THE GENETICS OF EARLY ONSET PARKINSONISM

SUMMARY

Parkinson's disease (PD) has a worldwide distribution, and is the second most common neurodegenerative disorder after Alzheimer's disease. Clinically, the disease is characterized by the classic triad of motor symptoms; bradykinesia (slowness of movements), muscular rigidity, and resting tremor, and by a wide range of non-motor symptoms. Pathologically, PD is characterized by a progressive loss of dopaminergic neurons in the substantia nigra pars compacta, a consequent reduction in striatal dopamine and, the presence of α-synuclein inclusions, i.e., Lewy bodies. Although dopamine replacement strategies can alleviate the motor symptoms, there is still a need to overcome the treatment associated side-effects and limited action on non-motor symptoms. Moreover, interventions to slow or prevent the disease progression remain an unmet clinical need. Success in that endeavour depends on a better understanding of the disease etiology and pathogenesis. Studies of the rare familial forms of PD provide extremely valuable insights into the pathogenesis (e.g. discovery of mutations in the SNCA gene), and may reveal molecular therapeutic targets for PD. A summary of the mutations identified (Table 1) and the putative functions of these genes are presented in chapter 2. The age at onset (AAO) of PD symptoms is typically after the age of 50, the late-onset PD (LOPD). More rare are the early-onset PD (EOPD) patients (AAO<50 years).

In the first half of this thesis (chapter 3, and 4), we assess the genetic contribution in an early-onset PD-Dutch cohort (EOPD-DC). Today mutations in SNCA, LRRK2, PARKIN, DJ-1, and PINK1 genes have been implicated in EOPD. In chapter 3, we assess the prevalence and nature of mutations in these genes by sequencing and exon dosage-analysis (the MLPA assay) in 187 EOPD-DC patients. This study represents the first large screen for both missense and copy number variants (CNVs) for these five EOPD-associated genes by a single center, and the first of its kind in a Dutch cohort. We detected mutations in 9% of the patients (including heterozygous). Our results suggest that the use of different patient recruitment criteria and screening methods, bound the mutation screenings to report different mutation prevalence.
Creation and application of genetic screening guidelines would significantly improve data quality and allow for easier comparison between reports. Most of the EOPD patients analysed (62%), carry a single mutation in PARKIN, PINK1, or DJ-1 gene. We discussed the possible explanations for the apparent pathogenic role of heterozygous mutations in recessive genes. The relatively low prevalence of mutations (4%) together with the significant proportion of copy number variants (CNVs) (48%) suggests that CNVs in yet non-associated genes remain to be discovered in the EOPD population. Thus in chapter 4, in search for novel loci associated with PD, we identified CNVs and runs of homozygosity (ROHs) in 60 patients from the EOPD-DC, 25 patients from an isolate (the Walcheren group), and 47 individuals of Dutch origin by using a whole genome high-density SNP array (SNP 5.0 Affymetrix). We identified a set of rare CNV regions (CNVRs) specific of PD patients, five of which overlapped with genes, which function is potentially associated with the PD phenotype, such as the HLA-DRB5, the L-type dependent calcium channel subunit CACNA1C, the deubiquination enzyme DUB-3, and olfactory receptor genes. In addition, an association analysis of the CNVRs present in both patients and controls revealed thirty regions significantly associated with the PD phenotype. The two most significant regions affect the glutathione S-transferase theta-1 (GSTT1) (EOPD-DC: p=6.6E-05; Walcheren: p=2.5E-07), and part of the T cell receptor (TCR) (EOPD-DC: p=5.3E-05; Walcheren: p=1.1E-07) genes, and other CNVRs affecting genes involved in the regulation of the immune response or olfaction. The HLA finding may be supported by the recent association of HLA-DRB5 with LOPD. The association of GSTT1 is of particular interest as these enzymes are involved in detoxification of xenobiotic compounds and also because GST polymorphisms have been suggested as potential risk factors for PD. The results of this study are preliminary and further studies are needed to confirm in an independent study the association of this variant with the disease phenotype. We also analysed the runs of homozygosity (ROHs) but none of the ROH cluster tested reached significance levels of association with the PD phenotype.

In the second half of this thesis (chapter 5 and 6), we focused on the study of the physical properties of DJ-1 protein and the effect of mutations in order to understand its pathogenic role. In chapter 5, using gel filtration assays we demonstrate that the L166P pathogenic mutation has multiple effects on DJ-1 protein that render it non-functional. At the structural level, the changes of DJ-1L166P protein include, unfolded protein conformation, incapacity to form homo-dimers, and higher propensity to form higher molecular weight (HMW) protein complexes. In addition, using pulse-chase
and protein-synthesis inhibition experiments we show that this mutation renders the protein unstable and rapidly degraded. Furthermore the cytoplasmic localisation of this mutant protein is reduced to the mitochondrial fraction. We propose that the apparent “preferential” mitochondrial localization of DJ-1L166P mutant protein reflects both the rapid degradation and the presence of HMW insoluble nuclear aggregates.

In chapter 6, to further contribute to the characterization of DJ-1 function, we searched for the DJ-1 protein interacting partners by performing a yeast two-hybrid screening using human DJ-1WT as bait. The screening identified one novel DJ-1 interactor, CHD3 (chromohelicase domain protein 3), and four previously identified interactors, DJ-1, DJBP, Piasxα, and Ubc9. We showed that the capacity of DJ-1 to interact with these proteins was altered by pathogenic mutations. Using the UbFC fluorescence complementation assay we confirmed DJ-1 interaction with the E2 SUMO conjugation enzyme Ubc9, and with SUMO-1, and visualized the resulting protein conjugates in living cells by confocal microscopy. Furthermore, our results indicate of a non-covalent interaction between DJ-1 and SUMO-1 and suggest that DJ-1 sumoylation results in DJ-1 translocation to subnuclear compartments, of which some were identified as Promyelocytic leukemia protein-Nuclear bodies (PML-NBs). Future studies should solve the functional consequence of DJ-1 localization at the PML-NBs, and investigate role of DJ-1 non-covalent interaction with SUMO-1 in the neurodegeneration process of PD. In addition the novel DJ-1 interactor, CHD3, may underlie the mechanism by which DJ-1 regulates transcription of genes, namely those involved in apoptosis. Therefore, a follow up study on this novel interaction may reveal important insights into the anti-apoptosis function of DJ-1. Based on the results of this thesis, we proposed additional experiments to study DJ-1 interactions under different genetic and environmental background that could provide further advances in etiopathogenesis and offer the opportunity to identify potential therapy targets.