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Retinal Dystrophies and the Road to Treatment: Clinical Requirements and Considerations

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Retinal dystrophies (RDs) comprise relatively rare but devastating causes of progressive vision loss. They represent a spectrum of diseases with marked genetic and clinical heterogeneity. Mutations in the same gene may lead to different diagnoses, for example, retinitis pigmentosa or cone dystrophy. Conversely, mutations in different genes may lead to the same phenotype. The age at symptom onset, and the rate and characteristics of peripheral and central vision decline, may vary widely per disease group and even within families. For most RD cases, no effective treatment is currently available. However, preclinical studies and phase I/II/III gene therapy trials are ongoing for several RD subtypes, and recently the first retinal gene therapy has been approved by the US Food and Drug Administration for *RPE65*-associated RDs: voretigene neparvovec-rzyl (Luxturna). With the rapid advances in gene therapy studies, insight into the phenotypic spectrum and long-term disease course is crucial information for several RD types. The vast clinical heterogeneity presents another important challenge in the evaluation of potential efficacy in future treatment trials, and in establishing treatment candidacy criteria. This perspective describes these challenges, providing detailed clinical descriptions of several forms of RD that are caused by genes of interest for ongoing and future gene or cell-based therapy trials. Several ongoing and future treatment options will be described.

Key Words: antisense oligonucleotides, gene therapy, genetics, inherited retinal dystrophies, stem cell therapy

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Retinal dystrophies (RDs) comprise a collection of degenerative diseases characterized by the usually progressive and sometimes stationary dysfunction of rods and/or cones. With a prevalence of 1:3000 individuals,[1,2] RDs are not particularly rare. However, due to their genetic heterogeneity, with >200 disease genes identified to date, each genetic subtype may be exceedingly rare. These diagnoses have a profoundly distressing impact on patient's lives, progressively affecting their mobility, professional functioning, and independence. Patients are often uncertain of their prognosis, questioning whether and when they will go blind, and whether they will pass this disease onto their children. Children or adolescents diagnosed with RD need to be informed on their prognosis, to make sound decisions on life planning such as future career paths. Special lighting or magnification requirements need to be tended to at their home, school, or professional environment, or they may need to visit a special needs school altogether.

THE EVOLUTION OF GENE-BASED THERAPEUTIC TRIALS

Due to the monogenic nature of most RDs, and the relative immune privilege of the eye, its accessibility, and the ability to noninvasively monitor its function and structure, the eye is a particularly suitable target for investigational gene therapy. The blood-retinal barrier restricts the degree of vector dissemination outside the eye, and limits immune responses to the viral vector and gene product. Another advantage of the eye over other organs is the lack of cell division in most retinal cells. Thus, the viral vector DNA does not have to integrate into the host cell genome to remain available in daughter cells after cell division, and the risk of malignancy is reduced.

Autosomal recessive disorders are characterized by loss of function or even (near) absence of the protein produced encoded by the gene. Therefore, for autosomal recessive disorders, gene therapy can be based "simply" on gene augmentation or replacement through the delivery of the normal gene. However, in autosomal dominant disease, such as rhodopsin (RHO)-associated retinitis pigmentosa (RP), the phenotype is typically the result of gain-of-function mutations, where one gene copy expresses a normally functioning protein, and the other gene copy expresses a detrimental protein that needs to be suppressed. For autosomal dominant disease, therapeutic intervention generally focuses on the suppression or inactivation on the gain-of-function gene.

Important advances have been made with the turn of the millennium in the development of (gene) therapies that aim to slow or (temporarily) halt the disease progression in RDs, or even to restore some visual function. The first successful gene therapy was applied in patients with *RPE65*-RD.[3] Several trials have found compelling results in other RD subtypes, such as choroideremia,[4,5] and many other trials are ongoing (Table 1) or in the basic experimental or preclinical phase.[6,7] However, an imbalance exists between the rapid advances in (gene) therapy development and the available literature on the clinical disease course and the phenotypic spectrum for each specific gene of interest. Until relatively recently, longitudinal studies on the detailed clinical characteristics and disease progression were scarce for several RD subtypes, which had been the focus of interest in gene therapy development. Prospective phenotyping study had been

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TABLE 1. An Overview of Ongoing or Recently Completed Human Gene Therapy Trials for Inherited Retinal Degenerations

Route of Administration/ Vector	Target Gene	Disease Group	Study Phase	Primary Outcome Measure*	Secondary Outcome Measure*	Clinicaltrials.Gov Number	Sponsor
Adeno-associated virus gene replacement therapy							
Subretinal AAV8-RPGR	*RPGR*	RP	2/3	Dose-limiting toxicities; treatment emergent AE; MP	BCVA; MP; OCT (EZ); FAF; VF	NCT03116113	NightstaRx Ltd, a Biogen Compay
Subretinal rAAV2tYF-GRK1-RPGR	*RPGR*	RP	1/2	AE, abnormal clinically relevant hemotology/chemistry paremeters	BCVA; perimetry; retinal structure by imaging; QoL questionnaire	NCT03316560	Applied Genetic Technologies Group
Subretinal AAV-RPGR	*RPGR*	RP	1/2	Adverse events	Visual function; retinal function; QoL questionnaire	NCT03252847	MeiraGTx UK II Ltd
Subretinal AAV2-REP1	*CHM*	CHM	2 Completed 2019	BCVA	Macular FAF; MP; AE	NCT02553135	Univ. of Miami, USA
Subretinal AAV2-REP1	*CHM*	CHM	1/2 Completed 2018	Ocular and systemic AE	Goldmann VF; MP; ERG; full-field scotopic threshold; SD-OCT; FAF; fundus photography	NCT02077361	Univ. of Alberta, Canada
Subretinal AAV2-REP1	*CHM*	CHM	1/2	Safety and tolerability	Not mentioned	NCT02341807	Spark Therapeutics; Children's Hospital of Philadelphia; Univ. of Pennsylvania, USA; Massachusetts Eye and Ear Infirmary
Subretinal AAV2-REP1	*CHM*	CHM	2	Treatment emergent AE	BCVA; FAF; OCT (EZ); MP	NCT03507686 (GEMINI TRIAL)	NightstaRx Ltd (now Biogen)
Subretinal AAV2-REP1	*CHM*	CHM	2	BCVA	MP; FAF	NCT02407678 (REGENERATE TRIAL)	Univ. of Oxford; Moorfields Eye Hospital, UK; University College London, UK
Subretinal AAV2-REP1	*CHM*	CHM	3	BCVA	FAF; OCT; MP; contrast sensitivity; color vision; reading performance; QoL questionnaire	NCT03496012 (STAR TRIAL)	NightstaRx Ltd (now Biogen)
Subretinal rAAV2.REP1	*CHM*	CHM	1/2 Completed 2018	BCVA	MP; OCT; FAF	NCT01461213	Univ. of Oxford, UK; multicenter†
Subretinal rAAV2.REP1	*CHM*	CHM	2 Completed 2018	BCVA	AE; FAF; MP; contrast sensitivity; color vision	NCT02671539 (THOR TRIAL)	Univ. of Tubingen, Germany
Subretinal rAAV2.REP1	*CHM*	CHM	Observational	AE	BCVA; FAF; EZ on OCT; MP	NCT03584165 (SOLSTICE TRIAL)	NightstaRx Ltd (now Biogen); multicenter
Subretinal rAAV2-CBSB-hRPE65	*RPE65*	LCA	1	Ocular examination; toxicity	Visual function	NCT00481546	Univ. of Pennsylvania; NEI
Subretinal AAV2-hRPE65v2, voretigene neparvovec-rzyl	*RPE65*	LCA	1/2	Safety and tolerability	Visual function	NCT00516477	Spark Therapeutics
Subretinal tgAAV76 (rAAV 2/2.hRPE65p.hRPE65)	*RPE65*	Severe early-onset RD	1/2 (Completed)	Intraocular inflammation	Visual function	NCT00643747	UCL, Moorfields Eye Hospital NHS Foundation Trust, Targeted Genetics Corporation
Subretinal rAAV2-CB-hRPE65	*RPE65*	LCA	1/2 (Completed)	Ocular and nonocular AE	VF in central 30°; BCVA	NCT00749957	Applied Genetic Technologies Corp; Oregon Health and Science Univ.; Univ. of Massachusetts, Worcester, USA
Subretinal rAAV2-hRPE65	*RPE65*	LCA	1 (Completed)	Ocular and systemic safety	Visual function	NCT00821340	Hadassah Medical Organization
Subretinal AAV2-hRPE65v2, voretigene neparvovec-rzyl	*RPE65*	LCA	3	MLMT	FST white light; MLMT; BCVA	NCT00999609	Spark Therapeutics, Children's Hospital of Philadelphia, University of Iowa, USA
Subretinal AAV2-hRPE65v2, voretigene neparvovec-rzyl	*RPE65*	LCA	1/2	Safety and tolerability	BCVA; VF; pupillary light response; mobility testing; FST; contrast sensitivity	NCT01208389	Spark Therapeutics
Subretinal rAAV2/4.hRPE65	*RPE65*	LCA or severe early-onset RD	1/2 (Completed)	Drug safety evaluation	Different efficacy parameters and immune parameters; global ERG; patient efficacy questionnaire, far and near BCVA; color vision, pupillometry, MP, DA	NCT01496040	Nantes University Hospital
Subretinal AAV RPE65	*RPE65*	RD	1/2 (Completed)	AE	Visual function; retinal function; QoL questionnaire	NCT02781480	MeiraGTx UK II Ltd
Subretinal SAR422459	*ABCA4*	STGD	1/2 (Study discontinued; not for safety reaseons)	AE; ocular safety (BCVA; IOP; MP; static and kinetic VF; OCT; ERG)	BCVA; MP; static and kinetic VF; OCT; FAF	NCT01367444	Sanofi
Subretinal rAAV.hCNGA3	*CNGA3*	ACHM2	1/2	AE	Visual function; patient reported outcomes; retinal imaging	NCT02610582	STZ eyetrial; University Hospital Tuebingen; Ludwig-Maximillians – Univ of Munich

TABLE 1. (Continued)

Route of Administration/ Vector	Target Gene	Disease Group	Study Phase	Primary Outcome Measure*	Secondary Outcome Measure*	Clinicaltrials.Gov Number	Sponsor
Subretinal AGTC-402	*CNGA3*	ACHM2	1/2	AE	BCVA; light discomfort testing; color vision	NCT02935517	Applied Genetic Technologies Corp
Subretinal AAV-CNGA3	*CNGA3*	ACHM2	1/2	AE	BCVA; MP; perimetry; QoL questionnaire	NCT03758404	MeiraGTx UK II Ltd
Subretinal AAV-CNGA3	*CNGB3*	ACHM3	1/2	AE	Visual function; retinal function; QoL questionnaire	NCT03001310	MeiraGTx UK II Ltd; EMAS Pharma; Syne Qua Non Limited
Subretinal rAAV2tYF-PR1.7-hCNGB3	*CNGB3*	ACHM3	1/2	AE	BCVA; light discomfort testing; color vision	NCT02599922	Applied Genetic Technologies Corp; NEI
Subretinal AAV2/8-hCARp.hCNGB3 and AAV2/8-hG1.7p.coCNGA3 (follow-up)	*CNGB3* and *CNGA3*	ACHM2 and ACHM3	1/2	AE	Visual function; retinal function; QoL questionnaire	NCT03278873	MeiraGTx UK II Ltd; Syne Qua Non Limited; EMAS Pharma
Subretinal AAV2/5-hPDE6B	*PDE6B*	RP	1/2	Ocular and nonocular AE	Mobility test; VF; reading speed; NEI-VFQ-25	NCT03328130	Horama S.A.
Subretinal CPK850	*RLBP1*	RP	1/2	AE, SAE, deaths, DA	DA; static VF; contrast sensitivity; MP; multifocal and full-field ERG; reading speed; eye dominance; mobility test; NEI-VFQ-25; low-luminance questionnaire	NCT03374657	Novartis Pharmaceuticals
Subretinal rAAV2-VMD2-hMERTK	*MERTK*	RP	1	Ocular safety	ETDRS BCVA; FST	NCT01482195	Fowzan Alkuraya; King Khaled Eye Specialist Hospital; King Faisal Specialist Hospital & Research Center
Subretinal SAR421869	*MYO7A*	USH1B	1/2	AE	Visual function	NCT01505062	Sanofi
Antisense oligonucleotides							
Intravitreal QR-1123	*RHO*	RP	1/2	Ocular and nonocular AE	BCVA; low-luminance BCVA; DAC perimetry; static VF; MP, SD-OCT; FST; FAF; contrast sensitivity; color vision; serum levels of QR-1123	NCT04123626	ProQR Therapeutics
Intravitreal QR-110	*CEP290*	LCA	1/2 (Completed), phase 2/3 started	Ocular AE	Nonocular AE; ophthalmic examination findings; BCVA; infrared imaging; OCT; vital safety parameters; serum level parameters	NCT03140969; NCT03913130; NCT03913143	ProQR Therapeutics
Intravitreal QR-421a	*USH2A*	RP	1/2	Ocular and nonocular AE	DAC perimetry; static VF; EZ area/width; BCVA; low luminance BCVA; MP; ERG; FAF; serum levels/clearance/half-time	NCT03780257	ProQR Therapeutics
CRISPR-based genome editing medicine							
Subretinal AGN-151587	*CEP290*	LCA	1/2	AE; dose limiting toxicities	Maximum tolerated dose; mobility course score; BCVA; pupillary response; FST; macula thickness; contrast sensitivity; MP; color vision; QoL score; kinetic VF; gaze tracking	NCT03872479	Allergan; Editas Medicine, Inc.

Optogenetic treatment methods were not included in this table.

AAV indicates adeno-associated viral vector; ACHM, achromatopsia; AE, adverse events; BCVA, best-corrected visual acuity; DA, dark adaptation; DAC, dark-adapted chromatic; ERG, electroretinogram; ETDRS, Early Treatment of Diabetic Retinopathy Study; EZ, ellipsoid zone; FAF, fundus autofluorescence; FST, full-field light sensitivity threshold testing; IOP, intra-ocular pressure; LCA, leber congenital amaurosis; MLMT, multi-luminance mobility testing; MP, microperimetry; NEI, National Eye Institute; NEI-VFQ-25, National Eye Instittute-Visual function questionnaire 25 items; OCT, optical coherence tomography; QoL, quality of life; rAAV, recombinant adeno-associated viral vector; RD, retinal degeneration; RP, retinitis pigmentosa; RPGR, retinitis pigmentosa GTPase Regulator; SD-OCT, spectral domain optical coherence tomography; SAE, serious adverse events; STGD, Stargardt disease; UK, United Kingdom; USH, Usher Syndrome; VF, visual fields.

*As noted on clinicaltrials.gov. Accessed January 17th 2020.

†Oxford University Hospitals NHS Trust; Moorfields Eye Hospital NHS Foundation Trust, University College, London; Manchester University NHS Foundation trust; University of Manchester; University Hospital Southampton NHS Foundation Trust, University of Southampton, UK.

even rarer in these often relatively small patient populations. However, such information is crucial in determining the window of therapeutic opportunity, patient eligibility criteria, and clinical endpoints in ongoing and future trials to assess treatment efficacy.

This perspective focuses on the clinical characteristics of RDs and the different ongoing and emerging treatment options, focusing on RDs caused by mutations in the *CRB1* gene, the *RPGR* gene, the *CHM* gene, and the *LRAT* gene as an example.

Clinical Perspectives in Autosomal Recessive RDs

An autosomal recessive inheritance mode is observed in 50% to 60% of all RP cases,[2,8] and in most cases of Leber congenital amaurosis.[9] The most common form of macular dystrophy, Stargardt disease, is inherited in the autosomal recessive form.[10] After the success of subretinal gene therapy in *RPE65*-associated RDs, which has led to the market approval of Luxturna by the Food and Drug Administration,[11] preclinical and clinical (gene) therapy studies are ongoing for several genes responsible for autosomal recessive RD subtypes, such as *ABCA4*, *CNGA3*, *CNGB3*, *PDE6B*, *RLBP1*, *MERTK*, and *MYO7A*.[12]

Clinical Perspectives in CRB1-Associated RDs

Particularly interesting advances have been made in RDs caused by mutations in the *CRB1* gene,[6] which account for 3% to 9% of nonsyndromic cases of autosomal recessive RP,[13] and 7% to 17% of Leber congenital amaurosis (LCA) cases.[13,14] With the ongoing development of human *CRB1* gene therapy,[6,7,15] a detailed understanding of the phenotypic and genotypic characteristics of *CRB1*-associated RDs is essential. Until relatively recently, studies from literature had mostly been case reports, case series, or genetic studies with only brief descriptions of the clinical phenotype, providing limited detail.[16–30]

A recent Dutch retrospective cohort is the largest described to date, which allowed for statistical analysis and robust results on clinical signs and course of visual decline, which were further validated in a Belgian population, although the phenotypic and genotypic variability was higher in the Belgian population.[31] Furthermore, in the Belgian population, a larger proportion of patients had a more severe diagnosis of LCA or early-onset severe retinal dystrophy (EOSRD), as compared with the Dutch population, where most patients had RP. Although the classic RP features, such as optic disc pallor, vascular attenuation, and bone-spicule-like pigmentation, were commonly found in *CRB1*-RP, certain characteristics outline a specific and typical *CRB1*-associated phenotype, such as hyperopia, nanophthalmos, a shallow anterior chamber, peri-arteriolar preservation of the retinal pigment epithelium (RPE), optic disc drusen, and Coats'-like exudative vasculopathy.[24,25,29,31–42] Furthermore, these large studies highlighted the need to monitor this patient group for the risk of developing acute angle-closure glaucoma. In the Dutch cohort, optic disc drusen were found in the genetic isolate only, which prompted the suggestion of a potential genotype–phenotype correlation. However, in the Belgian cohort, optic disc drusen and hamartomas were found in patients with several different genotypes. Coats'-like exudative vasculopathy had been described before in *CRB1*-assocatied disease,[35,39,42–45] and the large studies in the cohorts described earlier have shown cohort-wide prevalence of these vasculopathies in 10% of Dutch RP-patients, and 13% of Belgian patients.

It should be noted that although these features together form a "typical" *CRB1*-associated phenotype, each feature may be found in other RD subtypes as well. Optic disc drusen have been described, for example, in Usher syndrome,[46] albeit to a much rarer degree, and hyperopia has been a classic feature of *BEST1*-associated phenotypes,[47,48] where it can also be associated with angle-closure glaucoma.[49] Mutations in *MFRP* are associated with RP along with nanophthalmos, optic disc drusen, and foveoschisis.[50–53] Aside from its association with *CRB1*,[16,31,54,55] an initial diagnosis of uveitishas has also been described in association with *PRPF31*,[54] *RP1*,[54] Stargardt disease,[56] and Usher syndrome.[54,57] Although the exact mechanism of uveitis in RP remains unknown, several explanations have been suggested for the association between uveitis and RP. Circulating immune complexes have been detected in 43.5% of patients in a study, along with reduced levels of complement C3 and C4.[58] A B-lymphocyte-mediated autoimmune response against retinal S-antigen, which is present in rod photoreceptors, has been shown in some RP patients, showing a low-level auto-immune responsiveness in RP.[59] An as of yet unidentified genetic or auto-immune factor—or a combination thereof—may play a role.

An interesting recurrent finding is the Coats'-like exudative vasculopathy, which has a strong association with *CRB1*. In one case, it has been described in an RP patient from a pedigree where an *RPGR* ORF15 mutation segregated with disease, and where no other genes were tested.[60] It has also been reported in a single case of *RHO*-associated RP, where no *CRB1* mutations were found.[61] Otherwise, it has not been associated with another RD gene, although it has regularly been described in genetically undifferentiated case reports or series,[62–64] particularly in older studies where genetic analysis had not been performed.[65] In some studies wherein the associated gene had not been identified, other features, such as perivascular retinal sparing,[62] or nanophthalmos,[66] point toward an association with *CRB1*. Vasoproliferative retinal lesions have been reported in association with Usher syndrome type I or II, based on the presence of RP and congenital hearing impairment, but not on genetic analysis.[67,68] Few other reports, again lacking genetic analysis, have found Coats'-like vasculopathy in RP thought to be X-linked or autosomal dominant, based on pedigree analysis.[69,70] The underlying mechanism of Coats'-like exudative vasculopathy includes an abnormal vascular permeability, which may be an element of *CRB1*-RDs. The CRB1 protein is crucial in the regulation of the number and size of Muller glia cells.[71] Since Muller cells function as regulators of the tightness of the blood-retinal barrier,[72] this may in some way relate to the vascular abnormalities seen in some patients with *CRB1*-RDs.

Another distinctive finding that we frequently observed in the Dutch and Belgian *CRB1*-RD patient cohorts, was thickening of the inner retina on spectral-domain optical coherence tomography (SD-OCT), which was in line with earlier reports,[23,24,32,34,39,43,73–75] although some other studies have reported retinal thinning.[20,37,76,77] Mouse studies have shown retinal thickening to be caused by proliferating retinal progenitor cells, resulting in an increase in the number of rod photoreceptors, Müller cells, and bipolar cells,[78] or by ectopic photoreceptors.[79] In both studies, the Crb2 protein has been postulated to play a role in the retinal thickening mechanism. Some other studies have suggested inner retinal thickening to be due to a remodeling process in association with loss of the outer nuclear layer,[80,81] which could hinder efficacy of gene therapy. However, we found no correlation between outer retinal thinning and inner retinal thickening.

A crucial aspect of the *CRB1*-associated phenotype, is the (dis)organization of the normal retinal layers (lamination), and the degree of preservation of the external limiting membrane, which is assumed to include the Crumbs (CRB) complex and thus is at least in part *CRB1* gene therapy's target. The CRB complex plays a role in the adhesion between photoreceptors and Müller cells, and also between photoreceptors.[82,83] Reports on the laminar structure have varied, with some describing loss of lamination,[19,23] and others reporting normal lamination.[27,84] Reasonably well-preserved lamination was a frequent observation in our cohorts, with 91% and 41% of the Dutch and Belgian patients with available SD-OCT scans, respectively.[31] This again confirmed a generally more severe phenotype in the Belgian *CRB1* cohort.

Findings of *CRB1*-associated disease do not only involve the retina, but also other ocular structures, pointing to a role of protein CRB1 in the ocular development, as has been suggested before in *BEST1*-associated disease.[48,85] In the retina, Crumbs proteins have a crucial role in the retinal vascular development,[86] in the photoreceptor-to-photoreceptor adhesion and photoreceptor-to-Müller cell adhesion.[79] Müller cells span throughout the entire neuroretina, from the inner limiting membrane to the external limiting membrane, and they are responsible for the structural stabilization of the retina.[87] They are essential for the survival of photoreceptors and neurons. Furthermore, they take up neurotransmitters, such as glutamate and *gamma*-Aminobutyric acid (GABA), and thus are involved in regulating the synaptic activity in the inner retina. As for the role of Crumbs proteins outside of the retina, current knowledge remains limited, and this role is suggested mostly by the clinical findings. In *Drosophila*, crumbs proteins are involved in the development and organization of epithelial cells.[88] Further research is necessary in mammalian eyes to elucidate the role of the Crumbs complex outside of the retina.

The only truly robust genotype–phenotype correlation that we were able to elucidate, is the link between the p.Ile167_Gly169del *CRB1* mutation, either in homozygous or compound heterozygous form, and an isolated maculopathy. This association was observed in both Dutch and Belgian populations, and has been described in British patients as well.[89] In fact, this mutation has been present in at least one allele in all patients with *CRB1*-associated maculopathy described so far, and also in cases of *CRB1*-associated foveal retinoschisis.[77]

A striking degree of interfamilial variability was observed in both cohorts, even in the Dutch genetic isolate, where most patients had RP with variable visual results, as some patients became blind at a relatively early age, whereas others maintained ambulatory vision well into the later decades of life, and 1 patient had a cone-rod dystrophy, with macular atrophy and barely any (mid-)peripheral retinal changes. Although interindividual variability in *CRB1*-associated RDs has been described,[18] the particularity here is that this variability occurred despite the same homozygous mutation, and the same origin from a village.[90–92] This may indicate the involvement of genetic and possibly environmental modifiers, which have been implicated before in several RD subtypes.[93,94] Although RDs are monogenic, the retina is a complex tissue involving numerous proteins to survive and function normally, that may influence the phenotypic outcome of monogenic diseases considerably.[95] Mouse studies may give direction on possible research on genetic modifiers in human *CRB1*-RDs.[96]

Clinical Perspectives in LRAT-Associated RDs

In light of emerging therapeutic options, few studies have also focused on an extremely rare RD subtype: *LRAT*-associated RDs. Having previously only been described in a small number of case reports or series,[14,30,97–100] *LRAT*-associated RDs are estimated to account for <1% of RD cases, usually exhibiting Leber congenital amaurosis, early-onset severe RD, or retinitis punctata albescens. A retrospective study in 13 patients—to our knowledge the largest series described in literature so far—has broadened the phenotypic spectrum, by describing a subset of patients with relatively preserved vision into mid- and late adulthood.[101] As this study consisted largely of patients from a genetic isolate, carrying the same homozygous c.12del *LRAT* mutation, it was able to elucidate the intrafamilial variability, with some patients carrying an RP phenotype and other a cone-rod dystrophy (CORD) phenotype. It also provided a comparison with one patient from outside the genetic isolate, who had an overall more severe phenotype of panretinal dysfunction. The specific mutation in the genetic isolate may be the most prominent cause of the relatively slow disease course in these patients, as opposed to the early blindness described in literature. However, this remains a suggestion, as the size of the cohort did not allow for sound statistical comparisons.

LRAT encodes protein lecithin:retinol acyltransferase (LRAT), one of the retinoid cycle proteins. Protein LRAT forms a complex with RPE65 to act as the isomerol hydrolase in the regeneration of visual pigment in the retinoid cycle. Having this closely connected biological function, both *LRAT*-RD and *RPE65*-RD have been targeted in a single treatment phase I trial investigating the safety and efficacy of oral QLT091001, a synthetic chromophore 11-cis-retinal.[102,103] This study enrolled patients with Leber congenital amaurosis due to mutations in *RPE65* or *LRAT*. Although mouse studies have shown phenotypic similarities between *Rpe65*(-/-) and *Lrat*(-/-) mice, human studies comparing these phenotypes are lacking. In one study, clinical findings in a retrospective cohort of patients with *LRAT*-RD and earlier literature in *LRAT*-RD patients were compared with the available literature on *RPE65*-RD, and this assessment demonstrated that there is a degree of phenotypic variability, with considerable overlap in this spectrum. To further substantiate any conclusion drawn from our study, a natural history study would ideally include extensive phenotyping of patients with *LRAT*-RDs and *RPE65*-RDs in the same study.

Clinical Perspectives in X-Linked RDs

X-linked RDs comprise a clinically heterogeneous group. X-linked RP, which accounts for 10% to 20% of RP cases,[2,8] has often been found to be more severe than most autosomal recessive or autosomal dominant forms of the disease. X-linked RP is caused by mutations in the *RPGR* gene in approximately 75% of cases, whereas most remaining cases are caused by *RP2* mutations.

Cone dystrophies (COD) and CORD may be inherited in all modes of Mendelian inheritance, but the autosomal recessive form is the most common. However, the underlying genetic cause is often unknown in cases of autosomal recessive inheritance,[104–106] whereas the most common genetic association with X-linked COD/CORD is known to be the *RPGR* gene.[107,108]

Another X-linked RD subtype that has been a focus of interest in the development of gene therapy is X-linked juvenile retinoschisis caused by mutations in the *RS1* gene.[109,110] This entity is characterized by a "spoke-wheel" pattern of retinal fluid collections in the macula. However, in 50% of patients, abnormalities occur in the periphery, such as schisis or neovascularization.

Clinical Perspectives in RPGR-Associated RDs

As several human gene therapy trials for *RPGR*-associated RP emerge (NCT03116113; NCT03252847; NCT 03316560),[111,112] recent studies have also focused on the clinical and genotypic characteristics of *RPGR*-associated RDs. A recurring finding in literature is the association between the ORF15 mutational hotspot and a COD or CORD phenotype, particularly if the mutation is located at the 3' end of ORF15.[107,113–115] Symptom onset was in the first decade of life in RP. In COD/CORD, the median age at symptom onset was 23 years, approximately 10 years later than some earlier reports of COD/CORD,[104] although reports have varied, some describing a much later symptom onset.[116] This study showed a particularly high variability in the age at symptom onset in COD/CORD, followed by rapid decline of visual acuity, and a probability of being blind, defined by the World Health Organization as a best-corrected visual acuity of <20/400, at the age of 40 of 55%, as opposed to 20% in RP patients. Cystoid macular edema, an otherwise relatively common finding in RP, was not observed at any point during follow-up in this cohort of *RPGR*-RD patients, in line with other studies of *RPGR*-RD.[116,117]

We previously described that intrafamilial variability was particularly apparent in 2 families that comprised patients of RP and CORD phenotypes within the same family.[113] A pivotal factor here is time, as in later disease stages, both RP and CORD progressed to panretinal dysfunction and became indistinguishable from each other in some cases. Still, this variability in the early disease stage is striking. An earlier report has even shown variability in a pair of dizygotic twins, one with RP and the other with CORD,[118] and in other siblingships.[119]

A prominent finding in all subtypes of *RPGR*-associated RD is myopia.[113,120,121] Mild, moderate, or high myopia was present in 84% of male *RPGR* patients and 73% of female carriers. Patients became more myopic with increasing age. The Dutch study in male patients showed high myopia to be an evident risk factor for visual acuity loss in all RD subtypes, and for visual field loss in RP. *RPGR* mutations have been shown to coincide with the highest degree of myopia in RDs,[47] and this study elucidated the quantitative effect of myopia on disease progression in *RPGR*-RDs. Other studies have followed, confirming the link between myopia and more severe retinal degeneration.[117] Refractive errors are not uncommon in RDs,[47] and in some cases, the location and function of the protein product have been postulated to explain the refractive error. Although mutations in some genes such as *RPGR* are associated with (high) myopia, other genes (eg, *CRB1* and *BEST1*) are associated with hyperopia. The protein retinitis pigmentosa GTPase regulator (RPGR) is located in the connecting cilium of the photoreceptor, the transport area between the inner and outer segment. Several genes that encode connecting cilium proteins have been linked to myopia, such as *RP1* and *RP2*, although this does not apply to all connecting cilium proteins.[122,123] A study on induced refractive errors in a chick model has implicated a range of photoreceptor-related proteins involved in the development of myopia or hyperopia, and these implicated proteins were primarily linked to photoreceptor dystrophies, such as CNGB1, RS1, RPE65, and RLBP1.[124] The study unfortunately did not shed light on RPGR, and further studies are needed.

An important consideration is the phenotypic spectrum in female carriers of *RPGR* mutations.[125] Like in affected males, myopia has a deleterious effect on visual acuity. In one large study, visual symptoms were relatively common, being present in 40% of subjects, and complete expression of a disease, that is, RP or CORD was found in 23% of subjects. Likewise, some earlier studies have identified *RPGR* mutations in disease-affected female patients,[121,126–129] some of whom were presumed to have sporadic or autosomal dominant RP.[130] This sheds light on multiple essential questions: Why do some female carriers develop disease and others don't, and can we predict either outcome? And what implications do these findings hold in the clinical and genetic counselling of female carriers? Regarding the first matter, random X-inactivation and variable mosaicism could account for the phenotypic variation observed among female individuals,[131] or skewed X-inactivation,[132,133] as the relatively family-based aggregation of affected female carriers makes random X-inactivation unlikely as a sole factor. In symptomatic female carriers of choroideremia, another X-linked RD, severely skewed X-inactivation has indeed been demonstrated.[134] Genetic modifiers may also play a role. It is not yet possible to predict which female carrier will develop disease, although the phenotype in other heterozygotes from the same family may be predictive.[125] Presence of the tapetal-like reflex does not hold predictive value, and is not associated with symptoms or pigmentary retinal changes. Regarding the counseling of female carriers of *RPGR* mutations, it is vital that clinicians convey the risk of developing disease, while acknowledging that a complete disease expression does not occur in most heterozygotes.

When looking at genotype–phenotype correlations, studies have found a robust correlation between *RPGR*-ORF15 mutations and the COD/CORD phenotype.[108,113,114,116] Mutations in the ORF15 region have been associated with a higher degree of myopia.[113] In Dutch male RP patients, an *RPGR*-ORF15 mutation signified a higher hazard (twice as high) of reaching low vision or severe visual impairment than a mutation in exon 1–14.[113,135] Also, *RPGR*-ORF15 mutations were associated with higher myopia, a significantly thinner central retina, and a significantly faster visual field decline. Previous literature, however, has shown the opposite finding of more severe disease in patients with mutation in exon 1–14 than those with mutations in *RPGR*-ORF15.[136,137] This discrepancy may be explained by the higher degree of clinical variability in patients with mutations in *RPGR*-ORF15,[125] which may lead to skewed findings in one population compared with the next. Peculiarly enough, in female heterozygotes, mutations in *RPGR*-ORF15 were associated with a less severe phenotype.[125] Previous literature on genotype–phenotype correlations in female subjects is limited, but has shown the opposite effect, with worse visual function in female subjects with *RPGR*-ORF15 than those with mutations in exon 1–14.[138] Genetic and/or environmental modifiers may again play a role in this clinical variability. Some proteins, such as RPGRIP1L and CEP290, have been shown to biochemically interact with RPGR,[94,137] but additional research on such potential modifiers is needed.

Clinical Perspectives in Choroideremia

Choroideremia is a rare X-linked RD caused by mutations in the *CHM* gene. It has been proposed that this dystrophy primarily affects the retinal pigment epithelium, and secondarily the photoreceptors and choroid.[139] Advances in gene therapy have resulted in multiple human gene therapy trials worldwide (Table 1), which have recently reached phase III.[4,140–144] These advances prompted several studies on the associated phenotype and long-term clinical course. Symptoms of choroideremia are usually noticed in the 1st or 2nd decade of life, and the degeneration usually starts in the midperiphery, after which it gradually extends centripetally toward the periphery and the fovea.[145–148] Prolonged relative sparing of foveal structure and function accounts for the long-term preservation of visual acuity, which usually remains until the 5th decade of life. This striking feature of foveal sparing, which is also typical for eg, late-onset Stargardt disease and central areolar choroidal dystrophy,[149,150] remains of unknown etiology. One study investigated the kinetics of the progression of macular atrophy in several macular diseases, and found nearly identical patterns across several RD subtypes and age-related macula degeneration, suggesting a disease-independent mechanism.[150] One proposed mechanism has been the metabolic difference between different macular regions in susceptibility to atrophy of rods and cones, RPE, and choroid.[151] One longitudinal clinical study in a cohort of choroideremia patients showed a stable plateau of good vision until the 5th decade of life, and generally a turning point in the visual acuity decline from the 4th decade of life onwards.[145] Outer retinal tubulations, which have been associated with age-related macular degeneration and various other degenerative conditions,[152] were found in the majority of choroideremia patients (69%–94%) in several studies,[145,153–157] although smaller numbers have been reported as well.[158] They have also been demonstrated in symptomatic carriers, where they colocalized with areas of RPE atrophy and severe hypo-autofluorescence.[134] Outer retinal tubulations presumably result from the rearrangement of degenerating photoreceptors,[159] and areas containing them may be prone to surgical complications, such as macular hole formation as a result of subretinal injection of gene therapy vector solution. Moreover, areas containing outer retinal tubulations in choroideremia patients reportedly lack visual sensitivity despite the concomitant presence of viable cone inner segments in that same area.[158] This may guide in assessing which retinal areas of a particular patient are amenable to (gene) therapy. Choroideremia patients in general are at risk of developing a macular hole, the surgery of which seems to be effective in achieving anatomic closure.[160,161] No clear genotype–phenotype correlations have been established in choroideremia.[162]

Clinical Perspectives in Autosomal Dominant RDs

Autosomal dominant RDs, which are caused by mutations in the *rhodopsin* (*RHO*) gene in up to 30% to 40% of cases,[163] present another challenge for gene therapy development. After all, the dominant disease is usually the result of a deleterious “gain-of-function” mechanism, for example, where the altered gene product adversely affects the normal gene product from the wild-type allele. Mere gene supplementation would not suffice in slowing the disease process that is caused by a toxic gain-of-function mutant protein, and the gain-of-function effect leading to disease would have to be diminished. For *RHO*-associated RP, momentous advances have been made, with knockdown-and-replacement strategies,[164] CRISPR/Cas9 gene editing,[165,166] and antisense oligonucleotides.[167] “Simple” gene augmentation could still provide some therapeutic benefit in *RHO*-associated RP, even when the disease is caused by a dominant-negative effect.[168]

Considering these advances, several studies aimed to establish a detailed clinical profile and natural history in a large cohort of patients with *RHO*-associated RP. One study in Dutch and Belgian patients found an appreciable difference in disease progression between patients with sectorial RP (25% of our cohort) and those with generalized RP, as visual acuity decline was relatively stationary in the sectorial form, with the first case of blindness occurring after the 8th decade of life (*manuscript accepted*). In previous literature, an initial sectorial RP phenotype has relatively rarely been reported to progress to the generalized form.[169] In the study of Dutch and Belgian patients, no patients were found with sectorial RP whose phenotype progressed to generalized RP, although this study was restricted by the limited availability of follow-up full-field fundus photographs in its retrospective study design.

In the Dutch and Belgian *RHO*-RP populations (sectorial and generalized forms), best-corrected visual acuity generally remained well-preserved, with a median age of reaching mild visual impairment of 72 years (*manuscript accepted*). Based on visual fields, the median ages to reaching low vision and blindness were 52 and 79 years, respectively. This is in line with previous studies that have shown that *RHO*-associated RP is a slowly progressive disease where patients generally maintain a good central visual function,[170–173] as opposed to, for example, *RPGR*-associated or *CRB1*-associated RP. This points to a particularly lengthy window of therapeutic opportunity for ongoing and future (gene) therapy trials. On the contrary, the slow disease progression may complicate ways to clearly show a potential treatment effect. Some studies have referred to the sectorial disease phenotype as a “class B” phenotype, defined by an altitudinal (hemifield) loss of photoreceptor function.[170,174,175] The degree of light exposure of the retina has been suggested to play a role in the retinal degeneration, and has been hypothesized due to the altitudinal degeneration mostly affecting the inferior retinal hemisphere.[169,176,177] In support of this theory, animals with *RHO*-RP, including the RHO^{P23H}mouse and rat,[178,179] and the RHO^{T4R} dog,[180] that have been reared in complete darkness, have shown slower retinal degeneration. Mice that remain in red-tinted cages that filter short-wavelength light (<600 nm) have been shown to maintain a thicker photoreceptor layer and higher amplitudes of electroretinography responses than mice in non-tinted cages.[181] However, this effect has not been proven in humans, and would be challenging to prove in a clinical trial setting. Thus, it remains a controversial claim.

Over 150 mutations have been reported in *RHO*. Several studies have been performed in the $Rho^{P23H/+}$ mouse, as the p.Pro23His mutation is historically the first *RHO* mutation discovered, and one of the most common mutations in patients in the United States of America.[182] To our knowledge, this mutation has not been reported in European studies, including ours. In our Dutch cohort, 37% of patients had the p.Glu181Lys mutation. With regard to genotype-phenotype correlations, several studies found an association between the mild or sectorial RP form, and mutations that correspond to the extracellular domain (ie, the intradiscal domain),[174,175] particularly in comparison with mutations in the transmembrane domain.[183] However, the study in Dutch and Belgian patients still found variation in the phenotype, for example, sectorial versus generalized RP, in patients with identical genotypes, such as the p.Glu181Lys mutation. Conversely, mild and sectorial phenotypes have been reported in association with mutations in other domains.[184,185] Extreme intrafamilial variability in the *RHO*-RP phenotype has been reported.[186] In fact, both RP and congenital stationary night blindness have been reported in the same family carrying the c.337G>A (p.Glu113Lys) mutation,[187] which was not present in the Dutch and Belgian patients cohorts.

Clinical Heterogeneity and Potential Modifiers in RDs

A recurring finding in nearly all RD subtypes, including the ones studied in this thesis, is clinical *heterogeneity*. The same gene or even the same mutation may cause different phenotypes, and a nearly identical phenotype may be caused by different genes. The presence of a genetic isolate in a Dutch cohort of *CRB1*-RDs,[31,32,91] consisting of patients carrying the same homozygous p.Met1041Thr mutation, provided the opportunity to investigate not only genotype-phenotype correlations, but also the intrafamilial variability. Although the phenotype was generally severe, and some hallmark features of *CRB1*-RDs were elucidated, one 41-year old patient had a mild CORD phenotype, whereas age-matched relatives had advanced RP. Even more variability was observed in the Dutch cohort of *LRAT*-RDs, again consisting largely of a genetic isolate. Similarly, in 2 families of *RPGR*-RD, some had RP, whereas others had CORD. This last finding should be nuanced by the idea that different RDs may not be entirely different entities, but members of a continuum. Advanced stages of CORD may be indistinguishable from RP, and it may prove difficult to retrieve early medical records in a retrospective setting. Nonetheless, variable degrees of intrafamilial variability were evident in several cohorts,[31,32,101] and patients at roughly similar ages may still have different phenotypes (CORD or RP).[113] Environmental and genetic modifiers, such as heterozygous mutations in other RD genes or single nucleotide polymorphisms, may have a role, and may influence the degree of severity. In conclusion, although RDs are typically monogenic diseases, and rare cases of putative digenic inheritance have been reported[188,189] or suggested,[190] the retina and RPE are complex tissues, whose survival and function depend on the proteins encoded by >18,000 genes for each tissue.[95]

Current Patient Management

Before the advances made in gene therapy studies in this millennium, the management of RD patients consisted of the regular follow-up and monitoring of disease progression, genetic and prenatal counselling, low vision aids where needed, and potential enrollment in a clinical trial. For patients who are blind due to outer retinal degeneration, but have maintained the inner retinal structure and an intact optic nerve, 2 retinal prostheses, the Argus II epiretinal prosthesis system, and the Alpha IMS (first generation) and Alpha AMS (second generation) subretinal prostheses, may aid in gaining some mobility or performing specific daily tasks. However, they require careful preoperative screening and expectation management, counseling, and a comprehensive postoperative rehabilitation program at a specialized center.[191,192] The 2 most studied epiretinal implants, the Argus II and alpha-IMS/AMS, have shown performance results that can overall be considered similar, despite large differences in implant design.[193] While most patients with a retinal prosthesis show an improvement in mobility and orientation tasks, approximately one-third experiences measurable visual acuity improvement.[194] Reading speed can be improved in a subset of patients, although single-letter recognition may still take up to several minutes.[195] Preoperative counseling should comprise the advice that the output from the prosthesis is an entirely new type of functional vision rather than the recovery of previous vision.[196] Due to the guarded benefit, and the frequent visits and intensive rehabilitation required to achieve it, patient selection and expectation management are key.

The recent approval of voretigene neparvovec (Luxturna), a prescription gene therapy for *RPE65*-RD, has marked the dawn of a new era: the availability of an RD treatment to preserve and improve retinal function. However, for other RD forms, therapeutic options, if applicable, are being investigated in a clinical trial setting, or are in an earlier preclinical investigative phase.

Associated ocular conditions, such as cystoid macular edema (CME), should be monitored for development and treated. CME has been treated with different modalities. Topical and oral carbonic anhydrase inhibitors have shown morphological improvement with reduction of the CME,[197] although the effect on visual acuity has been inconsistent between studies and remains inconclusive.[198–202] One study has found that CME in the outer nuclear layer showed a better response to treatment with topical or oral carbonic anhydrase inhibitors than CME in the inner nuclear layer, where CME in RD is commonly found.[203] An intravitreal dexamethasone implant (Ozurdex) has shown improvement of visual acuity and edema resolution,[197,204] whereas intravitreal triamcinolone acetonide showed anatomical improvement without improvement in visual acuity.[205,206] When using steroids, the development of cataract, and perhaps more importantly, elevation of intraocular pressure should be closely monitored in these patients, who are at an increased risk of developing both.[31] Intravitreal injection of anti-vascular endothelial growth factor (VEGF) has shown inconsistent results with resolution of CME in some studies,[207,208] and no effect in other studies.[209] No evident visual acuity improvement was established with the use of anti-VEGFs.[208] Intravenous immunoglobulin therapy has been reported in the treatment of concomitant CME and uveitis in 1 patient, and has shown complete resolution of CME at 4 months and 1 year.[210] Octreotide has been postulated to have a role in the treatment of uveitis-associated CME,[211] and has been successful in reducing CME and stabilizing visual acuity in dominant cystoid macular dystrophy.[212] Its effect on CME in retinitis pigmentosa has not been reported to date.

Treatment options for Coats'-like exudative vasculopathy have included laser photocoagulation or cryotherapy. This can lead to regression of the exudates and to improved or stabilized vision,[60,64,69,213,214] but it has also been complicated by a vitreous hemorrhage requiring vitrectomy.[64] In the case of an exudative retinal detachment, treatment with vitrectomy and endolaser has been described, with the aim of salvaging the eye and maintaining any remaining vision.[70,215] More recently, the intravitreal injection of conbercept, a new anti-VEGF, has been described in RP patients with exudative retinal detachment due to Coats'-like exudative vasculopathy.[216] This led to complete resolution of the subfoveal serous detachment and improvement of the visual acuity. In a patient with *RHO*-associated RP and Coats'-like exudation, along with treatment-resistant CME, the intravitreal injection of a dexamethasone implant (Ozurdex) led to resolution of the exudation, along with a reduction in the CME, and maintenance of a well-preserved visual acuity.[61] All these case reports appear to meager to establish a clear guideline for the treatment of CME in the context of RDs.

Implications of Natural History Studies for Gene Therapy Trials

The findings in this thesis have several implications for ongoing and future gene therapy trials. Crucial factors in the design of a (gene) therapy trial, are the determination of:

a) a window of therapeutic opportunity
b) patient eligibility criteria
c) disease symmetry between eyes and the suitability of the contralateral eye as the untreated control; and
d) defining endpoints for the evaluation of clinical efficacy.

Window of Opportunity

The window of therapeutic opportunity refers to the time span within which potential treatments may still prevent disease or positively modify the natural history. As gene therapy uses viral vectors that need to infect viable retinal cells, the window of opportunity closes when no viable photoreceptors remain, and no useful vision remains to be rescued. In a trial setting, the therapy is ideally applied in an early or intermediate disease stage, when enough vision remains to be rescued, and the natural disease progression is fast enough for a therapeutic effect to be detected, that is, a change in the rate of disease progression. However, in treatment settings, intervening as early as possible in the disease course may provide the best protective effect. In the Dutch cohort of patients with *CRB1*-RP, the median ages for reaching visual acuity-based low vision, severe visual impairment, and blindness were 18, 32, and 44 years, respectively. Thus, the window of therapeutic opportunity spans the first 3 decades of life, and could be expanded in some patients to the fourth decade of life. In *CRB1*-LCA or EOSRD, intervention would ideally be much earlier, within the first decade of life, as any remaining useful vision usually degenerates in this period. In contrast, the window of opportunity is considerably broader in patients with *RHO*-RP. In *RPGR*-RDs, the window of opportunity depends on the phenotype intended to treat in the trial: patients with COD/CORD have a 55% likelihood of being blind at the age of 40, as opposed to 20% in patients with RP. Patients with mutations in the ORF15 region had a higher risk of becoming blind at an earlier age, and would thus also require earlier therapeutic intervention, according to our study. In Dutch patients with *LRAT*-associated RDs, the window of therapeutic opportunity may be particularly broad as well.[101]

In *RPGR*- and *RHO*-associated RDs, the presence of a hyperautofluorescent ring may aid in determining which retinal area is most likely to benefit from a subretinal gene therapy injection, as this ring signifies the transitional zone between degenerated retina and relatively preserved—and thus rescuable—retina. In *RPGR*-RP, this ring was present in 47% of patients with *RPGR*-RP and 71% of patients with *RPGR*-COD/CORD. Although the ring provides useful information on the location of the transitional zone between atrophic and relatively preserved retina, it is unknown whether it has additional value in determining the likelihood of benefit from therapeutic intervention.

Patient Eligibility Criteria

Patient eligibility criteria for inclusion in a future trial are largely dependent on the window of therapeutic opportunity, and thus the patient age and remaining visual function. The presence of CME may render the macula more susceptible to the formation of a secondary macular hole, when subretinal injection of a viral vector in gene therapy increases the retinal stretching.[217] Even if such a complication would not occur, the natural fluctuation in the extent of CME and the visual acuity may confound any potential therapeutic effect. On the contrary, successful gene augmentation via gene therapy may also have a beneficial effect on the resolution of CME. Patients with *CRB1*-RDs should be assessed for the risk of developing acute angle-closure glaucoma, and a prophylactic peripheral iridotomy or, if appropriate, cataract extraction may be warranted to reduce this risk before enrollment in a clinical trial that requires frequent mydriasis.

An extremely important point for consideration is the a priori amenability of the retina to (gene) therapy. A point of concern, particularly in some patients with *CRB1*-RD, would be the retinal disorganization, which would indicate a limited availability of viable cells for the viral vector to infect and/or the inability for the gene to function due to structural disintegration. Therefore, the degree of laminar disorganization was an area of focus in our retrospective and prospective studies. In the baseline report of our prospective study, the retinal laminar organization was preserved in 24% and showed only mild coarsening without disorganization in 38% of patients, indicating an amenability of the retina for gene therapy in 64% of patients. In the other 38% of patients, the retinal laminar organization was relatively disorganized, indicating a decreased amenability.

In choroideremia, the lengthy preservation of central visual function and foveal sparing affords a broad window of therapeutic opportunity for gene therapy.[4,145,148] Outer retinal tubulations, when present, may provide clues of areas retaining viable photoreceptors and remaining visual function, as they have been found to be present around areas of surviving retina.[145] Full-thickness macular holes have sporadically been described in choroideremia,[160,161] and although successful closure may be achieved surgically, these patients may be at a higher risk of iatrogenic damage in a gene therapeutic setting.

Gene therapy trials for male patients with *RPGR*-associated RP may take the additional detrimental effect of the associated high myopia into consideration when assessing patient eligibility and when interpreting safety and efficacy data, as high myopia is associated with worse visual function and a thinner retina.[113] High myopia may thus be a complicating factor in the rescue of the remaining photoreceptors.

Gene therapy trials for *RPGR* may consider enrolling affected female heterozygotes in future trial phases, as women may express a full disease phenotype. Most female heterozygotes are mildly affected or asymptomatic, and treatment in these patients may not be necessary.

The usefulness of gene therapy is impeded in cases of extensive atrophy of the photoreceptors, RPE, and choriocapillaris. In these patients, stem cell-based therapeutic options, which are not gene-specific, may provide more benefit. Examples include the intravitreal or subretinal administration of induced pluripotent stem cells or retinal progenitor cells.[218] These studies are in the early stages: one phase 1/2 clinical trial on human embryonic stem cell-derived RPE cells has been completed in age-related macular degeneration and Stargardt disease,[219] and has shown an acceptable safety profile and possible improvement in visual function. Clinical trials using induced pluripotent stem cells are expected, but are yet to be initiated. In patients with advanced atrophy, stem cells may need to differentiate into multiple cell types, such as RPE and choriocapillaris, and this may require multiple injections with each treatment session. The injected cells then have to successfully convert into each mature and functional cell structure individually, and organize into a structurally and functionally intact unit. Although these challenges complicate the treatment options for these patients, in vitro and in vivo studies have shown some promising results.[220,221]

So far, subretinal gene augmentation therapy trials have treated the posterior pole/macular region,[3,4,222,223] whereas patients with RP or choroideremia may experience visual field constriction as a major problem. Indeed, 1 study has surveyed patient-reported visual complaints and their effects on daily life, and has found that most choroideremia patients (70%) reported peripheral visual field constriction as the most debilitating symptom.[145] In these patients, expectation management before enrollment in a clinical gene therapy trial is crucial, as the peripheral rods responsible for the visual field are not targeted through conventional subretinal gene therapy. Intravitreal gene therapy administration may theoretically provide a better outcome in the peripheral visual function these patients, although it currently holds a higher risk of inflammation and systemic biodistribution,[224–226] and a lower degree of efficacy than subretinal administration in the eyes of primates.[227] Should intravitreal gene therapy administration develop a better profile in the future, intervention would ideally happen at a much earlier stage, as rods degenerate already in the earlier disease stages, while central cone function and visual acuity may remain preserved for many years.

Interocular Symmetry

As most retinal (gene) therapy studies have treated one eye, usually the worse-seeing eye, inter-eye symmetry within the same patient is an important aspect. Interocular symmetry enables the use of the contralateral eye as an ideal untreated control. A high degree of inter-eye symmetry has been confirmed in several RD subtypes of interest for ongoing and future gene- and cell-based therapy trials.[31,32,101,113,148,170,173,228–231] Interocular symmetry or lack thereof should be determined before enrollment in an interventional trial, and investigators should aim to identify a potential cause of significant asymmetry, if this can be determined.

Defining Endpoints for Evaluation of Treatment Efficacy

For many RD subtypes, it has proven to be challenging to define clinical endpoints for the evaluation of treatment efficacy. A thorough understanding and quantification of important parameters in the natural disease course is crucial, as this may help define the most appropriate efficacy endpoints. Using the appropriate endpoint may be pivotal in the process of for market-approval of gene therapy by regulatory bodies. To be an expeditious efficacy endpoint for a treatment aimed at slowing disease progression, a parameter would have to be expected to show significant decline within the clinical trial period, and a faster decline than any expected test–retest variability. Visual acuity, a measure of central cone function, usually shows significant decline over several decades of life, but may remain relatively stable over the course of a few years, whereas the duration of a clinical treatment trial is usually not much longer than 2 years. Visual acuity survival curves in *CRB1*-RP in the Dutch cohort have shown a relative plateau during the second decade of life. Meanwhile, the visual acuity decline rate was 0.03 logMAR per year, corresponding to 7.2% per year. Similar rates were demonstrated in the decline of the visual field area. To calculate how long a trial should last in order for a true treatment effect to be detected, test–retest variability in the visual function values should be determined in the study population. The estimated time needed to detect a significant change may be longer than the trial period in most patients, but longitudinal prospective studies must further investigate this. In patients with *RPGR*-RP, visual acuity did not show any significant decline before the age of 20 years in one study,[113] indicating that in these young patients, visual acuity is not a sensitive marker for change. However, it would be a judicious safety marker, as any significant visual acuity decline may be an indicator of iatrogenic damage to the retina.

Several studies have indicated that the ellipsoid zone width and ellipsoid zone area on SD-OCT may be sensitive biomarkers for disease progression,[229,232] even within a time span of 2 years of follow-up.[233–235] In our study of *RHO*-RP, we found similar results for ellipsoid zone width. Several challenges accompany this particular biomarker: while this biomarker appears to be useful for instance in *RHO*-RP or *RPGR*-RDs, in *CRB1*-RDs, the ellipsoid zone disintegration will probably be at a too advanced stage to be able to sensitively detect a significant change in decline rate. Moreover, regulatory bodies such as the US Food and Drug Administration (FDA) and the European Medicines Agency, have not yet approved structural biomarkers as defining parameters for the approval of a therapy for retinal disease.[236] For such structural biomarkers to serve as surrogate endpoints, their reliability, and their strong correlation to direct measures of the patient's visual function (eg, visual acuity), should be established. In a study on *CRB1*-RP (*manuscript under review*), the ellipsoid zone width did not maintain its significant correlation with visual acuity after correction for multiple testing. The thickness of the photoreceptor and RPE complex (ie, as measured from the external limiting membrane to the RPE at the fovea), however, did correlate with visual acuity. Its rate of decline (−0.6%/year), however, was much slower than that of the ellipsoid zone band width (−3.8%/year), which means that the expected time needed to detect a treatment effect is much longer.

Looking back at the *RPE65* gene therapy trial that led to market approval of Luxturna, useful endpoints have included the full-field stimulus testing,[237] which we have also employed in our prospective natural history study of *CRB1*-RD. Full-field stimulus testing is a psychophysical measure to determine the maximum retinal sensitivity in the full field, and chromatic stimuli can be added to determine whether this sensitivity is rod-mediated, cone-mediated, or mediated by a combination of the two.[238,239] It may be employed in patients with nondetectable dark-adapted and light-adapted responses on the electroretinogram, and is therefore particularly helpful in patients who are (nearly) blind. Another useful endpoint in studies leading to marked approval of voretigene neparvovec (Luxturna) was the multi-luminance mobility test. This is a navigation course, wherein patients must maneuver past obstacles at different levels of environmental illumination, ranging from 1 lux (a moonless night) to 400 lux (a brightly lit office). It provides a reliable measure of functional vision, that is meaningful with regard to the patient's daily life. Although an impractical measure in natural history studies, it has proven useful in interventional trials, and its validity has been demonstrated in a nontrial setting.[240] Other mobility courses and artificial platforms for mobility and for the simulation of daily activities have been developed, such as The StreetLab and HomeLab platforms designed by the Institut de la Vision (Paris).[241]

In gene therapy trials, primary outcome measures should ideally focus not only on the objective improvement in visual acuity and other visual and structural parameters, but also on the efficacy of treatments to significantly improve parameters that are important of patients' daily lives, such as level of independence, quality of life, and other patient-reported outcomes. This is challenging, as no standardized questionnaires have been established for such quality of life and social functioning aspects for this specific population with severe visual impairment due to RDs.

Emerging Therapies and Future Perspectives

Before the emergence of gene therapeutic trials, no evidence-based treatment options existed for RDs that led to a clinically measurable improvement in visual function. The development of therapies for rare diseases has historically been challenging due to small patient populations for trials, and the challenges in post-approval marketing.

The great advances in gene therapy in the last 2 decades have led to market approval of voretigene neparvovec (Luxturna) subretinal gene therapy for *RPE65*-associated early-onset RD/Leber congenital amaurosis. This success, along with other advances in gene therapy development,[6] has led to a spectacular expansion in the field of retinal gene therapy. Subretinal gene therapy is under development for *CRB1*-RDs,[6] and clinical trials ongoing for *RPGR*-associated RP, choroideremia, achromatopsia (associated with *CNGB3* and *CNGA3*), Stargardt disease (associated with *ABCA4*), X-linked retinoschisis (associated with *RS1*), and several other entities (Table 1), are in the pipeline.[6,164]

Gene Replacement and Gene Silencing

Gene transfer to the target cells in the retina may happen through viral vectors, mostly adenoviruses, lentiviruses, or adeno-associated viruses (AAV), the latter representing the most efficient and stable gene transfer in most RD forms.[11,15] AAV vectors are currently the most used viral vectors in gene therapy, due to the extensive experience with AAV, and their excellent safety profile: in the retina, the risk for immunogenicity is low,[242] and they have low inflammatory and low retinal toxicity potential.[242,243] Furthermore, they do not integrate their genome into the host-cell genome,[243] thus eliminating the risk of iatrogenic activation of oncogenes. Virtually all AAV serotypes are able to infect the RPE, and serotypes 2, 5, and 7 to 9 are able to infect photoreceptors.[244] Drawbacks of AAV vectors include their small size, which leads to a limited transgene capacity of 4.5 to 5.0 kb. In contrast, the larger lentivirus vectors have a transgene capacity of up to 10 kb.[245] However, they integrate their genome into the host-cell genome with great efficiency, although it has been shown that they do not preferentially integrate their genome in the vicinity of oncogenes.[246] Although the potential of viral vectors has been demonstrated repeatedly, nonviral gene delivery systems have been investigated as well. These transfer methods, using for instance nanoparticles, liposomes, or naked plasmid DNA, are cheaper and easier to produce, and have a lower risk of inducing an immune response. However, as of yet, they have not shown promising potential for safe gene delivery, due to, for example, lack of persistent transgene expression (naked DNA and nanoparticles), or the potential for retinal toxicity (liposomes).[247,248]

Although gene replacement or supplementation should be sufficient in autosomal recessive RDs, in which a lack of gene expression leads to a deficit in the gene product, (additional) gene silencing is necessary in autosomal dominant RDs. In autosomal dominant RPs, the gene mutations often lead to mutant gene expression resulting in altered protein products that impair normal function of the wild-type protein, leading to a toxic effect. In such cases, gene therapy is aimed at repairing or silencing the mutated gene, and gene supplementation in the case of additional haploinsufficiency.

Such gene silencing has been proposed through the use of allele-specific inhibitors that induce the degeneration of the mutated messenger RNA (mRNA).[249] Another approach is the suppression of both the mutated and wild-type allele, and their replacement by a wild-type nonsilenced allele.[250] Both strategies can be mediated for instance by small RNA inhibitors or ribozymes,[251–253] each with their own set of advantages and disadvantages,[254] such as a need for repeated injections.

Antisense Oligonucleotides

Antisense oligonucleotides (AONs) consist of small DNA or RNA molecules that are able to modulate splicing after binding to pre-mRNA. Preclinical studies using, for example, fibroblasts from affected patients, and animal studies have shown promising results for *CEP290*-LCA,[255] and for *RHO*-RP.[167] AONs can be administered "naked" through intravitreal injections, or through subretinal injections with an adenoviral-associated viral vector, and have shown minimal toxic or immunological adverse effects.[256] As naked AONs are small-sized molecules, they may be able to reach their destination cells, the photoreceptors, more easily after intravitreal injections. This approach would require repeated injections throughout life, whereas a subretinal injection of an AAV-mediated AON may give a considerably more durable therapeutic benefit. However, intravitreal AONs target the entire retina, and the need for a vitrectomy and its associated complications is circumvented. A recent phase 1/2 trial investigating the effect of intravitreal AONs in the treatment of 10 patients with *CEP290*-associated LCA found no serious adverse events, and a clinically meaningful improvement in vision, defined in the study as 0.3 logMAR, in 5 patients.[257] These encouraging results are followed up in a phase 2/3 trial, the ILLUMINATE study (NCT03913143).

Gene Editing: CRISPR/Cas9

An exciting potential alternative to gene replacement strategies is the therapeutic approach of gene editing. In gene editing, the genome can be altered by inducing double-stranded DNA breaks, single-stranded DNA breaks, or specific base changes in the DNA at target sites to correct the deleterious gene mutation. This can be achieved using several methods, such as zinc finger nucleases, meganucleases, and, more recently, clustered regularly interspaced short palindromic repeats CRISPR-Cas9-associated protein 9 (Cas9).[258] CRISPR/Cas9 gene editing is a fast, cheap, and relatively efficient method to edit the genome and repair genetic mutations, typically by inducing double-stranded breaks.

CRISPR is guided by RNA sequences, and multiple guide RNA sequences may be packaged into one targeted delivery system (eg, a viral vector). Thereby, CRISPR has the unique ability to target >1 genetic location.[259]

CRISPR/Cas9-based therapies have been used successfully in mouse models, for instance *PDE6B*,[260] *CEP290*,[261] and *RHO*.[165,262] In mouse models of *RHO*-RP, CRISPR/Cas9 has been used in a mutation-independent “ablate-and-replace” technique. Moreover, CRISPR/Cas9 has been used to generate accurate mouse models for RP and LCA.[263,264]

Drawbacks of the CRISPR/Cas9 gene editing system include concerns on its accuracy and the potential of off-target effects.[265] Additionally, its efficiency may vary. In induced pluripotent stem cells of a patient with *RPGR*-RP, CRISPR-Cas9 was applied to correct the gene mutation and convert it to the wild-type allele.[266] This succeeded in 13% of *RPGR* gene copies, which still spectacularly exceeds previous gene correction rates of 1% to 3%.[267] Furthermore, it is a large-sized system that cannot be packaged into a single viral vector, and typically a dual vector system is employed.[268]

The challenges associated with the CRISPR/Cas9 approach have driven the exploration of alternative precision gene editing approaches. One such approach is the recently published prime editing strategy,[269] which can alter DNA with single-nucleotide precision, potentially with greater safety, and with great versatility. It combines Cas9-mediated RNA-guided DNA breakage (or nicking) with reverse transcriptase-mediated DNA synthesis at the same target site. Different types of mutations, including insertions and deletions, can be corrected. It has been proposed that it can correct up to 89% of pathogenic human variants that have been described in the ClinVar archive of genetic variants in any part of the genome, which spectacularly broadens the range of mutations that can be corrected. The promising results of prime editing, as demonstrated in vitro, remain to be investigated in vivo.

Stem Cell-Based Strategies

When retinal cells have already died, genetic therapies to correct mutated genes in the affected target cells appear useless as an isolated therapeutic approach. In these cases, replacement of these dead cells by new functional cells may prove to be a viable future treatment option.[220,270] Human embryonic stem cells have been investigated as a treatment for several retinal disorders, and have shown some visual improvement in RD rat models.[271,272] In humans, a phase 1/2 trial transplanting human embryonic stem cells to the subretinal space in patients with Stargardt disease or atrophic age-related macular degeneration has shown some modest visual improvement in more than half of the treated eyes.[219] However, the use of human embryonic stem cells as a therapy has raised ethical concerns, and concerns over immunological responses and/or the need for immunosuppression.

Fibroblast-derived induced pluripotent stem cells (iPSCs) are derived from the patient, and have been used in the treatment of several mouse and rat models of RD, where they have led to potential preservation of the visual function.[273,274] Concerns regarding the use of iPSCs as a treatment modality include immunogenicity,[275] and tumor formation due to incompletely differentiated iPSCs.[276] A safer and particularly exciting application of iPSCs has been in the generation of retinal organoids,[277] where they aid in the examination of underlying disease mechanism and in the in-vitro study of treatment options, such as in *CRB1*-RDs.[15] In autologous iPSC-based cultured retinal cells of patients with RDs, the genetic defect may be corrected in vitro, using, for instance, AAV-based gene replacement or gene editing techniques,[221] in preparation for subretinal administration. A key challenge may be not only to achieve a correct anatomical integration of such stem cells into the retina after surgical administration, but certainly also subsequent cellular function and interaction, leading to genuine functional improvement that matters to the patients.[220,270,278]Another important aspect when considering cell transplantation for advanced RD is the fact that such cases do not only have photoreceptor atrophy, but also atrophy of the photoreceptor’s “nursing cells,” the RPE, and choriocapillaris. After all, the photoreceptor-RPE-Bruch membrane-choriocapillaris interface normally forms a closely connected and interdependent functional unit. This means that administration of such a combination of cells, possibly using cell sheets and/or a cell-carrying scaffold, may be mandatory to achieve a (close to) normal cellular interaction for a durable and functionally relevant treatment effect.

Bone-marrow-derived mesenchymal stem cells have been used in intravitreal injections in phase I clinical trials for RD patients, and in commercial “stem cell clinics” in the United States, where resulting vision-threatening complications, such as vitreous hemorrhage and rhegmatogenous retinal detachment, and blindness have been reported in patients with RD and with age-related macular degeneration.[279,280]

Optogenetics

In patients who are blind due to photoreceptor degeneration while still retaining a relatively intact inner retina, optogenetics may be a tool to reintroduce light perception. Optogenetics is a strategy whereby a gene encoding a photosensitive protein (an opsin) is introduced in inner retinal cells, that is, retinal ganglion cells and bipolar cells, with the aim of sensitizing these inner retinal cells to light in the absence of photoreceptors.[281] It thus provides an alternative visual cycle to improve retinal activity. Opsins may have a microbial origin (type 1), such as channelrhodopsins or halorhodopsins, which function as light-gated ion channels, or an animal origin (type 2), such as melanopsin or rhodopsin. Preclinical data have suggested that blind patients with preservation of the photoreceptor nuclei, as visible on OCT, may be eligible for functional photoreceptor restoration through optogenetics.[282]

Portable Vision Devices

Among new low-vision aids that are aimed at improving patients’ quality of life, is the OrCam, a portable and spectacle-attached device capable of recognizing optical characters, text, currency denominations, and, as programmed, faces and objects. The device is activated when pointed, pressed, or tapped on. In small series of visually impaired patients, usage of this device led to an increase in scores on a daily function test.[283]

Expectation Management in Interventional Clinical Trials

Although the advances of the last 2 decades have propelled research forward toward clinical application, with exciting new treatment possibilities for RD patients, expectations should be managed and critically reconsidered. Gene supplementation therapy is notably costly to develop, to test, and to implement, and of the several gene replacement therapy trials that have been performed in the *RPE65*-RD population, only one has led to considerable long-term success and market approval to date: voretigene neparvovec (Luxturna), priced at approximately US $850.000. Although the bench-to-bedside success of this first commercially available retinal gene therapy has further energized patient and research communities alike, the long-term effects of the therapy on visual function in the other *RPE65*-gene therapy trials have been more guarded.[222,223] For example, it has been found that retinal degeneration may continue, and the longevity of interventional therapy will be limited if the degree of photoreceptor degeneration has exceeded a certain limit before treatment.[284] Indeed, in most patients, retinal degeneration will have progressed to intermediate or advanced stages at the time of intervention. Although any degree of visual restoration and/or preservation is a revolutionary move forward in an otherwise untreatable disease entity, FDA documents have revealed that approximately half of treated patients met the FDA criteria for minimally meaningful improvement.[285] The other half did not achieve the criteria for meaningful improvement, and 2 patients had permanent vision loss, due to injection-related macular thinning in one patient, and irreversible optic nerve atrophy due to increased intraocular pressure in the other patient, who received ocular steroids for the treatment of a *Staphylococcus* infection.[285] These results may be particularly disappointing to the patient, having undergone the surgical procedure and a period of recovery and frequent hospital visits.

In choroideremia, gene therapy has led to a median gain in visual acuity of 4.5 letters in the treatment cohort, versus a visual acuity loss of 1.5 letters in the untreated eye at the 2-year post-treatment point. In some patients, this vision improvement was sustained at up to 5 years of follow-up.[141] Nonetheless, complications arose in 2 of 14 patients (14%)—surgery-related retinal thinning and incomplete vector dosing in one patient, and post-operative inflammation in the other. This has led to the prolongation of the postoperative immune suppression regimen.

The irreversibility of disease in cell populations that have already degenerated should be stressed to any potential participants in gene therapy trials. In the case of subretinal gene therapy that only targets the posterior pole, the treatment effect will be confined largely to the macula. Therefore, it should be explained to patients that peripheral visual field preservation is not expected when this is not the targeted area.

Issues regarding the cost of gene therapy remain a point of concern. As gene therapies for orphan indications, defined as diseases affecting $<$200,000 people in the United States, target specific genetic entities, and thus pertain to small patient populations, they remain among the most expensive drugs.

These considerations indicate that clinicians and researchers should exert caution not to oversell the capacities of investigative (gene) therapeutic strategies to patients, who are often driven by hope. In the context of informed consent, it is evident that eligible patients—who may already be small in number—are to be informed well on the risks of intervention, its investigative nature, and thus uncertain outcome, and on the lengthy post-intervention trajectory.

CONCLUDING REMARKS

New treatment opportunities emerge for RDs at an exceedingly rapid pace, offering hopeful perspectives to many RD patients worldwide. Given these developments and the need to approve effective treatments for clinical use, prospective natural history studies are of eminent importance. However, this thesis has shown that retrospective studies, despite their inherent limitations, can provide robust and useful information on important disease characteristics, variability, and course of many years. National collaborations, such as the RD5000 consortium in the Netherlands, or international collaborations, as within the European context of European Reference Network - Eye Diseases (ERN-EYE), are important to further strengthen the outcome of such studies in these relatively small patient populations. For example, access to the Delleman archive for hereditary eye diseases at the Amsterdam University Medical Centers/Academic Medical Center in Amsterdam, and access to the database for hereditary eye diseases at the Ghent University Hospital, have provided the unique opportunity to ascertain large sample sizes, and to assemble some of the largest retrospective cohorts described to date. Indeed, prospective studies will not be able to provide all the answers on disease progression and visual survival, and they will still have limitations, such as a limited capacity to include many patients, and a limited study duration. However, the limitations of retrospective research are well-described and include the lack of standardization of patient visits, interval censoring, and a limited availability of multimodal imaging. Improvement of phenotyping and genetic characterization remain of critical importance. Ongoing and future prospective studies should be geared at further assessing the rate of disease progression through different visual function parameters and biomarkers on multimodal retinal imaging, and at investigating correlations between these measures. In the end, retrospective and prospective studies have the powerful capacity to augment each other. Such studies are pivotal for well-balanced decision-making on patient eligibility for treatments, and endpoint selection to test treatment efficacy.

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