Summary

Frontotemporal lobar degeneration (FTLD) is a presenile neurodegenerative disorder characterized by progressive behavioural disturbances, aphasia and eventually cognitive decline. A proportion of patients show an autosomal dominant pattern of inheritance with linkage to chromosome 17q21. In a consensus conference it was recognized that these families suffered from a distinct disorder with comparable clinical and neuropathological features, designated as Frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17). In 1998 genetic analysis from several groups (including ours) showed that in most of these FTDP-17 families mutations in the microtubule associated protein tau (MAPT) were linked to this hereditary form of FTLD. Several exonic and intronic mutations were found in most FTDP-17 families and now over 100 families carrying more than 40 different MAPT mutations are known.

In all families with MAPT mutations, pathology is characterised by cellular inclusions of MAPT protein. Characterisation of FTDP-17 patients showed that different MAPT mutations can cause a variety of specific clinical phenotypes providing valuable information for both diagnostic and research purposes. In this thesis we describe studies to characterise the effects of both the G272V and ∆K280 MAPT mutations in patients. In these studies clinical, pathological and biochemical data, together with functional studies measuring the in vivo effects of MAPT mutations on (aggregated) protein composition and RNA expression are described.

The first mutation, G272V, was found in a large family with hereditary Pick’s disease, which description dates back from the beginning of last century. In this thesis a detailed overview of two brains with the G272V mutation that had become available is described. Both brains showed severe neuronal loss and abundant Pick bodies, characteristic for Pick's disease pathology. Furthermore all biochemical, immunohistochemical and electron microscopic results further confirmed that this family met all criteria to be diagnosed as hereditary Pick disease. This makes this family, ideal for providing valuable information for diagnosis of and research on the aetiology of Pick’s disease. Furthermore model systems using G272V mutant MAPT may provide valuable data about the mechanism causing this disabling and lethal disorder.

The second mutation, ∆K280, was identified in a single patient only. This mutation is important due to the duality in its predicted disease evoking mechanism. Alternative splicing in human adult brain gives rise to six alternatively spliced MAPT proteins. Because one of the alternatively spliced exons encodes for one of the four microtubule
Summary

binding repeats, three of the six isoforms have three (those that do not contain exon 10) and three of them (those containing exon 10) contain four microtubule binding repeats. In healthy human adult brain, there is equilibrium in the alternative use of exon 10 in MAPT transcripts and the ratio between the three repeat containing (3R) and four repeat containing (4R) MAPT proteins is approximately 1. Some mutations affect the alternative splicing of exon 10 and therefore alter the equilibrium between 3R and 4R MAPT, which subsequently leads to disease. In vitro experiments suggest that the ΔK280 mutation decreases both microtubule binding and increases the induction of self-aggregation of MAPT into filaments; and secondly, affects alternative splicing, changing MAPT isoform equilibrium. In the latter case, the dramatic effect of the ΔK280 mutation on microtubule binding or self-aggregation would be overruled by the postulated absence of mutant MAPT and relative increase of other MAPT isoforms, which may cause disease by itself. Our results confirmed a decrease in 4R isoform alternative splicing and 4R protein isoforms, which provided evidence for the latter hypothesis and support for the aetiologic significance of excess of MAPT isoforms to the disease process.

Except for FTDP-17 patients carrying MAPT mutations, MAPT pathology is also a characteristic for several other neurodegenerative disorders, covered by the name “Tauopathies”. Tauopathies include illnesses like Alzheimer’s disease, Pick’s disease or Progressive Supranuclear Palsy (PSP). To study the influence of the MAPT protein in different neurodegenerative processes we analysed snap frozen post-mortem tissue from the medial temporal lobe of patients with four different Tauopathies (Alzheimer’s disease, PSP, Pick’s disease and Frontotemporal dementia [FTD]) for gene expression and compared results with those from a control group. All patients were matched for age, gender, ApoE-ε and MAPT haplotype. From all groups a total of 790 probes (representing ~730 genes) were shown to be differently expressed when compared to control background levels. From the 790 identified probes we were able to extract a gene set of 166 genes that made it possible to distinguish PSP patients and Pick’s disease/FTD patients from each other and from controls. Since PSP patients could be differentiated as a distinct group we chose to study disease linked pathways in this illness. We demonstrated that expression of genes of the insulin receptor signalling, IFG-1 and PTEN signalling pathways were significantly changed. These pathways show large overlap of involved genes and affect the same transcription factors (i.e. FOXO1A and FOXO3A).

Interestingly insulin receptor signalling is linked to lifespan increase in various
species including mouse, by affecting regulation of FOXO transcription factors. According to our microarray experiments FOXO1A and FOXO3A were significantly upregulated. Furthermore in all other tested neurodegenerative diseases, except Alzheimer’s disease at least one of these genes was shown to be upregulated, stressing the importance of these genes in the neurodegenerative disease progresses.

The exact involvement of this signalling pathway on PSP however is still speculative. Possibly, FOXO factors in combination with heat shock factors regulate aggregation and disaggregation activities to promote cellular survival in response to constitutive toxic protein aggregation. This pathway would provide a mechanistic link between aggregation-mediated proteotoxicity and PSP.

Although in 1998 genetic analysis showed that in most FTDP-17 families mutations in MAPT were linked to this patient group, in part of these families, no MAPT mutations could be detected. Furthermore, most patients showed characteristic MAPT-negative but ubiquitin-positive neuronal inclusions. Only recently genetic defects (mutations in the GRN [granulin precursor protein; Progranulin] gene) were found in several families linked to chromosome 17q21.

Progranulin encodes a biologically active precursor glycoprotein of 7,5 tandem repeats of highly conserved motifs of 12 cystein residues called in granulin domains. Progranulin is suggested to be a multifunctional growth factor that in order to become functionally active is cleaved into separate granulins that may be involved in development, inflammation and wound repair.

To determine the possible involvement of GRN mutations in our FTLD cohort, we systematically screened for mutations in 77 cases with positive family history of dementia consistent with autosomal dominant pattern of inheritance and with no CHMP2B (another gene involved in FTLD) or MAPT mutations. Here we report on the finding of two novel frameshift mutations and three possible pathogenic missense mutations in the GRN gene. Although it cannot be excluded that the latter changes are benign variants as they are located in granulin domains, that were suggested to be functional redundant, recently studies have shown that separate repeats may have alternative binding capacities and also the precursor protein itself may have a distinct function, which highlights the possibility that these variants may be pathogenic.

In addition, we determined GRN mutation frequency in a large cohort of familial Frontotemporal lobar degeneration patients from the Netherlands. Recently, GRN mutations were suggested to be a very common cause of FTLD, however our and other studies couldn’t confirm these data, therefore regional differences may exist.
Summary

In conclusion, this work provides substantial contribution to the knowledge of Frontotemporal lobar degeneration (FTLD) and dementia in general. The results presented in this thesis show that research based upon the G272V MAPT mutation provides valuable information for diagnosis of and research on the aetiology of Pick’s disease; the ΔK280 MAPT mutation provides evidence that relative overexpression of 3R MAPT contributes to disease; characterisation of pathology defined patient groups on gene expression is possible and provides a basis for pathway analysis in different groups of Tauopathies; and also in the Netherlands GRN mutations present a significant risk on FTLD.