Inherited retinal degenerations: clinical characterization on the road to therapy

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1.

GENERAL INTRODUCTION

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1. ANATOMY AND PHYSIOLOGY OF THE EYE

As light enters the eye, its waves are refracted by several structures. These waves cross a path through the cornea, the aqueous humor of the anterior chamber, the lens, the vitreous humor, eventually reaching the retina, where the focal point lies in the macula. In the retina, photons are converted to an electrical signal through a cascade of processes, and the collection of electrical signals is converted to an image in the visual cortex of the brain.

Two major components that determine the focal point at which the light waves converge, are the corneal radius of curvature, and the axial length of the eye. In emmetropic eyes, the focal point to which light waves converge lies on the retina. In eyes with a long axis (>26 mm), i.e. myopic eyes, the focal point lies in front of the retina. In eyes with a short axis (<20 mm), i.e. hyperopic eyes, this point lies behind the retina.

1.1 The vitreous

Occupying 80% of the eye volume, the healthy vitreous is a clear substance composed of water, hyaluronic acid, and collagen. Its anterior surface is firmly attached to the lens of the eye, and its posterior surface is attached to the retinal vessels, the macula of the retina, and the optic nerve. Other sites of attachment include the vitreous base, which is located posterior to the junction between the retina and the pars plana of the ciliary body (the ora serrata). Various pathological processes, such as inflammation, may affect the clarity of the vitreous.

1.2 The neurosensory retina

The neurosensory retina consists of 8 distinct layers (Figure 1), each consisting of synapses or nuclear bodies, along with supporting cells. Furthermore, the neuroretina contains two "membranes", the inner limiting membrane and external limiting membrane, which are not true membranes but junctional systems connecting retinal cells. The central 5.5 mm of the neurosensory retina is the macula, and the fovea is the central 1.5 mm of the macula. Light travels through all retinal layers in order to reach the light-sensitive cells of the retina, i.e. the photoreceptors, which may be rods (95% of photoreceptors; responsible for light-sensitivity in low-luminance environments and for peripheral vision), or cones (5% of photoreceptors; responsible for spatial acuity and color vision).[1] A third type of photoreceptor, the retinal ganglion cell, is not involved in the visual pathway but is involved in mechanisms such as pupillary responses, and circadian rhythm. Figure 2 shows the normal spatial distribution of rods and cones. The highest cone density is in the very center of the macula, the fovea, which contains no rods, allowing the fovea to enable high visual acuity, high contrast vision, and color vision. Although the cone density decreases rapidly outside the fovea, 90% of cones lie outside the fovea. The highest rod density is approximately 15° away from the fovea. As the peripheral retina contains predominantly rod cells, the peripheral retina provides night vision and the peripheral visual field.

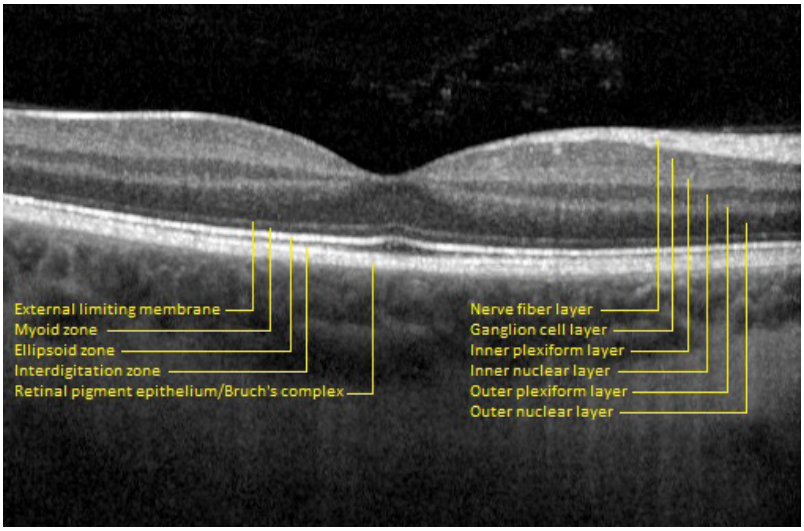

Figure 1. A cross section of the retina on spectral-domain optical coherence tomography, showing (from top-down) the inner limiting membrane, the retinal nerve fibre layer, the ganglion cell layer (containing the cell bodies of the ganglion cells), the inner plexiform layer (containing the synapses between ganglion cells and the bipolar cells), the inner nuclear layer (containing the cell bodies of the bipolar cells), the outer plexiform layer (containing the synapses between the bipolar cells and the photoreceptor cells), the outer nuclear layer (containing the cell bodies of photoreceptor cells), the external limiting membrane, the myoid zone (consisting of endoplasmic reticulum), the ellipsoid zone (i.e. the inner/outer segment junction, consisting of mitochondria, cilia, and inner discs), the outer segments, the interdigitation zone, and the retinal pigment epithelium/Bruch's complex.

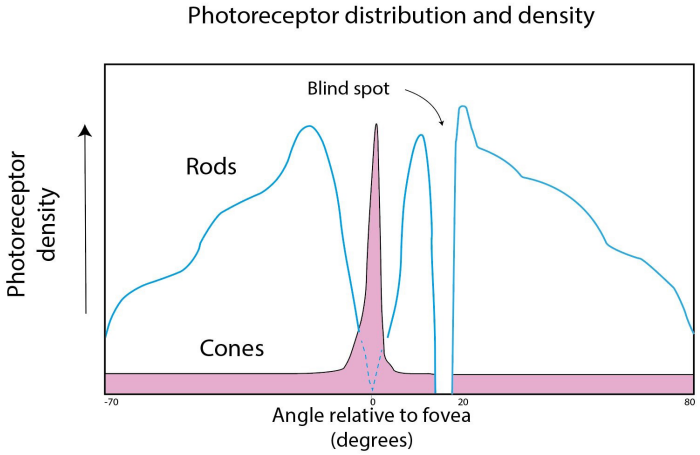

Figure 2. The spatial distribution of rod and cone photoreceptors in the normal human retina. Density curves show the highest cone density in the fovea centralis, where rods are absent. Away from the fovea centralis, cone density rapidly decreases, while rod density rapidly increases, reaching its maximum at approximately 15° eccentricity from the fovea. Rods further spread over a large area of the retina. (Adapted from © Brian Wandell, Foundations of Vision, Stanford University [http://foundationsofvision.stanford.edu/chapter-3-the-photoreceptor-mosaic])

Photoreceptors consist of four major compartments (Figure 3): the outer and inner segments, the cell body, and the synaptic terminal. The outer segment contains up to 1000 discs, stacked like pancakes, which contain the light-sensitive visual pigments. The outer and inner segments are separated by a ciliary transition zone, or the connecting cilium. The connecting cilium is the site of transport of lipids and proteins from the inner to the outer segment, and functions as a barrier between the differing plasma membrane compositions of the inner and outer segment. The photoreceptor inner segment contains mitochondria, which produce chemical energy (adenosine triphosphate).[2] These mitochondria, along with the outer segment discs, form the inner-outer segment junction. The photoreceptor inner segment further contains the Golgi apparatus, and endoplasmic reticulum, both responsible for protein synthesis. This area, visible on spectral domain optical coherence tomography as the "ellipsoid zone",[3] has been demonstrated to be of particular clinical relevance in predicting visual outcome.

Important support cells for retinal neurons are the Müller glial cells, which support homeostatic and metabolic cell functions, and maintain structural stability by regulating the tightness of the blood-retina barrier.[4] Their cell bodies are located in the inner nuclear layer, and their endfeet attach to the photoreceptors, forming adherens junctions at the external limiting membrane, at the level between the photoreceptor inner segment and the photoreceptor cell body.

1.3 The retinal pigment epithelium

The retinal pigment epithelium, or RPE, is a single layer of cells containing melanin pigment. It contributes to retinal function through a) visual pigment regeneration; b) light absorption; c) forming of the outer blood-retinal barrier through tight junctions; d) phagocytosis of rod and cone outer segments, regenerating the outer segments with a daily approximately 10% renewal rate; and e) molecular exchanges at the apical villi, such as water and ion transport ensuring the proper ionic environment. The maintenance of the ionic homeostasis occurs through various electrogenic pumps, such as the Na^+/K^+-ATPase pump.[5] The basal surface of the RPE lies on the Bruch's membrane, a thin elastin- and collagen-rich layer between the RPE and the fenestrated choroidal capillaries. Bruch's membrane acts as a molecular sieve, regulating the exchange of molecules and fluids, nutrients, oxygen, and metabolic waste.[6]

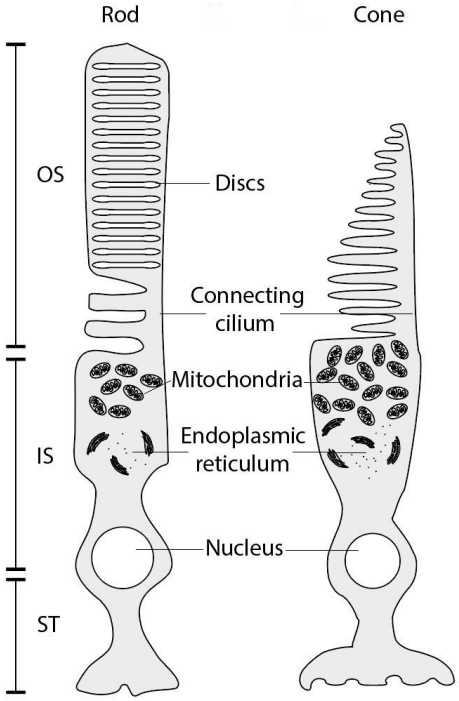

Figure 3. Schematic overview of the rod (left) and cone (right) photoreceptor anatomy. Light molecules, or photons, enter the photoreceptors through the inner segment first, and are then transmitted to the outer segment, which contains the visual pigments. OS = outer segment. IS = inner segment. ST = synaptic terminal. (Adapted from Cote RH, 2006[7])

1.3.1 The visual cycle

Visual pigment (chromophore: rhodopsin in rods; cone opsin in cones) is used in the process of photo-activation, and it must be regenerated in order to enable the next cycle of photo-activation. This regeneration occurs via the visual cycle, which takes place at the level of the photoreceptors and the RPE. As a photon enters the rod or cone photoreceptor, this stimulates a configurational change of 11-cis retinal opsin (the chromophore) into all-trans retinal.[8] In the RPE, this is converted to all-trans retinol, which is then modified to all-trans retinyl ester by lecithin retinol acyl transferase (protein LRAT). All-trans retinyl ester is in turn converted to 11-cis retinol by RPE-specific 65 kDa protein (protein RPE65). In the final step of the cycle, 11-cis retinol is converted back into 11-cis retinal by retinol dehydrogenase 5 (protein RDH5), which is then transported to the photoreceptor, where the next cycle starts upon activation by a photon.

This classical visual cycle is present in rods and cones, however in cones, an additional cone-specific, or non-canonical visual cycle, takes place. This alternative cycle is independent of the RPE, and instead relies on Müller glial cells in the retina.[9]

1.3.2 The choroid

Blood enters the choroid, the vascular layer of the eye, through the posterior ciliary arteries. The choroid supplies the retina, one of the most metabolically demanding tissues in the human body. The choroid has the highest blood flow rate of any tissue in the body. The outer layer of the choroid is known as the Haller layer, which contains large-caliber choroidal vessels. These vessels branch into smaller-caliber vessels and precapillary arterioles, forming the Sattler layer. These non-fenestrated vessels distribute blood over the breadth of the choroid, debouching into the capillary meshwork, or choriocapillaris, the most inner layer of the choroid. After passing the fenestrated choriocapillaris, blood drains into venules, which fuse to form the collecting channels of the four or five vortex veins, which eventually drain into the superior and inferior ophthalmic veins. The choroid is at its thickest (normally 0.22 mm) posteriorly, and thins as it moves anteriorly towards the ora serrata (normally 0.1 mm).

2. INHERITED RETINAL DEGENERATIONS

Inherited retinal degenerations (IRDs) comprise a collection of degenerative diseases, generally leading to visual dysfunction. IRDs are characterized by the usually progressive and sometimes stationary dysfunction of rods and/or cones. Patients may experience an array of symptoms, such as night blindness (nyctalopia), visual field deterioration, blurriness of vision, and a disturbance in color vision. These diagnoses generally have a profound impact on patient's lives, progressively affecting their social interactions, independence, professional functioning, and mobility.[10] Patients are often uncertain of their prognosis, questioning if and when they will go blind, and whether they will pass this disease onto their children.

More than 260 disease genes have been identified to date in association with IRD, each gene accounting for a portion of patients [RetNet, update of November 11, 2019, URL: sph.uth.edu/retnet/]. Moreover, IRDs can be categorized based on the phenotype and/or the type of photoreceptor which is primarily affected, the most common form being retinitis pigmentosa (RP), a rod-cone dystrophy. Other progressive forms include cone(-rod) dystrophy, isolated macular dystrophies, choroideremia, and Leber congenital amaurosis. Each IRD may be caused by different genes, and individual genes may be associated with several distinct forms of an IRD. Due to this clinical and genetic overlap, establishing a distinct diagnosis may be challenging in some cases, and can be subject to debate. While each IRD can be roughly distinguished clinically depending on the type of photoreceptor cells that are primarily affected, together they form a spectrum with variable presentation and severity, with an underlying broad range of different affected genes.

2.1 Retinitis pigmentosa

Retinitis pigmentosa (RP) is a group of progressive inherited retinal dystrophies (IRDs) in which rod photoreceptor degeneration precedes cone photoreceptor degeneration. Its worldwide incidence is estimated at 1:3500-1:4000,[11] but depending on the geographic location, incidence reports have varied between 1:9000 and 1:750.[12, 13] In addition to non-syndromic forms of RP, that encompass 65-70% of all RP cases, there are forms also displaying non-ocular signs, and more than 30 different RP-associated syndromes have been described.[11, 14] The most frequent syndromic form is Usher syndrome, which manifests with congenital hearing impairment followed by the development of RP in early adolescence.[15] Non-syndromic RP is a clinically and genetically heterogeneous disease entity, and its presentation may partially overlap with other IRDs, such as advanced cone-rod dystrophy. Furthermore, over 87 genes have been identified in association with RP, and individual genes may be associated with several distinct forms of an IRD, such as RP and Leber congenital amaurosis.

2.1.1 Presentation

The age at which symptoms present and the speed at which they evolve are variable and depend at least partially on the mode of inheritance and on the gene involved. The autosomal dominant forms of RP (ADRP) are usually the mildest, often with patients first experiencing symptoms in mid-adulthood, although symptom presentation in early childhood has also been described.[16, 17] Autosomal recessive (ARRP) and X-linked RP (XLRP) generally have a more severe disease course with an earlier onset, often within the first decade of life.[18] Typically, the initial symptom noticed by patients and/or their parents is decreased night vision (nyctalopia). In today's artificially lit environments, it may take years for patients to notice a disturbance in night vision. Moreover, these symptoms may be subtle, and patients may recognize these symptoms only when comparing their night vision to that of unaffected individuals. Often, patients report having first noticed nyctalopia during adolescence.[11] Loss of (mid-)peripheral visual field usually becomes apparent in adolescence or (young) adulthood, and patients may compensate for visual field loss by scanning their environment. In later stages, a central vision decrease, color vision disturbances, and light aversion may be present. Patients may also experience photopsia, which can manifest as static noise, background glow, flashes, or as static or moving phosphenes or shapes.[19] Depending on the location and frequency of these photopsia, they could disturb remaining vision. Photopsia may occur in early and in advanced disease stages,[19] and they have been postulated to be manifestations of spontaneous self-activation of impaired retinal cells or potentially as a result of retinal remodeling due to photoreceptor degeneration.[20]

2.1.2 Fundus features

Fundus examination in the early stage of disease can be normal, as changes may not yet be present or may be subtle. Typical features on fundoscopy in mid-stage RP include waxy optic disc pallor, which may be limited to the temporal optic disc in the early disease stage, vascular attenuation,

variable degrees of retinal atrophy, intraretinal bone-spicule-shaped pigment migrations, and retinal pigment epithelium (RPE) alterations or atrophy that typically start in the midperiphery and progress in a centripetal fashion (Figure 4). As the disease advances, RPE alterations appear in the posterior pole. The central macula is often spared until the late disease stages. In ADRP, the retinal degenerative changes may be sectorial, usually affecting the lower quadrants. Additional fundus features that may be present include cystoid macular edema (up to 50% of overall RP cases),[21, 22] cellophane maculopathy due to an epiretinal membrane (up to 35%),[23, 24] optic disc drusen (approximately 9% of RP cases) or hamartomas.[25] These features can be present in larger proportions depending on the gene-specific subtype of RP. Female carriers of XLRP may exhibit the pathognomonic tapetoretinal reflex, a golden-metallic sheen in the posterior pole. Some female carriers of XLRP develop symptoms and typical RP-associated fundus changes with advancing age.[26-30]

Non-retinal features that may be present include posterior subcapsular cataract (approximately 45% of RP patients),[31] and refractive error which, depending largely on the gene involved, could be myopia or hyperopia.[32] Vitreous abnormalities may be present, such as dust-like particles. Although nystagmus has been described, this is more closely associated with severe early-onset retinal dystrophies, such as Leber congenital amaurosis, rather than RP.[33]

2.2 Leber congenital amaurosis

Leber congenital amaurosis, or LCA, is the most severe form of IRD, with an onset that usually occurs congenitally or in infancy. Both rod and cone function are not detectable from infancy. The prevalence of LCA has been estimated at 1:100.000-1:30.000 births,[34-36] depending on the geographic location, and comprising approximately 5% of all IRDs. LCA is more prevalent in countries where consanguinity is more common.[37, 38]

To date, over 25 genes have been implicated in association with LCA. It is mostly inherited in the autosomal recessive form, but autosomal dominant (*IMPDH1* gene) and X-linked inheritance (*CRX* gene) have been described.

In literature, the terms LCA, early-onset IRD, and early-onset severe IRD, have been used interchangeably, to a certain extent. However, in the latter two phenotypes, more preservation of visual function into childhood is observed, along with some preservation of rod and/or cone responses on the electroretinogram. Establishing an LCA diagnosis is complicated by several factors, including difficulties associated with the ophthalmological examination of infants, and patient delay. This renders it nearly impossible in some cases to retrospectively distinguish between LCA and early-onset severe IRD, and a considerable clinical and genetic overlap between these entities should be taken into account.

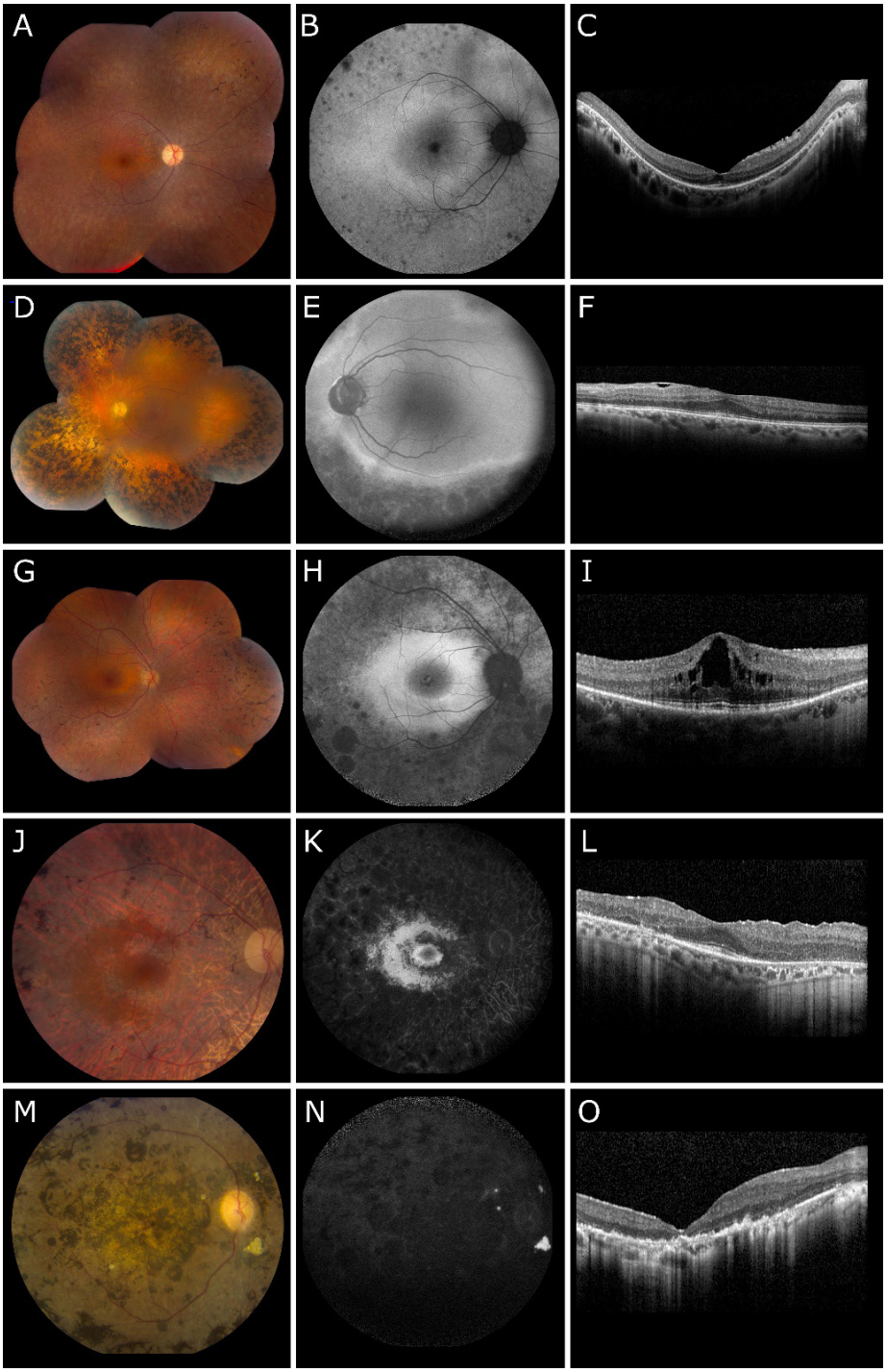

Figure 4. Multimodal imaging in non-syndromic retinitis pigmentosa (RP). A-C. A 20-year old North-African woman with autosomal recessive RP due to a homozygous mutation in the *IDH3A* gene (c.524C>T; p.(Ala175Val)).

Her nyctalopia and visual field symptoms started at the age of 14 years. Fundus photography (**A**) showed optic disc pallor, attenuated arteries, and bone-spicule-like pigmentation and mottled retinal pigment epithelium in the periphery. Fundus autofluorescence (FAF; **B**) shows round and mottled hypo-autofluorescence in the midperiphery, with sparing of the posterior pole. Spectral-domain optical coherence tomography (SD-OCT; **C**) showed an epiretinal membrane, and some relative foveal sparing of the outer nuclear layer (ONL), external limiting membrane (ELM), and ellipsoid zone (EZ), with thinning of these layers in the para- and perifovea. Corresponding best-corrected visual acuity (BCVA) was 20/22 in OD and 20/25 in OS. **D-F.** A 60-year old Caucasian man with RP. Next generation sequencing revealed no conclusive molecular result on the genetic basis for RP. Fundus photography (**D**) showed a pale optic disc with peripapillary atrophy, vascular attenuation, a relatively spared posterior pole, with atrophy and dense bone-spicule-like pigmentation in the periphery. FAF (**E**) revealed abnormalities in regions appearing relatively spared on fundoscopy, showing hypo-autofluorescent lesions with a hyperautofluorescent border, encroaching upon the posterior pole from the inferior retina. SD-OCT (**F**) showed an epiretinal membrane, relative preservation of the ONL, ELM, and EZ, with some disorganization of the interdigitation zone. He had visually significant posterior subcapsular cataract in both eyes, but considerably more in OS, and BCVA was 20/25 in OD and 20/29 in OS. After cataract surgery 2 years later, his BCVA improved to 20/20 in OD and 20/25 in OS. **G-I.** A 38-year old female patient with autosomal dominant RP due to a mutation in the *RHO* gene (c.541G>A; p.(Glu181Lys)). Fundus photography (**G**) revealed the typical RP-associated changes, which were more abundant in the inferior hemisphere. The posterior pole and the superior retina were relatively spared. FAF (**H**) showed mottled hypo-autofluorescent changes in the central macula and in the midperiphery extending into the posterior pole. In the inferior midperiphery, sharply circumscribed patches of absent autofluorescence are visible. A perimacular hyperautofluorescent ring is visible. The SD-OCT (**I**) shows relative preservation of the outer retina, with cystoid macular edema mostly in the inner nuclear layer, with a few cystoid spaces in the ONL and the ganglion cell layer. **J-L.** A 60-year old Middle-Eastern man with autosomal recessive RP due to a homozygous mutation in the *FAM161A* gene (c.1138T>C; p.(Arg380*)). He had been experiencing nyctalopia complaints since the age of 35-40 years. The fundus (J) showed optic disc pallor, vascular attenuation, bone-spicule-like pigmentation extending into the posterior pole, and generalized retinal atrophy with relative sparing in the central macula and the temporal posterior pole. The latter is also visualized on FAF (**K**). Due to the relative foveal sparing of the ONL, ELM, and EZ, as seen on SD-OCT (**L**), the corresponding best-corrected visual acuity was 20/20 OU. **M-O.** A 37-year old man with RP, and no other affected family members. Molecular analysis however revealed autosomal dominant RP due to a pathogenic mutation in the *NRL* gene (c.654del; p.(Cys219Valfs*4)). This incongruence with the pedigree raises suspicion that this is a de novo mutation. Fundus examination (**M**) revealed end-stage RP, with atrophy and dense intraretinal pigment migration in the posterior pole, and several hamartomas around the optic disc and in the posterior pole. FAF (**N**) showed barely any remaining autofluorescence, but hyperautofluorescence of the peripapillary hamartomas. SD-OCT (**O**) showed profound atrophy of the ONL and atrophy and disorganization of the hyperreflective outer retinal bands. The corresponding BCVA was 20/400 in OD and finger counting vision in OS.

2.2.1 Presentation

The typical presentation of LCA consists of severe and early loss of visual function, sensory nystagmus, amaurotic pupils, and non-detectable rod and cone function on the electroretinogram. Visual acuity usually ranges between 20/400 to light perception or even no light perception. A patient's parents usually notice the nystagmus or a lack of fixation. Patients may exhibit the oculodigital sign of Franceschetti, which is a repetitive and deep poking or pushing into the eye. This may lead to orbital fat atrophy, resulting in enophthalmos. Other signs include photoaversion and nyctalopia. Associated phenotypic features include a high refractive error, either hyperopia

or myopia, again depending on the gene involved, cataracts, and keratoconus. The latter may be resulting from the oculodigital sign.
Mental retardation has been described in a subset of patients with LCA.[39] The association between mental retardation and LCA has been arguable, as congenitally blind and mentally retarded patients may have a syndromic disease, such as Batten disease or peroxisomal diseases, which may initially only exhibit an LCA-like ocular phenotype, with other features occurring later in life.[40, 41]

Several forms of LCA, most notably *RPE65*-associated LCA, have been a focus of interest for gene-based therapeutic studies, as the relatively preserved retinal structure indicates the presence of viable photoreceptors, despite the severely affected visual function.[42] This dissociation of structure and function has also been described in *LRAT*-associated and *CEP290*-associated LCA.[43] The extensive studies in gene therapy for *RPE65*-associated LCA have resulted in the development of the first-ever FDA-approved clinical gene therapy, voretigene neparvovec (Luxturna®).

2.2.2 Fundus features

In infancy, patients may have a normal appearing fundus. Early abnormalities include macular atrophy, which may later progress to an appearance resembling retinitis pigmentosa, such as optic disc pallor and retina arteriolar narrowing. However, some patients maintain a relatively normal appearance of the retinal vessels and optic disc, into the third decade of life, despite an early maculopathy.[41] Other fundus features include a macular coloboma, choroidal sclerosis, optic disc drusen, and salt-and-pepper-like changes of the RPE.

2.3 Cone dystrophies and cone-rod dystrophies

Cone dystrophies are a heterogeneous group of disorders involving the cone photoreceptor system, while the rod system remains normal. In some patients, the disease progression involves secondary rod involvement in later life, developing into a cone-rod dystrophy. In cone-rod dystrophies, rods are affected relatively early in the disease process, albeit to a lesser degree than cones, but both rod and cone systems are abnormal. The prevalence of cone and cone-rod dystrophies is estimated at 1:30.00 to 1:40.000.[44, 45]

Over 42 genes have been implicated in cone and cone-rod dystrophies.[46] Inheritance may be autosomal recessive, autosomal dominant, and X-linked.[47-50] Cone and cone-rod dystrophies are mostly non-syndromic, but they may occur in syndromic form, as in Bardet-Biedl syndrome.[51]

Cone dystrophies differ from macular dystrophies, such as classic Stargardt disease or vitelliform degenerations, where the degeneration is confined to the macular cones, and where the degeneration is not usually detectable on full-field electroretinography.[52]

Although impaired color vision is a feature of cone dystrophies, cone dystrophies differ from congenital color blindness (protanopia, deuteranopia, and tritanopia), as patients with congenital color blindness maintain a normal visual acuity, do not have associated retinal degeneration, and do not show progression of their disease.[53]

2.3.1 Presentation

Cone dystrophies usually present in childhood, but may present in the teenage or adult years, and are characterized by the progressive loss of visual acuity and color discrimination in all three color axes. Patients' fixation may be eccentric, as they deviate their gaze to project images on the parafoveal regions that are less affected. Other signs are hemeralopia (day blindness) and photophobia. Peripheral vision remains normal in isolated cone dystrophies, but may be affected in cone-rod dystrophies. Patients with cone-rod dystrophies may also experience nyctalopia, and in later stages, their disease may be difficult to distinguish from RP. However, the clinical course of cone-rod dystrophies has been described as more severe and more rapid than many forms of RP.[44] Patients may be myopic, particularly in X-linked cone or cone-rod dystrophies.

2.3.2 Fundus features

Fundus appearance may be fully or near-normal in early disease stages. In cone dystrophies, abnormalities may range from mild RPE alterations, to a bull's eye pattern of macular atrophy, to more extensive and severe macular atrophy. Temporal pallor of the optic disc may be seen. In cone-rod dystrophies, these central abnormalities are accompanied by vascular attenuation, and various degrees of retinal atrophy and bone-spicule-like hyperpigmentation in the peripheral retina.

2.4 Choroideremia

Choroideremia is an X-linked chorioretinal dystrophy, with an estimated prevalence of 1:50.000-100.000, depending on the geographic location, and with a preponderance in the European population.[54] It is caused by mutations in *CHM*, a gene encoding Rab escort protein 1. This protein is an essential mediator of intracellular protein trafficking in photoreceptors and the RPE.[55]

2.4.1 Presentation

Symptoms usually present in childhood or adolescence. Male patients experience nyctalopia, followed by progressive visual field restriction. Visual acuity decline is slow, but rapidly ensues in the fifth to sixth decade of life.[56] Although more severe cases have been reported, patients usually reach legal blindness in mid-to-late adulthood.[56, 57]

Female carriers of *CHM* mutations typically remain asymptomatic and do not have abnormal electroretinographic signals.[58] However, some female carriers may experience nyctalopia.[56, 59, 60]

2.4.2 Fundus features

In affected male patients, the retina initially shows mottled areas of pigmentary changes in the equatorial region and in the macula. The degeneration gradually progresses and eventually manifests as widespread and sometimes serrated areas of atrophy of the retina, RPE, and choriocapillaris, in combination with scattered coarse intraretinal clumps of hyperpigmentation, which may even be observed in early childhood in severe cases.[61, 62] This results in a characteristic pale color of the fundus, due to the translucence of the sclera. Typically, larger choroidal vessels are preserved. The fovea is spared until later in the disease, and a residual island of foveal sparing is a frequent finding, although marked vision loss due to progression of foveal atrophy may ensue above the age of 50.[63] Female carriers of *CHM* mutations may show some retinal abnormalities, such as patchy or mottled pigmentary changes, or even RPE and choriocapillary atrophy,[58, 59] despite having no symptoms.

2.5 Macular dystrophies

The group of macular dystrophies encompasses a wide range of retinal dystrophies in which the degeneration is – at least initially – confined to the macular photoreceptors.[64] Therefore, this degeneration is usually not detectable on full-field electroretinography, but may be detectable on the multifocal electroretinogram. The most prevalent inherited macular dystrophy is Stargardt disease.[65,66] Best vitelliform macular dystrophy and adult-onset foveomacular vitelliform dystrophy are frequently encountered forms of autosomal-dominant macular dystrophy.[67]

2.5.1 Presentation

The presentation of symptoms may differ between each type of macular dystrophy. Stargardt macular dystrophy shows a highly variable age at symptom onset and variable severity.[65, 66, 68] Most cases present in childhood or early adulthood with central vision loss,[69] but onset in late-adulthood (age ≥ 45 years) has been described as well, usually accompanied by metamorphopsia rather than visual acuity loss.[65, 68, 70] In autosomal dominant Best vitelliform macular dystrophy, the age at onset is variable, and patients may present with reduced visual acuity, or with photophobia, metamorphopsia, and even night blindness.[67] In adult-onset foveomacular vitelliform dystrophy, the age at diagnosis is usually after the age of 40.[71] In central areolar choroidal dystrophy, early disease stages may be difficult to diagnose, due to the discrete and aspecific retinal changes, but later disease stages may be accompanied by profound central vision loss.[72] In North Carolina macular dystrophy, symptoms present in childhood, and are, along with the fundus features, typically stable throughout later years.

2.5.2 Fundus features

In Stargardt disease, characteristic irregular yellow-white fundus flecks are visible in the posterior pole, which later progress to chorioretinal atrophy.[65]

Best vitelliform macula dystrophy consists of several stages, in which the earliest is characterized by a normal fovea or subtle RPE alterations, followed by the "scrambled-egg" stage, a pseudohypopyon stage, ultimately progressing to chorioretinal atrophy. In 2-9% of patients with Best vitelliform macular dystrophy, choroidal neovascularization occurs.[67] The typical feature in adult-onset foveomacular vitelliform dystrophy is a solitary, slightly elevated, yellow-white, round to oval lesion. It may be complicated by choroidal neovascularization.

In some forms of macular dystrophy, drusen may be present, which may be small (≤63 μm), intermediate-sized (>63 μm and ≤125 μm), or large (>125 μm). Some forms of macular dystrophy may mimic age-related macular degeneration (AMD), as in Mallatia Leventinese, characterized by drusen, or Sorsby fundus dystrophy, characterized by choroidal neovascularization.[64] Central areolar choroidal dystrophy presents with aspecific RPE changes in its early stages, but progresses to a profound atrophy of the RPE and outer retina in the macula, that may easily be confused with geographic atrophy in AMD.[72]

3. CLINICAL EVALUATION OF RETINAL STRUCTURE IN INHERITED RETINAL DYSTROPHIES

The advent of novel therapeutic opportunities necessitates a thorough clinical insight in the natural history of inherited retinal dystrophies, further emphasizing the importance of longitudinal clinical examinations. The arrival of experimental therapeutic options has led to a paradigm shift: where, in the past, extensive examination at regular intervals in this patient population may have seemed unnecessarily burdensome to the patient, with no significant impact on the ophthalmologist's treatment policy, nowadays the regular and extensive examination of these patients can aid in natural history studies investigating the phenotypic disease spectrum, potential windows of opportunity, and the most sensitive parameter for documenting disease progression and treatment effect. The most common clinical parameters are described below.

3.1 Spectral-domain optical coherence tomography

Spectral-domain optical coherence tomography (SD-OCT) enables near-histological evaluation of the retinal architecture and the integrity of the photoreceptor structures in the macula. The photoreceptor nuclei are located in the outer nuclear layer. The external limiting membrane (ELM), which is the first hyperreflective outer retinal band, is thought to represent the adherens junctions between Müller cells and outer part of the photoreceptors.[73] The second hyperreflective outer retinal band, the so-called ellipsoid zone, represents the mitochondria-rich photoreceptor inner segments,[3, 74] but was previously thought to represent the inner segment/outer segment junction.[75] The photoreceptor outer segments co-localize with the interdigitation zone, the third hyperreflective outer retinal band, previously known as the cone outer segment tips line.

As photoreceptors degenerate, disorganization of the hyperreflective outer retinal bands occurs, corresponding with visual acuity loss,[76] accompanied by thinning of the outer nuclear layer. These changes are observed in the peripheral macula first, and encroach upon the fovea, showing a "transitional zone" between degenerated and relatively spared outer retina. Retinal sensitivity has been shown to decline faster in this transition zone than in other regions of the retina.[77] While the inner retinal layers may remain well-preserved, thinning of the ganglion cell layer has been described in advanced disease stages,[78] as well as thickening of the inner retina.[79, 80] The latter may be due to neuroglial remodeling reactive to photoreceptor loss, and may precede eventual inner retinal thinning in end-stage RP. With advanced retinal and RPE atrophy, and more so in cases with associated high myopia (such as in with XLRP), choroidal thinning and even posterior staphylomas may be observed.[81]

Optical coherence tomography angiography (OCTA) is a novel non-invasive imaging technique, that applies motion contrast between sequential OCT b-scans captured at the same cross-section. As a result, capillary blood flow can be visualized in the inner vascular plexus, the deep retinal vascular plexus, and the choriocapillaris.[82] Although it is prone to artifact, and may miss areas of slow blood flow (threshold artefacts), it is particularly useful in visualizing certain pathologies, such as choroidal neovascularization, or central serous chorioretinopathy.[83] Its utility in retinal dystrophies is undetermined.

3.2 Fundus autofluorescence

Short-wavelength fundus autofluorescence (FAF) provides information on the integrity and function of the RPE, through the visualization of lipofuscin (Figure 4). Lipofuscin is material derived from degraded photoreceptor outer segments that have been shed from photoreceptors and phagocytosed by the RPE, as part of a physiological outer segment turnover. Short excitation wavelengths (488 nm) of blue light will cause lipofuscin to autofluoresce, and light emitted at wavelengths between 500-800 nm is captured on the image. Areas of lipofuscin accumulation will appear hyperautofluorescent, while areas of atrophic RPE will appear hypo-autofluorescent. In RP, a perimacular concentric hyperautofluorescent ring or arc is seen in some patients, and is considered the transition zone between degenerated and relatively spared retina.[84] When overlaying a FAF image with a concurrent SD-OCT scan, the hyperreflective ring indeed colocalizes with an area of outer nuclear layer thinning and severe attenuation of the ellipsoid zone, with relative sparing of the photoreceptor structures internal to the ring. This ring often constricts progressively over time.[84] Double concentric hyperautofluorescent rings have also been described.[85] The origin of the hyperautofluorescence remains under debate. It has been postulated to stem from the increased rate of outer segment phagocytosis at the RPE level,[84, 86] or, more recently, from the accelerated lipofuscin synthesis pathway in compromised photoreceptors or photooxidation of liposfuscin.[87] Other RP-associated abnormalities are hypo-autofluorescent changes that can be granular, mottled, or bone-spicule-shaped.

An alternative mode of FAF is near-infrared-autofluorescence, which visualizes ocular melanin (as opposed to lipofuscin) through a longer excitation wavelength (787 nm). In turn, emitted light at wavelengths above 810 nm is captured on image.[88] Near-infrared-FAF may be particularly useful in patients with cataracts, severe photophobia, or a decreased capacity for attention (e.g. children), as image acquisition with this modality is faster, excites using less visible light, at wavelengths that better penetrate media opacities. The physiological autofluorescence pattern in near-infrared FAF differs from that in short-wavelength FAF, but in some patients with RP, a hyperautofluorescent ring can be observed with both imaging modalities.[89]

3.3 Adaptive optics

Adaptive optics high-resolution imaging is a method of in vivo histology, allowing the visualization of microscopic structures in the living human retina.[90] It has been used to visualize and quantify the human cone and rod photoreceptor mosaic, RPE cells and leukocytes. Techniques for imaging rods and cones differ, and this difference is thought to be based on the smaller size of rods and/or their reduced waveguide capabilities.[91] In severe retinal degeneration, adaptive optics may confirm the presence and integrity of target cells in the retina for gene therapy, thus estimating the therapeutic potential of gene therapy on a given retina. This estimate may be based on cell density and cell reflectivity. Adaptive optics may also be useful in monitoring the safety and efficacy of experimental therapeutic strategies. As gene therapy is not expected to add photoreceptors, but rather protect the remaining photoreceptors, cell reflectivity rather than cell density has been postulated as an indicator of treatment efficacy.[92]

3.4 Fluorescein angiography and indocyanine green angiography

Fluorescein angiography allows the study of the retinal and choroidal circulation. Retinal photographs are taken following the intravenous injection of fluorescein, an orange-red fluorescing molecule that diffuses through most body tissues. Its fluorescence is visualized on camera by blue light excitation (465-490 nm). The inner and outer blood-retinal barriers, formed by the tight junctions of the retinal capillary endothelial cells, and the RPE, respectively, normally prevent fluorescein from entering the retinal vessels or the subretinal space. However, when the capillary endothelium is damaged, fluorescein leaks into the retina. When RPE is damaged, fluorescein leaks into the subretinal space and the retinal interstitium.

Indocyanine green angiography allows the study of the choroidal circulation, through the use of indocyanine green dye. This dye is almost completely (95-98%) protein-bound after intravenous injection, and consists of relatively large molecules. As a result, diffusion through the small fenestration of the choriocapillaris is limited, and indocyanine green is mostly retained in the choroid, although diffusion through the choroidal stroma and accumulation within the RPE have also been demonstrated.[93] The fluorescence of indocyanine green occurs in the infrared range (790-805 nm).

In the diagnosis and follow-up of IRDs, fluorescein angiography may be useful in the visualization of cystoid macular edema, or the classic "dark choroid" in Stargardt disease, but is otherwise of limited added use.

4. CLINICAL EVALUATION OF RETINAL FUNCTION IN INHERITED RETINAL DYSTROPHIES

4.1 Central visual function

Measures of central visual function, such as best-corrected visual acuity (BCVA) and color vision, are usually normal in early disease stages. With the progression of central cone photoreceptor degeneration, BCVA will eventually decline. The age at which the visual acuity is expected to have declined to low vision or blindness, defined by the World Health Organization as BCVA<20/67 and BCVA<20/400, respectively, depends on the diagnosis. In RP, it depends partially on the mode of inheritance and the involved gene, but may differ even within the same family. ADRP generally has the best visual prognosis, with some patients maintaining good visual acuity well into the 6th and 7th decade of life.[17, 18] XLRP and ARRP have a worse visual prognosis. Some genetic subtypes of ARRP have been shown to lead to visual impairment within the first two decades of life in half the patient population.

While determining BCVA with a Snellen chart is the most conventional way of assessing central macular function, this method uses high-contrast charts that may not detect subtle changes in central visual function. Patients can experience difficulties with central vision in lower-luminance environments and may need visual aids or computer screen adjustments in their daily life, while still maintaining a well-preserved BCVA. A potentially more sensitive but less routinely examined parameter of central visual function is contrast sensitivity, which can be measured using e.g. the Pelli-Robson contrast sensitivity chart or sophisticated grating tests. Reports on contrast sensitivity in large populations of RP patients are limited, but RP patients with normal BCVA may still have significantly reduced contrast sensitivity.[94]

Color vision disturbances may be subtle and may be observed starting from the intermediate stages of RP. Different testing modalities have different sensitivities in detecting the presence, type, and severity of the color vision disturbance. Generally, tritanopia (blue-yellow deficiency) is the most frequently and first-observed color deficiency. In later stages, protanopia (red-green deficiency) may develop, and errors may become more chaotic with progressing central macular atrophy, advancing to total color blindness.

4.2 Visual field testing and microperimetry

Visual field impairment is a hallmark symptom of RP, typically beginning with patches of sensitivity loss in the midperiphery, which gradually form a ring scotoma. This ring scotoma then extends centripetally towards the far periphery and center. In advanced disease stages, a central island of vision and a peripheral wedge of vision are seen, which may or may not be connected to each other (Figure 5). In end-stage disease, some patients retain a central island of vision. Other visual field loss patterns have been described, such as progressive concentric visual field constriction without a preceding ring scotoma, or a predilection for visual field loss in the superior hemisphere.[95] These abnormalities and their progression with time should be examined periodically with kinetic perimetry and documented. In kinetic perimetry, visual stimuli of fixed size and intensity are moved by an operator from non-seeing areas into seeing areas of the visual field. Commonly, the Goldmann perimeter is used, and in recent years semi-automated perimeters such as the Octopus 900 (Haag-Streit International, Switzerland) are also used.

Central visual field measurements in the form of static perimetry or microperimetry map the sensitivity of the central macula, using stationary stimuli at different locations within the central 10° or 30° radius of the macula, adjusting the stimulus intensity based on the patient's response. This may be particularly useful in cone (-rod) dystrophies and macular dystrophies. However, in retinitis pigmentosa, central visual field measurements may remain within normal range in early disease stages. In microperimetry, this sensitivity is correlated to the exact location in the macula in real-time, and fixation stability is assessed. Microperimetry is particularly advantageous over conventional static perimetry in patients with fixation loss,[96] and it may detect macular sensitivity changes before BCVA changes occur.[97]

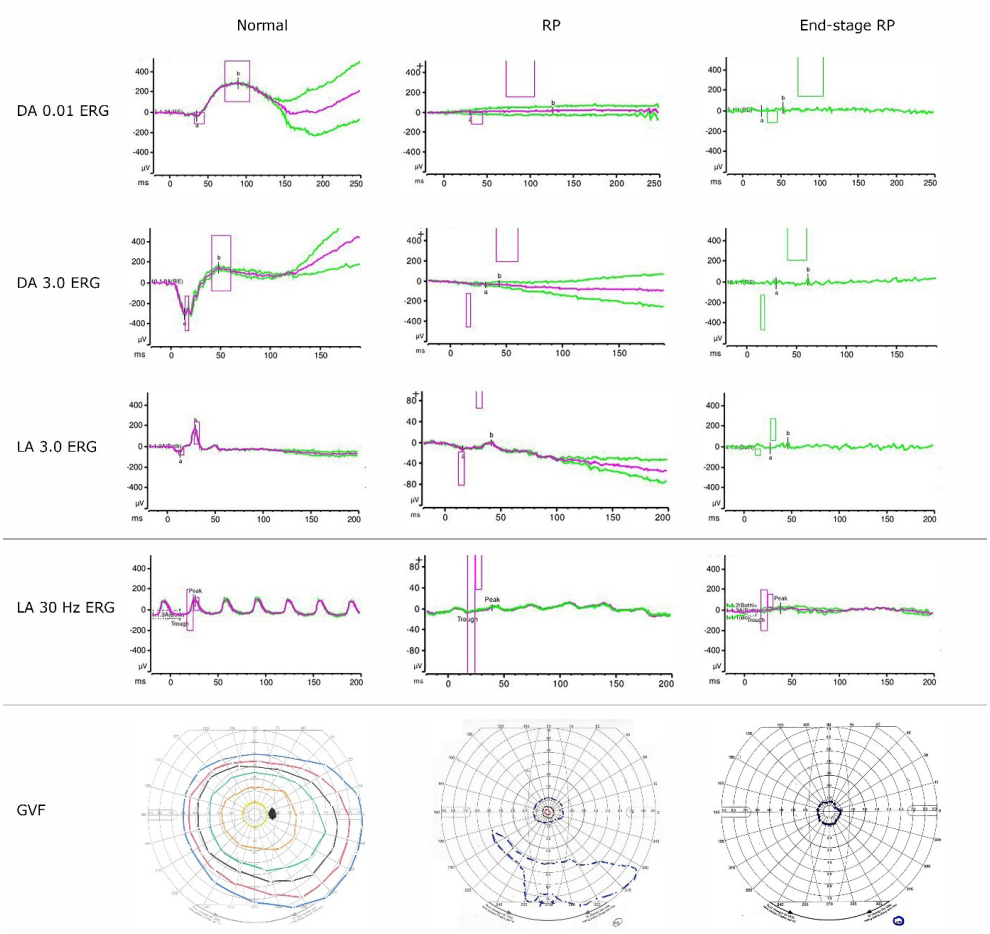

Figure 5. Overview of representative electrophysiological and visual field examination results in retinitis pigmentosa (RP). Each row represents the stimulus used in electroretinography (ERG) testing based on recommendations by the International Society for Clinical Electrophysiology of Vision (ISCEV). The dark-adapted (DA) 0.01 flash elicits a rod-driven response of ON-bipolar cells; the DA 3.0 flash elicits a mixed rod- and cone-driven yet rod-dominated response of both photoreceptors and bipolar cells; the light-adapted (LA) 3.0 flash elicits cone-driven responses from ON- and OFF-bipolar cells; and the 30 Hz flicker elicits a cone-driven response. Rod-driven responses are often non-detectable in early disease stages, while a 30 Hz flicker response progressively diminishes with advancing disease, i.e. a rod-cone pattern of dysfunction, as is seen in the 2nd and 3rd columns. The Goldmann visual fields (GVF) may show various patterns of (mid)peripheral visual field loss, eventually resulting in a small central remnant of vision.
Credit: the Department of Ophthalmology at the Leiden University Medical Center in Leiden, The Netherlands.

4.3 Electrophysiological testing

A full-field electroretinogram (ffERG) is an objective tool to measure the functional integrity of the inner and outer retina, and quantifies rod and cone dysfunction. This method measures dark- and

light-adapted responses to light flashes at different intensities, from which rod and cone electrical responses can be deduced, respectively. Isolated cone responses are measured after light-adaptation with a 30 Hz flicker stimulus (i.e. 30 stimuli per second), where rapidly repeating flashes stimulate the retina. Rods cannot respond to flickering light with a frequency above 20 Hz. The ffERG is usually performed for diagnostic purposes, and less commonly to clinically monitor disease progression. FfERGs should be evaluated on the amplitudes of the a-wave, a negative deflection that is generated by both rod and cone photoreceptor activation, and the b-wave, a positive deflection generated by activation from cells in the inner retina (i.e. bipolar cells, with some contribution from Müller cells), and on the implicit time, which is a time interval measured between stimulus onset and b-wave peak. The International Society for Clinical Electrophysiology of Vision (ISCEV) has established guidelines for a basic ffERG testing protocol, but also recommends extension of this protocol where relevant.[98]

FfERGs in RP show reduced dark-adapted (scotopic) responses from the early disease stages when light-adapted (mixed rod-cone) or cone-flicker responses can still be within normal limits. Wave amplitudes are reduced, and rod implicit times are often delayed. As the disease advances, rod and cone amplitudes further diminish and the implicit times are further delayed, but the attenuation is more marked for rod responses (a rod-cone pattern; Figure 5). In advanced stages of RP, the ffERG becomes extinguished, i.e. residual responses have become too small to be detectable. In sectorial RP, the ffERG can remain with the normal range, as a smaller retinal area is affected. Cone dystrophies are characterized by reduced light-adapted and/or cone-flicker responses. Dark-adapted responses remain normal, and may be reduced in end-stage cone dystrophy. In cone-rod dystrophy, both light-adapted and dark-adapted responses are reduced in the early disease stages, with a more profound reduction of the light-adapted and cone-flicker responses than the dark-adapted responses. As cone-rod dystrophy advances, it may be difficult to distinguish from RP. In macular dystrophies, typically no panretinal cone- and/or rod dysfunction is detectable on ffERG.

Multifocal electroretinography (mfERG) measures the retinal function across the central 40°-50° of the macula, mapping electrical responses to specific regions and requiring stable fixation, as opposed to the full-field approach. It is less commonly used as a diagnostic tool, as the cone function in the central macula is often spared in RP, but it can be used as a complementary tool in documenting disease progression and macular function.[99] It may be of particular use in cone-(rod) and macular dystrophies. The pattern electroretinogram (PERG) is another complementary tool in measuring the extent of macular function, as it measures responses from the macular retinal ganglion cells.

4.4 Full-field stimulus testing

In the most severe cases of IRD, where ffERG responses are non-detectable and where patients may have ultra-low vision or light perception vision, full-field stimulus threshold testing (FST) provides

a psychophysical outcome measure.[100] White light stimuli are used for testing the lowest luminance threshold a patient can detect. As the test measures the lowest luminance threshold, it detects the sensitivity of the most sensitive area of the retina, without further specification of the location of this area. Blue and red stimuli may be used to further specify whether the vision in this area of retina is cone- or rod-mediated.

This test is not part of the clinical workup routine in IRD patients, but is a useful tool in natural history studies and gene therapy trials, where the most severely affected IRD patients are usually the first patients to undergo safety testing in phase I. In such severely affected groups, FST may still be able to provide a measure of photoreceptor (rod and/or cone) function.

5. GENETICS

5.1 Inheritance patterns

All forms of Mendelian inheritance have been described in retinal dystrophies. For example, non-syndromic RP can be inherited in an autosomal dominant (15-25% of RP cases), autosomal recessive (5-20% of RP cases) or X-linked fashion (5-15% of RP cases).[14] The remaining 30-55% of RP cases cannot be classified as they have no reports of affected family members, and are denoted as isolated or simplex RP. Although the majority of simplex cases are predicted to be ARRP, approximately 15% of male patients with simplex RP have a mutation in the XLRP genes *RPGR* or *RP2*.[101] *De novo* ADRP mutations account for at least 1-2% of simplex RP cases.[102]

In XLRP, mild to severe phenotypic expression can occur in female carriers, probably due to non-random or skewed X-inactivation.[103] Consequently, some XLRP pedigrees may be misclassified as ADRP.[104] Approximately 8.5% of families initially considered to display ADRP are caused by mutations in XLRP genes.[105]

A very small proportion of cases have been reported to result from a non-Mendelian inheritance, such as digenic RP.[104]

5.2 Genetic heterogeneity

In the past two decades, mutations in at least 260 different genes have been identified in inherited retinal degenerations, with over 96 different genes in RP, 35 different genes in cone or cone-rod dystrophies, and 27 different genes in Leber congenital amaurosis [RetNet, update of June 21, 2021, URL: sph.uth.edu/retnet/]. Despite this high number of known disease-associated genes (locus heterogeneity), the mutation detection rate in e.g. RP is, depending on the RP type and the molecular technique used, 30-80%, leaving a considerable fraction of cases unexplained. Apart from this locus heterogeneity, allelic heterogeneity is also strikingly present, with over 3000

different mutations reported in non-syndromic RP. Within the same gene, different mutations can result in a different phenotypic outcome or variation in disease severity, illustrating a large clinical heterogeneity.[106, 107]

In some ADRP families, individual mutation carriers may not exhibit clinical signs of RP. Incomplete or reduced penetrance and variable expression has been reported for dominant mutations in a number of genes (e.g. *PRPH2*, *PRPF31, PRPF8, SNRNP200*).[108, 109]

5.3 Molecular analysis

Choosing the right molecular test for patients with a retinal dystrophy can be challenging, especially with the plethora of disease genes and the different tests that are provided by molecular laboratories. Several factors need to be taken into account, including the clinical findings, family history, presence or absence of consanguinity, previous molecular testing, the reimbursement options by the patients' insurance, and the expertise of the laboratory. An overview of the available genetic tests provided by accredited laboratories can be found on the orpha.net portal.

Since whole exome sequencing (WES) has become an affordable routine test, it competes with targeted gene-panel based next-generation sequencing (NGS) approaches. Despite screening of protein coding parts of a large part or all known inherited retinal dystrophy genes, many cases remain unsolved. In these cases, several considerations should be taken into account:

(1) Some panels only target a limited set of genes (e.g. the most prevalent RP genes). Furthermore, some regions of a specific gene might not be targeted using NGS. A molecular report should provide information about the targeted regions. It is expected that more RP-associated genes will be elucidated in the coming years, each accounting for a small percentage of cases.

(2) Some regions might not be well-covered using WES or targeted-NGS, especially GC-rich or highly repetitive sequences. The *RPGR* open reading frame (ORF) 15, a mutation hotspot for XLRP, is such an example. For XLRP it is recommended to start with a targeted approach that also provides coverage of the entire ORF15 sequence, especially since nearly 60-70% of the disease-causing *RPGR* mutations are located in this region.[81, 110]

(3) Inherent to the screening technique used, some specific genetic alterations might be missed, such as deep-intronic variants and structural variations (SVs).[111, 112] Whole genome sequencing (WGS) has proven to be a sensitive method for screening of both deep-intronic variants and SVs and will likely in the near future replace WES as a standard diagnostic test.[113, 114]

(4) Variants of unclassified significance (VUS), i.e. class 3 mutations, can be reported. The evidence of pathogenicity of these variants can evolve over time to benign or likely

pathogenic based on more biological evidence or the presence of the variant in multiple patients with an inherited retinal dystrophy. For partially solved cases, segregation analysis in affected and unaffected family members, may provide more insight in the pathogenicity of the VUS.[115]

6. PATIENT MANAGEMENT

For most forms of inherited retinal dystrophy, no effective and commercially approved treatment is available. Patient management should therefore consist mostly of elaborate counselling on the disease background and heredity, prognosis, low vision aids and advice on lighting where applicable, particularly at school or at the work place, and management of additional ocular conditions. Psychosocial counselling should also focus on a patient's coping strategy regarding the diagnosis and prognosis. However, as avenues for gene-specific and gene-independent treatments are investigated, establishing a reliable genetic diagnosis becomes increasingly important. Moreover, a molecular diagnosis provides the possibility to screen family members at risk, to perform carriership testing in partners, and to offer the option of prenatal testing or preimplantation genetic diagnosis. The visual function and retinal imaging should preferably be evaluated at regular intervals, such as every 1-3 years, also taking into account the patient's needs and preferences. Patients with associated ocular conditions such as cataract and glaucoma may require more frequent assessments. Careful documentation of the disease progression allows for a tailored estimate of the prognosis in any given patient. Moreover, with the relatively rapid advent of experimental therapeutic strategies, this documentation may prove invaluable in the setting of (retrospective) natural history studies. In prospective natural history studies and in gene therapy trials, retrospective documentation may prove a helpful aid, e.g. in determining the length of visit intervals.

Some patients may wish to be involved in patient groups or societies. Where possible, these patients should be referred to a tertiary expertise center, where the disease progression can be monitored, and where patients can be kept up to date on ongoing and future international clinical trials.

6.1 Family management and counselling

Determining the inheritance pattern is essential in family management and counselling. A detailed family history should be taken, including the number of potentially affected family members and their relation to the proband, as well as the presence of consanguinity in the family. Drawing a pedigree is advisable. Patients are usually referred to the genetic counsellor such as a clinical geneticist for queries regarding the reliability and implications of genetic test results. Usually, genetic testing is refrained from in presymptomatic children, as genetic testing should entail a well-considered decision process for which the child may be too young. Molecular testing can be

offered to family members at risk. If there is a higher risk of having affected children, the option of preconception counselling diagnosis and preimplantation genetic diagnosis should be discussed.

6.2 Treating associated ocular conditions

Ocular conditions associated with RP, such as refractive errors, macular edema or visually significant cataract, should be monitored and managed. Cystoid macular edema may be treated with systemic or topical carbonic anhydrase inhibitors, although this condition is often refractory in RP, and reports on potential visual benefit are inconsistent.[116] If the edema is unresponsive to carbonic anhydrase inhibitors, other treatments, including topical, oral or intravitreal steroids, intravitreal anti-vascular endothelial growth factors, laser photocoagulation, or pars plana vitrectomy, also appear of limited success.[115] Corrective glasses with specific color filters may be helpful in patients with light aversion or decreased contrast sensitivity. Visually significant cataracts should be surgically treated, as both visual outcome and patient satisfaction are generally favorable,[117, 118] but in the case of pre-operatively present macular atrophy, patients should be informed on the potentially limited visual benefit. In order to reduce potential light-induced toxicity to the retina, the intra-operative microscope light intensity can be reduced, and between different surgical steps the microscope light may be switched off in order to minimize light exposure.[118] Increased risks associated with cataract surgery in the RP population include zonular weakness, and the postoperative development of anterior capsular rhexisis phimosis, posterior capsule opacification and cystoid macular edema.[118, 119]

6.3 Retinal prostheses

Retinal prostheses can have a role in legally blind patients with end-stage RP, and require careful pre-operative screening and expectation management, counselling, and a comprehensive post-operative rehabilitation program at a specialized center.[120, 121] The two most studied epiretinal implants, the Argus II and alpha-IMS, have shown performance results that can overall be considered similar, despite large differences in implant design.[122] While most patients with a retinal prosthesis show an improvement in mobility and orientation tasks, approximately one third experiences measurable visual acuity improvement.[123] Reading speed can be improved in a subset of patients, although single-letter recognition may still take up to several minutes.[124] Pre-operative counselling should comprise the advice that the output from the prosthesis is an entirely new type of functional vision rather than the recovery of previous vision.[125] Due to the guarded benefit, and the frequent visits and intensive rehabilitation required to achieve it, patient selection and expectation management are key.

6.4 Oral nutritional supplements

The role of vitamin A supplementation remains debated.[126] At age-adjusted dosages, vitamin A supplementation has been shown to slow the decline of the cone amplitudes on ffERG in a recent study.[127] However, this result was described in a small, retrospective, observational study, where loss-

to-follow-up was higher among the group of RP patients who did not receive vitamin A, obscuring an already sparse finding. The randomized, controlled, double-blinded trial that prompted this recent study, was published in 1993 and found no significant beneficial effect of vitamin A supplementation.[128] As any effect has been shown in genetically undifferentiated RP populations, it remains unclear whether vitamin A supplementation works for all RP subtypes, or possibly be harmful to certain subgroups. Mouse studies have indicated that vitamin A supplementation might be avoided in *ABCA4*-associated retinal dystrophies, which do not entail RP, but nonetheless raise caution in prescribing vitamin A supplementation.[129] Moreover, hypervitaminosis A can lead to toxicity in several organ systems, and adverse reactions should be assessed at intervals. A synthetic vitamin A derivative, 9-cis-retinyl acetate, has shown efficacy in preserving visual field area and visual acuity in a clinical trial with patients with *RPE65*-associated and *LRAT*-associated retinal dystrophies, including RP, which comprise 5% of cases of childhood-onset RP.[130]

Care should be taken before prescribing oral supplements. Docohexaenoic acid (fish oils) has shown no clear efficacy in slowing disease progression, and oral valproic acid has been shown to lead to a worse visual outcome than placebo.[131,132] Vitamin E supplementation has been associated with a faster decline of light-adapted electroretinogram amplitude.[128]

6.5 Therapeutic advancements

As for treatments aimed at halting the degenerative process, the approval of subretinal injections of gene therapy (voretigene neparvovec, Luxturna®) for *RPE65*-associated retinal dystrophies in 2017 by the United States Federal Drug Administration offers promising perspectives for trials in retinal dystrophies associated with other genes.[133] With the advent of trials investigating therapeutic options, patients should be kept updated on ongoing or upcoming trials that may be relevant to them. All interventional efficacy trials in humans, including gene therapy studies, are registered (e.g. on the ClinicalTrials.gov platform). These trials will provide evidence on the safety and efficacy of a medical intervention. For several genes associated with ARRP or XLRP, clinical gene augmentation therapy trials are ongoing or being prepared for in the pre-clinical phase. For ADRP, gene augmentation alone is probably not enough, as the disease is based on a deleterious gain-of-function mechanism, and genome-editing approaches are under investigation, for example using the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and their associated genes (Cas) in a gene-replacement strategy. As these approaches usually need a viral vector, such as adeno-associated viral vectors, they are applicable in patients with remaining functional photoreceptor cells. Therefore, certain early-onset and rapidly progressive forms of RP would ideally require therapeutic intervention in childhood or adolescence.[134]

Another therapeutic strategy utilizes antisense oligonucleotides (AONs), which are short, synthetic, single-stranded oligodeoxynucleotides. They can be delivered as "naked" oligonucleotides or via a viral vector, and can interfere with splicing through several mechanisms.[135] AONs are useful

in blocking aberrant splice events and redirecting normal splicing. They are not applicable to all mutations, but preclinical efficacy studies have shown great potential for treating specific cases of e.g. Stargardt disease, and *CEP290*-associated LCA.[136, 137] In animal studies, AONs have shown promising results in the treatment of autosomal dominant IRD, such as *RHO*-associated RP.[138]

Expectations of the outcomes of such trials should be managed, as cell death is principally irreversible, and the highest aim is the stabilization of current vision, which may already be severely impaired. In rare cases, visual improvement has been reported with gene replacement therapy or antisense oligonucleotide therapy.[139, 140] Patients should be advised that these options are gene-specific or mutation-specific.

For more advanced disease stages, cell-based therapeutic options are investigated, which are not gene-specific. Examples include the intravitreal or subretinal administration of retinal progenitor cells or induced pluripotent stem cells.[141] As of yet, most studies investigating these methods have been performed in animal populations, and several drawbacks should be taken into consideration, including limited graft survival and limited in vivo graft function. Human cases of severe vision loss have been described after intravitreal injection of autologous adipose-tissue derived stem cells in the treatment of age-related macular degeneration at a "stem cell clinic" in the United States.[142] Intravitreal implants containing ciliary neurotrophic factor have shown short-term visual field sensitivity loss compared to sham treatment, and no long-term benefit.[143]

6.6 From bench to bedside: the gaps in clinical knowledge and the hurdles they present

The rapid evolution of therapeutic advancements is in stark contrast to the available clinical knowledge on the different disease phenotypes associated with each gene, and the rate of disease progression. This information is important in order to better understand the disease and to better inform patients on their prognosis, but it is also essential in the implementation of gene or cell-based therapy. Ideally, the preclinical development of a (gene) therapy should be paralleled by the available knowledge on the natural disease course, the identification of the patient subpopulation most likely to benefit from this therapy, and the identification of outcome parameters. However, the limited information available about the natural course of disease in many genetic subtypes of IRD is based predominantly on case reports or case series of single-visit patient data collected from single centers. Due to the relative rarity of some genetic subtypes, quantitative analysis often lacks from the available literature. Also, the available data from different reports may be inconsistent, as some studies may describe early blindness, while others report preservation of vision until relatively later years. Genotype-phenotype correlations are a particularly interesting point of focus in the potential explanation of these differences, and they remain to be elucidated.

Advances in retinal imaging, such as fundus autofluorescence and spectral-domain optical coherence tomography, and in functional testing, such as microperimetry, present new possibilities

for the definition of markers for disease progression. As most clinical trials last several years, the progression markers used as outcome parameters are ideally sensitive to change in a relatively short time-span. Thorough knowledge of the clinical aspects of a particular genetic IRD subtype is crucial in defining the window of therapeutic opportunity, patient candidacy criteria, and outcome parameters. These factors may be pivotal in the success of a gene therapy trial and the approval of a gene therapy by the FDA or EMA, further underlining the necessity of natural history studies.

AIMS AND OUTLINE OF THIS THESIS

As several studies investigating gene therapy trials and other cell-based therapeutic options have shown promising results, questions arise on the most appropriate implementation of these novel methods. The successful development and implementation of any new therapy requires an in-depth understanding of the disease, its phenotypic spectrum, and the speed at which it progresses. This theses aims to answer to these key issues, addressing the following questions for retinopathies associated with mutations in *CRB1*, *RPGR*, *RHO*, *LRAT*, and *CHM*:

- What is the window of opportunity for intervention through gene- or cell-based therapy?
- Which functional parameters are most time-sensitive to change? And which parameters will thus be the most appropriate clinical endpoints in a gene therapy trial?
- Which structural parameters correlate strongly to functional parameters, and may potentially serve as surrogate endpoints in a gene therapy trial?
- What are the genotype-phenotype associations, if any? I.e. can we identify characteristics that predict a specific phenotype, or that predict slower or faster disease progression?

Chapter 1 is the general introduction of this thesis, and provides the reader with information on the basic retinal anatomy and physiology. It introduces the reader to the general aspects of inherited retinal dystrophies.

Chapter 2.1. describes the largest longitudinal cohort of *CRB1*-associated autosomal recessive retinal dystrophies to date, combining patients from a large Dutch genetic isolate with patients from outside this genetic isolate. This study investigates the phenotypic spectrum and natural disease course in these patients. The size of this cohort allows for one of the first statistical analyses performed in this subgroup of patients. Yearly decline rates of the visual acuity and the visual field area are calculated, as well as time-to-event analyses for visual impairment. Some genotype-phenotype associations are described.

Chapter 2.2. contains a comprehensive description of several clinical parameters in the second largest longitudinal cohort of *CRB1*-associated retinal dystrophies, in a Belgian population. Some earlier findings from the Dutch population are corroborated in this Chapter, although the

phenotypes and, to a greater extent, the genotypes in the Belgian population were even more variable.

Chapter 2.3. is an extensive cross-sectional study of prospectively enrolled Dutch patients with *CRB1*-associated retinal dystrophies. This chapter focuses on the elucidation of structure-function correlations, in order to identify appropriate clinical endpoints and possible surrogate endpoints for future gene therapy trials for *CRB1*-associated retinal degenerations.

Chapter 3.1. describes the long-term clinical course and the visual outcome of (X-linked) choroideremia, with the longest follow-up time published to date, to our knowledge. Unique aspects of this Chapter include the focus on the effect of disease on the social participation of patients.

Chapter 3.2. describes the outcome of full-thickness macular hole surgery in a patient with choroideremia.

Chapter 4.1. describes a large study on the clinical characteristics and natural history of *RPGR*-associated X-linked retinitis pigmentosa and cone-/cone-rod dystrophy. Genotype-phenotype correlations are investigated, elucidating some differences between patients with mutations in the ORF15 mutational hotspot, and those with mutations in exons 1-14. The presence of high myopia and its effect on the disease course is featured in this chapter.

Chapter 4.2. studies female carriers of *RPGR* mutations, and uniquely highlights the phenotypic spectrum, and describes the effects of myopia and age on visual function. The complete expression of disease is demonstrated in a portion of subjects.

Chapter 5 describes the clinical spectrum and natural disease course in patients with *LRAT*-associated autosomal recessive retinal dystrophy, which is a particularly rare disease population. Inter- and intrafamilial differences are detailed.

Chapter 6 is a study of 100 patients with *RHO*-associated autosomal dominant RP. Detailed statistical analysis is performed, investigating the natural disease course as well as possible genotype-phenotype correlations in one of the largest studies on *RHO*-associated RP published to date.

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