Inherited retinal degenerations: clinical characterization on the road to therapy

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ENGLISH SUMMARY

Retinal dystrophies 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, e.g. retinitis pigmentosa or cone dystrophy. Conversely, mutations in different genes may lead to the same phenotype. The age at symptom onset, as well as the rate and characteristics of vision decline, may vary widely per disease group and even within families. For most IRD cases, no effective treatment is currently available. However, preclinical studies and phase I/II/III gene therapy trials are ongoing for several IRD subtypes, and recently the first retinal gene therapy has been approved by the United States Food and Drug Administration for *RPE65*-associated IRDs: voretigene neparvovec-rzyl (Luxturna®).

Despite rapid advances in gene therapy studies, insight into the phenotypic spectrum and long-term disease course remains limited for several IRD 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 thesis responds to these challenges, aiming to provide detailed clinical descriptions of several forms of IRD that are caused by genes of interest for ongoing and future gene or cell-based therapy trials. Adequate insight into the clinical characteristics, variability, and long-term outcome of these IRDs is crucial in establishing an adequate diagnosis and in counselling the patient on the prognosis. Moreover, this knowledge is essential in the design of ongoing and future gene therapy trials.

Chapter 1 is a general introduction to the retinal anatomy and function, and the techniques commonly used to evaluate these features. It also features a description of several common subtypes of IRDs, their presentation and clinical signs, and a brief overview of several emerging experimental therapeutic options.

One of the genes of interest in the development of gene therapy is *CRB1*, which has been described in Leber congenital amaurosis (LCA), retinitis pigmentosa (RP), and macular dystrophy, and has been suggested to cause disorganization of the normal laminar structure of the retina, which may complicate gene therapy efficacy. **Chapter 2** discusses the clinical characteristics and phenotypic spectrum of *CRB1*-associated retinal dystrophies. **Chapter 2.1** presents the largest retrospective cohort of patients with *CRB1*-RD described published to date. Most patients (91%) were reported to have *CRB1*-RP, with a symptom onset at the median age of 4 years, and a subsequent mean yearly visual acuity decline rate and mean visual field decline rate of 0.03 logMAR and 5%, respectively. The median ages for reaching low vision and blindness were 18 and 44 years, respectively. Frequent characteristics were non-detectable rod and cone responses on full-field electroretinography (50%), cystoid fluid collections in the macula (50%), hyperopia (98%), vitreous abnormalities such as cells and/or veils (54%), and optic disc drusen (20%). Interestingly, the macula showed organization in identifiable retinal tissue layers on OCT in 91% of patients with available OCT scans. In patients with LCA, severe visual impairment or blindness occurred early in life, with

early degeneration of the macula. As in *CRB1*-RP, frequent characteristics were hyperopia (100%), and vitreous abnormalities such as cells and/or veils (40%). The available imaging was limited, and therefore the presence of cystoid fluid collections and the degree of retinal organization could not be evaluated in these LCA patients. Unlike in *CRB1*-RP, where 92% of patient had bone spicule-like pigmentation, in *CRB1*-LCA the pigmentary changes were limited to a granulated or "powdered" aspect of the retina. This could be due to the young *CRB1*-LCA population (age range 3-15 years at the last examination) versus the *CRB1*-RP population (age range 2-78 years at the last examination).

Chapter 2.2 reports the baseline cross-sectional results of an extensive prospective phenotyping study in Dutch patients with *CRB1*-RD. Patients had RP, cone-rod dystrophy, or an isolated macular dystrophy. SD-OCT scans were performed in nearly all patients, and showed fully discernible retinal layers in 62% of patients, with mild to moderate laminar disorganization of the retina in the remaining patients. Nanophthalmos was present in 36% of patients, and associated with a risk of acute angle-closure glaucoma. Full-field sensitivity testing provided a subjective outcome measure for retinal sensitivity in eyes with (nearly) extinguished amplitudes on the full-field electroretinogram. Using white, red, and blue light stimuli, we were able to determine the origin of the retinal response (rod, cone, or mixed response).

Chapter 2.3 describes a large Belgian cohort of patients with *CRB1*-associated IRDs, in which blindness was seen in the 1st decade of life in LCA and early-onset severe retinal dystrophy, and in the 5th decade of life in the group of RP patients. All patients with macular dystrophy carried the same mutation on at least 1 allele, confirming a genotype-phenotype correlation. Disorganization of the lamellar retinal structure was observed in 52% of patients, a higher proportion than in the Dutch cohort (described in Chapter 2.2).

The combined results from Chapter 2 indicate a window of therapeutic opportunity in the first 3 to 4 decades of life in *CRB1*-RP, but within the first years of life in *CRB1*-LCA.

Chapter 3 focuses on choroideremia, an X-linked form of rod-cone dystrophy, caused by mutations in the *CHM* gene. Choroideremia has been treated relatively successfully in phase I/II human gene therapy trials, with phase III clinical trials currently being conducted. Still, relatively few longitudinal studies have been conducted for this disease.

Chapter 3.1 evaluates the long-term clinical course and visual outcome of choroideremia patients, with a mean follow-up time of 25 years. Symptoms started at a mean age of 15 years. Visual acuity remained stable until the age of 35, after which a significantly faster deterioration occurred, albeit with a high variability between individual patients. A unique aspect of this study is its focus on the patient's experience of the disease. Patients reported discontinuing professional work at a mean age

of 48, which was vision-related in 60% of cases. Visual field constriction was reported as the most disabling symptom by 70% of patients. This study is the first to report scleral pits and tunnels as a novel finding on SD-OCT. **Chapter 3.2** describes the successful outcome of full-thickness macula hole surgery in choroideremia, followed by structural and functional improvement.

Chapter 4 focuses on *RPGR*-associated phenotypes. Several human gene therapy trials are ongoing for *RPGR*-associated RP, and the first reports from phase I and II/III trials have shown a promising safety and efficacy profile (https://clinicaltrials.gov/: NCT03116113). However, remarkably limited information was available on the phenotypic spectrum, long-term clinical course, prognostic factors, and genotype-phenotype correlations, while *RPGR* gene therapy development accelerated at a rapid pace.

Chapter 4.1 investigates the phenotype and clinical course in a large cohort of 74 male patients with *RPGR*-associated IRD, which consisted of RP in 70%, cone dystrophy (COD) in 7%, and cone-rod dystrophy (CORD) in 23%. This study found an earlier age at symptom onset in RP patients than in patients with COD/CORD (5 years vs. 23 years). However, the ensuing visual acuity decline was faster in COD/CORD than in RP, with the probability of being blind at the age of 40 being 20% in RP and 55% in COD/CORD. Mutations in the ORF15 hotspot region were associated with high myopia, and a faster visual acuity decline, both in RP and COD/CORD, and a faster visual field decline in RP.

Chapter 4.2 examines the phenotype in heterozygous (female) *RPGR* mutation carriers. While the X-linked inheritance mode of *RPGR*-RD places the focus on affected male patients, we also studied the spectrum of abnormalities in female mutation carriers. This chapter describes the clinical and genetic findings in 125 female *RPGR* mutation carriers, who were from RP pedigrees, COD/CORD pedigrees, or mixed pedigrees. Retinal pigmentary changes and visual symptoms were present in 58% and 40% of female subjects, respectively. Complete expression of the RP or CORD phenotype was observed in 23% of heterozygotes, although usually in milder forms than in their affected male relatives. Blindness was found in 9% of female subjects. Myopia was a frequent feature (73%), and was associated with a lower visual acuity. Ongoing and future *RPGR* gene therapy trials may consider including such severely affected female patients, as gene therapy may be a viable treatment option, as has been reported in affected male patients.

The results from Chapter 4 indicate a window of therapeutic opportunity ideally in the first 4 decades of life in men with *RPGR*-associated RP and the first 3 to 4 decades of life in men with *RPGR*-associated COD/CORD.

In **Chapter 5**, a retrospective cohort of 13 patients with *LRAT*-associated IRDs, the largest series to date, is described, over a mean follow-up time of 25 years. Symptoms occurred in the first decade

of life, and 54% of patients reached low vision, at the mean age of 49.9 years, illustrating a more favorable disease course than in earlier case reports in literature. In an earlier treatment trial of an oral supplement, patients with *LRAT*-RD and those with *RPE65*-RD were grouped together, due to the close-knit function of their respective protein products in the retinoid cycle. We therefore aimed to study the comparability of *LRAT*-associated phenotypes and *RPE65*-associated phenotypes, using our clinical data of *LRAT*-RDs, as well as earlier literature of phenotypes associated with each gene. There was considerable variability in the phenotypic findings as RP phenotypes and cone-rod phenotypes were present in the same genetic isolate. This variability complicated the ability to make a clear comparison between phenotypes associated with *LRAT* mutations and *RPE65* mutations.

Chapter 6 characterizes the natural history of *RHO*-associated RP in 100 patients from Dutch and Belgian cohorts. The phenotype was relatively favorable compared to the autosomal dominant IRDs and X-linked IRDs studied in our earlier chapters, with a sectorial RP in 25% of patients, and generalized RP in 75% of patients. Median ages to reaching low vision and blindness were 52 and 79 years, respectively. In sectorial RP, visual acuity and visual field decline was significantly slower than in those with generalized RP. The thickness of the photoreceptor-retinal pigment epithelium complex correlated with visual acuity, and may thus provide a surrogate endpoint to evaluate treatments in future clinical trials. The results from Chapter 6 indicate a wide window of therapeutic opportunity, ideally within the first 6 decades of life.