Summary

Vanishing White Matter (VWM) is a severe brain disease. It is caused by mutations in genes encoding the eIF2B complex, which is involved in translation initiation; it is conditional for the translation of virtually all mRNAs to protein and a key regulator of protein synthesis rates under various stress conditions. Patients have progressive neurological dysfunction with ataxia and spasticity and also cognitive decline. The disease displays sensitivity to cellular stressors, like febrile infections, which cause rapid decline. Astrocytes and oligodendrocytes of the brain white matter are mainly affected. There is no treatment for VWM and many patients die early in life. My thesis involved the development and characterization of new model systems for preclinical testing and showed effectiveness in selecting candidate treatments for VWM. We present proof-of-concept for successful recovery after cell transplantation and Guanabenz treatment. Both are important steps in advancing therapy development for VWM.

In Chapter 2 we characterized new mouse models for VWM, and used these mice to study the role of astrocytes in VWM disease pathomechanisms. We demonstrated that our VWM mice recapitulate major aspects of VWM; they have a shortened lifespan, affected motor function and show abnormalities of astrocytes and oligodendrocytes. The homozygous mutations in *Eif2b5* or *Eif2b4* result in a lifespan of respectively 7-10 and 18-20 months. To model ultra-severe VWM we crossbred the mutant mice into double mutant mice with mutations in both *Eif2b5* and *Eif2b4*. Single homozygous *Eif2b5* mice were studied in depth and showed affected astrocytes starting at postnatal day 14, before onset of oligodendrocyte pathology and motor dysfunction. In co-cultures of astrocytes and oligodendrocyte progenitor cells (OPCs) of different compositions, we tested the contribution of each cell type. We showed that astrocytes of VWM mice secrete factors that inhibit OPC maturation, while VWM OPCs show normal maturation in the presence of wild-type (WT) astrocytes. So, VWM oligodendrocytes do not appear to have an intrinsic maturation problem. Consistent with VWM patients, mainly the white matter astrocytes in VWM mice were affected, while the grey matter astrocytes appeared normal on immunostainings on brain sections. A new finding was that two additional astrocytic populations were found to be affected in VWM mice and patients: the Müller cells of the retina and the Bergmann glia in the cerebellum. The mice recapitulate human VWM, both clinically and pathologically. The range of mouse mutants also recapitulate the spectrum of disease severity observed in human patients. They are therefore excellent models for further studies on VWM disease mechanisms and therapy development. Already the results of this chapter revealed new information by showing that specific astrocytic subpopulations are affected by VWM mutations and that these astrocytes may drive pathology in other cell types like the oligodendrocytes.

In Chapter 3 we developed new induced pluripotent stem cell (iPSC)-based models for VWM. Patient-derived iPSCs provide an autologous cell source for cell replacement therapies and are valuable tools to study disease mechanisms, such as the identification of intrinsically affected cell types by eIF2B mutations. In this chapter we aimed to study VWM astrocytic pathology *in vitro* using iPSCs of both VWM mice and VWM patients. After differentiation of human iPSCs we confirmed the negative effect of VWM astrocytes on OPC maturation previously observed with mouse cells in Chapter 2, indicating this mechanisms is shared between species. To study specifically the white matter astrocytes, which are mostly affected in VWM brain tissue, we differentiated iPSCs into astrocytes with two different protocols, one containing FBS and the other based on CNTF. These protocols led
to different astrocytic subtypes: the FBS astrocytes were more consistent with grey matter astrocytes while the CNTF astrocytes were more consistent with white matter astrocytes. Strikingly, VWM CNTF astrocytes inhibited OPC maturation, while VWM FBS astrocytes did not. We performed RNA sequencing analysis to identify differences between VWM and control astrocytes. Pathways related to “extracellular space”, “immune response” and “cell development” were consistently affected in VWM astrocytes. Human VWM CNTF astrocytes showed specifically affected pathways regarding “neuronal functioning” and “vasculature related”, which were not affected in FBS astrocytes or mouse CNTF astrocytes. So, in this chapter we present protocols to generate specific astrocytic subtypes in vitro, show the value of using mouse and human protocols in parallel, and give insight into differential intrinsic defects in astrocyte subtypes in VWM iPSC culture models.

In Chapter 4 we searched for new measures of the severity of VWM pathology with the aim of being able to quantitatively assess disease deterioration or improvement with treatment. With the purpose of modulating disease severity, the effects of Guanabenz, a potential therapeutic compound for VWM, were tested. The cerebellum of untreated VWM mice showed a decreased number of mature oligodendrocytes, and the cell bodies of the Bergmann glia were translocated from the Purkinje cell layer to the molecular layer. This translocation was apparent in VWM mice and patients, and could be quantified and used as a disease marker. As a proof of principle we treated VWM mice with Guanabenz, a compound that is FDA approved for the treatment of hypertension. Recent studies have shown that Guanabenz targets the integrated stress response (ISR), a pathway affected by VWM mutations. For this reason we expected Guanabenz to affect the VWM pathology and used the drug to validate the different disease markers. We showed that Guanabenz treatment improved markers of VWM pathology. The results of this study indicate that Guanabenz treatment may be beneficial for VWM patients. Because it is a known, FDA-approved drug, studies to explore the effects of application in patient can be initiated.

In Chapter 5 VWM mice were transplanted with (macro) glial progenitor cells (GPCs) neonatally, to study the therapeutic effects of glial cell replacement therapy for VWM. We transplanted different types of GPCs to test optimal cell populations. However, after transplantation no difference in astrocyte and oligodendrocyte differentiation potential was observed, although PDGFαR-sorted GPCs showed increased survival compared to GLAST- or A2B5-sorted GPCs. We showed that cell transplantation let to clinical improvement, i.e. improvement in time to cross the balance beam compared to saline-treated mice. Furthermore, a subset of animals showed improvements of VWM pathology after cell transplantation, which was correlated to a higher percentage of transplanted cells that differentiated into astrocytes. This is in line with the conclusion of chapter 2 regarding the central role of astrocytes in VWM. This study showed for the first time that glia cell transplantation could be effective in treatment of VWM, and that success of cell therapy for VWM depends on the astrogenic differentiation potential of the cell population.

In Chapter 6 we explored ways to further optimize cell transplantation for VWM. One option is to lower hyaluronan (HA) levels in the brain; high HA levels can be detrimental for cell migration and maturation, and are likely involved in VWM pathology. We treated VWM mice with HA lowering compounds Vitrase and Dexamethasone. Long term treatment did not lead to adverse side effects, although no improvements of VWM phenotype were observed. Dexamethasone even worsened the number of Nestin+ cells in the corpus callosum, suggesting that Vitrase might be a better candidate. Future studies combining Vitrase with cell transplantation are currently being carried out, and will show whether
lowering HA levels is beneficial in VWM cell-based treatment paradigms.

Chapter 7 discusses the importance of the brain microenvironment for the success of stem cell therapy. Many leukodystrophies have an adverse brain microenvironment, which can affect the ability of transplanted cells to survive and mature. It is important to test this in relevant disease models that replicate etiology and clinical disease as observed in patients. As human glial cells have a phylogenetic advantage over rodent glia, we argued that preclinical studies should be performed with rodent glia as well. Although further optimization and testing with appropriate models are necessary before proceeding with clinical trials, current preclinical cell therapy studies look promising.

In summary, experiments performed in this thesis confirmed the hypothesis that astrocytes are the primary affected cell type in VWM (1-4) (Figure 1). Mouse studies showed that astrocytes are affected early on, and inhibit OPC maturation. Additionally, specific astrocytic populations, like the white matter astrocytes, Bergmann glia and Müller cells, are affected, while grey matter astrocytes appear normal. By using human and mouse iPSC-based models, we showed that the white matter astrocytes in VWM are intrinsically more affected than grey matter astrocytes in terms of differential gene expression and OPC maturation support. We further provided proof-of-concept for successful treatment effects of Guanabenz administration and cell therapy. The success of cell therapy was correlated with the amount

Figure 1. White matter astrocytes are central in VWM pathology. This figure shows a graphical representation of how neural cells are affected by VWM mutations, based on the results of this thesis. VWM mutations appear to affect mainly white matter astrocytes, which show a decreased maturation and abnormal morphology. We showed that VWM astrocytes secrete factors that inhibit OPC maturation in vitro, which is a likely cause for the observed block in OPC maturation in vivo. Reactivity of both astrocytes and microglia is surprisingly low considering the severity of the disease. VWM microglia do not show overt pathology but defective cross talk between astrocytes and microglia might be present. Some neuronal pathology is present, but is relatively mild and likely secondary to glial abnormalities. Grey matter astrocytes are not as severely affected as white matter astrocytes: they show normal maturation and morphology and grey matter like iPSC-derived VWM astrocytes do not inhibit OPC maturation in vitro. iPSC-derived astrocytes show affected pathways on RNA sequencing analysis (RNA seq) regarding “extracellular space”, “immune response” and “cell development”. Additionally, human white matter like astrocytes show affected gene expression involved in “neuronal functioning” and “vasculature related”.

RNA seq analysis of iPSC derived astrocytes showed affected pathways:
- Extracellular space
- Immune response
- Cell development

High levels of HA and unknown secreted astrocytic factors inhibit OPC maturation

VWM astrocytes produce high amounts of HA

Neuronal pathology is relatively mild and likely secondary to glial pathology

It is unknown how microglia-astrocyte crosstalk influences VWM pathology

Grey matter astrocytes do not seem to be affected by VWM mutations:
- Normal maturation
- Normal morphology
- No effect on OPC maturation

RNA seq analysis of human white matter like astrocytes showed specifically affected pathways:
- Neuronal functioning
- Vascularity related

White matter astrocytes are affected by VWM mutations:
- Decrease maturation
- Abnormal morphology

HA

VWM astrocytes produce high amounts of HA

It is unknown how microglia-astrocyte crosstalk influences VWM pathology

RNA seq analysis of iPSC derived astrocytes showed affected pathways:
- Extracellular space
- Immune response
- Cell development
of injected cells that differentiated into astrocytes, again pointing to the central role of astrocytes in VWM. Further optimization of treatment strategies is necessary, but it is clear that astrocytes should be the main target of therapy development for VWM.