRD	4	VA loss	30.5	+0.9	22.2	Clear	CRD	710.1	Clear	×	✓	×

The mutation notation is based on the NM_201253.2 nomenclature. Patients P1-P10 originate from the same genetic isolate who were all homozygous carriers of the p.(Met1041Thr) mutation. Patients P14 and P15 are siblings. Patients P11 and P22 had milder forms of retinitis pigmentosa, as they had better preserved visual acuity and retinal structure compared to other patients in this cohort. Measurements were averaged between eyes. CRD = cone-rod dystrophy; Dx = diagnosis; MD = macular dystrophy; MR = minimal responses; ND = non-detectable; ONH = optic nerve head; PF = pseudophakic; PPRPE = para-arteriolar preservation of the retinal pigment epithelium; PRH = pre-retinal hemorrhage; PSC = posterior subcapsular cataract; RP = retinitis pigmentosa; RCD = rod-cone dystrophy; SER = spherical refractive error; V4e = V4e retinal seeing areas derived from Goldmann kinetic perimetry; VA = visual acuity; VF = visual field. [a]Patient P15 developed acute-angle closure glaucoma during mydriatic dark-adaption at baseline, and received immediate peripheral laser iridotomy. [b] Patients underwent cataract surgery prior to the baseline visit. [c]Patients underwent pars plana vitrectomy with inner limiting membrane peeling due to cystoid macular edema.

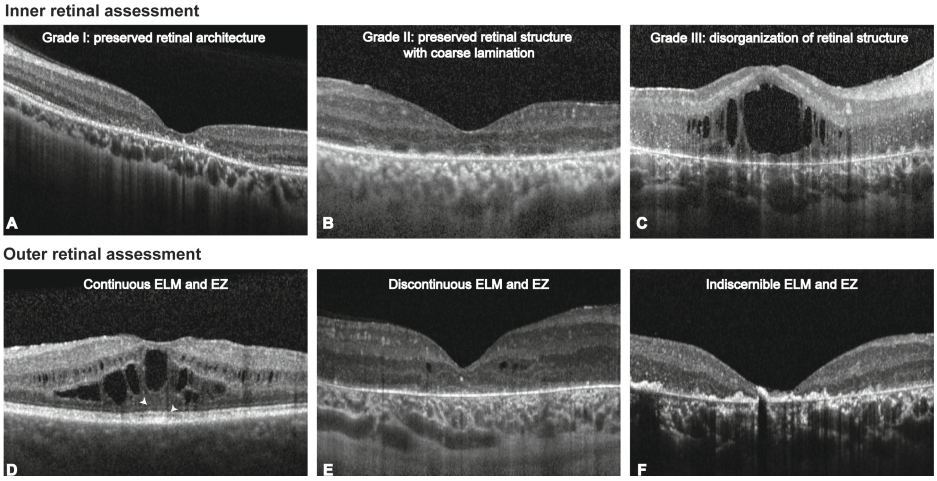
Table 2. Retinal imaging findings in patients with *CRB1*-associated retinal dystrophies.

ID	SD-OCT: retinal architecture		SD-OCT: integrity of outer retinal bands				Fundus autofluorescence
	Laminar organization	Presence of CME	Parafovea		Perifovea		
			EZ	ELM	EZ	ELM	
P1	Normal organization and lamination	No CME	Discontinuous	Discontinuous	Indiscernible	Discontinuous	Optic nerve drusen, mottled hypo-AF with preservation of AF between fovea and optic disc
P2	Disorganization	CME ODS	Indiscernible	Discontinuous	Indiscernible	Discontinuous	Optic nerve drusen, generalized hypo-AF with residual AF at the fovea
P3	Normal organization with coarse lamination	Parafoveal cysts	Discontinuous	Discontinuous	Discontinuous	Discontinuous	Generalized hypo-AF
P4	Disorganization	No CME	Discontinuous	Discontinuous	Discontinuous	Discontinuous	Generalized hypo-AF
P5	Disorganization	No CME	Discontinuous	Discontinuous	Discontinuous	Discontinuous	Optic nerve drusen, generalized hypo-AF
P6	Disorganization	CME ODS; resolved at follow-up	Discontinuous	Discontinuous	Discontinuous	Discontinuous	Optic nerve drusen; hypo-AF with residual AF at central macula
P7	Disorganization	No CME	Discontinuous	Discontinuous	Discontinuous	Discontinuous	Generalized hypo-AF
P8	Disorganization	CME ODS	Discontinuous	Indiscernible	Discontinuous	Discontinuous	Generalized hypo-AF with hyper-AF speckles
P9	Normal with coarse lamination	Parafoveal cysts OD; resolved at follow-up	Indiscernible	Discontinuous	Discontinuous	Discontinuous	Optic drusen, mottled hypo-AF in central macula with relatively preservation in perimacula
P10	Normal with coarse lamination	Parafoveal cysts OD; resolved at follow-up	Discontinuous	Discontinuous	Discontinuous	Discontinuous	Perimacular hypo-AF ring with foveal sparing
P11	Normal organization and lamination	Macular hole OD and CME OS	Continuous	Continuous	Continuous	Continuous	Normal AF in the posterior pole with hypo-AF in the periphery
P12	Normal with coarse lamination	CME ODS; CME OS resolved at follow-up	Discontinuous	Discontinuous	Discontinuous	Discontinuous	Optic nerve drusen, mottled hypo-AF in the periphery and central macula with preservation of the perimacula

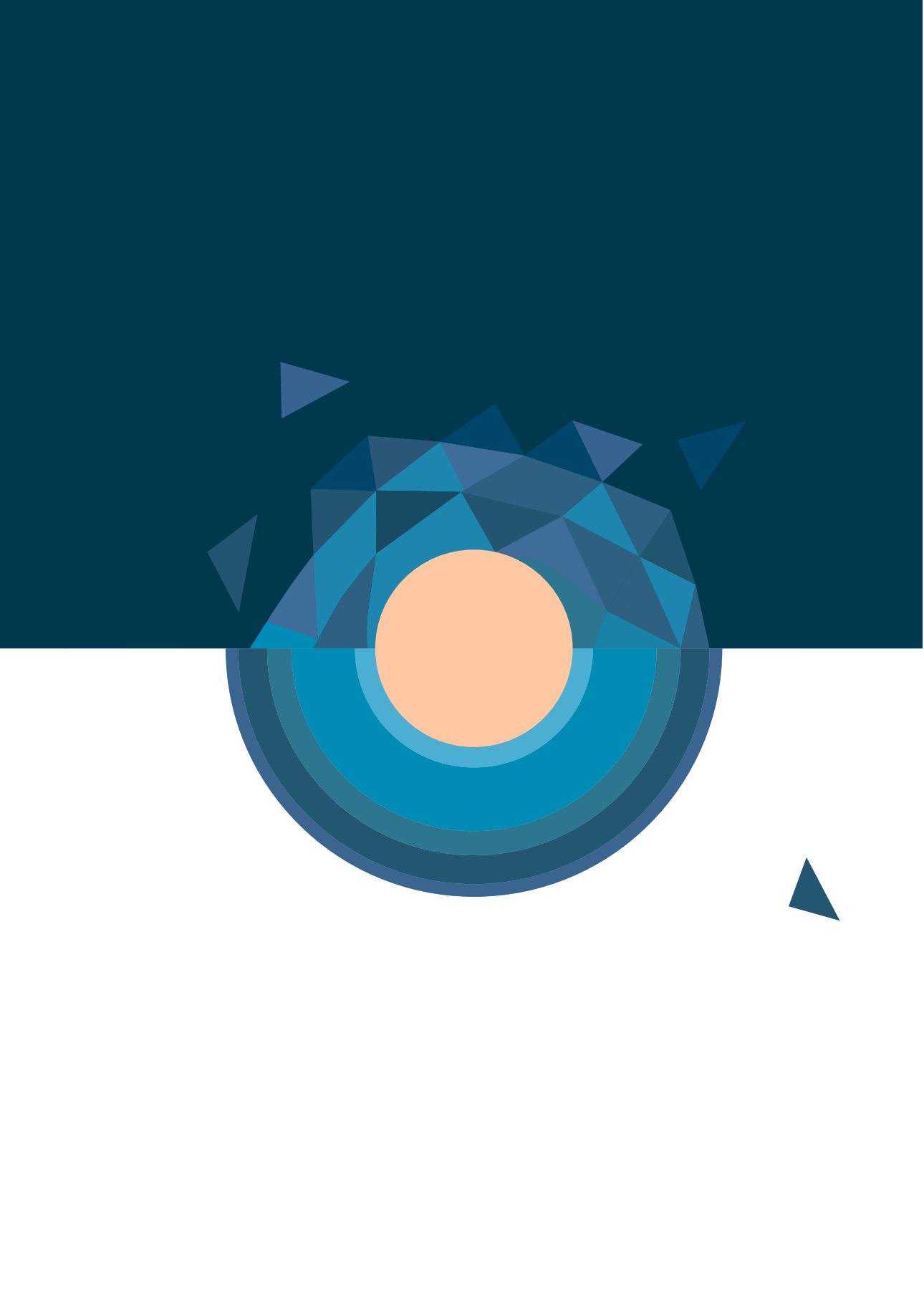
Table 2. Retinal imaging findings in patients with *CRB1*-associated retinal dystrophies. (continued)

ID	SD-OCT: retinal architecture		SD-OCT: integrity of outer retinal bands				Fundus autofluorescence
	Laminar organization	Presence of CME	Parafovea		Perifovea		
			EZ	ELM	EZ	ELM	
P13	Normal organization and lamination	No CME	Discontinuous	Discontinuous	Discontinuous	Discontinuous	Generalized hypo-AF with AF preservation around the vascular arcades
P14	Disorganization	No CME	Discontinuous	Discontinuous	Discontinuous	Discontinuous	Generalized hypo-AF with relatively preserved AF at posterior macula
P15	Disorganization	No CME	Discontinuous	Discontinuous	Discontinuous	Discontinuous	Generalized mottled hypo-AF
P16[a]	-	-	-	-	-	-	-
P17	Normal with coarse lamination	No CME	Discontinuous	Discontinuous	Discontinuous	Discontinuous	Mottled hypo-AF at central macula
P18	Normal with coarse lamination	No CME	Discontinuous	Discontinuous	Discontinuous	Discontinuous	Generalized hypo AF with hyper-AF spots around vascular arcades
P19	Normal with coarse lamination	Parafoveal cyst OD at follow-up	Discontinuous	Discontinuous	Discontinuous	Discontinuous	Mottled hypo-AF with preservation of AF between fovea and optic disc
P20	Normal organization and lamination	No CME	Continuous	Continuous	Continuous	Continuous	Mottled hypo-AF with preservation of central macula
P21	Normal organization and lamination	No CME	Indiscernible	Indiscernible	Discontinuous	Discontinuous	Mottled hypo-AF changes at the central macula with preservation outside the posterior pole
P22	Normal organization and lamination	No CME	Discontinuous	Discontinuous	Continuous	Continuous	Parafoveal hypo-AF with hyper-AF foveal spot.

Clinical findings were similar between eyes, unless specifically mentioned. No qualitative changes were seen between baseline and 2-year follow-up in laminar organization, retinal band integrity and autofluorescence findings. AF = autofluorescence; CME = cystoid macular edema; ELM = external limiting membrane; EZ = ellipsoid zone; FAF = fundus autofluorescence; hypo-AF = hypo-autofluorescence; OD = right eye; ODS = right and left eyes; OS = left eye; SD-OCT = spectral domain optical coherence tomography. [a]OCT and FAF could not reliably be performed in this patient.

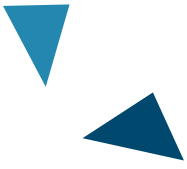


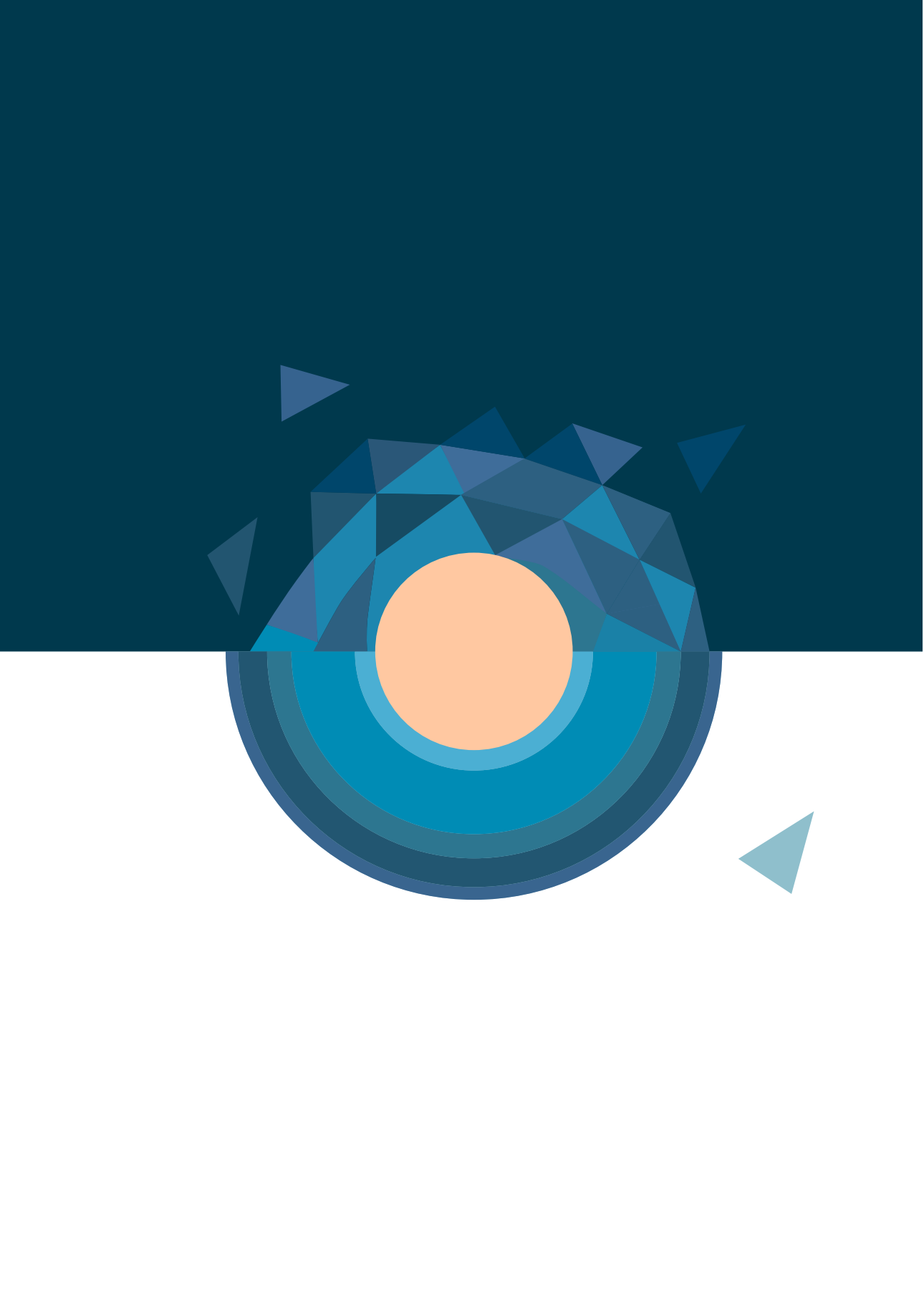
Supplemental Figure 1. Assessment of the inner retinal structure and the outer retinal band integrity using spectral-domain optical coherence tomography (SD-OCT). **A**. Grade I included patients with normal inner retinal structure and clear segmentation of individual layers. Note that the integrity of the outer retinal structures were not taken into consideration during grading. **B**. Patients with visible retinal delineation, but with a coarse aspect of individual layers were classified as grade II. **C**. Disorganization was defined as the inability to differentiate between adjacent inner retinal layers, which could be caused by the presence of cystoid macular edema. **D-F**. The external limiting membrane (ELM; white arrowhead) and ellipsoid zone (EZ; yellow arrowhead) were assessed at the peri- and parafovea. Retinal bands were classified as either continuous (relatively homogeneous reflectivity of the given band), discontinuous (heterogenous reflectivity and/or disruption of the given band) or indiscernible.



PART II

CLINICAL MANAGEMENT OF RETINITIS PIGMENTOSA





CHAPTER 3.1

OUTCOME OF CATARACT SURGERY IN PATIENTS WITH RETINITIS PIGMENTOSA

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ABSTRACT

Purpose
To assess the visual outcome of cataract surgery in patients with retinitis pigmentosa (RP).

Design
Retrospective, non-comparative clinical study.

Methods
Preoperative, intraoperative and postoperative data of patients with RP undergoing cataract surgery were collected from several expertise centers across Europe.

Result
In total**,** 295 eyes of 225 patients were included in the study. The mean age at surgery of the first eye was 56.1 ± 17.9 years. Following surgery, best-corrected visual acuity (BCVA) improved significantly from 1.03 to 0.81 logMAR (i.e. 20/214 to 20/129 Snellen) in the first treated eye (-0.22 logMAR; 95% CI: -0.31 to -0.13; $p < 0.001$), and from 0.80 to 0.56 logMAR (i.e. 20/126 to 20/73 Snellen) in the second treated eye (-0.24 logMAR; 95% CI: -0.32 to -0.15; $p < 0.001$). Marked BCVA improvements (postoperative change in BCVA of ≥ 0.3 logMAR) were observed in 87 out of 226 patients (39%). Greater odds for marked visual improvements were observed in patients with moderate visual impairment or worse. The most common complications were zonular dialysis (n = 15; 5%), and (exacerbation of) cystoid macular edema (n = 14; 5%), respectively. Postoperative posterior capsular opacifications were present in 111 out of 295 (38%) eyes.

Conclusion
Significant improvements in BCVA are observed in most patients with RP following cataract surgery. Baseline BCVA is a predictor for visual outcome. Preoperative evaluation should include the assessment of potential zonular insufficiency and the presence of CME, as they are relatively common, and may increase the risk for complications.

INTRODUCTION

Retinitis pigmentosa (RP) is a collective term for inherited retinal dystrophies that are characterized by degeneration of primarily rod photoreceptors, followed by loss of cone photoreceptors.[1, 2] As result of photoreceptor degeneration, patients may experience symptoms of night blindness, concentric loss of peripheral visual fields, and ultimately, central vision loss.[2] There is great variability in patients with RP with regards to their disease onset, severity, progression and their potential complications. The most common anterior segment complication described in RP is cataract, with the most common type being posterior subcapsular cataract (PSC).[3-5] Cataract develops at a relatively younger age in patients with RP compared to patients with age-related cataract, which is presumably caused by an early inflammation response invoked by RP.[3, 4, 6-8] Depending on the severity and morphology of cataract, loss of visual function is accompanied or preceded by visual disturbances, including symptoms of glare, photophobia and decreased contrast sensitivity, among others.[9-11] In patients with RP, cataracts may cause disproportionate functional symptoms due to the presence of both lenticular and retinal pathology.[7]

Surgical removal of cataract, followed by the implantation of an intraocular lens (IOL), is the primary treatment to improve visual function and/or to alleviate perceived visual disturbances.[7, 12] In absence of ocular comorbidities and surgical complications, cataract surgery leads to significant improvements in objective and subjective visual function.[13, 14] However, in patients with RP, the visual prognosis following cataract surgery is uncertain, as gradual loss of visual function can be attributed either to the development of lens opacities or to the ongoing retinal degeneration process by RP.[3, 15, 16] Furthermore, patients with RP are predisposed to intraoperative phototoxic retinal damage, cystoid macular edema (CME), and weakened lens zonules, which may negatively influence the visual prognosis and increase the risk of surgical complications.[7, 15, 17-19] Postoperatively, higher incidences of posterior capsular opacifications (PCO) and anterior capsular contraction have also been reported in RP, which may also impact the visual outcome if left untreated.[3, 4, 6, 7, 12, 15]

Despite the uncertain visual prognosis and surgical risks, cataract surgery should still be considered in patients with RP.[6, 7, 9, 12, 15] However, there is limited information to date on the outcome of cataract surgery in RP.[3, 7, 12, 17, 20] Evaluating factors that are predictive of visual outcome in patients with RP undergoing cataract surgery could prove beneficial in selecting those who are most likely to benefit from surgery. Also, this information will provide some insights into the necessary precautions that can be taken in order to minimize intra-operative and/or postoperative complications. For this purpose, this study aimed to evaluate the outcome of cataract surgery using retrospective data from multiple expertise centers across Europe. We report the objective and subjective visual benefit of cataract surgery, the impact of risk factors on the visual outcome, and the

incidence of intraoperative and postoperative complications. Using this knowledge, recommendations and considerations will be provided that may aid the counselling and clinical management of cataract in patients with RP.

METHODS

Data collection

This international, multicenter, retrospective study was performed in collaboration with several academic centers across Europe, which included Leiden University Medical Center (Leiden, The Netherlands), Amsterdam University Medical Centers (Amsterdam, The Netherlands), Erasmus University Medical Center (Rotterdam, The Netherlands), Radboud University Medical Center (Nijmegen, The Netherlands), University Medical Center Groningen (Groningen, The Netherlands), Leeds Teaching Hospital (Leeds, United Kingdom), Ghent University Hospital (Ghent, Belgium), Charles University and General University Hospital (Prague, Czech Republic), and the Hospital of Lithuanian University of Health Sciences (Kaunas, Lithuania). This study adhered to the tenets of the Declaration of Helsinki, and the protocol was approved the Medical Ethics Committee of the Leiden University Medical Center. Informed consent was not required due to the anonymized nature of this study.

Clinical data on cataract surgery in patients with RP were extracted from electronic patient medical records. Inclusion criteria were: clinical diagnosis of RP; medical history of cataract extraction surgery; and available pre-operative and postoperative visual acuity data. Patients were excluded if cataract surgery did not include the implementation of an IOL, or if patients underwent a combined anterior and posterior segment surgery (e.g. phacovitrectomy). Genetic confirmation of RP was not a requirement for this study.

When available, preoperative data closest to surgery were collected and included: patient demographics, ocular comorbidities, best-corrected visual acuity data (BCVA) and refraction, lens morphology, ocular biometry, and target refraction. Intraoperative clinical data obtained were: anesthetic procedure, IOL type and material, the use of a capsular tension ring during surgery, and intraoperative complications (e.g. posterior capsule tears, vitreous prolapse, dropped nucleus). Postoperative data included: BCVA and refraction, subjective visual improvement, multimodal imaging, and postoperative complications (e.g. corneal edema, CME, and PCO).

Statistical analysis

Data analysis was performed using the R statistical program (version 3.6.2). The normality of data was visually inspected and tested by using the normality tests. In case of violation of the normality assumption, continuous data were presented as

median, interquartile ranges (IQR) and range; otherwise data were reported as mean ± standard deviation (SD). Categorical data were shown as frequencies (n =) and percentages (%). Preoperative BCVA of patients were classified into different categories of visual impairment based on the guidelines of the World Health Organization: no visual impairment (BCVA ≥ 20/40), mild visual impairment (20/67 ≤ BCVA < 20/40), low vision (20/200 ≤ BCVA < 20/67), severe visual impairment (20/400 ≤ BCVA < 20/200) or blindness (BCVA < 20/400).[21] For statistical analysis, BCVA data were converted to Logarithm of the Minimum Angle of Resolution (logMAR) values. Vision classifications of counting fingers, hand motion, light perception and no light perception were given the logMAR values 2.1, 2.4, 2.7 and 3.0, respectively.[22, 23] Logistic regression models were estimated to investigate the association between risk factors and visual outcome. The level of significance was set at 0.05.

RESULTS

Patient characteristics

A summary of the clinical characteristics of this cohort is provided in Table 1. A total of 295 eyes from 226 patients with RP were included in the study. The mean age at time of first surgery was 56.1 ± 17.9 years. In patients that also underwent surgery in the fellow eye (n = 69; 31%), the median waiting time between surgeries was 0.8 years (IQR 0.9, range 0.0 – 10.0 years). The mode of inheritance was reported in 89 out of 226 patients (39%), which included autosomal recessive (n = 47, 53%), autosomal dominant (n = 32, 36%) and X-linked (n = 10, 11%) inheritance forms (Supplemental Table 1). In addition to RP, 38 out of 226 patients (17%) had other ocular comorbidities that could compromise visual outcome (Table 1). Cataract morphology was described in 180 out of 295 eyes (61%), with PSC (n = 109; 61%) being the most common, followed by mixed (n = 38, 21%), nuclear (n = 23, 13%), mature (n = 6, 3%) and cortical (n = 4, 2%) cataract, respectively. The mean preoperative BCVA of the first treated eye was 1.03 ± 0.79 logMAR (approximately 20/214 Snellen), and the mean preoperative BCVA of the second treated eye was 0.80 ± 0.71 logMAR (approximately 20/129 Snellen). SD-OCT imaging data was available at pre-operative intake in 36 out of 226 patients (16%), revealing CME in 27 out of 72 eyes (38%).

Table 1. Baseline characteristics of patients with retinitis pigmentosa planned for cataract surgery.

Characteristic	
Total no. of patients	226
Total no. of operated eyes	295
Follow-up time in years	
Mean ± SD	0.8 ± 1.6 years
Median (IQR, range)	0.2 years (IQR 1.0, range 0.1 – 15.6 years)
Sex	
Male	112 (49%)
Female	113 (50%)
Not recorded	1 (1%)
Visual impairment at baseline	
No VI (BCVA ≥ 20/40)	39 (17%)
Mild VI (20/67 ≤ BCVA < 20/40)	37 16%)
Moderate VI (20/200 ≤ BCVA < 20/67)	77 (34%)
Severe VI (20/400 ≤ BCVA < 20/200)	7 (3%)
Blindness (BCVA ≤ 20/400)	66 (30%)
SER at baseline in diopters (D)	
Mean ± SD	-1.2 ± 4.3 D
Median (IQR, range)	-0.5 D (IQR 2.3, range -28.3 to 25.5 D)
Ocular comorbidities	
None (n, %)	187 (83%)
Glaucoma (n, %)	12 (5%)
Epiretinal membrane (n, %)	1 (<1%)
Diabetic retinopathy (n, %)	2 (1%)
Fuchs endothelial dystrophy (n, %)	2 (1%)
Age-related macular degeneration (n, %)	2 (1%)
Retinal vascular occlusion (n, %)	2 (1%)
High myopia (n, %)	1 (<1%)
Corneal pathology (n, %)	2 (1%)
Retinal detachment (n, %)	2 (1%)
Amblyopia (n, %)	1 (<1%)
Laser refractive surgery (n, %)	2 (1%)
Optic nerve disease (n, %)	1 (<1%)
Unspecified macular pathology (n, %)	4 (2%)
Other (n, %)	4 (2%)

BCVA = best-corrected visual acuity; IQR = interquartile range; SD = standard deviation; SER = spherical equivalent of the refractive error; VI = visual impairment.

Intraoperative assessment

Cataract surgery was performed under general anesthesia (n = 91; 31%), or via local anesthesia using subtenon (n = 84; 29%), topical (n = 71; 24%), retrobulbar (n = 32; 11%) or unspecified (n = 17; 6%) techniques. All cataract extractions were performed using phacoemulsification techniques. The types of implanted IOLs, available for 287 cases, included monofocal IOLs (n = 279; 97%), and toric IOLs (n = 8; 3%). The

biomaterial of the implemented IOL lenses was hydrophobic acrylic (n = 271; 92%), hydrophilic acrylic (n = 13; 4%), silicon (n = 2; 1%), or unspecified (n = 9; 3%). Nineteen surgeries (6%) reported the use of blue-light filtering IOLs. The prophylactic use of a capsular tension ring was recorded in 17 out of 295 surgeries (6%). In total, 19 (6%) intraoperative complications were recorded, of which 15 (79%) were incidences of zonular dialysis; 2 were posterior capsular ruptures without vitreous loss; 1 case of intraoperative miosis; and 1 case reported a broken haptic IOL during surgery.

Postoperative assessment

Figure 1 shows a comparison between preoperative and postoperative BCVA after surgery. Following surgery, BCVA improved from 1.03 to 0.81 logMAR (-0.22 ; 95% CI: -0.31 to -0.13; $p < 0.001$) in the first eye, or approximately from 20/214 to 20/129 Snellen. In patients that also underwent surgery in the fellow eye, BCVA improved from 0.80 to 0.56 logMAR (-0.24; 95% CI: -0.32 to -0.15; $p < 0.001$), or approximately from 20/126 to 20/73 Snellen. Visual improvements, defined as a postoperative improvement in logMAR BCVA ≥ 0.1, were seen in 166 eyes (56%). In the remaining eyes, 36 eyes (12%) showed worse BCVA (reduction of logMAR BCVA ≥ 0.1), and remained unchanged in 93 eyes (32%, BCVA changes in logMAR < 0.1). These patients showed a postoperative change in BCVA of +0.19 logMAR (95% CI -0.28 to -0.11; $p < 0.001$). The clinical characteristics and the complications of patients having worse postoperative BCVA are summarized in Supplemental Table 2. Marked BCVA improvements, defined as a BCVA change equal or greater than 0.3 logMAR (i.e., 15 ETDRS letter change), were observed in 87 out of 226 cases after first surgery (39%), and in 22 out of 69 eyes (32%) in patients with surgery in the contralateral eye. Odds of achieving marked BCVA improvements, adjusted for age and gender, were seen in patients with moderate visual impairment (odds ratio: 3.60; 95% CI: 1.74 – 7.46) and in patients with severe visual impairment to blindness (odds ratio: 4.36; 95% CI: 2.0 – 9.46) compared to patients with mild to no visual impairment (Table 2). Ocular comorbidities, intraoperative, or postoperative complications did not influence the presence of marked BCVA improvements. Patient-reported outcome data were available for 101 out of 226 patients (45%). Seventy-four out of 101 patients (73%) reported a subjective improvement in visual function following their first surgery, of whom 5 patients had showed no objective postoperative change in BCVA. The remaining patients with patient-reported outcome data reported no change (n = 20; 20%) or worse subjective visual function (n = 7; 7%) after surgery. In these patients with stable or worse subjective visual function, there was no marked BCVA improvement in 24 patients (89%). The remaining 3 patients with no subjective changes who did demonstrate marked BCVA changes postoperatively included 2 severely visually impaired or blind patients with minimal improvements in BCVA, and 1 patient who developed marked anterior capsular phimosis after surgery. Postoperative complications, excluding PCO, were reported in 32 out of 295 cases (11%). The most common postoperative complication was the exacerbation of pre-existing CME (n = 8; 25%), followed by the development of new CME (n = 6; 19%),

corneal edema (n = 5; 16%), capsular phimosis (n = 5; 16%), increase of intraocular pressure (n = 4; 13%), IOL subluxation (n = 3; 9%) and endophthalmitis (n = 1; 3%). In total, 111 out of 295 eyes (38%) developed significant PCO that required laser posterior capsulotomy during follow-up. Figure 2 shows that the median time for patients to undergo laser posterior capsulotomy was 15.0 months (95% CI: 12.8 – 17.2) after cataract surgery.

Table 2. Unadjusted and adjusted odds ratios of patients with retinitis pigmentosa following cataract surgery using marked BCVA improvement (postoperative improvement of > 0.3 logMAR) as dependent variable .

Characteristic	Unadjusted OR (95% CI:)	Adjusted OR (95% CI:)
Sex		
Male	1.00 [reference]	1.00 [reference]
Female	1.09 (95% CI: 0.64 – 1.87)	1.15 (95% CI: 0.66 – 1.99)
Age at surgery		
< 20 years	1.00 [reference]	1.00 [reference]
20 to 39 years	0.20 (95% CI: 0.02 – 2.02)	0.23 (95% CI: 0.02 – 2.27)
40 to 59 years	0.64 (95% CI: 0.20 – 2.00)	0.70 (95% CI: 0.22 – 2.67)
60 to 79 years	0.90 (95% CI: 0.34 – 2.39)	1.02 (95% CI: 0.37 – 2.77)
≥ 80 years	0.34 (95% CI: 0.23 – 1.75)	0.72 (95% CI: 0.255 – 2.03)
Preoperative BCVA		
Mild VI or better (BCVA ≥ 20/67)	1.00 [reference]	1.00 [reference]
Moderate VI (20/200 ≤ BCVA < 20/67)	**3.36 (95% CI: 1.65 – 6.83)**	**3.60 (95% CI: 1.74 – 7.46)**
Severe VI or worse (BCVA < 20/200)	**4.32 (95% CI: 2.05 – 9.08)**	**4.36 (95% CI: 2.0 – 9.46)**
Ocular comorbidities		
Not present	1.00 [reference]	1.00 [reference]
Present	1.55 (95% CI: 0.77 – 3.13)	1.51 (95% CI: 0.73 – 3.13)
Intraoperative complications		
Absent	1.00 [reference]	1.00 [reference]
Present	0.91 (95% CI: 0.26 – 3.2)	0.94 (95% CI: 0.26 – 3.32)
Postoperative complications		
Absent	1.00 [reference]	1.00 [reference]
Present	0.53 (95% CI: 0.20 – 1.4)	0.78 (95% CI: 0.22 – 1.54)
Posterior capsular opacifications		
Absent	1.00 [reference]	1.00 [reference]
Present	0.78 (95% CI: 0.47 – 1.27)	0.84 (95 % CI: 0.50 – 1.40)

BCVA = best-corrected visual acuity; CI = confidence interval; OR = odds ratio; VI = visual impairment. Adjusted for age and gender. Analysis was performed using the first eye of each patient undergoing surgery. Intraoperative complications included zonulolysis, capsule rupture, dropped nucleus. Postoperative complications included cystoid macular edema, corneal edema, capsular phimosis, increased intraocular pressure, lens (sub)luxation, and endophthalmitis. Significant findings are shown in bold.

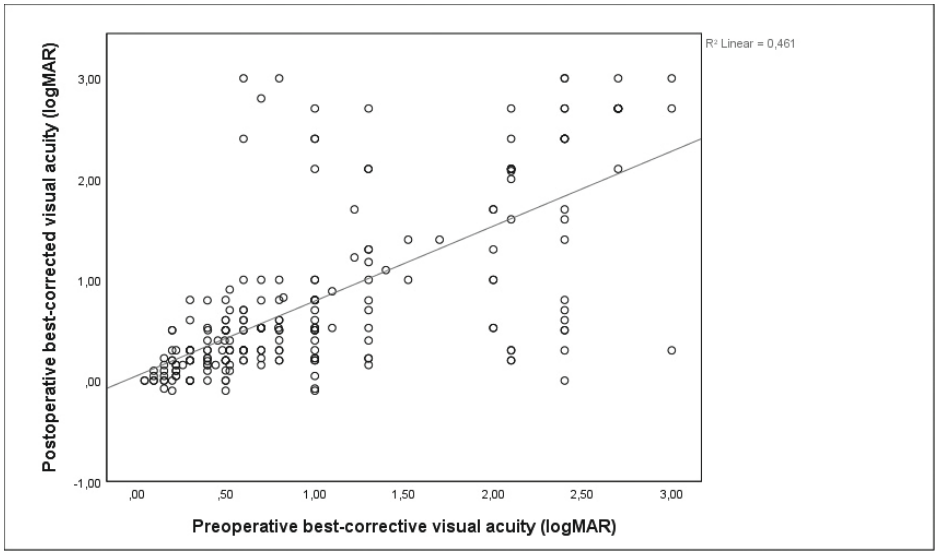

Figure 1. Scatterplot of preoperative best-corrected visual acuity using logarithm of the minimum angle of resolution (logMAR) values and postoperative best-corrected visual acuity in patients with retinitis pigmentosa that underwent cataract surgery. Analysis was performed using the first eye of each patient undergoing surgery.

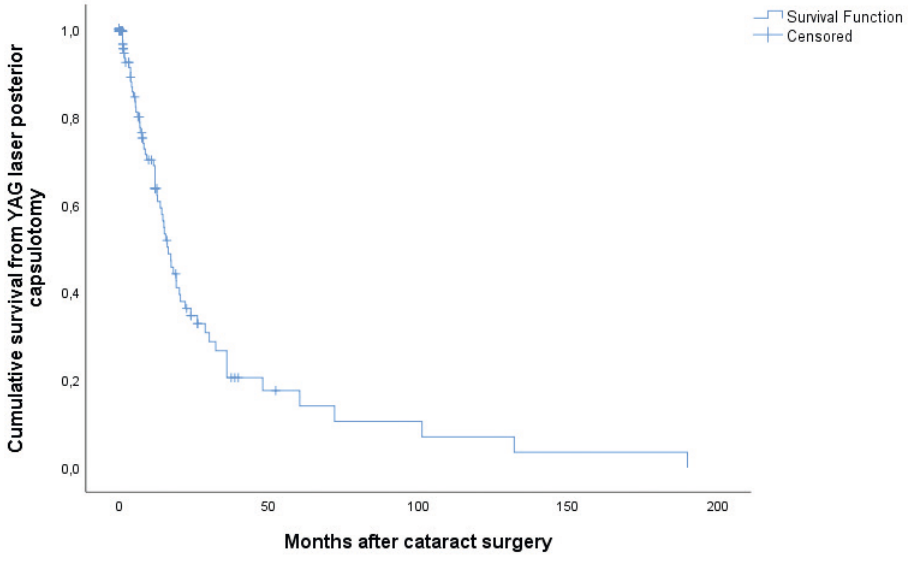

Figure 2. Kaplan-Meier survival curve showing the association between neodymium-doped yttrium-aluminum-garnet (YAG) laser posterior capsulotomy and the time after cataract surgery in patients with retinitis pigmentosa.

DISCUSSION

Our study demonstrates that cataract surgery in patients with RP leads to significant improvements in visual acuity, with more than half of the eyes included having better BCVA postoperatively than preoperatively. On average, BCVA improvements of 0.22-0.24 logMAR were observed, which is comparable to a 2- to 3-line BCVA increase measured on Snellen charts. These observations are consistent with BCVA outcomes in from previous smaller studies, who have reported postoperative BCVA improvements between 0.09-0.47 logMAR.[3, 6, 7, 12, 17, 24] Despite the impact of RP on visual acuity, we found that marked BCVA improvements (greater than 0.3 logMAR) were also possible in this cohort following cataract surgery. We found that preoperative BCVA was a predictor for marked BCVA improvements, as groups with a BCVA of 20/200-20/67 Snellen (i.e. moderate visual impairment) and 20/200 or worse (i.e. severe impairment/blindness) had better odds of achieving marked BCVA improvements than patients with a BCVA of 20/67 or better (i.e. mild/no visual impairment). This would be expected, as patients preserved visual acuities are limited in their BCVA improvements due to a ceiling effect.[7] Another possible and additional explanation is that patients with poor preoperative BCVA have more severe vision-impairing cataracts, which, in turn, yields higher gains in BCVA following surgery.

The severity of cataracts was not possible to ascertain in the current study, as cataract grading systems such as the Lens Opacities Classification System III are not routinely used in the clinical setting of the involved centers.[11, 25] Still, in the remaining 44% of operated eyes, we found no BCVA improvements following surgery. We postulate that patients with no BCVA improvements may have extensive macular involvement causing irreversible vision loss, which precludes any BCVA improvement following cataract surgery.[26] Despite yielding no BCVA improvements, removal of significant cataract can be useful to rule out the contribution of cataract in disease progression. Additionally, removal of optically significant cataracts may allow for better visualization of retinal structures on fundoscopy and multimodal imaging. While the majority of patients underwent cataract surgery under local anesthesia, a relatively large group of patients (31%) in this study underwent surgery under general anesthesia. From our own clinical experience, the vast majority of cataract surgeries can be performed under local anesthesia. However, local anaesthesia may not be desirable in relatively younger patients and patients with significant associated extra-ocular abnormalities e.g. cognitive or hearing impairments, in whom general anesthesia is a viable alternative.[27-29]Although our study did not investigate the degree of photoreceptor integrity on SD-OCT, it is well-known that preserved photoreceptor layers are associated with better visual acuity in patients of RP.[26, 30-32] In addition, a previous study by Yoshida and colleagues demonstrated that postoperative BCVA was significantly better in patients with intact photoreceptor layers on OCT imaging following cataract surgery.[3] Further investigation is needed into the potential predictive value of OCT imaging on visual

outcome following cataract surgery in patients with RP, as it was limited performed in this study.

In patients with available subjective data, subjective visual improvements were reported by most patients (73%), despite some having no objective change in BCVA following surgery. This suggests that objective BCVA improvement is not the sole indicator for successful cataract surgery. Due to the presence of both cataract and RP, it is possible that patients may perceive visual disturbances, such as glare, halo's and decreased contrast sensitivity, potentially at greater levels than those with age-related cataract.[3] Alleviation of these visual disturbances through cataract surgery may prove more important than potential improvements in BCVA. Therefore, patients should be assessed for any perceived visual disturbances at preoperative and postoperative assessment.

The most common intraoperative complication was zonular dialysis, occurring in 15 (5%) of all cases. The occurrence of zonular dialysis is rare in surgeries for age-related cataract, as a previous large cohort study reported an incidence rate for zonular dialysis of 0.5%.[20] While the underlying mechanism remains unclear, it is believed that an inflammatory process may be the main cause for weakened zonular attachments.[7] It has been postulated that increased levels of cytokines and chemokines are released into the aqueous humor and vitreous fluid as response to the diffusion of toxins derived from the degenerating retina by RP.[33] In turn, these may cause damage to zonular attachments, weakening their stability.[7, 19] When zonular weakness is present, this may result in complications including zonular dialysis, capsule tears, intraoperative miosis, and IOL (sub)luxation, which were all reported to some degree in the current study.[17] Therefore, preoperative assessment in patients with RP should include the identification of possible signs of zonular weakness, such as phacodonesis and lens subluxation, which are indicative of severe zonular weakness.[34] Ultrasound biomicroscopy has also been used for zonular evaluation in previous studies, and allows for the assessment of the extent of zonular damage pre-operatively.[35, 36] Intraoperative signs include prominent anterior capsule stria during capsulorrhexis and difficulties with nucleus rotation after hydrodissection.[37] Necessary precautions can then be taken during surgery, such as extra attention to avoidance of unnecessary manipulation and strain on the lens zonules, for instance by optimal hydrodissection, and bimanual rotation of the nucleus; or by using nuclear fragmentation techniques that require minimal rotation such as the 'cross chop' technique.[17, 38] In this regard, creating a larger-than average capsulorrhexis can also be helpful for better mobilization of lens fragments, and may also help to reduce the risk of postoperative capsular phimosis. Some studies advocate the use of a capsular tension ring to provide stability to the lens equator, although this remains to be a subject of controversy.[19, 39] The use of a capsular tension ring may decrease the risk for IOL (sub)luxation, anterior capsule phimosis, and

PCO.[17, 40] The use of a capsular tension ring was reported in 6% of our cases, although the use of this ring may be underreported in our current retrospective study.

Postoperatively, the most common complications were the development of new CME, or the exacerbation of pre-existing CME, which collectively were found in 14 out of 295 eyes (5%). CME is a well-known complication associated with RP, and has been reported in 10-50% of all patients with RP.[41] We reported the presence of CME in 16% of the patients at pre-operative intake, although its prevalence is likely underreported in this study as this information may not be automatically extracted from electronic health records. A recent study by Antonio-Aguirre and colleagues suggested that patients with RP were 4 times more likely to develop CME following cataract surgery.[42] The mechanism of CME in RP remains unclear, but is presumably due to a dysfunction of the retinal pigment epithelium pumping mechanism and/or breakdown of the blood-retina-barrier.[2, 43] Persistent CME may result in photoreceptor damage, and subsequently in loss of visual function if left untreated.[41] Identifying the presence of CME at preoperative assessment on fundoscopy or SD-OCT imaging can aid in pre-emptive management (e.g. administering topical non-steroidal anti-inflammatory drops postoperatively) and recognition of this complication. While limited evidence is available on the treatment of CME in patients with RP, several studies suggest the use of topical or oral carbon anhydrase inhibitors as a first-line approach.[41, 44] Our study revealed that 38% of cases developed PCO that required Nd:YAG posterior capsulotomy, which reiterates that PCO is a common complication in patients with RP after cataract surgery.[3, 4, 7, 12] The occurrence of PCO in this study is lower than reported rates of 43-52% by previous studies, although not all patients were available for follow-up, and thus the exact rate of PCO is probably underreported.[6, 7] We reported a median time of 15 months for patients to require Nd:YAG posterior capsulotomy, which is comparable to the period of 12 months described by Dikopf and co-workers.[7] It has also been previously shown that the IOL biomaterial is one of the main risk factors for the development of PCO, with hydrophobic IOLs showing lower PCO rates than hydrophilic IOLs after cataract surgery.[45] We were not able to investigate the phenomenon in this study, as the majority of patients received hydrophobic IOLs. Nevertheless, patients with RP should be informed of the relatively high occurrence of PCO and its possible symptoms, in order to facilitate early intervention.

The results of this study should be interpreted with caution in light of its study design. An inherent flaw of a retrospective study design is the lack of complete and standardized data, and several parameters of interest (e.g. OCT imaging, patient-reported outcome data, or genetic data) were unavailable for a sizeable proportion of patients, as they were not routinely performed per standard care.[7] Additionally, several comorbidities that may compromise visual acuity or pre-operative planning (e.g. presence of epiretinal membranes, CME or laser refractive surgery) showed unexpected low incidences and were most likely underreported, as these parameters

are not automatically recorded and extracted from electronic patient records. Nevertheless, this large study highlights some important features and outcomes in patients with RP, which may aid patient counselling and perioperative management.

In conclusion, this study suggests that cataract surgery leads to significant improvements in BCVA in the majority of patients with RP, with baseline BCVA being a potential predictive factor. Patients may experience subjective visual improvement, irrespective of their visual outcome. Surgeons should be aware of the high prevalence of zonular weakness and CME, which may warrant additional preparation. A high rate of PCO is also present, which requires early treatment with laser posterior capsulotomy. Further studies should include SD-OCT imaging, and the use of patient-reported outcome measures, as they are potentially important parameters for the evaluation of visual outcome.

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REFERENCES

1. Hamel CP. Retinitis pigmentosa. *Orphanet J Rare Dis*. 2006;1:40-40. doi:10.1186/1750-1172-1-40
2. Verbakel SK, van Huet RAC, Boon CJF, et al. Non-syndromic retinitis pigmentosa. *Progress in Retinal and Eye Research*. 2018/03/27/ 2018;66:157-186. doi:https://doi.org/10.1016/j.preteyeres.2018.03.005
3. Yoshida N, Ikeda Y, Murakami Y, et al. Factors Affecting Visual Acuity after Cataract Surgery in Patients with Retinitis Pigmentosa. *Ophthalmology*. 2015/05/01/ 2015;122(5):903-908. doi:https://doi.org/10.1016/j.ophtha.2014.12.003
4. Fujiwara K, Ikeda Y, Murakami Y, et al. Risk Factors for Posterior Subcapsular Cataract in Retinitis Pigmentosa. *Investigative Ophthalmology & Visual Science*. 2017;58(5):2534-2537. doi:10.1167/iovs.17-21612
5. Liew G, Strong S, Bradley P, et al. Prevalence of cystoid macular oedema, epiretinal membrane and cataract in retinitis pigmentosa. *British Journal of Ophthalmology*. 2019;103(8):1163. doi:10.1136/bjophthalmol-2018-311964
6. Jackson H, Garway-Heath D, Rosen P, Bird AC, Tuft SJ. Outcome of cataract surgery in patients with retinitis pigmentosa. *British Journal of Ophthalmology*. 2001;85(8):936. doi:10.1136/bjo.85.8.936
7. Dikopf MS, Chow CC, Mieler WF, Tu EY. Cataract Extraction Outcomes and the Prevalence of Zonular Insufficiency in Retinitis Pigmentosa. *American Journal of Ophthalmology*. 2013/07/01/ 2013;156(1):82-88.e2. doi:https://doi.org/10.1016/j.ajo.2013.02.002
8. Gwon A, Mantras C, Gruber L, Cunanan C. Concanavalin A-induced posterior subcapsular cataract: a new model of cataractogenesis. *Investigative Ophthalmology & Visual Science*. 1993;34(13):3483-3488.
9. Allen D, Vasavada A. Cataract and surgery for cataract. *BMJ*. 2006;333(7559):128-132. doi:10.1136/bmj.333.7559.128
10. Chua BE, Mitchell P, Cumming RG. Effects of cataract type and location on visual function: The Blue Mountains Eye Study. *Eye*. 2004/08/01 2004;18(8):765-772. doi:10.1038/sj.eye.6701366
11. Skiadaresi E, McAlinden C, Pesudovs K, Polizzi S, Khadka J, Ravalico G. Subjective Quality of Vision Before and After Cataract Surgery. *Archives of Ophthalmology*. 2012;130(11):1377-1382. doi:10.1001/archophthalmol.2012.1603
12. Bastek JV, Heckenlively JR, Straatsma BR. Cataract Surgery in Retinitis Pigmentosa Patients. *Ophthalmology*. 1982;89(8):880-884. doi:10.1016/S0161-6420(82)34700-4
13. Lundström M, Barry P, Henry Y, Rosen P, Stenevi U. Visual outcome of cataract surgery; Study from the European Registry of Quality Outcomes for Cataract and Refractive Surgery. *Journal of Cataract & Refractive Surgery*. 2013;39(5)
14. Wang J, Su F, Wang Y, Chen Y, Chen Q, Li F. Intra and post-operative complications observed with femtosecond laser-assisted cataract surgery versus conventional phacoemulsification surgery: a systematic review and meta-analysis. *BMC Ophthalmol*. 2019;19(1):177-177. doi:10.1186/s12886-019-1190-2
15. Davies EC, Pineda R. Cataract surgery outcomes and complications in retinal dystrophy patients. *Canadian Journal of Ophthalmology*. 2017/12/01/ 2017;52(6):543-547. doi:https://doi.org/10.1016/j.jcjo.2017.04.002
16. De Rojas JO, Schuerch K, Mathews PM, et al. Evaluating Structural Progression of Retinitis Pigmentosa After Cataract Surgery. *American Journal of Ophthalmology*. 2017;180:117-123. doi:10.1016/j.ajo.2017.05.026

17. Bayyoud T, Bartz-Schmidt KU, Yoeruek E. Long-term clinical results after cataract surgery with and without capsular tension ring in patients with retinitis pigmentosa: a retrospective study. *BMJ Open*. 2013;3(4):e002616. doi:10.1136/bmjopen-2013-002616

18. Jin-Poi T, Shatriah I, Khairy-Shamel ST, Zunaina E. Rapid anterior capsular contraction after phacoemulsification surgery in a patient with retinitis pigmentosa. *Clin Ophthalmol*. 2013;7:839-842. doi:10.2147/OPTH.S42122

19. Hong Y, Li H, Sun Y, Ji Y. A Review of Complicated Cataract in Retinitis Pigmentosa: Pathogenesis and Cataract Surgery. *Journal of Ophthalmology*. 2020/12/21 2020;2020:6699103. doi:10.1155/2020/6699103

20. Trikha S, Agrawal S, Saffari SE, Jayaswal R, Yang YF. Visual outcomes in patients with zonular dialysis following cataract surgery. *Eye (Lond)*. Oct 2016;30(10):1331-1335. doi:10.1038/eye.2016.108

21. World Health Organization. International Classification of Diseases 11th Revision. Accessed December 25th, 2020, https://icd.who.int/en

22. Day AC, Donachie PHJ, Sparrow JM, Johnston RL, on behalf of all surgeons contributing towards The Royal College of Ophthalmologists' National Ophthalmology D. The Royal College of Ophthalmologists' National Ophthalmology Database study of cataract surgery: report 1, visual outcomes and complications. *Eye*. 2015/04/01 2015;29(4):552-560. doi:10.1038/eye.2015.3

23. Buchan JC, Donachie PHJ, Cassels-Brown A, et al. The Royal College of Ophthalmologists' National Ophthalmology Database study of cataract surgery: Report 7, immediate sequential bilateral cataract surgery in the UK: Current practice and patient selection. *Eye*. 2020/10/01 2020;34(10):1866-1874. doi:10.1038/s41433-019-0761-z

24. Chan TCY, Lam SC, Mohamed S, Wong RLM. Survival analysis of visual improvement after cataract surgery in advanced retinitis pigmentosa. *Eye*. 2017/12/01 2017;31(12):1747-1748. doi:10.1038/eye.2017.164

25. Chylack LT, Jr., Wolfe JK, Singer DM, et al. The Lens Opacities Classification System III. The Longitudinal Study of Cataract Study Group. *Arch Ophthalmol*. Jun 1993;111(6):831-6. doi:10.1001/archopht.1993.01090060119035

26. Sakai D, Takagi S, Hirami Y, Nakamura M, Kurimoto Y. Use of ellipsoid zone width for predicting visual prognosis after cataract surgery in patients with retinitis pigmentosa. *Eye (Lond)*. Jan 1 2022;doi:10.1038/s41433-021-01878-3

27. Kumar CM, Seet E, Eke T, Irwin MG, Joshi GP. Peri-operative considerations for sedation-analgesia during cataract surgery: a narrative review. https://doi.org/10.1111/anae.14845. *Anaesthesia*. 2019/12/01 2019;74(12):1601-1610. doi:https://doi.org/10.1111/anae.14845

28. Pierrottet CO, Zuntini M, Digiuni M, et al. Syndromic and non-syndromic forms of retinitis pigmentosa: a comprehensive Italian clinical and molecular study reveals new mutations. *Genet Mol Res*. Oct 27 2014;13(4):8815-33. doi:10.4238/2014.October.27.23

29. Ferrari S, Di Iorio E, Barbaro V, Ponzin D, Sorrentino FS, Parmeggiani F. Retinitis pigmentosa: genes and disease mechanisms. *Curr Genomics*. Jun 2011;12(4):238-49. doi:10.2174/138920211795860107

30. Aizawa S, Mitamura Y, Hagiwara A, Sugawara T, Yamamoto S. Changes of fundus autofluorescence, photoreceptor inner and outer segment junction line, and visual function in patients with retinitis pigmentosa. *Clinical & Experimental Ophthalmology*. 2010/08/01 2010;38(6):597-604. doi:10.1111/j.1442-9071.2010.02321.x

31. Kim YJ, Joe SG, Lee DH, Lee JY, Kim JG, Yoon YH. Correlations between spectral-domain OCT measurements and visual acuity in cystoid macular edema associated with retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. Feb 1 2013;54(2):1303-9. doi:10.1167/iovs.12-10149

32. Liu G, Li H, Liu X, Xu D, Wang F. Structural analysis of retinal photoreceptor ellipsoid zone and postreceptor retinal layer associated with visual acuity in patients with retinitis pigmentosa by ganglion cell analysis combined with OCT imaging. *Medicine*. 2016;95(52):e5785-e5785. doi:10.1097/MD.0000000000005785

33. Yoshida N, Ikeda Y, Notomi S, et al. Clinical Evidence of Sustained Chronic Inflammatory Reaction in Retinitis Pigmentosa. *Ophthalmology*. 2013/01/01/ 2013;120(1):100-105. doi:https://doi.org/10.1016/j.ophtha.2012.07.006

34. Yaguchi S, Yaguchi S, Yagi-Yaguchi Y, Kozawa T, Bissen-Miyajima H. Objective classification of zonular weakness based on lens movement at the start of capsulorhexis. *PLoS One*. 2017;12(4):e0176169-e0176169. doi:10.1371/journal.pone.0176169

35. Bhatt V, Bhatt D, Barot R, Sheth J. Ultrasound Biomicroscopy for Zonular Evaluation in Eyes with Ocular Trauma. *Clin Ophthalmol*. 2021;15:3285-3291. doi:10.2147/opth.S323349

36. Salimi A, Fanous A, Watt H, Abu-Nada M, Wang A, Harasymowycz P. Prevalence of zonulopathy in primary angle closure disease. https://doi.org/10.1111/ceo.13983. *Clinical & Experimental Ophthalmology*. 2021/12/01 2021;49(9):1018-1026. doi:https://doi.org/10.1111/ceo.13983

37. Shingleton BJ, Neo YN, Cvintal V, Shaikh AM, Liberman P, O'Donoghue MW. Outcome of phacoemulsification and intraocular lens implantion in eyes with pseudoexfoliation and weak zonules. https://doi.org/10.1111/aos.13110. *Acta Ophthalmologica*. 2017/03/01 2017;95(2):182-187. doi:https://doi.org/10.1111/aos.13110

38. Fontana L, Coassin M, Iovieno A, Moramarco A, Cimino L. Cataract surgery in patients with pseudoexfoliation syndrome: current updates. *Clin Ophthalmol*. 2017;11:1377-1383. doi:10.2147/OPTH.S142870

39. Hasanee K, Butler M, Ahmed IIK. Capsular tension rings and related devices: current concepts. *Current Opinion in Ophthalmology*. 2006;17(1)

40. Takimoto M, Hayashi K, Hayashi H. Effect of a capsular tension ring on prevention of intraocular lens decentration and tilt and on anterior capsule contraction after cataract surgery. *Jpn J Ophthalmol*. Sep-Oct 2008;52(5):363-367. doi:10.1007/s10384-008-0570-6

41. Strong S, Liew G, Michaelides M. Retinitis pigmentosa-associated cystoid macular oedema: pathogenesis and avenues of intervention. *Br J Ophthalmol*. 2017;101(1):31-37. doi:10.1136/bjophthalmol-2016-309376

42. Antonio-Aguirre B, Swenor B, Canner JK, Singh MS. Risk of cystoid macular edema following cataract surgery in retinitis pigmentosa: an analysis of United States claims from 2010 to 2018. *Ophthalmology Retina*. doi:10.1016/j.oret.2022.04.018

43. Scorolli L, Morara M, Meduri A, et al. Treatment of Cystoid Macular Edema in Retinitis Pigmentosa With Intravitreal Triamcinolone. *Archives of Ophthalmology*. 2007;125(6):759-764. doi:10.1001/archopht.125.6.759

44. Bakthavatchalam M, Lai FHP, Rong SS, Ng DS, Brelen ME. Treatment of cystoid macular edema secondary to retinitis pigmentosa: a systematic review. *Surv Ophthalmol*. May-Jun 2018;63(3):329-339. doi:10.1016/j.survophthal.2017.09.009

45. Pérez-Vives C. Biomaterial Influence on Intraocular Lens Performance: An Overview. *Journal of ophthalmology*. 2018;2018:2687385-2687385. doi:10.1155/2018/2687385

SUPPLEMENTAL CONTENT

Supplemental Table S1. Mode of inheritance and molecular diagnosis of this cohort.

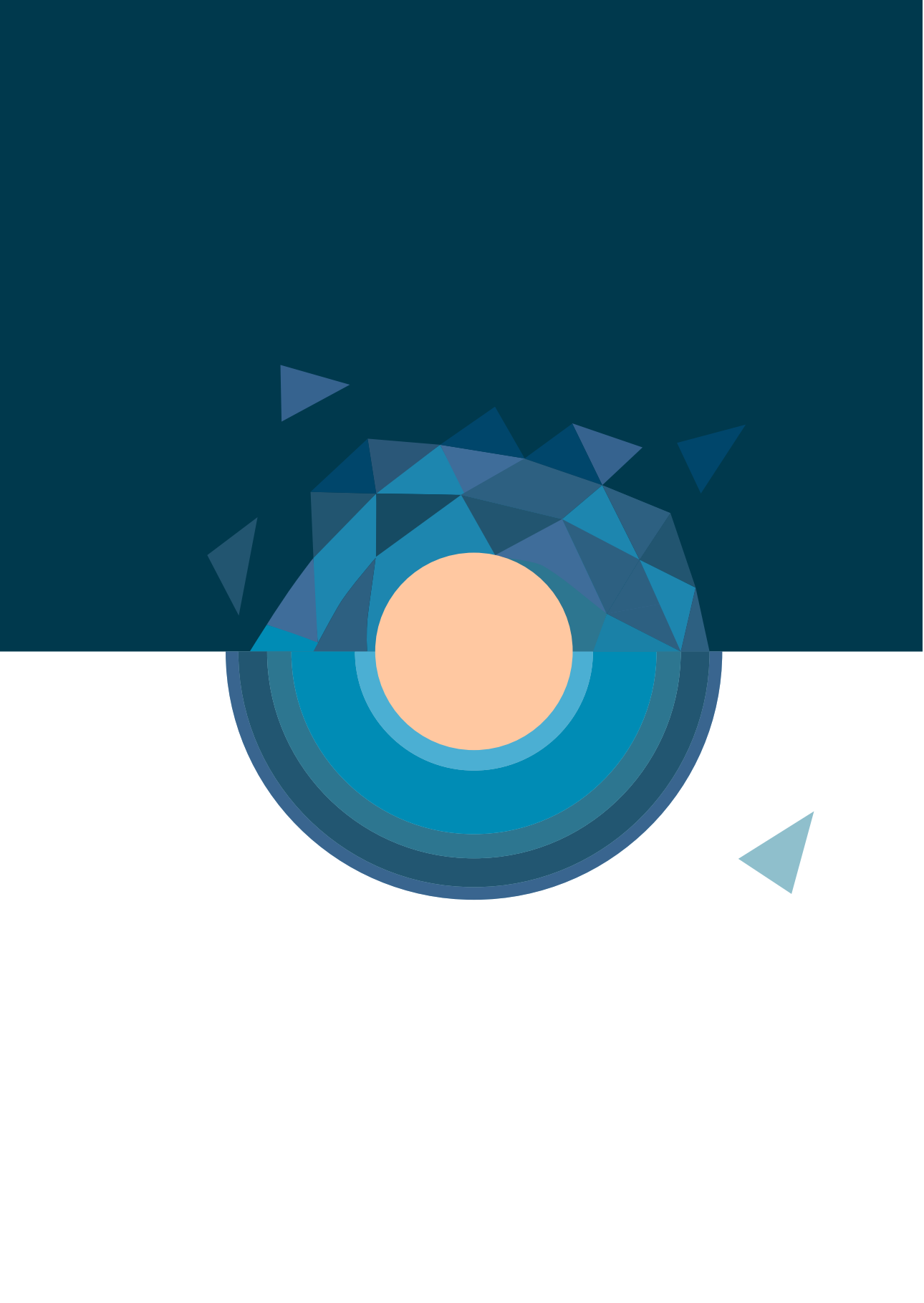
Characteristic	Frequency (n)
Total patients	226
Mode of inheritance available	89
Mode of inheritance unavailable	137
Autosomal recessive	47
USH2A	16
EYS	5
PDE6A	2
PDE6B	2
MERTK	1
IFT140	1
Unknown/not recorded	20
Autosomal dominant	32
RHO	3
PRPF31	3
RP1	2
SRNP200	2
CA4	1
IMPHD1	1
KLHL7	1
PRPF8	1
Unknown/not recorded	18
X-linked	10
RPGR	6
Unknown/not recorded	4

The mode of inheritance and molecular diagnosis were extracted anonymously from electronic patient records and were available for a limited amount of patients.

Supplemental Table S2. Clinical characteristics of patients with RP that had worse visual acuity following cataract surgery.

Characteristic	
Total no. of cases	36
Visual impairment at baseline	
No VI (BCVA ≥ 20/40)	6 (17%)
Mild VI (20/67 ≤ BCVA < 20/40)	4 (11)
Moderate VI (20/200 ≤ BCVA < 20/67)	15 (42%)
Severe VI (20/400 ≤ BCVA < 20/200)	3 (8%)
Blindness (BCVA ≤ 20/400)	8 (22%)
Preoperative BCVA in logMAR	
Mean ± SD	1.00 ± 0.75 logMAR
Postoperative BCVA in logMAR	
Mean ± SD	1.71 ± 0.97 logMAR
Ocular comorbidities	
None (n, %)	27 (75%)
Glaucoma (n, %)	4 (10%)
Fuchs endothelial dystrophy (n, %)	1 (3%)
Retinal detachment (n, %)	1 (3%)
Optic nerve disease (n, %)	1 (3%)
Unspecified macular pathology (n, %)	1 (3%)
Other unspecified disease (n, %)	1 (3%)
Surgical complications	
None (n, %)	35 (97%)
Zonulysis (n, %)	1 (3%)
Postoperative complications	
None (n, %)	28 (78%)
Increase of CME (n, %)	2 (6%)
New CME (n, %)	2 (6%)
Increase of intraocular pressure (n, %)	3 (8%)
Corneal edema (n, %)	1 (2%)
Posterior capsular opacifications	
None (n, %)	27 (75%)
Present (n, %)	9 (25%)

Worse visual acuity was defined as a postoperative worsening of best-corrected visual acuity (BCVA) greater than 0.1 logarithm of the minimum angle of resolution (logMAR). CME = cystoid macular edema, IQR = interquartile range; SD = standard deviation; SD = standard deviation; SER = spherical equivalent of the refractive error; VI = visual impairment.



CHAPTER 3.2

ARTIFICIAL VISION: THE EFFECTIVENESS OF THE ORCAM IN PATIENTS WITH ADVANCED INHERITED RETINAL DYSTROPHIES

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ABSTRACT

Purpose

To investigate the impact of the OrCam MyEye 2.0 (OrCam) on the quality of life and rehabilitation needs in patients with advanced retinitis pigmentosa (RP) or cone-rod dystrophies (CRD). The OrCam is a wearable low vision aid that converts visual information to auditive feedback (e.g. text-to-speech, barcode and facial recognition).

Methods

Patients with a clinical diagnosis of RP (n = 9, 45%) or CRD (n = 11; 55%), and a best-corrected visual acuity of ≤20/400 Snellen were invited to participate in this study. Questionnaires were administered at baseline and after 5.2 (standard deviation ± 1.5) weeks, which included the Dutch version of the National Eye Institute Visual Functioning Questionnaire (NEI-VFQ), the Participation and Activity Inventory (PAI), and the OrCam Function Questionnaire (OFQ).

Results

Following OrCam testing, significant improvements were observed in the 'near activities' subscale of the NEI-VFQ ($p < 0.001$); the 'visual functioning' subscale of the re-engineered NEI-VFQ ($p = 0.001$); the 'reading' rehabilitation goal of the PAI ($p = 0.005$); and the overall score of the OFQ ($p < 0.001$). The observed changes in questionnaire scores did not differ between phenotypes. Advantages and limitations of the OrCam were reported by patients. Three patients (15%) continued rehabilitation with the OrCam after completion of this study.

Conclusions

The OrCam mainly improves reading domains in patients with advanced stages of RP or CRD. Further improvements in the OrCam are needed to address current limitations, which may enhance its utility for patients with RP or CRD.

INTRODUCTION

Inherited retinal dystrophies (IRDs) comprise a diverse group of rare eye diseases characterized by progressive loss of photoreceptor function, ultimately leading to severe visual impairment.[1] IRDs can be differentiated, in part, through the order of which cells are lost.[1] In retinitis pigmentosa (RP), degeneration of rods precedes that of cones, resulting in initial symptoms of nyctalopia and peripheral visual field loss.[2-4] Ultimately, central vision is also lost. Conversely, in cone-rod dystrophies (CRD), the process of photoreceptor degeneration follows the opposite sequence of events than in RP, causing predominant symptoms of central vision loss, photophobia, and color vision impairment, followed by peripheral vision loss and night blindness in later stages of the disease.[5, 6] Loss of visual function due to RP or CRD has detrimental effects on a patient's well-being and on their ability to perform daily activities, although the extent and areas of difficulties may vary between these phenotypes.[7]

For most patients with IRDs, the visual prognosis remains poor, as curative treatments are unavailable or are still under investigation. Therefore, emphasis should be on assisting patients with managing their disease, e.g. through low-vision rehabilitation services.[8] The goal of low-vision rehabilitation is not to restore vision, but to utilize residual vision to its maximum potential.[9] This may be achieved by low-vision centers through the prescription of low-vision aids (LVAs), ranging from (non-)optical aids to electronic assistive technologies. The selection of appropriate LVAs for an individual patient is complex, and several factors need to be considered prior to prescription, such as a patient's visual and cognitive ability, disease stage, occupation, and own rehabilitation goals.[10, 11]

The OrCam MyEye (https://www.orcam.com), or OrCam in short, is a relatively recent addition to the list of commercially available LVAs. The OrCam is a portable LVA that can be attached to the frame of a patient's eyeglasses. It contains a small camera that converts digital or printed text to real-time auditive feedback using optical character recognition technology. As such, the intended audience for the OrCam consists of severe visually impaired or blind patients that have lost the ability to read independently. Aside from text-to-speech capabilities, the OrCam also contains color, object, barcode, money, and facial recognition. Thus, the OrCam has the potential to improve the performance of multiple daily activities in visually impaired patients. However, the impact of a single LVA remains unclear, as low-vision rehabilitation programs typically offer multiple LVAs and multidisciplinary services over the course of rehabilitation. This makes it difficult to distinguish the contribution of a single device or service on a patient's rehabilitation progress.[12, 13] Insights into the effectiveness of the OrCam will provide knowledge on which patients are most likely to benefit from the device, and will also inform us on which daily activities may improve when using devices such as the OrCam. In addition, as the target of interest has to be within

the OrCam's field of view, we also investigated whether the feasibility of the OrCam differed in those with different visual abilities, e.g. patients with peripheral blindness or central blindness. For this purpose, this study investigated the effectiveness of the OrCam on the quality of life and the perceived difficulties in daily activities in severe visually impaired or blind patients caused by either RP or CRD.

METHODS

Participants

Patients that were scheduled for one of the two Dutch low-vision rehabilitation centers, Bartiméus (Amsterdam, the Netherlands) or Royal Dutch Visio (Amsterdam, the Netherlands), were invited to participate in this study. Inclusion criteria for this study were a clinical diagnosis of RP or CRD based on full-field electroretinography data, and a best-corrected visual acuity (BCVA) of 20/200 Snellen acuity or worse. An additional inclusion criterion for patients with RP was a constricted peripheral visual field on Goldmann kinetic perimetry (<20° around point of fixation using a V4e stimulus) at the most recent examination, whereas for patients with CRD, an absolute central scotoma with residual peripheral fields was present in all. Identification of a causative gene was not a requirement for this study. Exclusion criteria for this study included the presence of other ocular diseases, significant cognitive impairment, insufficient understanding of the Dutch language, and tremor-inducing conditions that could impede gesture recognition by the OrCam (e.g. Parkinson's disease). Ethical approval for this study was obtained from the Medical Ethics Committee at the Leiden University Medical Center. The study adhered to the tenets of the Declaration of Helsinki, and informed consent was signed by all participants.

OrCam study protocol

Questionnaires were administered in patients using a personal interview-format at initial visit and at follow-up (mean follow-up: 5.2 weeks ± standard deviation [SD] 1.5). Additionally, patients underwent visual acuity testing using a Snellen letter chart and received instructions on the OrCam at first visit. Both centers followed a similar OrCam instruction protocol, performed by experienced instructors, to ensure identical training between centers. Different models of the OrCam exist, which differ in price and their available features (https://www.orcam.com). For this study, the OrCam MyEye 2.0 was tested by all patients (Figure 1), and instructions were given on the following functions: text recognition, facial recognition, barcode recognition, object recognition, money recognition, color recognition, and telling time.[12]

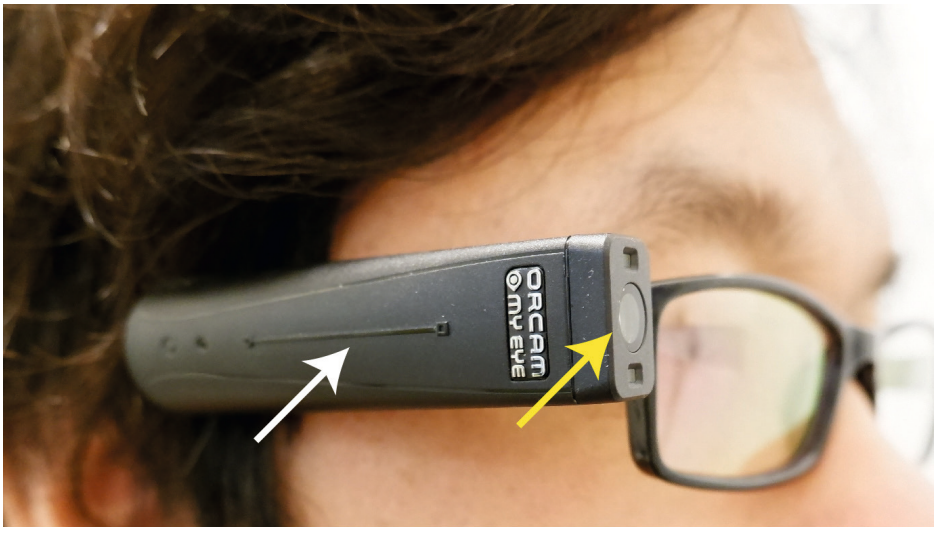

Figure 1. The OrCam MyEye 2.0 is a portable low vision aid that can be mounted to the arms of a pair of glasses. The processor unit has an internal speaker, charge port, power button, and a touch bar for activation and menu navigation (white arrow). Furthermore, the OrCam contains an optical sensor (yellow arrow), that returns scanned text or objects to auditive feedback via the internal speaker or through a Bluetooth connected earpiece. A mini flashlight is also present to aid in lower light situations. In addition to text-to-speech functions, the OrCam also contains color, (selective) barcodes, money, person and object recognition features. In order for person and object recognition features to function, it is required to scan the desired target in advance, subsequently storing this information in the internal memory of the OrCam. The OrCam is activated via the touch bar, or hands-free via automatic target recognition, or by performing gesturing motions (e.g. pointing at an target).

The OrCam's features are activated by pressing the touch bar located on the device itself; or hands-free via automatic target recognition, or by performing gesturing motions (e.g. pointing at an object for recognition features or flicking the wrist for time telling functions). After receiving detailed instructions, patients were lent the OrCam for personal use without any restrictions. Patients were called after approximately 1 week to assess whether they required changes in personal settings, or if any technical difficulties with the OrCam were encountered. At follow-up, patients returned the OrCam and the same questionnaires as at baseline were administered. Optionally, patients were able to share their overall experience with the OrCam using an open-ended question format. Remarks on the (dis)advantages of the OrCam that were mentioned by ≥25% of the cohort were included in the results.

Questionnaires

Three questionnaires were used in this study, which included the National Eye Institute Visual Function Questionnaire (NEI-VFQ), the Participation and Activity Inventory (PAI), and the OrCam Function Questionnaire (OFQ). Patients were instructed to answer all

questionnaires as if they were using their own LVAs, with the addition of the OrCam as a LVA at follow-up assessment.

The NEI-VFQ is a 25-item questionnaire with 14 supplemental items, and is one of the most common vision-related quality of life questionnaire used in ophthalmic research. The NEI-VFQ is designed to evaluate aspects of daily living, which can be categorized into 12 different subscales.[14] For our study, the driving subscale was omitted, as none of the patients were permitted to drive. Answers given by patients were subsequently recoded into a 100-point scale, where a higher score represents better (visual) functioning, as suggested by the original authors.[14] An overall composite score was calculated by averaging the scores of all subscales, while excluding the 'general health' subscale.

The PAI, formerly known as the Dutch Activity Inventory, is a validated questionnaire that is used in Dutch low vision rehabilitation centers to systematically assess the rehabilitation goals of patients.[15-18] The PAI is based on the Activity Inventory designed by Massof and colleagues,[19] which was modified in order to extend to the European population.[7, 15] For this study, a shortened version of the PAI was used, which included 11 rehabilitation goals related to central or peripheral vision (Supplemental Table 1).[7] Patients were instructed to rate each goal on 2 aspects: importance and difficulty. Importance is rated on a Likert scale ranging from 0 (not important) to 3 (very important), whereas the difficulty scale goes from 0 (not difficult) to 4 (impossible). Subsequently, a priority score is calculated as the product of importance and difficulty for each included goal. The maximum achievable priority score is 12, with a higher priority score signifying a greater rehabilitation need for this specific rehabilitation goal.

The OFQ is a non-validated questionnaire that was developed solely for this study. The questionnaire contained 14 items regarding vision-related daily activities. The OFQ uses a 5-level Likert scale, with possible difficulty scores being 1 (no difficulty), 2 (some difficulty), 3 (moderate difficulty), 4 (very difficult) or 5 (impossible due to disease). The activities included on the OFQ are as follows:

1. Reading a newspaper or book.
2. Reading for longer than 30 minutes without getting tired.
3. Reading an e-mail.
4. Reading text from a distant sign such as a street sign.
5. Reading handwritten text.
6. Identifying different money bills.
7. Recognizing colors on clothing pieces.
8. Recognizing familiar objects, such as your keys or phone, at home.
9. Recognizing a familiar product in the grocery store.

10. Finding your way in the grocery store.
11. Reading a product label.
12. Recognizing familiar faces at home.
13. Recognizing familiar faces within an unfamiliar environment
14. Telling time

Rasch analysis

Rasch analysis was performed exploratively on the NEI-VFQ and OFQ using the Andrich rating scale model (Winsteps 4.6.0).[20-22] Rasch analysis converts ordinal scores into an interval scale, and provides patient's ability and item difficulty using logit values for the underlying construct. In our study, patients with higher (visual) ability and items of greater difficulty are placed more negatively of the logit scale, whereas more positive logit values reflect patients with lower (visual) ability and items with less difficulty. For NEI-VFQ, re-engineering of the questionnaire was guided by previous authors, who proposed a two subscale structure: visual functioning and socioemotional subscales (Supplemental Table 1).[20, 21] For the OFQ, 3 items were removed to fit Rasch analysis, demonstrating reliable person and item separation values (reliability >0.8), scale targeting (difference between mean item and person measures <1.0 logit) and unidimensionality (variance accounted by the principal component >60%) (Supplemental Table 1**)**. Changes in person measures after OrCam rehabilitation were assessed using a stacked analysis.[23]

Statistical analysis

Data were analyzed using the SPSS version 25.0 (IBM Corp, Armonk, NY). Visual acuity data were converted to Logarithm of the Minimum Angle of Resolution (logMAR) values. For hand movement vision, light perception vision and no light perception, logMAR values of 2.7, 2.8 and 2.9 were used, respectively.[24] BCVA in the better-seeing eye of included patients were categorized into two groups: severe visual impairment (SVI; 20/400 ≤ BCVA < 20/200) or blindness (BCVA < 20/400), based on criteria set by the World Health Organization.[25] As data were normally distributed, a paired 2-tailed *t*-test was used to determine significant changes in raw scores for each instrument. The effect of age, vision categories (SVI or blindness) and phenotypes (RP or CRD) on the likelihood of change were also investigated using a linear mixed model. A p-value of 0.05 or less was considered clinical significant, and correction for multiple testing using the Bonferroni method was applied where appropriate.

RESULTS

Clinical characteristics of the patients are presented in Table 1. Twenty patients with IRD were enrolled in the study, of which 9 patients were clinically diagnosed with RP (45%), and 11 patients with CRD (55%). Patients had an average BCVA of 1.5 logMAR (SD ± 0.4), which is equivalent to 20/640 Snellen visual acuity. Aside from visual

field patterns, there were no differences in clinical characteristics between the two phenotypes. All patients had previously undergone low vision rehabilitation, and the majority of patients (n = 19; 95%) included in this study were in possession of at least 1 LVA with text-to-speech capabilities (Table 1).

Table 1. Clinical characteristics and prescribed visual aids in patients of this cohort.

Variable	Total (n = 20)	Retinitis pigmentosa (n = 9)	Cone-rod dystrophies (n = 11)	p-value
Age in years (mean ± SD)	47.6 ± 16.3	51.3 ± 16.5	44.5 ± 16.2	0.366
Male (n, %)	12 (60%)	7 (78%)	5 (45%)	0.197
Disease duration in years (mean ± SD)*	30.8 ± 12.8	33.5 ± 13.6	28.6 ± 12.3	0.406
Follow-up in weeks (mean ± SD)	5.2 ± 1.5	5.0 ± 0.9	5.3 ± 1.9	0.634
logMAR BCVA (mean ± SD)	1.5 ± 0.4	1.5 ± 0.4	1.5 ± 0.5	0.881
Visual impairment (n, %)				
Severe impairment	9 (45%)	4 (44%)	5 (45%)	
Blindness	11 (55%)	5 (56%)	6 (55%)	0.999
Visual field pattern				
Central island	9 (45%)	9 (100%)	0 (0%)	
Central scotoma with peripheral remnants	11 (55%)	0 (0%)	11 (100%)	<0.001
Optical aids (n, %)				
Glasses	13 (65%)	6 (67%)	7 (64%)	
Telescopes	3 (15%)	1 (11%)	2 (18%)	
Hand or stand magnifiers	9 (45%)	3 (33%)	6 (55%)	
Non-optical aids (n, %)				
Filter glasses	11 (55%)	6 (67%)	5 (46%)	
Illumination control	8 (40%)	4 (44%)	4 (36%)	
Braille	6 (30%)	2 (22%)	4 (36%)	
White cane	13 (65%)	8 (89%)	5 (46%)	
Text-to-speech products (n, %)†				
Screen reading software	14 (70%)	7 (56%)	9 (82%)	
Daisy reader (physical or digital)	14 (70%)	7 (78%)	7 (64%)	
Text-to-speech mobile applications	16 (80%)	8 (89%)	8(73%)	

*P-values were derived from the independent t-test, χ2 test or Fisher's exact test. BCVA, best-corrected visual acuity; logMAR, Logarithm of the Minimum Angle of Resolution; SD, standard deviation. *Disease duration was defined as the difference between age at baseline and age at first symptom onset. † Text-to-speech products included software (e.g. JAWS, SuperNova, Window Eyes, VoiceOver), equipment, and mobile applications that convert digital or printed text to auditive feedback (e.g. Seeing AI or KNFB reader).*

National Eye Institute Visual Function Questionnaire

At initial visit, the NEI-VFQ showed a significantly lower score on the peripheral vision subscale in patients with RP compared to patients with CRD (p = 0.014). Other subscales on the NEI-VFQ were found to be comparable between subgroups, including the overall composite score (Supplemental Table 2**).** Rasch analysis revealed mean person measures of 0.53 (SD ± 0.64) and -0.18 (SD ± 0.59) logits for the visual functioning

and socio-emotional subscales, respectively. At follow-up, significant improvements were observed in the raw scores of the near activities' subscale (+23.5, 95% CI: 13.2 to 33.9; $p < 0.001$), which was not found for other subscales after correction for multiple testing (adjusted p-value = 0.004; Figure 2). The observed change was not affected by phenotype ($p = 0.798$), initial age ($p = 0.089$) or vision classification ($p = 0.317$). A significant change was also observed on the Rasch-calibrated visual functioning subscale, showing an improvement of -0.65 logits (95% CI: -0.97 to -0.32; $p = 0.001$) after OrCam use. No significant change was found in the socio-emotional subscale (-0.14, 95% CI: -0.40 to 0.11; $p = 0.257$) after rehabilitation.

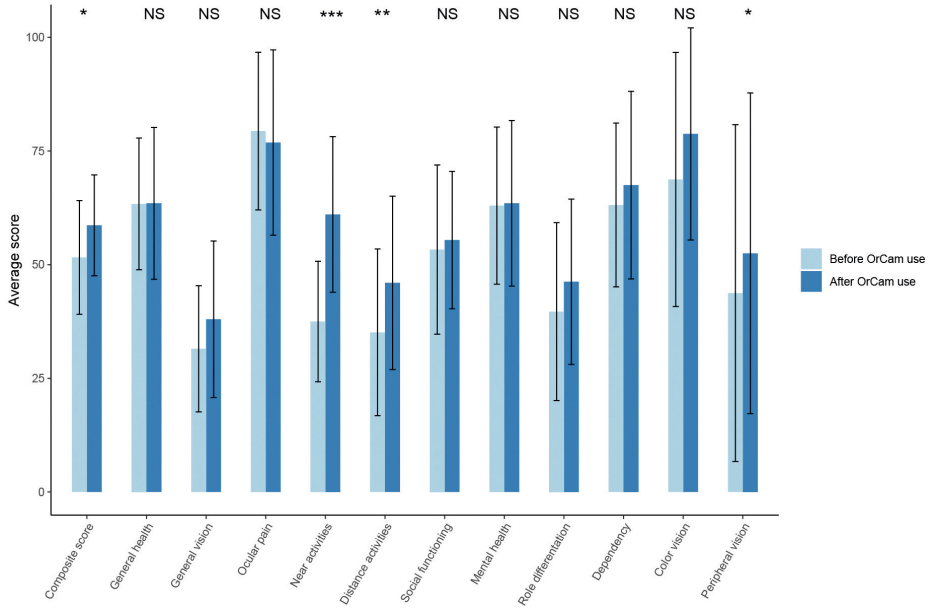

Figure 2. Average scores on the subscales of the National Eye Institute Visual Functioning pre- and post-rehabilitation with the OrCam. The bar heights represent the mean scores of each subscale, and the black error bars indicate the corresponding standard deviation. Higher scores indicate better functional performance. Critical value of significance was set at 0.004 following correction for multiple testing (0.05/11). NS, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Participation and Activity Inventory Questionnaire

A summary of the priority scores for each goal on the PAI is provided in Table 2. Goals with the highest priority scores, indicating goals with the highest rehabilitation needs, were 'mobility indoors within an unfamiliar environment' and 'personal administration' for patients with RP; whereas the highest priority scores were found in the 'reading' and 'personal administration' goals for patients with CRD (Table 2). While the order of priority for rehabilitation goals differed between phenotypes, there was no significant difference in average scores for each goal **(**Supplemental Table 2**)**. Bivariate analysis

revealed a correlation between the priority score of the 'mobility indoors within an unfamiliar environment' goal and age at initial visit (r = 0.570; p = 0.009), suggesting that the rehabilitation need for the 'mobility indoors within an unfamiliar environment' goal becomes greater with increasing age.

Out of the 11 rehabilitation goals included, 'reading' was the only goal that improved after rehabilitation with the OrCam, as shown as a lower priority score at follow-up (-2.6, 95% CI: -4.2 to -0.9; p = 0.005). When analyzing the underlying tasks of the 'reading' goal, a significant lower priority score was found for the task 'reading ordinary-sized print' (-3.9, 95% CI: -6.4 to 1.3; p = 0.005), which was not found for other tasks related to the 'reading' goal.

Table 2. Priority scores as measured on the Participation and Activity Inventory questionnaire in patients with retinitis pigmentosa and cone-rod dystrophies.

Retinitis pigmentosa		**Cone-rod dystrophies**	
Rehabilitation goal	Priority score	Rehabilitation goal	Priority score
Mobility indoors within an unfamiliar environment	6.6 ± 4.8	Reading	7.1 ± 3.4
Personal administration	6.0 ± 4.0	Personal administration	7.0 ± 3.7
Grocery shopping	5.4 ± 4.0	Grocery shopping	5.4 ± 4.2
Public transportation	5.2 ± 3.0	Mobility indoors within an unfamiliar environment	5.1 ± 2.5
Reading	5.2 ± 3.0	Computer use	4.5 ± 2.0
Writing	4.6 ± 5.1	Public transportation	4.2 ± 3.2
Mobility outdoors	4.4 ± 4.0	Mobility outside	4.0 ± 3.1
Computer use	3.7 ± 3.6	Writing	3.1 ± 2.5
Recognition and communication	2.3 ± 2.9	Mobility indoors at home	2.5 ± 2.9
Mobility indoors at home	1.7 ± 2.6	Keeping time and following a schedule	1.9 ± 2.8
Keeping time and following a schedule	0.7 ± 2.0	Recognition and communication	1.5 ± 2.1

Rehabilitation goals for patients with retinitis pigmentosa or cone-rod dystrophies are shown in descending order of priority. Priority scores are shown as means ± standard deviation. The maximum achievable priority score was 12, indicating a goal with the highest rehabilitation need.

OrCam Function Questionnaire

An item-person map based on Rasch analysis of the OFQ questionnaire is shown in Figure 3. Items on the OFQ that were considered most difficult for this cohort were: 'recognizing familiar faces within an unfamiliar environment' (-1.67 logits),' reading text from a distant sign' (-1.40 logits), and 'reading a product label' (-0.90 logit); whereas 'reading an e-mail' (1.18 logits) and 'recognizing familiar objects at home'(1.15 logits) were considered the least difficult tasks. The average person measure was 0.43 logits (SD ±

0.92), which improved significantly following OrCam rehabilitation (-1.11, 95% CI: -1.61 to 0.61; p < 0.001). The observed change did not differ between phenotypes (p = 0.696).

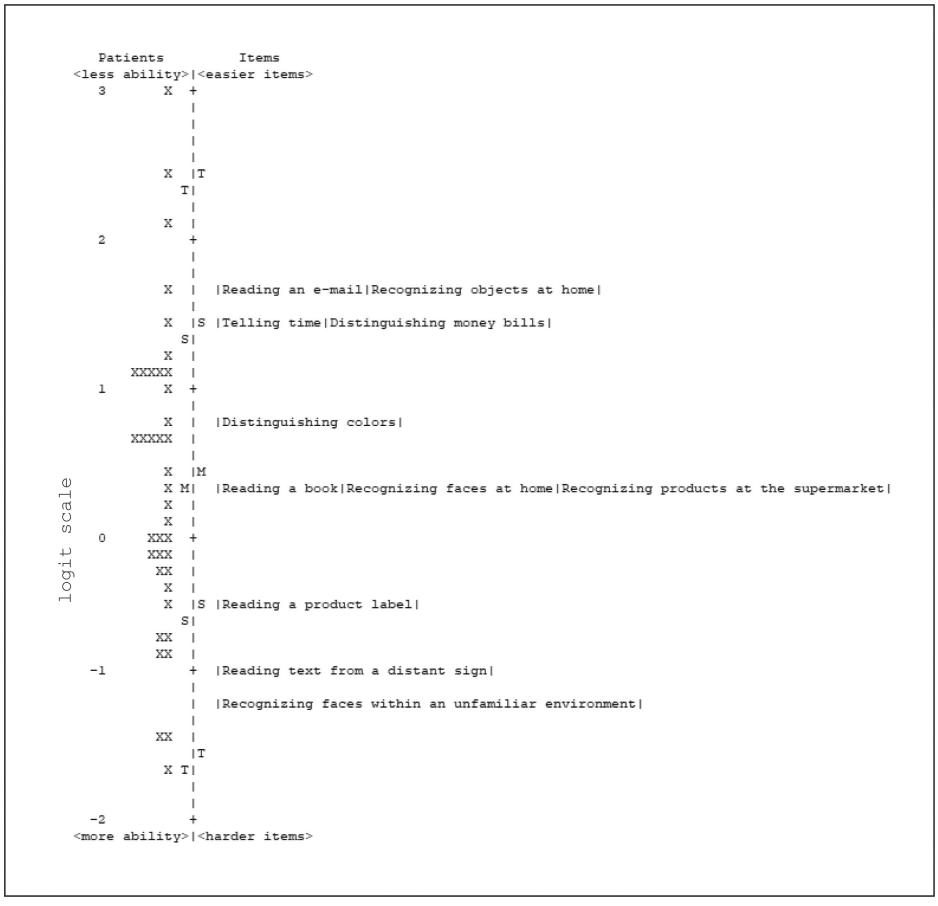

Figure 3. Stacked person-item map of the OFQ questionnaire. Patients are shown as crosses and are mapped across the vertical line based on their (visual) ability measured in logits. For context, a patient with high abilities (i.e. no difficulty in performing activities) would be placed at the bottom of the logit scale. Similarly, item are also mapped according to their measure in logits, with the hardest items placed at the bottom of the scale. M, mean; S, 1 standard deviation from the mean; T, 2 standard deviations from the mean.

Overall experience with the OrCam

At final visit, patients shared their overall experience with the OrCam. Fifteen patients (75%) reported that the OrCam's text recognition features functioned well in optimal light conditions. However, these features were less reliable in poorly lighted or dark rooms. Object and facial recognition features were not tested by most patients (n = 16; 80%), as patients reported that the current study period was too short to adequately

test these features, or they did not consider these features necessary for their daily activities. Main advantages and limitations of the OrCam MyEye 2.0 provided by this cohort are summarized in Table 3. After completion of this study, 2 patients with RP (10%; aged 24 and 60) and 1 patient with CRD (5%; aged 51) continued with rehabilitation with the OrCam. The remaining patients (n = 17; 85%) did not resume rehabilitation with the OrCam. Reasons for not continuing with the OrCam, that were mentioned by at least 5 patients, were: 1) having text-to-speech products with similar functions as the OrCam (e.g. Seeing AI or KNFB reader); 2) pricing of the OrCam; 3) and lack of features that were considered important to a patient (e.g. assistance with navigation). We found no significant differences in baseline age (p = 0.845), disease duration (p = 0.258), mean logMAR BCVA (p = 0.765), visual functioning subscale score on the NEI-VFQ (p = 0.616), 'reading' goal priority score on the PAI (p = 0.616), or person measure score on the OFQ (p = 0.546) between those who did and those who did not resume rehabilitation with the OrCam.

Table 3. Advantages and limitations of the OrCam reported by patients with retinitis pigmentosa or cone-rod dystrophies

Advantages	Limitations
(+) Text recognition in optimal light conditions	(-) Difficulties with text recognition in low light
(+) Portability	(-) Heavy and unbalanced on lightweight frames
(+) Hands-free	(-) Short battery life
(+) Color recognition	(-) No connectivity capabilities with your smartphone
(+) Barcode recognition	
(+) Bluetooth connectivity with earpieces	(-) Lack of desired features*

*Remarks that were mentioned by at least 5 patients are listed. *Example of features that were requested in this patient cohort included: assistance with navigation, voice activation, and internet connectivity.*

DISCUSSION

The objective of this study was to investigate whether the OrCam could assist in performing daily activities and subsequently improve the quality of life in patients with RP or CRD. As visual function gradually declines in patients with IRDs, so does their ability to perform daily activities, which, in turn, results in reduced vision-related quality of life.[26] As such, our cohort with severely visually impaired and blind patients with IRDs presented with markedly impaired of quality of life, as measured on the NEI-VFQ.

When assessing the priority scores on the PAI, we found that the highest scores were found in the 'mobility indoors within an unfamiliar location' rehabilitation goal for patients with RP, whereas 'reading' and 'personal administration' were the most important rehabilitation goals in patients with CRD. These findings coincide with the different visual abilities present in patients with RP and CRD, with patients with

RP most often facing challenges with mobility due to loss of peripheral vision, and patients with CRD experiencing difficulties with reading due to loss of central vision.[4, 6]

The Rasch-calibrated OFQ revealed that the most difficult tasks were 'reading a distant sign', 'reading a product label' and 'recognizing familiar faces within an unfamiliar environment', as they required the highest visual ability of patients. These tasks share a common theme in that they all involve visual search behavior, which is defined as the perceptual ability to actively scan the environment to locate the target of interest amongst other visual distractors.[27] Visual search requires input from central and peripheral vision, both of which are lost, to various degrees, in our patient cohort.[27, 28]

After rehabilitation with the OrCam, significant improvements were seen in the 'near activity' subscale of the NEI-VFQ. Similar results were found in a previous study with the OrCam, showing improvements in the 'near vision' subscale of the NEI-VFQ in patients with end-stage glaucoma.[13] As previous studies have demonstrated that the NEI-VFQ suffers from multidimensionality, we also obtained Rasch estimates from visual functioning and socio-emotional subscales.[20, 21] Using this method, we found significant improvements in the visual functioning subscale, but no improvements in the socio-emotional subscale at follow-up. Significant improvements were also observed in the 'reading' goal on the PAI and the person measure score on the OFQ. These findings altogether suggest that the OrCam primarily improves reading abilities in patients with RP or CRD. The improvements after OrCam usage did not differ between phenotypes, which may be due to our limited sample size, impeding more in-depth subgroup analysis. As suggested previously, it is possible that the level of visual acuity loss rather than visual field loss is important when selecting eligible patients for the OrCam.[13] Other features, such as facial and object recognition, were not tested by all patients during this relatively short follow-up, and the impact of these features on the quality of life in patients with IRDs remains uncertain. For these features, patients are required to store the person or object into the memory of the OrCam, a process that could take more than several minutes for the current version of the OrCam for each person or object, which is potentially exhaustive and time-consuming for severely visually impaired or blind patients over a study period of 5.2 weeks.

Most patients (85%) did not continue with rehabilitation, as they were in possession of other text-to-speech products, such as mobile applications with text recognition features (e.g. Seeing AI or the KNFB reader). These products share similar features with the OrCam, although, unlike the OrCam, most of these products often cannot be controlled hands-free or through gesturing motions. However, these products are typically less expensive compared to the OrCam MyEye 2.0, which is currently available for approximately €3,500 in Europe or $4,500 in the US. The higher costs of the OrCam may pose as an entry barrier for patients that wish to rehabilitate with the

device. In order for the OrCam to be serviceable to more patients with IRDs, further improvements in the OrCam are needed. Examples of improvements suggested by patients include: improved text-recognition in low light conditions, connectivity capabilities with a smartphone, and inclusion of additional features (e.g. navigation assistance), among others. Recently, a new version of the OrCam, the OrCam MyEye Pro, was released, which contains additional features such as smart reading and orientation features.

Several limitation and confounding factors were present in this study. This study included a relatively small sample size of patients with advanced stages of IRDs. Therefore, our findings may not be generalizable to other populations or to patients with higher visual abilities. Furthermore, this study only included one follow-up assessment, as not all rehabilitation goals were met with OrCam rehabilitation, and withholding patients from receiving adequate rehabilitation for all their rehabilitation needs would be considered unethical.[29] The possibility exists that patients overestimated or underestimated their functional changes with the OrCam, as they may not have accumulated enough real-life experiences with the device within our relatively short study period. Additionally, as patients were aware of being observed, the possibility of a more positive response to rehabilitation with the OrCam to appease clinical researchers, i.e. a Hawthorne effect, should not be disregarded.[8, 30] Future studies that include longer follow-up visits, different phenotypes, and a wider range of visual abilities would be invaluable to extend the current findings.

In conclusion, this study has provided a comprehensive overview of the OrCam MyEye 2.0 addressing both advantages and disadvantages of this device when prescribed to patients with RP or CRD. This knowledge may inform patients about the possibilities with the OrCam, while also setting realistic expectations, which, in turn, will facilitate the decision-making process regarding the OrCam. The OrCam is an useful LVA to improve reading abilities in patients with RP or CRD. Further improvements in the OrCam may enhance its utility in the rehabilitation process of patients with RP or CRD.

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REFERENCES

1. Cremers FPM, Boon CJF, Bujakowska K, Zeitz C. Special Issue Introduction: Inherited Retinal Disease: Novel Candidate Genes, Genotype-Phenotype Correlations, and Inheritance Models. *Genes (Basel)*. 2018;9(4):215. doi:10.3390/genes9040215
2. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. *The Lancet*. 2006/11/18/ 2006;368(9549):1795-1809. doi:10.1016/S0140-6736(06)69740-7
3. Ferrari S, Di Iorio E, Barbaro V, Ponzin D, Sorrentino FS, Parmeggiani F. Retinitis Pigmentosa: Genes and Disease Mechanisms. *Curr Genomics*. 02/14/received. 03/28/revised. 04/11/accepted 2011;12(4):238-249. doi:10.2174/138920211795860107
4. Hamel CP. Retinitis pigmentosa. *Orphanet J Rare Dis*. 2006;1:40-40. doi:10.1186/1750-1172-1-40
5. Thiadens AAHJ, Soerjoesing GG, Florijn RJ, et al. Clinical course of cone dystrophy caused by mutations in the RPGR gene. *Graefe's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie*. 2011;249(10):1527-1535. doi:10.1007/s00417-011-1789-3
6. Hamel CP. Cone rod dystrophies. *Orphanet J Rare Dis*. 2007;2:7-7. doi:10.1186/1750-1172-2-7
7. Latham K, Baranian M, Timmis MA, Pardhan S. Difficulties With Goals of the Dutch ICF Activity Inventory: Perceptions of Those With Retinitis Pigmentosa and of Those Who Support Them. *Invest Ophthalmol Vis Sci*. 2015;56(4):2381-2391. doi:10.1167/iovs.14-16237
8. Lamoureux EL, Pallant JF, Pesudovs K, Rees G, Hassell JB, Keeffe JE. The Effectiveness of Low-Vision Rehabilitation on Participation in Daily Living and Quality of Life. *Invest Ophthalmol Vis Sci*. 2007;48(4):1476-1482. doi:10.1167/iovs.06-0610
9. Langelaan M, de Boer MR, van Nispen RMA, Wouters B, Moll AC, van Rens GHMB. Change in quality of life after rehabilitation: prognostic factors for visually impaired adults. *International Journal of Rehabilitation Research*. 2009;32(1)
10. Lorenzini M-C, Wittich W. Factors related to the use of magnifying low vision aids: a scoping review. *Disability and Rehabilitation*. 2020/11/19 2020;42(24):3525-3537. doi:10.1080/09638288.2019.1593519
11. Das K, Gopalakrishnan S, Dalan D, Velu S, Ratra V, Ratra D. Factors influencing the choice of low-vision devices for visual rehabilitation in Stargardt disease. https://doi.org/10.1111/cxo.12867. *Clinical and Experimental Optometry*. 2019/07/01 2019;102(4):426-433. doi:https://doi.org/10.1111/cxo.12867
12. Moisseiev E, Mannis MJ. Evaluation of a Portable Artificial Vision Device Among Patients With Low Vision. *JAMA Ophthalmology*. 2016;134(7):748-752. doi:10.1001/jamaophthalmol.2016.1000
13. Waisbourd M, Ahmed OM, Newman J, et al. The Effect of an Innovative Vision Simulator (OrCam) on Quality of Life in Patients with Glaucoma. *Journal of Visual Impairment & Blindness*. 2019/07/01 2019;113(4):332-340. doi:10.1177/0145482X19869797
14. Mangione CM, Lee PP, Gutierrez PR, et al. Development of the 25-list-item National Eye Institute Visual Function Questionnaire. *Archives of Ophthalmology*. 2001;119(7):1050-1058. doi:10.1001/archopht.119.7.1050
15. Bruijning J, van Nispen R, Verstraten P, van Rens G. A Dutch ICF Version of the Activity Inventory: Results from Focus Groups with Visually Impaired Persons and Experts. *Ophthalmic Epidemiology*. 2010/12/01 2010;17(6):366-377. doi:10.3109/09286586.2010.528133
16. Bruijning JE, van Nispen RMA, van Rens GHMB. Feasibility of the Dutch ICF Activity Inventory: a pilot study. *BMC Health Services Research*. 2010/11/26 2010;10(1):318. doi:10.1186/1472-6963-10-318

17. Macnaughton J, Latham K, Vianya-Estopa M. Rehabilitation needs and activity limitations of adults with a visual impairment entering a low vision rehabilitation service in England. https://doi.org/10.1111/opo.12606. *Ophthalmic and Physiological Optics*. 2019/03/01 2019;39(2):113-126. doi:https://doi.org/10.1111/opo.12606

18. Elsman EBM, van Rens GHMB, van Nispen RMA. Psychometric properties of a new intake questionnaire for visually impaired young adults: The Participation and Activity Inventory for Young Adults (PAI-YA). *PLoS One*. 2018;13(8):e0201701. doi:10.1371/journal.pone.0201701

19. Massof RW, Ahmadian L, Grover LL, et al. The Activity Inventory: an adaptive visual function questionnaire. *Optometry and vision science : official publication of the American Academy of Optometry*. 2007;84(8):763-774. doi:10.1097/OPX.0b013e3181339efd

20. Stelmack JA, Stelmack TR, Massof RW. Measuring Low-Vision Rehabilitation Outcomes with the NEI VFQ-25. *Invest Ophthalmol Vis Sci*. 2002;43(9):2859-2868.

21. Pesudovs K, Gothwal VK, Wright T, Lamoureux EL. Remediating serious flaws in the National Eye Institute Visual Function Questionnaire. *Journal of Cataract & Refractive Surgery*. 2010;36(5)

22. Massof RW, Fletcher DC. Evaluation of the NEI visual functioning questionnaire as an interval measure of visual ability in low vision. *Vision Research*. 2001/02/01/ 2001;41(3):397-413. doi:https://doi.org/10.1016/S0042-6989(00)00249-2

23. Anselmi P, Vidotto G, Bettinardi O, Bertolotti G. Measurement of change in health status with Rasch models. *Health and Quality of Life Outcomes*. 2015/02/07 2015;13(1):16. doi:10.1186/s12955-014-0197-x

24. Talib M, van Schooneveld MJ, van Genderen MM, et al. Genotypic and phenotypic characteristics of *CRB1*-associated retinal dystrophies. *Ophthalmology*. 2017;124(6):884-895. doi:10.1016/j.ophtha.2017.01.047

25. World Health Organization. International Classification of Diseases 11th Revision. Accessed December 25th, 2020, https://icd.who.int/en

26. Chaumet-Riffaud AE, Chaumet-Riffaud P, Cariou A, et al. Impact of Retinitis Pigmentosa on Quality of Life, Mental Health, and Employment Among Young Adults. *American Journal of Ophthalmology*. 2017/05/01/ 2017;177:169-174. doi:https://doi.org/10.1016/j.ajo.2017.02.016

27. Timmis MA, Allsop J, Baranian M, et al. Visual Search Behavior in Individuals With Retinitis Pigmentosa During Level Walking and Obstacle Crossing. *Invest Ophthalmol Vis Sci*. 2017;58(11):4737-4746. doi:10.1167/iovs.17-21573

28. Sullivan B, Jovancevic-Misic J, Hayhoe M, Sterns G. Use of multiple preferred retinal loci in Stargardt's disease during natural tasks: a case study. *Ophthalmic Physiol Opt*. 2008;28(2):168-177. doi:10.1111/j.1475-1313.2008.00546.x

29. Wang BZ, Pesudovs K, Keane MC, Daly A, Chen CS. Evaluating the Effectiveness of Multidisciplinary Low-Vision Rehabilitation. *Optometry and Vision Science*. 2012;89(9)

30. McCambridge J, Witton J, Elbourne DR. Systematic review of the Hawthorne effect: new concepts are needed to study research participation effects. *J Clin Epidemiol*. 2014;67(3):267-277. doi:10.1016/j.jclinepi.2013.08.015

SUPPLEMENTAL CONTENT

Supplemental Table 1. Questionnaires used in this study and their included items.

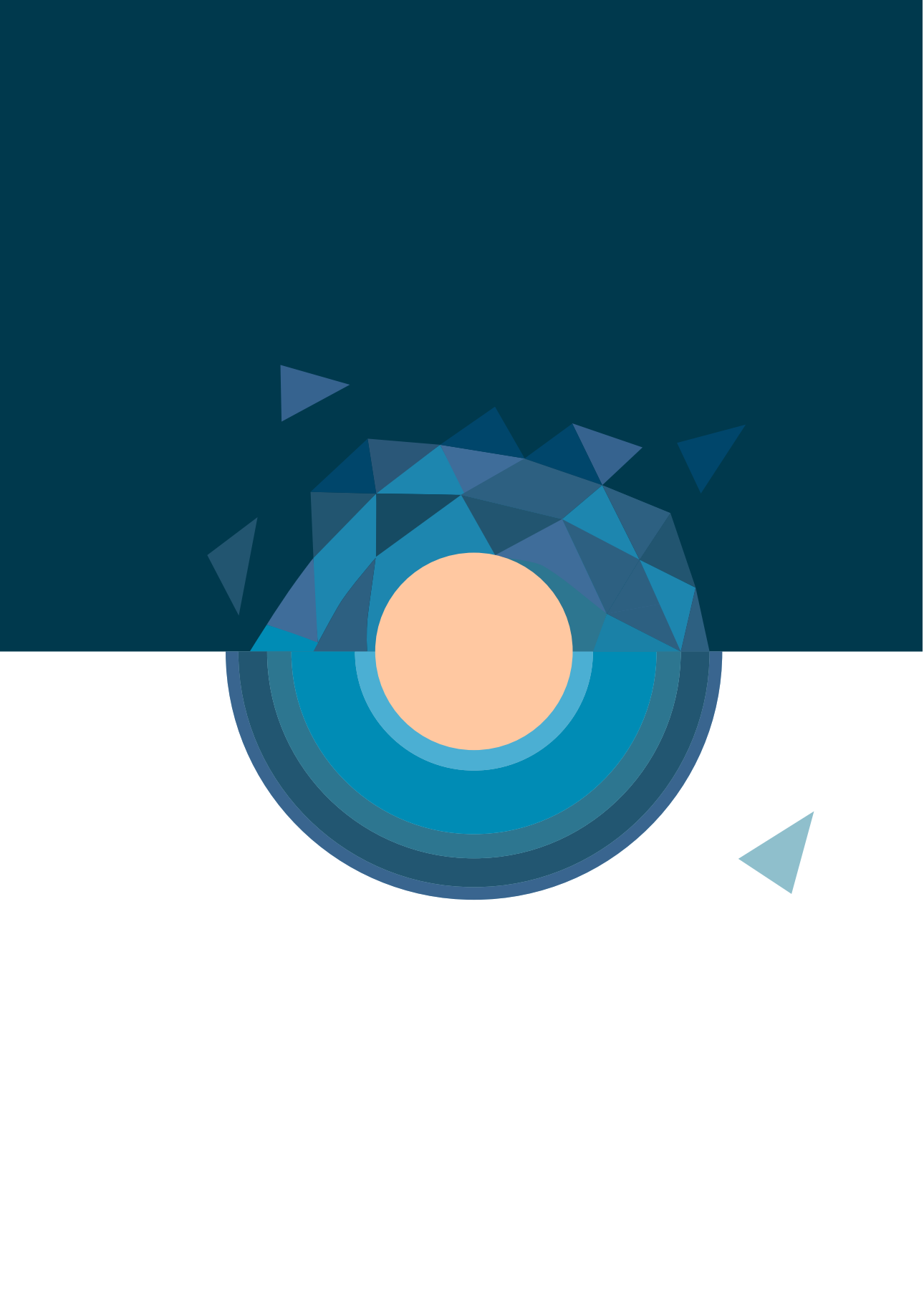
NEI-VFQ visual functioning subscale (original item #)*	NEI-VFQ socio-emotional subscale (original item #)*	Participation and Activity Inventory	OrCam Function Questionnaire (original item #)*
Eyesight (2)	Seeing how people react (11)	Reading	Reading a page from a book (1)
Reading ordinary print in newspapers (5)	Visiting people at their home, parties or restaurants (13)	Writing	Reading an e-mail (3)
Seeing well up close (6)	Accomplishing less (17)	Personal administration	Reading text from a distant sign, such as a street sign (4)
Finding something on a crowded shelf (7)	Staying at home most of the time (20)	Keeping time and following a schedule	Distinguishing different monetary bills (6)
Reading street signs or names of stores (8)	Having much less control (22)	Computer use	Distinguishing colors on a clothing piece (7)
Going down steps, stairs, or subs in dim light or at night (9)	Relying too much on what other people tell (23)	Mobility indoors at home	Recognizing objects, such as your keys or phone, at home (8)
Noticing objects off to the side while you are walking along (10)	Needing a lot of help from others (24)	Mobility indoors within an unfamiliar location	Recognizing a familiar product in the grocery store (9)
Picking out and matching your own clothes (12)	Worrying about doing something embarrassing (25)	Mobility outside	Reading a product label (11)
Going out to see movies, plays or sport events (14)	Receiving more help from others (A11a)	Public transportation	Recognizing familiar faces at home (12)
Read small print in a telephone book or medicine bottle (A3)	Being limited in things to do (A11b)	Grocery shopping	Recognizing familiar faces outdoors (13)
Checking accuracy of bills (A4)	Not leaving home alone (A13)	Recognition and communication	Telling time (14)
Shaving, styling and putting on make-up (A5)			
Recognizing people across the room (A6)			
Take part in sports or outdoor activities (A7)			
See and enjoy programs on TV (A8)			

*All questionnaires were administered in Dutch. Patients were instructed to answer all questions as if they were using all of their relevant visual aids. NEI-VFQ-25, National Eye Institute Visual Function Questionnaire-25. *These questionnaires were administered in full, but were subsequently re-engineered to fit Rasch analysis. Re-engineering of the NEI-VFQ was guided by previous studies (Stelmack et al. 2002; Pesudovs et al. 2010).*

Supplemental Table 2. Baseline scores of the NEI-VFQ, PAI and OFQ questionnaires.

	Mean score ± SD			
	Study group (n = 20)	**Retinitis pigmentosa (n = 9)**	**Cone-rod dystrophies (n = 11)**	**p-value**
NEI-VFQ subscale scores (max score = 100; higher score = better functioning)				
General health	63.4 ± 14.5	64.7 ± 11.3	62.3 ± 17.2	0.718
General vision	31.5 ± 13.9	28.3 ± 13.2	34.1 ± 14.5	0.370
Ocular pain	79.4 ± 17.3	84.7 ± 18.5	75.0 ± 15.8	0.221
Near activities	37.5 ± 13.2	36.6 ± 17.7	38.3 ± 22.0	0.786
Distance activities	35.1 ± 18.3	38.0 ± 22.0	37.8 ± 15.4	0.543
Social functioning	53.3 ± 18.6	48.1 ± 23.5	57.6 ± 13.2	0.271
Mental health	63.0 ± 17.3	61.1 ± 18.7	65.5 ± 18.7	0.670
Role limitation	39.7 ± 19.6	37.5 ± 20.7	41.5 ± 19.4	0.674
Dependency	63.1 ± 18.0	66.7 ± 22.8	60.2 ± 13.5	0.441
Color vision	68.8 ± 28.0	69.4 ± 32.5	68.2 ± 25.2	0.923
Peripheral vision	43.8 ± 37.1	22.2 ± 23.2	61.4 ± 37.7	0.014
Composite score	51.6 ± 12.5	49.3 ± 13.9	53.5 ± 11.5	0.465
PAI priority scores (max score = 12; higher score = higher rehabilitation need)				
Reading	6.3 ± 3.3	5.2 ± 3.0	7.1 ± 3.4	0.213
Writing	3.7 ± 4.0	4.6 ± 5.1	3.1 ± 2.5	0.430
Personal administration	6.6 ± 3.9	6.0 ± 4.0	7.0 ± 3.7	0.571
Keeping time and following a schedule	1.4 ± 2.5	0.7 ± 2.0	1.9 ± 2.8	0.276
Computer use	4.2 ± 2.9	3.7 ± 3.6	4.5 ± 2.0	0.544
Mobility indoors at home	2.1 ± 2.8	1.7 ± 2.6	2.5 ± 2.9	0.541
Mobility indoors within an unfamiliar location	5.8 ± 3.7	6.6 ± 4.8	5.1 ± 2.5	0.883
Mobility outside	4.2 ± 3.4	4.4 ± 4.0	4.0 ± 3.1	0.783
Public transportation	4.7 ± 3.1	5.2 ± 3.0	4.2 ± 3.2	0.488
Grocery shopping	5.9 ± 4.2	5.4 ± 4.0	5.4 ± 4.2	0.967
Recognition and communication	1.9 ± 2.5	2.3 ± 2.9	1.5 ± 2.1	0.483
OFQ difficulty scores (max score = 5; higher scores = more difficulty)				
Reading a page from a book	3.5 ± 1.5	3.0 ± 1.6	4.0 ± 1.3	0.143
Reading an e-mail	2.0 ± 1.5	1.3 ± 0.7	2.5 ± 1.7	0.061
Reading text from a distant sign, such as a street sign	4.5 ± 0.8	4.4 ± 0.7	4.5 ± 0.8	0.977
Distinguishing different monetary bills	2.4 ± 1.2	2.6 ± 1.1	2.3 ± 1.3	0.622
Distinguishing colors on a clothing piece	3.2 ± 1.4	3.4 ± 1.4	2.9 ± 1.4	0.405
Recognizing objects at home	2.0 ± 1.0	1.9 ± 1.2	2.1 ± 0.9	0.673
Recognizing products in the grocery store	3.7 ± 0.9	3.9 ± 0.9	3.5 ± 0.9	0.423
Reading a product label	4.5 ± 0.5	4.4 ± 0.5	4.6 ± 0.5	0.418
Recognizing familiar faces at home	3.6 ± 1.5	3.4 ± 1.5	3.6 ± 1.5	0.780
Recognizing familiar faces within an unfamiliar environment	4.6 ± 0.8	4.7 ± 0.7	4.5 ± 0.7	0.731
Telling time	2.7 ± 1.5	2.3 ± 1.3	3.0 ± 1.6	0.333

NEI-VFQ-25, National Eye Institute Visual Function Questionnaire-25; OFQ, OrCam Function Questionnaire; PAI, Participation and Activity Inventory Questionnaire; SD, standard deviation.



CHAPTER 3.3

QUALITY OF LIFE IN PATIENTS WITH *CRB1*-ASSOCIATED RETINAL DYSTROPHIES: A LONGITUDINAL STUDY

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ABSTRACT

Purpose

To assess the longitudinal vision-related quality of life among patients with *CRB1*-associated inherited retinal dystrophies.

Methods

A longitudinal questionnaire study included 22 patients with pathogenic *CRB1* variants. The National Eye Institute Visual Function Questionnaire (39 items, NEI VFQ-39) was applied at baseline, two-year follow-up, and 4-year follow-up. Classical test theory was performed to obtain subdomain scores and in particular 'near activities' and 'total composite' scores. The Rasch analysis based on previous calibrations of the NEI VFQ-25 was applied to create visual functioning and socio-emotional subscales.

Results

In total, 22 patients with pathogenic *CRB1* variants were included, with a median age of 25.0 years (IQR: 13–31 years) at baseline and mean follow-up of 4.0 ± 0.3 years. A significant decline at 4 years was observed for 'near activities' (51.0 ± 23.8 vs 35.4 ± 14.7, p = 0.004) and 'total composite' (63.0 ± 13.1 vs 52.0 ± 12.1, p = 0.001) subdomain scores. For the Rasch-scaled scores, the 'visual functioning' scale significantly decreased after 2 years (−0.89 logits; p = 0.012), but not at 4-year follow-up (+0.01 logits; p = 0.975). The 'socio-emotional' scale also showed a significant decline after 2 years (−0.78 logits, p = 0.033) and 4 years (−0.83 logits, p = 0.021).

Conclusion

In the absence of an intervention, a decline in vision-related quality of life is present in patients with pathogenic *CRB1* variants at 4-year follow-up. Patient-reported outcome measures should be included in future clinical trials, as they can be a potential indicator of disease progression and treatment efficacy.

INTRODUCTION

Inherited retinal dystrophies (IRDs) are a spectrum of hereditary degenerations of the retina which can result in photoreceptor loss. Leber congenital amaurosis (LCA) and retinitis pigmentosa (RP) are among the more severe phenotypes within IRDs. LCA usually presents within the first year of life with nystagmus, severe visual impairment, and (nearly) non-detectable responses on electroretinography. RP is caused by a degeneration of photoreceptors, initially rod photoreceptors followed by cone photoreceptors. Accordingly, patients suffer from reduced night vision and progressive loss of the peripheral visual field. As cone photoreceptors deteriorate and macular function is lost in later stages, patients notice a loss of visual acuity, limited contrast sensitivity, and loss of colour discrimination. Both LCA and RP patients are significantly affected by their IRD in daily life and substantially limited in their everyday activities.

LCA and RP can both be caused by relatively common pathogenic variants in the crumbs cell polarity complex component 1 (*CRB1*) gene. Studies have shown that patients with *CRB1*-associated IRD are likely to be classified as having low-vision by the median age of 18 years, and being blind by the median age of 40 years.[1, 2] With the current advances in gene augmentation research and the advent of gene therapy for LCA caused by mutations in *RPE65*,[3] treatment for *CRB1*-associated IRDs seems feasible. Experiments in mice and retinal organoids using adeno-associated viral vectors for *CRB1*-*CRB2* have shown encouraging results to facilitate upcoming gene therapy trials for *CRB1*-associated IRDs. Such trials require sensitive and clinically-relevant outcomes which have been identified using retrospective studies and a prospective natural history study. The latter has described the progression and course of *CRB1*-associated IRDs based on ophthalmological examinations including visual acuity, perimetry, microperimetry, full-field stimulus threshold (FST) testing, and multimodal imaging.[4, 5]

These clinical measurements are useful for detecting possible treatment effects, but do not reflect patient experiences in their daily life and activities. For instance, patients may have an improved visual acuity on psychophysical measurements without a meaningful treatment effect from a patient's perspective. Additionally, clinical measurements are often variable and fail to address daily obstacles associated with the retinal dystrophy.

Assessing patient context and quality of life is a critical part of treatment evaluation, but has been relatively scarcely addressed in therapeutic trials for IRDs to date. Without proper measurements for patient experiences, clinical trials may fail in capturing the most meaningful outcome. Patient-reported outcome measures (PROMs) are recognised and encouraged by the US Food and Drug Administration (FDA) as clinical trial outcome measures.[6, 7] An IRD PROM should ideally be based on patient input, and should be reliable, validated, and able to detect changes, before it can be used

for clinical trials. The National Eye Institute developed a 25-item questionnaire called the National Eye Institute Visual Function Questionnaire (NEI VFQ-25), which assesses difficulties with visual activities and condition-specific symptoms for a wide range of chronic eye diseases.[8] Fourteen additional items were added in an appendix to enhance the reliability of the various domains and resulted in the NEI VFQ-39.[9]

The NEI VFQ-25 and NEI VFQ-39 are well-established questionnaires that focus on different domains specific to a patient's day-to-day functioning and well-being.[8] Thus far, several clinical gene therapy trials have used the NEI VFQ-25 as PROM.[10-12] However, these questionnaires do not meet the requirements of the FDA for a PROM as they are not specifically developed for IRD patients. Moreover, the analysis of the NEI VFQs is flawed according to current methodology standards, as it is based on classical test theory which assumes equal difficulty per question.[13-15] Instead, expert consensus suggests using questionnaires based on item response theory, such as the Rasch analysis, which is based on the notion that some questions are more difficult than other questions and which enables estimates from ordinal responses on an invariant scale.[16] One method to maintain the use of the NEI VFQ is to apply the Rasch analysis to calibrate item measures to an invariant scale as was done by Goldstein *et al.* (2022).

The present study reports on the physical and social functioning and well-being of patients with a *CRB1*-associated IRD based on the NEI VFQ-39 over the course of 4 years in order to provide an insight into the quality of life using the pre-calibrated item measures of the NEI VFQ to meet current psychometric standards.

METHODS

Subjects

Participants were recruited from the RD5000 database, a Dutch registry of patients with IRDs,[17] and from the Delleman archive for inherited ophthalmic disorders at Amsterdam University Medical Centers. This study is part of a larger cohort study, which investigated the natural history of *CRB1*-associated IRDs.[4, 5] As such, inclusion criteria have been described earlier, but shortly include the following: (1) biallelic pathogenic variants in the *CRB1* gene, and (2) a Snellen best-corrected visual acuity (BCVA) of 1.3 logMAR (equivalent to 20/400) in the better-seeing eye.[4] Initially, 22 patients were included of which 10 patients originated from a Dutch genetic isolate that has been previously described.[18] This study adhered to the tenets of the Declaration of Helsinki and has obtained approval from the Erasmus Medical Center Medical Ethics Committee and from the Leiden University Medical Center Ethics Review Board. Informed consent was given by all patients, and obtained from their caretakers if applicable.

Clinical assessment

For this study we focused on BCVA, microperimetry, and the NEI VFQ-39.[4, 5] Refraction and BCVA were determined using the Early Treatment Diabetic Retinopathy Study (ETDRS) letter charts. BCVA was initially noted in decimal as is common in the Netherlands, and later converted to logMAR values. Microperimetry was used to determine mesopic macular sensitivity with the Macular Integrity Assessment System (MAIA, CenterVue) and a standard 37-stimuli grid. The follow-up function was used at every follow-up visit. Some patients were not able to complete all measurements due to young age. All patients were asked to complete the NEI VFQ-39 before each visit at baseline, at the two-year follow-up, and at the 4-year follow-up. During the second follow-up the questionnaire was not completed by two patients due to their young age and during the final follow-up, one questionnaire was not completed by one elderly patient due to the COVID-19 pandemic.

NEI VFQ-39 questionnaire

The NEI VFQ-39 is a 25-item questionnaire with 14 supplemental items and consists of the following subdomains: general health', 'general vision', 'ocular pain', 'near activities', 'distance activities', 'social functioning', 'mental health', 'role differentiation', 'dependency', 'colour vision', and 'peripheral vision'. Per classical test theory, composite scores for each domain of the NEI VFQ-39 were calculated as the sum of all items for each patient at each time point, resulting in separate scores per subdomain, and a total composite score which was calculated as an average of all subdomain scores (excluding 'general health'). A higher score reflects a better quality of life relating to that specific subdomain. The subdomain scores range from 0 to 100 units. Of specific interest were the subdomain 'near activities' and total composite score for the other analyses.

Modification and calibration of item measures of the NEI VFQ-25

The NEI VFQ-25 is a well-known and often-used questionnaire; however it also has inherent problems with multidimensionality, item fit validity, and differential item functioning.[13-15] To resolve these problems and to bypass Rasch analysis on this small test sample, Goldstein *et al.* suggested using modified versions and calibrated item measures.[19] In brief, the NEI VFQ-25 was modified to two separate questionnaires: NEI VFQ-VF and NEI VFQ-SE, focusing on visual function and on socio-emotional functioning respectively. A third modification led to the NEI VFQ-25C, which excludes general health and eyesight quality, and serves as an overall measure. Goldstein *et al.* combined the data of 3342 patients with retinal diseases (mostly age-related macular degeneration, diabetic retinopathy, and retinal vein occlusion) into a single dataset which was used for Rasch analysis and the method of successive dichotomisation to estimate person and item measures. For this study, the calibrated item measures based on the NEI VFQ-25 provided by Goldstein *et al.* were used to estimate person measures. ranging from −3 to +3, which translate to low and high perceived visual function, respectively. [19] There are currently no calibrated item measures for the NEI VFQ-39 available.

Statistical analysis

Data were analysed in SPSS (version 25.0.0; IBM Corp) and in R software using the R package 'msd'. Normal-distributed data are presented as mean ± standard deviation (SD), and non-normal distributed data are presented as median and interquartile range (IQR). Changes in scores were assessed using a linear mixed model. *p*-Values of ≤0.05 were considered statistically significant, unless *p*-values were corrected for multiple testing.

RESULTS

Baseline measurements

We identified 22 patients with a *CRB1*-associated IRD. Most patients (n = 20; 90%) had (early-onset) RP, followed by cone-rod dystrophy (n = 1; 5%) and macular dystrophy (n = 1; 5%). Study participants ranged in age at first visit from 6 to 74 years, with a median age of 25.0 years (IQR: 13.0–31.0 years) (Table 1). At the time of the first visit, the estimated mean disease duration was 19.0 ± 10.6 years based on the age of the first symptoms. Median BCVA was relatively low with 1.0 logMAR (0.6–1.2 logMAR), equivalent to 20/200 Snellen. Retinal sensitivity on mesopic microperimetry was performed on 13 patients at baseline with median sensitivity of 6.9 dB (2.7–12.8 dB). At baseline measurement, the NEI VFQ-39 was not completed by six patients, due to a variety of reasons including young age (n = 3), incomplete questionnaire (n = 2), and the development of acute glaucoma after topical use of mydriatics (n = 1).

Table 1. Baseline characteristics of patients with *CRB1*-associated retinal dystrophies included in this study

Baseline characteristics	Number (%) or median (IQR)
Gender	
Male	13 (41%)
Female	9 (59%)
Age at first visit	25.0 (13.0 – 31.0 years)
Follow-up time	4.0 ± 0.3 years
IRD diagnosis	20 (90%)
(Early-onset) RP	1 (5%)
Cone-rod dystrophy	1 (5%)
Macular dystrophy	
Estimated duration of disease at time of first visit	19.0 ± 10.6 years
Part of genetic isolate	
Yes	10 (45%)
No	12 (55%)
logBCVA ODS	1.0 logMAR (0.6 – 1.2 logMAR)
Retinal sensitivity on microperimetry* (n= 13, 3 missing at baseline)	6.9 dB (2.7 – 12.8 dB)
IRD = inherited retinal dystrophy; RP = retinitis pigmentosa; logMAR = logarithm of the minimum angle of resolution. *n=13, 3 missing at baseline	

*IRD = inherited retinal dystrophy; RP = retinitis pigmentosa; logMAR = logarithm of the minimum angle of resolution. *n=13, 3 missing at baseline*

Follow-up after 2 and 4 years

Disease progression in patients was evaluated on a biennial basis up to 4 years (4.0 ± 0.3 years) with mean follow-up times of 2.0 ± 0.1 years and 2.0 ± 0.3 years between the first two and the last two visits, respectively. At 2 years, the median BCVA of both eyes at 0.9 logMAR (0.6–1.1 logMAR; i.e. 20/160 Snellen) did not significantly differ from baseline (p = 0.069), whereas at 4 years; median BCVA (1.1 logMAR; IQR: 0.8–1.3; i.e. 20/240 Snellen) significantly decreased compared to baseline (p = 0.003). Microperimetry was performed on 20 patients during the 2-year follow-up with two patients being too young to perform reliably. During the 4-year follow-up, microperimetry was performed on 19 patients with one patient being too young and two patients being unable to visit the hospital due to the COVID-19 pandemic. Median retinal sensitivity changed significantly from baseline over 2 and 4 years, from 6.9 dB (2.7–12.8 dB) to 4.3 dB (1.4–9.9 dB, p = 0.001) and 3.7 dB (0.0–7.9 dB, p = 0.004), respectively.

Composite scores NEI VFQ

Individual questions on the NEI VFQ were clustered into the following generally accepted domains: 'general health', 'general vision', 'ocular pain', 'near activities', 'distance activities', 'social functioning', 'mental health', 'role differentiation', 'dependency', 'colour vision', and 'peripheral vision'. Finally, a final score was produced by compressing all domain scores into one score 'total composite score' (Table S1). Overall, there was a downward trend in most domains from baseline until final visit, especially those that relate best to IRD symptoms, such as 'general vision', 'near and distance activities', and 'peripheral vision' (Figure 1). However, after correction for multiple testing, none of these downward trends was statistically significant. The other domains remained stable over 4 years.

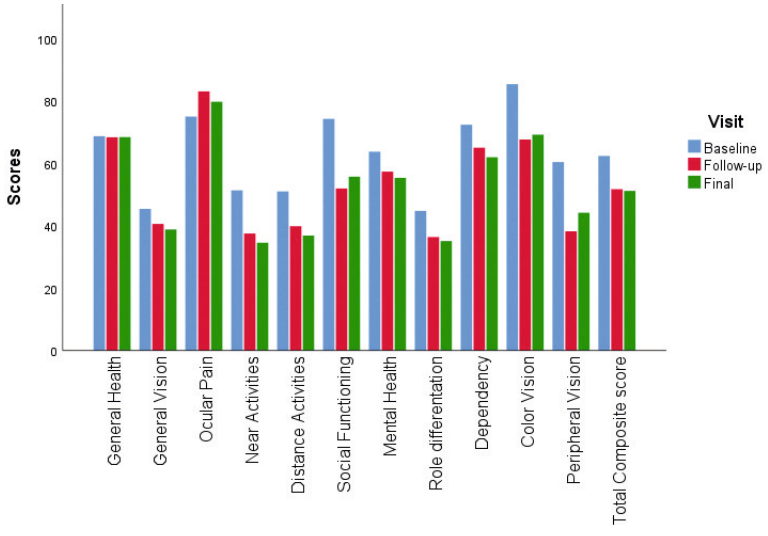

Figure 1. Bar chart demonstrating the different subdomains of the National Eye Institute Visual Function Questionnaire, and the mean scores given by patients with *CRB1*-associated retinal dystrophies at each visit.

Relationship between BCVA and microperimetry vs. scores for near activities (V_{NA}) and total score (V_{TOT})

We were interested in studying the relationship between the scores on the VFQ-39, for near activities (VNA) and total score (VTOT), and functional measurements of BCVA and macular sensitivity on microperimetry. Using a linear mixed model, we found significant positive relationships between BCVA and the composite score for 'near activities' (VNA), where every 0.002 increase in visual acuity ($p = 0.004$) and every 0.086 dB increase in microperimetry ($p = 0.002$) resulted in a unit increase in VNA (Figure 2a,b). In addition, with every 0.002 increase in visual acuity ($p = 0.008$) and every 0.095 dB on microperimetry ($p = 0.0003$), total composite score (VTOT) increases one unit (Figure 2c,d).

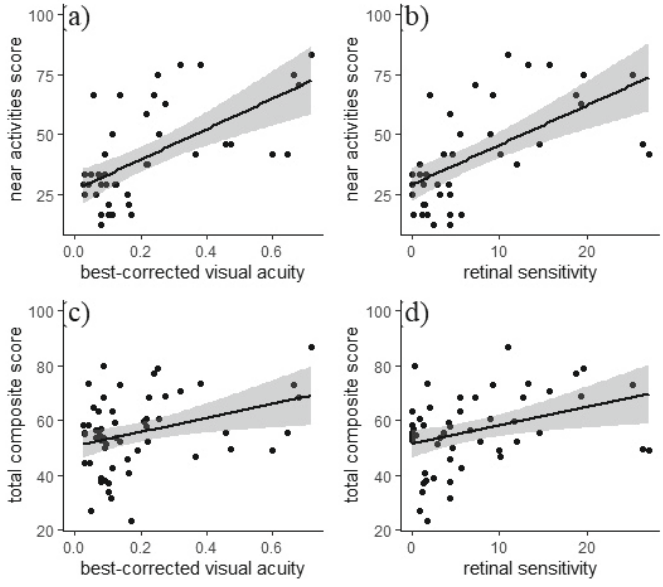

Figure 2. Significant positive relationships between BCVA, macular sensitivity and scores for 'Near Activities' and 'Total composite score' based on linear mixed model analysis. **A**) Significant relationship between BCVA and NEI VFQ-25 score for 'Near Activities' (V_{NA}). **B**) Significant relationship between retinal sensitivity as measured on microperimetry and V_{NA}. **C**) Significant relationship between BCVA and total composite score of NEI VFQ-25. **D**) Significant relationship between retinal sensitivity and total composite score of NEI VFQ-25. Significance is denoted as $p \leq 0.05$.

Inter-visit progression

Overall progression was determined by comparing baseline measurements with the measurements taken at the final visit, revealing a significant drop in score of 15 units in VNA ($p = 0.005$) and of 10 units in VTOT ($p = 0.001$). Interestingly, these declines appear to occur between the first and second visits, with VNA decreasing with 13 points after the first 2 years ($p = 0.008$) and VTOT with nine points ($p = 0.004$). There

was no significant decrease in VNA and VTOT between the 2-year follow-up and 4-year follow-up, p = 0.684 and p = 0.626, respectively.

MSD person measures

The calibrated item measures were developed specifically for the VFQ-25, and using these, we found a mean ± SD person measure of 0.428 ± 0.360 logit. The calculated person measures were plotted against their standard errors of the person measure (Figure S1). Extreme person measures, that is greater visual function, generally demonstrate lower precision, that is higher standard errors. Like the authors of the original paper, we also found a slight increase in standard error for higher person measures when using the calibrated item measures (Goldstein et al., 2022). Figure 3 plots the estimated person measures against the NEI VFQ-25 composite scores, demonstrating a near-linear relationship with a correlation of $R^2 = 0.905$. This indicates that the visual ability of a patient, as estimated with the person measures, is well-described by the composite scores of the NEI VFQ-25.

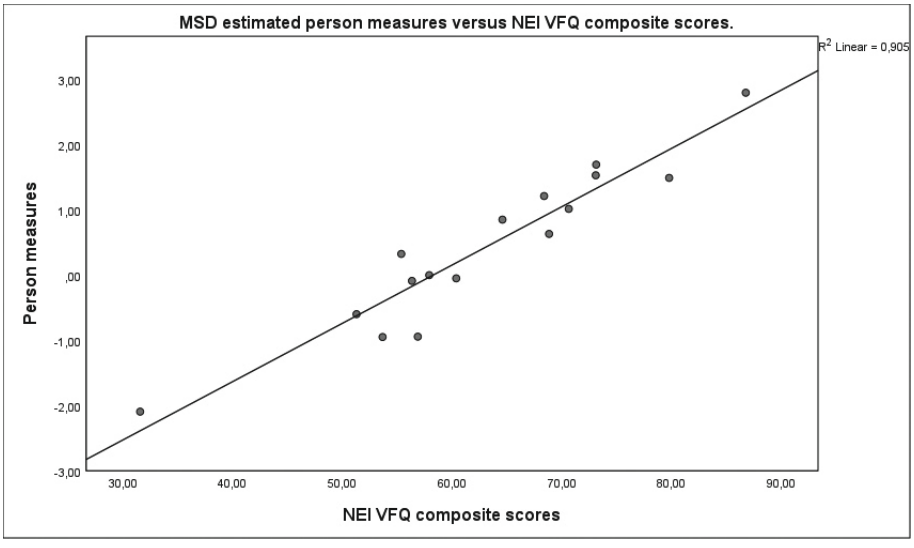

Figure 3. Near-linear relationship between estimated person measures and composite score of the NEI VFQ-25, R^2=0.905.

Quality of life based on visual function and socio-emotional impact

Goldstein et al. suggested domain-specific versions for the NEI VFQ-25 based on visual function, NEI VFQ-VF, and on socio-emotional impact, NEI VFQ-SE. We investigated the quality of life based on three modified versions of the NEI VFQ-25. Overall quality of life based on the VFQ-25C score declined significantly after two (–0.756; p = 0.006) and 4 years (–0.839; p = 0.002) after baseline. Visual function as measured with VFQ-VF was significantly decreased after 2 years (–0.889; p = 0.012), although this decrease did

not reach statistical significance over 4 years (0.010; p = 0.975). Scores on the socio-emotional domain as determined by the NEI VFQ-SE were also significantly lower after two (−0.776; p = 0.033) and 4 years (−0.831; p = 0.021).

DISCUSSION

In this study, we investigated the quality of life in patients with *CRB1*-associated retinal dystrophies based on the NEI VFQ-25 questionnaire and its appendix, and compared it to the functional measures BCVA and retinal sensitivity on microperimetry. Currently, many research groups are developing therapies for retinal dystrophies, including RP.[20, 21] Many of the functional outcome measures assessed in these studies may not reflect a patient's experience. Thus, PROMs are becoming increasingly important outcome measures. Being able to reliably assess key quality of life aspects in RP patients and correlating this knowledge to the disease course, may help in the design of future clinical trials.

Similar to previous RP studies, our patient cohort showed a significant general decline in quality of life over the course of 4 years.[22-24] Furthermore, we found a strong correlation between the deterioration of BCVA and macular sensitivity, and a decreasing total quality of life-score (V_{TOT}). More specifically, BCVA and macular sensitivity were found to be closely related to quality of life score for 'near activities' (V_{NA}). Interestingly, some earlier reports did not find a correlation between NEI VFQ-25 scores and visual acuity in RP, and visual field loss has been proposed as a better estimate of quality of life.[23, 24] Instead of visual field loss, we investigated macular sensitivity on microperimetry as an even more sensitive outcome measure in the present *CRB1*-IRD cohort. Here, we found another strong relationship between the deterioration of macular sensitivity and a decreasing quality of life based on V_{NA} and V_{TOT}.[5] This finding is corroborated by another study with 30 RP patients with a relatively high visual acuity of >0.2 logMAR (equivalent to 20/32 Snellen) who performed microperimetry on the Nidek MP1 microperimeter.[25] The NEI VFQ-39 scores deteriorated over time, but we found that the greatest significant decline occurred between baseline and second visit. The reason for the disparity in decline between the first 2 years and the second 2 years remains uncertain.

Although the NEI VFQ-39 has been shown as a marginally more informative questionnaire in IRD patients compared to the NEI VFQ-25, the latter has been a popular ophthalmic PROM since its first introduction in 2001 due to its simplicity and widespread use which facilitates international comparison.[8] However, to accommodate for its simplicity, the NEI VFQ-25 does not meet current psychometric standards as it lacks unidimensionality, has poor item fit validity, and has crude differential item functioning.[13-15] Rasch analysis has been proposed as a modern psychometric

technique to enable estimates on an invariant scale from ordinal responses. However, this form of item response theory can only be applied on sufficiently large patient cohorts. As IRDs are relatively rare and we have focused specifically on *CRB1*-associated IRDs, our cohort is too small for the Rasch analysis. So, we applied the Rasch-calibrated item measures provided by Goldstein *et al.* to analyse the quality of life in this cohort.

In addition to providing calibrated item measures, Goldstein *et al.* suggest adopting domain-specific versions to resolve the problem of multidimensionality of the NEI VFQ-25.[19] Two of these versions focus on visual function (VFQ-VF) and socio-emotional impact (VFQ-SE), and a third version excludes overall health and eyesight quality (VFQ-25C). Using these versions, we found that visual function as measured with the VFQ-VF decreased significantly after 2 years, but not after 4 years. Interestingly, these significant decreases did not follow the visual function parameters BCVA which decreased significantly after 4 years, but not after 2 years. This finding implies that patients are able to notice deterioration in experienced quality of life earlier, than BCVA as an objective visual function parameter can. Additionally, the socio-emotional impact of the *CRB1*-associated IRD was substantially higher after 2 years ($p = 0.033$) and after 4 years ($p = 0.021$) compared to the baseline visit. Likewise, macular sensitivity on microperimetry was significantly decreased after 2 and 4 years. Thus, macular sensitivity reflects visual function and socio-emotional impact as measured on the domain-specific VFQ-VF and VFQ-SE.

Our study has some limitations, mainly related to the content validity of the calibrated item measures and the NEI VFQ-25. The calibrated items and the resulting modified versions of the NEI VFQ-25 were estimated based on different patient populations with retinal disease diagnoses ranging from low-vision and age-related macular degeneration to retinal vein occlusion and diabetic retinopathy. As such, the calibrations may not be entirely applicable for inherited retinal dystrophies, and we suggest a calibration of item measures based on data sets of IRD patient cohorts. Moreover, the heterogeneity of our patient population, including adults and children, and IRD diagnoses of RP, cone-rod dystrophy, and macular dystrophy may hinder the generalisability of our findings, although the great majority of patients constituted of adult RP patients. As our patient cohort was too small for an individual Rasch analysis, the most suitable option for analysis was using the calibrated item measures. In doing so, we aim to provide some guidance in applying this method for future clinical IRD trials with relatively small patient cohorts. Regarding content validity of the questionnaire, the NEI VFQ-25 and the appendix focus on difficulties caused by general visual impairment, rather than consequences specifically caused by an IRD. Patients with a *CRB1*-associated IRD do not only experience progressive loss of visual acuity but also constriction of visual fields, nyctalopia, photophobia, and loss of colour and contrast discrimination.[2] These symptoms each result in a different set of obstacles which are currently not investigated in the NEI VFQ-25 or in the NEI VFQ-39. On the

other hand, ocular pain is a separate item on these questionnaires, but is not a regular symptom of an IRD and is thus not relevant to our study population.

At the time of assessment, the NEI VFQ-25 was widely used in ophthalmological clinical trials and accepted by the regulatory authorities as a means to evaluate vision-related quality of life. However, as the flaws of the NEI VFQ-25 questionnaire become more apparent and IRD research advances towards clinical applications, experts agree on the need for a specific IRD questionnaire that can evaluate meaningful changes for a patient.[26-28] A few PROMs have been developed for specific IRDs, such as Stargardt macular dystrophy and congenital stationary night blindness, but these tools have not been validated and are of limited use in larger IRD populations.[29, 30] IRD patients experience many similar symptoms; thus, a non-gene specific PROM may be useful for different IRD patient populations. Some questionnaires focus on a single domain, such as mobility for the Mobility Difficulties Questionnaire, Independent Mobility Questionnaire, or daily tasks for the Daily Task Performance Questionnaire and Everyday Task Questionnaire.[27] While informative, these single-domain questionnaires cannot be used to give an overall perspective of a patient's quality of life. Other IRD PRO instruments do not meet the current quality criteria or were not based on in-depth interviews with patients.[26, 27] A novel questionnaire, the Michigan Retinal Degeneration Questionnaire (MRDQ) has been developed and psychometrically validated by Lacy *et al.* that conforms to the FDA guidelines for the use in IRD clinical trials.[6] The MRDQ items were derived from both expert and patient interviews, and seven relevant domains were identified: central vision, colour vision, contrast sensitivity, scotopic function, photopic peripheral vision, mesopic peripheral vision, and photosensitivity. Item response theory techniques were applied for psychometric validation and test-retest variability was assessed. Since its introduction, the MRDQ has already been incorporated into several clinical trials for IRDs such as RP and LCA (NCT05203939, NCT05176717).

Investigating the vision-related quality of life in patients with gene-specific IRDs such as those caused by variants in the *CRB1* gene is important as it may form a key outcome measure for the upcoming gene therapy. This study is meaningful in that it investigated the use of calibrated item measures for the NEI VFQ-25 questionnaire in patients with IRDs and correlated these to visual function measures. The results from this study are useful for the design of future clinical trial designs for small IRD patient cohorts.

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REFERENCES

1. Talib M, Van Cauwenbergh C, De Zaeytijd J, et al. CRB1-associated retinal dystrophies in a Belgian cohort: genetic characteristics and long-term clinical follow-up. *Br J Ophthalmol*. Feb 12 2021;doi:10.1136/bjophthalmol-2020-316781
2. Talib M, van Schooneveld MJ, van Genderen MM, et al. Genotypic and Phenotypic Characteristics of CRB1-Associated Retinal Dystrophies: A Long-Term Follow-up Study. *Ophthalmology*. Jun 2017;124(6):884-895. doi:10.1016/j.ophtha.2017.01.047
3. Maguire AM, Bennett J, Aleman EM, Leroy BP, Aleman TS. Clinical Perspective: Treating RPE65-Associated Retinal Dystrophy. *Molecular Therapy*. 2021/02/03/ 2021;29(2):442-463. doi:https://doi.org/10.1016/j.ymthe.2020.11.029
4. Talib M, van Schooneveld MJ, Wijnholds J, et al. Defining inclusion criteria and endpoints for clinical trials: a prospective cross-sectional study in CRB1-associated retinal dystrophies. *Acta Ophthalmologica*. 2021;99(3):e402-e414. doi:https://doi.org/10.1111/aos.14597
5. Nguyen X-T-A, Talib M, van Schooneveld MJ, et al. CRB1-associated retinal dystrophies: a prospective natural history study in anticipation of future clinical trials. *American Journal of Ophthalmology*. 2021/07/25/ 2021;doi:https://doi.org/10.1016/j.ajo.2021.07.021
6. Guidance for industry: patient-reported outcome measures: use in medical product development to support labeling claims: draft guidance. *Health Qual Life Outcomes*. Oct 11 2006;4:79. doi:10.1186/1477-7525-4-79
7. Varma R, Richman EA, Ferris FL, III, Bressler NM. Use of Patient-Reported Outcomes in Medical Product Development: A Report from the 2009 NEI/FDA Clinical Trial Endpoints Symposium. *Invest Ophthalmol Vis Sci*. 2010;51(12):6095-6103. doi:10.1167/iovs.10-5627
8. Mangione CM, Lee PP, Gutierrez PR, Spritzer K, Berry S, Hays RD. Development of the 25-item National Eye Institute Visual Function Questionnaire. *Arch Ophthalmol*. Jul 2001;119(7):1050-8. doi:10.1001/archopht.119.7.1050
9. Clemons TE, Chew EY, Bressler SB, McBee W. National Eye Institute Visual Function Questionnaire in the Age-Related Eye Disease Study (AREDS): AREDS Report No. 10. *Arch Ophthalmol*. Feb 2003;121(2):211-7. doi:10.1001/archopht.121.2.211
10. Russell S, Bennett J, Wellman JA, et al. Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial. *Lancet*. Aug 26 2017;390(10097):849-860. doi:10.1016/s0140-6736(17)31868-8
11. Dimopoulos IS, Hoang SC, Radziwon A, et al. Two-Year Results After AAV2-Mediated Gene Therapy for Choroideremia: The Alberta Experience. *American Journal of Ophthalmology*. 2018/09/01/ 2018;193:130-142. doi:https://doi.org/10.1016/j.ajo.2018.06.011
12. Weleber RG, Pennesi ME, Wilson DJ, et al. Results at 2 Years after Gene Therapy for RPE65-Deficient Leber Congenital Amaurosis and Severe Early-Childhood-Onset Retinal Dystrophy. *Ophthalmology*. Jul 2016;123(7):1606-20. doi:10.1016/j.ophtha.2016.03.003
13. Khadka J, McAlinden C, Pesudovs K. Validation of the National Eye Institute Visual Function Questionnaire-25 (NEI VFQ-25) in Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci*. 2012;53(3):1276-1276. doi:10.1167/iovs.12-9541
14. Marella M, Pesudovs K, Keeffe JE, O'Connor PM, Rees G, Lamoureux EL. The Psychometric Validity of the NEI VFQ-25 for Use in a Low-Vision Population. *Invest Ophthalmol Vis Sci*. 2010;51(6):2878-2884. doi:10.1167/iovs.09-4494
15. Stelmack JA, Stelmack TR, Massof RW. Measuring low-vision rehabilitation outcomes with the NEI VFQ-25. *Invest Ophthalmol Vis Sci*. Sep 2002;43(9):2859-68.

16. Boone WJ. Rasch Analysis for Instrument Development: Why, When, and How? *CBE Life Sci Educ*. Winter 2016;15(4)doi:10.1187/cbe.16-04-0148

17. van Huet RAC, Oomen CJ, Plomp AS, et al. The RD5000 Database: Facilitating Clinical, Genetic, and Therapeutic Studies on Inherited Retinal Diseases. *Invest Ophthalmol Vis Sci*. 2014;55(11):7355-7360. doi:10.1167/iovs.14-15317

18. van den Born LI, van Soest S, van Schooneveld MJ, Riemslag FC, de Jong PT, Bleeker-Wagemakers EM. Autosomal recessive retinitis pigmentosa with preserved para-arteriolar retinal pigment epithelium. *Am J Ophthalmol*. Oct 15 1994;118(4):430-9. doi:10.1016/s0002-9394(14)75792-7

19. Goldstein JE, Bradley C, Gross AL, Jackson M, Bressler N, Massof RW. The NEI VFQ-25C: Calibrating Items in the National Eye Institute Visual Function Questionnaire-25 to Enable Comparison of Outcome Measures. *Transl Vis Sci Technol*. May 2 2022;11(5):10. doi:10.1167/tvst.11.5.10

20. Schneider N, Sundaresan Y, Gopalakrishnan P, et al. Inherited retinal diseases: Linking genes, disease-causing variants, and relevant therapeutic modalities. *Progress in Retinal and Eye Research*. 2022/07/01/ 2022;89:101029. doi:https://doi.org/10.1016/j.preteyeres.2021.101029

21. Botto C, Rucli M, Tekinsoy MD, Pulman J, Sahel J-A, Dalkara D. Early and late stage gene therapy interventions for inherited retinal degenerations. *Progress in Retinal and Eye Research*. 2022/01/01/ 2022;86:100975. doi:https://doi.org/10.1016/j.preteyeres.2021.100975

22. Levinson JD, Joseph E, Ward LA, et al. Physical Activity and Quality of Life in Retinitis Pigmentosa. *J Ophthalmol*. 2017;2017:6950642. doi:10.1155/2017/6950642

23. Altinbay D, Taskin I. Evaluation of vision-related quality of life in retinitis pigmentosa patients with low vision. *Jpn J Ophthalmol*. Nov 2021;65(6):777-785. doi:10.1007/s10384-021-00875-z

24. Sugawara T, Hagiwara A, Hiramatsu A, Ogata K, Mitamura Y, Yamamoto S. Relationship between peripheral visual field loss and vision-related quality of life in patients with retinitis pigmentosa. *Eye*. 2010/04/01 2010;24(4):535-539. doi:10.1038/eye.2009.176

25. Sugawara T, Sato E, Baba T, Hagiwara A, Tawada A, Yamamoto S. Relationship between vision-related quality of life and microperimetry-determined macular sensitivity in patients with retinitis pigmentosa. *Japanese Journal of Ophthalmology*. 2011/11/01 2011;55(6):643-646. doi:10.1007/s10384-011-0080-9

26. Lacy GD, Abalem MF, Musch DC, Jayasundera KT. Patient-reported outcome measures in inherited retinal degeneration gene therapy trials. *Ophthalmic Genet*. Feb 2020;41(1):1-6. doi:10.1080/13816810.2020.1731836

27. Prem Senthil M, Khadka J, Pesudovs K. Assessment of patient-reported outcomes in retinal diseases: a systematic review. *Survey of Ophthalmology*. 2017/07/01/ 2017;62(4):546-582. doi:https://doi.org/10.1016/j.survophthal.2016.12.011

28. Selvan K, Abalem MF, Lacy GD, Vincent A, Héon E. The State of Patient-Reported Outcome Measures for Pediatric Patients with Inherited Retinal Disease. *Ophthalmology and Therapy*. 2022/06/01 2022;11(3):1031-1046. doi:10.1007/s40123-022-00514-x

29. Bijveld MM, van Genderen MM, Hoeben FP, et al. Assessment of night vision problems in patients with congenital stationary night blindness. *PLoS One*. 2013;8(5):e62927. doi:10.1371/journal.pone.0062927

30. Miedziak AI, Perski T, Andrews PP, Donoso LA. Stargardt's macular dystrophy--a patient's perspective. *Optometry*. Mar 2000;71(3):165-76.

SUPPLEMENTAL CONTENT

Table S1. NEI VFQ-39 mean total and subscale scores at baseline.

NEI VFQ-39 score	Baseline (mean ± SD, n=16)	2-year follow-up (mean ± SD, n=19)	4-year follow-up (mean ± SD, n=21)
General health	69.4 ± 10.0	70.1 ± 14.1	67.5 ± 15.8
General vision	46.3 ± 15.2	41.6 ± 21.2	38.1 ± 16.9
Ocular pain	80.5 ± 18.2	84.9 ± 18.9	83.3 ± 21.8
Near activities	51.0 ± 23.8	37.5 ± 20.3	35.4 ± 14.7
Distance activities	51.6 ± 20.7	40.3 ± 17.1	40.0 ± 16.9
Social functioning	72.2 ± 19.1	53.7 ± 23.1	53.9 ± 20.9
Mental health	66.6 ± 19.2	57.2 ± 17.0	56.2 ± 17.2
Role difficulties	48.4 ± 19.6	36.2 ± 17.6	37.5 ± 13.4
Dependency		65.1 ± 14.3	58.0 ± 19.2
74.2 ± 16.0			
Colour vision	84.4 ± 22.1	67.1 ± 25.1	70.2 ± 26.9
Peripheral vision	53.1 ± 30.1	38.2 ± 30.5	40.5 ± 31.1
Total composite score	63.0 ± 13.0	52.3 ± 14.0	52.0 ± 12.1

SD = standard deviation; NEI-VFQ = National Eye Institute Visual Function Questionnaire. A higher score reflects a higher visual function.

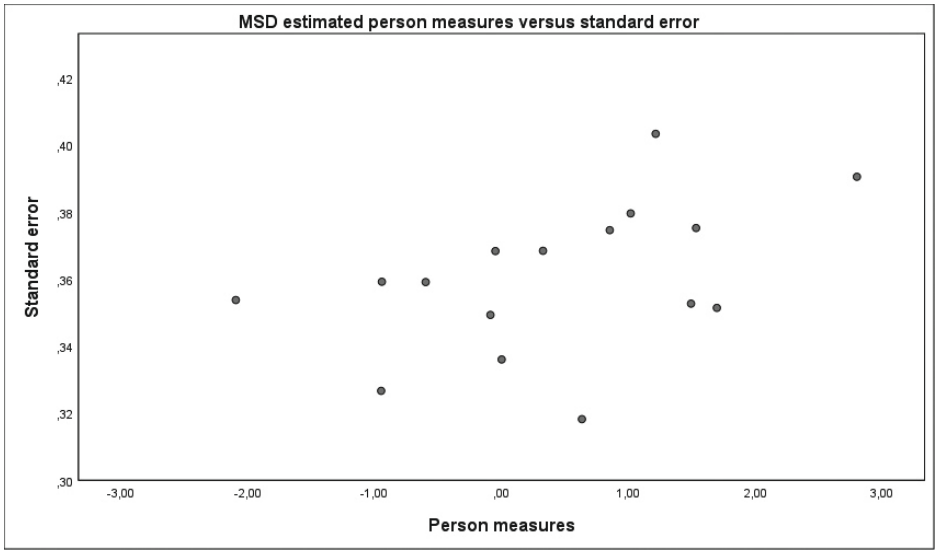
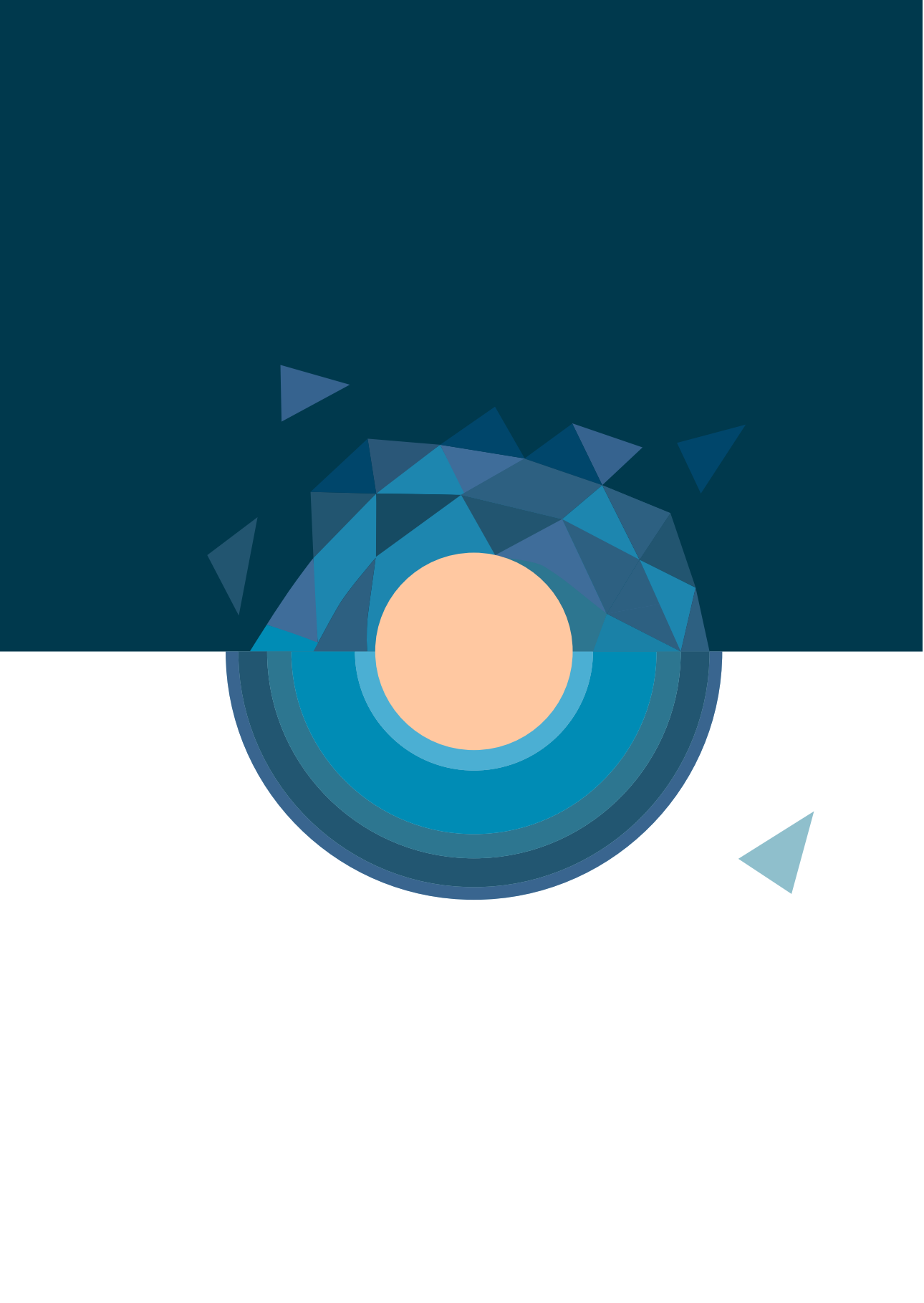


Figure S1. MSD estimated person measures vs standard error person measure at baseline.



CHAPTER 4

GENERAL DISCUSSION

Partly adapted from:

Retinitis Pigmentosa: Current Clinical Management and Emerging Therapies
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GENERAL DISCUSSION

In view of current and future clinical trials, this thesis aims to provide a better understanding of the clinical and genetic characteristics of several subtypes of RP. An in-depth knowledge on the natural history of RP is important for trials and often required by regulatory agencies to determine the appropriate patient sample size, and to determine sensitive endpoints. By comparing treated and non-treated eyes, treatment efficacy and safety may be evaluated. Moreover, surrogate endpoints or biomarkers may also be identified based on these natural history studies, which can accelerate the measurement of progression or treatment response.

In this thesis, we also explored the benefit of several treatment or supportive options that are currently available for RP. In this exciting era of emerging gene therapy, it remains imperative to continue supporting patients with RP using all available options to manage their condition. Patients with a progressive retinal disease such as RP may experience a wide variety of physical, mental and social-emotional challenges over time that need to be timely addressed if possible.

Below, the findings of our previous chapters are discussed, and we explore current management options and future perspectives.

CLINICAL CHARACTERISTICS AND NATURAL HISTORY STUDIES

The natural course of *RHO*-associated RP indicates the need for surrogate endpoints

The *RHO* gene was the first causal gene to be linked with RP.[1] *RHO* encodes the rod visual pigment rhodopsin that is located in the outer segments of rod photoreceptors. To date, over 150 variants have been found associated with *RHO*-associated RP, and this gene is responsible for over 30% of all cases of autosomal dominant RP. Variants in *RHO* are not only associated with generalized RP, but can also cause sectorial RP, and rarely congenital stationary night blindness.[2] The protein rhodopsin has been thoroughly investigated, and it has been highly suggested that variants in specific protein domains have distinct consequences on the protein's structure and function.[2]

In **Chapter 2.1**., the phenotype and genotype of patients with *RHO*-associated RP were characterized, based on one of the largest cohorts to date. Two separate phenotypes were present in the study: generalized RP and sectorial RP, the latter characterized by retinal degeneration confined to the inferior quadrant of the retina.[3] In patients with generalized RP, visual function was relatively preserved, as the first occurrences of low vision, several visual impairment and blindness based on BCVA or VF were observed from the 5th decade onward in patients with generalized RP. This was even later for

patients with sector RP, with the first instance of blindness found in the 8th decade of life. This is in line with previous studies, suggesting that *RHO* has slower disease course with a more favorable visual prognosis compared to other genetic subtypes of RP.

A slow disease course is also favorable with respect to therapies, as it means that there is a broader window for therapeutic intervention. Based on the occurrence of visual impairment in our study, we proposed that the optimal window for intervention in *RHO*-associated RP is before the 5th decade of life.

In the context of developing future therapies, the use of surrogate markers is an important factor to consider. Since *RHO*-associated RP is a disease that progresses slowly based on BCVA and VF, it would be challenging to demonstrate treatment efficacy using these conventional outcome measures. Clinical trials are typically conducted in a period between 2 to 4 years. Based on our studies, we suggest that the outer retinal thickness may be used as a surrogate outcome measure, because it highly correlated with visual acuity. Surrogate markers may facilitate the measurement of change within a shorter period of time, which can be particularly helpful for relatively slowly-progressing IRDs such as *RHO*-associated RP.

***RPGR*-associated retinal dystrophies: clinical and histopathological features**

Variants in *RPGR* are the major genetic cause of XLRP (70-90%), and XLRP is considered one of the most severe forms of RP. While young males are typically affected, clinical symptoms and fundus findings may also be present in female carriers.[4] Both RP and CRD phenotypes have been described, with the latter typically found in the mutational hotspot ORF15.

In **Chapter 2.2**, we report the early onset of symptoms in patients with *RPGR*-associated RP, and a much later symptom onset in patients with CRD. Multimodal imaging revealed a hyper-AF ring, which correlates with the presence of the EZ band on OCT, suggesting that this demarcates the transition between healthy and degenerated retina. An important criterion for gene therapy is the preservation of intact photoreceptors in order to provide successful gene delivery. We measured the retinal sensitivity using microperimetry, and showed that despite macular atrophy, some residual sensitivity (i.e. remaining photoreceptors) may remain which can be targeted for therapy. However, we also noticed a limitation of microperimetry: greater variation in microperimetry results is found in patients with end-stage disease, which decreases the reliability of this testing method. Another psychophysical test is FST, which measures the sensitivity of the entire retina, and can be performed in all patients regardless of fixation capabilities, and even in those without measurable photoreceptor function on full-field ERG. A limitation of the FST is the inability to provide spatial information, as it can only give an indication of total photoreceptor function.

Our data supports the use of microperimetry and FST in clinical trials, as they provide complementary information on the residual visual function. This allows for the possible inclusion of patients in advanced stages of RP that were previously excluded based on non-detectable photoreceptor responses. Additionally, these tests can be used to measure treatment efficacy, allowing researchers to assess local and global improvements in visual function.

The knowledge on the retinal histopathology of patients with *RPGR*-IRDs is scarce. We obtained histopathology sections of a patient carrying an *RPGR* mutation with an advanced CRD/sector RP phenotype. Immunohistochemical analysis showed that in regions with degeneration of photoreceptors, reactive gliosis had taken place in the inner retina, also in regions without bone-spicule pigmentary changes. This process of reactive inner retinal gliosis may prove to be detrimental for treatments that require the use of the remaining neurons following the loss of photoreceptors, such as optogenetic strategies or retinal prostheses. Most therapies should therefore be applied at the earliest convenience for the best chance for successful therapy.

PHARC syndrome: a rare syndromic form of RP

In **Chapter 2.3**, we describe PHARC (polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract) syndrome, a rare syndromic form of RP that is associated with neurological, audiological and ophthalmological manifestations. It is caused by variants in the *ABHD12* gene, which plays a key role in lipid metabolism. With only 50 cases described in the literature, PHARC syndrome is one of the rarest forms of RP, and much is still unknown about this disease entity. We describe that PHARC syndrome shows great clinical variability with respect to onset of disease and rate of progression. Because of the great clinical variability in presentation, many other diagnoses may precede before establishing PHARC syndrome.

From an ophthalmic perspective, patients do not exhibit the clinical hallmarks of RP (bone-spicule hyperpigmentation, optic disc pallor and vessel attenuation), but the fundus findings resemble an atypical variant of RP without hyperpigmentation. The presence of panretinal rod-cone degeneration could be established using ffERG, highlighting the importance of electrophysiological testing for this disease. Multimodal imaging showed loss of the outer retina similar as seen in non-syndromic RP. Another important finding is the presence of cataract in PHARC syndrome, between the 2nd and 4th decade of life. Interestingly, in some patients, cataracts were star-shaped and delineated the crystalline sutures of the lens, suggesting a congenital component.

The complexity of PHARC syndrome and the rarity of this disease make it a difficult diagnosis to establish. Genetic testing is necessary to confirm the presence of variants associated with *ABHD12*, but these tests may not be available in all hospitals. A multidisciplinary approach involving specialists from multiple different fields is

necessary to obtain a timely diagnosis of PHARC syndrome. An accurate diagnosis and a better understanding of the pathophysiological mechanisms in PHARC syndrome will facilitate the development of therapeutic strategies.

CRB1-associated IRDs: paving the path towards gene therapy

Proof of concept for gene therapy for *CRB1*-associated IRDs has been achieved in murine and human iPSC-derived retinal organoid models, with possibility of clinical gene therapy trials in the future. However, before making the translation to clinical studies, many knowledge gaps need to be addressed first, for example the natural course and identification of the most sensitive potential outcome measures.

In **Chapter 2.4**, we describe the first prospective natural history study in patients with *CRB1*-associated IRDs. Variants in *CRB1* causes a variety of phenotypes including early-onset RP, LCA, CRD and macular dystrophy. Many patients with C*RB1*-associated IRDs are affected at a young age, with progressive loss of visual function. In our 2-year investigation, we demonstrate that conventional parameters, such as BCVA and visual fields, remain relatively stable over time. However, microperimetry showed significant decline of retinal sensitivity over 2 years, thus preceding BCVA and VF, suggesting that this outcome measure might be appropriate to monitor efficacy for clinical trials. Also, FST was able to measure residual photoreceptor function in this cohort, which is beneficial in patients with *CRB1*-associated IRDs as ffERG responses are typically minimal or non-detectable.

We observed great variability in visual acuity, visual fields, and microperimetry; most likely due to nystagmus, severe loss of visual acuity and poor fixation capabilities. In future studies, it is important that the amount of variability is investigated, as this will inform us whether changes in outcome parameters are due to disease progression, treatment effect, or to test-retest variability in patients.

CLINICAL MANAGEMENT OF RP

Management of RP-associated complications

In the majority of patients, clinical management of RP remains symptomatic and is not curative in nature. There are several complications commonly found in association with RP, which should be closely monitored, and, if possible, managed timely to minimize their impact. Below, we list several common and uncommon complications associated with RP, their potential impact on RP and suggested treatment options.

Cataract

Cataract is a common anterior segment complication in RP patients.[5-8] Cataract associated with RP is present at a younger age than those with age-related cataract,

and most commonly is posterior subcapsular cataract (PSC), suggesting differences in the etiology of cataract formation between these two groups.[6, 7, 9] Previous studies have demonstrated that increased levels of pro-inflammatory cytokines and chemokines are present in the aqueous humor and vitreous fluid of patients with RP compared to the controls.[9, 10] These increased inflammatory levels were mainly observed in younger patients and in those with significantly lower visual function, suggesting that a pro-inflammatory environment may play an important role in cataractogenesis in RP.[9]

Significant cataract impairs visual function and additionally causes visual disturbances that may exacerbate existing functional symptoms in patients with RP.[11-13] The type of visual disturbances varies with the morphology of the lens opacity and includes symptoms of glare, photophobia and decreased contrast sensitivity, among others.[11] Straylight effects caused by cataract can aggravate visual disability.[14, 15] Considering the impact of cataract in patients with RP, surgical removal of the lens opacity can be offered to improve visual function and to relieve any functional symptoms. Currently, the most used surgery technique for cataract removal is phacoemulsification of the natural lens and implantation of an artificial intraocular lens (IOL).[16, 17] In the absence of other (ocular) comorbidities, cataract surgery leads to significant improvements in visual function. However, in patients with RP, visual prognosis is less certain as the cause of progressive vision loss can be caused by the increased clouding of the lens, by the ongoing retinal degeneration by RP or a combination thereof. Patients with RP are also at increased risk for intra- and postoperative complications, including intraoperative phototoxic damage to the retina, (increase in existing) CME and zonular dialysis, among others.[18-23] Furthermore, higher rates of posterior capsular opacification and anterior capsule phimosis have been described following cataract surgery in patients with RP, which may also negatively influence visual outcomes if left untreated.[24]

In our study (**Chapter 3.1.**), we also demonstrated significant visual improvements in the majority of patients with RP following cataract surgery. Patients with lower baseline BCVA had higher odds of achieving marked BCVA improvements (> 15 ETDRS letter gain). A possible explanation is that patients with poor preoperative BCVA have more extensive, vision-impairing cataract that allows for higher BCVA gains. Also, patients with preserved preoperative BCVA are limited by a ceiling effect. Previous studies on cataract surgery in RP have reported similar findings (Table 1).[7, 24-33]

Table 1. Overview of studies on cataract surgeries in patients with retinitis pigmentosa.

Study	Pts	Eyes	Follow-up	Baseline BCVA	Postoperative BCVA	BCVA change	Complications
Jackson et al. 2001	89	142	32.7 months	1.05 ± 0.38 logMAR	0.63 ± 0.49 logMAR	-0.42 logMAR	PCO (63%), CME (14%), CCS (10%)
Dikopf et al. 2013	47	80	23.3 months	1.23 ± 0.99 logMAR	0.81 ± 0.87 logMAR	-0.42 logMAR	PCO (83%), IOL dislocation (3%)
Bayyoud et al. 2013	52	46	26.0 months	1.45 ± 0.85 logMAR	1.32 ± 0.95 logMAR	-0.13 logMAR	PCO (44%), CME (4%), CCS (4%)
Garcia-Martin et al. 2013	35	35	1.0 month	0.10 ± 0.23 Snellen	0.48 ± 0.21 Snellen	0.38 Snellen	N/A
Nakamura et al. 2015	43	58	3.0 months	0.81 ± 0.51 logMAR	0.34 ± 0.43 logMAR	-0.47 logMAR	None
Yoshida et al. 2015	40	56	37.5 ± 22.6 months	0.76 ± 0.65 logMAR	0.45 ± 0.53 logMAR	-0.31 logMAR	PCO (84%), CCS (23%)
Davies et al. 2017	18	30	3.7 ± 3.3 months	1.09 ± 0.69 logMAR	0.61 ± 0.45 logMAR	-0.47 logMAR	CME (13.3%), PCO (66.7%)
Chan et al. 2017	42	67	6.9 ± 4.4 years	1.27 ± 0.42 logMAR	1.18 ± 0.49 logMAR	-0.09 logMAR	N/A
De Rojas et al. 2017	19	19	259 days	0.33 ± 0.20 logMAR	0.19 ± 0.17 logMAR	-0.14 logMAR	CME (32%), PCO (95%)
Lu et al. 2017	52	101	5.09 ± 2.2 months	0.12 ± 0.09 Snellen	0.21 ± 0.16 Snellen	0.09 Snellen	CCS (2%), increased IOP (2%)
Mao et al. 2018	70	109	3 months	0.80 ± 0.59 logMAR	0.45 ± 0.41 logMAR	-0.35 logMAR	N/A
Chatterjee et al. 2021	103	132	13.5 ± 25.1 months	1.21 ± 0.87 logMAR	0.66 ± 0.64 logMAR	-0.55 logMAR	PCO (17%), CME (5%), zonulolysis (3%). PCR (2%), uveitis (4%)
Chen et al. 2021	63	84	6 months	1.3 ± 0.7 logMAR	0.91 ± 0.88 logMAR	-0.39 logMAR	CCS (5%)
Miura et al. 2021	62	62	3 months	0.45 ± 0.25 logMAR	0.11 ± 0.19 logMAR	-0.33 logMAR	None
Nakamura et al. 2022	64	96	5.8 ± 2.4 years	0.64 ± 0.52 logMAR	0.61 ± 0.52 logMAR	-0.03 logMAR	PCO (53%), CME (3%), ERM (2%), macular hole (1%), VMT (1%)
Nguyen et al. 2022	225	295	0.8 ± 1.6 years	1.03 ± 0.79 logMAR	0.81 ± 0.87 logMAR	-0.22 logMAR	PCO (38%), CME (5%), zonulolysis (5%), CCS (2%), IOL dislocation (1%), PCR (<1%), endophthalmitis (<1%)

BCVA = best-corrected visual acuity; PCO = posterior capsule opacification; CME = cystoid macular edema; CCS = capsular contraction syndrome; IOP = intraocular pressure; N/A = not available; PCR = posterior capsule rupture; Pts = number of patients; ERM = epiretinal membrane; VMT = vitreomacular traction

Subjectively, visual improvement was reported between 44.8%-96.7% of patients included in these studies.[23] Possible predictors for visual outcomes suggested by previous studies include the integrity of the EZ and ELM in the fovea, and baseline BCVA.[34-36] Extensive loss of macular EZ integrity, often seen in patients with advanced

stages of RP, may cause irreversible vision loss, leading to no or only modest visual gains after cataract surgery.[25] Some authors have advocated the use of low-light settings during surgery and the use of blue-light filtering IOLs in an attempt to limit additional retinal phototoxicity, although the evidence to support these preventive measures in RP is very limited.[14, 37]

The presence of new CME or the exacerbation of existing CME, with reported rates of up to 32% (Table 1), can negatively influence the visual outcome, and chronic CME may even aggravate photoreceptor loss in patients and thus should be timely managed.[34] For patients with RP, a previous study recommended the simultaneous postoperative use of topical nonsteroidal anti-inflammatory drugs and CAIs for at least 3 months to prevent the risk of CME.[20] Alternatively, or in addition, parabulbar steroids may be administered at the end of the operation in an attempt to reduce the likelihood of postoperative (increase in) CME. SD-OCT imaging in the pre- and postoperative care of patients with RP-associated cataracts is useful for monitoring CME.

Incidence rates of up to 13% of zonular dialysis following surgery have been reported. This increased risk of zonular dialysis is believed to be caused by a low-grade intraocular inflammation process in RP that causes weakened zonular attachments.[19] During preoperative intake, signs of zonular weakness can be present, including phacodonesis and lens subluxation, indicative of moderate to severe zonular weakness. However, zonular weakness is best observed while maneuvering the nucleus intraoperatively. Surgeons should avoid unnecessary manipulation and strain on the lens zonules, by using optimal hydrodissection and bimanual rotation of the nucleus. Large capsulorrhexis can assist with optimal maneuvering, while also reducing the risk of capsular phimosis. The use of a capsular tension ring may also provide stability and decrease the risk of IOL (sub)luxation and anterior capsular phimosis, although the insertion itself of the capsular tension ring may also cause strain on the lens zonule system, so prophylactic insertion of such a ring may not be indicated.[23, 28] IOL (sub) luxation at short- or long-term follow-up in RP has been reported in several case studies and these cases were managed using scleral suture fixation or by replacing them with a range of anterior chamber IOLs after the (sub)luxated IOL had been removed, often requiring accompanying vitrectomy.[38-40]

Posterior capsular opacification is another common complication after cataract surgery and is believed to develop faster in patients with RP, with a significant posterior capsular opacification occurring after a median time of 12-15 months postoperatively, reported by two studies.[19, 24] It may already be pre-existent because of the presence of residual posterior capsular cataract remnants at the end of the cataract surgery. Posterior capsular opacification can be treated with neodymium-doped-yttrium-aluminum-garner laser capsulotomy, preferably using low energy levels, considering that this procedure can also induce CME.[41]

In summary, the current literature suggests that cataract surgery is beneficial for a large group of patients with RP, provided that there is a good preoperative diagnostic evaluation, with postoperative improvements in both objective and subjective visual outcome measures.[23] Ophthalmologists are advised to employ SD-OCT imaging pre- and postoperatively to evaluate EZ integrity and CME, and to be aware of signs of zonular weakness. Patients should be counseled about the increased risk of complications and the guarded visual prognosis following surgery in order to set realistic expectations.

Cystoid Macular Edema

The presence of CME has been variably reported, with prevalence rates from 10% to up to 70% in at least one eye between different study populations.[42-44] While CME can occur in every genetic subtype of RP, it is more commonly found in patients with adRP.[45] Significant CME in RP may cause reduction in visual acuity, and if left untreated, it might cause further degenerative changes in the retina, including macular hole formation.[46] However, the short-term and long-term additional visual impact and detrimental influence of CME in RP has not been firmly established.[43] The advent of OCT imaging in clinical practice has made the detection of CME more efficient, allowing for earlier diagnosis and treatment. It should be noted that cystoid changes in patients with RP are not necessarily consistent with active fluid leakage and CME.[47-52]

The specific pathophysiology of RP-CME remains to be elucidated, but multiple mechanisms have been proposed: leakage of fluid through the RPE due to insufficient RPE pumping fluid function; vitreomacular traction; breakdown of the blood retina barrier; Müller cell dysfunction; and antiretinal antibodies.[42, 43] Previous studies demonstrated that RP-CME typically resides within the inner nuclear layers (INLs) of the retina and does not cause significant disruption of the vascular plexus.[53, 54] These findings support the hypothesis that RP-CME is more likely to be related to Müller cell dysfunction, rather than being vasculogenic, although other possible underlying mechanisms cannot be excluded to date. In the case of postoperative occurrence of CME, which occurs in up to 20% of RP patients after cataract extraction, there may be a more important role for a vasculogenic factor and active leakage.[5, 18, 34]

Because the pathophysiology is not completely understood and different gene mutations are associated with different likelihoods of RP-CME, the appropriate treatment remains a subject of debate.[55-58] An in-depth review by Bakthavatchalam and colleagues on the treatment of RP-CME suggested that the oral carbonic anhydrase inhibitor (CAI) acetazolamide is an effective first-line treatment.[44, 57-60] The exact mechanism of CAIs on RP-CME remains to be elucidated, but it has been postulated that CAIs selectively inhibit different carbonic anhydrase isozymes located in RPE cells, improving the polarity of RPE cells and improving fluid transport.[61, 62] Several relatively small prospective and retrospective studies showed that oral intake of acetazolamide

causes a significant reduction in central macular thickness in up to 80% of patients with CME.[58, 60, 63] While CAIs may restore retinal structure, its effect on retinal function, i.e., visual acuity, appears to be limited, and the long-term functional and anatomical benefit of reducing CME in RP remains to be observed.[42, 44, 57, 60, 62, 64] In addition, there is a range of potential adverse effects of systemic CAIs, including paresthesia, malaise, nausea, altered taste, depression and drowsiness, as well as potential serum biochemical changes, including decreased serum potassium and increased chloride levels, thus discouraging prolonged use of CAIs.[65, 66] In rare cases, patients also develop renal stones as a consequence of prolonged CAI use.[67, 68]

As an alternative to systemic CAIs, topical CAIs such as dorzolamide and brinzolamide can be used for the treatment of CME. Previous studies have shown a significant decrease in CME in 30-81% of study eyes following the use of topical CAIs, although the efficacy of systemic CAIs in reducing CME was higher than that of topical medication.[44, 57, 63, 69, 70] Topical CAIs can be prescribed if patients experience any adverse effect from systemic medication. Despite the significant reduction in CME, re-occurrence of CME after a period of discontinued use of CAIs is common.[44, 63, 69-74] Therefore, patients need to be actively monitored for recurrent CME, which requires restarting CAIs.

Furthermore, intravitreal injections with anti-vascular endothelial growth factor (anti-VEGF) have also been proposed as treatment for RP-CME.[42, 75] Vascular endothelial growth factor (VEGF) is a protein important for angiogenesis, as well as for vasculogenesis.[76, 77] Thus far, given the limited evidence of efficacy as well as the patient burden, there is no indication for anti-VEGF treatment for uncomplicated RP-CME.

Intravitreal injection of a dexamethasone implant has also been used for the treatment of RP-CME. A prospective study by Veritti and colleagues compared the efficacy of dexamethasone implants versus oral acetazolamide (30 eyes in each arm), demonstrating that dexamethasone implants caused more reduction in central macular thickness and a higher BCVA letter gain compared to oral acetazolamide.[78] While the use of dexamethasone implants for RP-CME may be promising, current evidence on its usage and long-term effects in RP-CME is limited.[79-81] Furthermore, intravitreal injections of dexamethasone implants can cause increased intraocular pressure, cataract formation and subconjunctival hemorrhages, as well as more severe and rare complications such as retinal detachments, misplacement of the implant, and endophthalmitis.[82]

Based on the available literature, if there is an indication for the treatment of RP-CME, CAIs are currently the preferred choice, with systemic CAIs preferred over topical CAIs because of their comparatively superior efficacy, provided that the profile of side effects are acceptable for the patient. Oral acetazolamide can be prescribed when

there is significant central (fovea-involving) CME and patients should be informed of the common adverse effects, the possibility of refractory CME and the uncertainty regarding long-term benefit for visual function. More studies are needed on the long-term natural course of RP-CME, the use of anti-VEGF and steroid implants, the potential detrimental effect of cystoid fluid in the macula of RP patients and if treatment of CME has a short-term and long-term functional benefit.

Other Macular Abnormalities and Retinal Detachments

The prevalence of macular abnormalities, such as epiretinal membrane (ERM), macular hole and vitreomacular traction syndrome, has been estimated to be around 1.9% in patients with RP.[83] Significant epiretinal membranes cause visual disturbances (e.g., visual acuity loss, metamorphopsia and diplopia) and can also result in macular holes. The exact etiology behind epiretinal membrane formation remains unknown, although elevated inflammatory factors have been observed in the vitreous of patients with RP, suggesting that inflammation is likely a contributing factor.[84] Surgical outcomes for the treatment of the ERM in RP are limited; a study involving 10 RP patients that underwent pars plana vitrectomy and inner limiting membrane peeling for ERM showed improvements in retinal morphology for the majority of cases (82%), but no significant improvement in BCVA was observed.[85]

Similarly, the occurrence of macular holes is rare in RP and as a consequence, outcome rates of vitreoretinal surgery in patients with RP have only been reported in a select few case studies involving a small number of eyes.[46, 86-88] The study by Jin and colleagues showed an improvement in visual acuity and structural integrity of the retina following pars plana vitrectomy in three out of five treated eyes, as well as an improvement in the sealing of the macular hole. The remaining patient, who also had extensive retinal detachment, showed no change in visual acuity.[86] A different case report by Garcia-Fernandez and colleagues showed that primary surgery resulted in closure of the macular hole in the treated patient, but reopening of the hole occurred after two years.[88]

The prevalence of retinal detachments (RDs) in RP has been reported to be between 0.7% and 1.3%.[89-91] Retinal detachments occur at a relatively younger age in patients with RP than in those without RP. Retinal detachments are often rhegmatogenous in nature, although exudative and tractional forms have also been described.[89] In the study of Chan and colleagues, exudative RDs were mainly seen in patients with *CRB1*-associated IRDs.[89] In three previous studies, final reattachment rates between 86% and 96% were reported, using scleral buckling or vitrectomy, suggesting a favorable anatomical outcome.[89-91] An overview of surgical outcomes for retinal detachments in RP can be found in Supplemental Table S1.

Uveitis

Uveitis in patients with RP is relatively rare, with a prevalence estimated in one study at approximately 0.26%, although this is likely an underestimation as most patients have milder forms of uveitis and/or are asymptomatic.[92] Uveitis in RP most commonly presents as anterior uveitis, followed by intermediate uveitis and, even more rarely, as posterior uveitis.[92-95] Some forms of uveitis, such as acute zonal occult outer retinopathy and (atypical) advanced birdshot chorioretinopathy may mimic features of RP, such as pigment clumping and retinal vessel attenuation, which leads to initial misdiagnosis.[96, 97] A specific form of uveitis found in patients with RP is Fuchs' heterochromic uveitis, which has been reported in several case series.[98-104] The co-occurrence of uveitis in RP can be coincidental, but there may also be a role for underlying immunological abnormalities that play a role in the disease etiology of RP, which is supported by several animal and immunohistochemical studies.[95, 98, 105, 106]

Currently, there is limited evidence on the treatment of uveitis in RP. Only a few studies describe treatment modalities, and these case reports seem to show a low efficacy in preventing uveitis relapse.[94, 95] Majumder and colleagues have described the use of topical, periocular and oral corticosteroids for the treatment of 22 patients with anterior and/or intermediate uveitis, with varying results. Two patients with anterior uveitis developed CME, which was resolved using topical nonsteroidal anti-inflammatory drugs. The management of uveitis did not show improvements in visual acuity at follow-up.[92] While the treatment of uveitis does not necessarily improve visual function, monitoring the activity of inflammation remains important to prevent further complications that may worsen visual function such as CME formation, and leakage of the optic nerve and/or retinal vessels, findings which have all been described in patients with RP.[107-109]

Glaucoma

A common form of glaucoma found in RP is primary angle-closure glaucoma (PACG), with prevalence rates between 1.0% and 2.3%.[110-112] Previous studies have shown that the association between RP and PACG are related to nanophthalmos, short axial length, cataract and lens subluxation.[111] Anatomically, patients with a short axial length and/or cataract have a relatively shallow anterior chamber more prone to occlusion. Furthermore, the presence of zonular insufficiency and ectopia lentis in RP may cause forward displacement of the lens, which may also induce closing of the anterior chamber angle.[112] As PACG can cause irreversible optic nerve damage that may lead to further loss of remaining visual function in patients with RP, clinical work-up and timely intervention is crucial. In the acute setting, the overall goal for the management of PACG is to reduce intraocular pressure and to relieve angle closure. Glaucoma medications are given to lower intraocular pressure, to reduce pain and in preparation for laser peripheral iridotomy, which is the definitive treatment for PACG.

Fellow eyes should also prophylactically receive an iridotomy as they are also at risk for developing PACG.[113]

Rehabilitative and Psychological Management

The visual impairment caused by RP and the progressive nature of this disease may have detrimental effects on patients' general health, self-sufficiency and independence, which can profoundly impact their own quality of life and that of their caretakers.[114] The impact of RP is diverse and may result in physical, mental, emotional and social disabilities. The extent to which the lives of patients are affected by RP varies greatly between individuals and relies on several factors including their functional ability, age, daily activities, work, education, family, support networks and coping mechanisms.[114] Not all patients are aware of the rehabilitation services that can provide assistance for some of these aspects, and thus are left with unmet clinical needs.[114] Healthcare providers should screen patients for rehabilitation needs and, if desired, refer them to the appropriate services, such as low vision rehabilitation, psychological counseling and mobility training services, which are commonly present in visual rehabilitation centers. The aim of these services is to help patients manage the consequences of their disease and to lead a lifestyle as autonomous as possible, optimizing their quality of life.[115] Low-vision rehabilitation services (LVRSs) encompass a multidisciplinary team that aims to achieve the maximum potential of a patient's residual vision.[116, 117] The composition of this multidisciplinary team varies between different countries and may include, but is not limited to ophthalmologists, optometrists, occupational therapists, social workers and psychologists.[115, 118] Multiple studies have demonstrated improvements in the quality of life in patients with visual impairment following LVRSs.[119, 120] Rehabilitation services are tailored to a patient's individual situation, which are based on a patient's current visual abilities and their own rehabilitation goals.[121] Several instruments exist that can be used at the intake to screen for important rehabilitation needs, and to measure the efficacy of rehabilitation services. Common tools used at initial assessment within LVRSs may include variations of the National Eye Institute Visual Function Questionnaire, an instrument to measure vision-related quality of life, as well as the Activity Inventory, which systematically assesses the most important life domains and specific tasks for a patient.[121-123] A limitation of these aforementioned questionnaires is that they are not tailored to patients with RP, who may experience different difficulties than those, for example, with glaucoma. New questionnaires are being developed specifically for patients with IRDs in light of new upcoming therapies as a subjective outcome measure, such as the Michigan Retinal Degeneration Questionnaire.[124]

Without rehabilitation, patients with visual impairment may have to abandon tasks, for instance, those that require detailed vision, such as reading.[125] A low-vision aid (LVA) yields improvement in visual performance and encompasses corrective glasses; filtering lenses; optical and non-optical LVAs (e.g., magnifiers, telescopes, reading

stands); electronic assistive technologies, such as closed-circuit television, screen readers; and, more recently, portable electronic devices (e.g., Orcam or eSight).[126-128]

In **Chapter 3.2**, we explore impact of one electronic assistive device, the OrCam MyEye 2.0, on the quality of life in patients with IRDs. The OrCam is a wearable assistive technology device that utilizes artificial intelligence and computer vision to assist individuals with visual impairments. The device consists of a small camera mounted on a pair of glasses that can identify and read text, recognize faces, and provide audio descriptions of objects and environments. The OrCam is designed to enhance the independence and quality of life of individuals with visual impairments by providing them with additional visual information.

As visual function gradually becomes less in patients with IRDs, so does their ability to do their daily activities independently, and in turn their quality of life. In our study of 19 patients with advanced IRDs, we noticed that important rehabilitations goals were mobility indoors for patients with RP, and reading and administration for patients with cone-based dystrophies, which corresponds with the affected photoreceptors in each of these diseases. Following OrCam usage, we observed a significant increase in near reading abilities, as measured on 3 different questionnaires (NEI-VFQ, D-AI and the OFQ). Other subdomains or rehabilitation goals, remains unchanged after the test period of 5.2 weeks. These findings suggested that devices such as the OrCam can be particularly useful for improving reading abilities in patients with advanced IRDs, and less so for other activities. Further improvements are needed to improve the serviceability of the OrCam and similar devices to a broader audience. Several upgrades in the hardware and software of the OrCam have already been made to address these limitations.

The efficacy of LVAs is demonstrated by improvements in reading speed and acuity in clinical studies, although knowledge on other important factors such as the subjective preference and cost of LVAs, can also play an considerable role in the recommendation of these devices.[125] Simple adaptations can also be made at home, at school or at work to improve autonomous function and to create a safe environment.[129] Examples of these adjustments include improving lighting control, removing trip hazards, and creating contrasts between objects for easier identification.

Blindness is often ranked as one of the worst medical conditions by the general population among other very severe diseases, as well as being considered the medical condition with the highest impact on day-to-day life.[130] Nevertheless, the psychological consequences may be under-recognized. Loss of vision has been associated with depression, social isolation, sadness, anxiety and fear.[131-133] Few studies have investigated the psychological impact of LVRSs which showed improvements in mental well-being following rehabilitation.[134] Further studies are needed to understand

the effectiveness of LVRSs on mental health and whether the implementation of psychological interventions such as cognitive behavioral therapy, should be routinely embedded in LVRSs.[135]

The ongoing degeneration of the retina due to RP causes progressive loss of visual function, which can have a significant impact on a person's daily life, the ability to perform tasks, and emotional well-being. While objective outcome parameters measure a patient's visual function, they do not necessarily reflect a patient's own experience, for which subjective outcome measures are more appropriate.

In **Chapter 3.3**, we explore the longitudinal changes in patients with *CRB1*-associated IRDs over the course of 4 years using the most commonly ophthalmic patient reported outcome measure to date, the NEI-VFQ. While praised for its simplicity, the NEI-VFQ in its classical test theory form does not meet the current standard for psychometric testing due to lack of unidimensionality and poor item fitting. In our study, we employed the use of Rasch analysis to tackle some of the limitations, and we made use of previous item measure calibrations to analyze the quality of life in this cohort. When looking at objective outcome measures, we observed a decline in BCVA and microperimetry over the course of 4 years. For subjective measures, we observed a decline in the 'visual function' domain over 2 years, but not over 4 years. These findings illustrate that changes in objective visual function do not necessarily reflect a patient's experience. By including both subjective and objective outcome measures, a more complete and nuanced assessment of treatment effectiveness can be achieved. For subjective outcome measures, the NEI-VFQ can be relevant and meaningful for the design of future clinical trials. New questionnaires such as the Michigan Retinal Degeneration Questionnaire, are also being developed that meet current psychometric standards.

For individuals with extensive visual field loss such as in RP, traveling independently can become increasingly difficult, especially in unfamiliar and poorly lit environments.[136] Many aspects of life are impeded by the inability to travel, such as social interaction and work; therefore, mobility impairment may also significantly impact an individual's quality of life. In such cases, orientation and mobility training can be useful, which aims to teach patients to ambulate (un)known environments safely and independently. Examples of mobility training objectives include training on the use of a white cane when using public transport, riding a bike and using navigation devices while traveling.[137]

LVRSs should be an integral part of the care for eye diseases, especially in patients with significant visual impairment, such as those caused by RP, to improve their independence and overall well-being. It is advisable to refer patients to LVRSs when unmet needs are evident as well as when these needs are not so apparent, as low-

vision centers provide many helpful services that are not necessarily known to a patient.

Investigational Treatment Modalities

Improved understanding of the underlying mechanisms of RP has driven current research, resulting in the dawn of novel treatment strategies. The timing and underlying mechanism causing retinal degeneration determines a patient's eligibility for treatment. Below, we briefly explain the key features of current and emerging treatment modalities, their relevance in the treatment of RP and IRDs and their advantages and limitations.

Gene-Dependent Strategies

Ocular genetic therapies have become an emerging treatment modality for a wide variety of IRDs and have been successfully used in mice, dogs and now clinically in patients.[138-140] Retinal diseases appear to be excellent targets for gene-based therapies as the eye is highly compartmentalized, immune privileged, and are relatively accessible for local administration, while there is an elaborate armamentarium of structural and functional tests to evaluate treatment efficacy. Gene-based strategies are most effective in the early stages of disease, as they aim to prevent further degeneration of the surviving target cells, whereas they are unable to restore cells that have already degenerated.[141] The term gene therapy encompasses different strategies based on the transfer and application to different nucleic acids.

Gene Augmentation Therapy

The most straightforward strategy is gene augmentation therapy, in which a wild-type (normal) copy of the mutant gene is delivered to the site of interest with the use of a vector in which the correct gene is packaged for delivery at the target cells. The vector that is generally used is an adeno-associated virus (AAV), which has been extensively researched, has high transduction efficiency and exhibits relatively low immunogenicity.[142] However, other viral and non-viral vectors are also studied, and each has its advantages and disadvantages.[143] The correct copy of the gene carried by the vector aims to compensate for the disease by restoring wild-type expression, thus preventing further disease. This method can be particularly useful for autosomal recessive and X-linked RP as these variants typically result in loss-of-function. In contrast, adRP may result in gain of function or dominant-negative variants, which may require alternative approaches, such as gene silencing or knockdown-and-replacements strategies.[144] In patients with *RPE65*-associated IRDs, subretinal administration of functional copies of *RPE65* using an adeno-associated virus vector resulted in functional improvements (e.g., BCVA, FST blue, and multi-luminance mobility test).[139, 145-148] A meta-analysis revealed that changes in BCVA were significant at 1 year after treatment, but afterwards declined to baseline BCVA 2-3 years post-treatment. It is possible that photoreceptors continue to degenerate due to

insufficient delivery of functional genes, or that photoreceptors had already reached a pre-apoptotic state at the moment of therapeutic intervention.[149] A recent review demonstrated that the treatment effects of *RPE65*-gene therapy lasts up to 7.5 years after administration, which suggests that multiple gene therapy doses are needed to provide clinical stability during a patient's lifetime.[150] A single dose of FDA-approved Luxturna costs approximately USD 425,000 per eye per treatment. Furthermore, a subset of *RPE65* patients developed chorioretinal atrophy as a side effect of the subretinal administration of gene therapy.[151, 152]

The challenges in gene augmentation strategies lie in the fact that it is a gene-specific therapy and thus cannot be universally applied for all IRDs. Each gene in RP varies in its clinical course, affected cell types and size, among other factors. Therefore, each gene may differ in its optimal timing for therapeutic intervention, the method of administration and its therapeutic delivery. While subretinal delivery has a more direct effect on photoreceptor cells, it provides treatment only for a limited region of the retina, thus requiring multiple or larger treatment zones for better outcomes.[153] Furthermore, intravitreal and subretinal delivery can induce immune and inflammatory responses which can typically be managed with steroid therapy, but in rare cases may result in significant ocular inflammation with sight-threatening complications.[154] For many large genes in RP, such as *USH2A, ABCA4* and *EYS*, AAV vectors cannot be used as a vehicle considering the limited packaging capacity of approximately 4.7 Kb.[145, 155] Different viral vectors have been suggested, which differ in their gene-carrying capacity, cellular tropism, immunogenicity and mutagenicity.[140] Aside from *RPE65*, a range of RP-associated genes are currently targeted in gene-therapy trials, including but not limited to *RPGR, GUCY2D, XLRS* and *CRB1*.[140, 149]

CRISPR/Cas9-Based Therapy

Gene editing strategies, such as repurposing the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 system, have recently emerged as a potential solution for the limitations brought by gene augmentation strategies.[145, 149, 156-158] In CRISPR-Cas9 gene therapy, a Cas9 endonuclease is delivered to the target region via guide RNA, which causes double-strand breaks in the predefined regions of the genome. Subsequently, DNA-repair mechanisms are activated, namely non-homologous end joining (NHEJ) or homology-directed repair (HDR). Based on these two repair mechanisms, several types of gene editing can be performed. Using NHEJ, the ends of the cleaved DNA are ligated with or without the addition of base pairs, often resulting in gene inactivation. If multiple guide RNAs are introduced that target separates sites, NHEJ can be used to delete specific sequences. If a DNA template homologous to the target region is introduced alongside the CRISPR-Cas9 system, cells can even correct a gene, or insert a new gene using HDR mechanisms.[156]

As with any form of gene therapy, the main challenges of CRISPR-Cas9 include the delivery of the CRISPR-Cas9 complex, and the potential risk of an immune response. In addition, a major drawback for the use of CRISPR-Cas9 therapies are potential off-target effects. When using the CRISPR-Cas9 system, the guide RNA may target different regions than intended due to similarities within the genome, subsequently resulting in unwanted genomic modifications.[159] Furthermore, HDR efficiency, which is required to correct IRD-causing variants, in retinal cells is low. HDR functions mainly in dividing cells and is not highly efficient in post-mitotic retinal cells.[157]

Antisense Oligonucleotide Therapy
RNA therapies, such as antisense oligonucleotides (AONs), are an interesting treatment modality for IRDs, as they provide a possible solution for some patients with genetic variants not suited for AAV-gene therapy, e.g., patients with splice-site defects.[160, 161] AONs are short chains of nucleic acids that bind to a specific complementary messenger RNA (mRNA) to modify the expression of a given nucleotide sequence. The exact working mechanism differs between AONs, as they can be used for example, to correct pre-mRNA splicing, for exon skipping or for mRNA knockdown.[149]

There are some potential advantages of AONs over DNA-based therapies: AONs are relatively small in size and can fit current vectors; they do not directly modify DNA; and they do not induce double-strand breaks, thus not interfering with the endogenous expression of the target gene.[162] A limitation is that AONs have a limited duration effect based on their half-life and multiple intravitreal injections over the course of disease are likely needed.[161] Currently, no approved RNA therapies are available for IRDs and more data are needed to support the efficacy in this group of diseases.[161]

Gene-independent strategies

Optogenetics
In late-stage RP, degeneration of photoreceptors may reach a point in which the window of therapeutic opportunity for ocular genetic therapies is surpassed. The remaining neurons, such as dormant cones, bipolar and retinal ganglion cells, are typically preserved until end-stage disease, making them possible targets for optogenetic therapies.

The key idea of optogenetic therapy is to deliver and express genetically encoded light-sensitive proteins called opsins to the remaining light-insensitive neurons in the inner retina of patients with RP via viral vectors.[163] Once opsins are expressed in these target cells, they can be stimulated by light and invoke a visual response, thus bypassing lost or damaged photoreceptors. If the targeted cells are connected to other cell types in the retinal circuit, light also modulates the activity of these cells.

Optogenetic therapy can theoretically be applied to all patients with end-stage RP, regardless of genotype.[164]

Several human clinical trials are ongoing that involve optogenetic therapy in patients with RP (NCT02556736, NCT03326336, NCT04919473, and NCT04278131). Different types of opsins have been used; however, all studies use an AAV2 or similar variant as a viral vector via intravitreal injections, targeting retinal ganglion or bipolar cells. In the study by Sahel and colleagues, partial recovery of visual function was observed in a patient with light perception vision that received the AAV vector containing the light-sensitive protein ChrimsonR. With light stimulation via engineered goggles, the patient was able to locate and perceive different objects in a controlled environment, demonstrating proof-of-concept for the use of optogenetic therapy in RP, although further optimization is likely needed.[165]

Stem cell therapy

Stem cell therapy involves the use of stem cells to replace or repair cells in the retina and can be applied in patients with end-stage RP regardless of genotype.[157] The treatment can be categorized by effect, i.e., replacement or preservation of cells, and stem cell type as follows: embryonic stem cells (ESCs); induced pluripotent stem cells (iPSCs); hematopoietic stem cells; mesenchymal stem cells (MSCs); and retinal progenitor cells (RPCs).[166-172] Stem cells with a higher cell potency, such as pluripotent ESCs and iPSCs, come with more extensive differentiation properties and can be used for the replacement of retinal cells.[166] These cells, as well as their derivatives, have a higher risk of tumorigenesis and uncontrollable cell migration when compared to lower-cell-potency stem cells.[166, 173] The tumorigenesis of a treatment dose is closely monitored before administrating it to a patient, but no extensive long-term data are currently available.[166] RPCs can be derived from ESCs, iPSCs and MSCs, among others. These cells show promising results with increased BCVA outcomes in injected eyes, but are relatively self-limiting regarding expansion compared to pluripotent cell lines. [174, 175] RPCs also retain their capacity to differentiate in preclinical studies, which poses challenges post-transplantation.[166, 174, 176] MSCs, with their lower cell potency, are considered safer and have more long-term data on the risk of tumorigenesis. Patients treated with bone-marrow-derived MSCs showed initial improvements in BCVA, although their vision reverted to baseline at 12-month follow-up.[168, 169] Stem cell therapy is still in the early stages of development, and further research is needed to refine and optimize its technique and to determine its safety and effectiveness in the treatment of IRDs. Important hurdles of stem cell therapy include potential immune rejection, tumorigenicity and surgical complications.[176] Nevertheless, it can be a promising treatment option for patients with end-stage retinal disease.[168, 177]

Retinal Prostheses

Electronic retinal implants are designed to provide a basic sense of visual function in severely visually impaired patients.[149] In essence, retinal prostheses stimulate remaining retinal neural cells with electrical pulses via an electrode array. This treatment is primarily intended for patients with little to no visual function as the current resolution of vision is low.[178] The number of electrodes, amount of stimulation and the remaining retinal function all play a role in the quality of perception created by retinal prostheses. Furthermore, patients require a relatively intact posterior visual pathway to ensure correct visual processing of light stimulation.[178] Retinal prostheses can be utilized via direct electrical stimulation, where an external processing unit (e.g., a digital camera mounted on eyeglasses) captures real-time images which are then transmitted to the retinal implant, or via photodiodes arrays, which are directly imbedded into the retinal space and convert projected light patterns into local electric currents.

Retinal implants can be installed in the epiretinal, subretinal or suprachoroidal space.[179, 180] In the epiretinal configuration, the implant is placed in the near vicinity and directly interacts with the retinal ganglion cells. In the subretinal configuration, the implant is positioned between the outer retinal layer and retinal pigment epithelium, at the site of the photoreceptors. The suprachoroidal approach was developed to prevent damage to the neural retina, as the stimulating electrode array is not directly attached to the retina. However, this meant that electrodes were placed further away from the intended cells, thus requiring higher currents for stimulation.[179, 181, 182]

Several retinal implants have been developed, of which three have been approved by regulatory authorities and implanted in over 500 patients over the past two decades as follows: The Argus II, developed by Second Sight Medical Products, which was an epiretinal implant with glasses paired to a processing unit; Retina Implant Alpha-AMS and the Retina Implant Alpha-IMS by Retina Implant AG, which used a subretinal electrode array. Up to 20/1260 Snellen vision was achieved using Argus II, and 20/546 Snellen was achieved with the Retinal Implant Alpha-AMS.[178]

The implants do not come without risks as up to 30-40% of Argus II users showed adverse events of conjunctival erosion, hypotony, conjunctival dehiscence or endophthalmitis within five months after implantation.[183, 184] Alpha-IMS (by Retina Implant AG) showed increased intraocular pressure caused by subretinal bleeding in 1 out of 19 patients (5.3%).[184] Retina Implant AG and Second Sight Medical Products, have withdrawn their current products, with the latter now testing a cortical visual prosthesis in an attempt to address a wider patient group.[178, 185]

Retinal protheses are intended for patients with limited visual function, although the visual benefit with current techniques appears modest. Future developments in retinal prostheses should focus on increasing resolution of vision, visual fields and to minimize

adverse effects as result of electrode array implantation, which require innovation from engineering, software and electrophysiological perspectives.

Neurotrophic Factors

Neurotrophic factors are proteins that promote the survival, differentiation and growth of neuronal cells. Several neurotrophic factors have been studied in animal models for the potential to treat retinitis pigmentosa, including ciliary neurotrophic, nerve growth, and brain-derived neurotrophic factors.[186] Improvements in scotopic and photopic responses were observed in eyes that received ciliary neurotrophic factor (CNTF) compared to control eyes. For clinical delivery, direct intravitreal or subretinal neurotrophic factor injections have been the most common route.[186] However, an implantable device has also been suggested as it allows for the long-term release of neurotrophic factors, minimizing the risk accompanied by repeated injections. Several clinical trials have been conducted to evaluate the safety and effectiveness of CNTF as a treatment for retinitis pigmentosa. In one phase 1/2 clinical trial, CNTF was administered to patients with retinitis pigmentosa via a slow-release implant in the eye. The results of this trial showed that CNTF was generally well-tolerated and may have some beneficial effects on visual function in patients with retinitis pigmentosa.[186] Further randomized clinical trials evaluated the use of encapsulated-cell-ciliary neurotrophic factor implants for RP, showing no significant improvements in BCVA and visual field sensitivity for patients in the short (12 months) or long term (60–96 months).[187, 188]

Neuroprotective Agents

In rod-specific retinal diseases, cone photoreceptors may still degenerate.[189] It is hypothesized that when large amounts of rods degenerate in RP, oxygen consumption in the retina is severely reduced, leading to the generation of large amounts of toxic free radicals.[189] These compounds are harmful to the remaining cone photoreceptors.[2] Additionally, the production of rod-derived cone viability factor is also affected, making cone receptors more vulnerable to degeneration.[190, 191] N-acetylcysteine (NAC) and its more potent version, N-acetylcysteine amide (NACA), are powerful antioxidants that have shown to preserve cone function in animal models of RP.[3,4] In the FIGHT-RP1 study, the therapeutic benefit of daily intake of NAC was investigated, which showed improvements in visual function over the study period of 6 months.[192] These improvements diminished once patients discontinued the study medication. A retrospective study by the same group found similar neuroprotective features in the macula, as measured on microperimetry.[193] Another studied neuroprotective factor includes cerium oxide nanoparticles (CeO_2-NPs), which are nanocrystals with antioxidative effects derived from the rare earth element cerium.[194] In rat models, these have been shown to be effective in preserving photoreceptor function as well as slowing down the loss of photoreceptors.[194, 195] So far, human clinical trials in RP

patients have not been conducted. Currently, no neurotrophic drugs have been approved by the regulatory authorities.

Nutritional Therapies

Dietary supplements, such as vitamin A, lutein and docosahexaenoic acid (DHA) supplements, have been previously studied in patients with RP. Berson and colleagues published their study in 1993, where they assigned 601 non-genotyped RP-patients with either 15,000 IU/d vitamin A, 15,000 IU/d vitamin A plus 400 IU/d vitamin E, trace amounts of both vitamins or 400 IU/d vitamin E.[196] The first two groups showed a slower decline in retinal function based on full-field cone electroretinography compared to the latter two. This group conducted a follow-up study in 2004, assigning RP-patients with either DHA plus vitamin A (treatment group) or fatty acid plus vitamin A (control group), with a follow-up of two years. The authors concluded that the DHA + vitamin A group slowed the disease course of retinitis pigmentosa compared to patients in the group not assigned to DHA.[197] Similar effects of vitamin A supplements were also found in children by Berson and colleagues.[198] It has been postulated that because vitamin A is an important chromophore in the visual cycle, vitamin A supplementation can compensate for deficiencies in patients with RP.[199] Currently, less than 10% of the genes in RP involve genes associated with vitamin A metabolism.[200]

A randomized clinical trial by Hoffman and colleagues (DHAX trial) investigated the use of high dose DHA in patients with X-linked RP over the course of 4 years.[201] The results of this study demonstrated that DHA was not effective in slowing down rod or cone ERG progression. A second analysis of the DHAX trial revealed that DHA might reduce the rate of progression in final dark-adapted thresholds and visual field sensitivity parameters.[201, 202]

Recent reviews concluded that there was no clear benefit of vitamin A and/or DHA for patients with RP, in terms of mean change in visual fields or ERGs.[203-205] An editorial by Massof and colleagues concluded that there was no convincing evidence that vitamin A is beneficial, and may even carry potential health risks.[206] Excess vitamin A compromises liver function and may cause birth defects.[206] Furthermore, careful consideration should be given to the possibility that RP is caused by specific genetic variants (e.g., in the *ABCA4* gene), as it has been shown in animal models that an excess of vitamin A may boost the accumulation of lipofuscin in the retina and accelerate disease progression.[200, 207, 208]

Taken together, there is no strong evidence that supports the use of nutritional supplements for patients with RP. Nutritional supplements may slow down disease progression in IRDs closely tied to the vitamin A pathway in the retina (e.g., *LRAT*, *RPE65*, *RLBP1*, *RDH5* and *RDH11*), although its clear benefit has not yet been sufficiently proven in studies.[200] Vitamin A should be avoided in patients with genetic subtypes

susceptible for excess vitamin A (e.g., variants in *ABCA4*) as this may potentially accelerate disease progression.[209] Patients who do receive high doses of vitamin A should undergo laboratory work-up prior to therapy as longstanding use of vitamin A can result in toxicity (e.g., birth defects, liver failure, osteoporosis and central nervous system disorders).[210] For these reasons, most ophthalmologists do not prescribe nutritional supplements to patients with RP as routine care.

CONCLUDING REMARKS

The paradigm of IRDs has shifted from a diagnostic field to one in which potential curative treatments are being developed. For patients with RP who are eligible for current or upcoming clinical trials, establishing the natural history and defining clinical endpoints is essential to measure treatment efficacy. As genetic subtypes may display differences in clinical characteristics, natural progression and disease severity, outcome measures need to be tailored to each subtype of RP. Therefore, establishing the clinical and genetic diagnosis of patients with RP should be the first step, as many of the consecutive management steps rely on a thorough knowledge on the genetic and clinical characteristics (Figure 1). As RP is a rare disease, an international collaboration to facilitate retrospective and prospective studies is highly recommended. Modern outcome measures such as microperimetry and FST should be considered in future trials, as they provide additional insight into the remaining photoreceptor function. Upcoming studies should also investigate the test-retest variability of key measurement tools, as common problems in RP, such as severe visual impairment and nystagmus, can influence testing results.

The most common comorbidities, such as cataract and CME, can be managed using current treatment options. Patients with difficulties in daily activities due to reduced functional vision can be referred to low vision rehabilitation centers where they may obtain assistance in performing daily activities, which includes the prescription of low vision aids, in order to maintain independence. Validated patient-reported outcome measures are helpful in detecting rehabilitation needs, and to measure treatment effect from a patient's perspective. Developing a RP-specific questionnaire will be beneficial for obtaining the most relevant information and accurate information for managing this disease, which should be a focus for future studies.

Patients should not only be informed about new treatment modalities, but also about currently available clinical management possibilities outside curative treatment, as they may provide relief of physical, psychological and social burden until early therapeutic intervention and prevention are possible. With a range of future treatments on the horizon, the current management options should not be overlooked.

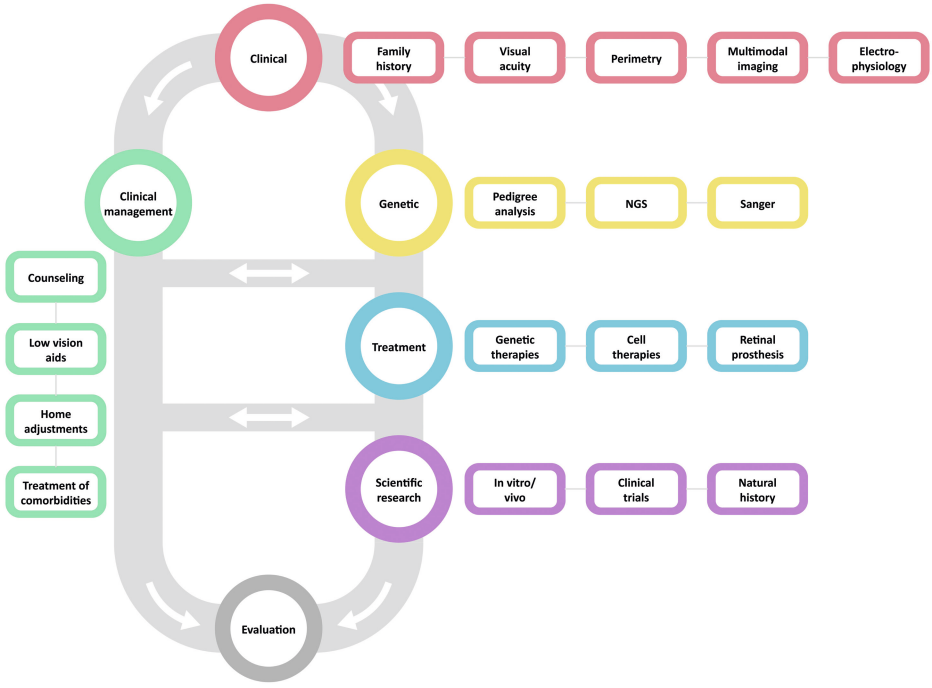


Figure 1. Flowchart demonstrating the clinical management of patients with retinitis pigmentosa (RP). The first step should be identifying patients with possible RP clinically, after which genetic testing should be performed, when available, if a diagnosis of presumed RP is made. Simultaneously, further clinical management should be offered through counseling, low-vision aids, home adjustments and treatment of comorbidities. Depending on the underlying causal gene, symptoms and severity of RP, treatment eligibility is assessed. Additionally, patients may opt to participate in ongoing research. The landscape for RP continues to change, and regular follow-up is advised to remain up to date with current clinical management and novel therapies.

REFERENCES

1. Dryja TP, McGee TL, Hahn LB, et al. Mutations within the rhodopsin gene in patients with autosomal dominant retinitis pigmentosa. *N Engl J Med*. Nov 8 1990;323(19):1302-7. doi:10.1056/nejm199011083231903
2. Athanasiou D, Aguila M, Bellingham J, et al. The molecular and cellular basis of rhodopsin retinitis pigmentosa reveals potential strategies for therapy. *Prog Retin Eye Res*. Jan 2018;62:1-23. doi:10.1016/j.preteyeres.2017.10.002
3. Georgiou M, Grewal PS, Narayan A, et al. Sector Retinitis Pigmentosa: Extending the Molecular Genetics Basis and Elucidating the Natural History. *American Journal of Ophthalmology*. 2021/01/01/ 2021;221:299-310. doi:https://doi.org/10.1016/j.ajo.2020.08.004
4. Talib M, van Schooneveld MJ, Van Cauwenbergh C, et al. The Spectrum of Structural and Functional Abnormalities in Female Carriers of Pathogenic Variants in the RPGR Gene. *Invest Ophthalmol Vis Sci*. Aug 1 2018;59(10):4123-4133. doi:10.1167/iovs.17-23453
5. Jackson H, Garway-Heath D, Rosen P, Bird AC, Tuft SJ. Outcome of cataract surgery in patients with retinitis pigmentosa. *British Journal of Ophthalmology*. 2001;85(8):936. doi:10.1136/bjo.85.8.936
6. Fujiwara K, Ikeda Y, Murakami Y, et al. Risk Factors for Posterior Subcapsular Cataract in Retinitis Pigmentosa. *Invest Ophthalmol Vis Sci*. 2017;58(5):2534-2537. doi:10.1167/iovs.17-21612
7. Yoshida N, Ikeda Y, Murakami Y, et al. Factors Affecting Visual Acuity after Cataract Surgery in Patients with Retinitis Pigmentosa. *Ophthalmology*. 2015/05/01/ 2015;122(5):903-908. doi:https://doi.org/10.1016/j.ophtha.2014.12.003
8. Liew G, Strong S, Bradley P, et al. Prevalence of cystoid macular oedema, epiretinal membrane and cataract in retinitis pigmentosa. *British Journal of Ophthalmology*. 2019;103(8):1163. doi:10.1136/bjophthalmol-2018-311964
9. Yoshida N, Ikeda Y, Notomi S, et al. Clinical Evidence of Sustained Chronic Inflammatory Reaction in Retinitis Pigmentosa. *Ophthalmology*. 2013/01/01/ 2013;120(1):100-105. doi:https://doi.org/10.1016/j.ophtha.2012.07.006
10. Lu B, Yin H, Tang Q, et al. Multiple cytokine analyses of aqueous humor from the patients with retinitis pigmentosa. *Cytokine*. 2020/03/01/ 2020;127:154943. doi:https://doi.org/10.1016/j.cyto.2019.154943
11. Chua BE, Mitchell P, Cumming RG. Effects of cataract type and location on visual function: The Blue Mountains Eye Study. *Eye*. 2004/08/01 2004;18(8):765-772. doi:10.1038/sj.eye.6701366
12. Allen D, Vasavada A. Cataract and surgery for cataract. *BMJ*. 2006;333(7559):128-132. doi:10.1136/bmj.333.7559.128
13. Skiadaresi E, McAlinden C, Pesudovs K, Polizzi S, Khadka J, Ravalico G. Subjective Quality of Vision Before and After Cataract Surgery. *Archives of Ophthalmology*. 2012;130(11):1377-1382. doi:10.1001/archophthalmol.2012.1603
14. van Bree MCJ, Pierrache L, Zijlmans BLM, Reus NJ, van den Born LI, van den Berg T. Straylight as an Indicator for Cataract Extraction in Patients with Retinal Dystrophy. *Ophthalmol Retina*. Nov-Dec 2017;1(6):531-544. doi:10.1016/j.oret.2017.02.010
15. van der Meulen IJ, Gjertsen J, Kruijt B, et al. Straylight measurements as an indication for cataract surgery. *J Cataract Refract Surg*. May 2012;38(5):840-8. doi:10.1016/j.jcrs.2011.11.048
16. Lundström M, Barry P, Henry Y, Rosen P, Stenevi U. Visual outcome of cataract surgery; Study from the European Registry of Quality Outcomes for Cataract and Refractive Surgery. *Journal of Cataract & Refractive Surgery*. 2013;39(5)

17. Wang J, Su F, Wang Y, Chen Y, Chen Q, Li F. Intra and post-operative complications observed with femtosecond laser-assisted cataract surgery versus conventional phacoemulsification surgery: a systematic review and meta-analysis. *BMC Ophthalmol*. 2019;19(1):177-177. doi:10.1186/s12886-019-1190-2

18. Antonio-Aguirre B, Swenor B, Canner JK, Singh MS. Risk of cystoid macular edema following cataract surgery in retinitis pigmentosa: an analysis of United States claims from 2010 to 2018. *Ophthalmology Retina*. 2022;doi:10.1016/j.oret.2022.04.018

19. Dikopf MS, Chow CC, Mieler WF, Tu EY. Cataract Extraction Outcomes and the Prevalence of Zonular Insufficiency in Retinitis Pigmentosa. *American Journal of Ophthalmology*. 2013/07/01/ 2013;156(1):82-88.e2. doi:https://doi.org/10.1016/j.ajo.2013.02.002

20. Davies EC, Pineda R. Cataract surgery outcomes and complications in retinal dystrophy patients. *Canadian Journal of Ophthalmology*. 2017/12/01/ 2017;52(6):543-547. doi:https://doi.org/10.1016/j.jcjo.2017.04.002

21. Bayyoud T, Bartz-Schmidt KU, Yoeruek E. Long-term clinical results after cataract surgery with and without capsular tension ring in patients with retinitis pigmentosa: a retrospective study. *BMJ Open*. 2013;3(4):e002616. doi:10.1136/bmjopen-2013-002616

22. Jin-Poi T, Shatriah I, Khairy-Shamel ST, Zunaina E. Rapid anterior capsular contraction after phacoemulsification surgery in a patient with retinitis pigmentosa. *Clin Ophthalmol*. 2013;7:839-842. doi:10.2147/OPTH.S42122

23. Hong Y, Li H, Sun Y, Ji Y. A Review of Complicated Cataract in Retinitis Pigmentosa: Pathogenesis and Cataract Surgery. *Journal of Ophthalmology*. 2020/12/21 2020;2020:6699103. doi:10.1155/2020/6699103

24. Nguyen X-T-A, Thiadens AAHJ, Fiocco M, et al. Outcome of cataract surgery in patients with retinitis pigmentosa. *American Journal of Ophthalmology*. 2022;doi:10.1016/j.ajo.2022.10.001

25. De Rojas JO, Schuerch K, Mathews PM, et al. Evaluating Structural Progression of Retinitis Pigmentosa After Cataract Surgery. *American Journal of Ophthalmology*. 2017;180:117-123. doi:10.1016/j.ajo.2017.05.026

26. Chan TCY, Lam SC, Mohamed S, Wong RLM. Survival analysis of visual improvement after cataract surgery in advanced retinitis pigmentosa. *Eye*. 2017/12/01 2017;31(12):1747-1748. doi:10.1038/eye.2017.164

27. Nakamura Y, Mitamura Y, Hagiwara A, et al. Relationship between retinal microstructures and visual acuity after cataract surgery in patients with retinitis pigmentosa. *British Journal of Ophthalmology*. 2015;99(4):508. doi:10.1136/bjophthalmol-2013-304819

28. Chen CX, Wang JD, Zhang JS, et al. Effect of lens capsular tension ring on preventing capsular contraction syndrome in the surgery of retinitis pigmentosa combined with cataract: Retrospective case series. https://doi.org/10.1111/ijcp.14272. *International Journal of Clinical Practice*. 2021/08/01 2021;75(8):e14272. doi:https://doi.org/10.1111/ijcp.14272

29. Garcia-Martin E, Rodriguez-Mena D, Dolz I, et al. Influence of cataract surgery on optical coherence tomography and neurophysiology measurements in patients with retinitis pigmentosa. *Am J Ophthalmol*. Aug 2013;156(2):293-303.e2. doi:10.1016/j.ajo.2013.03.019

30. Mao J, Fang D, Chen Y, et al. Prediction of Visual Acuity After Cataract Surgery Using Optical Coherence Tomography Findings in Eyes With Retinitis Pigmentosa. *Ophthalmic Surg Lasers Imaging Retina*. Aug 1 2018;49(8):587-594. doi:10.3928/23258160-20180803-06

31. Chatterjee S, Agrawal D, Agrawal D, Parchand SM, Sahu A. Cataract surgery in retinitis pigmentosa. *Indian J Ophthalmol*. Jul 2021;69(7):1753-1757. doi:10.4103/ijo.IJO_2916_20

32. Miura G, Baba T, Tatsumi T, Yokouchi H, Yamamoto S. The Impact of Cataract Surgery on Contrast Visual Acuity and Retinal Sensitivity in Patients with Retinitis Pigmentosa. *J Ophthalmol*. 2021;2021:2281834. doi:10.1155/2021/2281834
33. Lu QJ, Bi J, Dd BH, Wang D, Liu Q. Efficacy analysis of phacoemulsification combined with intraocular lens implantation in the treatment of retinitis pigmentosa complicated with cataract. *Recent Advances in Ophthalmology*. 2017:1064-1067.
34. He H, Song H, Meng X, et al. Effects and Prognosis of Cataract Surgery in Patients with Retinitis Pigmentosa. *Ophthalmology and Therapy*. 2022/09/04 2022;doi:10.1007/s40123-022-00563-2
35. Nakamura S, Fujiwara K, Yoshida N, et al. Long-term Outcomes of Cataract Surgery in Patients with Retinitis Pigmentosa. *Ophthalmol Retina*. Apr 2022;6(4):268-272. doi:10.1016/j.oret.2021.12.010
36. Sakai D, Takagi S, Hirami Y, Nakamura M, Kurimoto Y. Use of ellipsoid zone width for predicting visual prognosis after cataract surgery in patients with retinitis pigmentosa. *Eye (Lond)*. Jan 1 2022;doi:10.1038/s41433-021-01878-3
37. Leung TW, Li RW-H, Kee C-S. Blue-Light Filtering Spectacle Lenses: Optical and Clinical Performances. *PLoS One*. 2017;12(1):e0169114-e0169114. doi:10.1371/journal.pone.0169114
38. Masket S, Bostanci Ceran B, Fram NR. Spontaneous dislocation of posterior chamber intraocular lenses (PC IOLs) in patients with retinitis pigmentosa - Case series. *Saudi J Ophthalmol*. Jan 2012;26(1):61-5. doi:10.1016/j.sjopt.2011.09.003
39. Sudhir RR, Rao SK. Capsulorhexis phimosis in retinitis pigmentosa despite capsular tension ring implantation. *J Cataract Refract Surg*. Oct 2001;27(10):1691-4. doi:10.1016/s0886-3350(01)00869-0
40. Najjar DM, Igbre AO, Tsai FF. Late capsular bag contraction and intraocular lens subluxation in retinitis pigmentosa: a case report. *J Med Case Rep*. Feb 14 2011;5:65. doi:10.1186/1752-1947-5-65
41. Karahan E, Er D, Kaynak S. An Overview of Nd:YAG Laser Capsulotomy. *Med Hypothesis Discov Innov Ophthalmol*. Summer 2014;3(2):45-50.
42. Strong S, Liew G, Michaelides M. Retinitis pigmentosa-associated cystoid macular oedema: pathogenesis and avenues of intervention. *Br J Ophthalmol*. 2017;101(1):31-37. doi:10.1136/bjophthalmol-2016-309376
43. Kim YJ, Joe SG, Lee DH, Lee JY, Kim JG, Yoon YH. Correlations between spectral-domain OCT measurements and visual acuity in cystoid macular edema associated with retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. Feb 1 2013;54(2):1303-9. doi:10.1167/iovs.12-10149
44. Bakthavatchalam M, Lai FHP, Rong SS, Ng DS, Brelen ME. Treatment of cystoid macular edema secondary to retinitis pigmentosa: a systematic review. *Surv Ophthalmol*. May-Jun 2018;63(3):329-339. doi:10.1016/j.survophthal.2017.09.009
45. Hajali M, Fishman GA, Anderson RJ. The prevalence of cystoid macular oedema in retinitis pigmentosa patients determined by optical coherence tomography. *Br J Ophthalmol*. Aug 2008;92(8):1065-8. doi:10.1136/bjo.2008.138560
46. Vingolo EM, Valente S, Gerace E, Spadea L, Nebbioso M. Macular hole in retinitis pigmentosa patients: microincision vitrectomy with polydimethylsiloxane as possible treatment. *Eye (London, England)*. 2015;29(5):699-702. doi:10.1038/eye.2014.344
47. Hirakawa H, Iijima H, Gohdo T, Tsukahara S. Optical coherence tomography of cystoid macular edema associated with retinitis pigmentosa. *American Journal of Ophthalmology*. 1999/08/01/ 1999;128(2):185-191. doi:https://doi.org/10.1016/S0002-9394(99)00100-2
48. Fishman GA, Fishman M, Maggiano J. Macular lesions associated with retinitis pigmentosa. *Arch Ophthalmol*. May 1977;95(5):798-803. doi:10.1001/archopht.1977.04450050076006
49. Ffytche TJ. Cystoid maculopathy in retinitis pigmentosa. *Trans Ophthalmol Soc U K*. 1972;92:265-83.

50. Shahidi M, Fishman G, Ogura Y, Ambroz K, Zeimer R. Foveal thickening in retinitis pigmentosa patients with cystoid macular edema. *Retina*. 1994;14(3):243-7. doi:10.1097/00006982-199414030-00009

51. Apushkin MA, Fishman GA, Janowicz MJ. Monitoring cystoid macular edema by optical coherence tomography in patients with retinitis pigmentosa. *Ophthalmology*. Oct 2004;111(10):1899-904. doi:10.1016/j.ophtha.2004.04.019

52. Nussenblatt RB, Kaufman SC, Palestine AG, Davis MD, Ferris FL, 3rd. Macular thickening and visual acuity. Measurement in patients with cystoid macular edema. *Ophthalmology*. Sep 1987;94(9):1134-9. doi:10.1016/s0161-6420(87)33314-7

53. Yeo JH, Kim YJ, Yoon YH. OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY IN PATIENTS WITH RETINITIS PIGMENTOSA–ASSOCIATED CYSTOID MACULAR EDEMA. *RETINA*. 2020;40(12)

54. Makiyama Y, Oishi A, Otani A, et al. Prevalence and spatial distribution of cystoid spaces in retinitis pigmentosa: investigation with spectral domain optical coherence tomography. *Retina*. May 2014;34(5):981-8. doi:10.1097/iae.0000000000000010

55. Kjellström U. Reduced macular function in ABCA4 carriers. *Mol Vis*. 2015;21:767-82.

56. Burgess R, Millar ID, Leroy BP, et al. Biallelic mutation of BEST1 causes a distinct retinopathy in humans. *Am J Hum Genet*. Jan 2008;82(1):19-31. doi:10.1016/j.ajhg.2007.08.004

57. Grover S, Fishman GA, Fiscella RG, Adelman AE. Efficacy of dorzolamide hydrochloride in the management of chronic cystoid macular edema in patients with retinitis pigmentosa. *Retina*. 1997;17(3):222-31. doi:10.1097/00006982-199705000-00009

58. Fishman GA, Gilbert LD, Fiscella RG, Kimura AE, Jampol LM. Acetazolamide for treatment of chronic macular edema in retinitis pigmentosa. *Arch Ophthalmol*. Oct 1989;107(10):1445-52. doi:10.1001/archopht.1989.01070020519031

59. Orzalesi N, Pierrottet C, Porta A, Aschero M. Long-term treatment of retinitis pigmentosa with acetazolamide: A pilot study. *Graefe's archive for clinical and experimental ophthalmology*. 1993;231:254-256.

60. Liew G, Moore AT, Webster AR, Michaelides M. Efficacy and Prognostic Factors of Response to Carbonic Anhydrase Inhibitors in Management of Cystoid Macular Edema in Retinitis Pigmentosa. *Invest Ophthalmol Vis Sci*. 2015;56(3):1531-1536. doi:10.1167/iovs.14-15995

61. Wolfensberger TJ. The role of carbonic anhydrase inhibitors in the management of macular edema. *Documenta Ophthalmologica*. 1999/01/01 1999;97(3):387-397. doi:10.1023/A:1002143802926

62. Huang Q, Chen R, Lin X, Xiang Z. Efficacy of carbonic anhydrase inhibitors in management of cystoid macular edema in retinitis pigmentosa: A meta-analysis. *PLoS One*. 2017;12(10):e0186180-e0186180. doi:10.1371/journal.pone.0186180

63. Grover S, Apushkin MA, Fishman GA. Topical dorzolamide for the treatment of cystoid macular edema in patients with retinitis pigmentosa. *Am J Ophthalmol*. May 2006;141(5):850-8. doi:10.1016/j.ajo.2005.12.030

64. Moldow B, Sander B, Larsen M, Lund-Andersen H. Effects of acetazolamide on passive and active transport of fluorescein across the normal BRB. *Invest Ophthalmol Vis Sci*. Jul 1999;40(8):1770-5.

65. Lichter PR. Reducing Side Effects of Carbonic Anhydrase Inhibitors. *Ophthalmology*. 1981/03/01/1981;88(3):266-269. doi:https://doi.org/10.1016/S0161-6420(81)35040-4

66. Schmickl CN, Owens RL, Orr JE, Edwards BA, Malhotra A. Side effects of acetazolamide: a systematic review and meta-analysis assessing overall risk and dose dependence. *BMJ Open Respir Res*. Apr 2020;7(1)doi:10.1136/bmjresp-2020-000557

67. Ahlstrand C, Tiselius HG. Urine composition and stone formation during treatment with acetazolamide. *Scand J Urol Nephrol*. 1987;21(3):225-8. doi:10.3109/00365598709180326

68. Kass MA, Kolker AE, Gordon M, et al. Acetazolamide and Urolithiasis. *Ophthalmology*. 1981;88(3):261-265. doi:10.1016/S0161-6420(81)35041-6

69. Genead MA, Fishman GA. Efficacy of sustained topical dorzolamide therapy for cystic macular lesions in patients with retinitis pigmentosa and usher syndrome. *Arch Ophthalmol*. Sep 2010;128(9):1146-50. doi:10.1001/archophthalmol.2010.172

70. Ikeda Y, Yoshida N, Notomi S, et al. Therapeutic effect of prolonged treatment with topical dorzolamide for cystoid macular oedema in patients with retinitis pigmentosa. *Br J Ophthalmol*. Sep 2013;97(9):1187-91. doi:10.1136/bjophthalmol-2012-303005

71. Apushkin MA, Fishman GA, Grover S, Janowicz MJ. Rebound of cystoid macular edema with continued use of acetazolamide in patients with retinitis pigmentosa. *Retina*. Oct 2007;27(8):1112-8. doi:10.1097/IAE.0b013e31805f6b79

72. Fishman GA, Apushkin MA. Continued use of dorzolamide for the treatment of cystoid macular oedema in patients with retinitis pigmentosa. *Br J Ophthalmol*. Jun 2007;91(6):743-5. doi:10.1136/bjo.2006.107466

73. Ozdemir H, Karacorlu M, Karacorlu S. Intravitreal triamcinolone acetonide for treatment of cystoid macular oedema in patients with retinitis pigmentosa. *Acta Ophthalmol Scand*. Apr 2005;83(2):248-51. doi:10.1111/j.1600-0420.2005.00395.x

74. Srour M, Querques G, Leveziel N, et al. Intravitreal dexamethasone implant (Ozurdex) for macular edema secondary to retinitis pigmentosa. *Graefes Arch Clin Exp Ophthalmol*. Jun 2013;251(6):1501-6. doi:10.1007/s00417-012-2249-4

75. Strong SA, Gurbaxani A, Michaelides M. Treatment of Retinitis Pigmentosa-Associated Cystoid Macular Oedema Using Intravitreal Aflibercept (Eylea) despite Minimal Response to Ranibizumab (Lucentis): A Case Report. *Case Rep Ophthalmol*. May-Aug 2016;7(2):389-397. doi:10.1159/000448427

76. Apte RS, Chen DS, Ferrara N. VEGF in Signaling and Disease: Beyond Discovery and Development. *Cell*. Mar 7 2019;176(6):1248-1264. doi:10.1016/j.cell.2019.01.021

77. Melincovici CS, Boşca AB, Şuşman S, et al. Vascular endothelial growth factor (VEGF) - key factor in normal and pathological angiogenesis. *Rom J Morphol Embryol*. 2018;59(2):455-467.

78. Veritti D, Sarao V, De Nadai K, et al. Dexamethasone Implant Produces Better Outcomes than Oral Acetazolamide in Patients with Cystoid Macular Edema Secondary to Retinitis Pigmentosa. *J Ocul Pharmacol Ther*. Apr 2020;36(3):190-197. doi:10.1089/jop.2018.0153

79. Ahn SJ, Kim KE, Woo SJ, Park KH. The effect of an intravitreal dexamethasone implant for cystoid macular edema in retinitis pigmentosa: a case report and literature review. *Ophthalmic Surg Lasers Imaging Retina*. Mar-Apr 2014;45(2):160-4. doi:10.3928/23258160-20140131-03

80. Sudhalkar A, Kodjikian L, Borse N. Intravitreal dexamethasone implant for recalcitrant cystoid macular edema secondary to retinitis pigmentosa: a pilot study. *Graefes Arch Clin Exp Ophthalmol*. Jul 2017;255(7):1369-1374. doi:10.1007/s00417-017-3660-7

81. Park UC, Park JH, Ma DJ, Cho IH, Oh BL, Yu HG. A RANDOMIZED PAIRED-EYE TRIAL OF INTRAVITREAL DEXAMETHASONE IMPLANT FOR CYSTOID MACULAR EDEMA IN RETINITIS PIGMENTOSA. *Retina*. Jul 2020;40(7):1359-1366. doi:10.1097/iae.0000000000002589

82. Celik N, Khoramnia R, Auffarth GU, Sel S, Mayer CS. Complications of dexamethasone implants: risk factors, prevention, and clinical management. *Int J Ophthalmol*. 2020;13(10):1612-1620. doi:10.18240/ijo.2020.10.16

83. Hagiwara A, Yamamoto S, Ogata K, et al. Macular abnormalities in patients with retinitis pigmentosa: prevalence on OCT examination and outcomes of vitreoretinal surgery. *Acta Ophthalmol*. Mar 2011;89(2):e122-5. doi:10.1111/j.1755-3768.2010.01866.x

84. Fujiwara K, Ikeda Y, Murakami Y, et al. Association Between Aqueous Flare and Epiretinal Membrane in Retinitis Pigmentosa. *Invest Ophthalmol Vis Sci*. 2016;57(10):4282-4286. doi:10.1167/iovs.16-19686

85. Ikeda Y, Yoshida N, Murakami Y, et al. Long-term Surgical Outcomes of Epiretinal Membrane in Patients with Retinitis Pigmentosa. *Sci Rep*. Aug 13 2015;5:13078. doi:10.1038/srep13078

86. Jin Z-B, Gan D-K, Xu G-Z, Nao-I N. Macular Hole Formation in Patients With Retinitis Pigmentosa and Prognosis of Pars Plana Vitrectomy. *RETINA*. 2008;28(4)

87. Rao PK, Shah G, Blinder KJ. Bilateral macular hole formation in a patient with retinitis pigmentosa. *Ophthalmic Surg Lasers*. Mar-Apr 2002;33(2):152-4.

88. García-Fernández M, Castro-Navarro J, Bajo-Fuente A. Unilateral recurrent macular hole in a patient with retinitis pigmentosa: a case report. *J Med Case Rep*. Mar 14 2013;7:69. doi:10.1186/1752-1947-7-69

89. Chan WO, Brennan N, Webster AR, Michaelides M, Muqit MMK. Retinal detachment in retinitis pigmentosa. *BMJ Open Ophthalmol*. 2020;5(1):e000454. doi:10.1136/bmjophth-2020-000454

90. Dave VP, Jalali S, Nayaka A, Pappuru RR, Pathengay A, Das T. CLINICAL PRESENTATIONS AND OUTCOMES OF RHEGMATOGENOUS RETINAL DETACHMENT IN RETINITIS PIGMENTOSA. *Retina*. Jul 2016;36(7):1345-8. doi:10.1097/iae.0000000000000910

91. Rishi E, Rishi P, Bhende M, et al. Retinal Detachment in 31 Eyes with Retinitis Pigmentosa. *Ophthalmol Retina*. Jan 2018;2(1):10-16. doi:10.1016/j.oret.2017.03.012

92. Dutta Majumder P, Menia N, Roy R, et al. Uveitis in Patients with Retinitis Pigmentosa: 30 Years' Consecutive Data. *Ocul Immunol Inflamm*. 2018;26(8):1283-1288. doi:10.1080/09273948.2017.1348527

93. Li AS, Pasricha MV, Mishra K, Nguyen QD, Beres SJ, Wood EH. CRB1-associated retinal dystrophy presenting as self-resolving opsoclonus and posterior uveitis. *Am J Ophthalmol Case Rep*. 2022;26:101444-101444. doi:10.1016/j.ajoc.2022.101444

94. Murro V, Mucciolo DP, Sodi A, et al. Retinal capillaritis in a CRB1-associated retinal dystrophy. *Ophthalmic Genet*. Dec 2017;38(6):555-558. doi:10.1080/13816810.2017.1281966

95. Verhagen F, Kuiper J, Nierkens S, Imhof SM, Radstake T, de Boer J. Systemic inflammatory immune signatures in a patient with CRB1 linked retinal dystrophy. *Expert Rev Clin Immunol*. Dec 2016;12(12):1359-1362. doi:10.1080/1744666x.2016.1241709

96. Duncker T, Lee W, Jiang F, et al. ACUTE ZONAL OCCULT OUTER RETINOPATHY: Structural and Functional Analysis Across the Transition Zone Between Healthy and Diseased Retina. *Retina*. Jan 2018;38(1):118-127. doi:10.1097/iae.0000000000001513

97. Willermain F, Greiner K, Forrester JV. Atypical end-stage birdshot retinochoroidopathy. *Ocul Immunol Inflamm*. Dec 2003;11(4):305-7. doi:10.1076/ocii.11.4.305.18271

98. Chowers I, Zamir E, Banin E, Merin S. Retinitis pigmentosa associated with Fuchs' heterochromic uveitis. *Arch Ophthalmol*. Jun 2000;118(6):800-2. doi:10.1001/archopht.118.6.800

99. Díez-Cattini GF, Ancona-Lezama DA, Valdés-Lara C, Morales-Cantón V. The unusual association of inverse retinitis pigmentosa and Fuchs' heterochromic iridocyclitis. *Int J Retina Vitreous*. 2017;3:3. doi:10.1186/s40942-016-0056-5

100. van den Born LI, van Schooneveld MJ, de Jong PT, Bleeker-Wagemakers EM. Fuchs' heterochromic uveitis associated with retinitis pigmentosa in a father and son. *Br J Ophthalmol*. Jun 1994;78(6):504-5. doi:10.1136/bjo.78.6.504

101. Vuorre I, Saari M, Tiilikainen A, Rasanen O. Fuchs' heterochromic cyclitis associated with retinitis pigmentosa: a family study. *Can J Ophthalmol*. Jan 1979;14(1):10-6.

102. Yalvaç IS, Altintas AK, Gökdere A, Duman S. Fuchs' heterochromic uveitis associated with retinitis pigmentosa. *Acta Ophthalmol Scand*. Apr 1998;76(2):243-4. doi:10.1034/j.1600-0420.1998.760225.x

103. Sandinha T. Retinitis pigmentosa associated with Fuchs' heterochromic uveitis. *Eye*. 2003/08/01 2003;17(6):778-779. doi:10.1038/sj.eye.6700461

104. Lichtinger A, Chowers I, Amer R. Usher syndrome associated with Fuchs' heterochromic uveitis. *Graefes Arch Clin Exp Ophthalmol*. Oct 2010;248(10):1481-5. doi:10.1007/s00417-010-1429-3

105. Sevgi DD, Davoudi S, Comander J, Sobrin L. Retinal pigmentary changes in chronic uveitis mimicking retinitis pigmentosa. *Graefes Arch Clin Exp Ophthalmol*. Sep 2017;255(9):1801-1810. doi:10.1007/s00417-017-3689-7

106. Hettinga YM, van Genderen MM, Wieringa W, Ossewaarde-van Norel J, de Boer JH. Retinal Dystrophy in 6 Young Patients Who Presented with Intermediate Uveitis. *Ophthalmology*. Sep 2016;123(9):2043-6. doi:10.1016/j.ophtha.2016.03.046

107. Szabó E, Brichová M, Lišková P, Svozílková P, Říhová E. [Retinitis pigmentosa mimicking uveitis. A case report]. *Cesk Slov Oftalmol*. Mar 2013;69(1):32-6. Benigní maskující syndrom u pacientky s retinitis pigmentosa.

108. Latorre RH, Fernandez-perez S, Garcia-martin E, et al. Bilateral intermediate uveitis asociated with retinosis pigmentosa. *Acta Ophthalmologica*. 2012;249(90):0-0.

109. Kaufman M, Medina-Mendez C, Friberg T, Eller A. Evaluation of peripheral retinal vasculitis in retinitis pigmentosa using wide-field fluorescein angiography. *Invest Ophthalmol Vis Sci*. 2013;54(15):4018-4018.

110. Badeeb O, Trope G, Musarella M. Primary angle closure glaucoma and retinitis pigmentosa. *Acta Ophthalmol (Copenh)*. Dec 1993;71(6):727-32. doi:10.1111/j.1755-3768.1993.tb08591.x

111. Hung M-C, Chen Y-Y. Association between retinitis pigmentosa and an increased risk of primary angle closure glaucoma: A population-based cohort study. *PLoS One*. 2022;17(9):e0274066. doi:10.1371/journal.pone.0274066

112. Pradhan C, Khadka S, Joshi P. Angle Closure Glaucoma in Retinitis Pigmentosa. *Case Rep Ophthalmol Med*. 2020;2020:6023586. doi:10.1155/2020/6023586

113. Lai J, Choy BN, Shum JW. Management of Primary Angle-Closure Glaucoma. *Asia Pac J Ophthalmol (Phila)*. Jan-Feb 2016;5(1):59-62. doi:10.1097/apo.0000000000000180

114. Slade A, Isa F, Kyte D, et al. Patient reported outcome measures in rare diseases: a narrative review. *Orphanet J Rare Dis*. Apr 23 2018;13(1):61. doi:10.1186/s13023-018-0810-x

115. Wilkinson ME, Shahid KS. Low vision rehabilitation: An update. *Saudi Journal of Ophthalmology*. 2018/04/01/ 2018;32(2):134-138. doi:https://doi.org/10.1016/j.sjopt.2017.10.005

116. Langelaan M, de Boer MR, van Nispen RM, Wouters B, Moll AC, van Rens GH. Change in quality of life after rehabilitation: prognostic factors for visually impaired adults. *Int J Rehabil Res*. Mar 2009;32(1):12-9. doi:10.1097/MRR.0b013e3283063503

117. van Nispen RMA, Virgili G, Hoeben M, et al. Low vision rehabilitation for better quality of life in visually impaired adults. *Cochrane Database of Systematic Reviews*. 2020;(1)doi:10.1002/14651858.CD006543.pub2

118. Owsley C, McGwin G, Jr., Lee PP, Wasserman N, Searcey K. Characteristics of Low-Vision Rehabilitation Services in the United States. *Archives of Ophthalmology*. 2009;127(5):681-689. doi:10.1001/archophthalmol.2009.55

119. Lamoureux EL, Pallant JF, Pesudovs K, Rees G, Hassell JB, Keeffe JE. The Effectiveness of Low-Vision Rehabilitation on Participation in Daily Living and Quality of Life. *Invest Ophthalmol Vis Sci*. 2007;48(4):1476-1482. doi:10.1167/iovs.06-0610

120. Stelmack JA, Tang XC, Wei Y, Massof RW, Low-Vision Intervention Trial Study Group ft. The Effectiveness of Low-Vision Rehabilitation in 2 Cohorts Derived From the Veterans Affairs Low-Vision Intervention Trial. *Archives of Ophthalmology*. 2012;130(9):1162-1168. doi:10.1001/archophthalmol.2012.1820

121. Massof RW, Ahmadian L, Grover LL, et al. The Activity Inventory: an adaptive visual function questionnaire. *Optometry and vision science : official publication of the American Academy of Optometry*. 2007;84(8):763-774. doi:10.1097/OPX.0b013e3181339efd

122. Mangione CM, Lee PP, Gutierrez PR, et al. Development of the 25-list-item National Eye Institute Visual Function Questionnaire. *Archives of Ophthalmology*. 2001;119(7):1050-1058. doi:10.1001/archopht.119.7.1050

123. Bruijning J, van Nispen R, Verstraten P, van Rens G. A Dutch ICF Version of the Activity Inventory: Results from Focus Groups with Visually Impaired Persons and Experts. *Ophthalmic Epidemiology*. 2010/12/01 2010;17(6):366-377. doi:10.3109/09286586.2010.528133

124. Lacy GD, Abalem MF, Andrews CA, et al. The Michigan Retinal Degeneration Questionnaire: A Patient-Reported Outcome Instrument for Inherited Retinal Degenerations. *Am J Ophthalmol*. Feb 2021;222:60-68. doi:10.1016/j.ajo.2020.08.032

125. Virgili G, Acosta R, Bentley SA, Giacomelli G, Allcock C, Evans JR. Reading aids for adults with low vision. *Cochrane Database of Systematic Reviews*. 2018;(4)doi:10.1002/14651858.CD003303.pub4

126. Nguyen X-T-A, Koopman J, van Genderen MM, Stam HLM, Boon CJF. Artificial vision: the effectiveness of the OrCam in patients with advanced inherited retinal dystrophies. https://doi.org/10.1111/aos.15001. *Acta Ophthalmologica*. 2021/09/26 2021;n/a(n/a)doi:https://doi.org/10.1111/aos.15001

127. Lorenzini M-C, Wittich W. Factors related to the use of magnifying low vision aids: a scoping review. *Disability and Rehabilitation*. 2020/11/19 2020;42(24):3525-3537. doi:10.1080/09638288.2019.1593519

128. Wittich W, Lorenzini M-C, Markowitz SN, et al. The Effect of a Head-mounted Low Vision Device on Visual Function. *Optometry and vision science : official publication of the American Academy of Optometry*. 2018;95(9):774-784. doi:10.1097/OPX.0000000000001262

129. Markowitz M. Occupational therapy interventions in low vision rehabilitation. *Can J Ophthalmol*. Jun 2006;41(3):340-7. doi:10.1139/i06-020

130. Scott AW, Bressler NM, Ffolkes S, Wittenborn JS, Jorkasky J. Public Attitudes About Eye and Vision Health. *JAMA Ophthalmol*. Oct 1 2016;134(10):1111-1118. doi:10.1001/jamaophthalmol.2016.2627

131. Demmin DL, Silverstein SM. Visual Impairment and Mental Health: Unmet Needs and Treatment Options. *Clin Ophthalmol*. 2020;14:4229-4251. doi:10.2147/OPTH.S258783

132. Munaw MB, Tegegn MT. Visual impairment and psychological distress among adults attending the University of Gondar tertiary eye care and training center, Northwest Ethiopia: A comparative cross-sectional study. *PLoS One*. 2022;17(2):e0264113. doi:10.1371/journal.pone.0264113

133. Horowitz A, Leonard R, Reinhardt JP. Measuring Psychosocial and Functional Outcomes of a Group Model of Vision Rehabilitation Services for Older Adults. *Journal of Visual Impairment & Blindness*. 2000/05/01 2000;94(5):328-337. doi:10.1177/0145482X0009400509

134. Rees G, Ponczek E, Hassell J, Keeffe JE, Lamoureux EL. Psychological outcomes following interventions for people with low vision: a systematic review. *Expert Review of Ophthalmology*. 2010/06/01 2010;5(3):385-403. doi:10.1586/eop.10.32

135. Veldman MHJ, van der Aa HPA, Bode C, et al. E-nergEYEze, a vision-specific eHealth intervention based on cognitive behavioral therapy and self-management to reduce fatigue in adults with visual impairment: study protocol for a randomized controlled trial. *Trials*. 2021/12/28 2021;22(1):966. doi:10.1186/s13063-021-05935-w

136. Virgili G, Rubin G. Orientation and mobility training for adults with low vision. *The Cochrane database of systematic reviews*. 2010;2010(5):CD003925-CD003925. doi:10.1002/14651858.CD003925.pub3

137. Zijlstra GAR, Ballemans J, Kempen GIJM. Orientation and mobility training for adults with low vision: a new standardized approach. *Clin Rehabil*. 2013;27(1):3-18. doi:10.1177/0269215512445395

138. Acland GM, Aguirre GD, Bennett J, et al. Long-term restoration of rod and cone vision by single dose rAAV-mediated gene transfer to the retina in a canine model of childhood blindness. *Mol Ther*. Dec 2005;12(6):1072-82. doi:10.1016/j.ymthe.2005.08.008

139. Acland GM, Aguirre GD, Ray J, et al. Gene therapy restores vision in a canine model of childhood blindness. *Nat Genet*. May 2001;28(1):92-5. doi:10.1038/ng0501-92

140. Aguirre GD. Concepts and Strategies in Retinal Gene Therapy. *Invest Ophthalmol Vis Sci*. 2017;58(12):5399-5411. doi:10.1167/iovs.17-22978

141. Verbakel SK, van Huet RAC, Boon CJF, et al. Non-syndromic retinitis pigmentosa. *Progress in Retinal and Eye Research*. 2018/03/27/ 2018;66:157-186. doi:https://doi.org/10.1016/j.preteyeres.2018.03.005

142. Li C, Samulski RJ. Engineering adeno-associated virus vectors for gene therapy. *Nature Reviews Genetics*. 2020/04/01 2020;21(4):255-272. doi:10.1038/s41576-019-0205-4

143. Bulcha JT, Wang Y, Ma H, Tai PWL, Gao G. Viral vector platforms within the gene therapy landscape. *Signal Transduction and Targeted Therapy*. 2021/02/08 2021;6(1):53. doi:10.1038/s41392-021-00487-6

144. Cideciyan AV, Sudharsan R, Dufour VL, et al. Mutation-independent rhodopsin gene therapy by knockdown and replacement with a single AAV vector. *Proc Natl Acad Sci U S A*. Sep 4 2018;115(36):E8547-e8556. doi:10.1073/pnas.1805055115

145. DiCarlo JE, Mahajan VB, Tsang SH. Gene therapy and genome surgery in the retina. *J Clin Invest*. Jun 1 2018;128(6):2177-2188. doi:10.1172/jci120429

146. Cideciyan AV, Jacobson SG, Beltran WA, et al. Human retinal gene therapy for Leber congenital amaurosis shows advancing retinal degeneration despite enduring visual improvement. *Proc Natl Acad Sci U S A*. Feb 5 2013;110(6):E517-25. doi:10.1073/pnas.1218933110

147. Pierce EA, Bennett J. The Status of RPE65 Gene Therapy Trials: Safety and Efficacy. *Cold Spring Harb Perspect Med*. 2015;5(9):a017285-a017285. doi:10.1101/cshperspect.a017285

148. Wang X, Yu C, Tzekov RT, Zhu Y, Li W. The effect of human gene therapy for RPE65-associated Leber's congenital amaurosis on visual function: a systematic review and meta-analysis. *Orphanet J Rare Dis*. Feb 14 2020;15(1):49. doi:10.1186/s13023-020-1304-1

149. Botto C, Rucli M, Tekinsoy MD, Pulman J, Sahel JA, Dalkara D. Early and late stage gene therapy interventions for inherited retinal degenerations. *Prog Retin Eye Res*. Jan 2022;86:100975. doi:10.1016/j.preteyeres.2021.100975

150. Leroy BP, Fischer MD, Flannery JG, et al. Gene therapy for inherited retinal disease: long-term durability of effect. *Ophthalmic Res*. Sep 14 2022;doi:10.1159/000526317

151. Gange WS, Sisk RA, Besirli CG, et al. Perifoveal Chorioretinal Atrophy after Subretinal Voretigene Neparvovec-rzyl for RPE65-Mediated Leber Congenital Amaurosis. *Ophthalmol Retina*. Jan 2022;6(1):58-64. doi:10.1016/j.oret.2021.03.016

152. Reichel FF, Seitz I, Wozar F, et al. Development of retinal atrophy after subretinal gene therapy with voretigene neparvovec. *Br J Ophthalmol*. May 24 2022;doi:10.1136/bjophthalmol-2021-321023

153. Peng Y, Tang L, Zhou Y. Subretinal Injection: A Review on the Novel Route of Therapeutic Delivery for Vitreoretinal Diseases. *Ophthalmic Res*. 2017;58(4):217-226. doi:10.1159/000479157

154. Ghoraba HH, Akhavanrezayat A, Karaca I, et al. Ocular Gene Therapy: A Literature Review with Special Focus on Immune and Inflammatory Responses. *Clin Ophthalmol*. 2022;16:1753-1771. doi:10.2147/opth.S364200

155. Boon N, Wijnholds J, Pellissier LP. Research Models and Gene Augmentation Therapy for CRB1 Retinal Dystrophies. *Front Neurosci*. 2020;14:860. doi:10.3389/fnins.2020.00860

156. Bansal M, Acharya S, Sharma S, et al. CRISPR Cas9 based genome editing in inherited retinal dystrophies. *Ophthalmic Genetics*. 2021/07/04 2021;42(4):365-374. doi:10.1080/13816810.2021.1904421

157. Burnight ER, Giacalone JC, Cooke JA, et al. CRISPR-Cas9 genome engineering: Treating inherited retinal degeneration. *Prog Retin Eye Res*. Jul 2018;65:28-49. doi:10.1016/j.preteyeres.2018.03.003

158. Pulman J, Sahel J-A, Dalkara D. New Editing Tools for Gene Therapy in Inherited Retinal Dystrophies. *The CRISPR Journal*. 2022/06/01 2022;5(3):377-388. doi:10.1089/crispr.2021.0141

159. Rasul MF, Hussen BM, Salihi A, et al. Strategies to overcome the main challenges of the use of CRISPR/Cas9 as a replacement for cancer therapy. *Molecular Cancer*. 2022/03/03 2022;21(1):64. doi:10.1186/s12943-021-01487-4

160. Collin RWJ, Garanto A. Applications of antisense oligonucleotides for the treatment of inherited retinal diseases. *Current Opinion in Ophthalmology*. 2017;28(3)

161. Girach A, Audo I, Birch DG, et al. RNA-based therapies in inherited retinal diseases. *Therapeutic Advances in Ophthalmology*. 2022/01/01 2022;14:25158414221134602. doi:10.1177/25158414221134602

162. Collin RW, den Hollander AI, van der Velde-Visser SD, Bennicelli J, Bennett J, Cremers FP. Antisense Oligonucleotide (AON)-based Therapy for Leber Congenital Amaurosis Caused by a Frequent Mutation in CEP290. *Mol Ther Nucleic Acids*. Mar 27 2012;1(3):e14. doi:10.1038/mtna.2012.3

163. Duebel J, Marazova K, Sahel JA. Optogenetics. *Curr Opin Ophthalmol*. May 2015;26(3):226-32. doi:10.1097/icu.0000000000000140

164. Sakai D, Tomita H, Maeda A. Optogenetic Therapy for Visual Restoration. *Int J Mol Sci*. Nov 30 2022;23(23)doi:10.3390/ijms232315041

165. Sahel JA, Boulanger-Scemama E, Pagot C, et al. Partial recovery of visual function in a blind patient after optogenetic therapy. *Nat Med*. Jul 2021;27(7):1223-1229. doi:10.1038/s41591-021-01351-4

166. Shen Y. Stem cell therapies for retinal diseases: from bench to bedside. *J Mol Med (Berl)*. Oct 2020;98(10):1347-1368. doi:10.1007/s00109-020-01960-5

167. Bacakova L, Zarubova J, Travnickova M, et al. Stem cells: their source, potency and use in regenerative therapies with focus on adipose-derived stem cells - a review. *Biotechnol Adv*. Jul-Aug 2018;36(4):1111-1126. doi:10.1016/j.biotechadv.2018.03.011

168. Sharma A, Jaganathan BG. Stem Cell Therapy for Retinal Degeneration: The Evidence to Date. *Biologics*. 2021;15:299-306. doi:10.2147/btt.S290331

169. Tuekprakhon A, Sangkitporn S, Trinavarat A, et al. Intravitreal autologous mesenchymal stem cell transplantation: a non-randomized phase I clinical trial in patients with retinitis pigmentosa. *Stem Cell Res Ther*. Jan 9 2021;12(1):52. doi:10.1186/s13287-020-02122-7

170. Gagliardi G, Ben M'Barek K, Goureau O. Photoreceptor cell replacement in macular degeneration and retinitis pigmentosa: A pluripotent stem cell-based approach. *Prog Retin Eye Res*. Jul 2019;71:1-25. doi:10.1016/j.preteyeres.2019.03.001

171. Siqueira RC. Stem cell therapy in retinal diseases? *Rev Bras Hematol Hemoter*. 2012;34(3):222-6. doi:10.5581/1516-8484.20120054

172. Tang Z, Zhang Y, Wang Y, et al. Progress of stem/progenitor cell-based therapy for retinal degeneration. *J Transl Med*. May 10 2017;15(1):99. doi:10.1186/s12967-017-1183-y

173. Blum B, Benvenisty N. The tumorigenicity of human embryonic stem cells. *Adv Cancer Res*. 2008;100:133-58. doi:10.1016/s0065-230x(08)00005-5

174. Brown C, Agosta P, McKee C, et al. Human primitive mesenchymal stem cell-derived retinal progenitor cells improved neuroprotection, neurogenesis, and vision in rd12 mouse model of retinitis pigmentosa. *Stem Cell Res Ther*. Apr 8 2022;13(1):148. doi:10.1186/s13287-022-02828-w

175. Liu Y, Chen SJ, Li SY, et al. Long-term safety of human retinal progenitor cell transplantation in retinitis pigmentosa patients. *Stem Cell Res Ther*. Sep 29 2017;8(1):209. doi:10.1186/s13287-017-0661-8

176. Singh MS, Park SS, Albini TA, et al. Retinal stem cell transplantation: Balancing safety and potential. *Prog Retin Eye Res*. Mar 2020;75:100779. doi:10.1016/j.preteyeres.2019.100779

177. Musiał-Wysocka A, Kot M, Majka M. The Pros and Cons of Mesenchymal Stem Cell-Based Therapies. *Cell Transplant*. Jul 2019;28(7):801-812. doi:10.1177/0963689719837897

178. Ayton LN, Barnes N, Dagnelie G, et al. An update on retinal prostheses. *Clin Neurophysiol*. Jun 2020;131(6):1383-1398. doi:10.1016/j.clinph.2019.11.029

179. Miyoshi T, Morimoto T, Sawai H, Fujikado T. Spatial Resolution of Suprachoroidal-Transretinal Stimulation Estimated by Recording Single-Unit Activity From the Cat Lateral Geniculate Nucleus. *Front Neurosci*. 2021;15:717429. doi:10.3389/fnins.2021.717429

180. Shim S, Eom K, Jeong J, Kim SJ. Retinal Prosthetic Approaches to Enhance Visual Perception for Blind Patients. *Micromachines (Basel)*. May 24 2020;11(5)doi:10.3390/mi11050535

181. Ayton LN, Blamey PJ, Guymer RH, et al. First-in-human trial of a novel suprachoroidal retinal prosthesis. *PLoS One*. 2014;9(12):e115239. doi:10.1371/journal.pone.0115239

182. Fujikado T, Kamei M, Sakaguchi H, et al. Testing of semichronically implanted retinal prosthesis by suprachoroidal-transretinal stimulation in patients with retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. Jul 1 2011;52(7):4726-33. doi:10.1167/iovs.10-6836

183. White J, Knight L, da Cruz L, et al. Effects of the Argus II Retinal Prosthesis System on the Quality of Life of Patients With Ultra-Low Vision Due to Retinitis Pigmentosa: Protocol for a Single-Arm, Mixed Methods Study. *JMIR Res Protoc*. Jan 20 2021;10(1):e17436. doi:10.2196/17436

184. Chuang AT, Margo CE, Greenberg PB. Retinal implants: a systematic review. *Br J Ophthalmol*. Jul 2014;98(7):852-6. doi:10.1136/bjophthalmol-2013-303708

185. Stronks HC, Dagnelie G. The functional performance of the Argus II retinal prosthesis. *Expert Rev Med Devices*. Jan 2014;11(1):23-30. doi:10.1586/17434440.2014.862494

186. Sieving PA, Caruso RC, Tao W, et al. Ciliary neurotrophic factor (CNTF) for human retinal degeneration: phase I trial of CNTF delivered by encapsulated cell intraocular implants. *Proc Natl Acad Sci U S A*. Mar 7 2006;103(10):3896-901. doi:10.1073/pnas.0600236103

187. Birch DG, Weleber RG, Duncan JL, Jaffe GJ, Tao W. Randomized trial of ciliary neurotrophic factor delivered by encapsulated cell intraocular implants for retinitis pigmentosa. *Am J Ophthalmol*. Aug 2013;156(2):283-292.e1. doi:10.1016/j.ajo.2013.03.021

188. Birch DG, Bennett LD, Duncan JL, Weleber RG, Pennesi ME. Long-term Follow-up of Patients With Retinitis Pigmentosa Receiving Intraocular Ciliary Neurotrophic Factor Implants. *Am J Ophthalmol*. Oct 2016;170:10-14. doi:10.1016/j.ajo.2016.07.013

189. Campochiaro PA, Mir TA. The mechanism of cone cell death in Retinitis Pigmentosa. *Progress in Retinal and Eye Research*. 2018/01/01/ 2018;62:24-37. doi:https://doi.org/10.1016/j.preteyeres.2017.08.004

190. Aït-Ali N, Fridlich R, Millet-Puel G, et al. Rod-derived cone viability factor promotes cone survival by stimulating aerobic glycolysis. *Cell*. May 7 2015;161(4):817-32. doi:10.1016/j.cell.2015.03.023

191. Léveillard T, Sahel JA. Rod-derived cone viability factor for treating blinding diseases: from clinic to redox signaling. *Sci Transl Med*. Apr 7 2010;2(26):26ps16. doi:10.1126/scitranslmed.3000866

192. Campochiaro PA, Iftikhar M, Hafiz G, et al. Oral N-acetylcysteine improves cone function in retinitis pigmentosa patients in phase I trial. *J Clin Invest*. Mar 2 2020;130(3):1527-1541. doi:10.1172/jci132990

193. Kong X, Hafiz G, Wehling D, Akhlaq A, Campochiaro PA. Locus-Level Changes in Macular Sensitivity in Patients with Retinitis Pigmentosa Treated with Oral N-acetylcysteine. *Am J Ophthalmol*. Jan 2021;221:105-114. doi:10.1016/j.ajo.2020.08.002

194. Maccarone R, Tisi A, Passacantando M, Ciancaglini M. Ophthalmic Applications of Cerium Oxide Nanoparticles. *J Ocul Pharmacol Ther*. Jul/Aug 2020;36(6):376-383. doi:10.1089/jop.2019.0105

195. Wong LL, Pye QN, Chen L, Seal S, McGinnis JF. Defining the catalytic activity of nanoceria in the P23H-1 rat, a photoreceptor degeneration model. *PLoS One*. 2015;10(3):e0121977. doi:10.1371/journal.pone.0121977

196. Berson EL, Rosner B, Sandberg MA, et al. A randomized trial of vitamin A and vitamin E supplementation for retinitis pigmentosa. *Arch Ophthalmol*. Jun 1993;111(6):761-72. doi:10.1001/archopht.1993.01090060049022

197. Berson EL, Rosner B, Sandberg MA, et al. Further evaluation of docosahexaenoic acid in patients with retinitis pigmentosa receiving vitamin A treatment: subgroup analyses. *Arch Ophthalmol*. Sep 2004;122(9):1306-14. doi:10.1001/archopht.122.9.1306

198. Berson EL, Weigel-DiFranco C, Rosner B, Gaudio AR, Sandberg MA. Association of Vitamin A Supplementation With Disease Course in Children With Retinitis Pigmentosa. *JAMA Ophthalmology*. 2018;136(5):490-495. doi:10.1001/jamaophthalmol.2018.0590

199. Klaver CCW, Thiadens A. Vitamin A for Children With Retinitis Pigmentosa: An Unresolved Mystery. *JAMA Ophthalmol*. May 1 2018;136(5):496-497. doi:10.1001/jamaophthalmol.2018.0658

200. Sajovic J, Meglič A, Glavač D, Markelj Š, Hawlina M, Fakin A. The Role of Vitamin A in Retinal Diseases. *Int J Mol Sci*. Jan 18 2022;23(3)doi:10.3390/ijms23031014

201. Hoffman DR, Hughbanks-Wheaton DK, Pearson NS, et al. Four-year placebo-controlled trial of docosahexaenoic acid in X-linked retinitis pigmentosa (DHAX trial): a randomized clinical trial. *JAMA Ophthalmol*. Jul 2014;132(7):866-73. doi:10.1001/jamaophthalmol.2014.1634

202. Hoffman DR, Hughbanks-Wheaton DK, Spencer R, et al. Docosahexaenoic Acid Slows Visual Field Progression in X-Linked Retinitis Pigmentosa: Ancillary Outcomes of the DHAX Trial. *Invest Ophthalmol Vis Sci*. 2015;56(11):6646-6653. doi:10.1167/iovs.15-17786

203. Rayapudi S, Schwartz SG, Wang X, Chavis P. Vitamin A and fish oils for retinitis pigmentosa. *Cochrane Database Syst Rev*. Dec 19 2013;2013(12):Cd008428. doi:10.1002/14651858.CD008428.pub2

204. Schwartz SG, Wang X, Chavis P, Kuriyan AE, Abariga SA. Vitamin A and fish oils for preventing the progression of retinitis pigmentosa. *Cochrane Database Syst Rev*. Jun 18 2020;6(6):Cd008428. doi:10.1002/14651858.CD008428.pub3

205. Zhao Y, Feng K, Liu R, Pan J, Zhang L, Lu X. Vitamins and Mineral Supplements for Retinitis Pigmentosa. *Journal of ophthalmology*. 2019;2019:8524607-8524607. doi:10.1155/2019/8524607

206. Massof RW, Fishman GA. How strong is the evidence that nutritional supplements slow the progression of retinitis pigmentosa? *Arch Ophthalmol*. Apr 2010;128(4):493-5. doi:10.1001/archophthalmol.2010.46

207. Sofi F, Sodi A, Franco F, et al. Dietary profile of patients with Stargardt's disease and Retinitis Pigmentosa: is there a role for a nutritional approach? *BMC Ophthalmol*. Jan 22 2016;16:13. doi:10.1186/s12886-016-0187-3

208. Radu RA, Yuan Q, Hu J, et al. Accelerated accumulation of lipofuscin pigments in the RPE of a mouse model for ABCA4-mediated retinal dystrophies following Vitamin A supplementation. *Invest Ophthalmol Vis Sci*. Sep 2008;49(9):3821-9. doi:10.1167/iovs.07-1470

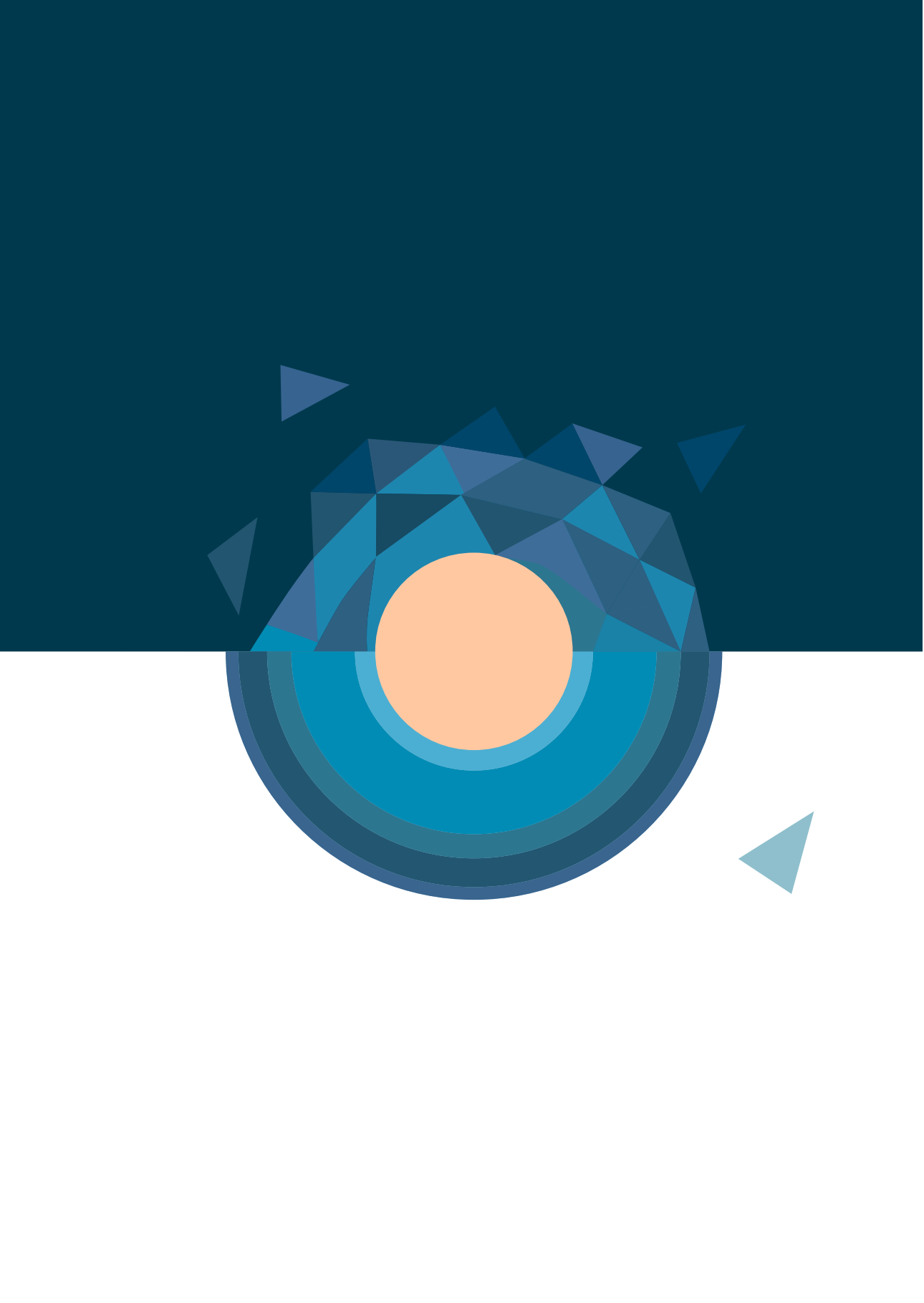
209. Federspiel CA, Bertelsen M, Kessel L. Vitamin A in Stargardt disease-an evidence-based update. *Ophthalmic Genet*. Oct 2018;39(5):555-559. doi:10.1080/13816810.2018.1488174

210. Shintani K, Shechtman DL, Gurwood AS. Review and update: current treatment trends for patients with retinitis pigmentosa. *Optometry*. Jul 2009;80(7):384-401. doi:10.1016/j.optm.2008.01.026

Supplemental Table S1. Overview of studies involving patients with retinitis pigmentosa that underwent surgery for retinal detachments.

	Chan et al. 2020	Dave et al. 2016	Rishi et al. 2018
Sample size	90 patients	17 patients	31 patients
Follow-up	15.4 years	8.52 ± 12.6	33 months (range 1-145)
Mean Age	Mean age 32.8 years	Mean age: 34.5 years	Mean age 22 years
Type of RD	Rhegmatogenous (68%) Exudative (21%) Tractional (3%) Combined (8%)	Rhegmatogenous (100%)	N/A
Intervention	Scleral buckling (3%) PPV (20%) Unknown surgical intervention (39%)* Conservative (28%) Not treated (10%)	Scleral buckling (30%) PPV (35%) Not treated (35%)	Scleral buckling (42%) PPV (32%) Not treated (26%)
Attachment rate	85%	91%	96%
Change in BCVA	1.52 vs 1.25 logMAR (p = 0.098)	1.4 ± 0.9 vs 1.1 ± 0.8 logMAR (p = 0.15)	1.63 ± 0.89 vs 0.87 ± 0.25 logMAR (p < 0.001)
Complications	Re-RD (n = 5) High IOP (n = 3) UGH (n = 1) Scleritis (n = 1) IOL subluxation (n = 1)	N/A	Re-RD (n = 3) Epiretinal membrane (n = 3) cataract (n = 2); Macular hole (n = 1); High IOP (n = 1)

*BCVA = best-corrected visual acuity; PPV = pars plana vitrectomy; IOP = intraocular pressure; UGH = uveitis-glaucoma-hyphaema syndrome; IOL = intraocular lens; RD = retinal detachment; re-RD = retinal re-detachment. *No information was available for the remaining surgical interventions in this study by Chan et al. Patients with exudative retinal detachments were treated conservatively. Patients that were not treated were due to poor visual prognosis.*



APPENDIX

ENGLISH SUMMARY

Chapter 1 provides a comprehensive overview of the anatomy of the eye and the retina, with a particular focus on the phototransduction and visual cycle processes. In addition, this chapter goes into detail on RP and sheds light on our current understanding of the pathophysiology behind this complex retinal disease. Given the clinical and genetic variability associated with RP, it often presents with overlapping symptoms with other IRDs, making an accurate diagnosis challenging. Hence, establishing the correct diagnosis through clinical and genetic testing is essential for effective management of RP. This chapter delves into the different clinical and genetic testing tools that should be utilized to confirm an accurate RP diagnosis, and the implications of genetic testing for family planning.

In **Chapter 2.1**, we evaluated the natural history of *RHO*-associated RP using one of the largest cohorts to date. One of the first genes to be discovered to cause RP was *RHO*, causing an autosomal dominant form of RP. We found that *RHO*-associated RP is associated with a mild form of RP with regards to disease onset, severity and progression. Also, *RHO* is known to cause a very peculiar form of RP called sector RP, which shows minimal to no progression. As it is difficult to assess treatment efficacy in a disease that is slowly progressive, we propose the use of surrogate endpoints, such as the outer retinal thickness for the measurement of treatment efficacy in future trials.

In **Chapter 2.2**, we investigated the clinical phenotype of male *RPGR* affected individuals using modern psychophysical testing tools, such as the microperimetry and FST. This information is pivotal since several clinical gene therapy trials are currently underway for this genetic subtype of RP. *RPGR* variants typically result in a severe, early-onset form of RP with visual impairment at a young age. Our results reveal several different phenotypes, RP, CRD and MD; with the latter two phenotypes associated with the '3 end of *RPGR*, more specifically, exon ORF15. In the retina of a donor patient with a *RPGR* variant, histopathological evaluation showed that inner remodeling may already take place in regions without macroscopic signs of degeneration. As such, it is important for therapies to be applied at the earliest signs of degeneration.

Chapter 2.3 describes the clinical and genetic characteristics of a rare syndromic form of RP called PHARC syndrome, with less than 50 patients described worldwide. It is caused by variants in *ABHD12*, which plays a vital role in lipid metabolism. There was a variable severity in terms of visual acuity, fundus findings and multimodal imaging with no clear genotype-phenotype correlations. We found that from an ophthalmic perspective, patients with PHARC syndrome did not exhibit characteristic signs of RP, such as bone-spicule-like pigmentation, although the diagnosis rod-cone degeneration could be established using ffERG. Early macular involvement was present in patients with PHARC syndrome. Given the variability of symptom onset

of ophthalmic, neurological and audiological symptoms, it will be difficult to initially diagnose PHARC syndrome. A multidisciplinary approach, consisting of neurologists, geneticists, audiologists and ophthalmologists, is required to accurately diagnose PHARC syndrome.

Chapter 2.4 reports the first prospective, multicenter natural history study for patients with *CRB1*-associated retinal dystrophies. Variants in the *CRB1* gene cause a spectrum of IRDs phenotypes, with the most common being early-onset RP. The first proof of concept of gene therapy for *CRB1* has been established in the LUMC, paving the way for a potential treatment for this form of RP. In preparation for future clinical trials, this study evaluated the 2-year clinical course of *CRB1*, in order to determine time-sensitive progression markers. We found that *CRB1* has a variable disease expression, but most commonly results in visual impairment at a young age. A common finding is the thickening of the retina – which is in contrast with other subtypes of RP – which is almost pathognomic for this specific type of RP. We found that conventional parameters, such as visual acuity and visual fields, did not show any progression during follow-up, and may not be suitable to measure treatment efficacy. Significant progression was observed on microperimetry, suggesting that microperimetry is a more sensitive outcome measure in this disease. As ffERG were not detectable in the majority of patients in this study, we employed the use of the FST, which allowed us to measure residual photoreceptor function, and thus FST can be used as treatment efficacy measure, or as inclusion criterion.

In **Chapter 3.1**, we evaluate the visual outcome of cataract surgery in patients with RP, and we discuss potential risk factors. Cataract is one of the most common complications found in patients with RP. Lens opacities, together with ongoing retinal degeneration can aggravate the loss of visual function, and in turn, significantly reduce patient's quality of life. In our study, cataract surgery leads to a significant improvement in visual acuity, and also improvements in subjective visual function in the majority of patients. However, surgeries should be performed with caution, as patients with RP are also relatively higher at risk for complications such as CME, PCO and zonulysis. If necessary precautions are undertaken for this population, cataract surgery can prove a valuable treatment.

In **Chapter 3.2,** we measured the effectiveness of the OrCam MyEye 2.0 on the quality of life in patients with IRDs. The OrCam MyEye is a low vision aid that converts visual stimuli to audio using an optical sensor mounted on a patient's glasses. Using the NEI-VFQ and D-AI, we demonstrated that the OrCam mainly improved activities related to 'near distance', and did not improve other subdomains of the NEI-VFQ or other rehabilitation goals on D-AI. Further improvements are needed in the OrCam to make the device serviceable for a broader audience.

In **Chapter 3.3**, we present a four-year report on the quality of life changes in patients with *CRB1*-associated retinal dystrophies. While objective outcome measures are essential for measuring treatment efficacy in upcoming trials, it's equally important to consider subjective outcome measures that reflect a patient's own experience. Unfortunately, subjective outcome measures are not adequately addressed in current clinical trials. Our study found that patients with *CRB1*-associated IRDs experienced a general decline in vision-related quality of life on the NEI-VFQ, in the absence of treatment. Therefore, we recommend that future clinical trials include patient-reported outcome measures as a relevant endpoint for disease progression and treatment efficacy.

NEDERLANDSE SAMENVATTING

Hoofdstuk 1 biedt een uitgebreid overzicht van de anatomie van het netvlies, met aandacht voor processen zoals de fototransductie en de visuele cyclus. Daarnaast gaat dit hoofdstuk dieper in op retinitis pigmentosa (RP) en de pathofysiologie achter deze complexe netvliesaandoening. Gezien de klinische en genetische variabiliteit van RP, manifesteert RP zich vaak met symptomen die overlappen met andere erfelijke aandoeningen, waardoor een nauwkeurige diagnose uitdagend blijft. Het is daarom essentieel om de juiste diagnose te stellen middels uitgebreide klinische en genetische diagnostiek. Dit hoofdstuk beschrijft de huidige mogelijkheden qua diagnostiek en gaat ook verder in op de implicaties die de testresultaten kunnen hebben voor een patiënt.

In **Hoofdstuk 2.1** hebben we de klinische karakteristieken en natuurlijke beloop van *RHO*-geassocieerde RP geëvalueerd. *RHO* was een van de eerste genen die werd ontdekt als oorzaak van RP en veroorzaakt een autosomaal dominante vorm van RP. *RHO*-geassocieerde RP leidt vaak tot een relatief milde vorm van RP met betrekking tot de ernst en progressie. Bovendien veroorzaakt RHO een unieke vorm van RP genaamd sector RP, die minimale tot geen progressie vertoont. Aangezien het moeilijk is om behandeleffectiviteit te beoordelen bij een ziekte die langzaam progressief is, stellen we het gebruik van surrogaat eindpunten voor, zoals de dikte van de neurosensorische retina, als uitkomstmaat voor toekomstige trials.

In **Hoofdstuk 2.2** hebben we de klinische fenotypen van mannelijke patiënten met een variant in het *RPGR* gen onderzocht middels moderne uitkomstmaten, zoals de microperimetrie en FST. Deze informatie is cruciaal omdat er momenteel verschillende onderzoeken aan de gang zijn voor dit specifieke subtype van RP. *RPGR*-varianten resulteren doorgaans in een ernstige vorm van RP met visuele beperking op jonge leeftijd. Onze resultaten laten zien dat er verschillende fenotypen kunnen ontstaan zoals RP, kegel-staaf dystrofieën en geïsoleerde kegeldystrofieën; waarbij de laatste twee fenotypen geassocieerd zijn met exon ORF15. Een donorpatiënt met een *RPGR*-mutatie toonde aan dat veranderingen van het netvlies mogelijk al plaatsvinden in gebieden zonder macroscopische tekenen van degeneratie. Het is daarom belangrijk dat therapieën worden toegepast bij de eerste tekenen van degeneratie.

Hoofdstuk 2.3 beschrijft de klinische en genetische kenmerken van een zeldzame syndromale vorm van RP genaamd het PHARC-syndroom, waarbij wereldwijd minder dan 50 patiënten beschreven zijn. Het wordt veroorzaakt door varianten in het *ABHD12* gen, dat codeert voor een eiwit dat een belangrijke rol speelt in het vetmetabolisme. In deze studie was er sprake van klinische variabiliteit met betrekking tot de gezichtsscherpte en beeldvorming zonder duidelijke genotype-fenotype correlaties. Vanuit een oogheelkundig perspectief, toonden patiënten met het PHARC-syndroom

geen karakteristieke tekenen van RP, zoals beenbalkjes, hoewel de diagnose staafkegeldystrofie wel kon worden vastgesteld middels elektrofysiologie. Gezien de variabiliteit in het ontstaan van oogheelkundige, neurologische en audiologische symptomen, zal het moeilijk zijn om PHARC-syndroom in eerste instantie te diagnosticeren. Een multidisciplinaire aanpak, bestaande uit neurologen, genetici, audiologen en oogartsen, is nodig om PHARC-syndroom tijdig vast te stellen.

Hoofdstuk 2.4 beschrijft de eerste prospectieve, multicenter studie voor patiënten met *CRB1*-geassocieerde netvliesdystrofieën. Varianten in het *CRB1* gen veroorzaken een spectrum van fenotypen, waarvan de meest voorkomende 'early-onset RP' is. Het eerste concept van gentherapie voor *CRB1* was aangetoond in het LUMC, en hiermee is er een weg gemaakt voor een mogelijke behandeling van deze vorm van RP. Ter voorbereiding op toekomstige klinische trials evalueerde deze studie het 2-jarige klinische verloop van *CRB1* patiënten om zo tijdsgevoelige progressiemarkers te bepalen. We ontdekten dat *CRB1* een variabele ziekte expressie heeft, maar meestal resulteert in visuele beperking op jonge leeftijd. Een veelvoorkomende bevinding is de verdikking van de verschillende lagen van het netvlies, wat karakteristiek is voor dit specifieke type van RP. We ontdekten dat conventionele parameters, zoals gezichtsscherpte en gezichtsvelden, geen progressie vertoonden tijdens follow-up en mogelijk niet geschikt zijn om de behandeleffectiviteit te meten. Er werd significante progressie waargenomen op de microperimetrie, wat suggereert dat microperimetrie een gevoelige uitkomstmaat is bij *CRB1*-geassocieerde netvliesdystrofieën. Aangezien elektroretinografie niet detecteerbaar was bij de meerderheid van patiënten met *CRB1* mutaties, maakten we gebruik de full-field stimulus threshold. De full-field stimulus threshold stelde ons in staat om resterende fotoreceptorfunctie te kwantificeren, en kan dus als uitkomstmaat of inclusiecriteria fungeren.

In **hoofdstuk 3.1** evalueren we het resultaat van cataractchirurgie bij patiënten met RP en bespreken we mogelijke risicofactoren. Cataract is een van de meest voorkomende complicaties bij patiënten met RP. Cataract in combinatie met de degeneratie van de fotoreceptoren, kunnen het verlies van visuele functie verergeren en daardoor de kwaliteit van leven van de patiënt aanzienlijk verminderen. In onze studie leidt cataractchirurgie tot een significante verbetering van de gezichtsscherpte en ook tot verbeteringen in de subjectieve visuele functie bij de meerderheid van de patiënten. Operaties moeten echter met uiterste voorzichtigheid worden uitgevoerd, omdat patiënten met RP ook relatief meer risico lopen op complicaties zoals macula-oedeem, nastaar en zonulolysis. Als er noodzakelijke voorzorgsmaatregelen worden genomen voor deze populatie, kan cataractchirurgie een waardevolle behandeling zijn.

In **hoofdstuk 3.2** hebben we de effectiviteit gemeten van de OrCam MyEye 2.0 op de kwaliteit van leven van patiënten met erfelijke netvliesdystrofieën. De OrCam MyEye is een hulpmiddel voor slechtzienden dat visuele prikkels omzet in geluid met behulp

van een optische sensor die op de bril van de patiënt is gemonteerd. Met behulp van de NEI-VFQ en D-AI vragenlijsten hebben we aangetoond dat de OrCam voornamelijk de activiteiten verbeterde die gerelateerd zijn aan “activiteiten op nabije afstand” en geen verbetering liet zien op andere subdomeinen van de NEI-VFQ of andere revalidatiedoelen op de D-AI. Verder verbeteringen zijn nodig in de OrCam om het hulpmiddel relevant te maken voor een breder publiek.

In **Hoofdstuk 3.3** presenteren we een rapport over de 4-jaars veranderingen in de kwaliteit van leven bij patiënten met *CRB1*-geassocieerde netvliesdystrofieën. Hoewel objectieve uitkomstmaten essentieel zijn voor het meten van de behandelingsdoeltreffendheid in aankomende onderzoeken, is het net zo belangrijk om subjectieve uitkomstmaten te overwegen die de ervaring van de patiënt weerspiegelen. Helaas worden subjectieve uitkomstmaten momenteel niet adequaat aangepakt in klinische onderzoeken. Onze studie toonde aan dat patiënten met *CRB1*-geassocieerde netvliesdystrofieën een algemene achteruitgang ervaren in de kwaliteit van leven met betrekking tot het gezichtsvermogen op de NEI-VFQ vragenlijst. Daarom raden we aan dat toekomstige klinische onderzoeken vragenlijsten opnemen als een relevante maatstaf voor progressie en mogelijk behandelingsdoeltreffendheid.

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CURRICULUM VITAE

Xuan-Thanh-An Nguyen was born on the 2nd of June 1992 in Rotterdam, The Netherlands. After graduating from the Erasmiaans Gymnasium in Rotterdam in 2010, he studied Business Administration at the Erasmus University for one year before applying for Medicine at the Leiden University Medical Center. During his college years, he was an active member of the International Federation of Medical Student Associations, where he organized multiple courses including first aid and medical sign language lessons. He also went on a clinical internship on Forensic Pathology at the Otto-von-Guericke-University in Magdeburg, Germany under supervision of prof. dr. A. Roessner.

After obtaining his M.D., the author went to pursue his Ph.D. on the clinical characteristics and management of retinitis pigmentosa under guidance of prof. dr. C.J.F. Boon and dr. J. Wijnholds at the Department of Ophthalmology in the Leiden University Medical Center. The results of this research are presented in this thesis.

During his PhD, Thanh has coordinated a prospective, observational study involving patients with *CRB1*-associated retinal dystrophies; and has also coordinated a prospective study on the impact of a low vision device called the Orcam at Bartiméus and Koninklijke Visio. He has presented at several international conferences and was an invited speaker for the EURETINA Winter Meeting 2019 in Prague, Czech Republic.

In August 2021, the author started his residency training in Ophthalmology under the supervision of prof. dr. N.E. Schalij-Delfos.

LIST OF PUBLICATIONS

1. de Guimaraes TAC, Georgiou M, Robson AG, Fujinami K, Vincent A, Nasser F, Khateb S, Mahroo OA, Pontikos N, Vargas ME, Thiadens AAHJ, Carvalho ER, **Nguyen XT**, Arno G, Fujinami-Yokokawa Y, Liu X, Tsunoda K, Hayashi T, Jiménez-Rolando B, Martin-Merida MI, Avila-Fernandez A, Salas EC, Garcia-Sandoval B, Ayuso C, Sharon D, Kohl S, Huckfeldt RM, Banin E, Pennesi ME, Khan AO, Wissinger B, Webster AR, Heon E, Boon CJF, Zrenner E, Michaelides M. ***KCNV2*-associated retinopathy: genotype-phenotype correlations - *KCNV2* study group report 3.**
Br J Ophthalmol. 2023 Oct 18:bjo-2023-323640. Epub ahead of print.

2. Moekotte L, Kuiper JJW, Hiddingh S, **Nguyen XT**, Boon CJF, van den Born LI, de Boer JH, van Genderen MM. ***CRB1*-Associated Retinal Dystrophy Patients Have Expanded Lewis Glycoantigen-Positive T Cells.**
Invest Ophthalmol Vis Sci. 2023 Oct 3;64(13):6.

3. Karuntu JS*, **Nguyen XT***, Talib M, van Schooneveld MJ, Wijnholds J, van Genderen MM, Schalij-Delfos NE, Klaver CCW, Meester-Smoor MA, van den Born LI, Hoyng CB, Thiadens AAHJ, Bergen AA, van Nispen RMA, Boon CJF. **Quality of life in patients with *CRB1*-associated retinal dystrophies: A longitudinal study.**
Acta Ophthalmol. 2023 Sep 25. Epub ahead of print.

4. **Nguyen XT***, Moekotte L*, Plomp AS, Bergen AA, van Genderen MM, Boon CJF. **Retinitis Pigmentosa: Current Clinical Management and Emerging Therapies.**
Int J Mol Sci. 2023 Apr 19;24(8):7481.

5. **Nguyen XT**, Thiadens AAHJ, Fiocco M, Tan W, McKibbin M, Klaver CCW, Meester-Smoor MA, Van Cauwenbergh C, Strubbe I, Vergaro A, Pott JR, Hoyng CB, Leroy BP, Zemaitiene R, Khan KN, Boon CJF. **Outcome of Cataract Surgery in Patients With Retinitis Pigmentosa.**
Am J Ophthalmol. 2023 Feb;246:1-9.

6. **Nguyen XT**, Koopman J, van Genderen MM, Stam HLM, Boon CJF. **Artificial vision: the effectiveness of the OrCam in patients with advanced inherited retinal dystrophies.**
Acta Ophthalmol. 2022 Jun;100(4):e986-e993.

7. **Nguyen XT**, Talib M, van Schooneveld MJ, Wijnholds J, van Genderen MM, Schalij-Delfos NE, Klaver CCW, Talsma HE, Fiocco M, Florijn RJ, Ten Brink JB, Cremers FPM, Meester-Smoor MA, van den Born LI, Hoyng CB, Thiadens AAHJ, Bergen AA, Boon CJF. ***CRB1*-Associated Retinal Dystrophies: A Prospective Natural History Study in Anticipation of Future Clinical Trials.**
Am J Ophthalmol. 2022 Feb;234:37-48.

8. Georgiou M, Fujinami K, Vincent A, Nasser F, Khateb S, Vargas ME, Thiadens AAHJ, de Carvalho ER, **Nguyen XT**, De Guimarães TAC, Robson AG, Mahroo OA, Pontikos N, Arno G, Fujinami-Yokokawa Y, Leo SM, Liu X, Tsunoda K, Hayashi T, Jimenez-Rolando B, Martin-Merida MI, Avila-Fernandez A, Carreño E, Garcia-Sandoval B, Ayuso C, Sharon D, Kohl S, Huckfeldt RM, Boon CJF, Banin E, Pennesi ME, Wissinger B, Webster AR, Héon E, Khan AO, Zrenner E, Michaelides M. ***KCNV2*-Associated Retinopathy: Detailed Retinal Phenotype and Structural Endpoints-*KCNV2* Study Group Report 2**.
Am J Ophthalmol. 2021 Oct;230:1-11.

9. **Nguyen XT***, Almushattat H*, Strubbe I, Georgiou M, Li CHZ, van Schooneveld MJ, Joniau I, De Baere E, Florijn RJ, Bergen AA, Hoyng CB, Michaelides M, Leroy BP, Boon CJF. **The Phenotypic Spectrum of Patients with PHARC Syndrome Due to Variants in *ABHD12*: An Ophthalmic Perspective.**
Genes (Basel). 2021 Sep 11;12(9):1404.

10. Georgiou M, Robson AG, Fujinami K, Leo SM, Vincent A, Nasser F, Cabral De Guimarães TA, Khateb S, Pontikos N, Fujinami-Yokokawa Y, Liu X, Tsunoda K, Hayashi T, Vargas ME, Thiadens AAHJ, de Carvalho ER, **Nguyen XT**, Arno G, Mahroo OA, Martin-Merida MI, Jimenez-Rolando B, Gordo G, Carreño E, Ayuso C, Sharon D, Kohl S, Huckfeldt RM, Wissinger B, Boon CJF, Banin E, Pennesi ME, Khan AO, Webster AR, Zrenner E, Héon E, Michaelides M. ***KCNV2*-Associated Retinopathy: Genetics, Electrophysiology, and Clinical Course-*KCNV2* Study Group Report 1.**
Am J Ophthalmol. 2021 May;225:95-107.

11. **Nguyen XT***, Talib M*, van Cauwenbergh C, van Schooneveld MJ, Fiocco M, Wijnholds J, Ten Brink JB, Florijn RJ, Schalij-Delfos NE, Dagnelie G, van Genderen MM, de Baere E, Meester-Smoor MA, De Zaeytijd J, Balikova I, Thiadens AA, Hoyng CB, Klaver CC, van den Born LI, Bergen AA, Leroy BP, Boon CJF. **Clinical Characteristics and Natural History of *RHO*-associated Retinitis Pigmentosa: A Long-Term Follow-up Study.**
Retina. 2021 Jan 1;41(1):213-223.

12. **Nguyen XT**, Talib M, van Schooneveld MJ, Brinks J, Ten Brink J, Florijn RJ, Wijnholds J, Verdijk RM, Bergen AA, Boon CJF. ***RPGR*-Associated Dystrophies: Clinical, Genetic, and Histopathological Features.**
Int J Mol Sci. 2020 Jan 28;21(3):835.

13. Karuntu JS, **Nguyen XT,** Boon CJF. **Correlations between the Michigan Retinal Degeneration Questionnaire and visual function parameters in patients with retinitis pigmentosa.**
Manuscript submitted.

14. Moekotte, L de Boer JH, Hiddingh S, de Ligt AM, **Nguyen XT**, Hoyng CB, Inglehearn CF, McKibbin M, Lamey TM, Thompson J, Chen F, McLaren T, Talbishi AMA, Panneman DM, Boonen EGM, Banf S, Bocquet B, De Baere E, Koenekoop R, Oldak M, Rivolta C, Roberts L, Strupaite R, Kohl S, Farrar GJ, van Vugt M, van Setten J, Roosing S, van den Born LI, Boon CJF, van Genderen MM, Kuiper JJW. **Elevated Plasma Complement Factors in *CRB1*-associated inherited retinal dystrophies.**
Manuscript submitted.

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