Clinical characteristics and management of retinitis pigmentosa

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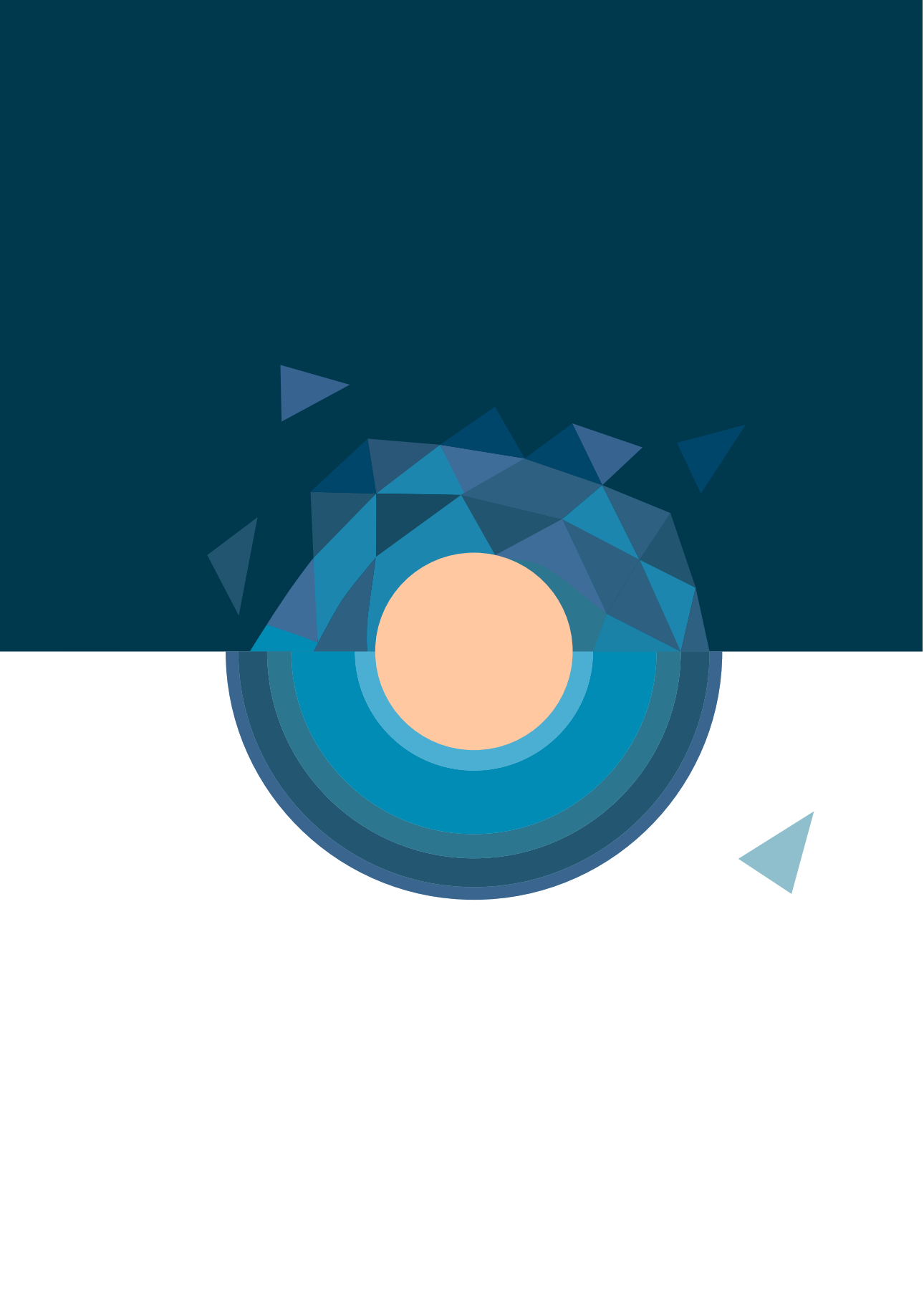

CHAPTER 1

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]

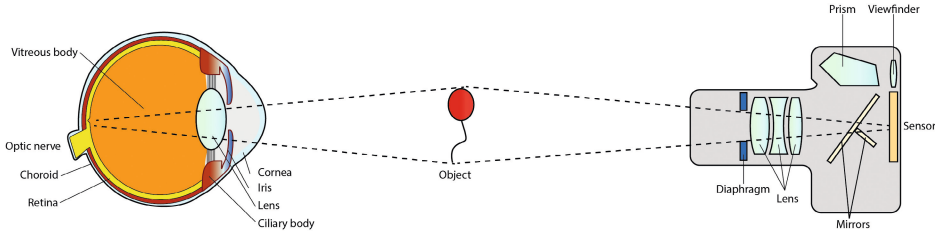

Figure 1. The shared similarities in structures between a human eye and a digital camera. An eye and a camera both have lenses and a light-sensitive region at the posterior segment. Also, an aperture is present in the eye (the pupil created by the iris) and the camera (created by the diaphragm) that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

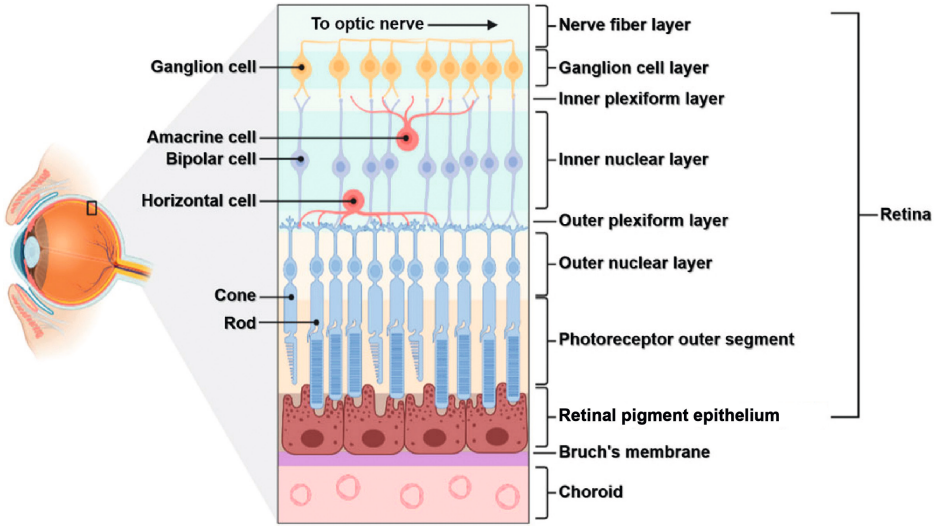

Figure 2. The layers of the retina (adapted from Yang et al., 2021[3]).

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]

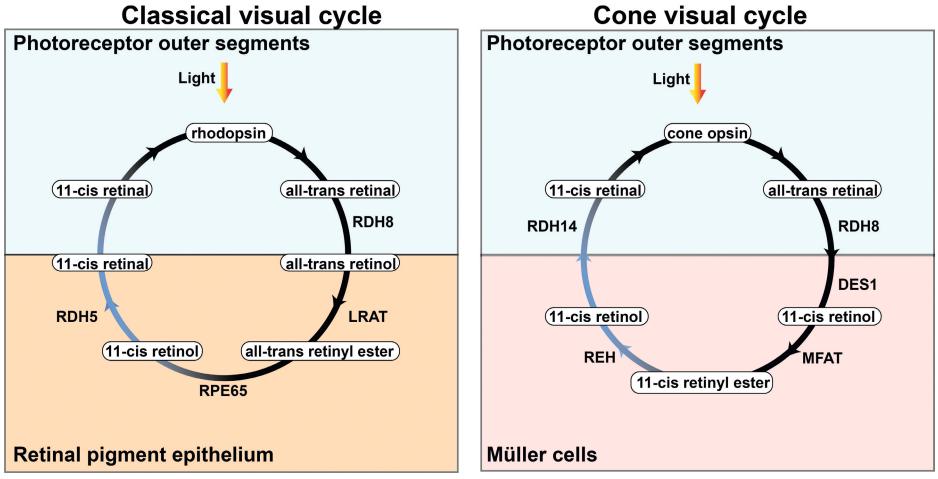

Figure 3. Overview of the classical and cone visual cycle (adapted from Tsin et al., 2018).[8] These visual cycles are needed to regenerate visual pigment, which is needed to detect photons. Cone visual pigment has access to an alternative visual cycle via Müller cells, making them less dependent on 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.

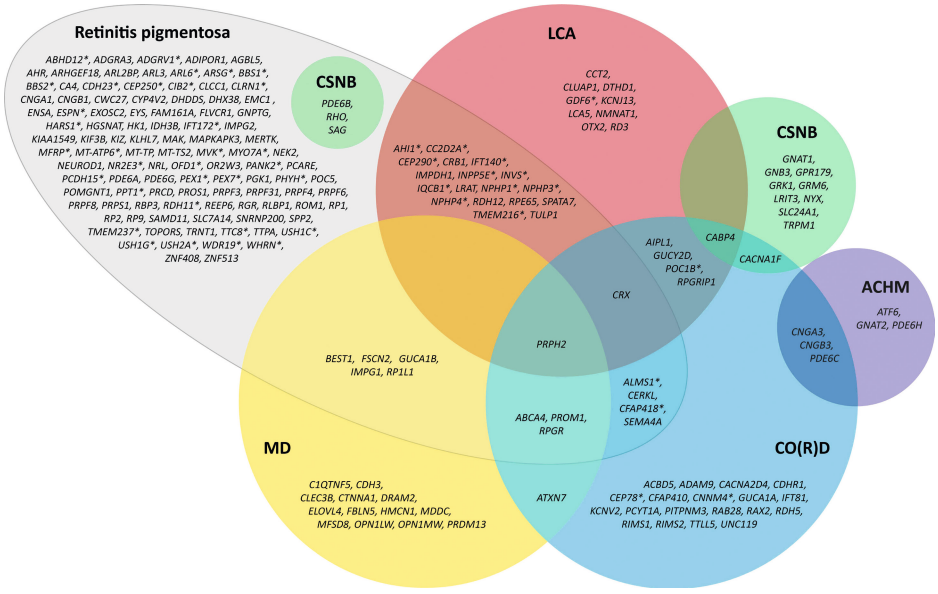

Figure 4. Venn diagram of currently identified genes associated with retinitis pigmentosa (RP) and their genetic overlap with other inherited retinal dystrophies. For example, variants in the *RHO* gene can manifest in either RP or congenital stationary night blindness phenotypes. All genes included 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]

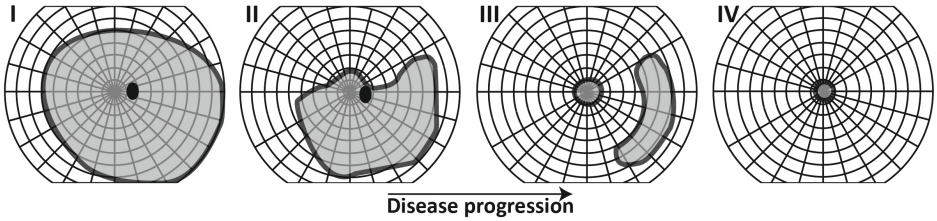

Figure 5. Illustrative example of typical visual field progression in a patient with retinitis pigmentosa using kinetic perimetry. Visual fields can be within normal limits in early stages of disease (I), although visual field defects may already be present but not detectable within the used target stimulus. 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.

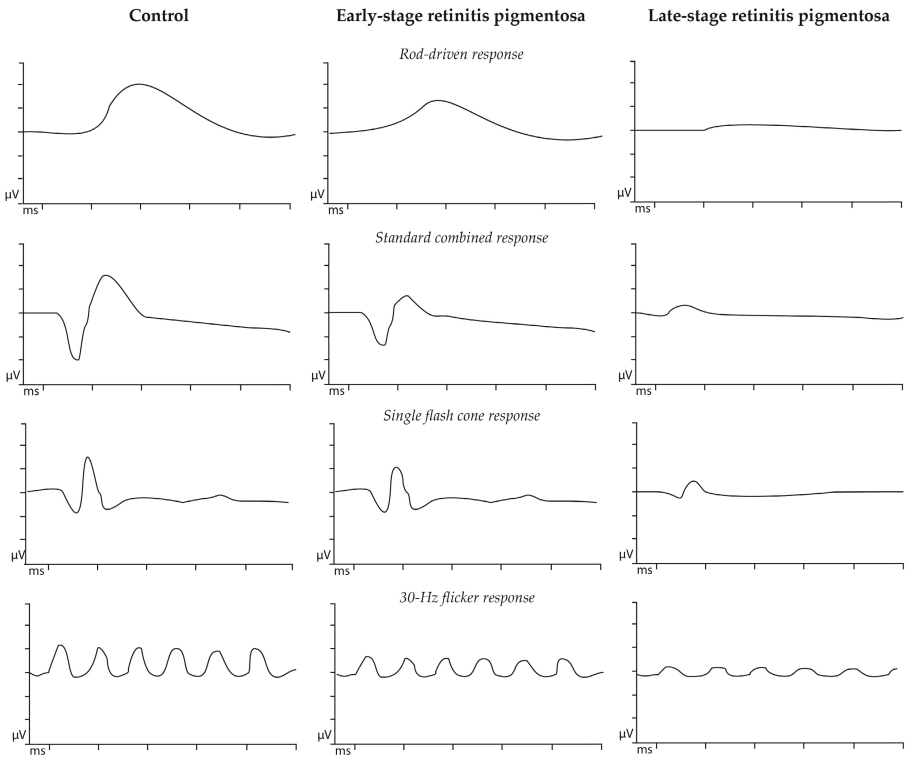

Figure 6. Example full-field electroretinography recordings in a healthy patient and in patients with different disease stages of retinitis pigmentosa. Different stimuli are used to establish the diagnosis of retinitis pigmentosa, which is based on the guidelines of the International Society for Clinical 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).

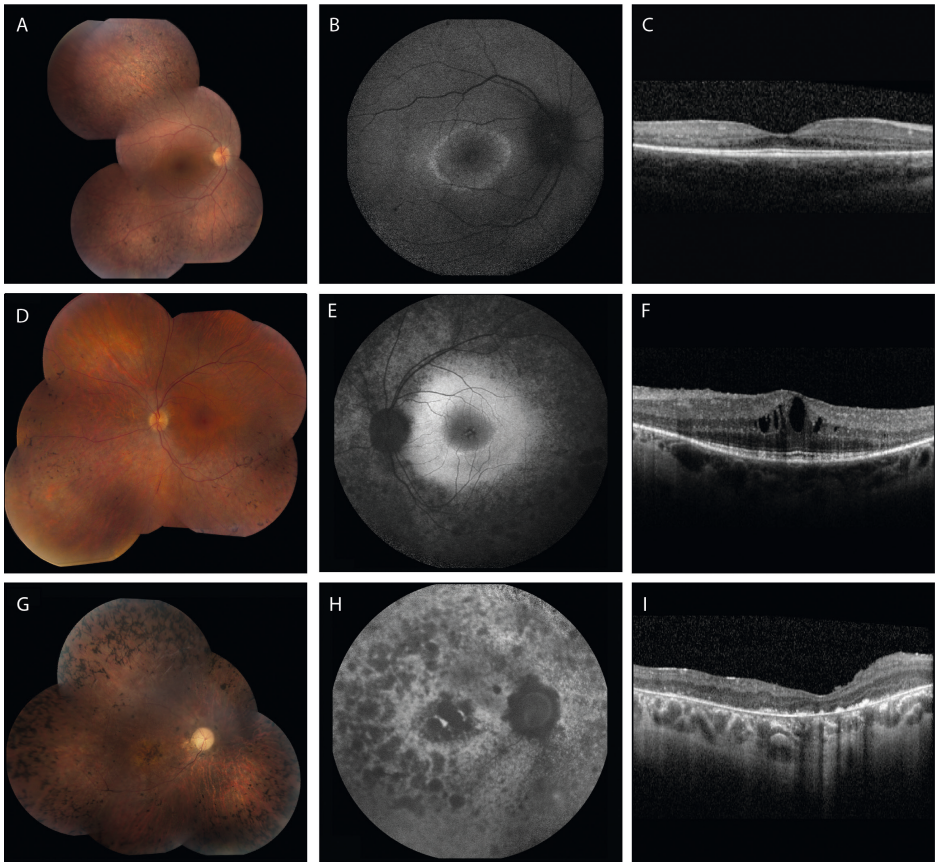

Figure 7. Multimodal imaging in three patients with retinitis pigmentosa (RP). (**A-C**): Multimodal imaging in a patient with RP caused by a variant in the *RHO* gene showing the clinical hallmarks of RP, including attenuated vessels and bone-spicule-like hyperpigmentation in the (mid)peripheral retina (**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.

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