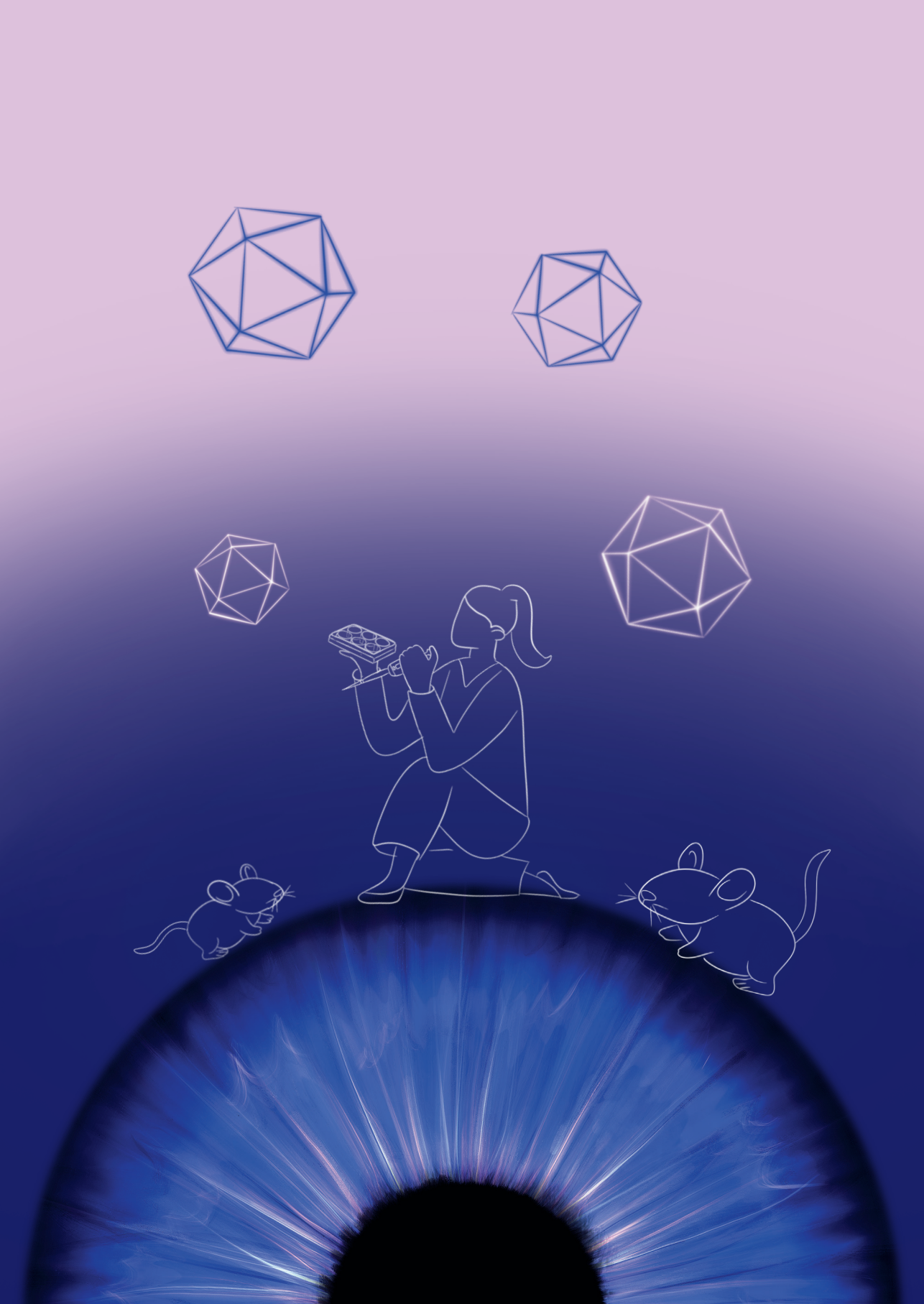
Viral gene therapy approaches for CRB1 retinal disease

Boon, N.

Chapter 7

Summary
Nederlandse Samenvatting

List of publications
Curriculum Vitae
Acknowledgements



Summary

Crumbs homologue 1 (CRB1) is a large transmembrane protein located in the retina. It consists of a large extracellular domain with multiple epidermal growth factor (EGF) and laminin-globular like domains, as well as a short intracellular domain with a conserved PDZ binding motif. CRB1, CRB2, and CRB3 are all members of the Crumbs family, with CRB1 and CRB2 being specifically localized in the retina. The *CRB1* gene has been associated with more than 200 different mutations, leading to retinal dystrophies such as retinitis pigmentosa (RP) and Leber congenital amaurosis (LCA), with no clear genotype-phenotype correlation. The clinical variability of disease onset and severity, even among patients with the same homozygous mutations, supports the hypothesis that other factors may modulate the phenotype. Currently, there are no treatment options available for patients with mutations in the *CRB1* gene.

Since *CRB1*-related RP and LCA are heterogenous diseases, various animal-derived models have been described to mimic different aspects of the heterogeneous disease phenotype. This diversity is represented by numerous distinct animal models previously generated by us and other researchers, including four LCA and ten RP-like mouse models. **Chapter 2** introduces two additional RP-like mouse models: *Crb2*$^{\Delta Rods}$ and *Crb1*^{KO}*Crb2*$^{\Delta Rods}$ mice. In this chapter, we demonstrate that the specific loss of CRB2 from rod photoreceptors leads toretinitis pigmentosa, and that concomitant loss of CRB1 exacerbates the retinitis pigmentosa phenotype. The impaired contrast sensitivity observe might serve as an outcome parameter to measure functional vision gain or maintenance following adeno-associated viral vector (AAV)-mediated gene augmentation therapy. Furthermore, in **Chapter 3**, we provide a more detailed description of the phenotype observed in a rat with a spontaneous mutation in the *Crb1* gene. The initial signs of retinal degeneration were observed at postnatal day 10 (p10), and a significant decrease in retinal function was already evident at 1 month of age in these *Crb1* mutant rats. Additionally, spectral domain optical coherence tomography (SD-OCT) was employed to monitor the retinal degeneration in a single rat over time.

To understand differences between rodent and humans, we determined the subcellular localization of CRB1 and CRB2 using immuno-electron microscopy in **Chapter 3**. We observed that the endogenous expression of CRB1 and CRB2 in rats is similar to what has been observed in the mouse retina. However, recent studies on non-human primates, human fetal retina, and human derived retinal organoids have shown a distinct localization of CRB1 and CRB2. This highlights the importance of utilizing human-derived models for gene augmentation therapies. In **Chapter 4 and 5**, we described the use of human induced pluripotent cells (hiPSC) derived retinal organoids. In **Chapter 4**, we presented *CRB1* RP patient-derived, and in **Chapter 5**, we described *CRB1*^{KO} and *CRB1*^{KO}*CRB2*$^{+/-}$ LCA retinal organoids. Both the *CRB1* patient-derived and the *CRB1*^{KO} and *CRB1*^{KO}*CRB2*$^{+/-}$ retinal organoids exhibit a significant reduction in the number of photoreceptor nuclei in a row and a significant increase in the number of photoreceptor cells above the OLM in compared to the isogenic controls. Single cell RNA sequencing in **Chapter 4** revealed similar *CRB1* transcript levels in patient-derived and isogenic control retinal organoids, while the CRB1 protein was clearly diminished. Additionally, in **Chapter 5**, the KO organoids showed less CRB1 protein at the OLM compared to the isogenic control. Collectively, the *CRB1* patient-derived and *CRB1*^{KO} and *CRB1*^{KO}*CRB2*$^{+/-}$ retinal organoids accurately mimic the phenotype observed in patients and are, therefore, a suitable model for studying gene augmentation therapy possibilities.

Before studying the possibility of gene augmentation therapy using AAVs, it is crucial to determine the tropism of different capsids in rats (**Chapter 3**) and in retinal organoids (**Chapter 4 and 5**). In rats, subretinal injection of AAV2/5 and AAV2/9 successfully transduced the RPE and photoreceptor cells, whereas AAV6 variant ShH10^{Y445F} was transducing the RPE, photoreceptor cells, MGCs, and other cell types in the INL. Intravitreal injection of AAV2/5 and AAV2/9 showed relatively poor transduction efficiency of both photoreceptors and other cells in the INL. However, an efficient transduction of mainly MGCs was observed after intravitreal injection of ShH10^{Y445F}. Due to these observations, ShH10^{Y445F}.h*CRB* was used for AAV-mediated gene augmentation therapy in rats (**Chapter 3**). In **Chapter 4**, AAV2/2 and AAV2/5 were used to define tropism of early organoids, while **Chapter 5** used AAV2/2 and AAV2/5

to define tropism of older retinal organoids. Both AAV2/2 and AAV2/5 efficiently transduced mainly photoreceptor cells and also some MGC and other cells in the INL, with AAV2/5 slightly more efficiently transducing cells in the INL. To target both the photoreceptor cells and MGC in the retinal organoids, AAV2/5.h*CRB* was used in **Chapter 4 and 5**.

Previous research demonstrated the successful preservation of retinal morphology and function in *Crb1* RP mouse models through AAV-mediated *CRB2* gene augmentation therapy. Based on these promising results, AAV-mediated gene augmentation therapy was initially explored in an animal model with a severe and early-onset phenotype: the *Crb1* mutant rat (**Chapter 3**). Unfortunately, timely rescue of the retinal phenotype using retinal function and visual acuity was not observed, likely due to the severity and early onset of the phenotype. This suggests the need for an earlier onset of recombinant hCRB protein expression to efficiently rescue the severe retinal phenotype in *Crb1* mutant rats. Next, AAV-mediated gene augmentation therapy was conducted on human derived induced pluripotent stem cells (hiPSC) derived retinal organoids (**Chapter 4 and 5**). In these chapters, the RP phenotype showed partial improved after treatment with AAV.h*CRB2*, as evidenced by a restored number of photoreceptor nuclei in a row. For the *CRB1* RP patient-derived retinal organoids (**Chapter 4**), a partial improvement was observed following treatment with AAV.h*CRB1*. Single cell RNA sequencing analysis of AAV treated *CRB1* patient-derived retinal organoids demonstrated a partial restoration of transcript expression levels of genes related to the endosomal system back to isogenic control levels. These findings indicate the potential efficacy of AAV-mediated gene augmentation therapy for treating CRB1-related retinal dystrophies.

In conclusion, in this thesis has provided valuable insights into several aspects of *CRB1* related retinal dystrophies. Specifically, we have demonstrated (1) the phenotypic characteristics of *CRB1* mutant mice, rats, and human-derived retinal organoids, shedding light of the disease progression in various models. (2) We have investigated the distinct tropism of AAV capsids in different species, which is crucial for developing targeted gene therapy approaches. (3) And we have achieved successful AAV-mediated gene augmentation therapy in *CRB1* patient-derived as well as *CRB1*^{KO} and

CRB1^{KO}*CRB2*$^{+/-}$ retinal organoids, suggesting the potential therapeutic relevance of this approach. Altogether, these findings represent a significant advancement in understanding the pathogenesis and potential treatment options for patients with mutations in the CRB1 gene.