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Monitoring progression of retinitis pigmentosa: current recommendations and recent advances

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Abstract

Introduction—Retinitis pigmentosa (RP) is the most common form of inherited retinal degenerations with an estimated prevalence of 1 in 4,000 and more than 1 million individuals affected worldwide. With the introduction of the first retinal gene therapy in 2017 the importance of understanding the mechanisms of retinal degeneration and its natural progression has shifted from being of academic interest to being of pivotal for the development of new therapies.

Areas covered—This review covers standard and innovative diagnostic techniques and complementary examinations needed for the evaluation and treatment of RP. It includes chapters on the assessment of visual function, retinal morphology, and genotyping.

Expert Opinion—Monitoring the progression of RP can best be achieved by combining assessments of both visual function and morphology. Visual acuity testing using ETDRS charts should be complemented by low-luminance visual acuity and colour vision tests. Assessment of the visual field can also be useful in less advanced cases. In those with central RP involvement measuring retinal sensitivity using microperimetry is recommended. Retinal morphology is best assessed by OCT and autofluorescence. Genetic testing is pivotal as it contributes to the pathophysiological understanding and can guide clinical management as well as identify individuals that could benefit from retinal gene therapy.

Keywords

Autofluorescence; ETDRS letters; genotyping; inherited retinal degeneration; IRD; microperimetry; optical coherence tomography; OCT; retinitis pigmentosa; RP

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1.0 Introduction

Inherited retinal degenerations (IRDs) are neurodegenerative diseases that lead to dysfunction of the retinal metabolism and/or cell death of various outer retinal cells (generally photoreceptors), and affect about 1 in 3,000 people worldwide.[1] Retinitis pigmentosa (RP), which describes a group of genetically heterogenous rod-cone dystrophies, is the most common form of IRD with an estimated prevalence of 1 in 4,000 and more than 1 million individuals affected worldwide.[2–4] More than 250 genes have been identified to cause IRD of which 80 genes have been linked to RP (Leiden Open Variation Database www.databases.lovd.nl; RetNet www.sph.uth.edu). The genetic trait of RP is usually inherited as autosomal recessive (50-60%), autosomal dominant (30-40%) or X-linked (5-15%).[4–7] The diagnosis is established clinically through a variety of examinations, which include both assessment of function and morphology that is then confirmed genetically. In addition to addressing any potential complications of the disease, the monitoring of the progression of RP was generally limited to re-assessments of visual function and provision of low vision aids as well as patient and family counselling. The recent approval of Luxturna® (voretigene nepavorec) for the *RPE65* associated Leber congenital amaurosis (LCA) both by the FDA and the EMA has heralded the start of a new era, and boosted the research and development of many other retinal gene therapies of which some have already entered clinical trial phase 3 (choroideremia: NCT03496012, *RPGR* X-linked RP: NCT03116113). The development of retinal gene therapies requires an understanding of the mechanisms of retinal degeneration and a way to monitor response to treatment. Developing standards for clinical trials in ophthalmology are of paramount importance and help with obtaining timely approval of new therapies arising from clinical research. This review covers standard and innovative diagnostic techniques and complementary examinations needed for the evaluation and treatment of RP. The focus will be put on highlighting methods that can serve as surrogate endpoints in clinical trials. A surrogate endpoint, as defined by the FDA is a biomarker that is “reasonably likely, based on epidemiologic, therapeutic, pathophysiologic, or other evidence to predict clinical benefit”.[8] It shall also highlight the importance of genotyping not only in establishing the correct diagnosis, but also to understand the pathophysiology and ultimately the phenotype.

2.0 Retinitis Pigmentosa Diagnostics and Monitoring

2.1 Assessment of Visual Function

2.1.1 ETDRS letters—Visual acuity (VA) is a reliable marker of visual function, and is the primary measure of visual function both in clinical and research settings. It is used as an entry criterion, outcome or efficacy measure as well as safety endpoint. VA is measured as minutes of arc and calculated as the reciprocal of the minimal angle of resolution (MAR). The ETDRS (Early Treatment Diabetic Retinopathy Study) letter charts with a retro-illuminated box were developed in the 1980s by Ferris et al.[9,10] with the goal of standardization of VA measurements, and have become the standard logMAR chart used in many multicentre clinical trials.[11] The characteristic of ETDRS letter charts is that a three-line worsening of visual acuity is equivalent to a doubling of the visual angle regardless of the baseline visual acuity used, which allows for correct interpretation of changes in visual

acuity over time.[9] Each ETDRS letter chart line has five letters created from the ten Sloan letters[12] with similar optotype difficulty scores with a geometric progression of letter height from line to line of 0.1 log unit. The 0.1 logMAR unit between the five letter lines allows to assign a 0.02 logMAR unit to each letter, which leads to a high repeatability and accuracy of the EDTRS letter charts.[13] Sensitivity and specificity analyses of the EDTRS charts in normal subjects however show that only a difference of 0.2 logMAR units (2 lines on the chart) reliably indicates a meaningful clinical change.[13] In patients with RP the reliability of EDTRS letter charts can vary even to a larger amount, which means that the precision to determine whether a true change in visual acuity has occurred may be reduced. The assessment of patients with visual acuities <20/200 (legal blindness) and visual field diameter of <20° showed twice the variability reported for normal sighted individuals.[14,15] Factors like glare and sensitivity to light as well as motivation and the typically higher rate of depression in the RP population increase the variability of VA assessment even in patients with good central vision, and have to be kept in mind when evaluating VA changes. The ETDRS letter charts are internally illuminated, which allow for consistent testing conditions. However, room illumination levels also must be considered since changes in illuminance levels have been found to give rise to greater changes in VA in the presence of myopic refractive errors, which are found more commonly in RP patients.[16] Another possible explanation for the reduced precision in patients with RP is the impact of the visual field loss on the ability to track letters on the chart. Increased VA test precision can be achieved by using electronic visual acuity (EVA) systems, in particular when the visual field loss affects the fovea.[17] It is certainly impossible to control fully for all psychophysical factors involved in VA testing, but efforts should be made to maximise standardisation of the procedure. In monitoring the progression of RP both in clinical routine and for trials we recommend using the standardized ETDRS charts (Precision Vision Inc., Woodstock, IL, USA) with consistent room illuminance to assure uniform contrast and luminance of the optotypes. The ETDRS-Fast method can be employed in the clinical routine setting to reduce test time, while maintaining reasonable accuracy.[18]

2.1.2 Low-luminance visual acuity, colour vision and contrast sensitivity—

Visual acuity decreases significantly with luminance.[19] Low luminance visual acuity (LLVA) is performed by using 2.0 log unit neutral density filters (reduction in luminance by 10^2 times) while reading a normally illuminated ETDRS chart. The low luminance deficit is defined as the difference between the LLVA and the standard VA level in logMAR units. It is a well-established fact that patients with RP show a more pronounced visual acuity deficit at low luminance.[20] In patients with geographic atrophy associated with age-related macular degeneration (AMD) the low luminance visual dysfunction was strongly predictive for future visual acuity loss.[21] However, care must be taken when interpreting LLVA changes since it might not only indicate a reduction in foveal cone function, but also a shift to eccentric fixation.[22]

Many patients with RP can retain good visual acuity until late in the disease course, while colour vision deficits can be present before the onset of degeneration. The physiologic substrate of the trichromatic colour vision is the cone photoreceptor, of which there are three classes: the short- (S-), the medium- (M-), and the long- (L-) wavelength sensitive cones.

[23,24] Colour discrimination can be tested using the Farnsworth-Munsell 100-Hue arrangement test under optimum lighting conditions. Patients with RP generally present with acquired S-mechanism deficiency (Verriest type III).[24] In choroideremia colour vision defects were detected prior to loss of central visual function indicating a general retinal functional impairment occurring earlier than cell loss.[25] Similarly, in a cohort of RP patients carrying the same homozygous *LRAT* mutation severe deficiency in all colour vision axes was found in a majority of patients despite non impaired visual acuity.[26] While colour vision testing is suitable to detect early subtle functional changes before deterioration in visual acuity, tracking of the colour discrimination is not a useful measure of disease progression.

Contrast sensitivity testing, over a range of spatial frequencies, is an alternate method of testing central visual function. Its usefulness lies in understanding the impact of visual impairment on the functional ability, and patients with IRDs often complain of reduced central vision, despite having normal visual acuity. There is no pattern of contrast sensitivity function that is unique to any particular disorder, although it could be a useful tool in monitor progression in Stargardt disease.[27]

2.1.3 Visual Field—Visual acuity tends to be an insensitive measure of disease severity, and other tests of visual function are required to diagnose and monitor RP. In static perimetry (i.e., automated perimetry), a stationary target is changed in size and brightness until seen, while in kinetic perimetry (i.e., Goldmann visual field) a target of predefined size and luminance is moved from a non-seeing to a potentially seeing area. The Goldmann visual field (GVF) is particularly useful for monitoring peripheral visual field defects, large scotomata, and the progression of visual field loss over time (Fig. 1).[28] The reliability of the GVF is however highly operator dependent, and prone to erroneous results from variable target speeds, particularly for small and dim targets.[29,30] The original Goldmann perimeter is no longer produced, and has been replaced by semi-automated kinetic perimetry (SKP) devices with standardization of target velocity and test pattern. The SKP Octopus 900 (Haag Streit AG, Koeniz, Switzerland) has shown strong agreement with the GVF, and is considered a viable alternative to the GVF in clinical trials and for clinical care.[30,31] Studies testing the quantitative analysis of OCT as a structural measure for disease progression have shown that perception of the Goldmann I4e isopter correlates with retinal areas showing an intact inner segment ellipsoid zone.[32] The usefulness of the Goldmann visual field as a clinical trial outcome measure however is limited by its high test-retest variability and its operator dependence.[28,33,34] Computerized wide-field static perimetry may however be a more reliable approach to assess the absolute sensitivity across the retina and ultimately enable an estimation of the functional change over time.[35] Assessment of the paracentral retinal sensitivity and the scotoma size can be achieved by static automated perimetry either using the Octopus 900® M pattern or the Humphrey® visual field 10-2 (Zeiss Medical Technology, Jena, Germany). Macular perimetry has also been shown to correlate well with loss of the ellipsoid zone in patients with RP[36], however does not allow accurate co-registration between the sensitivity map and the fundus image. The correlation between retinal sensitivity and fundus changes is achieved with microperimetry. The two current generation devices are the CenterVue MAIA (Macular Integrity Assessment) and the

Nidek MP-3 microperimeters. The devices are essentially similar and enable tracking of eye movement and assessment of fixation stability along with quantification of retinal sensitivity. Inter-device comparison on healthy subjects showed however differing retinal sensitivity values.[37] Robust normative database that would allow inter-relating both devices are still lacking. Currently, the most widely used microperimeter in clinical trials is the CenterVue MAIA (CenterVue, Padova, Italy), which is especially useful in the evaluation of central involvement of RP (Fig.2). The Nidek MP-3 has however also proven to detect significant changes in central retinal sensitivity over time.[37,38] Both devices are excellent clinical tools to monitor the progression of RP. Microperimetry may prove to be a valuable outcome measure in assessing retinal gene therapy responses in ciliopathies in which regeneration of outer segments have been linked to improvements in central retinal sensitivity.[39]

2.1.4 Electrophysiology—The full-field electroretinogram (ERG) enables a global assessment of photoreceptor function, and has traditionally played an important role both in the diagnosis and characterization of RP. The ERG represents a mass electrical response generated by light-induced changes in extracellular electrolytes (mainly Na^+ and K^+) at the level of the photoreceptor outer segments.[40] Comprehensive information on ERG is given by the International Society for Clinical Electrophysiology of Vision.[41] According to the ERG responses, retinal degenerations may be characterized as causing rod-cone, cone-rod, or second-order neuron dysfunction. RP is a typical example of rod-cone degeneration. The amplitude of the responses is proportional to the area of functioning retina.[42] It is estimated that on average, RP patients lose about 16-18.5% per year of remaining ERG amplitudes.[28] The ERG has its greatest value in the diagnosis of RP and other IRDs, however is less useful for monitoring the disease progression. Figure 3 shows an example of a 10 year-old asymptomatic boy with a strong family history of *PRPF31* associated RP, and a marked dysfunction both in rod and cone photoreceptors. In addition, ERG can be very useful in confirming the pathogenicity of novel mutations of uncertain pathogenicity, such as in a case of nuclear hormone receptor gene (*NR2E3*) associated with enhanced S-cone syndrome.[43] However, as an objective measure of retinal function in response to new therapies such as gene replacement, the ERG is insensitive to changes in macular function and subtle changes in photoreceptor survival because it is a pan-retinal response. The multifocal ERG (mfERG) permits assessment of cone diseases affecting local regions of the retina, and represents retinal function from the central 40-50 degrees of the macula corresponding with automated perimetry tests of the central macula. mfERG is a useful tool in screening for toxic maculopathies or diseases characterized by interocular and intraretinal variation, such as white dot syndromes, however to monitor changes in function over time, macular visual field sensitivity or microperimetry are much more readily accessible and accurate.

The measurement of the sensitivity threshold during the adaptation to darkness is known to show a characteristic biphasic function with an initial cone response, followed by the rod-cone break after about 5 to 10 minutes, and the rod response that reaches a plateau after 40 to 50 minutes.[44] The dark adaptation function reflects the ability of the rod and cone photoreceptors to regenerate photopigment, and thus sensitivity after exposure to light.[44] Dark adaptometry is particularly useful characterizing patients with congenital stationary

night blindness (CSNB) and normal fundi. In patients without a scotopic ERG, an absent rod-cone break on the dark adaptation curve is indicative for a molecular defect at the rod photoreceptor level.[45] Prolonged dark adaptation is furthermore the most characteristic feature of the *RHO* gene Pro23His genotype.[46]

2.1.5 Multi-luminance mobility test (MLMT), Full-field stimulus threshold (FST)—

The multi-luminance mobility test (MLMT) was designed to allow quantifiable measurement of functional vision in severely affected RP patients unable to perform other forms of functional visual assessments. It tests the ability of a subject to navigate a course accurately and at a reasonable pace at different levels of illumination.[47]

The full-field stimulus threshold is an alternative to measure the dark adapted light-sensitivity in RP patients unable to perform visual field tests or with undetectable ERG. The FST is fast to perform and determines the luminance threshold for detection of a single stimulus flash without requiring patients' fixation.[48]

Both functional tests have been used as outcome measures for the voretigene neparvovec phase III and IV pivotal trial, and in fact the MLMT represented a novel functional primary outcome.[49] Despite being FDA approved new clinical trial end-points, it would however be difficult for to adopt these as practical, widely available clinical tests, and thus neither tests are essential for monitoring.

2.2 Assessment of Retinal Structure

2.2.1 Optical Coherence Tomography—

Optical coherence tomography (OCT) has revolutionized the way ophthalmologists evaluate retinal diseases and treat patients, and about two decades after its introduction it has become indispensable both for research and clinic. The introduction of spectral domain OCT (SD-OCT) has further enhanced the visualization of retinal structures with an axial resolution of approximately 5 μm for commercial devices and 2 μm for research devices, permitting correlation with histological features of the retina.[50] In high quality, high resolution SD-OCT scans at least 13 different retinal layers can be identified. The first hyper-reflective band at the vitreoretinal interface is the internal limiting membrane (ILM), followed by the hyper-reflective nerve fiber layer (NFL) and by a less hyper-reflective band composed of the ganglion cell layer (GCL) and inner plexiform layer (IPL). Both nuclear layers (inner and outer) are hypo-reflective and separated by the hyper-reflective outer plexiform layer (OPL). With variation of the incident light beam, Henle's fiber layer (HFL) can further be visualized adjacent to the OPL either as a hyper-reflective or hypo-reflective band, depending on the recording angle.[51] In the outer retina, the SD-OCT resolves four distinct bands. The innermost band is thought to be the external limiting membrane (ELM), which is composed of junctional complexes between Müller cells and photoreceptors.[50] The origin of the second and third bands has been a source for debate in recent years. The terminology suggested by an international nomenclature panel is ellipsoid zone (EZ) for the second, and interdigitation zone (IZ) for the third band.[52] However, the nomenclature remains controversial since the inner segment ellipsoid is around 16 to 20 μm and thus too thick and also too proximally located to produce the second band.[53] The origin of the third band has similarly been challenged,

and attributed to the cone outer segment tips.[54,55] The fourth hyper-reflective outer retinal band is attributed to the RPE with possible contributions from Bruch's membrane and the choriocapillaris.[56]

The OCT has been used to aide with the diagnosis of common RP features such as cystoid macular oedema and epiretinal membrane since its introduction. More recently, detailed structural information, such as the integrity and the extent of the ELM and EZ as well as ONL thickness, have been shown to correlate well with function, and are thus used to monitor disease progression and serve as outcome measures in therapeutic trials.[32,36,57–62] Figure 3A shows a progressive decrease in the lateral extend of the ELM and EZ in a left eye of a 28 year-old male patient with autosomal recessive RP during a follow up period of six years. Figure 3B is an example of a 26 year-old female patient with *CRB1* associated RP that showed clinically meaningful improvement of her right eye cystoid macular oedema upon systemic acetazolamide therapy.

2.2.2 Autofluorescence—Fundus autofluorescence, classically using blue-light excitation, is a non-invasive imaging technique that can give indirect information on the level of metabolic activity of the RPE through visualizing fluorophores, such as lipofuscin granules, which accumulate in the RPE as a byproduct of constant phagocytosis of shed photoreceptor outer segments.[63–65] Extracellular ocular fluorophores from shed photoreceptor outer segment debris may accumulate subretinally due to RPE dysfunction and loss of apposition between the photoreceptor tips and the RPE, and clinically be seen as vitelliform material.[66] The normal fundus autofluorescence pattern is characterized by a physiological foveal hypo-autofluorescence from absorption of the short-wave length excitation light by lutein and zeaxanthin. Increasing accumulation of lipofuscin granules in the RPE cytoplasm occurs naturally with aging.[67] In IRDs changes in the autofluorescence pattern can be appreciated prior to any functional deficit and can be diagnostic.[64,68] In Stargardt disease with bi-allelic mutations in the ATP-binding cassette transporter (ABCA4), the excessive accumulation of bisretinoids leads to a quantifiable early increased lipofuscin-related autofluorescence, and later to characteristic hyperautofluorescent fundus flecks.[69] In patients with RP a central ring of increased autofluorescence intensity has been shown to surround regions of preserved central photopic function, visual field sensitivity, and preservation of the inner segment EZ, while visual sensitivity was reduced across the ring and markedly decreased outside the ring of hyperautofluorescence with associated loss of the EZ.[70–72] In choroideremia the preserved central retinal island reveals a unique autofluorescence pattern of sharply demarcated edges.[73] In *RPGR* X-linked RP it has been recently shown that the hyperautofluorescent ring correlates nicely with retinal regions that have lost the EZ, but still retain the ELM.[74] The decrease in the extent of the ELM and EZ, observed in the patient featured in Fig. 3A, correlates nicely with the concentric constriction of the physiological foveal hypoautofluorescence. Fig. 3C features autofluorescence and OCT images of the right eye of a 61 year-old male with *PRPF8* associated autosomal dominant RP. The inner hyperautofluorescent circle shows good correlation with the loss in ELM, while the outer larger hyperautofluorescent ring correlates with retinal area of absent ELM and highly thinned ONL. Absent or severely reduced autofluorescence can be observed in patients with RPE65-associated retinal dystrophy (Fig.

4D).[75] Melanin is another naturally occurring ocular fluorophore that is located at the apical end of the RPE cell compared to the basolateral accumulation of lipofuscin.[76] In contrast to lipofuscin with a peak excitation wavelength of 470nm, melanin has a peak excitation wavelength of 787nm, and is the primary fluorophore seen in near-infrared fundus autofluorescence.[77] The most striking feature in near-infrared autofluorescence imaging is increased autofluorescence in the fovea as opposed to the short-wavelength autofluorescence, which reveals a dark area at the fovea.[77] Melanin in the RPE cell is thought to have anti-oxidative properties and to protect against lipofuscin accumulation.[78,79] Having been introduced to overcome the potential phototoxic properties of short-wavelength autofluorescence, near-infrared autofluorescence does not have the same clinical applicability and diagnostic usefulness as the classical short-wavelength autofluorescence.

Fundus autofluorescence cannot only be characterized by the spatial distribution of the autofluorescence signal, and by the emission spectrum, but also by quantifying the average time between excitation and reaching the fluorescent ground state. Fluorescence lifetime imaging ophthalmoscopy (FLIO) is an emerging imaging modality that measures the lifetime of retinal fluorophores independent of concentration and intensity, and can thus be used to detect retinal fluorophores other than lipofuscin.[80] In choroideremia FLIO helps identify retinal areas that retain photoreceptors in the absence of RPE by showing shorter fluorescence lifetimes in comparison to areas with combined RPE and photoreceptor loss.[81] In *ABCA4* associated Stargardt disease FLIO has been shown to change over time thus possibly being a promising imaging modality to both monitor natural progression and response to treatments.[82]

2.2.3 Adaptive Optics Scanning Laser Ophthalmoscopy—Adaptive Optics uses a wavefront sensor to measure the ocular aberrations and compensates for them with a deformable mirror, generating non-invasive, high-resolution images of the retina.[83] The transverse resolution is approximately 2μm, which permits visualization of photoreceptors. Visualization of a single photoreceptor with the conventional confocal AOSLO (Adaptive Optics Scanning Laser Ophthalmoscopy) is enabled by the high refractive index of the photoreceptor relative to the surrounding interphotoreceptor matrix, but requires intact photoreceptor outer segments.[84] As reflectivity from the RPE can be confounded as having a photoreceptor origin, the source of photoreceptor signal continues to be debated.[85,86] In non-confocal methods, such as split-detector AOSLO, the signal is believed to arise from the photoreceptor inner segment, which can be acquired simultaneously with confocal AOSLO, and help guide image analysis.[87] By enabling direct visualization of single photoreceptors AOLSO has the potential to directly quantify the extent of photoreceptor degeneration in RP, and to monitor therapeutic interventions. However, the lack of validation of photoreceptor-based biomarkers (i.e. density, size, reflectivity) and the difficulty of resolving single cones in the fovea as well as the absence of normative database have limited the clinical applicability of AOSLO and its usefulness in monitoring the progression of RP. Furthermore, AOSLO requires significant time resources, and commercial machines that enable equal quality to the investigational devices are still missing.

2.3 Genotyping

A striking feature of RP is its genetic heterogeneity, which makes attempts to identify the causative genetic variant often challenging. Genetic testing can however direct clinical management, yield prognosis and most importantly help identify individuals that could benefit from retinal gene therapy. Next generation sequencing (NGS) has significantly reduced time and cost needed to analyse the DNA, and thus more patients with RP are being genotyped. Historically the diagnosis of RP has been a clinical one. Genotyping has helped us understand that a same phenotype can be associated with variants in different genes, and that one single gene can give rise to a multitude of phenotypes. A genetic variant is a permanent change in the nucleotide sequence of the DNA (deoxyribonucleic acid), which can be mutated either by substitution, deletion or insertion of base pairs. Single base variants are called point mutations. Depending on the impact the single base substitution has on the codon and thus on the amino acid it codes for, a point mutation can be silent, missense, or nonsense. Deletions or insertions may lead to the translational frame being altered, which typically leads to a non-functional product. A clinical example where variants in the same gene can give rise to different phenotypes is the *BEST1* gene mutation. Missense mutations give rise to the less severe phenotype of autosomal dominant Best macular dystrophy, while splicing defect can cause the more generalized ADVIRC (autosomal dominant vitreoretinochoroidopathy) syndrome.[88] Variants in *ABCA4*, a gene encoding a retina specific ATP-binding cassette (ABC) transporter protein (subfamily A, member 4), give rise to retinal dystrophies that are genotypically and phenotypically very heterogeneous.[89] The disease phenotype, macular dystrophy (Stargardt disease) or cone-rod dystrophy, correlates with the severity of the variant.[89–91] Knowing which genetic variant gives rise to which disease phenotype is important both for genetic counselling and identification of patients that might qualify for therapies. Variants in the *PRPH2* gene can lead to a remarkable variation of retinal phenotypes ranging from macular dystrophies to RP.[92] It has been recently highlighted that *PRPH2* point mutations can affect mRNA splicing with a different effect on rods and cones, respectively.[93] The *PROM1* gene, may have dominant or recessive phenotypes that influence disease onset and severity. Thus, the dominant cases associated with a specific missense variant (c.1117C>T) show milder, cone-driven phenotype, suggesting that the dominant disease is preferentially associated with cone photoreceptors.[94] The M- and L-cone opsin genes situated on the long arm of the X chromosome are another example of highly variable severity of the phenotype, which can vary from mild colour deficiency to blue cone monochromacy (BCM) depending on the variant.[95] USH2A is a large gene located on chromosome 1 that causes about 30% of all Usher type 2 cases, but also about 20% of autosomal recessive RP.[2] Initially believed to be always associated with hearing impairment and visual loss, in 2000 Rivolta *et al.* identified a missense mutation c.2276G>T (p.Cys759Phe) which was thought to cause non-syndromic RP only.[96] *RHO* mutations, affecting the amino acid sequence of the rod specific protein rhodopsin, account for up to 40% of all autosomal dominant RP cases (source RetNet www.sph.uth.edu). The severity and progression rate of *RHO* associated autosomal dominant RP can vary depending on the affected portion of the rhodopsin molecule, where the most severe disease is thought to be associated with the cytoplasmatic edge.[97] Iannaccone *et al.* showed that distinct point mutations at codon position 135 can cause different disease severity and progression.[97] The arginine to lysine (R135L) missense

change showed a less severe disease with a slower progression rate than the arginine to tryptophan (R135W) substitution.

Since the introduction of the first retinal gene therapy for biallelic *RPE65* associated retinal dystrophy (voretigene neparvovec) in 2017, genotyping is essential to confirm the mutation and thus the eligibility for treatment. With retinal gene therapies for choroideremia (NCT03496012) and *RPGR* X-linked RP (NCT03116113) having reached phase 3, and other emerging exciting therapeutic fields such as optogenetics, genotyping plays a key role in the management of all patients with IRDs where early and accurate diagnosis are crucial.

3.0 Conclusion

The new era launched by the clinical approval of Luxturna® (voretigene neparvovec) and the multitude of emerging therapies for IRDs demands accurate clinical characterization and genetic testing of all patients with RP. Precise phenotyping includes assessment of both function and morphology. When assessing visual acuity, it is of paramount importance to be aware of the impact low vision and advanced visual field loss have on the ability to track letters on the chart, especially when the visual field loss affects the fovea. When choosing visual acuity as a clinical trial outcome measure, ETDRS letter charts should be used along with standardization of room illumination to achieve uniform contrast and luminance of the optotypes. A minimum of at least a three-line worsening of visual acuity over time to allow for correct interpretation of changes in visual acuity should be employed in any trials with RP patients. In addition, assessment of LLVA should be included since RP patients show a more pronounced visual acuity deficit at low luminance and the test result could be predictive for future visual acuity loss. Colour vision testing should also be included in the clinical assessment of patients with RP as good visual acuity can be retained until late in the disease course, while colour vision deficits can be present before the onset of degeneration. As a clinical trial outcome measure however colour vision performance should be handled with care as little information is known on the specificity and sensitivity in RP patients, and also both low vision and advanced field loss can have a significant impact on the results, because the Farnsworth-Munsell 100 test requires comparing and selecting coloured discs spaced across a relatively wide visual field.

The Goldmann visual field remains very useful in characterizing the extent of gross visual field loss (e.g. as a marker of toxicity), which seems to correlate well with morphological OCT markers. The central visual field is best assessed by MAIA microperimetry, which allows the simultaneous observation of the retina during the perimetric testing. MAIA microperimetry has been shown to be reliable in monitoring the central retinal sensitivity over time and is thus an important surrogate endpoint in clinical trials.

The ERG plays an important role both in the diagnosis and characterization of RP through enabling global assessment of photoreceptor function. As a clinical trial endpoint however to monitor changes in function over time, visual field sensitivity is much more readily accessible and accurate.

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Multimodal imaging with OCT and fundus autofluorescence used routinely in the clinical evaluation in retinal clinics plays a pivotal role in the accurate characterization of RP patients as well. The morphological information that can be extracted from OCT images is unmatched by any other imaging tool and is best described as *in vivo histological dissection* of the retinal anatomy. Fundus autofluorescence gives indirect information on the level of metabolic activity of the RPE through visualizing fluorophores, and has also proven to be indispensable in the management of retinal degenerations. Detailed structural information seen in OCT images, such as the integrity and the extent of the EZ, has been shown to correlate well with function, and is thus used as outcome measure in therapeutic trials, while the lack of readily available quantification of the autofluorescence signal still limits its applicability as trial biomarker.

AOLSO has the potential to directly quantify the extent of photoreceptor degeneration in RP, and as such be of highest value to monitor therapeutic interventions. However, the lack of validation and the absence of normative database have limited the clinical applicability of AOSLO and its usefulness in monitoring the progression of RP.

Genetic testing has become absolutely essential in the clinical characterization of RP patients and is vital to determine subjects suitable for retinal gene therapy. Matching the detailed genetic information with the clinical phenotype has triggered a vast expansion of the molecular mechanisms behind RP and allows the quest for tailored, even mutation specific, therapies. The information acquired through genotyping should however always be meticulously scrutinized, and its validity be matched with the clinical picture.

4.0 Expert Opinion

State-of-the-art management of patients with RP requires a super-specialized centre with a readily available visual function assessment unit and up to date multimodal imaging. Medical supporting staff, such as optometrist, nurses and technicians should all be acquainted with the specific challenges and requirements of assessing RP patients. Initial clinical characterization must include standardized visual acuity testing using ETDRS charts as well as OCT and autofluorescence imaging. Assessment of visual field should also be part of any initial assessment, and ideally include both kinetic and static measures. While genotyping is also crucial wherever available, we believe that the importance of the ERG has diminished. Certainly, electrophysiological testing still is very valuable whenever there is diagnostic uncertainty.[43] Monitoring the progression of RP can best be achieved by adhering to the same standardized assessments of both visual function and retinal morphology. Standardized visual acuity testing using ETDRS charts and multimodal imaging with OCT and autofluorescence reassures accurate monitoring of the disease course. Assessment of the visual field can also be useful in less advanced cases. In very advanced subjects visual field testing is of low clinical utility, and has the potential to induce significant psychological stress in a patient. Similarly, we also do not advise using ERG routinely to monitor disease progression.

In the setting of a clinical trial the choice of accurate and repeatable biomarkers is essential. We recommend always using standardized ETDRS charts to test visual acuity, and to

consider LLVA as well. Functional assessment should be complemented with visual field tests, ideally using MAIA microperimetry. Colour vision testing should be chosen judiciously in a clinical trial situation. Morphology must be assessed by OCT imaging where detailed structural information such as the integrity and the extent of the EZ can serve as surrogate endpoint. Fundus autofluorescence can provide additional information, however quantification still remains challenging. The usefulness of AOSLO as a surrogate marker also warrants critical judgement as it still lacks validation despite being able to directly visualize photoreceptors.

In summary, the armamentarium of clinical assessment tools with recent advances seen in multimodal imaging as well as in the efficacy of genotyping allows for most accurate characterization of patients with RP and fine monitoring of the disease course. Most importantly the detailed audit of all currently emerging therapies in the field of RP is not only driving innovation, but puts all retinal specialists at a point where realistic hope can be offered.

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Article highlights box

- Comprehensive discussion of standard and innovative techniques to diagnose and monitor RP
- Article discusses both functional and morphological parameters
- Excellent guideline for the retinal specialist interested in the clinical management of RP patients
- Excellent overview of outcome measures pertinent to the design of clinical trials in retinal degenerative diseases

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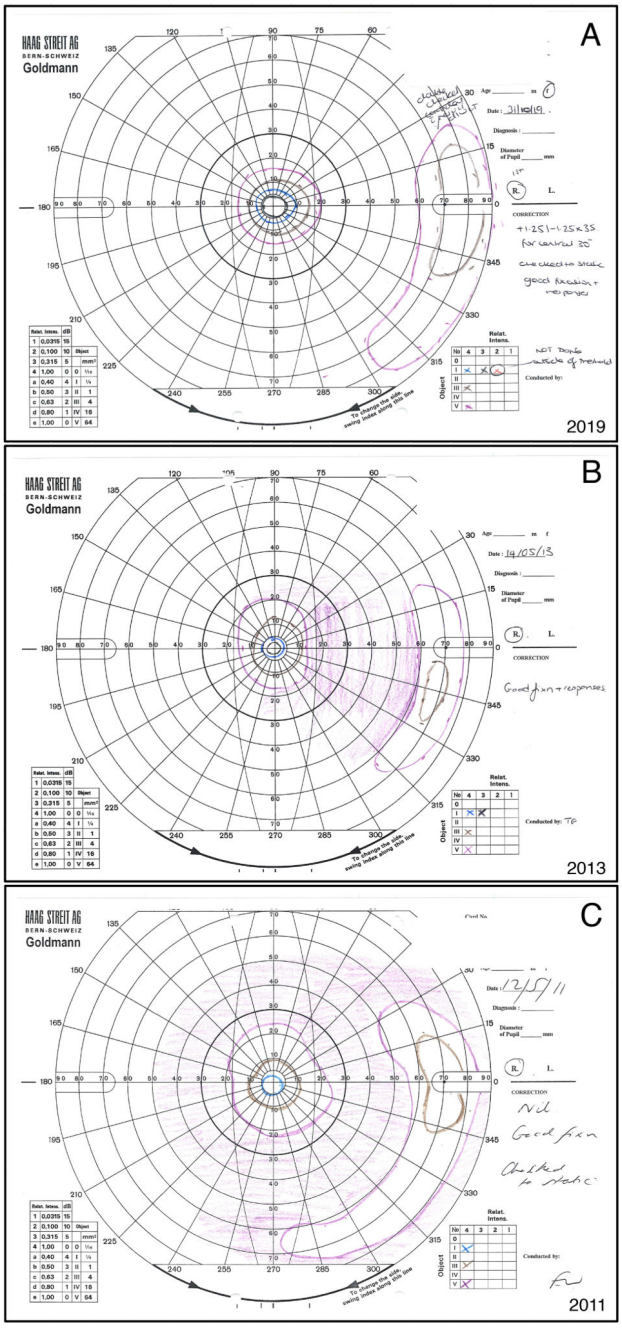

Figure 1.

Goldmann visual fields of the right eye of a 37 year-old female with *PDE6B* associated autosomal recessive RP. Figure 1A shows the most recent visual field from 2019, while 1B is from 2013, and 1C from 2011. The normal Goldmann visual field usually reaches about 90° temporally, 50° nasally, and 50-60° superiorly and inferiorly for the largest and brightest stimulus. All fields show a concentric constriction with a peripheral remnant of visual field, which is typical for RP. Significantly increased constriction and decrease in the size of the peripheral remnant can be noted from 2011 to 2013. However, the visual field from 2019

seems to not have worsened in comparison to the previous exams. Indeed, both the central island and the peripheral remnant appear larger. A test-retest variability of up to 20% even when using a single experienced operator has been known for Goldmann visual fields, and might explain the apparent improvement in visual field seen in this patient. The clinical improvement seen in the cystoid macular oedema between 2013 and 2019 (not shown) could however have contributed to a real improvement in visual field sensitivity in this case.
The purple lines represent the Goldmann stimulus V4e. The Roman number indicates the stimulus size of 64mm^2 for V. The Arabic number and the lower case letter indicate the light intensity of 1000 apostilb (315 cd/m^2). Brown = III4e (4mm^2, 1000 asb); blue = I4e (1mm^2, 1000 asb); black = I3e (1mm^2, 315 asb).

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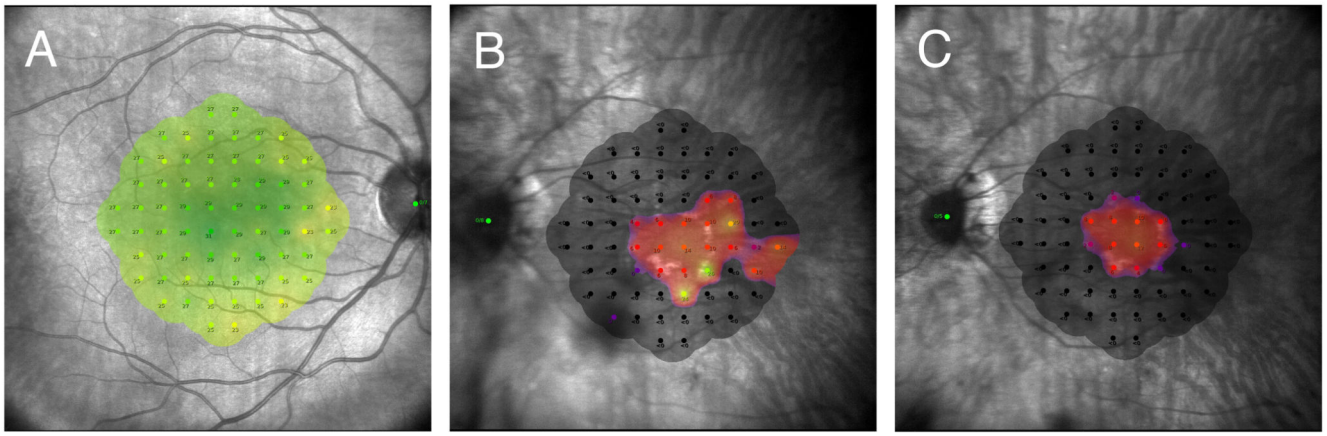

Figure 2.

Standard 10-2 grid consisting of 68 retinal points arranged in a Cartesian pattern covering the central 20°. The threshold sensitivity value at each retinal location is colour coded and shown as an overlay on the near-infrared image. A) Macular sensitivity heat map of a healthy 27 year-old male. Green indicates normal sensitivity (maximum 36dB). B and C) Macular sensitivity heat map of a 38 year-old male with *RPGR* X-linked RP taken two years apart. In 2017 (B) the patient still showed few retinal points of nearly normal sensitivity, while two years later (C) both the central visual field constriction, and sensitivity threshold had worsened.

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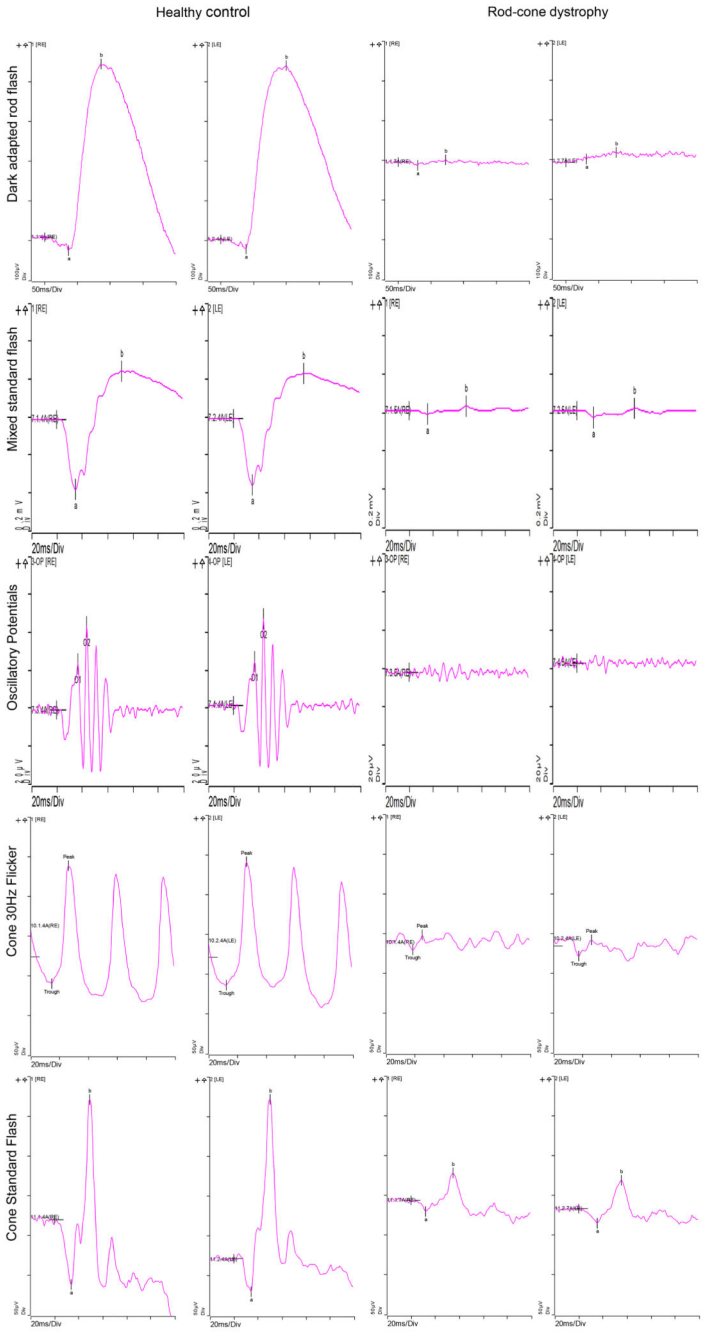

Figure 3.

ISCEV Standard ERG of a 10 year-old boy with a strong family history of *PRPF31* associated RP. Normal values of a healthy control are shown on the left columns. The right columns show the ERG of the 10 year-old boy. The dark-adapted rod flash (0.01 cd*s/m^2) shows barely measurable components. The mixed standard flash (3 cd*s/m^2) reveals reduced amplitudes, whilst peak times are normal. The third row shows barely recordable dark-adapted (3 cd*s/m^2) oscillatory potentials. The light-adapted 30 Hz flicker ERG and the cone standard flash (3 cd*s/m^2) both show markedly reduced responses. In summary, the

ERG confirms rod and cone dysfunction in both eyes. Overall rods seem to be more affected than cones at this stage.

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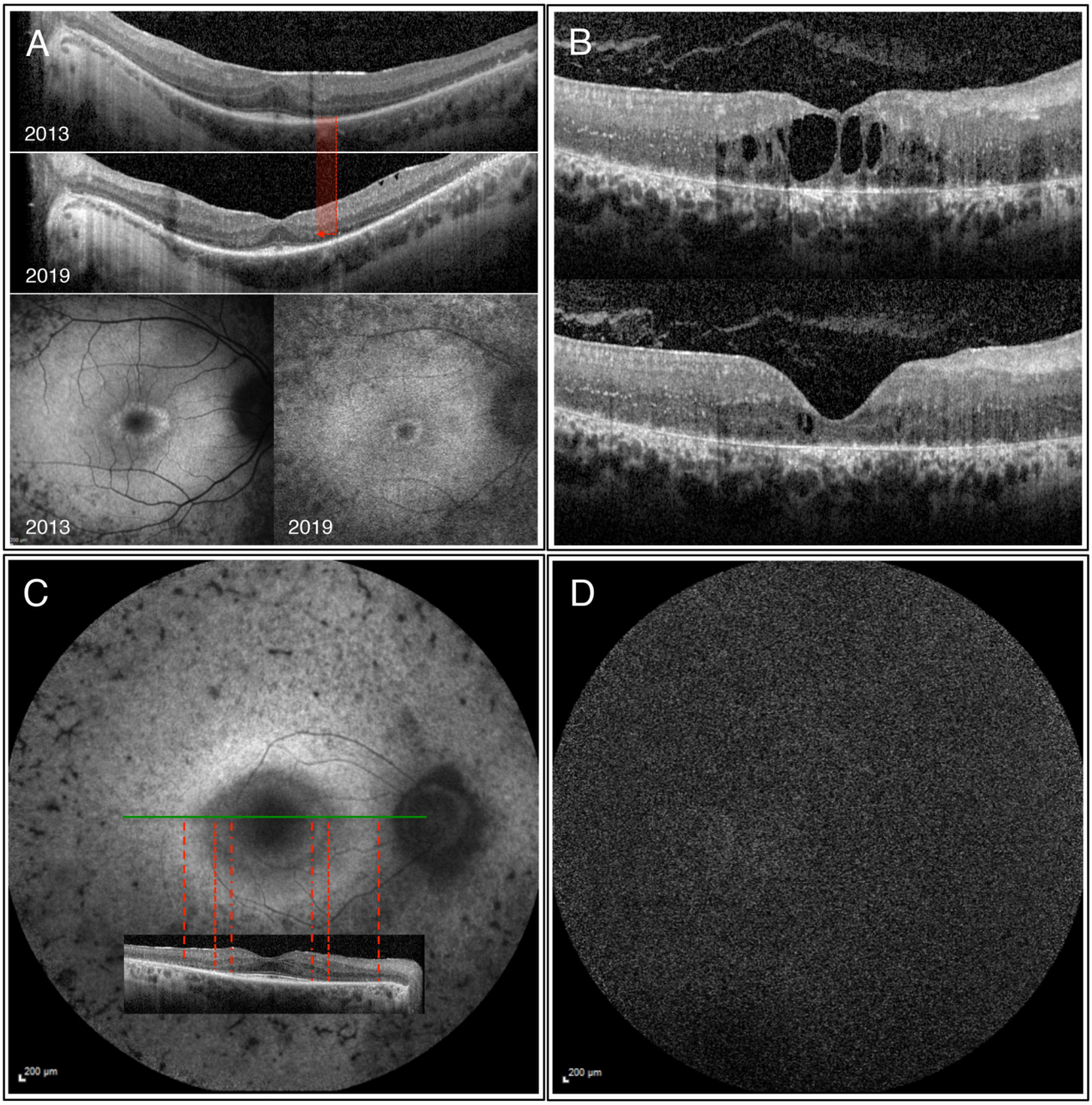

Figure 4.

OCT and autofluorescence images of various patients with RP. A) Images of the right eye of a 28 year-old male with autosomal recessive RP (compound heterozygous mutations in *DFNB31* and *USH2A* found). Significant decrease in the width of both the ELM and EZ paralleled by diminishing foveal hypoautofluorescence can be observed between 2013 and 2019. Visual acuity in 2019 was 6/7.5, which translates to 20/25 (20 ft) or 0.8 (decimal) Snellen equivalent. B) OCT images of the right eye of a 26 year-old female patient with *CRB1* associated RP showing clinically meaningful improvement of the cystoid macular

oedema upon systemic acetazolamide therapy with an increase in visual acuity from 3/60 (20/400, 0.05) to 6/38 (20/125, 0.16). C) Autofluorescence and OCT images of the right eye of a 61 year-old male with *PRPF8* associated autosomal dominant RP. The inner hyperautofluorescent circle shows good correlation with the loss in ELM, while the outer larger hyperautofluorescent ring correlates with retinal area of absent ELM and highly thinned ONL. D) Short-wavelength autofluorescence image of a 48 y/o male patient with biallelic *RPE65* (c.11+5G>A, c.1543C>T) showing the characteristic absent autofluorescence.