The Extraordinary Phenotypic and Genetic Variability of Retinal and Macular Degenerations: The Relevance to Therapeutic Developments

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Inherited retinal diseases (IRDs) are a clinically and genetically heterogeneous group of rare conditions leading to various degrees of visual handicap and to progressive blindness in more severe cases. Besides visual rehabilitation, educational, and socio-professional support, there are currently limited therapeutic options, but the approval of the first gene therapy product for *RPE65*-related IRDs raised hope for therapeutic innovations. Such developments are facing obstacles intrinsic to the disease and the affected tissue including the extreme phenotypic and genetic variability of IRDs and the fine tuning of visual processing through the complex architecture of the postmitotic neural retina. A precise phenotypic characterization is required prior to genetic testing, which now relies on high-throughput sequencing. Their challenges will be discussed within this article as well as their implications in clinical trial design.

Inherited retinal diseases (IRDs) are a large group of rare genetic conditions with a prevalence of about 1/3000 subjects worldwide (Bessant et al. 2001) that may be isolated or part of a syndromic disorder. IRDs may affect the entire retina such as in the most common one, rod-cone dystrophies (RCDs) also known as retinitis pigmentosa (RP), or only the macular region (i.e., macular dystrophies) with various degrees of visual handicap up to progressive blindness. Besides visual rehabilitation, educational, and socio-professional support, there are currently limited therapeutic options for IRDs (Sahel et al. 2015). Nevertheless, the approval of the first gene therapy product for the treatment of *RPE65*-related IRDs after successful phases 1/2 (Maguire et al. 2008) and 3 (Russell et al. 2017) and ongoing clinical trials (Sahel et al. 2015) raised hope for therapeutic innovations. Such developments are facing obstacles intrinsic to

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the disease and the affected tissue including the extreme phenotypic and genetic variability of IRDs and the fine tuning of visual processing through the complex architecture of the postmitotic neural retina. We will be discussing some of these issues within this article.

PHENOTYPIC VARIABILITY OF IRDs MIRRORED BY AN EXTREME GENETIC HETEROGENEITY

IRDs are characterized by an extreme phenotypic variability, which is difficult to accurately capture within a clinical classification, an attempt being presented in Table 1. IRDs range from congenital, usually stationary disorders to early-, juvenile-, or adult-onset degenerative disorders. Each of these disorders will have a variable impact and degree of severity on visual function. Some dystrophies will progressively affect the entire retina usually leading to blindness, whereas disorders affecting only the central part, the macula, will be associated with a severe impact on visual acuity but with preserved peripheral vision. Most of these disorders primarily affect photoreceptor cells, namely, rod photoreceptors responsible for dim light vision, and cone photoreceptors associated with daylight, color, and precise vision (Lamb 2022). The primary site of dysfunction can also be the retinal pigment epithelium (RPE), which, among other functions, provides metabolic support essential to photoreceptor functioning and survival (Caceres and Rodriguez-Boulan 2020). The respective prevalence of each of these entities is not always precisely known but, by far, RCD (RP) is the most prevalent one, affecting about 1/4000 individuals worldwide, representing up to 70% of IRDs (Bocquet et al. 2013). This entity itself is associated with some phenotypic variability in the age at onset, the disease course, and the potential association with additional alterations as part of syndromic diseases. Usher syndrome, associated with variable degrees of deafness and vestibular dysfunction, is the most common syndromic form of RCD (Delmaghani and El-Amraoui 2022) followed by Bardet–Biedl syndrome, a ciliopathy that associates variable degrees of cognitive difficulties, obesity, hexadactilia, and kidney disease (Mockel et al. 2011). The management and support of patients affected with these syndromic disorders is therefore not only challenged by the visual handicap but also by the other systemic alterations, which require a multidisciplinary management. Of note, despite the clinical heterogeneity of RCD/RP, symptoms and ophthalmic alterations may be consistent within this clinical heterogeneity with night blindness often being the first symptom followed by progressive peripheral visual field constriction and eventually the loss of central vision late in the disease leading to blindness in most severe cases. Clinical examination will typically reveal visual field constriction, generalized rod-cone dysfunction on the full-field electroretinogram (ff-ERG) and cardinal signs on fundus examination including a waxy pallor of the optic disc, narrowed retinal vessels, and pigmentary changes in the retinal periphery (Fig. 1). Macular dystrophies will manifest differently starting with decrease in visual acuity, color vision disturbances, and some pathognomonic macular alterations on fundus examination or fundus autofluorescence imaging (Fig. 2). With a more severe visual outcome, cone and cone-rod dystrophies have overlapping visual symptoms at onset in addition to photophobia. ff-ERG is instrumental for the differential diagnosis and visual prognosis (i.e., macular dystrophies have normal retinal function on ff-ERG while cone and cone-rod dystrophies show variable degrees of generalized cone and rod dysfunction) (Cornish et al. 2021).

The clinical heterogeneity of IRDs is mirrored by the genetic heterogeneity. Indeed, since the initial discovery of mutations in *RHO* underlying autosomal-dominant RCD as the first gene defect identified underlying IRDs (Dryja et al. 1990a,b), more than 250 genes have been associated with some forms of IRDs (web.sph.uth.edu/RetNet/home.htm). These genes encode proteins of various expression profiles and functions, some being specific to photoreceptors (e.g., involved in the phototransduction cascade) or the RPE (e.g., involved in the visual cycle), whereas others are associated with a more ubiquitous expression profile such as splicing factors (e.g., *PRPF31*, *PRPF8*, *PRPF3*) or proteins involved in the primary cilium maintenance, mutations in the latter potentially leading to syn-

Table 1. Attempt for a simplified clinical classification showing the phenotypic variability of inherited retinal diseases

Phenotypic variability	Cellular alterations	Diagnosis	Mode of inheritance	Prevalence	References
Stationary	Rod dysfunction	Riggs-type CSNB	AD, AR	Unknown	Zeitz et al. 2015
	Post-phototransduction dysfunction	Schubert–Bornschein-type CSNB	AR, XL	Unknown	Zeitz et al. 2015
	Cone dysfunction	Achromatopsia	AR	1/50,000	Aboshiha et al. 2016
		BCM	XL	1/100,000	De Silva et al. 2021
Degenerative	Generalized progressive rod then cone dysfunction and affiliated disorders	RCD, isolated	AD, AR, XL	1/4000	Verbakel et al. 2018
		RCD-type LCA/EORD	AR	1/100,000	Hanein et al. 2004
		Syndromic (e.g., Usher syndrome, BBS)	AR	1/20,000	Boughman et al. 1983; Mockel et al. 2011
		ESCS/Goldman–Favre syndrome	AR	<1/1,000,000	Yzer et al. 2013
		Chorioretinopathies			
		CHM	XL	1/100,000	Zeitz et al. 2021
		Bietti chorioretinal dystrophy	AR	Unknown	García-García et al. 2019
		Gyrate atrophy	AR	Unknown	Sergouniotis et al. 2012
	Generalized progressive cone +/− rod dysfunction	CD and CRD, isolated	AR, AD, XL	1/100,000	Gill et al. 2019
		CRD-type LCA/EORD	AR	1/100,000	Hanein et al. 2004
		Syndromic cone and cone-rod dystrophies (e.g., SCA7, BBS)	AD, AR	<1/1,000,000	Gill et al. 2019
	Inner retina	XL-retinoschisis	XL	1/100,000	Molday et al. 2012
	Macular dystrophies	Stargardt disease	AR	1/10,000	Rahman et al. 2020)
		Best disease	AD	1/10,000	Boon et al. 2009
		Pattern dystrophy including maternally inherited diabetes and deafness	AD, mitochondrial	Unknown	Rahman et al. 2020
		North Carolina macular dystrophy	AD	<1/1,000,000	Rahman et al. 2020
		Doyne Honeycomb retinal dystrophy	AD	<1/1,000,000	Rahman et al. 2020
		Sorsby fundus dystrophy	AD	<1/1,000,000	Rahman et al. 2020

(AD) Autosomal-dominant, (AR) autosomal-recessive, (XL) X-linked, (CSNB) congenital stationary night blindness, (BCM) blue cone monochromacy, (SCA7) spinocerebellar ataxia type 7, (BBS) Bardet–Biedl syndrome, (ESCS) enhanced S-cone syndrome, (CHM) choroideremia, (LCA) Leber congenital amaurosis, (EORD) early-onset retinal dystrophies, (RCD) rod-cone dystrophies, (CRD) cone-rod dystrophy.

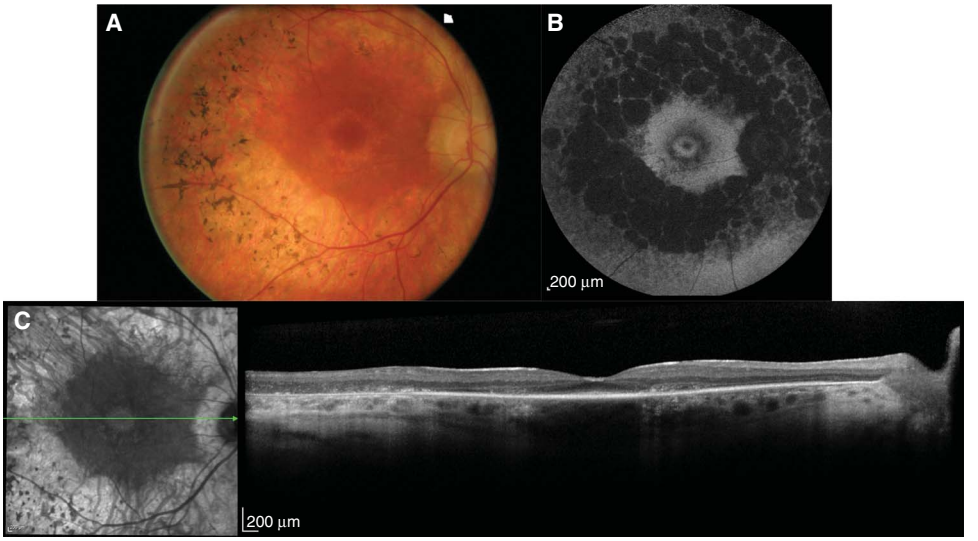

Figure 1. Characteristic retinal alterations of rod-cone dystrophy, also known as retinitis pigmentosa, in a 50-yr-old man with biallelic *USH2A* variants (allele 1: c.1036A > C, p.(Asn346His); allele 2: c.2276G > T, p.(Cys759Phe) (right eye). (*A*) Fundus photograph showing a pale optic disc, narrowed retinal vessels, chorioretinal atrophy, and pigmentary changes in the mid-periphery with perifoveal changes. (*B*) Short-wavelength fundus autofluorescence showing peripheral loss of autofluorescence. (*C*) Spectral domain optical coherence tomography showing outer retinal alteration outside the foveal region.

dromic disease defined as ciliopathies (Bujakowska et al. 2017).

The genetic complexity goes even further since distinct mutations within the same gene can lead to distinct phenotypes or inheritance (Fig. 3), while a similar phenotype can be associated with distinct genotypes. For instance, an RP phenotype may be associated with genetic variants in more than 70 distinct genes (web.sph.uth.edu/RetNet/home.htm) while some of these genes may be associated with several distinct IRDs depending on the genetic variants. Similarly, maculopathies with flecks are more commonly associated with *ABC44* variants following an autosomal-recessive inheritance, but similar phenotypes may also be associated with variants in *PRPH2* or less commonly in *ELOVL4*, both gene defects following an autosomal-dominance inheritance.

Biallelic variants in the ATP-binding cassette, subfamily A, member 4 (*ABCA4*, OMIM* 601691) are underlying Stargardt disease (OMIM#248200), the most common form of macular dystrophy, but may also lead to cone, cone-rod dystrophy (CORD3, OMIM#604116), and possibly RP (RP19, OMIM#601718) with some degree of phenotype/genotype correlation, genetic variants leading to a more severe ABCA4 dysfunction being associated with more severe phenotypes (Cremers et al. 2020). Similarly, monoallelic *BEST1* (OMIM*607854) variants were initially associated with the second-most-common macular dystrophy, autosomal-dominant Best vitelliform macular dystrophy (OMIM# 153700) (Petrukhin et al. 1998), whereas monoallelic-specific splice site variants were found underlying autosomal-dominant vitreoretinochoroidopathy (ADVIRC, OMIM#193220) (Burgess et al. 2009) and biallelic changes in autosomal-recessive bestrophynopathy (ARB, OMIM# 611809) (Burgess et al. 2008). *RPGR* (OMIM*312610) variants are the most common cause of X-linked RCD (RP3, OMIM#300029) (Meindl et al. 1996) but also X-linked CRD (OMIM#304020) (Yang et al. 2002; Nassisi et al. 2022), both having distinct impact on visual function. Even more relevant for patients' management and counseling, certain genes may be asso-

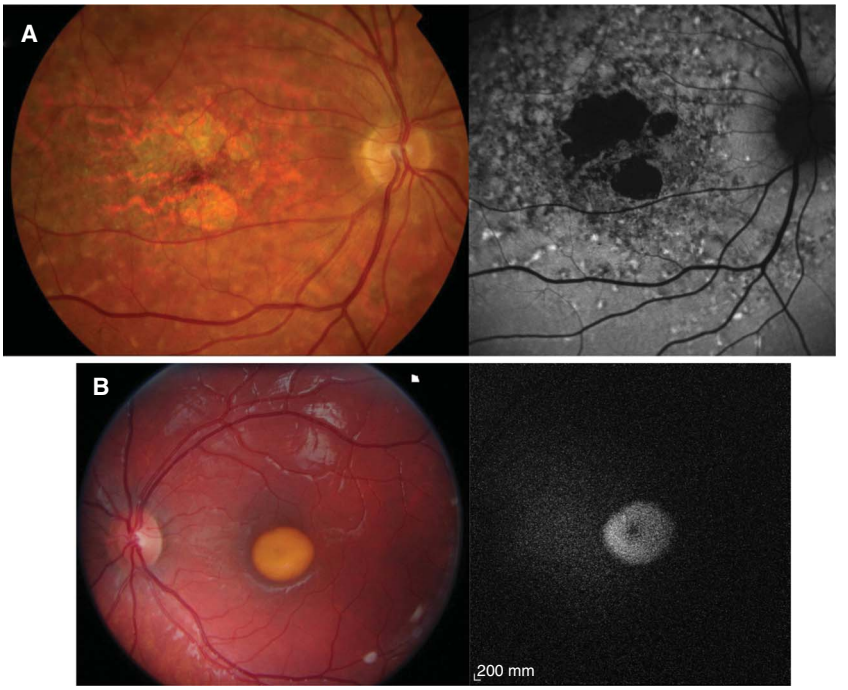

Figure 2. Characteristic retinal alterations for the two most common macular dystrophies on fundus photographs and short-wavelength fundus autofluorescence: (*A*) Stargardt macular dystrophy, and (*B*) Best vitelliform dystrophy (*BEST 1* heterozygous variant: c.889C > T, p.(Pro297Ser)).

ciated with either isolated retinal disorders or syndromic diseases (e.g., biallelic *USH2A* variants leading either to autosomal-recessive RCD or Usher type 2 [Eudy et al. 1998; Bernal et al. 2003]; *CLN3* variants can lead to a severe and eventually lethal syndromic neurological disorder—Batten disease—or to an isolated retinal dystrophy [Smirnov et al. 2021]). Finally, even within the same gene defect, there is a high inter- and intrafamilial variability, variable expression, and incomplete penetrance (Farrar et al. 2017).

A precise delineation of phenotype/genotype correlation is therefore essential for a better understanding of IRD, an improved patients' management, but also to support therapeutic research.

MASSIVE PARALLEL SEQUENCING APPLIED TO IRDs

The past two decades have seen tremendous technological developments in the field of massive parallel sequencing accelerating gene discovery and delivering high-throughput analytic tools particularly suited to encompass the genetic heterogeneity of IRDs. Indeed, nearly 300 genes have been associated with specific forms of IRDs (web.sph.uth.edu/RetNet/sum-dis.htm#D-graph, last accessed March 13, 2023). Targeted next-generation sequencing (NGS) has indeed passed from a research setting (Audo et al. 2012) to diagnostic laboratories with a genetic resolution rate reaching about 70% (Shah et al. 2020; Britten-Jones et al. 2023). In addition, whole-exome sequencing (WES) and further whole-genome sequencing (WGS) have also entered the diagnostic area and provide a more comprehensive genetic analysis. In addition to providing a better coverage of coding regions, the latter covers also noncoding intronic and regulatory regions and allows a more precise analysis of DNA structural alterations such as copy number variations and other large genomic changes or repeat expansions (Dolzhenko et al. 2017; Chen et al. 2019). This massive

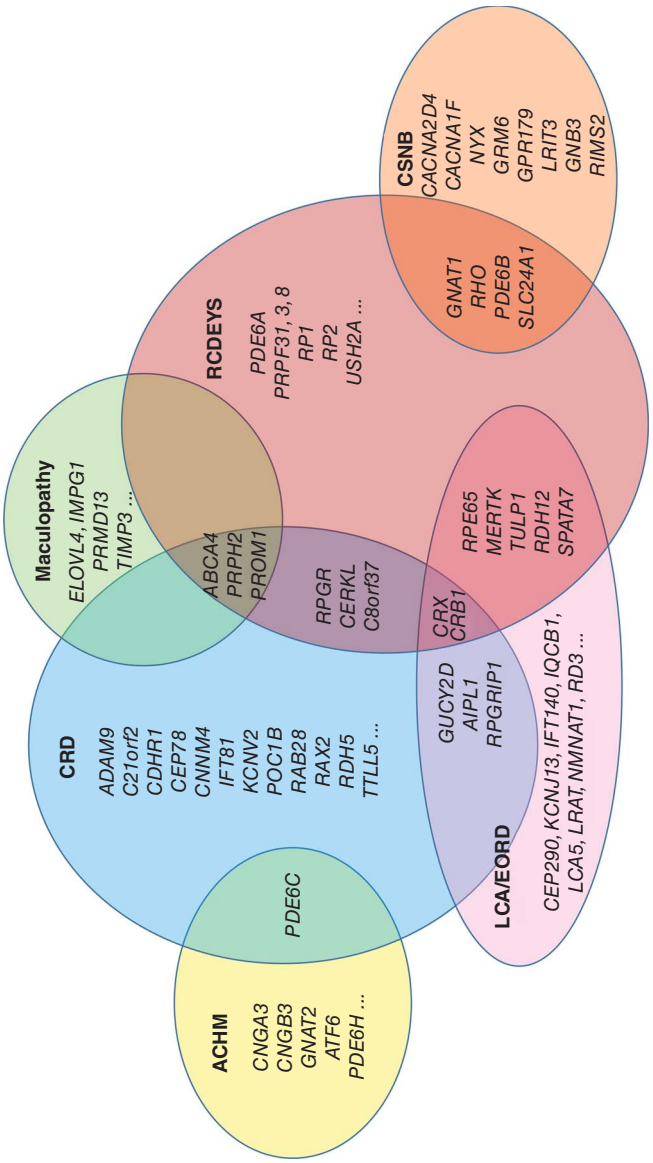

Figure 3. Genetic heterogeneity of inherited retinal dystrophies and overlapping genotype/phenotype correlation. (ACHM) Achromatopsia, (CRD) cone-rod dystrophy, (CSNB) congenital stationary night blindness, (LCA/EORD) Leber congenital amaurosis/early-onset retinal degeneration, (RCD) rod-cone dystrophy, also known as RP.

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parallel sequencing however generates a significant volume of data to be stored and analyzed through relevant bioinformatic algorithms, which became more efficient along with the development of the techniques and the reduction of their cost. In this context, a detailed phenotypic characterization and a precise phenotype/genotype delineation has become increasingly essential to pinpoint the genetic cause among the numerous genetic variants obtained with high-throughput sequencing. The issue of unsolicited or incidental genetic findings, which should be discussed with the patient prior to genetic testing, can be resolved by performing phenotype-based in silico panels on a predefined list of genes associated with the disease of interest. This option reduces the number of variants to analyze and limits the interpretation to genomic regions within the strict expertise of the interpreting biologist. A variant interpretation has been refined with the American College of Medical Genetics and Genomics guidelines (Richards et al. 2015). Nevertheless, variants of unknown significance remain a real issue when it comes to accurate genetic counseling and access to therapies or selection for clinical trials (Hoffman-Andrews 2017). To improve variant classification, a precise phenotypic characterization may help in addition to variant segregation within a given family, which should be sought systematically. The implementation of functional tests, such as mini-gene assays to validate putative RNA mis-splicing, for instance, cilia-based assays, or biochemical tests (Sangermano et al. 2018; Yang et al. 2019; Westin et al. 2021; Lange et al. 2022), when relevant, are essential to refine variant classification. But these require an additional technology that is not always present in a diagnostic setting. Finally, an incentive should be given to report any new variant in available databases, such as the Leiden Open Variation Database (databases.lovd.nl/shared/genes) or ClinVar (www.ncbi.nlm.nih.gov/clinvar/), a requirement for certain genetic journals prior to publication (e.g., *Human Mutation*), which should be made mandatory for any diagnostic genetic laboratory. Indeed, an exhaustive reporting of all identified variants would help further develop the genetic landscape of IRD, which still needs to be completed. In this respect, a recent meta-analysis of targeted NGS panels applied to IRDs revealed a genetic diagnostic yield between 52% and 74% (Britten-Jones et al. 2023). More recently applied, WGS constitutes an unprecedented powerful tool to solve additional cases, although recent studies suggest that this added improvement may be limited (Wen et al. 2023) with only 2.1% additional cases for which WGS identified structural variants that were missed by NGS or WES (Wen et al. 2023). Expanding IRD cohorts analyzed through WGS, with improved bioinformatic pipelines and variant classification, will help better address the impact of WGS to resolve genetically unsolved cases. Newer technologies such as long-read sequencing may bring further insight in providing a better genome mapping of structural variants and highly repetitive regions as well as providing important phase information (Amarasinghe et al. 2020).

Finally, there are so far very few studies addressing the role of epigenetics in IRDs, which could account, to some extent, for the intrafamilial phenotypic variability or for some of the genetically unresolved cases (Dvoriantchikova et al. 2022). Further studies would be needed to establish the role of epigenetics in the clinical and genetic heterogeneity of IRDs, with a major obstacle residing in the lack of direct access to the diseased tissue.

A better understanding of the genetic landscape of IRDs, along with the development of deep phenotyping and relevant phenotype/genotype correlation, is essential to support the development of therapeutic trials.

NEED FOR A BETTER PHENOTYPIC DELINEATION AND OUTCOME MEASURE TO FACILITATE CLINICAL TRIALS

The past decades have seen an increased number of clinical trials in IRDs, which led to the approval of the first gene-augmentation therapy for *RPE65*-related IRDs (Russell et al. 2017). This active translational research is challenged by the phenotypic and genetic heterogeneity of IRDs. In this context, a panel of experts in the field recently established the priorities needed to accelerate therapeutic research facing these challenges (Thompson et al. 2015, 2020). The first priority was identified as "the use of natural history studies

to guide clinical trial design" (Thompson et al. 2015). These natural history studies will have to face the intrinsic phenotypic variability of IRDs, even within a single genotype, by including a significant number of patients to reach statistical power. They will provide relevant information on disease progression and determine biomarkers and clinical end points better suited to document disease severity and progression, while potentially identifying a relevant window of intervention, which will be essential for trial design.

The selection of relevant outcomes and end points for clinical trials may directly affect the success of innovative therapeutic strategies. While for other acute diseases (e.g., exudative neovascular age-related macular degeneration, uveitis) (Schmetterer et al. 2023) improvement of visual acuity is generally considered the most important clinical outcome, for IRDs it may not be the most relevant criteria. In most cases, a reasonable objective of a therapy in IRDs would be delaying disease progression and stabilizing visual acuity over time, improving it being elusive when associated with photoreceptor degeneration. Indeed, the phase 2/3 studies for X-linked RP (NCT03116113) and for choroideremia (NCT03496012) did not meet the primary outcome of a 15-letter gain in visual acuity from baseline, an outcome accepted by the FDA, whereas other end points, such as no change in visual acuity or visual field from baseline, may have been relevant for the disease and the patients. A close dialogue with regulatory agencies prior to clinical trial design to better discuss the specificity of IRDs, in light of the phenotypic and genetic heterogeneity, along with natural history data may help to better define outcome measures and end points in IRDs. The development of standardized testing guidelines would take into account (1) the different definition of "therapeutic efficacy" for specific types of IRDs and interventions; (2) the correlation of each surrogate outcome with accepted clinically meaningful outcomes for assessing efficacy; and (3) the reproducibility and reliability of the outcome measures evaluated (Thompson et al. 2020). Furthermore, the current advancing use of artificial intelligence and machine learning is leading to the opportunity of standardizing outcome measures across multiple trial sites and may take into account genetic heterogeneity (Sumaroka et al. 2019, 2020). Nevertheless, while highly standardized clinical tests are important for the evaluation of potential treatments, the development of new clinical outcomes that reflect the needs of patients (e.g., patient-reported outcomes and performance-based tests) would be highly relevant (Thompson et al. 2020).

CONCLUDING REMARKS

IRDs are characterized by a significant phenotypic and genetic heterogeneity. The advent of genomic testing along with deep phenotyping offers a unique opportunity to better delineate phenotype/genotype correlations and subsequently support improved management of IRD patients, but also to provide more accurate biomarkers for successful clinical trials.

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