Functional Evaluation in Inherited Retinal Disease

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Abstract

Functional assessments are a fundamental part of the clinical evaluation of patients with inherited retinal diseases (IRD). Their importance and impact have become increasingly notable given the significant breadth and number of clinical trials and studies investigating multiple avenues of intervention across a wide range of IRD, including gene, pharmacological and cellular therapies. Moreover, the fact that many clinical trials are reporting improvements in vision, rather than the previously anticipated structural stability/slowing of degeneration, makes functional evaluation of primary relevance. In this review, we will describe a range of methods employed to characterise retinal function and functional vision, beginning with tests variably included in the clinic, such as visual acuity (VA), electrophysiological assessment and colour discrimination; and then discuss assessments often reserved for clinical trials / research studies such as photoaversion testing, full-field static perimetry and microperimetry, and vision-guided mobility testing; discussing perimetry in greatest detail given it is commonly a primary outcome metric. We will focus on how these tests can help diagnose and monitor particular genotypes - also noting their limitations/challenges, exploring analytical methodologies for better exploiting the functional measurements, as well as how they facilitate patient inclusion and stratification in clinical trials and serve as outcome measures.
**Introduction**

Inherited retinal diseases (IRD) are a complex group of conditions with a wide genotypic and phenotypic spectrum.\textsuperscript{1–4} Detailed functional assessment is valuable in the diagnosis and monitoring of IRD, in both clinical and research settings. A wide range of tests and devices have been developed to record and quantify retinal function and functional vision, which vary in their degree of objective measurement and subjective patient response, all having significant benefits and limitations. In these regards, functional characterisation is similar to structural characterisation, in that for both a 'multi-modal' evaluation is most informative. The degree of change in any of these measurements that is universally agreed to be clinically meaningful remains to be established; although for inexorably progressive IRD, any change that is greater than test-retest variability for the metric may be clinically meaningful. Functional testing, whilst subject to concomitant ocular disorders such as media opacity, myopic retinopathy and aging, significantly contributes to our understanding of disease pathophysiology, informs advice on prognosis, assists monitoring the impact of interventions, and increasingly underpins clinical trial endpoints. An overview of the functional assessments included in this review is shown in Tables 1 and 2.

**Best Corrected Visual Acuity (BCVA)**

Visual acuity (VA) represents the ocular spatial resolving capacity.\textsuperscript{5} Quantification of VA is usually the first assessment in clinic, and by far the most commonly performed. Knowing the VA and the BCVA of an individual is essential in the evaluation of the function and integrity of the visual system. The first and most widespread chart was developed by Snellen in 1862.\textsuperscript{5} However, it has an imprecise scoring method that uses lines instead of letters and lacks standardization, leading to difficulties in statistical analysis.\textsuperscript{6} Hence the current gold standard is the retro-illuminated logarithm of the minimum angle of resolution (LogMAR) chart, following the Early Treatment Diabetic Retinopathy Study (ETDRS) optotype,\textsuperscript{7} which has high repeatability, and is therefore the method of choice in clinical trials.\textsuperscript{8} Other commonly used methods to assess VA include
the Tumbling E-chart, Landolt C optotypes (both for illiterate or non-Latin language speaking patients and children), and those specifically tailored for children such as Kay Pictures, Lea Symbols and Allen Figures.9

BCVA is typically reduced early in cone dysfunction syndromes (e.g. achromatopsia (ACHM)), cone and cone-rod dystrophies (COD/CORD), macular dystrophies (MD) and early-onset severe retinal dystrophy/Leber congenital amaurosis (EOSRD/LCA), but is often preserved until late stages in rod-cone dystrophies (RCD).4,10–12 BCVA has been shown to significantly correlate with the width and integrity of the ellipsoid zone (EZ) on optical coherence tomography (OCT),13 as well as with visual field (VF).14,15 However, BCVA can show notable disconnect with structural measures (both better or worse respectively, than predicted from anatomy alone), including in certain genotypes such as RDH12 and CEP290, and also in cone density measured with adaptive optics (AO) imaging can be up to 60% decreased and yet acuity remains normal – highlighting the redundancy in the visual system and potentially boding well for cell replacement strategies.16 BCVA is an outcome measure included in all IRD trials.

Low Luminance VA (LLVA)

LLVA can be measured by placing a 2.0 log unit neutral density filter over the patient’s best correction or over the ETDRS chart, while the latter is read.17 Other options include using a U23 NoIR 4% transmission filter to simulate mesopic conditions.18 Patients with RCD have difficulties in dim environments and have reduced LLVA from the earliest stages of disease.19 Consequently, changes in LLVA are secondary outcome measures in gene therapy trials for the following RCDs: USH2A (NCT03780257), RHO (NCT04123626), CHM (NCT03496012) and RPGR (NCT03252847). The measurement of LLVA is an inexpensive and simple procedure, although more data regarding its correlation with other parameters are needed.
**Contrast Sensitivity**

Reduced contrast sensitivity (CS) is a frequent symptom in IRD – significantly impairing central vision; even in those with normal or near-normal BCVA.\(^{20,21}\) Multiple methods have been used to assess CS, but Pelli-Robson (PR) charts\(^{20}\) are currently the most frequently used, both in clinical and research settings.\(^{21,22}\) However, the PR chart has relatively sparse spatial frequencies and stimulus contrast, which may lead to imprecision. Newer computer-based methods to evaluate CS (e.g. the Quick Contrast Sensitivity Function test and photoreceptor-specific temporal contrast sensitivity) are continuously evolving, with early evidence suggesting higher resolution assessments and thereby more capability to detect change over time.\(^{23–26}\)

A decrease in CS has been documented in patients with RCD,\(^ {27}\) ACHM,\(^ {4,10,22}\) and CORD.\(^ {26}\) Higher spatial frequencies (6.0 to 18.0) are usually more severely affected, as reported in individuals with USH2A-RCD, ABCA4 retinopathy and BEST1 maculopathy.\(^ {21,29,30}\) An association between mean retinal sensitivity (MS) and CS has been reported in patients with RCD and ACHM.\(^ {22,31}\) Moreover, CS was significantly associated with reading speed in patients with ABCA4 retinopathy and RCD.\(^ {27,28}\) CS assessment is an easy and clinically important method to monitor visual function, being currently a secondary outcome measure in many gene therapy trials for the following RCDs: PDE6A (NCT04611503), RLBP1 (NCT03374657) and RPGR (NCT04671433); as well as in multiple pharmacological trials for Stargardt disease (STGD; ABCA4).\(^ {1}\)

**Colour Vision**

Colour vision (CV) defects are typically observed at an early stage with CORD, ACHM and cone dysfunction syndromes.\(^ {3,10}\) Individuals with RCD may also report early issues with CV,\(^ {32,33}\) and certainly at later stages of disease as cone function becomes compromised. CV can be assessed by a wide range of tests. The most commonly used in the clinic is one of the oldest: the Ishihara pseudoisochromatic plates.\(^ {34}\) However, whilst easy to use, it lacks evaluation of the tritan axis.\(^ {34}\) Hardy-Rand-Rittler pseudoisochromatic plates are as easy to administer and assess discrimination along all 3 colour axes.\(^ {35}\)
Another option are the Farnsworth-Munsell tests, where the patient sorts coloured caps according to their chromaticity. The 100-Hue version consists of 85 caps and now also exists as a computer-based test; while the D15 has only 15 caps (with the PV-16 being a low vision version with enlarged caps). These tests are more challenging to administer and more time consuming, but especially useful when assessing and monitoring acquired CV defects. Computerised systems are primarily employed in research and offer a quantitative and more comprehensive characterisation of colour discrimination. The Cambridge Colour Test (CCT) is the first popular computer-based test. It consists of pseudoisochromatic plates at decreasing luminance levels and also has a low vision version (lvvCCT), suitable for visually impaired individuals. Other computerized tests available are the Rabin Cone Contrast Test and the Universal Colour Discrimination Test (UCDT), the latter being suitable for individuals with low vision.

CV testing helps to discriminate between cone dysfunction syndromes, including between complete and incomplete ACHM - one of the features of the latter being residual colour perception. In addition, tests probing the tritan axis, including that created by Berson et al. are valuable in helping to identify males with blue cone monochromacy. By detailed testing of colour discrimination in individuals with IRD, we can infer how different cone classes are affected and this can help with the differential diagnosis and suggest a genetic basis. CV is a secondary outcome measure in on-going ACHM gene therapy trials, including NCT03001310 and NCT02599922 (both CNGB3), and also NCT03758404 and NCT02935517 (both CNGA3).

**Visual Field (VF) and Retinal Sensitivity**

In 1927, Traquair first described the VF as “an island of vision in a sea of darkness.” Loss of peripheral VF as occurs in early forms of RCD results in symptoms such as tripping, bumping into people/obstacles, struggling to find objects, or difficulty navigating in dim or crowded/unfamiliar environments. In contrast, loss of central VF in COD/CORD, MD and EOSRD/LCA, usually leads to difficulties in recognizing faces, reading signs and identifying objects.
VF evaluation, as performed using kinetic and static perimetry, has evolved significantly over the last two decades. Kinetic VF testing has been used to monitor progression in patients with RCD and Usher syndrome - with semi-automated kinetic perimetry (SKP) being more frequently employed. However, there is no consensus or standard method of conducting KP, making it challenging to compare results from one centre to another. It requires a higher level of skill, greater training, knowledge about expected field defects in specific diseases, and experience. KP has a higher test-retest variability (up to 20-30%) and its quality and efficiency can vary substantially even within the same clinical centre, from one examination to another, as well as due to patient cooperation. Whilst test-retest variability is less for SKP, the major drawback of all KP remains that, because the shape and height of the hill of vision depends upon the existing pathology for the individual patient, it is not possible to fully automate it for all clinical situations. KP is a valuable tool to define sharp borders of blind areas, however it is less able to detect mild slopes or transitions between seeing and unseeing parts, or to distinguish shallow islands of remaining sensitivity.

Semi-automated static perimetry (SP) by Octopus 900 is a robust method to comprehensively evaluate retinal sensitivity/visual field integrity and has been applied in a broad range of genotypes in both adults and children, including RPE65, ABCA4, RPGR and USH2A. It also has lesser dependency than kinetic testing on the technician’s expertise, and less inherent test-retest variability. A major advantage of static over KP is the availability of parameters that evaluate the reliability and validity of patient test responses, such as the frequency of false positive and false negative responses, and the quantification of a reliability factor (RF). Variability and inconsistencies between test sessions and among test subjects can be reduced and validity of testing increased by specific instructions read to the patient by the perimetrist before each test as to how to respond to the test stimulus presentations. Static testing is better than kinetic testing at detecting and defining gradual changes of either lesser or greater sensitivity and isolated regions of residual sensitivity in advanced disease. The Humphrey perimetry has been extensively used for clinical studies and trials for glaucoma and to a lesser extent, mostly in the past, for IRD. The fast integrated SITA Standard thresholding algorithm available on the Humphrey...
The normal 4-2-1 strategy on the Humphrey takes much longer and is, therefore not suited for full-field static testing.

The Octopus 900 perimeter, using the fast German Adaptive Thresholding Estimation (GATE) algorithm, is currently the most robust and optimal system for static testing the entire visual field of patients with IRD. The GATE algorithm is as fast as the SITA Standard and has a validity and precision comparable to the normal 4-2-1 strategy. Octopus 900 perimetry using the GATE strategy has become the most commonly used device for clinical studies and treatment trials for IRD. The Octopus system allows (i) use of custom color test targets, (ii) a validated, retina-specific optimized testing strategy to be employed, i.e., GATE, and arguably most importantly (iii) exportation of all raw retinal sensitivity data, which can then be comprehensively and robustly analyzed, using Visual Field Modelling and Analysis (VFMA) methodology (Figure 1), from which topographic displays and hill-of-vision volumetric outputs can be derived; including the total hill-of-vision ($V_{TOT}$) or any subset e.g. the central 30 degree field of vision ($V_{30}$). These volumetric analyses afforded by VFMA can be applied equally as well to VF data obtained from microperimetry, potentially allowing game-changing state-of-the-art retinal function evaluation in IRD and other retinal diseases, and enabling incorporation of all data in a non-biased fashion, truly representing the full impact of disease natural history or treatment effect. Assessment of retinal sensitivity using VFMA with creation of volumetric endpoints, such as $V_{TOT}$, $V_{30}$, $V_{10}$, and $V_{3}$, allow direct comparison of values between subjects, at different regions with a given test, and between baseline and follow-up testing. Octopus perimetry is thereby increasingly the static perimeter of choice, both in clinic and in studies/trials - and is being applied as a primary or secondary endpoint in multiple studies and trials including RPGR (NCT04671433), USH2A (NCT03780257) and RPE65 (NCT02781480).

Fundus-guided perimetry/microperimetry (MP) consists of a static perimetry device with eye tracking and fixation stabilization features, that allows measurement of the sensitivity threshold of individual macular loci under direct retinal visualization, facilitating correlation between structure (especially OCT) and function, and allowing quantification of fixation...
stability and topographical localization of retinal loci. However, the presence of unstable/poor fixation, which is commonplace in IRD, can lead to registration difficulties. Furthermore, despite their popularity, there is no consensus on the type of retinal sensitivity parameters that should be used to monitor progression and responses to therapeutic intervention. For these reasons, it is arguably less reliable than SP; also, it only tests macular function. MP devices with a broad range of testing abilities (mesopic, photopic and dual-colour scotopic testing) and dynamic ranges are available, including the most commonly used Macular Integrity Assessment (MAIA; CenterVue, Padova, Italy) and Nidek microperimeters (Nidek Technologies Srl, Padova, Italy). MP has been used to characterize and monitor the progression of multiple IRDs, including STGD, ACHM, and both syndromic and non-syndromic USH2A-retinopathy, as well as in clinical trials of gene therapy for RPE65-LCA (NCT00643747) and RPGR-RCD (NCT03252847), pharmacological trials for STGD (NCT03735810, NCT03033108, NCT02402660, NCT03364153), and transplantation of human embryonic stem cell-derived (hESC-) retinal pigment epithelial (RPE) cells in STGD (NCT01469832).

**Dark Adaptometry**

Measuring dark adaptation (DA) provides insight into photoreceptor thresholds and kinetics. Canonical and rapid adaptometers exist, with Goldmann-Weekers being the most commonly employed. DA curves show how retinal sensitivity changes at set locations, after switching from photopic to scotopic conditions. DA is typically biphasic, with an initially cone-mediated phase, followed by a cone-rod breakpoint, and a final, longer phase representing rod function. Elevated thresholds of DA have been reported in a broad range of conditions, including RCD, CORD, ACHM, congenital stationary night blindness, and STGD.

Newer devices have been developed; portable, LED-based dark adaptometers such as the Scotopic Sensitivity Tester (SST-1) from LKC Technologies Inc. (Gaithersburg, MD, USA), and instruments with increased testing efficiency.
Among the latter, the AdaptDx (MacuLogix, Hummelstown, PA) has been used to study delayed DA mainly in age related macular degeneration (AMD).\textsuperscript{78} Higgins et al. have recently proposed a novel ‘time-to-event’ analysis method that can be applied to this data, providing better statistical power.\textsuperscript{79}

**Assessment of Photoaversion**

Testing of light discomfort threshold has been implemented in several conditions such as migraine, blepharospasm, LCA and ACHM.\textsuperscript{80–84} The technique used for the first three entities was similar: increasing luminance stimuli were presented to the subject until he/she pressed a button, indicating that the stimulus was uncomfortable and ending the test.\textsuperscript{80} For ACHM, an arguably more objective and precise approach has been proposed.\textsuperscript{85} This involves video-recording the subject’s reaction to different light exposures and capturing various metrics such as average distance between the eyelids (palpebral fissure aperture).\textsuperscript{81,83} This method has been included to monitor efficacy in two on-going ACHM gene therapy trials: CNGB3-NCT03001310 and CNGA3- NCT03758404. Other gene therapy trials, such as CNGA3- NCT02935517 and CNGB3-NCT02599922, have implemented a device called the Ocular Photosensitivity Analyser (OPA) as a secondary outcome measure. The OPA uses a concave LED and measures patient indication of pain threshold, along with several further metrics such as inter-blink interval and pupil diameter.\textsuperscript{85,86}

Identifying the most sensitive way to measure and compare photoaversion is certainly challenging. Different groups have proposed their own method, with different approaches regarding adaptation to light levels (Verriotto et al. adapt at 100 lux, while Aboshiha et al. use total darkness),\textsuperscript{84,85} stimuli intensity and colour, and outcome metrics. A consensus is yet to be established. Qualitative assessments of photoaversion are also being explored and will no doubt be complimentary to the aforementioned objective assessments; these include the questionnaire developed for the CNGA3- NCT02610582 trial, ‘A3-PRO’,\textsuperscript{87,88} and the Visual Light Sensitivity Questionnaire-8, designed by Verriotto et al.\textsuperscript{85}
Visual Electrophysiology

The electroretinogram (ERG) can be a valuable tool in the diagnosis and characterisation of IRD, especially those with pathognomonic ERG features such as IRD associated with NR2E3 and KCNV2, being able to probe the extent, degree and cellular nature of dysfunction objectively. Electrophysiological assessment is also helpful in providing better informed advice on prognosis (particularly in STGD), in the differential diagnosis of childhood nystagmus/poor vision from birth/early infancy, and helping to distinguish between late-onset IRD and autoimmune retinopathy. However, the test-retest variability of ERG is high (20-30%), making it insensitive to measuring change overtime clinically or in clinical trials; with patients also often reporting reluctance to have serial electrophysiological testing.

Full-field (ff) ERG measures the global retinal electrical potential changes provoked by light stimuli, under light- and dark-adapted conditions, to provide information on generalized retinal function of both rod and cone systems. The International Society for Clinical Electrophysiology of Vision (ISCEV) recommends a minimum of six stimuli for a complete clinical ERG assessment. Two of the most important components of the ERG are the a and b waveforms. The a wave corresponds to the initial negative deflection and originates from the light-induced hyperpolarization of rod and cone outer segments. The b wave is the positive deflection following the a wave, and represents bipolar cell depolarization.

Photoreceptor disorders (e.g. RCD, CORD, ACHM) affect both the a and b wave, whereas conditions involving the post-photoreceptor signal transduction (e.g. X-linked retinoschisis) selectively reduce the b wave, causing an 'electronegative waveform' (b/a ratio <1.0). Macular function can be explored with a range of electrophysiological assessments, including multifocal (mf) ERG, focal ERG, and pattern ERG (PERG). Such testing may be helpful in the diagnosis of e.g. RP1L1-occult macular dystrophy, and structure-function correlations including between PERG-mfERG and the high intensity autofluorescence perimacular ring often seen in RCD and CORD.

The electrooculogram (EOG) evaluates the RPE and the photoreceptor-RPE complex, measuring photopic and scotopic changes in the resting potential between the cornea and the retina. It is expressed as a ratio of the peak light-
adapted amplitude to the minimum dark-adapted amplitude (Arden ratio, ≥1.8 in normal eyes). The EOG ratio is often reduced when the ffERG is abnormal, and is generally abnormal in autosomal dominant Best disease, where a decreased Arden ratio with normal ffERG is characteristic.\textsuperscript{102}

Visual Evoked Potentials (VEP) are used to evaluate the integrity of the complete visual pathway, and depend highly on central visual function. ISCEV recommends three basic stimuli: flash (useful for media opacity), pattern reversal (for both pre- and post-chiasmal lesions), and pattern on/off (provides estimates of potential VA).\textsuperscript{103}

Lastly, full-field light sensitivity threshold (FST) testing is a dark-adapted assessment with white, blue, green and red full-field stimuli, providing a psychophysical assessment of luminance thresholds; which unlike the aforementioned electrophysiological assessments lacks international standardisation.\textsuperscript{104} By comparing the responses to stimuli of different wavelengths, inference can be made about which mechanisms are primarily mediating the response.\textsuperscript{104} FST has been correlated with dark-adapted perimetry derived retinal sensitivity in a cohort of subjects with a range of IRD.\textsuperscript{105} FST has also been correlated with OCT parameters and BCVA in patients with STGD,\textsuperscript{106} with disease duration in individuals with USH2A-associated retinopathy (both syndromic and isolated),\textsuperscript{107} and with ffERG amplitude in patients with RCD.\textsuperscript{108} FST has a test-retest variability of around 0.3 log cd/m\textsuperscript{2} and has been used as a secondary outcome measure in gene therapy clinical trials for IRD, including the pivotal trial leading to approved treatment for RPE65-associated retinal dystrophy.\textsuperscript{109,110}

**Patient-Reported Outcome Measures**

Comprehensively understanding the patient experience while living with an IRD is key to fully measuring the impact of IRD including emotionally, psychologically, socially and financially, and is critical to the provision of appropriate management and the development and approval of treatments. Several standardised questionnaires have shown significant reliability and validity and are included in research settings as patient-reported outcome measures (PROs).\textsuperscript{111–113}
The National Eye Institute Visual Function Questionnaire (NEI-VFQ) is one of the most commonly applied instruments to evaluate vision-related quality of life in visually impaired individuals. A version consisting of 25 items (VFQ25) has been validated and used in the CHM gene therapy clinical trial (NCT01461213) and pivotal RPE65-RCD trial, among others.\textsuperscript{114–116} The Impact of Vision Impairment (IVI) questionnaire is another option and is available in adult (IVI-A) and child-friendly (IVI-C) versions, and is being used as a secondary outcome measure in RPGR-RCD and ACHM gene therapy trials (NCT04671433, NCT03001310 and NCT03758404).\textsuperscript{117} Particularly for RCD, Szlyk et al. have developed questionnaires that showed strong correlation with BCVA, CS and VF.\textsuperscript{118,119} The Vision Function Scale-plus (19 items) survey, initially developed for cataract, has also provided promising results in RCD.\textsuperscript{120} Recently, the Michigan Retinal Degeneration Questionnaire was also validated as a PRO for patients with IRD, employing 59 items in 7 domains.\textsuperscript{121}

Whilst there remains no consensus on the most appropriate PRO tools in IRD and whether they need to be disease/genotype specific given the extreme clinical heterogeneity of IRD, they provide clinically meaningful information for both patients and researchers and are an integral assessment to fully evaluate treatment efficacy and calculate cost-effectiveness.

**Functional Magnetic Resonance Imaging (fMRI)**

MRI can provide anatomical, physiological and functional information in a single, non-interventional setting. Functional MRI commonly uses the blood oxygenation level-dependent (BOLD) technique, which shows increased signal as deoxyhemoglobin concentration decreases, and vice versa.\textsuperscript{122} fMRI has allowed the delineation of retinotopic and population-receptive field maps, which connect visually stimulated retinal regions with a corresponding visual cortex area that responds to this stimulation with an increased BOLD signal.\textsuperscript{123} BOLD fMRI has been used to assess how the visual cortex responded to retinal gene therapy in patients with RPE65-LCA,\textsuperscript{124} and has also recently identified new cone-driven
signals in visual cortical areas in a child with ACHM, following gene therapy (NCT03758404 and NCT03001310), with plans for fMRI to be incorporated into other ACHM gene therapy trials.\textsuperscript{125}

**Vision-guided mobility**

Orientation and Mobility testing (MT) is a way of assessing functional vision, and can be defined as the physical ability to move efficiently and safely in an environment. Assessments of vision-guided mobility can be helpful in exploring the impact of vision on everyday function, with impaired mobility having been associated with reduced wellbeing. Constricted VF, as well as nystagmus, seen in RCD and other IRD, are known to markedly impair mobility.\textsuperscript{126–128}

Increasingly IRD trials, including the pivotal trial for *RPE65*-LCA (where mobility was the primary outcome), employ assessments to quantify vision-guided mobility before and after intervention; with multiple mobility assessments developed to date, including with or without obstacles, with or without visual acuity dependent prompts, performed under a range of different lighting conditions, and of varying sizes and complexities.\textsuperscript{110,129,130} One of the most important MT assessments was the one custom designed for the *RPE65* gene therapy pivotal trial (NCT00999609), which was named multi-luminance mobility testing (MLMT) and had a dimension of 7 × 12 ft (equivalent to 2.1 x 3.6 m);\textsuperscript{131} for which patients were dark adapted and asked to navigate a path, making turns and avoiding obstacles, with one and/or both eyes open. Other groups have employed a mixed indoors and outdoors setting,\textsuperscript{132} while others have directly utilized true real-life scenarios such as shopping malls\textsuperscript{128} and sections of hospitals.\textsuperscript{133} *RPE65*- NCT02781480 and *RPGR*- NCT03252847 have chosen a different type of MT, with a dimension of 7.2 × 10.8 m, and an adjustable modular platform at decreasing, standardised lighting levels (Figure 2). This test can also include obstacles and has been validated for use in subjects with *RPE65*-LCA.\textsuperscript{134}

The metrics used to quantify performance on these mobility assessments have varied, however the most commonly employed are the time taken to navigate the course and/or the errors made during navigation, at a given illumination level. These have been used as either continuous variables or incorporated into a pass/fail criterion; and have been included as
both inclusion criteria and primary outcome measures in several clinical trials and validation studies.\textsuperscript{129,131,134,135} An association between MT parameters and VF has been most strongly established,\textsuperscript{134,136} with a correlation with BCVA\textsuperscript{135} and CS also reported.\textsuperscript{132} Of note, central field loss has not appeared to be as limiting for mobility as peripheral loss.\textsuperscript{51} It remains likely that these correlations will be partly disease dependent and/or severity related.

The capability to navigate independently in dim environments contributes to quality of life and productivity.\textsuperscript{137} Decreased mobility has also been associated with depression.\textsuperscript{138} Innovative MT assessments provide an accurate way of understanding how patients perform on a daily basis and how treatments can help improve their quality of life and increase their independence.

**Virtual Reality and New Methodologies**

Virtual reality (VR) represents an additional opportunity to capture aspects of functional vision under real-life-like conditions. VR technology has become readily available, providing flexibility, reproducibility, participant engagement, safety, and the ability to tailor countless scenarios with excellent ecological validity (highly accurate designs, displaying the relevant features of the environment).\textsuperscript{139} A recent study has tested a VR MT in patients with \textit{RPE65}-LCA, providing proof-of-concept of the utility of this approach and encouraging further broader application to IRD, and potentially resulting in mobility assessments being more accessible and varied.\textsuperscript{140}

Another interesting field has been the development of tools and applications (apps) that can assess aspects of vision while we use our own digital devices.\textsuperscript{141} Information about VF, tracking, CV, CS and VA can be estimated through the use of apps, again potentially providing a more accessible (and arguably more directly functionally relevant) way of characterizing and monitoring vision\textsuperscript{141,142} Standardization and validation of such approaches will be necessary.
Conclusions

Functional testing in IRD has gained increasing relevance over the last decade, superseding structural assessments in providing evidence of efficacy in clinical trials of treatments,\textsuperscript{143} given that improvement in function is generally being recorded, thereby shifting the emphasis away from slowing/halting retinal degeneration which is often focused on structure.\textsuperscript{115,131} Although some of the assessments provide unique, novel information such as real-life mobility performance, most clinically meaningful features can be assessed through a range of modalities e.g. macular function can be evaluated through static perimetry, microperimetry, CV, BCVA, CS, etc. Ideally, following a genetic diagnosis, patients with IRD should have both a structural and functional multimodal evaluation, to fully characterize their disorder, help to provide better informed advice on prognosis, as well as facilitate determination of eligibility and end-points for interventional clinical trials.
Legends

Figure 1: Example of semi-automated static Octopus perimetry and corresponding Visual Field Modelling and Analysis (VFMA), displaying the total hill-of-vision volumetric output ($V_{TOT}$). A) Baseline assessment of an individual with $RPGR$-RCD, with a $V_{TOT}$ of 35.68 decibel-steradians (dB-sr). The latter combines the magnitude and extent of the sensitivity across the test grid. B) Four-year follow up of the same patient, demonstrating a decreased $V_{TOT}$ of 17.28 dB-sr. C) Subtraction analysis of VFMA at baseline (A) and follow-up (B), allowing direct comparison. The 3D image enables us to visualize the representation from above, below, and different angles, to also qualitatively assess the areas where sensitivity has changed, while quantitative analysis reveals a $\Delta V_{TOT}$ of -17.07 dB-sr between both time-points.

Figure 2: Example of mobility assessment. “Fisheye” view from overhead camera showing the Visual Mobility Assessment configuration used in NCT02781480 and NCT02714816 to evaluate individuals affected by $RPE65$-associated retinal dystrophy.
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**Table 1: Summary of the most common methods used in clinic for IRD functional evaluation.**

<table>
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<th>Imaging Modality</th>
<th>Characteristics</th>
<th>Use in Inherited Retinal Disorders (IRD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best Corrected Visual Acuity (BCVA)</td>
<td>Usually, the first assessment in clinical practice, and the most commonly performed.</td>
<td>BCVA is a fundamental parameter with significant correlation with Optical Coherence Tomography (OCT) parameters, as well as with visual field (VF). BCVA is an outcome measure in several gene therapy trials.</td>
</tr>
<tr>
<td>Contrast Sensitivity (CS)</td>
<td>Multiple methods to assess CS are available, with the Pelli-Robson charts being the most common. Newer methods that test a wide range of spatial frequencies and stimulus contrasts are increasingly being employed.</td>
<td>CS is notably reduced in most IRD and has been correlated with OCT features, retinal sensitivity and reading speed. CS is a secondary outcome measure in many gene therapy trials.</td>
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<tr>
<td>Color Vision (CV)</td>
<td>Colour can be assessed by a wide range of tests, complex and simple, computer and paper-based, and specifically tailored for visually impaired individuals.</td>
<td>Particularly useful in specific differential diagnoses such as discrimination between complete and incomplete achromatopsia (ACHM). Also, helpful to infer how cone systems are affected and potentially measure differences in specific cone response to intervention.</td>
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</tbody>
</table>
| VF and Retinal Sensitivity        | • Kinetic VF testing largely superseded by static perimetry.  
  • Octopus to a greater extent than Humphrey automated static perimetry is better suited to the evaluation of retinal sensitivity cross-sectionally and longitudinally in IRD. | Evaluating VF and retinal sensitivity is key to monitoring disease progression, as well as impact of interventions. Recent advances include modelling and Hill-of-Vision analysis software, |
- **Microperimetry** = fundus-guided perimetry allows assessment of central macular sensitivity and improved correlation between structure and function. Some devices also have a range of testing conditions (photopic, mesopic and scotopic) and dual-colour testing. From which topographic information and volumetric assessments can be derived.

Testing under a range of conditions and 2-colour microperimetry provides differential information on rod, cone and mixed mechanisms, with a high correlation with OCT parameters. Static perimetry and microperimetry are very common outcome measures in a wide range of clinical trials.

<table>
<thead>
<tr>
<th>Visual Electrophysiology</th>
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<tr>
<td>- Full-field (ff) electroretinogram (ERG): measures the retinal electrical potential changes provoked by light stimuli, under light and dark-adapted conditions.</td>
<td>• ffERG provides information on generalised retinal function.</td>
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<td>- Multifocal (mf) ERG: measures retinal function in the central macula and paramacula.</td>
<td>• mfERG assesses localized macular function.</td>
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<td>- Pattern ERG (PERG): typically uses a contrast-reversing checkerboard stimulus to detect macular dysfunction. It reflects the integrity of bipolar cells, retinal ganglion cells, and macular photoreceptors.</td>
<td>• PERG: assesses macular and optic nerve function.</td>
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<tr>
<td>- Electrooculogram (EOG): evaluates the RPE and the photoreceptor-RPE complex.</td>
<td>• EOG: valuable in the diagnosis of disorders of the RPE such as Best disease, where a normal ffERG and abnormal EOG are characteristic.</td>
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</tr>
<tr>
<td></td>
<td>• FST: provides information on which cell type is primarily mediating the responses. First</td>
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</table>
• Full-field light-sensitivity threshold (FST): provides a psychophysical assessment of luminance thresholds using white, blue, green and red full-field stimuli. developed for use in patients with profound visual impairment unable to perform perimetry. Has been shown to correlate with perimetry, OCT parameters, BCVA, disease duration and ffERG amplitude. It is a secondary outcome measure in several gene therapy trials.
Table 2: Summary of the current and developing methods for IRD functional evaluation used in research and clinical trial settings.

<table>
<thead>
<tr>
<th>Imaging Modality</th>
<th>Characteristics</th>
<th>Use in Inherited Retinal Disorders (IRD)</th>
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<tr>
<td>Low Luminance Visual Acuity (LLVA)</td>
<td>Can be measured by placing a filter over the patient’s best correction or over the letter chart, to simulate mesopic conditions.</td>
<td>Changes in LLVA are secondary outcome measures in several IRD gene therapy trials.</td>
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<tr>
<td>Dark adaptometry</td>
<td>Yields insights into photoreceptor function, measuring change in retinal sensitivity during transition from photopic to scotopic conditions.</td>
<td>Provides information on rod and cone kinetics and thresholds - which are variably abnormal in many IRD.</td>
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<td>Photoaversion Testing</td>
<td>Both qualitative and quantitative assessment of light discomfort and / or its associated impact on vision e.g. BCVA and CS. Known as photosensitivity, photoaversion and photophobia.</td>
<td>Particularly useful in cone dysfunction syndromes such as ACHM, and COD/CORD. Currently a secondary/exploratory outcome measure in gene therapy trials for ACHM.</td>
</tr>
<tr>
<td>Patient Reported Outcome Measures</td>
<td>General and disease-specific questionnaires designed to better evaluate the impact of IRD on patients’ lives.</td>
<td>Invaluable instruments to help fully evaluate treatment efficacy and calculate cost-effectiveness.</td>
</tr>
<tr>
<td>Functional Magnetic Resonance Imaging (fMRI)</td>
<td>Provides anatomical, physiological and functional information in a single, non-interventional setting.</td>
<td>fMRI has allowed the delineation of retinotopic and population-receptive field maps; providing objective visual function data and being currently used to measure gene therapy outcomes.</td>
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<tr>
<td>Vision-guided Mobility</td>
<td>Mobility testing (MT) is a way of assessing functional vision, which refers to the impact played by vision on</td>
<td>MT is able to differentiate between controls and patients and to capture longitudinal changes. It is</td>
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<tr>
<td>Virtual reality (VR) and new trends</td>
<td>VR represents a cost efficient and readily available opportunity to capture aspects of functional vision under real-life-like conditions. Tools and apps that assess our vision while we use our own digital devices are also under development.</td>
<td>VR assessed-mobility performance has been shown in a proof-of-concept study to be a useful measure of functional vision in individuals with RPE65-LCA. Apps potentially allow VF, tracking, CV, CS and VA to be estimated whilst using commonplace digital devices.</td>
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