SUMMARY

The molecular mechanisms underlying frontotemporal dementia (FTD) and Parkinson's disease (PD) are still poorly understood and the progress in promising therapeutic targets is limited. Genetics has identified pathogenic mutations in genes that cause these neurodegenerative diseases, which increased our understanding and provided new starting points for developing therapeutics. New genetic technologies such as next-generation sequencing (NGS) of whole-exome DNA and NGS-based RNA sequencing are replacing the classical "positional cloning" approaches and consequently, the attention is shifting from studying single genes to gene networks for genetically complex diseases.[1] In FTD and PD, these technological advancements have already contributed to identifying a number of genetic risk factors for sporadic forms of disease. [2,3] However, there is an urgent need to understand the molecular networks that link these genetic risk factors to disease phenotypes and the traditional reductionist approach, focusing on individual components, would be time-consuming and insufficient to interpret the full complexity of the gene networks. Multi- "omics" approaches are now being explored to understand how genetic risk factors contribute to the pathogenic mechanisms underlying neurodegenerative diseases. In this thesis I focused on functional genomics, transcriptomics and cellomics approaches to investigate pathogenic aspects of FTD and PD. The overall aim was to gain insight into gene regulation and molecular mechanisms underlying these neurodegenerative diseases. To this end, I studied the regulation of *C9orf72* expression in **Chapter 2**. In an attempt to find splicing regulators and to examine the potential of two RNA-based technologies to specifically decrease endogenous 4R tau levels, splicing of *MAPT* exon 10 was studied in **Chapter 3 and 4**. In addition, I prioritized candidate genes for PD in **Chapter 5** with RNA-interference (RNAi) screens in human cell culture and experimental animal models.

In **Chapter 1**, I reviewed the literature regarding cell-based screening approaches in the context of neurodegeneration and outlined the current progress in the field. The advancements in automation of human cell culture, in combination with methods to systematically modify gene expression on a genome-wide scale, makes high-throughput cell-based screens feasible and has the potential to bridge the gap between *in vitro* and animal models. [4-6] I discussed the genetics of FTD with a focus on the disease-causing genes *MAPT* [7-9] and *C9orf72* [10,11] which, together with granulin (*GRN*) [12,13], explain the majority of familial FTD cases. [29,50] Furthermore, I reviewed the genetics of PD and outlined the general aim of this thesis.

In **Chapter 2**, I studied the regulation of *C9orf72* expression. Cap Analysis of Gene Expression sequence (CAGEseq) data was used and we observed that *C9orf72* expression is mostly low with the exception of a subset of myeloid cells, especially CD14+ monocytes, which are involved in innate and adaptive immunity. This suggests that *C9orf72* might play a role in immune-related processes. Transcription at the *C9orf72* locus showed a complex architecture and the annotated *C9orf72* transcripts are differentially expressed across samples. We detected novel TSSs in both sense and antisense strands of the *C9orf72* locus. Notably, *C9orf72* coding transcripts show a consistent decrease not only in brain tissue and monocytes from *C9orf72*-HRE patients, but also in several brain

regions from *MAPT* and *GRN* mutation carriers together with an increase in antisense transcripts. This suggests that the reduction of *C9orf72* expression is not entirely dependent on the repeat expansion and might play a more general role in neurodegeneration. Moreover, I explored the possibility of a functional interaction and performed knockdown experiments in BE(2)-M17 cells with lentiviral constructs targeting *C9orf72*, *MAPT* and *GRN*. I observed a clear influence on expression of *C9orf72* after knockdown of *MAPT* and *GRN* separately compared to a non-targeting scrambled control, which implicates that *MAPT*, *GRN* and *C9orf72* might interact in a yet unknown common pathway.

In **Chapter 3**, we performed a shRNA-based RNAi screen of the human spliceosome to determine alterations in *MAPT* exon 10 inclusion with a qPCR assay to specifically detect endogenous 4R and 3R tau levels in BE(2)-M17 neuroblastoma cell lines. To optimize the assay, knockdown of known modulators of tau exon 10 inclusion were tested. Knockdown of *STOX1* led to a consistent 2-fold increase in 4R tau transcript levels and with an average robust Z' factor of 0.57, our assay was considered "excellent". The library contained 324 shRNA pools targeting splicing-related factors and we identified 27 pools that significantly changed 4R tau transcript levels compared to non-targeting scrambled control wells, without changing total tau. Notably, we found several splicing factors previously identified to modify tau exon 10 splicing, demonstrating the strength of our approach. We then reconstructed a splicing network and revealed a total of 51 interactions among 27 genes with high confidence through a protein-protein interaction network analysis. Validation experiments must be devised to further validate and prioritize candidate genes.

In **Chapter 4**, we explored the potential of two RNA-based technologies to specifically decrease the levels of 4R tau. We designed several partly overlapping antisense oligonucleotides (AONs) to cover exon 10 and the exon/intron and exon/exon boundaries with the help of the Human Splicing Finder algorithm. The potential of both AON and short hairpin RNA (shRNA) was assessed at the endogenous level in BE(2)-M17 cells. We identified 7 AONS and 3 shRNAs decreasing 4R tau transcript levels specifically. The RNAi findings were translated to the protein level and primary cortical neurons of humanized *MAPT* mice were used to select 2 shRNAs for further studies. As proof-of-principle, we demonstrated the effect of one of the shRNAs targeting the exon 10/ exon 11 boundary in cortical neurons derived from human induced pluripotent stem cells (iPSCs).

In **Chapter 5**, we used whole exome sequencing (WES) to identify rare PD susceptibility variants. Following Sanger sequencing confirmation, we identified a total of 27 candidate genes all predicted to cause a loss of gene function. To define potential links between the 27 candidate genes and well-established mechanisms of PD susceptibility and pathogenesis, we deployed RNAi-based functional studies in both mammalian cells and experimental animal models, including *Drosophila* and *C. elegans*. We quantified mitochondrial morphology after gene knockdown in BE(2)-M17 cell lines and used the well-established Parkin translocation assay based on neuroblastoma cell lines stably expressing Parkin-GFP. Furthermore, developmental lethality and survival were tested in *C. elegans* and α-synuclein mediated toxicity was tested in *Drosophila*, the latter recapitulating features of PD-related neurodegenerative pathology. Taken together, we identified genetic evidence consistent with

replication of 7 genes that were implicated by WES analysis, of which 5 were further validated based on functional screening in PD-relevant model systems. By integrating human genetic and functional evidence, interesting PD susceptibility gene candidates were identified for further investigation.