Genetic Risk Factors of Parkinson's Disease

Thesis submitted as requirement to fulfill the degree

„Doctor of Philosophy" (Ph.D.)

at the

Faculty of Medicine

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Nanjing, China

2018

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Chapter 1 Introduction

Outlook

Parkinson's disease (PD) is one of the most common neurodegenerative diseases, second only to Alzheimer's disease (AD) in prevalence. Approximately 1-2% of the population older than 65 years of age are affected, with prevalence reaching 4% by the age of 95 (de Lau and Breteler, 2006). Age adjusted incidence rates are similar between countries of Asian ethnicity versus countries with Caucasian ethnicity (Asian versus Europe and the Americas), while African countries have lower prevalence, adjusted both for mortality and incidence, than in Europe or North and South America (de Lau and Breteler, 2006). As the average age of the population in many developed and developing nations increases, the number of PD cases is also expected to increase dramatically in the next two decades, from approximately four million cases to over nine million cases worldwide (Dorsey et al., 2007).

PD is a heterogeneous disease, both genetically and clinically. Physicians diagnose PD based on a series of clinical characteristics and exclusion criteria as developed by the United Kingdom Brain Bank (UKBB), with the diagnostic criteria focusing on motor symptoms including resting tremor, bradykinesia, and rigidity (Gibb and Lees, 1988). In addition to the motor symptoms, non-motor symptoms such as hyposmia, depression, sleep disorders, and constipation have also proven useful as prodromal symptoms with predictive qualities to determine the progression of PD before the onset of motor symptoms in patients (Witjas et al., 2002, Schrag et al., 2000). The more recent Movement Disorder Society Clinical Diagnostic criteria also increased recognition to non-motor

systems, while maintaining motor parkinsonism as the central feature of the disease (Postuma et al., 2015).

Technological advancements over the last 20 years along with the availability of comprehensive catalogue of genetic variations from multi-ethnic populations available in various publically available data bases such as the HapMap project and the 1000 Genomes project have enabled researchers to decipher the genetic landscape of complex diseases including PD (Lander et al., 2001, Genomes Project et al., 2010, International HapMap, 2005, Simon-Sanchez et al., 2009, Lill, 2016). The development of PCR amplification allowed for the processing of multi-allelic markers to map disease loci in families using linkage-based studies. (Elston, 1998, Singleton et al., 2003). This approach led to successful identification of a number of genes involved in monogenic forms of PD (Shulman et al., 2011). However, monogenetic forms of PD only explain a fraction of PD cases. Out of the diagnosed PD cases, approximately 5-10% of patients develop PD as a result of either autosomal dominant or autosomal recessive forms of PD. In contrast, the development of array based approaches led to start of genome wide association studies (GWAS). GWAS followed an unbiased approach in identifying novel loci for a given phenotype (Mullin and Schapira, 2015). This approach examines a genome-wide set of genetic variants across individuals to identify loci associated with a given trait, successfully leading to the identification novel loci for PD (Lill, 2016). Despite the success of GWAS, the majority of genetic architecture still remains elusive, and thus indicating the complex genetic architecture of PD (Obeso et al., 2017). The thesis presented herein aims to plug this gap by following a multi-dimensional strategy that investigates the complex disease origins of PD.

Scope of the thesis:

The scope of this thesis has three broad sections. The first section, Chapter 1, describes the current status of PD literature, and explores the possible avenues of influence on risk of disease as the literature currently stands. The second section, Chapter 2, presents the original work of the thesis,

the projects undertaken in investigating the varied risk factors of PD, consisting of parts 1, 2, and 3.

Part 3 investigated the influence of genes on possible cross-phenotype effect, by investigating the effect of ataxia genes on PD risk. Recent evidence has shown that although the various late-onset neurodegenerative diseases such as ataxias, AD, and PD present with clinically heterogeneous characteristics, for example differential pathogenesis and degeneration of the motor system, they may have common genetic risk factors that influence the predisposition to disease development. Previous clinical and pathologic findings have emphasized the need to evaluate the significance of polyglutamine repeat expansions in PD worldwide (Gwinn-Hardy et al., 2001, Wang et al., 2009). Of particular interest is the possible connection between SCA genes, including SCA2 (ATXN1), SCA3 (ATXN3), SCA6 (CACNA1A), and SCA17 (TBP) and their influence on the probability of developing idiopathic PD (Charles et al., 2007, Furtado et al., 2004, Lim et al., 2006, Kim et al., 2009). Previous studies have screened for SCA2, SCA3, SCA6 and SCA17 genes in cohorts of autosomal dominant PD and identified carriers, suggesting that intermediate repeat structure may influence the clinical variability in autosomal dominant forms of PD and cerebellar ataxia. We address the question of possible risk due to intermediate polyglutamine repeats in idiopathic PD.

Part 2 addresses the influence of genetic interaction by investigating how known PD risk-affecting variants encompassing the LRRK2 and PARk16 loci mediate each other and thus in turn modulate total disease risk. Recent studies have demonstrated a functional interaction between the retromer and lysosomal pathways in PD pathogenesis, and interestingly two genes, namely LRRK2 and RAB7L1 have shown to functionally interact (MacLeod et al., 2013). Studies have also demonstrated that overexpression of the RAB7L1 gene rescues the LRRK2 mutant phenotype, thus suggesting that both RAB7L1 and LRRK2 genes are bound together and functionally interact with each other in regulating neurite length both in vitro and in-vivo (Chuang and Gitler, 2013). Chapter 4 dealt with this issue to understand the impact of interaction in world-wide populations using the tagged single nucleotide polymorphisms (SNPs) encompassing these two loci.

Part 3 of the results investigated the epidemiological interaction between PD and diabetes, a disease of the adaptive immune system. As more is learned about PD pathogenesis, researchers have also found evidence that the neuronal degeneration may result from destructive immune responses. There is a compelling argument that PD pathogenesis is modulated and influenced by the adaptive immune system (Surendranathan et al., 2015). Type 1 Diabetes Mellitus (T1DM) is also characterized by an autoimmune response, thereby destroying the body's capability of insulin production (Singh et al., 2011). Due to the similarities in the two diseases and their relationships to immune dysfunction, we utilized the largest German diabetes database to investigate possible differential disease pathogenesis between T1DM patients with PD and neurologically normal T1DM patients.

History of Disease

Parkinson's disease (PD) was mentioned in medical writings as early as Galen's documents (Larner, 2014). The first unambiguous description of the disease is attributed to James Parkinson, an English doctor who published in 1817 a set of six case studies titled "An Essay on the Shaking Palsy" (Parkinson, 2002). Parkinson characterized the disease of his namesake, describing in detail the abnormal posture, altered gait, paralysis, weakness, and resting tremor observed in his six cases, focusing on the physical manifestation of the disease, with limited hypothesis regarding its origins (Parkinson, 2002). Nonetheless, his seminal essay contains the first definitive expositions about this debilitating neurodegenerative disease. In 1912, Frederic Lewy discovered the common presence of protein bundles in the brains of PD patients, later called "Lewy bodies", advancing the pathological characterization of the disease (Rodrigues e Silva et al., 2010). Later on in 1997, Spillantini, Trojanowski, and Goedert et al found that the main component of Lewy bodies is alpha-synuclein (SNCA), thus bringing our understanding of PD into modern times (Goedert et al., 2017).

PD primarily affects the elderly population, with prevalence at approximately 1-2% for patients older than 65, rising to 4% for patients older than 85 (Brooks, 2012). Mean age of diagnosis is mid 60's, with range of disease diagnosis from the 2nd to the 8th decade (Baumann, 2012). The main pathology of PD is the defining loss of neurons in the substantia nigra pars compacta (SNPc), the reduction in neurons leading to striatal dopamine deficiency (Obeso et al., 2017).

Non-motor symptoms develop first, with degradation of the olfactory system, constipation, and sleep problems. Non-motor symptoms of PD can be grouped into the following categories: neuropsychiatric symptoms, sleep disorders, autonomic symptoms, gastrointestinal symptoms, and sensory symptoms (Chaudhuri et al., 2006). Depression affects up to 45% of PD patients (Schrag et al., 2000). Causes of depression include both disease pathogenesis and psychological reactions due to the progressive debilitation. Excessive daytime sleepiness and disruption of REM sleep is also commonly observed, affecting up to 50% of patients (Schapira, 2004). Most likely, disease

advancement, levodopa therapy, and nighttime sleep disruption together cause the sleep disorders seen in PD patients (Schapira, 2004). Dementia is much more prevalent in PD cases, with patients 6 times more likely to develop dementia than in a healthy population, affecting approximately 40% of patients (Titova et al., 2017b). Constipation, pathologically linked to loss of colonic and central dopaminergic neurons, is a common pro-dromal PD symptom that has been linked to PD prediction (Singaram et al., 1995). Hyposmia, the most common prodromal symptom affecting up to 90% of patients, also increases the risk of a person receiving a PD diagnosis within 2 years follow-up (Lee and Koh, 2015).

Further neuronal degeneration causes the characteristic motor symptoms of bradykniesia, rigidity, and resting tremor (Gelb et al., 1999). Bradykinesia is strongly correlated with dopamine deficiency and is strongly related to basal ganglia disorders (Berardelli et al., 2001, Vingerhoets et al., 1997). Rigidity, defined as stiff or inflexible muscles, is another primary motor symptom, manifesting itself by severely limiting movement such as turning or standing from a seated position, or causing a fixed or mask-like facial expression. Often initially appearing as a stiff shoulder, the increased resistance that characterizes rigidity goes on to also affect locomotion and axial coordination of the trunk. A prospective study of 6038 persons (mean age 68.5) found a hazard ratio of 2.11 relating the presence of stiffness to the risk of developing PD (de Lau et al., 2006). Resting tremors in PD are characterized by unilateral onset in the fingers at a frequency between 4 and 6 Hz, and progression to bilateral symptoms as disease progresses. In a prospective study with autopsy proven disease, 100% of autopsy certified patients at some point also experienced tremors (Rajput et al., 1991). In another study, Hughes et al found that 69% of patients exhibited resting tremor at point of disease onset, and 75% of patients experienced the symptom at some point over the course of the disease (Hughes et al., 1993). Response to levodopa treatment is the third tenet of PD characterization, a defining trait of PD and used in the determination of disease (Baumann, 2012) .

Parkinson's disease staging system

The traditional staging system of PD as laid out by the United Kingdom Brain Bank (UKBB) diagnostic criteria focuses on the motor symptoms of PD (Gibb and Lees, 1988). The important indications of PD include pathological degeneration of the projection neurons of the substantia nigra pars compacta. The disease progression of idiopatic PD is well-documented, with well-defined induction sites that develop in a predicable sequence (Gibb and Lees, 1988). The traditional disease pathogenesis with on-set characterized by degeneration of dopaminergic neurons in the substantia nigra has been challenged by Braak and colleagues, who developed from autopsy studies a PD pathogenesis trajectory composed of a six-stage process with clearly defined induction sites of Lewy-body formation (Braak et al., 2002, Braak et al., 2003), with full manifestation of the motor symptoms occurring in the last two stages, and pre-motor symptoms dominating in the initial staging. Diagnosis of idiopathic PD is relatively accurate when using the conventional criteria of asymmetrical onset of akinesia rigidity and tremor in conjunction with lack of atypical symptoms (93 %) (Hughes et al., 1993). However, atypical features such as several early dementia, lack of response to levodopa, confusion, or early autonomic degeneration was also found in 12% of autopsy confirmed idiopathic PD cases (Hughes et al., 1992)

Genetics of PD

Twin Studies

Twin and familial studies allowed for the studying of both shared genetic or environmental components prior to the "genomics era". Monozygotic twins share complete genetic information while dizyogtic twins share approximately 50% of their genetic information (Wirdefeldt et al., 2011b). Because both types of twins share environmental and familial factors, one can differentiate between the genetic inheritability of the disease versus the influence of environmental factors on the disease. Twin studies compare the concordance rates in monozygotic versus dizygotic twins; a higher rate of concordance in monozygotic twins demonstrates a genetic component to the disease. While cross-sectional twin studies did not demonstrate different concordance rates by zygosity in

normal onset PD cases, several studies including the National Academy of Sciences/National Research Council World War II veteran twin registry and the Swedish Twin Registry found increased concordance rates in early (before age 50) onset (Wirdefeldt et al., 2011b). A prospective longitudinal study by Mayo Clinic Rochester also followed monozygotic and dizygotic twin pairs for clinical PD and found evidence suggesting a substantial role in inheritance in sporadic PD (Piccini et al., 1999).

Autosomal dominant forms of PD

SNCA (PARK1)

The first autosomal-dominant gene, SNCA, was discovered in 1997 in 4 separate families of Greek and Italian/American origins (Polymeropoulos et al., 1997). All families presented with autosomal-dominant mode of inheritance. Since then, a number of families have been identified across different populations, which led to the identification of additional missense mutations. The SNCA mutations (A53T, A30P, E46K, G51D, H50Q, and A53E), located the long arm of chromosome 4q21 (Kalinderi et al., 2016).

The different SNCA missense mutations present with a wide range of clinical symptoms, from classical PD to atypical PD including severe autonomic dysfunction and dementia in addition to Parkinsonism (Kalinderi et al., 2016). Phenotypes also differentiate by mutations. The A53T mutation typically presenting with earlier age of onset and rapid progression in addition to high prevalence of psychiatric and dementia symptoms (Goedert, 2001). The A30P mutation is associated with milder disease and later age of onset (Kruger et al., 1998). The E46K mutation presents with a phenotype more typical of diffuse Lewy body (DLB) disease (Zarranz et al., 2004). A dosage effect has also been demonstrated in the mutation, with duplicates and triplicates showing earlier age of onset, more rapid disease progression, and a higher prevalence of dementia (Singleton et al., 2003, Chartier-Harlin et al., 2004). SNCA triplicates experience on average ~10 year earlier age of onset as opposed SNCA duplicates (Lill et al., 2016).

SNCA is the main component of Lewy bodies, which are one of the characeristic neuropathological biomarkers of PD (Goedert, 2001). Additionally, glial cytoplasmic inclusions as observed in multiple system atrophy (MSA) have also been shown to be strongly immunoreactive for alpha-synuclein (Goedert, 2001). It is hypothesized that aggregation of the protein plays an important role in the neurodegeneration seen in Lewy body diseases and MSA. Mitochrondrial dysfunction and oxidative stress has also been associated with mutations in SNCA gene (Smith et al., 2005).

The concentration of alpha-synuclein increases with the number of replicates of the gene: duplicates present with 150% concentration of the protein, while triplicates present with 200% concentration of the protein (Farrer et al., 2004). Point mutations of SNCA appear to lead to increased aggregation behavior of the proteins (Conway et al., 1998). Synuclein expression has also been found to be substantially increased in PD patients versus neurologically normal patients, with one study recording levels of on average 4 times in PD patients despite substantial loss and degeneration of the substantia nigra (Chiba-Falek et al., 2006). The development of alpha-synuclein filaments has been hypothesized to be both necessary and sufficient for dopamine nerve cell degeneration, a defining pathology of PD (Goedert, 2001).

LRRK2 (PARK8)

The second autosomal dominant gene discovered was LRRK2 (leucine-rich repeat kinase 2). Appearing with much higher frequencies than SNCA, the gene has 6 confirmed highly penetrant pathogenic mutations (R1441C, R1441G, R1441H, Y1699C, G2019S, and I2020T) (Healy et al., 2008a, Healy et al., 2008b). The LRRK2 protein encodes a large multi-domain protein containing guanosine triphosphatase (GTPase), kinase, and protein-protein interaction domains (Zimprich et al., 2004). While the exact function is not known, emerging evidence indicates that it plays a role in the intracellular signaling pathways and vesicle formation and transport (Tan et al., 2007). Widely expressed throughout the brain and other systems, it has also been the subject of many functional

and genetic interaction studies that have investigated the synergistic interaction between itself and the PARK16 locus (Chuang and Gitler, 2013, MacLeod et al., 2013). LRRK2 appears with higher frequency than SNCA, with the G2019S mutation exhibiting frequency of up to 20% in Ashkenazi Jews and 40% in North African Arabs (Healy et al., 2008a). Average frequencies range between 5-15% in familial cases and approximately 1% in the general Caucasian population (Berg et al., 2005). Penetrance in general is considered moderate due to the large number of neurologically normal individuals with the mutation.

The G2019S mutation is of particular interest within the LRRK2 loci. It is the most common genetic risk factor to date identified for PD (Bouhouche et al., 2017). The G2019S mutation is defined by its high penetrance, between 30% to 70% in certain familial populations (Li et al., 2015, Goldwurm et al., 2007). In the African Berber population, where a recent study found the prevalence among autosomal dominant patients at 76% and up to 28% within sporadic patients, the clinical phenotype of the disease was marked by less severe tremor but higher degree of dystonia and dyskinesia and sleep disturbances (Bouhouche et al., 2017). The clinical phenotype from the Moroccan study are in line with findings in other clinical studies of LRRK2 associated PD (Pont-Sunyer et al., 2017).

Two Asian population-specific LRRK2 mutations have also been identified: G2385R and R1628P (Di Fonzo et al., 2006, Ross et al., 2008). The clinical phenotype of LRRK2 patients is typical for late-onset PD, with average age of onset of 59 with predominant symptom of tremor (Zimprich et al., 2004, Berg et al., 2005). While individual cases present non-differentially from sporadic idiopathic PD cases, LRRK2 PD cases as a whole trend towards less severe disease pathogenesis and lower rates of dementia and psychiatric symptoms (Berg et al., 2005). The G2019S kinase mutation, the most common mutation of the LRRK2 domain, is at the activation segment of the MAPKKK domain of LRRK2; thus mutations in G2019S alter the kinase activity of LRRK2 (Smith et al., 2006). Mutant LRRK2 is neuronally toxic and has been clinically shown to lead to neural degeneration (Smith et al., 2006).

EIF4G1 (PARK18)

Genome-wide analysis of a multi-incident autosomal-dominant PD family led to the discovery of a missense mutation (R1205H) in eukaryotic translation initiation factor 4-gammon (EIF4G1) (Chartier-Harlin et al., 2011). Linkage and disease segregation followed by sequencing and genotype analysis differentially identified 6 different missense mutations in affected patients with familial Parkinson's disease and idiopathic Lewy body disease versus none in control patients. Disease pathogenesis appears to be related to increased vulnerability of mutant cells to oxidative stress and mitochondrial dysfunction under duress. Further studies have found EIF4G1 to be a very rare cause of PD, and a large scale study in European cohorts found it to be a benign variant, neither a common nor high-risk locus for Parkinson's disease (Huttenlocher et al., 2015).

VPS35 (PARK17)

Vacuolar protein sorting 35 (VPS35) is an autosomal dominant PD gene discovered using whole-exome sequencing (WES) (Vilarino-Guell et al., 2011, Zimprich et al., 2011). Two groups both positively identified a mutation (D620N) in an Austrian and a Swiss kindred and it has been confirmed by additional independent datasets that the D620N mutation of the VPS35 gene is a causal autosomal dominant PD gene (Lill, 2016). The VPS35 protein functions as part of the retrograde transport of proteins from endosomes to the trans-Golgi network. Furthermore, VPS35 has been found to be active in the dopamine signaling pathway, interaction with the dopamine receptor D1 (Tian et al., 2015, Tang et al., 2015b). Accounting for approximately 1% of PD cases worldwide, the clinical profile of VPS35 patients resembles typical sporadic idiopathic PD patients, with average age of onset of 53 years and low rates of psychiatric and dementia symptoms in the disease pathogenesis.

DNJAC13 (PARK21)

Exome analysis within a large North American family identified the DNJAC13 mutation. The mutation was observed in all case family members of the identifying family, and further genotype analysis in a

multi-ethnic case control series also identified the mutation in cases (Vilarino-Guell et al., 2014). Carriers present with late age of onset and common motor symptoms (Rajput et al., 2015). Pathological studies showed staining consistent with LB disease. Preliminary functional analysis suggests that the mutation leads to difficulties in endosomal and retromer trafficking, a common theme among PD risk-mutations (Vilarino-Guell et al., 2014).

CHCHD2 (PARK22)

A genome wide linkage analysis followed by next-generation sequencing on a Japanese family with 8 affected individuals and 5 unaffected individuals identified the coiled-coil-helix-coiled-coil-helix domain containing 2 (CHCHD2) mutation (Funayama et al., 2015). Patients presented with late-onset autosomal dominant PD and typical parkinsonian symptoms. CHCHD2 mutations are associated with autosomal dominant PD (Funayama et al., 2015). CHCHD2 belongs to a protein family with small proteins that localize in the mitochondrial intermembrane space and are involved in mitochondrial respiration. Disruptions of the CHCHD2 gene results cause disruptions in mitochondrial respiration and oxidative activity (Funayama et al., 2015). However, functional studies are needed to understand the pathophysiology of CHCHD2 mutations within PD, and genetic studies in other populations are also need to confirm the results.

TMEM230

Transmembrane protein 230 (TMEM230) is the most recent autosomal-dominant gene discovered is TMEM230. The controversy surrounding it exemplifies the complexity of disentangling the genetic architecture of PD. A recent study in 2016 identified a novel missense variant in TMEM230 within a family with northern European ancestry located in North America, a gene that encodes a primarily transmembrane protein that localizes in synaptic vesicles (Deng et al., 2016). The same family was also used for the identification of DNAJC13 (Olszewska et al., 2016). TMEM230 signal was also found

to overlap with VPS35 signal, indicating that perhaps VPS35 and TMEM230 share a pathogenic pathway that results in defective synaptic vesicle transport. However, the results have not been reproduced since the original study in several large European and Chinese ancestry population studies with sporadic PD cases (Ibanez et al., 2017, Yan et al., 2017, Giri et al., 2017). Further studies are needed to determine the validity of TMEM230 gene, but the evidence thus far does not support the conclusion that it is a disease-causing mutation.

Autosomal Recessive Inheritance

PARKIN (PARK2)

PARKIN was first identified in Japanese families. Its subsequent mapping led to the identification of gene and has been shown to cause autosomal recessive PD cases. The protein PARKIN is an ubiquitin E3 ligase that interacts with the ubiquitin-conjugating enzymes E2s to mark proteins for autophagy (Shimura et al., 2000). Mutations (including A574G, A633T, C823T, G235T, G500A, C718T, C1319T), including deletions, insertions, and point mutations disrupt the function of the protein, causing loss of function of the catalytic activity of the parkin protein (Dawson and Dawson, 2014, Foroud et al., 2003). PARKIN is the most common cause of early onset PD, with average age of onset at 32 years for the Caucasian population and accounting for 50% of familial autosomal recessive early onset cases younger than 50 years of age (Periquet et al., 2003). It accounts for 0.4-0.7% of all non-familial PD cases in general. Prevalence of the PARKIN mutation in cases with onset younger than 25 years has been observed to up to 50%, while prevalence in the age category 30-45 years is observed to be 3-7% (Periquet et al., 2003, Bonifati et al., 2001). The mutation has been observed in sporadic cases as well as familial cases, and penetrance is observed to be 100% for homozygous carriers or compound heterozygous. First observed in a cohort of Japanese families with early-onset autosomal recessive PD, it has since then been seen in sporadic patients and in populations from all ethnic origins (Hattori et al., 1998).

PARKIN was first discovered in European and North African families with autosomal recessive juvenile parkinsonism (Lucking et al., 2000), and the majority of PARKIN mutations have been found in the early onset or juvenile cohort early on. However, subsequent studies have discovered that the heterozygous mutations of PARKIN in normal age on of onset patients and families with pseudo-dominant inheritance patterns (Sun et al., 2006). Thus, the current range of age of onset for PARKIN mutations now range from juvenile to post-70 years of age (Sun et al., 2006). New evidence suggests that homozygous and multiple heterozygous mutations result in early onset PD whereas single heterozygous mutations result in increased risk of late-onset PD (Sun et al., 2006). It has also been observed that there is a high frequency of heterozygous mutations in the general population (Klein et al., 2007). While their role as risk factors is less clear, it is hypothesized that effect of heterozygous mutations on conferring PD phenotype is a result of interactions with other genetic, epigenetic, and environmental factors (Klein et al., 2007).

PINK1 (PARK6)

PINK1 (PTEN-induced kinase 1) is another autosomal recessive PD gene. First discovered in two Italian and one Spanish family, it was subsequently found to have prevalence rates between 0.5-9% in the general population, and 3.7% in early onset PD (<50 years of age) (Valente et al., 2004, Valente et al., 2001). Autosomal recessive mutations include G309D, W437X, and L347P (Beilina et al., 2005).

The clinical profile of PINK1 is similar to that of homozygous PARKIN, with earlier onset paired with slower disease progression (Bonifati et al., 2005, Li et al., 2005). Heterozygous mutations of PINK1 (such as A339T, Y431H, N451S, C575R) showed a similar clinical profile to idiopathic PD regarding age of onset, asymmetrical motor degeneration, and L-dopa responsive (Gandhi et al., 2006). PINK1 patients, however, show increased incidences of psychiatric disorders such as anxiety and depression (Bonifati, 2012).

The pathology of PINK1 cases is relatively unestablished, however, Lewy bodies typical for PD was found in the few cases that were investigated (Gandhi et al., 2006). The PINK1 protein is found in the mitochondrial membranes of cells across all organ systems, not just limited to the brain, and it plays a role in the mitochondrial response to cellular and oxidative stress and mitochondrial quality control (Valente et al., 2004). Mutations and deletions to the gene decreases the functional ability of the protein. Like PARKIN, PINK1 has highly penetrant, with homozygous and compound heterozygous mutation carriers showing 100% penetrance (Li et al., 2005).

DJ1 (PARK7)

DJ1 is an autosomal recessive gene identified using kindred families (one Dutch and one Italian family)(Bonifati et al., 2003). It accounts for 0.4% of early onset cases (<50 years), and shows similar disease pathogenesis profile to PINK1 and PARKIN (Bandopadhyay et al., 2004). Unlike PARKIN and PINK1, it is theorized that DJ1 is involved in the mechanism to protect neurons from oxidative stress (Takahashi-Niki et al., 2004). Originally identified through a point mutation and a large deletion (both homozygous), the list of known DJ1 mutations now comprise of several splice-site mutations, missense mutations and exonic deletions (Schulte and Gasser, 2011, Abou-Sleiman et al., 2003). The phenotype for DJ1 patients is similar to other autosomal recessive disease pathogenesis, with earlier than average onset (20-40 years) and slower progression (Bonifati et al., 2003, van Duijn et al., 2001). Some patients have also presented with more psychiatric symptoms, shorter stature, and brachydactyly, and one family in addition presented with dementia and amyotrophic lateral sclerosis (AML) (Annesi et al., 2005).

ATP13A2 (PARK9)

This mutation related to juvenile onset Parkinsonism was first found in a Jordanian consanguineous family with Kufor-Rakeb disease (PARK9) with Parkinsonism and dystonia (Najim al-Din et al., 1994). Further studies used linkage analysis to confirm the mutation in a pedigree from Chile (Ramirez et al., 2006). A study with 46 patients with juvenile onset familial or sporadic PD also confirmed the

findings and discovered additional novel missense mutations of the same coding region (Di Fonzo et al., 2007). The ATP13A2 gene encodes a lysosomal protein active in the family of transmembrane active transporters (Di Fonzo et al., 2007). There is increasing evidence of the importance of lysosomes and cellular waste transport, in particular of alpha-synuclein, in playing a central role in PD pathogenesis and etiology (Ramirez et al., 2006).

FBX07 (PARK15)

Mutations in the F-box only protein 7 (FBXO7) gene causes autosomal recessive juvenile onset Parkinsonism with pyramidal disturbance (PARK15) (Bonifati, 2012). The mutation of the gene was characterized using a Dutch and an Italian family, and the protein isoforms were confirmed to be differentially expressed between affected PARK15 patients and WT proteins (Di Fonzo et al., 2009). Age of onset was in the teenage years, and patients presented with classic motor symptoms in additional to dysphagia and slowed saccades that responded to levodopa treatment (Di Fonzo et al., 2009). Functionally, it has been demonstrated that FBXO7 mutations disrupts mitophagy, leading to toxic aggregation of the FBXO7 protein in the mitochondria and decreasing cellular (and dopaminergic cellular) viability (Zhou et al., 2015).

DNAJC6 (PARK19)

DNAJC6 is an autosomal recessive PD gene also discovered using WES methods on two symptomatic brothers from a consanguineous family in Palestine (Elsayed et al., 2016, Edvardson et al., 2012). Additional familial studies found the mutation in 2 other juvenile onset families, confirming the mutation (Elsayed et al., 2016). Average age of onset for DNAJC6 PD patients with atypical symptoms is 10 years from the 7 cases across 3 families (range 7-11), while carriers of DNAJC6 mutations with typical PD symptoms had an average age of onset of 31 years (Koroglu et al., 2013, Olgiati et al., 2016). It is hypothesized that DNAJC6 encodes a protein auxilin that is important for vesicle trafficking, thus suggesting that PD pathology is strongly affected by this cellular mechanism(Eisenberg and Greene, 2007).

SYNJ1 (PARK20)

This novel missense mutation in SYNJ1 is the most recent discovery of an autosomal recessive gene causing Parkinsonism. It has been reported in Italian, Iranian, and Indian consanguineous families with autosomal recessive juvenile Parkinsonism (Quadri et al., 2013, Krebs et al., 2013, Kirola et al., 2016). While the mutations are rare, the mutation discovered in the Indian family was absent in 570 additional PD samples, the gene is highly conserved across species and missense mutations are highly damaging. SYNJ1 encodes a phosphatase highly expressed in brain nerve terminals and facilitates endocytosis in the adult brain; it also interacts with proteins responsible for signaling, actin nucleations, and synaptic vesicle recycling (Kirola et al., 2016). It has also been implicated in Alzheimer's disease and Down syndrome, with higher levels reported in patient brains versus healthy controls (Voronov et al., 2008, McIntire et al., 2012).

VPS13C (PARK23)

VPS13C is an autosomal recessive early-onset PD gene that is associated with early-onset parkinsonism defined by severe disease with rapid disease progression and cognitive decline (Lesage et al., 2016). It was first discovered using whole-exome sequencing in conjunction with whole-exome linkage mapping using a patient population with early-onset non-familial and familial PD across European, North African, Turkish, and Lebanese origins (Lesage et al., 2016). The three subjects in which the mutation was discovered all presented with Levodopa-responsive parkinsonism marked by accelerated degeneration. Disease pathology also showed Lewy body disease in the brain. The VPS13C silencing mutation is associated with changes to the mitochondrial function, namely decreased membrane potential, increased mitochondrial fragmentation, and increased cellular respiration (Lesage et al., 2016).

Candidate gene studies

Discovery of the aforementioned genes occurred through a variety of different genetic methods, in step with the technological status of genetic studies at a given time. The earliest of the genetic

studies used the candidate gene approach. Candidate gene studies use preselected groups of genes in case-control studies to determine if there exists correlation between the gene(s) and the disease factor. This early genetic study design identified the first set of risk genes, including variants in SNCA, LRRK2, MAPT (microtubule-associated protein tau). More recently, glucocerebrosidase (GBA) mutations linked to PD were also identified after noting the presence of parkinsonism in a cohort of Gaucher's disease patients (Lwin et al., 2004, Bembi et al., 2003). SNCA was first studied via the candidate gene approach after its discovery as a risk variant in the early familial studies (Kruger et al., 1998).

Multiple candidate gene studies confirmed the significant correlation between SNCA and PD, and studies have shown that multiple single nucleotide polymorphisms (SNPs) spanning the SNCA gene are significantly involved with association (Kruger et al., 1998). The majority of SNCA studies focused on Rep1, a multi-allelic polymorphism located upstream from the SNCA transcription starting point. German and Japanese groups confirmed SNCA as a risk gene in respective candidate gene studies and also showed found associations for the same SNP rs356165 across their different ethnic populations respectively (Mueller et al., 2005, Mizuta et al., 2006), and Soldner et al found in 2016 that the transcriptional deregulation of SNCA is related to the neurotranscription factors EMX2 and NKX6-1 (Soldner et al., 2016). SNCA has been confirmed and established as a risk gene with numerous candidate gene studies following its original discovery.

A missense mutation in LRRK2 in an autosomal dominant Japanese family first indicated that it is a potential risk gene for PD (Funayama et al., 2005). Candidate gene sequencing also confirmed the findings in German-Canadian and American family (Zimprich et al., 2004). In 2009, LRRK2 candidate gene studies in Asian populations identified one of the strongest risk variants p.G2385R (rs3477838348) (Webber and West, 2009). Later studies found non-overlapping variants in Caucasian cohorts, although further GWA studies did not present strong statistical support (Healy et al., 2008b). More recently, studies using patients with G2019S mutations have identified common

genes for future candidate studies (Liu et al., 2011). LRRK2 is one of the most established PD risk genes, and has also become a gene of interest in genetic interaction studies, with the recent investigations by Macleod et al, and also by this thesis, into the functional and statistical interaction between PARK16 and LRRK2.

MAPT, a gene that encodes the microtubule associated protein tau was also discovered using candidate gene studies (Lill, 2016). Several neurodegenerative diseases, including "tauopathies", named as such due to the presence of tau inclusions in the diseased brain (Alzheimer's disease, progressive supranuclear palsy, corticobasal degeneration, and frontotemporal dementia) and PD, show increased concentration and deposits of intraneuronal hyperphosphorylated tau (Schulte and Gasser, 2011). The mutation only affects Caucasian populations, due to the existence of two haplotypes H1 and H2, where the primary haplotype H1 associated with PD risk is found only in Caucasians (Kwok et al., 2004). While MAPT has not been shown to be a monogenic cause of PD, genetic causality between frontotemporal dementia-17 (FTD-17) and MAPT has been demonstrated (van der Zee et al., 2006).

Candidate gene studies also identified GBA, the gene that encodes the enzyme glucocerebrosidase as PD risk variant. First noted as a candidate gene due to the increased incidence of PD in families with cases of type 1 Gaucher disease, clinicians discovered that certain mutations in GBA increases risk for PD (Bembi et al., 2003). Following meta-analysis studies also found that rare GBA variants were also risk factors for sporadic PD (Aharon-Peretz et al., 2004, Sidransky et al., 2009). The GBA mutation N370S is the one most commonly observed in PD, leading to a 3 fold risk of developing PD per allele (Schapira, 2015). The L144P mutation has also been observed in multiple cohorts, replicated in a Chinese and in French-Canadian cohort. Patients with GBA mutations show the typical clinical profile of PD, with also comparable age of onsets to the general patient population. It is hypothesized that degenerate lysosomal function due to GBA mutations may lead to SNCA aggregation and Lewy body formation, thus resulting in Parkinsonism and cognitive degeneration

(Mazzulli et al., 2011, Tsuang et al., 2012, Yap et al., 2013). While Gaucher's disease is an autosomal-recessive disorder caused by defective mutations in both copies of GBA alleles, the familial PD and autosomal-dominant PD cases carried one healthy GBA allele. This is in line with recently studies that show that a partial loss of function of the GBA mutation is causally related to PD development (Alcalay et al., 2014). Of note, the GBA mutations associated with Parkinson's are not observed in Gaucher disease patients, one hypothesized explanation is that the mutations are homozygous embryionically lethal, and thus only observed in PD cases with one functioning gene (Petrucci et al., 2014).

Candidate gene studies successfully identified the first set of PD risk variants. However, the studies also had a high rate of false positives when the genes were further investigated inside of GWAS studies. Many findings from the over 800 studies were later found to be false positives from GWAS studies. Candidate gene studies were limited by lack of power, bias from the patient cohort, and inappropriate statistical significance thresholds. Therefore, while candidate gene studies were able to successfully identify four major genetic risk genes, the usage of GWAS studies opened the doors to a deeper and more accurate understanding of the genetic landscape of PD.

GWAS

The genetic understanding of PD developed in step with the advancement of genotyping technology and analysis methods. The foundational projects of the human genome mapping provided the genetic maps necessary to design powerful, high resolution microarrays (Kalinderi et al., 2016). As of today, the most recent and largest GWAS PD study confirmed 26 PD risk loci, with moderate odds ratios ranging from 1.10 to 1.82 (Deng et al., 2017). GWA studies have been extremely useful in confirming and establishing previously identified SNPs of interest from candidate gene studies.

The previously discovered SNCA, LRRK2, MAPT, and GBA genes have all also been confirmed across the GWA studies (Lill et al., 2012). For SNCA, two independent SNPs were identified recently, one in the promoter region of the gene (rs7681154) and a more significant SNP approximately ~19 kb

downstream from SNCA (rs356182) (Pihlstrom et al., 2015, Mueller et al., 2005). The most recent GWAS found that the increased odds ratio for SNCA to be around 1.30 (Nalls et al., 2014), and SNCA as a risk factor has been consistently validated by GWAS studies (Lill, 2016).

GWAS also identified two rarer additional variants for GBA (minor allele frequency (MAF) is ~0.01) (Sidransky et al., 2009). One other important finding recently associated the human leukocyte antigen (HLA) region with PD risk, thus suggesting for the first time through genetic evidence that the immune system plays a pathogenic role in PD disease development and progression (McGeer and McGeer, 2011, Little et al., 2016). Lastly, GWAS recently also found a correlation between PD risk and a variant in intron 1 of GCH1, a known causative gene for levodopa-responsive parkinsonism (Mencacci et al., 2014). It also been demonstrated to have an effect across different ethnicities.

More recently, a group of Chinese researchers found rare GTP cyclohydrolase (GCH1) heterozygous variants with a risk association with PD (Xu et al., 2017). They identified 7 rare heterozygous variants (R57Q, S77C, S80N, M137V, R198Q, G203E, and G232D) in the GCH1 gene. The GCH1 gene is implicated in interactions with proteins within the same biosystem as PD; but more research is needed to understand the functionality and genotype-phenotype pathways.

The non-candidate-driven phenotypic approach of GWAS allows for the discovery of completely novel variants, as opposed to previous candidate-gene studies (Obeso et al., 2017). While GWAS studies have been instrumental in the confirmation and identification of new variants and SNPs in known regions of interest, there are still limitations to the study designs. GWAS require genotype imputation to estimate genotypes or genotype probabilities at markers that are not directly processed by a GWAS. Thus, by using a reference panel such as the 1000 Genomes project or the International HapMap Project or more densely sequencing only a subset of study participants as examples of complete genotypes, genotype imputations are utilized to include coverage of genotypes at positions not assayed in study samples and increase the proportion of common markers between different datasets (Martin et al., 2014). However, imputation also limits GWAS

studies from identifying rare, high-risk variants since it relies on finding shared haplotype segments between study participants. Another point of difficulty regarding GWAS is that the signals found tend to cover a relatively large region that includes many correlated SNPs, and thus it is difficult to tease out the pathogenic SNP from the region of interest identified by the studies.

GWAS has also been commonly used to identify and confirm susceptibility loci in AD (Bertram, 2009). The GWAS results have not been very consistent in validating previously discovered genes, with the exception of the ε4-allele of the apolipoprotein E gene (*APOE*) (Bertram and Tanzi, 2009). Until now, GWAS studies in AD have identified 23 risk loci (Lambert et al., 2013). The low degree of penetrance and diffuse findings suggest that sporadic late-onset AD has a complicated genetic architecture with many low-risk common alleles.

Next generation sequencing, including WES and whole-genome sequencing (WGS), are able to better grapple with rare variants and address many of the short comings of GWAS in identifying rare, high-risk variants (Bras et al., 2012). In particular. Whole genome studies provide increased coverage and reduces bias due to fewer amplification steps, and is given the current methods, the optimal method of identification of rare, high-risk variants (Jansen et al., 2017). It is our best method moving forward for discovering new genetic defects, although GWAS studies and candidate based exome-chip studies are still useful for the right questions, and much less resource-intensive.

Synucleinopathies and tauopathies

PD belongs to a larger diverse group of neurodegenerative proteinopathies defined by the presence of alpha-synuclein lesion formation. The major pathological characteristic of alpha-synucleinopathies (AS) is the presence of AS-positive lesions in the neuronal and glial cells. The protein was first discovered to be related to PD in 1998, when Spillantini et al. demonstrated that Lewy bodies, one of the defining histological traits of PD, displayed high immunoreactivity for AS (Goedert et al.,

2017). Additional studies demonstrated that Lewy bodies and Lewy body filaments obtained from PD patients also tested positive for alpha-synuclein through antibody staining. Lewy body formation without symptoms, believed to be prodromal for diseases with Lewy bodies, is observed in 5-10% of the generation population over 60 years of age (Gibb and Lees, 1988, Forno, 1969). Thus, the prevalence of neurodegenerative diseases correlated to alpha-synuclein is quite high in the elderly population and in neurodegenerative diseases, representing a significant disease burden.

MSA

MSA is another neurodegenerative disease characterized by alpha-synuclein deposits. However, the deposits in MSA are not Lewy bodies. Instead, the defining pathological features are alpha-synuclein-immunoreactive filamentous lesion deposits in primarily glial cells (Fellner and Stefanova, 2013). It is characterized by a diverse set of extrapyramidal, pyramidal, autonomic, and cerebellar symptoms, with parkinsonism seen in almost all patients as disease progresses (Jellinger, 2003). Genetic associations have been reported between MSA and alpha-synuclein, in particular with the cerebellar subtype of MSA (Al-Chalabi et al., 2009). GWAS failed to identify any genome wide significant risk loci but did find several regions of interest including SNPs in MAPT, FBXO47, EDN1, and ELOVL7 (Sailer et al., 2016).

Tauopathy

While alpha-synuclein is the primary protein associated with PD, the tau protein also features prominently in neurodegenerative diseases such as AD, CBD, Pick's disease (PiD), and FTD (Goedert, 2004). Tau is a protein that assists in the stabilization and assembly of microtubules. Commonly found on the axons of nerve cells, it also appears in diseased brains on cell bodies and dendrites. AD, the most prevalent neurodegenerative disease, is defined by the presence of beta-amyloid neuritic plaques and intraneuronal neurofibrillary lesions comprised of tau proteins (Hernandez and Avila, 2007). Tau lesions without the presence of beta-amyloid plaques are also the defining characteristic

of other neurodegenerative diseases such as progressive surpranuclear palsy (PSP), Pick's disease (PiD), and corticobasal degeneration (CBD) (Lee et al., 2001).

CBD is a neurodegenerative disorder, characterized by adult-onset, progressive disease pathogenesis, and marked neuronal achromasia, that affects the cerebral cortex, substantia nigra, and deep cerebellar nuclei (Goedert, 2004). Affected regions feature high concentrations of glia and neuronal intracytoplasmic filamentous tau deposits. One of the defining features of CBD is the extensive presence within the cortical and subcortical areas of tau-immunoreactive neuropil filaments. PSP is a related disease of CBD; both diseases display similar biochemical tau profiles. PSP is characterized by the symptoms of postural instability and supranuclear gaze palsy (Goedert, 2004). Its neurological disease profile includes neuronal atrophy, gliosis, and degradation of the basal ganglia, subthalamus, and brainstem. Genetic studies have demonstrated substantial correlation between the two diseases and the same A0 allele of the tau gene (Soto-Ortolaza and Ross, 2016). In particular, GWAS studies have identified shared variants between PSP and CBD in the myelin-associated oligodendrocyte basic protein (MOBP) and MAPT loci (Kouri et al., 2015). Given the similar biochemical profile and genetic correlation, it is possible that the two diseases are actually different physical manifestations of the same neurological disease pathogenesis (Soto-Ortolaza and Ross, 2016).

PiD is a variant of FTD, and is defined by the appearance of tau-immunoreactive Pick bodies (Goedert et al., 2001). The neurological disease pathogenesis is characterized by degradation of the frontotemporal lobes and the limbic system, with neuronal loss, gliosis, spongiosis, and Pick bodies. The tau found in PiD exhibits a different biochemical profile from the tau protein found in AD, CBD, and PSP (Lee et al., 2001).

The last group of tauopathies discussed is FTDP-17 with parkinsonism. Comprised of autosomal-dominant neurodegenerative diseases that all present with extensive tau filaments in nerve cells and in some cases also glial cells, the disease family is linked to a series of mutations in the same region

of chromosome 17 (Foster et al., 1997). Depending on the site of the disease burden in the brain, patients present with different syndromes and disease phenotypes of FTDP-17. However, all FTDP-17 syndromes present with neuronal atrophy in the affected area and extensive hyperphosphorylated tau filament pathology in neuronal and also possibly glial cells (Foster et al., 1997). This develops phenotypically with progressive degeneration of cognitive, motor, and executive neuronal functions. A GWA study for FTD identified risk variants in the HLA locus across entire and a new locus at RAB36/CTSC (related to lysosomal biology) in the FTD behavioral subtype (Ferrari et al., 2014).

Epidemiology of PD

MPTP

Langston and colleagues established in 1983 that 1-methyl-4-phenyl-1,2,3,6-tetra hydropyridine (MPTP) breaks down in the body into a pre-parkinsonian molecule, 1-methyl-4-phenyl pyridnium (MPP$^+$), and resulted in chronic parkinsonism and dopaminergic neural degeneration(Langston and Ballard, 1984). Furthermore, drug addicts accidentally injected with MPTP, a by-product of meperidine synthesis, showed clinical symptoms and pathology in-line with PD(Davis et al., 1979).

Environmental epidemiology has also linked the pesticide paraquat and Maneb, containing a chemical structured similar to MPTP, with PD (Desplats et al., 2012). The majority of the epidemiological pesticide studies treat exposure as a dichotomous variable; with most studies reporting ORs between 1.3-3.7 in increased risk of PD after exposure. Interaction effects between Paraquat and Maneb and the LRRK2 G2019S mutation has also been demonstrated (Desplats et al., 2012). Studies have found a 3-fold increase in risk of PD development in workers that carry one of the susceptibility alleles in the dopamine transport gene *SLC6A3* (rs2652510, rs2550956, VNTR) and a 4-fold increase in workers with more than risk allele (Ritz et al., 2009).

Smoking

PD is also inversely related to smoking through the nicotinic acetylcholine receptor, with smoking reducing risk of disease by up to 70% (Ritz et al., 2009). The nicotine receptor activates signaling molecules downstream, thus possibly slowing the progression and severity of PD by protecting the cells and decreasing cellular death, increasing neuronal survival, and also possibly attenuating immune activity (Quik et al., 2012). Thus, the resulting adaptations in the immune system responsiveness appears to ameliorate the damage from PD, reducing the severity of symptoms (Quik et al., 2012). Additionally, nicotine itself both improves cognition and attention and also has anti-depressant characteristics, thus countering the mental health and cognitive symptoms of PD (Quik et al., 2012). The protective effect has been confirmed by meta-analyses, and modeling methods have also observed a correlation between reduced risk and time-since-cessation, specifically that the protective effect diminishes the longer the time since cessation (Hernan et al., 2002). There have also been indications of gene-environment interaction between PD and smoking, with evidence that one or more GSTP1 polymorphisms modulates the risk of PD in conjunction with smoking, where carriers of the GSTP1*C haplotypes are more prevalent in PD patients that smoke (Checkoway et al., 1998). Additionally, the MAO-B gene displayed only male gender-specific interactions between smoking and the A/G polymorphism (OR=0.27, 95% CI (0.13-0.58) for smokers versus non-smokers, and OR=1.26 (0.60-2.63) for men of genotype A ($X2=8.14$, $p=0.004$)) (Polito et al., 2016). The epidemiological relationship with cigarette smoke and PD has been established. However, more research needs to be done to understand the functional relationship between cigarette smoke and PD.

Coffee

Coffee consumption has also been shown to significantly decrease PD risk, with consistent findings across multiple studies. Caffeine is a commonly consumed methylxanthine, thus belonging to a class of pharmacological molecules that have an antagonistic effect on the adenosine receptors (Yamada-Fowler and Soderkvist, 2015). Adenosine receptors modulate the nigrostriatal dopamine system, and

it has been shown that adenosine A2A antagonists reduce the amount of awake OFF time in PD

patients with motor control issues (Nicoletti et al., 2015). Furthermore, it appears that caffeine

attenuates the toxicity of alpha-synuclein aggregates, thus possibly reducing the impact of alpha-

synuclein related gene mutations (Yamada-Fowler and Soderkvist, 2015). A dose effect where

increasing cups leads to decreasing risk has also been observed. Odds ratio on the order of 0.44

(95% CI 0.23-0.83, P=0.01) were observed, with a further reduction in risk when the number of cups

per day increases (1-3 versus more than 3) (Polito et al., 2016). A recent GWAS identified GRIN2A, an

N-methyl-D-aspartate receptor (NMDA) glutamate-receptor subunit that is a part of the excitatory

pathways, as another disease modifier that reduces risk in conjunction with caffeine intake(Yamada-

Fowler and Soderkvist, 2015). In an ethnically homogenous Swedish cohort, there were both strong

joint effects of gene and caffeine on PD risk, and also a gene-caffeine interaction affect, which was

very mildly protective but significant (joint effect OR=0.38 95% CI (0.20-0.70), p=0.002 for TC heavy

caffeine versus CC light caffeine and gene-caffeine interaction (OR=0.998 95% CI (0.991-0.999) p-

value<0.001) (Polito et al., 2016, Hernan et al., 2002).The effect of caffeine also manifests itself in

tea consumption. However, contradictory findings between the protective effect of either black or

green tea indicate that further studies are needed to substantiate and clarify the role that tea plays

on the risk of developing PD.

Uric Acid

Uric acid (Urate) is found at high concentrations in the body. The metabolic end product of purines,

studies have shown it to have protective effects from degeneration of dopaminergic neurons. One

study investigated the effect of serum urate levels on PD risk, finding a protective effect from higher

serum urate levels on the likelihood of PD development. Mendelian Randomization (MR) has also

been utilized in the epidemiology of PD and serum urate levels (Simon et al., 2014). Using 80

patients with 3 SLC2A9 genotyped SNPs related to lower urate concentrations, Simon et al found an

increased hazard ratio for progression to dopaminergic treatment for patients with more minor

alleles of the three loci, thus tying more rapid progression of PD to lower serum urate levels.

Iron Levels

Pathological studies from post-mortem PD patients have found increased levels of iron in the SN and lateral globus pallidus (Dusek et al., 2012). MR is a powerful tool that that uses the genetic variant tied to a specific phenotype as an instrumental variable in epidemiological studies. Pichler et al use three genetic variables that affect iron levels, HFE rs1800562, HFE rs1799945, and TMPRSS6 rs855791 (Pichler et al., 2013). The three alleles are known to increase iron levels, confirmed with large scale meta-analyses of GWAS in general population (Pichler et al., 2013). Using MR, the study found a protective effect on the risk of PD development with one standard deviation unit increase in serum iron levels, with overall OR found to be 0.88 (95% CI: (0.82, 0.95), p=0.001). With a study size of over 21,000 individuals of European and Australia descent, the findings using MR suggest a causal relationship between iron levels and PD development (Pichler et al., 2013). The functional relationship remains unclear, but theories suggest that the reduction in free ferritin in the brain could decrease neuronal iron usage by lowering the amount of iron available for neuronal enzymes, thus possibly linking the commonly observed lower levels in the substantia nigra of iron in neurons in PD patients to a lower concentration of free iron (Zecca et al., 2004a, Zecca et al., 2004b). Mice studies similarly demonstrated that an iron-restricted diet related to the decline in the levels of striatal dopamine (Jellen et al., 2013). Thus, using MR, the study suggests a causal relationship between iron exposure and outcome, with more robust inference than one can make from a purely observational study.

Future directions

The epidemiology of PD has matured since its inception, however, we still only can account for a small fraction of the causality of PD cases. From the environmental perspective, while we have found strongly effective compounds that either increase or decrease disease risk, causality still remains an open question in many cases. Environmental factors do not have complete penetrance. From the genetics angle, only approximately 10% of sporadic PD cases are explained by monogenic

inheritance patterns of known genes. The incomplete penetrance of the mutations demonstrates that it is highly unlikely that the genes act alone to instigate disease. Unlike monogenic diseases such as Huntington's, one sees that the genetic basis of PD is complex and multi-faceted. Thus, further research in the field should not only concentrate on the discovery of novel SNPs, but also on the possibilities of genetic pleiotropy, cross-disease effects, gene-gene interactions, and gene-environment interactions and their modulation of the effect of the single SNPs.

Chapter 2 Results

The results section consists of the two published manuscripts, the relevant supplementary materials

pertaining to the publication, and the 3rd submitted manuscript. All results presented are first author

publications.

Large-scale assessment of polyglutamine repeat expansions in Parkinson disease

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ABSTRACT

Objectives: We aim to clarify the pathogenic role of intermediate size repeat expansions of SCA2, SCA3, SCA6, and SCA17 as risk factors for idiopathic Parkinson disease (PD).

Methods: We invited researchers from the Genetic Epidemiology of Parkinson's Disease Consortium to participate in the study. There were 12,346 cases and 8,164 controls genotyped, for a total of 4 repeats within the SCA2, SCA3, SCA6, and SCA17 genes. Fixed- and random-effects models were used to estimate the summary risk estimates for the genes. We investigated between-study heterogeneity and heterogeneity between different ethnic populations.

Results: We did not observe any definite pathogenic repeat expansions for SCA2, SCA3, SCA6, and SCA17 genes in patients with idiopathic PD from Caucasian and Asian populations. Furthermore, overall analysis did not reveal any significant association between intermediate repeats and PD. The effect estimates (odds ratio) ranged from 0.93 to 1.01 in the overall cohort for the SCA2, SCA3, SCA6, and SCA17 loci.

Conclusions: Our study did not support a major role for definite pathogenic repeat expansions in SCA2, SCA3, SCA6, and SCA17 genes for idiopathic PD. Thus, results of this large study do not support diagnostic screening of SCA2, SCA3, SCA6, and SCA17 gene repeats in the common idiopathic form of PD. Likewise, this largest multicentered study performed to date excludes the role of intermediate repeats of these genes as a risk factor for PD. *Neurology®* **2015;85:1283–1292**

GLOSSARY

AAO = age at onset; **CI** = confidence interval; **GEO-PD** = Genetic Epidemiology of Parkinson's Disease; **PD** = Parkinson disease; **SCA** = spinocerebellar ataxia.

Spinocerebellar ataxias (SCAs) represent a clinically and genetically diverse group of neurodegenerative diseases, which share degeneration of the cerebellum and its afferent and efferent connections, besides variable degeneration of multiple neurologic systems.[1] Expansions of trinucleotide repeats in the coding or untranslated regions of various genes cause several SCAs; these expansions also account for most of the clinical and genetic heterogeneity.[2] Emerging evidence provides tangible support to the growing consensus that clinically heterogeneous yet biologically overlapping late-onset neurodegenerative disorders may have common genetic risk factors that might change predisposition to the diseases.[2,3]

Whether polyglutamine repeat expansions in SCA genes such as SCA2, SCA3, SCA6, and SCA17 wield a similar effect in idiopathic Parkinson disease (PD) needs to be determined. Previous clinical and pathologic findings emphasize the need to evaluate the significance of polyglutamine repeat expansions of these genes in PD worldwide.[4–9] Most studies performed to date, including this study, are biased by case selection at specialist movement disorders clinics. However, to get a better estimate of the frequency of repeat expansions in such a setting, their relative contribution to disease worldwide, we performed a large multicenter study with members of the Genetic Epidemiology of Parkinson's Disease (GEO-PD) Consortium.

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METHODS Participants and samples. The GEO-PD Consortium includes researchers from 59 investigative sites, across 30 countries and 6 continents (http://www.geopd.org/about/); we invited all sites to participate in the study. Twenty-five sites from 20 countries and 4 continents contributed DNA samples and clinical data, resulting in 20,528 participants. Patients were diagnosed with PD by a movement disorders specialist using the standard criteria.[10–12] Controls at the date of examination were neurologically healthy, unrelated individuals free of PD or another associated movement disorder. Local sites collected demographically similar and sex-, age-matched neurologically healthy individuals as controls. Not all controls were given a detailed neurologic examination, but all were questioned about previous diagnoses or familial history of a neurologic disease. After quality control of data, a total of 20,510 samples were included (12,346 cases, 8,164 controls). The Caucasian series consisted of 16,819 (10,204 cases and 6,615 controls), and the Asian series consisted of 3,691 patients (2,142 cases and 1,549 controls). Patients with missing data were excluded from the relevant analysis. There were a total of 508 patients missing SCA2 genotype information, 445 missing SCA3, 861 missing SCA6, and 608 missing SCA17.

Genotyping. The SCA2, SCA3, SCA6, and SCA17 loci containing the CAG repeats were amplified with PCR using fluorescently labeled primers (primer sequences are available upon request). PCRs for SCA2, SCA3, and SCA17 were performed in one multiplex assay, SCA6 in a singleplex. All amplicons of one individual were pooled and separated by size using capillary electrophoresis on an ABI3730 sequencer. Data analysis was performed with GeneMapper 4.0 software. This included automatic sizing and allele calling. A total of 8 individuals (2 for each locus) were Sanger sequenced and the number of triplet repeats was counted. This information was used to convert amplicon lengths to repeat numbers.

Standard protocol approvals, registrations, and patient consents. The local ethics committee approved the study. All participants signed an informed consent.

Statistical analysis. We first generated distribution plots for SCA2, SCA3, SCA6, and SCA17 genes (see figure e-1 on the Neurology® Web site at Neurology.org) to estimate the repeats' cutoffs in our cohort. Based on our observation, expanding repeats for each gene were categorized into short, intermediate, and long repeats. Using the Monte Carlo simulation method (1,000 simulations) as implemented in the CLUMP, we compared the distribution of allele length of SCA genes to determine the significance of departure from the expected values between cases and controls.[13] Because CLUMP uses the Monte Carlo simulation method, all significances should be unbiased and robust to small expected values or continuity corrections.[13] We also assessed the correlation between age at onset (AAO) (5,310 cases) and polyglutamine repeats in our cohort. Likewise, in a subset of the data with the age at study available (14 sites, 4,400 controls, 5,310 cases), we analyzed the data with age at study as a covariate in the models. Finally, the association between SCA CAG expansion repeats and PD was evaluated using a logistic regression model with sex included as a covariate. Datasets from countries that included only cases were not included in the modeling because they lacked a proper control set, and thus could not be modeled using logistic regression. Fixed- and random-effects models estimated the odds ratios. Fixed-effect models assume that populations from different sites have the same risk effect from the repeat expansions and that observed differences are due to random

chance. For datasets containing between-study heterogeneity, fixed-effect estimates provide smaller confidence intervals (CIs) and p values, relative to random-effects models.[14–16] If, however, heterogeneity exists, the effects may diverge substantially across the populations. Random-effects models allow for random variation between the sites, therefore adjusting for genuine heterogeneity that may exist across different sites. We used the inverse variance method for fixed-effects models and the DerSimonian and Laird method for random-effects models.[17] To evaluate the between-site heterogeneity, we used the Cochran Q test of homogeneity and the I^2 metric. The I^2 parameter is bounded by 0 and 1 and estimates the proportion of heterogeneity that is highly unlikely due to random variation. A larger I^2 value implies more heterogeneity, with I^2 more than 0.75 or 75% indicating large heterogeneity. However, given that there exists significant imprecision in the estimation of I^2, particularly for variants with low minor allele frequency, we also provided the 95% CI of I^2.[16] The overall analysis considered all sites and populations regardless of ancestry. We then separately modeled the Caucasian and Asian sites. All statistical analyses were performed using R version 3.0.2, with package "metafor" for the random-effects logistic regression models. The p values are 2-tailed.

RESULTS A total of 25 sites contributed 12,346 patients with PD and 8,164 neurologically normal controls. Table 1 displays the characteristics of all participating sites. Nineteen sites contributed patients of Caucasian descent; 6 sites were from countries of Asian descent. The proportion of males ranged from 46% to 63% over the participating sites (table 1). The mean AAO of PD in this investigated population was 60 years. We excluded 2 sites that contributed only cases to avoid the influence of population substructuring (Japan and South Africa, 519 patients). Nevertheless, these 2 sites were analyzed independently to assess the expanded repeats. One German site contributed only cases, and allelic repeat density analysis did not show differences in repeat length between different German sites. Therefore, we decided to merge German sites into one data site titled "Germany" for further analyses, thus combining data from Deutschländer, Klein, and Gasser sites.

Expanding repeats of SCA genes in PD. Of 20,528 participants who were successfully genotyped, we did not observe any definite pathogenic repeat expansion for SCA2 (>32), SCA3 (>61), SCA6 (>19), and SCA17 (>47) genes in our cohort, thus excluding the role of definite pathogenic repeat expansion of these genes in PD.

Intermediate repeats and PD. The distribution of the cutoff repeat length of SCA genes as observed in the density distribution plots in our study is in agreement with previously published studies.[2,18–20] Furthermore, the histogram plots showed that the distribution of intermediate repeat length are similar for SCA2, SCA3, SCA6, and SCA17 genes independent of ethnicities.[20]

Using CLUMP, we did not observe differences in allele length distribution between cases and controls

Table 1 — Characterization of sites and overall database

Site	Country	Total	Cases	Controls	Male (%)	Mean AAO	Diagnostic criteria
Annesi	Italy	394	197	197	204 (51.8)	61.5	UKPDBB
Bardien/Carr	South Africa	398	398	0	246 (61.2)		UKPDBB
Bozi	Greece	218	114	104	105 (46.1)	69.9	UKPDBB
Brice	France	504	272	232	301 (59.7)	47.6	UKPDBB
Chung	Korea	1,900	1,200	700	876 (46.1)		UKPDBB
Deutschländer	Germany	140	70	70	80 (57.1)	69.7	UKPDBB
Garraux	Belgium	77	64	13	40 (51.9)	62.1	UKPDBB
Goldwurm	Italy	3,798	2,795	1,003	1,992 (52.4)		UKPDBB
Hadjigeorgiou	Greece	641	313	328	339 (52.9)	63.4	UKPDBB
Hattori	Japan	121	121	0	62 (51.2)		UKPDBB
Jeon	Korea	737	397	340	427 (57.9)		UKPDBB
Klein	Germany	320	317	3	185 (59.3)		UKPDBB
Krüger/Sharma/Gasser	Germany	1,909	1,219	690	1,149 (60.4)		UKPDBB
Lin	Taiwan	320	160	160	160 (50.0)	62.0	UKPDBB
Lynch/Ross	Ireland	700	339	361	322 (46.0)	50.5	UKPDBB
Mellick	Australia	1,809	893	916	929 (51.4)	59.0	Bower
Mok	China	390	214	176	232 (60.1)		UKPDBB
Morrison	United Kingdom	1,072	723	349	577 (53.9)	66.1	UKPDBB
Opala/Ross	Poland	614	352	262	358 (58.3)	50.2	UKPDBB
Rogaeva	Canada	562	391	171	296 (53.7)	49.7	UKPDBB
Tan	Singapore	344	171	173	217 (63.1)	59.7	UKPDBB
Toft	Norway	816	364	452	484 (59.3)		UKPBDD
Van Broeckhoven	Belgium	1,011	501	510	500 (49.6)	60.5	Pals/Gelb
Wirdefeldt	Sweden	260	67	193	128 (49.2)	65.8	Gelb
Wszolek/Ross	United States	1,455	69,4	761	764 (52.5)	64.4	UKPDBB
Total		20,510	12,346	8,164			

Abbreviations: AAO = age at onset; UKPDBB = United Kingdom Parkinson's Disease Brain Bank.

in the overall cohort (table e-1). Likewise, stratifying the analysis by ethnicity did not reveal associations; this suggests that intermediate repeats in SCA2, SCA3, SCA6, and SCA17 genes are not a major risk factor for PD (table e-2A).

Overall analysis. In the overall cohort, we observed no statistically significant associations between PD and intermediate repeat length for the SCA2, SCA3, SCA6, and SCA17 genes. The odds ratio ranged from 0.93 to 1.01 in the overall cohort (table 2, figure 1). We observed no heterogeneity for SCA3, SCA6, and SCA17 loci in our cohort, while SCA2 showed moderate heterogeneity; however, all heterogeneity 95% CIs contained 0 (table 2). Of note, we observed a p value of 0.013 (uncorrected) in our CLUMP

Table 2 — Overall analysis irrespective of ethnicity and influence of between-study heterogeneity

Locus	Gene name	Q test p value	OR (95% CI)	I²	RE p value	FE p value
SCA2	ATXN2	0.03	1.01 (0.78, 1.28)	37 (0, 82)	0.93	0.76
SCA3	ATXN3	0.66	0.93 (0.69, 1.26)	0 (0, 63)	0.64	0.64
SCA6	CACNA1A	0.87	1.00 (0.93, 1.06)	0 (0, 26)	0.90	0.90
SCA17	TBP	0.97	0.97 (0.56, 1.70)	0 (0, 6)	0.92	0.92

Abbreviations: ATXN2 = ataxin 2; ATXN3 = ataxin 3; CACAN1A = calcium channel, voltage dependent, P/Q type, alpha 1A subunit; CI = confidence interval; FE = fixed effects; OR = odds ratio; RE = random effects; TBP = TATA box binding protein.

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Figure 1 Forest plot of effect sizes of SCA2, SCA3, SCA6, and SCA17 loci in the overall cohort
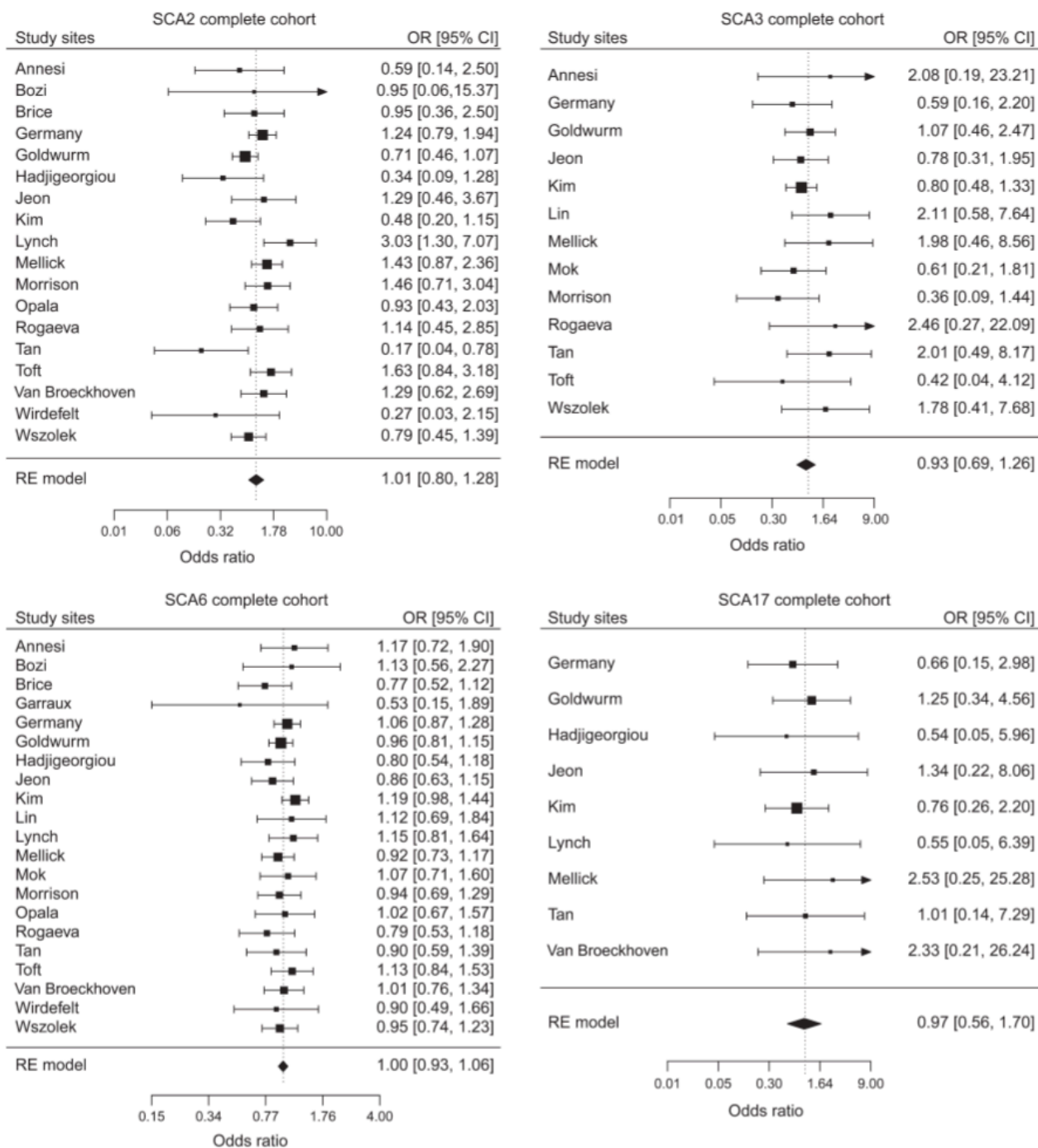
Boxes indicate the summary effect estimate. Germany site is a combination of Deutschländer, Klein, and Gasser sites. Axis scaled in relation to CIs. CI = confidence interval; OR = odds ratio; RE = random effects.

analysis for SCA6 in the overall cohort, but it was not significant after correcting for multiple testing (table e-1). The I^2 estimates ranged from 0% to 37% in the overall cohort. The Q test was not statistically significant for all SCA loci (table 2). Restricting the analysis to the Caucasian and Asian populations did not reveal an association between PD and intermediate repeat length. The odds ratio ranged from 0.97 to 1.09 for the Caucasian population, while for the Asian population, effect estimates ranged from 0.52 to 1.04 for SCA loci (figures 2 and 3 and table e-2A). We observed a trend for association for the SCA2 locus

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Figure 2 Forest plot of SCA2, SCA3, SCA6, and SCA17 loci in the Asian population
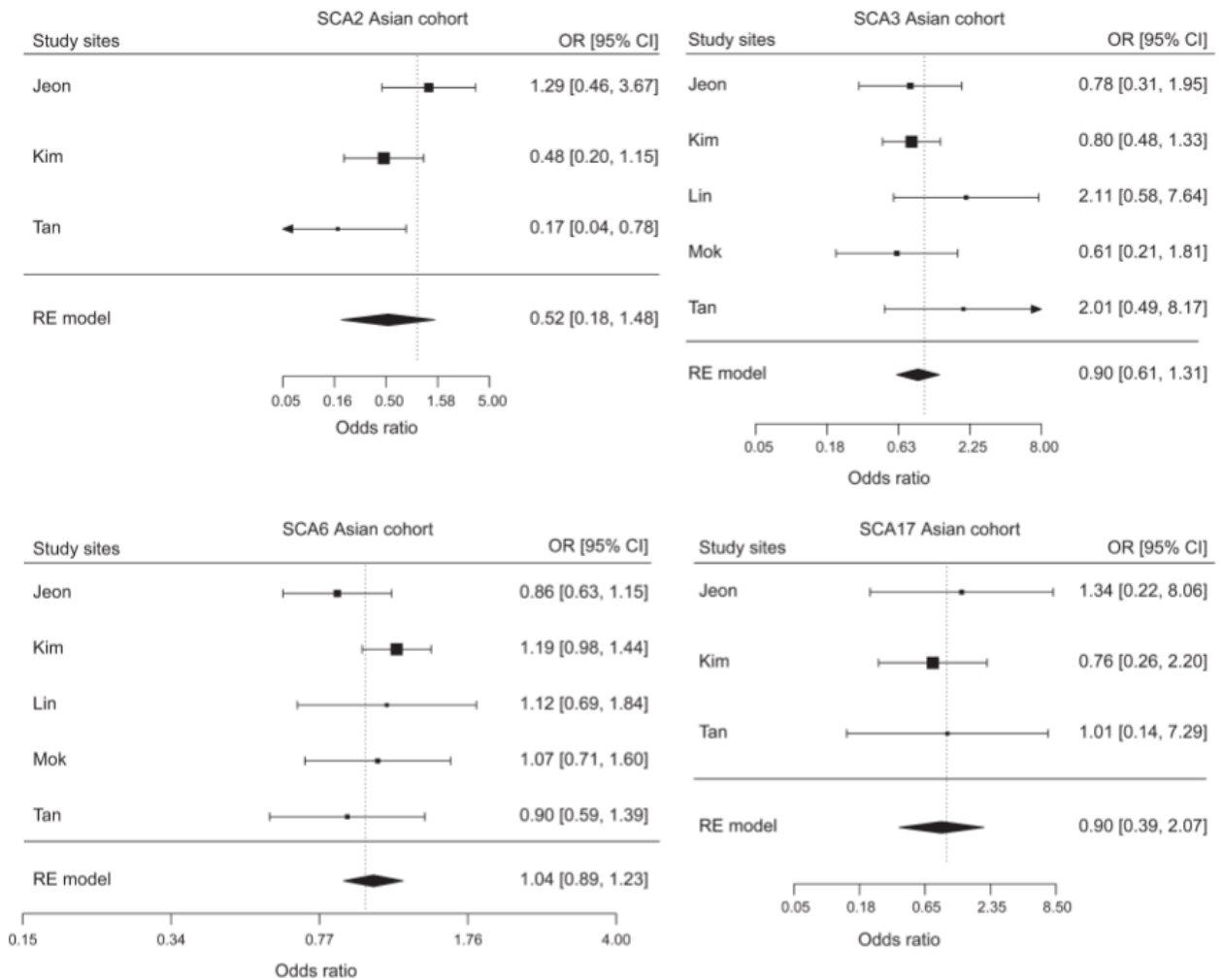
Boxes indicate the summary effect estimate. Germany site is a combination of Deutschländer, Klein, and Gasser sites. Axis scaled in relation to CIs. CI = confidence interval; OR = odds ratio; RE = random effects.

only in the Asian population with large heterogeneity, but this was not significant (table e-2A).

AAO analysis. In a subset of data with AAO available, we did not find any significance correlation between the SCA2, SCA3, SCA6, or SCA17 repeats and the AAO of PD (table e-2D). Likewise, stratifying by ethnicity, we did not observe any association between intermediate repeats. The effect estimates of SCA loci on AAO ranged from −0.79 to 2.13 for the overall cohort, and for the Caucasian population, effect estimates ranged from −0.13 to 4.76 (table e-2D). In addition, the age-adjusted analysis did not yield any significant association between SCA repeats and PD (table e-2C). We also performed random-effects models with the Student t test comparing the mean repeat length between cases and controls, and logistic regression models using repeat length as a quantitative trait. We did not observe a significant association between disease and repeat length ($p > 0.05$).

DISCUSSION The expansion of trinucleotide repeats has provided mechanistic explanations for human disorders. Besides defining autosomal dominantly inherited disease genes, variability in the distribution of repeat length as well as composition has remarkable influence on the disease phenotype; the longer the expansion, the earlier the AAO and the more aggressive the disease course.[1] Therefore, we performed a large-scale multicenter evaluation to assess the role of SCA2, SCA3, SCA6, and SCA17 gene repeats in PD. Our study excluded a major role of poly-(Q) repeat expansions for these genes in the causation of PD, at least in typical PD.

So far, there is no clear consensus on the appropriate threshold to understand the influence of

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Figure 3 Forest plot showing the comparison of effect of SCA2, SCA3, SCA6, and SCA17 loci in the Caucasian population

SCA2 Caucasian cohort

Study sites	OR [95% CI]
Annesi	0.59 [0.14, 2.50]
Bozi	0.95 [0.06, 15.37]
Brice	0.95 [0.36, 2.50]
Germany	1.24 [0.79, 1.94]
Goldwurm	0.71 [0.46, 1.07]
Hadjigeorgiou	0.34 [0.09, 1.28]
Lynch	3.03 [1.30, 7.07]
Mellick	1.43 [0.87, 2.36]
Morrison	1.46 [0.71, 3.04]
Opala	0.93 [0.43, 2.03]
Rogaeva	1.14 [0.45, 2.85]
Toft	1.63 [0.84, 3.18]
Van Broeckhoven	1.29 [0.62, 2.69]
Wirdefelt	0.27 [0.03, 2.15]
Wszolek	0.79 [0.45, 1.39]
RE model	1.09 [0.87, 1.38]

Odds ratio: 0.01 0.07 0.55 4.05 30.00

SCA3 Caucasian cohort

Study sites	OR [95% CI]
Annesi	2.08 [0.19, 23.21]
Germany	0.59 [0.16, 2.20]
Goldwurm	1.07 [0.46, 2.47]
Mellick	1.98 [0.46, 8.56]
Morrison	0.36 [0.09, 1.44]
Rogaeva	2.46 [0.27, 22.09]
Toft	0.42 [0.04, 4.12]
Wszolek	1.78 [0.41, 7.68]
RE model	1.00 [0.61, 1.64]

Odds ratio: 0.01 0.05 0.30 1.64 9.00

SCA6 Caucasian cohort

Study sites	OR [95% CI]
Annesi	1.17 [0.72, 1.90]
Bozi	1.13 [0.56, 2.27]
Brice	0.77 [0.52, 1.12]
Garraux	0.53 [0.15, 1.89]
Germany	1.06 [0.87, 1.28]
Goldwurm	0.96 [0.81, 1.15]
Hadjigeorgiou	0.80 [0.54, 1.18]
Lynch	1.15 [0.81, 1.64]
Mellick	0.92 [0.73, 1.17]
Morrison	0.94 [0.69, 1.29]
Opala	1.02 [0.67, 1.57]
Rogaeva	0.79 [0.53, 1.18]
Toft	1.13 [0.84, 1.53]
Van Broeckhoven	1.01 [0.76, 1.34]
Wirdefelt	0.90 [0.49, 1.66]
Wszolek	0.95 [0.74, 1.23]
RE model	0.98 [0.90, 1.05]

Odds ratio: 0.15 0.34 0.77 1.76 4.00

SCA17 Caucasian cohort

Study sites	OR [95% CI]
Germany	0.66 [0.15, 2.98]
Goldwurm	1.25 [0.34, 4.56]
Hadjigeorgiou	0.54 [0.05, 5.96]
Lynch	0.55 [0.05, 6.39]
Mellick	2.53 [0.25, 25.28]
Van Broeckhoven	2.33 [0.21, 26.24]
RE model	1.04 [0.48, 2.21]

Odds ratio: 0.05 0.59 7.00

Boxes indicate the summary effect estimate. Germany site is a combination of Deutschländer, Klein, and Gasser sites. Axis scaled in relation to CIs. CI = confidence interval; OR = odds ratio; RE = random effects.

intermediate repeat expansions in PD. We used our large cohort to estimate the global distribution of repeat length for SCA genes in PD. The allelic density as well as histogram distribution plots showed the threshold for intermediate repeats ranges from 24 to 32 for SCA2, 36 to 61 for SCA3, 11 to 19 for SCA6, and 42 to 47 for SCA17 in the PD cases. The intermediate range as observed in our study is in agreement with previously published studies.[21–26]

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In contrast, a recently published study from a Japanese population suggested that a population-specific SCA2 intermediate repeat cutoff length could influence the PD outcome.[27] Using a cutoff of 25, the authors observed a significant association for the autosomal dominant form of PD in their population.[27] By using this repeat length cutoff, as suggested by Yamashita et al.,[27] we did not observe significant association for the SCA2 locus, and thus our study did not support the notion that variability in cutoff repeat length varies from population to population (table e-2B). Likewise, our study excluded the role of population-specific intermediate repeat length variability on the risk of PD, at least in sporadic forms of PD. Of note, using the cutoff as observed in our study, we observed a trend (nonsignificant) for SCA2 locus in the Asian population. The proportion of intermediate carriers for SCA2 in our Asian population cohort is small (1.5%) and thus these results need to be interpreted cautiously.

Most, if not all, studies that have been published so far screened the SCA2, SCA3, SCA6, and SCA17 genes only in cohorts of autosomal dominant forms of PD,[21–26] and identified carriers for SCA2, SCA3, SCA6, and SCA17 repeats in different ethnic populations, which suggests that intermediate repeat structure influenced the clinical variability in autosomal dominant forms of PD and autosomal dominant cerebellar ataxia. For example, a previous French study identified 9 patients with PD who are carriers for SCA2 repeats.[28] They observed interrupted repeats for SCA2 as compared to the patients with autosomal dominant cerebellar ataxia who carry pure CAG repeats suggesting that differences in the repeat structure may lead to different phenotypes. Likewise, a study in Asian patients identified 7 SCA2 carriers that showed overlapping phenotype with ataxia such as dysarthria and postural instability.[7] However, such patients would not have been included in this study because the inclusion criterion was diagnosis of PD. Our study also did not investigate the role of interruptions in the repeats on PD, thus we cannot draw any conclusions for this subcategory of patients. It is worthwhile to mention that most of the participants in our cohort showed intermediate repeats in the normal range, and hence it will be unlikely that intermediate repeats will have an important role in PD. Nevertheless, deep sequencing of intermediate repeats should be pursued to resolve the role of intermediate repeats in PD, as emerging evidence has shown that genetic variations in these regions have an important role in explaining the missing heritability.[29,30]

Taken together, we examined the role of poly-(Q) repeats in PD using the largest sample size until now, and our results unequivocally show that polyglutamine repeats in SCA2, SCA3, SCA6, and SCA17 are unlikely to be clinically important risk factors for typical, idiopathic PD, without evidence of a family history of neurodegenerative disease (parkinsonism) or atypical signs (e.g., ataxia). Nevertheless, emerging genetic and functional evidence suggest that further studies of these genes in the context of other neurodegenerative diseases are justified.

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AUTHOR CONTRIBUTIONS

STUDY FUNDING

DISCLOSURE

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Comment:
CAG repeats in idiopathic Parkinson disease— To screen or not to screen

In this large study within the Genetic Epidemiology of Parkinson's Disease Consortium, Wang et al.[1] examined the relationship between idiopathic Parkinson disease (PD) and CAG repeat expansions in ataxia genes. They examined, on a larger scale, an issue that has been examined by several authors. The rationale for the study is that parkinsonian phenotypes, and even L-dopa–responsive PD, occur in carriers of SCA mutations, mainly in Asians, and that intermediate poly-Q expansions are predisposing factors for dominant PD.[2,3] The authors studied 12,346 patients with PD from Caucasian and Asian populations and 8,164 controls, seeking CAG expansions in SCA2, SCA3, SCA6, and SCA17 genes. The study is "negative" since they did not identify causative mutations or increased risk of PD attributable to long normal repeat alleles.

The major strength of the study is that this is the largest screening of ataxia loci in idiopathic PD and takes advantage of large samples of cases and controls from multiple international sites. The study is technically well executed: all samples were tested in one facility; quality monitored; internal standards run; and some samples were sequenced to confirm repeat length. Limitations of the study are that a similar number of patients and controls from the different centers would have been advisable, and that the full range of disease-associated CAG expansions (i.e., Huntington disease and SCA1) was not investigated.

These results suggest that the previously reported association between these loci and parkinsonism probably refers to familial or atypical forms and not to typical idiopathic PD.[3,4] The study therefore does not support genetic screening of SCA2, SCA3, SCA6, and SCA17 in idiopathic PD and excludes the role of intermediate alleles of these genes as risk factors for PD; this may redirect the field away from association studies of CAG repeats in ataxia genes and PD, a valuable contribution in and of itself.

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Study funding: No targeted funding reported.
Disclosure: The author reports no disclosures. Go to Neurology.org for full disclosures.

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Supplementary Materials

Figure e-1: Density Plots comparing Allelic Distribution of Repeat Lengths for SCA2, SCA3, SCA6, SCA17. Complete Cohorts.
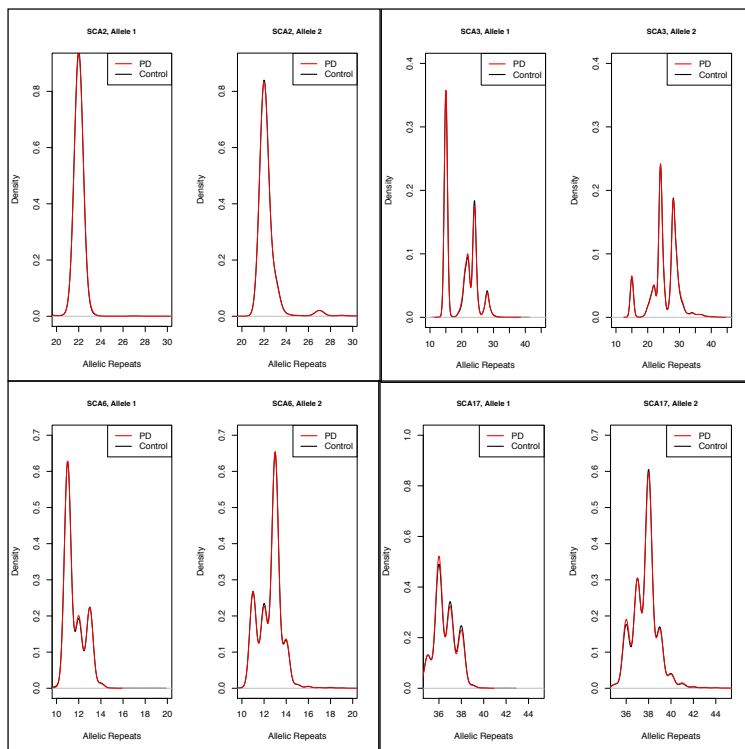
Table e-1a: Distribution of allele frequency of SCA loci

SCA	Cases (%)	Controls (%)	Chi-Square	All, pvalue	Asian, pvalue	Caucasian, pvalue
SCA2	8029 (41%)	11587 (59%)	2.1	0.35	0.15	0.31
SCA3	8005 (41%)	11662 (59%)	0.1	0.78	0.73	0.30
SCA6	7732 (40%)	11519 (50%)	6.2	0.01	0.39	0.13
SCA17	7926 (41%)	11578 (59%)	0.1	0.78	0.74	0.97

Comparison performed using CLUMP between cases and controls in overall cohort

Table e-2b: Overall effect estimates for SCA loci in Asian and Caucasian population

Cohort	Locus	Gene Name	Q test pvalue	OR (95% CI)	I^2	RE pvalue	FE pvalue
Asian	SCA2	ATXN2	0.08	0.52 (0.19, 1.48)	61 (0, 99)	0.22	0.07
	SCA3	ATXN3	0.45	0.90 (0.61, 1.31)	0 (0, 91)	0.57	0.57
	SCA6	CACNA1A	0.42	1.04 (0.87, 1.23)	21 (0, 83)	0.61	0.38
	SCA17	TBP	0.86	0.90 (0.39, 2.07)	0 (0, 80)	0.81	0.81
Caucasian	SCA2	ATXN2	0.12	1.09 (0.87, 1.38)	30 (0, 79)	0.46	0.40
	SCA3	ATXN3	0.58	1.00 (0.61, 1.64)	0 (0, 74)	0.99	0.99
	SCA6	CACNA1A	0.92	0.98 (0.90, 1.05)	0 (0, 29)	0.52	0.52
	SCA17	TBP	0.86	1.04 (0.49, 2.21)	0 (0, 63)	0.93	0.93

RE= Random Effects, FE= Fixed Effects, CI= Confidence Interval, OR= Odds Ratio

Table e-2c: Overall summary estimates for SCA2 stratified by ethnicity using repeat length cut-off of

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SCA2	Q test pvalue	OR (95% CI)	I^2	RE pvalue	FE pvalue
Complete	0.034	1.01 (0.80, 1.28)	37 (0, 83)	0.93	0.76
Asian	0.084	0.52 (0.19, 1.48)	61 (0, 100)	0.22	0.08
Caucasian	0.12	1.09 (0.87, 1.38)	30 (0, 79)	0.46	0.40

RE= Random Effects, FE= Fixed Effects, CI= Confidence Interval, OR= Odds Ratio

Part 2: Evaluation of the interaction between LRRK2 and PARK16 loci in determining risk of Parkinson's disease: analysis of a large multicenter study
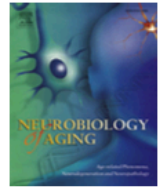
Negative results

Evaluation of the interaction between *LRRK2* and *PARK16* loci in determining risk of Parkinson's disease: analysis of a large multicenter study

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ARTICLE INFO

ABSTRACT

A recent study MacLeod et al. has shown that an interaction between variants at the *LRRK2* and *PARK16* loci influences risk of development of Parkinson's disease (PD). Our study examines the proposed interaction between *LRRK2* and *PARK16* variants in modifying PD risk using a large multicenter series of PD patients (7715) and controls (8261) from sites participating in the Genetic Epidemiology of Parkinson's Disease Consortium. Our data does not support a strong direct interaction between *LRRK2* and *PARK16* variants; however, given the role of retromer and lysosomal pathways in PD, further studies are warranted.

1. Introduction

Genetic discoveries made over the years either by using linkage, array, and/or exome-based approaches have helped in advancing our knowledge of the genetic underpinnings of Parkinson's disease (PD) (International Parkinson Disease Genomics Consortium et al., 2011; Lesage and Brice, 2009; Trinh and Farrer, 2013). As we discover new loci relevant to idiopathic PD pathogenesis, it has become imperative to also understand the gene–gene interaction effect in modulating PD risk in population (see Supplementary Information) (Elbaz et al., 2011). Although the results of most gene–gene interactions studies in PD to date have pointed toward independent effects for PD susceptibility variants, an exception to this has been an assessment of functional-genetic interaction between the *LRRK2* and *PARK16* loci in which overexpression of *RAB7L1*, a candidate gene for *PARK16* locus, reversed the effects of the *LRRK2* mutation and rescued the phenotypes (MacLeod et al., 2013). Therefore, this study aims to evaluate the interaction between several different *LRRK2* and *PARK16* variants in determining PD risk using a Caucasian series with more than 10,000 subjects from 14 different centers, and an Asian series with more than 5000 subjects from 5 different centers.

2. Methods

The Genetic Epidemiology of Parkinson's Disease (GEoPD) consortium includes investigators from 59 sites, across 30 countries and 6 continents, as of 2016. A total of 19 sites representing 17 countries and 4 continents agreed to contribute DNA samples and clinical data for the present study. In total, 15,976 subjects were included in this study, divided into a Caucasian series (5769 PD patients, 4988 controls) and an Asian series (1946 PD patients, 3273 controls). We selected 5 SNPs for the *PARK16* locus (rs823139 [*RAB7L1*], rs708725 [*RAB7L1*], rs823156 [*SLC41A1*], rs11240572 [*PM20D1*], and rs708723 [*RAB7L1*]) because previously published studies suggested associations with PD risk and the respective sites also provided coverage of the *PARK16* locus. We selected 2 SNPs from the *LRRK2* gene (rs1491942, rs7133914) due to previously demonstrated associations with PD and minor allele frequencies high enough to allow for reasonable interaction analysis. Analysis was performed separately for the Caucasian series, the Asian series,

and the combined series. We evaluated single variant associations using fixed effects logistic regression models adjusted for GEoPD site. Pairwise multiplicative interactions between *LRRK2* and *PARK16* variants were also examined using fixed effects logistic regression models. In addition to including terms for the given 2 individual variants and their interaction, these models were adjusted for the individual GEoPD site. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated. Subjects were coded as either 0 (absence of the minor allele) or 1 (presence of the minor allele) for each variant. Variants with a MAF of 10% or greater in both the Asian and Caucasian series were examined under an additive model, with the subject coded as (0,1,2), depending on the number of copies of the minor allele. To account for the 10 tests of *LRRK2-PARK16* interaction that were performed in each series (Caucasian, Asian, or combined), we utilized a Bonferroni correction for multiple testing separately in each series, after which 2-sided *p*-values of 0.005 or lower were considered as statistically significant. All statistical analyses were performed using R Statistical Software. The local Ethics Committee at each GEoPD site approved the study. All participants signed an informed consent.

3. Results

Of the 10 interactions that were examined between the *PARK16* and *LRRK2* variants, nonsignificant evidence of gene–gene interaction was observed between *LRRK2* rs1491942 and *PARK16* rs11240572 in the combined series (interaction OR: 0.97, 95% CI: 0.74–1.01, $p = 0.07$, Supplementary Table 1). *PARK16* rs11240572 appeared to have no effect on PD risk for individuals with the common GG genotype for *LRRK2* rs1491942, but a slight protective effect for those with GC and CC *LRRK2* rs1491942 genotypes (see Supplementary Information). Investigating this further in the stratified data (Supplementary Table 6), we observed for noncarriers of *PARK16* rs11240572, *LRRK2* rs1491942 a statistically significant higher risk of PD development in the Caucasian and combined series (OR 1.17 and 1.15, *p*-value <0.001). However, after correcting for multiple testing, it no longer approached statistical significance under the interaction model. There were no other noteworthy interactions between *LRRK2* rs1491942 and *PARK16* variants in any series (all interaction $p \geq 0.25$, Supplementary Tables 3–5), or between *LRRK2* rs7133914 and *PARK16* variants in the Caucasian series (all interaction $p \geq 0.096$,

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Supplementary Table 7). Interaction ORs ranged between 0.85 and 1.20, which supports the lack of a biologically meaningful interaction by lack of a notable deviation from an OR of 1. Between-site heterogeneity in interaction effects was generally relatively low (ranges between 0% and 35% with most around 0%), lending consistency to the lack of interaction. Models adjusted for age and gender using the subset of subjects with complete information and random effects models also produced similar results in gene−gene interaction analyses.

4. Discussion

The identification of genetic mutations in genes linked to familial forms of PD (e.g., *LRRK2*, *VPS35*, and *DNAJC13*), and genetic variability within the *PARK16* locus in genome wide association studies strongly implicates the role of retromer and lysosomal pathway in PD pathogenesis (Heckman et al., 2014; Soto-Ortolaza et al., 2013). Therefore, to understand the impact of interaction in world-wide populations, we performed a large multicenter study to assess the genetic evidence of interaction between *LRRK2* and *PARK16* locus. The results of our study do not provide evidence of a genetic interaction between *PARK16* and *LRRK2* variants with regard to risk of PD. Of note, the directionality of effect estimates, albeit with a much weaker effect size observed in the present study, involving the specific *LRRK2* rs1491942/*PARK16* rs11240572 interaction, are in agreement with previously published findings. Genetic interaction studies are limited by sample size and power because the variable of focus in an interaction study is the presence of the genotype of interest for both variants, and this occurs much less frequently than in the individual variant genotypes.

Therefore, even with our large sample size, power is still limited to detect moderate to small gene−gene interaction effects. Although there was some degree of concordance between our interaction findings and those that were previously reported, our results were much weaker than the strong *LRRK2-PARK16* interaction that was previously reported (Beilina et al., 2014; MacLeod et al., 2013). Even with the large GEoPD sample size, which we have accrued to perform the present study, we are likely underpowered to detect weaker interaction effects. In addition, lack of genetic interaction does not exclude the presence of cellular or functional interaction. However, such genetic studies will be critical if we are to understand the role of gene−gene interaction in disease susceptibility.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2016.09.022.

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Supplementary Materials

1.   **Introduction**

While a comprehensive evaluation of interactions of PD susceptibility has yet to be performed, several studies have investigated the interactions between various genes of interest[1-4]. Although the results of most gene-gene interactions studies in PD to date have pointed toward independent effects for PD susceptibility variants, an exception to this has been assessments of functional-genetic interaction between *LRRK2* and *PARK16* locus[5-7], with a previous study demonstrating through protein-protein interaction arrays that *RAB7L1* is a binding partner for *LRRK2*[5] More recently, Kuwahara et al also demonstrated that orthologues of *LRRK2* and *RAB7L1* in C. elegans neurons work together concomitantly in an ordered pathway to determine axonal length[6]. One recent study also found function evidence that overexpression of *RAB7L1,* a candidate gene for *PARK16* locus, reversed the effects of the *LRRK2* mutation and rescued the phenotype[8]. Furthermore, effect of the *LRRK2* risk variant (rs1176052) was negated in individuals with a copy of the protective allele for the *PARK16* variant (rs823114)[8]. This initial identification of an interaction between *LRRK2* and *PARK16* regarding susceptibility to PD leads to several important follow-up questions, such as whether the interaction is still evident when even larger series are examined, whether it is consistent for subjects of differing ethnicities and from different geographic regions, and whether it remains apparent for other variants in the two loci. Therefore, the aim of this study was to evaluate the interaction between several different *LRRK2* and *PARK16* variants in determining PD risk using a Caucasian series consisting of more than 10,000 subjects from 14 different centers, and an Asian series comprised of more than 5,000 subjects from 5 different centers.

2.   **Methods**

2.1 Participants

Diagnosis of PD was made according to standard criteria[9,10]. Controls

were individuals free of any extra-pyramidal disorder. All subjects were unrelated, and carriers of

pathogenic *LRRK2* mutations were excluded. Demographic information of PD patients and controls

for each GEoPD site is provided in Supplementary Table 1. We selected *LRRK2* rs1491942 and *LRRK2*

rs7133914 in concordance with previously demonstrated associations[11,12]. Of note, *LRRK2* rs7133914

(*LRRK2* p.R1398H) is part of a 3-SNP haplotype (p.N551K-R1398H-K1423K) that has been shown to

be protective for PD, and was selected for inclusion for this study as it has been shown to be the

most likely functional variant on the haplotype[13,14]. The Japanese site from our Asian series utilized

genotype data from a previous GWAS[15]. If the *LRRK2* and *PARK16* SNPs of interest in this study were

not directly genotyped in Japanese GWAS cohort, we used proxy SNPs with r^2 values >0.8 to fully

capture genetic information. Using these proxy criteria, we were able to match exact SNPs from the

Japanese GWAS cohort for four SNPs (rs708725, rs823139, rs823156, rs11240572) within the

PARK16 locus, and a single proxy SNP, rs2201144, was identified for *LRRK2* rs1491942. *LRRK2*

rs7133914 was not genotyped in the Asian series and was genotyped for only a subset of the

Caucasian series (3622 patients, 3042 controls). Furthermore, Haploview was used to measure the

LD coefficient between SNPs used in our study and with that of MacLeod et al[5]. All variants followed

Hardy Weinberg Equilibrium (HWE) in controls. All genotype call rates were >97%. We found limited

linkage disequilibrium between the variants within the same gene or locus (r^2<0.50 in all cases).

The Department of Human Genetics of the Helmholtz Center served as the genotyping core and

performed all genotyping (Munich). Each site sent 100–200 ng of DNA to the laboratory core. Case-

control status was blinded at the genotyping code for each site. We performed the genotyping on a

matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry on a

MassArray system (Sequenom, San Diego, CA). A mass spectrometer analyzed the cleaned extension

products (Bruker Daltronik, USA), and the MassArray Typer 4.0.2.5 software was used for peak

identification (Sequenom). The AssayDesigner software 4.0 (Sequenom) was used for assay design,

with the default parameters for the iPLEX Gold chemistry and the Human GenoTyping Tools ProxSNP and PreXTEND (Sequenom). One multiplex assay was used to genotype all of the variants. To check genotype clustering, an experienced investigator blinded to patient affection status visually reviewed the sample results.

2.4 Statistical analysis

Sensitivity of the results of gene-gene interaction analysis to the use of random effects models was also considered[16]. Not all of the sites had complete information on age and gender, and thus we performed secondary analyses with adjustment for age and gender. Cochran's Q test of homogeneity was used to evaluate between-site heterogeneity, along with the I^2 metric, which is interpreted as the proportion of variation in between-site interaction ORs that is due to heterogeneity beyond chance[17,18].

To aid in the interpretation of tests of interaction, we also combined the two variants involved in the given interaction into one variable, allowing for a different category for each different genotype combination. The association between this genotype combination variable and PD was then evaluated using a fixed effects logistic regression model adjusted for GEO-PD site, where the most common genotype combination was the reference category, and ORs and 95% CIs were estimated in relation to this reference category. P-values were also calculated for comparison of the reference category, though the ORs and 95% CIs are of most interest in interpreting the interaction (or lack thereof) between the given two variants and p-values are presented mostly for completeness. We refer to these analyses where the joint effect of the given two SNPs on PD risk is being examined as "tests of association". To be clear, these tests of association are presented only to assist in the interpretation of the aforementioned gene-gene interactions, and do not represent tests of interaction themselves[19].

Variants with a minor allele frequency (MAF) less than 10% in either one of the series (Caucasian or Asian) were evaluated under a dominant model (i.e. presence vs. absence of the minor allele) in all

analyses to maintain consistency of statistical models and allow for comparison of results between series. Subjects were coded as either 0 (absence of the minor allele) or 1 (presence of the minor allele) for each variant. Variants with a MAF of 10% or greater in both the Asian and Caucasian series were examined under an additive model (i.e. effect of each addition allele), with the subject coded as (0,1,2), depending on the number of copies of the minor allele. Under this rule, *LRRK2* variant rs7133914 and *PARK16* rs11240572 were coded under the dominant scheme, and *LRRK2* 1491942 and *PARK16* rs823139 [*RAB7L1*], rs70875 [*RAB7L1*], rs823156 [*SLC41A1*], and rs708723 [*RAB7L1*] were coded under the additive scheme. All statistical tests were two-sided, with multiple testing correction set at 0.005 for 2-sided p-value, due to the 10 different combinations of SNPs. All statistical analyses were performed using R Statistical Software (version 3.0.2, R Foundation for Statistical Computing, Vienna, Austria).

3. Results

A total of 19 sites contributed 7627 PD patients and 8261 neurologically normal patients (Supplementary Table 1). Out of the 19 sites, 14 sites contributed data of Caucasian ancestry and five sites contributed data of Asian ancestry. The proportion of men ranged from 41.6% to 67.8% in PD patients and 35.1% to 62.3% in controls (Supplementary Table 1). The mean age at onset was 68.1 years for PD patients, and comparable to the mean age of study of healthy controls was 67.5 years (Supplementary Table 1). Of note, given the homogenous nature of the Belgium population, we combined the data from the two Belgium sites (Garraux and von Broeckhoven) in all analyses due to the smaller sample size of the Garraux site (Supplementary Table 1).

Associations of each of the eight individual *PARK16* and *LRRK2* variants with risk of PD are shown in Supplementary Table 2. Although some of these associations have largely been shown previously, it is helpful to first understand single-variant associations before examining interactions. *LRRK2* rs1491942 was associated with an increased risk of PD in the Caucasian series (OR: 1.16, P=2.2E-05) but not the Asian series (OR: 1.10, P=0.03) (Supplementary Table 2). For *PARK16*, a risk effect was observed for rs708723 in the Asian series (OR: 1.76, P=0.00011) but not Caucasian series (OR: 1.03,

P=0.32), while protective effects were noted for both rs708725 in the Caucasian series and for

rs708725, rs823139, rs823156, and rs11240572 in the Asian series (Supplementary Table 2).

Supplementary Tables 3-5 contain the results for the multiplicative interaction analysis between

LRRK2 rs1491942 and *PARK16* SNPs, for both the combined and the individual Caucasian and Asian

series. Supplementary Table 6 shows the results for the stratified analysis between *LRRK2* rs1491942

and *PARK16* rs11240572, where we observe a trend in the stratified analysis; however the

multiplicative interaction is not significant after multiple testing correction. Supplementary Table 7

displays the results of the multiplicative interaction between *LRRK2* rs7133914 and the *PARK16*

SNPs, no interaction effects of note were observed.

4. Discussion

The identification of genetic mutations in genes linked to familial forms of PD (e.g. *LRRK2*, *VPS35*,

DNAJC13) and genetic variability within the *PARK16* locus in GWAS strongly implicates the role of

retromer and lysosomal pathway in PD pathogenesis[20,21]. Mutant forms of *LRRK2* have been shown

to affect *RABL71* dependent lysosomal clustering and thus linking endosomal pathway to PD.

Additionally, previous functional studies have demonstrated that *LRRK2* and *RAB7L1* work together

in regulating axonal elongation[6]. Interestingly, deficiency of the *PARK16* locus candidate gene,

RAB7L1, in mammalian or Drosophila dopamine neurons causes neurodegeneration; in contrast,

overexpression of the *RAB7L1* rescued the *LRRK2* mutant phenotype suggesting that both *RAB7L1*

and *LRRK2*genes bound together and functionally interact with each other in regulating neurite

length process in vitro and in-vivo[7]. Therefore, to understand the impact of interaction in world-wide

populations, we performed a large-multi-center study to assess the genetic evidence of interaction

between *LRRK2* and *PARK16* locus.

The results of our study do not provide evidence of a genetic interaction between *PARK16* and

LRRK2 variants with regard to risk of PD. Heterogeneity in between-site interaction OR estimates

was minimal, thus the lack of interaction was consistently observed. It is unlikely that our results are influenced by analyzing different SNPs as compared to MacLeod study. Indeed, we observed high D' prime (range 0.5 -1.0) between our *LRRK2* SNPs and SNPs analyzed in a previously published study, and similarly for our *PARK16* SNPs (range 0.15-1) indicating that our SNPs fully captured the genetic information covered by MacLeod et al.

Of note, the directionality of effect estimates, albeit with a much weaker effect size observed in the present study, involving the specific *LRRK2* rs1491942/*PARK16* rs11240572 interaction are in agreement with previously published findings[8]. Specifically, while both studies observed ORs very close to 1 for the *LRRK2* variant that was examined in the given study in carriers of the rare *PARK16* allele, ORs associated with the *LRRK2* variant were greater than 1 in our study (ORs of 1.13 and 1.17 in the Asian and Caucasian series) and in the MacLeod study (ORs ranging from 1.31 to 2.49 in the 4 different series examined) for non-carriers of the *PARK16* minor allele.

Several limitations of our study should be noted. Genetic interaction studies are limited by sample size and power due to the fact that variable of focus in an interaction study is the presence of the genotype of interest for both variants, and this occurs much less frequently than the individual variant genotypes. As a result, even with our large sample size, power is still limited to detect moderate to small gene-gene interaction effects. Therefore, the possibility of a false-negative finding is important to bear in mind, and 95% confidence limits for interaction OR estimates should be considered when interpreting results. Finally, we cannot exclude the possibility that population stratification could have had an impact on our results, however our analysis adjusting for GEO-PD site would have accounted for that to a large extent.

In conclusion, our study does not provide strong evidence to support previous findings that *LRRK2* and *PARK16* variants may interact in determining risk of PD for a given individual. However, there

was some degree of concordance between our interaction findings and those that were previously

reported, with the caveat that our results were much weaker than the strong *LRRK2-PARK16*

interaction that was previously reported[7]. Larger series will be needed to resolve whether a true

LRRK2-PARK16 interaction occurs. Of note, even with the large GEoPD sample size, which we have

accrued to perform current study, we are likely underpowered to detect weaker interaction effects;

however such studies will be critical if we are to understand the role of gene-gene interaction in

disease susceptibility.

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Supplementary Table 1: Descriptive Statistics of Individual Sites

Site	Country	No. of PD Cases	No. of Controls	No. (%) of Males in PD cases	No. (%) of Males in Controls	Mean(SD) Age in PD Cases	Mean(SD) Age in controls	Diagnostic Criteria
Caucasian series								
Aasly	Norway	527	510	314 (59%)	276 (54%)	73.8 (10.6)	71.1 (10.6)	UKPDBB
Annesi	Italy	178	182	98 (53.6%)	93 (51.1%)	65.6 (9)	61.9 (9)	UKPDBB
Bozi	Greece	113	104	55 (48.7%)	49 (47.1%)	75.7 (7.3)	73.0 (7.3)	UKPDBB
Brice	France	272	231	168 (61.8%)	132 (57.1%)	57.7 (11.5)	57.8 (11.5)	UKPDBB
Garraux	Belgium	64	14	34 (53.1%)	7 (50%)	70.5 (9.6)	66.1 (9.6)	UKPDBB
Hadjigeorgiou	Greece	313	328	170 (54.3%)	169 (51.5%)	64.7 (9.8)	63.2 (9.8)	UKPDBB
Lynch/Ross	Ireland	328	345	183 (55.8%)	121 (35.1%)	68.1 (10.3)	73.4 (10.3)	UKPDBB
Maragonore	USA	801	223	491 (62.4%)	109 (62.6%)	61.7 (11)	NA	UKPDBB
Mellick	Australia	893	916	556 (62.3%)	373 (40.7%)	73.6 (11.1)	71.7 (11.1)	Bower
Morrison	England	723	349	490 (67.8%)	87 (25.1%)	66.2 (10.8)	NA	UKPDBB
Opala/Ross	Poland	291	255	186 (63.9%)	113 (44.3%)	70.9 (10.5)	65.0 (10.5)	UKPDBB
van Broeckhoven	Belgium	497	510	286 (57.1%)	214 (42.1%)	68.1 (10.6)	64.7 (10.6)	Pals/ Gelb
Wirdefeldt	Sweden	77	194	41 (53.2%)	93 (47.9%)	72.3 (8.4)	71.0 (8.4)	UKPDBB
Wszolek/Ross	USA	692	827	440 (63.6%)	354 (42.8%)	74.6 (11.4)	70.1 (11.4)	UKPDBB
Total		7715	8261					
Asian series								
Chung	Korea	368	333	153 (41.6%)	140 (42%)	58 (9.6)	66.1 (9.6)	UKPDBB
Lin	Taiwan	160	160	80 (50%)	80 (50%)	70.8 (9.8)	70.8 (9.8)	UKPDBB
Mok	China	259	78	158 (61.5%)	52 (66.7%)	64.8 (4.1)	62.5 (4.1)	UKPDBB
Satake/Toda	Japan	988	2521	405 (41.0%)	1525 (60.5%)	58.8 (10.1)	49.9 (14.2)	UKPDBB
Tan	Singapore	171	181	110 (64.3%)	112 (61.9%)	62.4 (9.3)	45.2 (9.3)	UKPDBB
Total		1946	3273					

No.= number, NA indicates that data was unavailable

Supplementary Table 2: Associations and Minor Allele Frequency (MAF) of individual *LRRK2* and *PARK16* variants with risk of PD in Caucasian and Asian Series. MAF was calculated separately for Asian and Caucasian series.

Caucasian Series

SNP	MAF	Minor Allele	OR	Pvalue
LRRK2 rs1491946	21.2%	C	1.16 (1.10 ,1.24)	<0.001
LRRK2 rs7133914	6.9%	A	0.93 (0.81 ,1.08)	0.36
PARK16 rs708273	42.6%	T	1.03 (0.97 ,1.09)	0.32
PARK16 rs708725	48.4%	C	0.91 (0.86 ,0.97)	0.004
PARK16 rs823139	12.0%	T	0.91 (0.84 ,1.00)	0.038
PARK16 rs823156	18.2%	G	0.94 (0.87 ,1.01)	0.09
PARK16 rs11240572	3.3%	A	1.09 (0.93 ,1.28)	0.28

Asian Series

SNP	MAF	Minor Allele	OR	Pvalue
LRRK2 rs1491946	31.8%	C	1.10 (1.00 ,1.21)	0.03
PARK16 rs708723	14.0%	T	1.76 (1.30 ,2.35)	<0.001
PARK16 rs708725	47.3%	C	0.82 (0.75 ,0.90)	<0.001
PARK16 rs823139	14.1%	T	0.78 (0.69 ,0.88)	<0.001
PARK16 rs823156	20.7%	G	0.76 (0.68 ,0.85)	<0.001
PARK16 rs11240572	14.7%	A	0.78 (0.68 ,0.89)	<0.001

OR (Odds Ratio)

Supplementary Table 3: Evaluation of interactions of *LRRK2* rs1491942 and *PARK16* variants with regard to susceptibility to Parkinson's disease in the Combined Series

LRRK2 Variant/Genotype	*PARK16* Variant/Genotype	Sample genotype count and frequency	Test of Association OR	p-value	Models for the *LRRK2* and *PARK16* SNPs and Test of Interaction Results
LRRK2 rs1491942	rs708273				Additive/Additive Model
GG	GG	2601 (21.7%)	1.00 (reference)	N/A	OR: 1.0 (0.9, 1.11)
GG	GT	3288 (27.5%)	1.02 (0.92, 1.10)	0.75	p=0.98
GG	TT	1185 (9.9%)	1.02 (0.9, 1.15)	0.75	
GC	GG	1725 (14.4%)	1.11 (0.94, 1.30)	0.21	Heterogeneity: I^2=0, p=0.49
GC	GT	1824 (15.2%)	1.08 (0.94, 1.24)	0.25	
GC	TT	673 (5.6%)	1.2 (1.04, 1.37)	0.011	
CC	GG	315 (2.6%)	1.11 (0.9, 1.35)	0.32	
CC	GT	260 (2.2%)	1.37 (1.06, 1.78)	0.018	
CC	TT	93 (0.8%)	1.64 (1.21, 2.22)	0.001	
LRRK2 rs1491942	rs708725				Additive/Additive Model
GG	TT	2291 (15.8%)	1.00 (reference))	N/A	OR: 0.98 (0.90, 1.06)
GG	TC	3884 (26.7%)	0.85 (0.76, 0.95)	0.00365	p=0.55
GG	CC	2066 (14.2%)	0.82 (0.72, 0.94)	0.00332	
GC	TT	1579 (10.9%)	1.22 (1.06, 1.39)	0.00418	Heterogeneity: I^2=0, p=0.12
GC	TC	2510 (17.3%)	0.9 (0.8, 1.02)	0.0968	
GC	CC	1266 (8.7%)	0.91 (0.78, 1.06)	0.237	
CC	TT	249 (1.7%)	1.38 (1.05, 1.82)	0.0227	

CC	TC	448 (3.1%)	1.14 (0.92, 1.41)	0.232	
CC	CC	228 (1.6%)	1.15 (0.85, 1.54)	0.372	
LRRK2 rs1491942	rs823139				Additive/Additive Model
GG	GG	6825 (43.6%)	1.00 (reference)	N/A	OR: 1.01 (0.92, 1.11)
GG	GT	1945 (12.4%)	0.82 (0.74, 0.92)	0.00036	p=0.87
GG	TT	144 (0.9%)	1.08 (0.76, 1.53)	0.663	
GC	GG	4331 (27.7%)	1.12 (1.04, 1.22)	0.00483	Heterogeneity: I²=0, p=0.56
GC	GT	1295 (8.3%)	0.91 (0.8, 1.03)	0.154	
GC	TT	103 (0.7%)	1.08 (0.72, 1.62)	0.702	
CC	GG	762 (4.9%)	1.35 (1.15, 1.58)	0.000244	
CC	GT	218 (1.4%)	1.25 (0.94, 1.66)	0.127	
CC	TT	16 (0.1%)	0.7 (0.25, 1.96)	0.491	
LRRK2 rs1491942	rs823156				Additive/AdditiveModel
GG	AA	5734 (37.6%)	1.00 (reference)	N/A	OR: 0.99 (0.88, 1.11)
GG	AG	2580 (16.9%)	0.89 (0.81, 0.98)	0.0228	p=0.83
GG	GG	346 (2.3%)	0.76 (0.6, 0.96)	0.0204	
GC	AA	3687 (24.2%)	1.14 (1.05, 1.24)	0.00316	Heterogeneity: I²=33, p=0.56
GC	AG	1681 (11%)	0.98 (0.87, 1.1)	0.679	
GC	GG	234 (1.5%)	0.95 (0.72, 1.27)	0.744	
CC	AA	635 (4.2%)	1.32 (1.11, 1.57)	0.00148	
CC	AG	285 (1.9%)	1.29 (1.01, 1.67)	0.0453	
CC	GG	55 (0.4%)	0.97 (0.54, 1.75)	0.92	
LRRK2 rs1491942	rs11240572				Additive/Dominant Model
GG	CC	7839 (50%)	1.00 (reference)	N/A	OR: 0.87 (0.74, 1.01)
GG	CA+AA	1095 (7%)	0.99 (0.86, 1.13)	0.844	p=0.069
GC	CC	4903 (31.3%)	1.14 (1.06, 1.23)	0.000512	
GC	CA+AA	834 (5.3%)	0.91 (0.78, 1.06)	0.234	Heterogeneity: I²=0, p=0.99
CC	CC	834 (5.3%)	1.39 (1.19, 1.62)	2.16E-05	
CC	CA+AA	167 (1.1%)	1.13 (0.82, 1.57)	0.447	

OR (Odds Ratio), Tests of Interaction presents results of Fixed Effects Model with OR and 95% Confidence Interval (CI), Models were either *LRRK2* Additive/*PARK16* Additive, *LRRK2* Additive/*PARK16* Dominant, or *LRRK2* Dominant/*PARK16* Dominant (when MAF<15%, then Dominant scheme was used)

Supplementary Table 4: Evaluation of interactions of *LRRK2* rs1491942 and *PARK16* variants with regard to susceptibility to Parkinson's disease in the Caucasian Series

LRRK2 Variant/Genotype	*PARK16* Variant/Genotype	Sample genotype count and frequency	Test of Association OR	p-value	Models for the *LRRK2* and *PARK16* SNPs and Test of Interaction Results
			OR	p-value	
LRRK2 rs1491942	rs708273				Additive/AdditiveModel
GG	GG	2091 (20.2%)	1.00 (reference)	N/A	OR: 1.0 (0.88, 1.14)
GG	GT	3203 (30.9%)	1.02 (0.91, 1.14)	0.72	p=0.95
GG	TT	1130 (10.9%)	1.14 (0.99, 1.33)	0.074	
GC	GG	1111 (10.7%)	1.12 (0.96, 1.3)	0.15	Heterogeneity: I^2=0, p=0.48
GC	GT	1730 (16.7%)	1.19 (1.04, 1.36)	0.009	
GC	TT	625 (6%)	1.08 (0.9, 1.30)	0.39	
CC	GG	155 (1.5%)	1.61 (1.15, 2.27)	0.006	
CC	GT	230 (2.2%)	1.55 (1.17, 2.06)	0.002	
CC	TT	74 (0.7%)	1.52 (0.93, 2.48)	0.092	
LRRK2 rs1491942	rs708725				Additive/Additive Model
GG	TT	1626 (17.1%)	1.00 (reference)	N/A	OR: :1.02 (0.88, 1.18)
GG	TC	2728 (28.7%)	0.89 (0.78, 1.01)	0.066	p=0.83
GG	CC	1540 (16.2%)	0.85 (0.73, 0.99)	0.042	
GC	TT	960 (10.1%)	1.17 (1.00, 1.38)	0.058	Heterogeneity: I^2=0, p=0.16
GC	TC	1481 (15.6%)	0.95 (0.82, 1.10)	0.51	
GC	CC	770 (8.1%)	0.96 (0.79, 1.16)	0.66	
CC	TT	116 (1.2%)	1.33 (0.9, 1.96)	0.16	
CC	TC	194 (2%)	1.52 (1.11, 2.08)	0.009	
CC	CC	100 (1.1%)	1.26 (0.8, 1.98)	0.33	
LRRK2 rs1491942	rs823139				Additive/Additive Model
GG	GG	5035 (48.1%)	1.00 (reference)	N/A	OR: 0.99 (0.90, 1.10)
GG	GT	1374 (13.1%)	0.86 (0.76, 0.98)	0.018	p=0.59
GG	TT	91 (0.9%)	1.11 (0.72, 1.71)	0.63	
GC	GG	2708 (25.9%)	1.11 (1.01, 1.22)	0.03	Heterogeneity: I^2=0, p=0.54
GC	GT	742 (7.1%)	0.97 (0.83, 1.14)	0.71	
GC	TT	58 (0.6%)	1.19 (0.70, 2.02)	0.52	
CC	GG	361 (3.4%)	1.46 (1.17, 1.83)	<0.001	
CC	GT	91 (0.9%)	1.69 (1.08, 2.64)	0.021	
CC	TT	10 (0.1%)	0.65 (0.18, 2.32)	0.50	
LRRK2 rs1491942	rs823156				Additive/Additive Model
GG	AA	4151 (41.2%)	1.00 (reference)	N/A	OR: 0.95 (0.86, 1.05)
GG	AG	1882 (18.7%)	0.94 (0.84, 1.05)	0.27	p=0.87
GG	GG	209 (2.1%)	0.87 (0.66, 1.16)	0.35	
GC	AA	2278 (22.6%)	1.12 (1.01, 1.25)	0.034	Heterogeneity: I^2=23, p=0.37
GC	AG	996 (9.9%)	1.12 (0.97, 1.30)	0.11	
GC	GG	108 (1.1%)	0.84 (0.56, 1.24)	0.38	
CC	AA	301 (3%)	1.48 (1.15, 1.89)	0.002	
CC	AG	124 (1.2%)	1.59 (1.09, 2.32)	0.016	

CC	GG	17 (0.2%)	0.84 (0.31, 2.22)	0.72	
LRRK2 rs1491942	rs11240572				Additive/Dominant Model
GG	CC	6094 (57.9%)	1.00 (reference)	N/A	OR:0.89 (0.68, 1.17)
GG	CA+AA	434 (4.1%)	1.15 (0.94, 1.40)	0.17	p=0.25
GC	CC	3300 (31.4%)	1.12 (1.03, 1.23)	0.009	
GC	CA+AA	223 (2.1%)	1.06 (0.81, 1.40)	0.67	Heterogeneity: I²=0, p=0.87
CC	CC	433 (4.1%)	1.51 (1.23, 1.86)	<0.001	
CC	CA+AA	33 (0.3%)	1.65 (0.8, 3.40)	0.17	

OR (Odds Ratio), Tests of Interaction presents results of Fixed Effects Model with OR and 95% Confidence Interval (CI), Models were either *LRRK2* Additive/*PARK16* Additive, *LRRK2* Additive/*PARK16* Dominant, or *LRRK2* Dominant/*PARK16* Dominant (when MAF<15%, then Dominant scheme was used)

Supplementary Table 5: Evaluation of interactions of *LRRK2* rs1491942 and *PARK16* variants with regard to susceptibility to Parkinson's disease in the Asian Series

LRRK2 Variant/Genotype	*PARK16* Variant/Genotype	Sample genotype count and frequency	Test of Association OR	p-value	Models for the *LRRK2* and *PARK16* SNPs and Test of Interaction Results
LRRK2 rs1491942	rs708723				Additive/Additive Model
GG	GG	510 (31.6%)	1.00 (reference)	N/A	OR: 1.1 (0.89, 1.36)
GG	GT	85 (5.3%)	2.72 (1.53, 4.83)	<0.001	p=0.40
GG	TT	55 (3.4%)	1.96 (0.97, 3.95)	0.060	
GC	GG	614 (38%)	1.01 (0.8, 1.28)	0.91	Heterogeneity: I²=0, p=0.43
GC	GT	94 (5.8%)	4.11 (2.21, 7.65)	<0.001	
GC	TT	48 (3%)	2.94 (1.31, 6.56)	0.009	
CC	GG	160 (9.9%)	1.15 (0.81, 1.65)	0.43	
CC	GT	30 (1.9%)	2.69 (1.07, 6.8)	0.036	
CC	TT	19 (1.2%)	4.41 (1.22, 15.9)	0.024	
LRRK2 rs1491942	rs708725				Additive/Additive Model
GG	TT	665 (13.3%)	1.00 (reference)	N/A	OR: 0. 97 (0.86, 1.11)
GG	TC	1156 (23.1%)	0.76 (0.62, 0.94)	0.01	p=0.69
GG	CC	526 (10.5%)	0.74 (0.58, 0.96)	0.0237	
GC	TT	619 (12.4%)	1.25 (0.99, 1.58)	0.0614	Heterogeneity: I²=0, p=0.19
GC	TC	1029 (20.6%)	0.8 (0.65, 0.99)	0.0411	
GC	CC	496 (9.9%)	0.82 (0.63, 1.06)	0.124	
CC	TT	133 (2.7%)	1.35 (0.9, 2)	0.142	
CC	TC	254 (5.1%)	0.85 (0.62, 1.16)	0.302	
CC	CC	128 (2.6%)	1 (0.67, 1.51)	0.982	
LRRK2 rs1491942	rs823139				Additive/Additive Model
GG	GG	1790 (34.6%)	1.00 (reference)	N/A	OR: 1.08 (0.93, 1.26)
GG	GT	571 (11%)	0.72 (0.58, 0.89)	0.00223	p=0.31
GG	TT	53 (1%)	1 (0.55, 1.81)	0.993	
GC	GG	1623 (31.4%)	1.12 (0.97, 1.29)	0.133	Heterogeneity: I²=0, p=0.23
GC	GT	553 (10.7%)	0.81 (0.66, 1)	0.0508	
GC	TT	45 (0.9%)	0.92 (0.48, 1.74)	0.791	
CC	GG	401 (7.8%)	1.21 (0.96, 1.52)	0.112	
CC	GT	127 (2.5%)	0.96 (0.65, 1.42)	0.839	

CC	TT	6 (0.1%)	0.79 (0.14, 4.51)	0.791	
LRRK2 rs1491942	rs823156				Additive/Additive Model
GG	AA	1583 (30.6%)	1.00 (reference)	N/A	OR: 0.99 (0.82, 1.12)
GG	AG	698 (13.5%)	0.76 (0.62, 0.93)	0.00658	p=0.92
GG	GG	137 (2.6%)	0.53 (0.35, 0.82)	0.00388	
GC	AA	1409 (27.2%)	1.14 (0.97, 1.33)	0.103	Heterogeneity: I^2=16 p=0.29
GC	AG	685 (13.2%)	0.74 (0.61, 0.91)	0.00352	
GC	GG	126 (2.4%)	0.98 (0.64, 1.52)	0.94	
CC	AA	334 (6.5%)	1.13 (0.88, 1.46)	0.326	
CC	AG	161 (3.1%)	1.02 (0.72, 1.46)	0.893	
CC	GG	38 (0.7%)	0.92 (0.43, 1.99)	0.835	
LRRK2 rs1491942	rs11240572				Additive/Dominant Model
GG	CC	1745 (33.9%)	1.00 (reference)	N/A	OR:0.92 (0.75, 1.13)
GG	CA+AA	661 (12.8%)	0.85 (0.7, 1.04)	0.116	p=0.42
GC	CC	1603 (31.1%)	1.17 (1.01, 1.35)	0.039	
GC	CA+AA	611 (11.9%)	0.82 (0.67, 1.01)	0.0633	Heterogeneity: I^2=0, p=0.87
CC	CC	401 (7.8%)	1.23 (0.97, 1.55)	0.0825	
CC	CA+AA	134 (2.6%)	1 (0.68, 1.45)	0.981	

OR (Odds Ratio), Tests of Interaction presents results of Fixed Effects Model with OR and 95% Confidence Interval (CI), Models were either *LRRK2* Additive/*PARK16* Additive, *LRRK2* Additive/*PARK16* Dominant, or *LRRK2* Dominant/*PARK16* Dominant (when MAF<15%, then Dominant scheme was used)

Supplementary Table 6: Stratified Analysis of Effect of *LRRK2* rs1491942 on Carriers and Non-carriers of *PARK16* rs11240572 Minor Allele

	PARK16 rs11240572 allele					
	Carriers			Non-Carriers		
Ethnic Series	*LRRK2* OR (95% CI)	pval	n	*LRRK2* OR (95% CI)	pval	n
Asian	1.02 (0.85 ,1.22)	0.82	1409	1.13 (1.58 ,1.10)	0.17	3766
Caucasian	1.04 (0.80 ,1.35)	0.79	697	1.17 (1.10 ,1.25)	<0.000	9995
Combined	1.03 (0.88 ,1.19)	0.73	2106	1.15 (0.10 ,1.22)	<0.000	13761

Supplementary Table 7: Evaluation of interactions of *LRRK2* rs7133914 and *PARK16* variants with regard to susceptibility to Parkinson's disease in the Caucasian Series

LRRK2 Variant/Genotype	*PARK16* Variant/Genotype	sample genotype count and frequency	Test of Association OR	p-value	Models for the *LRRK2* and *PARK16* SNPs and Test of Interaction Results
LRRK2 rs7133914	rs708273				Dominant/Additive Model
GG	GG	1788 (27.9%)	1.00 (reference)	N/A	OR: 0.92 (0.69, 1.20)
GG	GT	3764 (58.7%)	1.02 (0.91, 1.16)	0.71	p=0.54
GG	TT	288 (4.5%)	1.10 (0.94, 1.30)	0.22	
GA+AA	GG	577 (9%)	1.07 (0.83, 1.39)	0.60	Heterogeneity: I^2=35 p=0.11
GA+AA	GT	1361 (22.7%)	0.88 (0.71, 1.10)	0.26	
GA+AA	TT	3834 (63.9%)	0.96 (0.69, 1.33)	0.79	
LRRK2 rs7133914	rs708725				Dominant/Additive Model
GG	TT	206 (3.4%)	1.00 (reference)	N/A	Additive Model
GG	TC	600 (10%)	0.92 (0.8, 1.06)	0.24	OR: 0.85(0.61, 1.18)
GG	CC	4317 (66.4%)	0.79 (0.66, 0.94)	0.008	p=0.33
GA+AA	TT	1306 (20.1%)	0.89 (0.66, 1.20)	0.44	
GA+AA	TC	688 (10.6%)	0.72 (0.57, 0.91)	0.006	Heterogeneity: I^2=0, p=0.61
GA+AA	CC	188 (2.9%)	1.02 (0.74, 1.40)	0.91	
LRRK2 rs7133914	rs823139				Dominant/Additive Model
GG	GG	3658 (58.1%)	1.00 (reference)	N/A	Additive Model
GG	GT	1799 (28.6%)	0.92 (0.81, 1.05)	0.22	OR: 1.20(0.97, 1.48)
GG	TT	573 (9.1%)	1.00 (0.65, 1.53)	0.99	p=0.096
GA+AA	GG	270 (4.3%)	0.98 (0.83, 1.15)	0.80	
GA+AA	GT	5486 (84%)	0.72 (0.53, 0.98)	0.034	Heterogeneity: I^2=13, p=0.25
GA+AA	TT	169 (2.6%)	1.26 (0.34, 4.64)	0.73	
LRRK2 rs7133914	rs823156				Dominant/Additive Model
GG	AA	848 (13%)	1.00 (reference)	N/A	Additive Model
GG	AG	26 (0.4%)	1.00 (0.89, 1.13)	0.96	OR: 0.88 (0.72, 1.09)
GG	GG	5281 (80.9%)	0.83 (0.60, 1.13)	0.24	p=0.25
GA+AA	AA	370 (5.7%)	0.97 (0.81, 1.17)	0.77	
GA+AA	AG	805 (12.3%)	0.79 (0.60, 1.03)	0.078	Heterogeneity:I^2=0. p=0.74
GA+AA	GG	70 (1.1%)	1.13 (0.49, 2.64)	0.77	
LRRK2 rs7133914	rs11240572				Dominant/Dominant Model
GG	CC	1730 (16.7%)	1.00 (reference)	N/A	OR: 1.20 (0.70, 2.07)
GG	CA+AA	625 (6%)	1.05 (0.84, 1.30)	0.69	p=0.51
GA+AA	CC	155 (1.5%)	0.91 (0.78, 1.06)	0.25	
GA+AA	CA+AA	230 (2.2%)	1.15 (0.71, 1.86)	0.57	Heterogeneity: I^2=0, p=0.61

Figure 1: Forest Plot of LRRK2 rs1491942 and PARK16 rs1124072 in PD cases and controls in Caucasian, Asian, and combined series
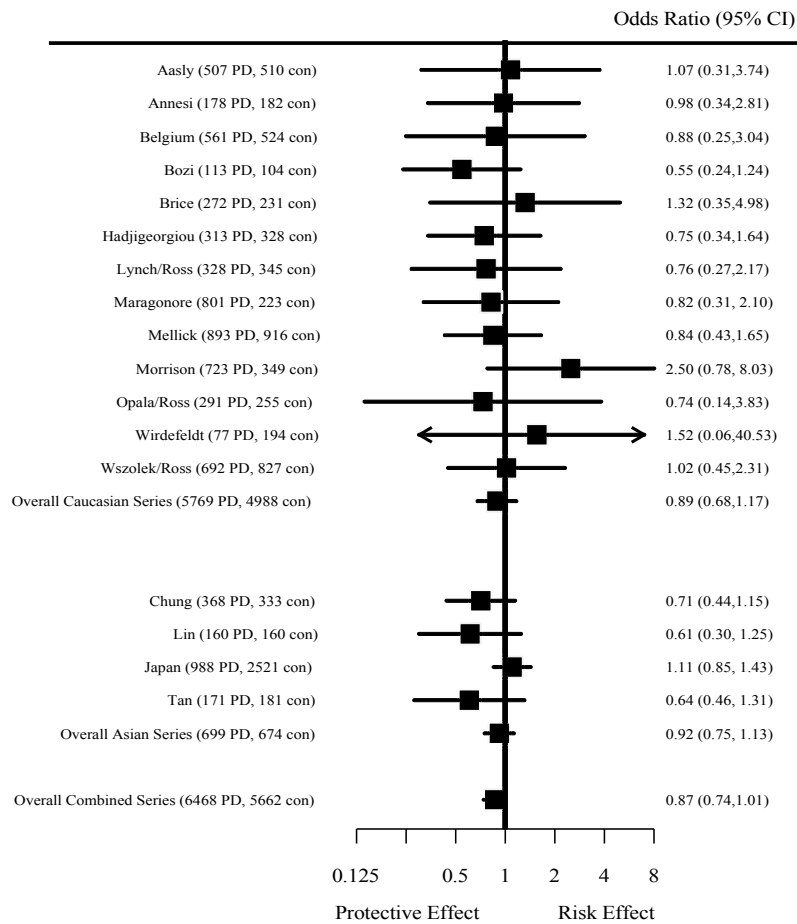
Part 3: Parkinson's disease and type 1 diabetes – An analysis on 19,864 patients from the DPV registry

Manuscript 3:

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Abstract

Introduction: The growing life expectancy increases the risk for comorbidities. Emerging evidence showed an involvement of immune dysfunction in seemingly diverse phenotypes such as Parkinson disease (PD) and type-1 diabetes (T1D). We examined differences in diabetes disease pathogenesis between PD and non-PD patients. We determined the prevalence and compared the diabetic end-points in T1D patients with or without PD.

Methods: We performed an epidemiological study with multivariable logistic regression. Data was retrieved from the German/Austrian prospective, multicenter diabetes patient follow-up registry, DPV. In total, 19,864 T1D patients aged ≥40 years were analyzed; 111 patients had a PD diagnosis and/or used specific anti-Parkinson medication. We compared clinical and biological endpoints between PD and non-PD T1D patients. To adjust for demographic differences, multivariable regression models with age, sex and duration of diabetes as covariates were used.

Results: PD patients were significantly older (74.2 vs 55.7 years, <0.0001) and had longer diabetes duration than non-PD patients (30.1 vs 21.9 years, p<0.0001). Demographically-adjusted analyses showed a significantly increased risk for renal failure for patients with PD and T1D (OR (95%CI): 3.3 (1.8-6.0), p<0.0001). Furthermore, increased risk for dementia (OR (CI): 2.0 (1.2-3.3), p=0.005), stroke (1.7 (1.1-2.8), p=0.028), and as well as a longer duration of hospital stay (5.19±0.18 vs 3.08±0.01, p<0.001) was observed for PD patients.

Conclusion: An increased risk for renal failure, dementia and stroke was observed in patients with T1D and PD as compared to patients with T1D alone. The clinical end-points should be taken into consideration in routine clinical practice.

Short summary: A large scale retrospective cohort study comparing comorbidies of Parkinson's disease patients with neurologically normal patients within a type 1 diabetes population.

Introduction:

Parkinson's disease (PD) is one of the most common neurodegenerative disorders, affecting between 0.1 and 3% of the general population, and second in prevalence only to Alzheimer's disease [1]. The disease is characterized by a loss of dopaminergic neurons in the substantia nigra, resulting in reduced striatal dopamine content, which leads to decline in the motor systems [2]. The non-motor symptoms such as depression, gastrointestinal dysfunction, and autonomic dysfunction precede onset of motor symptoms [2], and severity of non- motor symptoms increases with advancing disease [3,4]. While the degeneration of dopaminergic neurons remains central to disease pathogenesis, non-dopaminergic neurons (such as norepinephrinergic, cholinergic and serotoninergic neurons) in the basal forebrain, locus coeruleus, and raphei nuclei respectively are also affected indicating that disease progresses first through lower brainstem before spreading to the basal ganglia and cortex [4].

The exact mechanism of neuronal decay in PD is not fully understood, although emerging evidence links abnormal protein misfolding, mitochondrial dysfunction, and oxidative stress to PD pathogenesis [5]. Furthermore, neuroinflammatory mechanisms such as microglia activation, astrogliosis and lymphocytic infiltration may also contribute to the neuronal degeneration [6]. It is still not resolved whether neuroinflammation is a consequence or cause of cell death. Nevertheless, emerging evidences generated from postmortem, epidemiological and genetic studies indicate that neuroinflammation can be a risk factor for PD. For example, in post-mortem studies of PD patients, neuroinflammatory markers such as tumor necrosis factor (TNFα), interleukin (IL) 1β, 6, and 2 were found in the striatum and substantia nigra, suggesting that immune response plays a significant role in the disease progression [2]. Candidate gene studies assessing genetic variability of neuroinflammation genes such as (TNF, IFNɣ, IL) consistently showed increased risk for PD [7,8]. Likewise, data generated from epidemiological studies also showed protective effect of anti-

inflammatory drugs for PD patients [9]. Interestingly, data generated from genome wide association studies (GWAS) in PD established the role of HLA locus in PD, thus underscoring an involvement of neuroinflammation in PD [8,10]. In addition, alpha-synuclein deposits have been discovered in the pancreas in PD patients, and the protein itself has been implicated in binding of insulin-secretory granules and in insulin secretion inhibition [11]. The developing body of evidence suggests that the two diseases interact at on both the genetic and functional level [12].

The role of inflammatory mediators contributing to the pathogenesis of autoimmune disorders such as T1D is much wider than previously anticipated because of the polygenic nature of the disease [13,14]. It has been suggested that immune response, inflammatory mediators such as cytokines, apart from contributing to cell death also increases the risk for neurodegenerative diseases [14-16]. Recent studies have also shown that apoptosis-induced neuronal decay occurs in the brain of T1D BB/W rats, suggesting that the T1D phenotype is related the cognitive impairment seen in the rat models [17].

 Given the converging immune dysfunction evidence between T1D and PD [18,19], we aimed to investigate the prevalence and diabetic end-points in neurologically normal T1D patients in comparison to T1D patients with PD. We leveraged the prospective diabetes patient registry (DPV) cohort with over 19000 adult T1D patients to assess whether there are clinical and epidemiological differences between the two patient populations.

Methods:

The DPV initiative has been ethically approved by the Ethics Committee of Ulm University, Germany. The local review boards of each participating clinic, approved the anonymized data collection.

Study population

Specialized diabetes clinics in Germany and Austria document real-life clinical data on diabetes patients regularly in a joint initiative for over 20 years by using a standardized electronic record system. The locally stored data are transmitted twice yearly in anonymous form to the University of Ulm, Germany for central analyses as well as benchmarking [20,21]. All plausible data are

aggregated in order to set-up the prospective, multicenter diabetes patient follow-up registry, DPV.

Up to now, demographic and diabetes-related data of 453580 patients with any type of diabetes

documented by 478 clinics are available in the registry. The DPV initiative has been approved by the

ethics committee of Ulm University, Germany, and the anonymized data assessment by the local

review board of each participating clinic. Until September 2016, the DPV registry included 19864 T1D

patients aged 40 years or older with documented insulin dosage who were eligible for the study. To

identify patients with comorbid PD, a structured database search was performed which was

described in detail in the previous work on PD in T2D patients [22]. In brief, ICD-10-codes as well as

specific search terms for a diagnosis and/or treatment of PD were used to select patients with co-

existing PD. Excluded from analysis were patients with atypical or drug induced PD and subjects with

PD medication plus a documented diagnosis/treatment of restless-legs-syndrome, Huntington's

disease, multiple sclerosis or brain tumor. Finally, 111 patients were identified having both T1D and

PD.

In case of multiple datasets per patient, data was aggregated over the last year of treatment,

respectively.

Definitions of diabetes therapy and outcome

Hemoglobin A1c (HbA1c) was used to assess metabolic control. Due to the multicenter data

collection, HbA1c values were mathematically standardized to the DCCT reference range (20.7-42.6

mmol/mol, 4.05–6.05%) using the multiple of the mean (MOM) method [23,24].

Renal failure was diagnosed in patients with glomerular filtration rate (GFR)<15ml/min/1.73m²

during the last treatment year and/or renal transplantation or dialysis. GFR was estimated by the

Modification of Diet in Renal Disease (MDRD) formula according to Silveiro et al [25]. The definition

of renal failure is based on the official guidelines from the German Diabetes Association (Deutsche

Diabetes Gesellschaft) [26].

 We defined dementia by ICD-10 codes, DSM-IV and DSM-5 codes, or specific search terms for a

diagnosis of dementia, and/or drugs specific for dementia treatment. Data entries were made by

physicians and health care professionals at each site based on clinically available data from routine care. Dementia was either already diagnosed in patients, or diabetologists made the diagnosis jointly with neurologists. This method was previously applied in two other publications on dementia and diabetes [22,27].

ICD-10-codes or specific search terms were used to analyze the frequency of myocardial infarction, stroke and diabetic foot syndrome.

Statistical analysis

SAS 9.4 (Statistical Analysis Software, SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses. First, descriptive statistics were performed and results are given as median with interquartile range or proportion. Kruskal-Wallis test or χ^2-test was applied to compare data. For multiple comparisons, p-values were adjusted using Bonferroni stepdown correction (Holm method). Second, to consider demographic differences between groups, multivariable regression analyses with age, sex and duration of diabetes as covariates were used to compare groups regarding diabetes therapy and outcome. Separate models were built for the following outcome variables: i) glycemic control, ii) type of insulin therapy and iii) specific parameters of diabetes outcome (e.g. hypertension, dyslipidemia, stroke, renal failure, myocardial infarction). The covariate 'age' was categorized as 40-<50 y, 50-<60 y, 60-<70 y and ≥70 y, and duration of diabetes was divided into tertiles. Linear regression with residual maximum likelihood as estimation technique was used for continuous outcome variables, logistic regression with maximum likelihood estimation for binary data and Poisson regression again with maximum likelihood estimation for count data. For the outcomes of interest as determined by p-values in the general analyses, individual analyses stratified by age groups was also performed. Between-within technique was used to compute denominator degrees of freedom. Based on observed marginal frequencies, adjusted estimates (means ± SEM) were calculated. A two-sided p<0.05 was defined significant.

Results:

Study Population:

Baseline clinical characteristics of the patient population are presented in Table 1. Our T1D study population has PD diagnosis or treatment rate of 0.6% (111 PD patients). PD frequencies change between the age groups (Figure 1). In the youngest age category (40-<50 years), the prevalence is 0.1% versus 1.8% in the oldest age category (>=70), suggesting that PD is also age dependent in our T1D cohort (Figure 1). In the 50 to 60 year-olds, PD is more prevalent in T1D patients compared to the general population (Figure 1), whereas in the oldest age category PD prevalence tends to be higher in the general population (GP) than in T1D patients. Likewise, we observed more men than women with PD in the 50-60 age group (0.27% vs 0.16%) as well as in the oldest age group >=70 (2.0% vs 1.6%), and thus risk observed in our cohort for PD is consistent with overall incidence estimates as reported for PD worldwide[1], (Figure 2). Compared to national health claims data, PD prevalence seemed to be similar in male T1D patients aged 60 to <70 years, whereas in the other age groups for both sexes PD prevalence was either lower (>=70) or higher (50-<60 y) compared to the general population (Figure 2). Median age of onset of diabetes was later in T1D patients with PD than in patients without (40.5 vs 34.3 years, p=0.005). BMI was not related to PD (Table 1), in analysis adjusted for age, gender, and time since onset of T1D, (p=0.73); PD patients had a BMI of 26.34±0.50 kg/m² versus non-PD patients of 26.31±0.04 kg/m².

Comparison of metabolic control and diabetes treatment:

In analyses adjusted for age, gender, and diabetes duration, we found that PD and non-PD patients had similar HbA1c levels. In addition, insulin therapy, including pumps and medication usage, was also comparable between PD and non-PD patients, with p-values >0.05 (Table 2). Thus, PD patients did not differ from non-PD patients in the control and management of T1D.

Comparison of Diabetes Complications:

In the age, gender, and diabetes duration adjusted analyses, we found renal failure, stroke, and dementia at an increased rate in PD patients as opposed to non-PD patients, (Table 2). The odds ratio of renal failure occurring in PD patients was 3.34 (95% CI: 1.84, 6.05), with p-value < 0.0001. In PD patients, the OR of dementia was 2.03 (1.24, 3.33), with p-value=0.005 and of stroke 1.73 (1.06,

2.82), with p-value=0.028. Hospitalization was defined as a categorical yes/no variable, and there was also an increased odds of hospitalization in PD patients, although not statistically significant with OR of 1.54 (0.96, 2.46), p-value=0.076. However, duration of hospital stay was significantly longer in PD patients (Table 2).

Microvascular complications such as retinopathy and microalbuminuria tended to be more frequent in PD patients if adjusted for age, gender and diabetes duration (Table 2). By contrast, aside from stroke, cardiovascular health was comparable between the two groups, based on dyslipidemia, hypertension and rates of myocardial infarction (Table 2). Furthermore, diabetic foot syndrome also occurred at a similar rate between the two groups, p-value=0.12.

Age-stratified analysis:

Dementia, stroke, rate of hospitalization, and renal failure were selected for age-stratified analysis (Table 3). We chose them based upon the p-values in the regression models across the entire study population. In an age dependent manner, we observed an increased risk for dementia (OR=2.48 (1.38, 4.47), p-value = 0.0025), and stroke (OR=2.207 (1.241, 3.925), p-value=0.007) in >70 age group category (Table 3) in PD patients compared to T1D subjects without PD. Interestingly, increased risk of renal failure was consistently observed across a wide spectrum of age groups, from 40-50 (OR=10.4 (1.80, 59.9), p-value=0.009), to 50-60 age range (OR=8.7 (2.25, 33.6), p-value=0.002), and also in the >70 age group (OR=2.25, 0.04) indicating renal dysfunction as an early prognostic clinical marker for PD. Length of hospitalization (days) also increased with PD diagnosis. In the age group 40-50y, duration of stay is longer in PD patients compared to T1D patients without PD (4 (3.2, 5.3) days on average more for PD patients, p<0.001). In the 50-60y age category, we see an increase in expected number of days by 1.3 (1.02, 1.75) days (p=0.035) in PD patients versus neurologically normal patients. In the age group 60-70y, we seen an expected decrease in number days, 0.27 (0.18, 0.407) (p<0.001). In the oldest age group >70y, we seen an expected increase in of 2 days (1.84, 2.13), (p<0.001).

Discussion:

In this prospective, large, multi-center study of 202 separate sites across Germany and Austria, we compared clinical and demographic data between patients with T1D and PD and patients with only T1D. While median age of PD patients was dramatically older; renal failure, stroke and dementia were more frequent and duration of hospital stay was longer than in non-PD patients (median age of 74 versus 56). Most other clinical endpoints of diabetes management, including HbA1$_c$ levels, were comparable between the two groups. Of note, the study found no differences between BMI in the two groups, suggesting that there is no significant weight loss in PD patients due to increasing nutritional needs and or decreased food intake.

We observed that although men constituted 58% of the T1D population in patients between the ages of 40 to 50 years, the rate of PD was higher in women than in men (0.11% versus 0.08%, Figure 2). However, in the 50 to 60 years' category, the rate of PD is much higher in men than in women, (0.27% to 0.16%), consistent with male dominance of PD prevalence in the general population (Figure 2). In our study, we observed in the middle age group (60-70) an increased frequency of PD in female T1D patients besides a similar PD frequency in males compared to the general population, suggesting a potential gender dependent effect in patients with T1D and PD in the middle age group category. The increased risk for stroke, as observed in our study, indicates that cerebrovascular changes contribute to increased risk for PD. Interestingly, a previously published study observed an increase prevalence of diabetes and hypertension in PD [28], suggesting that orthostatic hypotension in PD may contribute to disease risk. However, aside from stroke the cardiovascular and circulatory systems were not differentially compromised; T1D patients with PD showed increased risk for renal failure and dementia. The age stratified analysis also suggested a synergistic effect between PD and dementia that is particular to the oldest age group (>70 years), again perhaps due to a worsening effect of PD on dementia.

Previously, patients with T1D have been shown to be associated with increased risk for acute renal failure [29]. The causes of acute renal failure observed in TID are more likely to include chronic renal impairment and urinary tract infection among others [30]. Interestingly, recently published studies

focusing on assessing non-motor symptoms in PD consistently highlighted the urinary dysfunction as one of the most common non-motor symptom observed in PD patients [31]. The high risk for renal dysfunction has been observed in PD patients undergoing non-neurological surgery [32]. Renal failure can be attributed to bladder dysfunction that can cause multiple urinary symptoms including urgency, increased frequency of urination, nocturia, and urinary retention. Thus the convergence of renal dysfunction in both phenotypes underscores the need for close surveillance of renal function in diabetic patients. In the age stratified analysis, a positive PD diagnosis increases risk of developing renal failure in the younger age categories, including 40-50 and 50-60, thus suggesting that renal dysfunction manifests during the early phase of the disease. Mechanistically, at least from PD perspective, it has been hypothesized that a failure of D1 activation led to the detrusor overactivity (DO), which might underline overactive bladder (OAB) [33]. Further studies using functional imaging of dopamine transporters are needed for additional assessment and to explore the correlation between urinary dysfunction and nigrostriatal dysfunction.

In our study, we also observed increased risk for dementia in T1D with PD indicating that an auto-immune component might underline the relationship between these two. Given that dementia is a common co-morbidity of PD, the increased risk observed here may tie into the common diseases pathogenesis of PD and T1D. Interestingly, a recently published study found a moderate level of pleiotropic enrichment between Alzheimer disease (AD) and auto-immune diseases including T1D suggesting that probably shared genetic factors might contribute to the disease pathogenesis [27,34].

We observed also a longer duration of hospital stay and a trend towards a higher incidence of hospitalization in PD patients versus non-PD patients, confirming the previously observed finding that PD and its complications (for example infections, falls, psychiatric disorders, and pneumonia) contributing to the complexity of patient care and thus demand more hands-on clinical care. We observed similar results, however, in diabetic co-morbidities such as stroke, diabetic foot syndrome, heart attacks, and retinopathy. Therefore, although care for PD patients is more complicated, the

overall quality of care is comparable and PD patients do not suffer disproportionately given their PD

diagnosis. In the age-stratified analysis, a PD diagnosis generally increased length of stay, with the

exception in the 60-70 age group.

In conclusion, our study highlighted renal failure, stroke, dementia and duration of hospital stay/

hospitalization as important clinical end-points for patients with T1D and PD, and found evidence

that supports the emerging relationship between PD and T1D, and thus future research in

delineating the clinical endpoints for comorbid phenotypes should be pursued to develop

personalized approaches for patient care.

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Tables:

Table 1 (Table 13): Characteristics of study population, Median with interquartile range or proportion

	All	PD	No PD	Females	Males
N	19864	111	19753	9075	10789
Sex, % Male	54.3%	54.1%	54.3%		
Age, years	55.8 (47.9-66.6)	74.2 (64.9-73.3)	55.7 (47.9-66.5)	57.1 (48.5-68.9)	54.7 (47.5-64.9)
Age at DM diagnosis, years	34.4 (22.3-46.0)	40.5 (28.2-53.2)	34.3 (22.3-46.0)	34.8 (21.5-48.7)	34.0 (22.9-44.6)
DM duration, years	22.0 (10.1-34.9)	30.1 (19.9-43.6)	21.9 (10.1-34.9)	22.4 (10.6-35.4)	21.5 (9.7-34.4)
BMI, kg/m²	25.5 (22.9-28.9)	26.3 (23.5-29.3)	25.5 (22.9-28.9)	25.2 (22.5-29.3)	25.7 (23.3-28.7)

Except for BMI and sex, all $p < 0.05$ for the comparison between PD and non-PD. Between females and males, all $p < 0.05$.

Table 2 (Table 14): Diabetes therapy and outcome compared between type 1 diabetes with or without PD

	PD	No PD	p-value
HbA1c, mmol/mol	63.0±2.1	64.4±0.2	0.49
Insulin use, %			
CT	23.0	19.7	0.38
MDI	58.4	63.5	0.36
CSII	18.6	16.8	0.67
Insulin dose, IU/kg*d	0.635±0.032	0.619±0.002	0.61
Acute complications, %			
Hypoglycemia			
Severe	13.4	16.5	0.39
With coma	5.7	7.9	0.38
DKA	5.0	5.4	0.87
Chronic complications, %			
Dyslipidemia	58.1	61.0	0.59

Lipid-lowering drugs	26.8	25.9	0.81
Hypertension	23.8	27.9	0.32
Antihypertensiva	54.4	47.6	0.19
Stroke	16.2	10.0	0.03
Myocardial infarction	12.4	10.6	0.50
Dementia	16.1	8.7	0.005
Diabetic foot syndrome	15.3	10.9	0.12
Retinopathy	47.0	34.8	0.12
Microalbuminuria	31.0	23.8	0.12
Renal failure	9.4	3.0	<0.001
Hospital care			
Frequency, %	16.3	11.3	0.08
Duration, days per year	5.19±0.18	3.08±0.01	<0.001

Data as adjusted mean with SEM or proportion. PJ patient year, CT conventional therapy, DKA diabetic ketoacidosis, MDI multiple daily injections, CSII continuous subcutaneous insulin infusion

Table 3 (Table 15): Age-stratified analysis for T1D with or without PD

Outcome	40-<50 years	50-<60 years	60-<70 years	>70 years
Stroke (OR, 95% CI)	0.88 (0.46, 16.5)	0.81 (0.11, 5.92, 1.78)	1.55 (0.54, 4.59)	2.21 (1.24, 3.93)
Dementia (OR, 95% CI)	0.98 (0.05, 18.5)	0.96 (0.13, 7.09)	2.10 (0.71, 6.20)	2.48 (1.37, 4.47)
Renal failure (OR, 95% CI)	10.4 (1.80, 59.9)	8.68 (2.25, 33.6)	1.97 (0.46, 8.55)	2.51 (1.06, 5.92)
Duration of hospitalization (expected difference in number of days)	4.0 (3.2, 5.3)	1.3 (1.02, 1.75)	0.27 (0.18, 0.407)	2 (1.84, 2.13)

Figure 1: PD prevalence rates between GP[35] and T1D patients

a) stratified by age group,
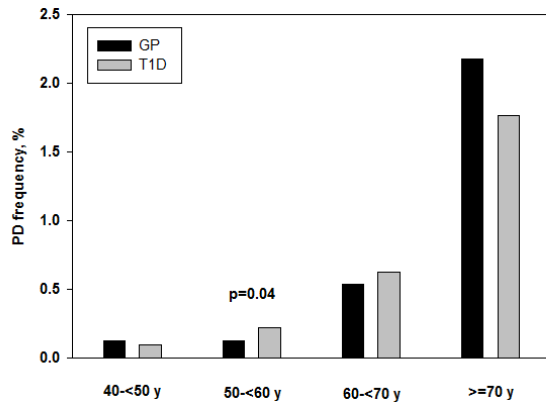
b) stratified by age and gender

Coesfeld/Dülmen Innere Med., Darmstadt Innere Medizin, Deggendorf Gemeinschaftspraxis, Deggendorf Medizinische Klinik II, Dortmund Knappschaftskrankenhaus Innere, Dortmund Medizinische Kliniken Nord, Dortmund-Hombruch Marienhospital, Dortmund-St. Josefshospital Innere, Dortmund-West Innere, Duisburg Evang. und Johanniter Krhs Innere, Duisburg Malteser Rhein-Ruhr St. Anna Innere, Duisburg Malteser St. Johannes, Duisburg-Huckingen, Duisburg-Huckingen Malteser Rhein-Ruhr ST. Johannes, Duisburg-St.Johannes Helios, Eberswalde Klinikum Barnim Werner Forßmann - Innere, Eisleben Lutherstadt Helios-Klinik, Erlangen Uni Innere Medizin, Essen Diabetes-Schwerpunktpraxis, Eutin St.-Elisabeth Innere, Forchheim Diabeteszentrum SPP, Frankfurt Diabeteszentrum Rhein-Main-Erwachsenendiabetologie (Bürgerhospital), Frankfurt Uni-Klinik Innere, Frankfurt-Sachsenhausen Innere, Frankfurt-Sachsenhausen Innere MVZ, Freiburg Uni Innere, Friedberg Innere Klinik, Fulda Innere Medizin, Geislingen Klinik Helfenstein Innere, Gelnhausen Innere, Gießen Ev. Krankenhaus Mittelhessen, Graz Uni Innere, Göppingen Innere Medizin, Göttingen Uni Gastroenterologie, Güstrow Innere, Halberstadt Innere Med. AMEOS Klinik, Halle-Dölau Städtische Kinderklinik, Hamburg Endokrinologikum, Hanau St. Vincenz - Innere, Hannover Henriettenstift - Innere, Heidelberg St. Josefskrankenhaus, Heilbronn Innere Klinik, Herdecke Kinderklinik, Herford Innere Med I, Herne Evan. Krankenhaus Innere, Herten St. Elisabeth Innere Medizin, Hildesheim GmbH - Innere, Hinrichsegen-Bruckmühl Diabetikerjugendhaus, Idar Oberstein Innere, Ingolstadt Klinikum Innere, Innsbruck Universitätsklinik Innere, Iserlohn Innere Medizin, Jena diabetol. Schwerpunktpraxis, Kaiserslautern-Westpfalzklinikum Kinderklinik, Kamen Klinikum Westfalen Hellmig Krankenhaus, Karlsburg Klinik für Diabetes & Stoffwechsel, Kassel Rot-Kreuz-Krankenhaus Innere, Kaufbeuren Innere Medizin, Kempen Heilig Geist - Innere, Kirchheim-Nürtingen Innere, Klagenfurt Innere Med I, Kleve Innere Medizin, Koblenz Kemperhof 1. Med. Klinik, Konstanz Innere Klinik, Krefeld Alexianer Innere, Krefeld Innere Klinik, Krefeld-Uerdingen St. Josef Innere, Landau/Annweiler Innere, Lilienthal Diabeteszentrum, Limburg Innere Medizin, Lindenfels Luisenkrankenhaus Innere, Lindenfels Luisenkrankenhaus Innere 2, Linz AKH - 2. Med, Linz Krankenhaus Barmherzige Schwestern Kardiologie Abt. Int. II, Ludwigshafen diabetol. SPP, Lübeck

Uni-Klinik Innere Medizin, Lünen Klinik am Park, Magdeburg Städtisches Klinikum Innere, Mannheim Uniklinik Innere Medizin, Marburg - UKGM Endokrinologie & Diabetes, Marktredwitz Innere Medizin, Marpingen-SPP, Memmingen Internistische Praxis, Moers - St. Josefskrankenhaus Innere, Murnau am Staffelsee - diabetol. SPP, Mölln Reha-Klinik Hellbachtal, Mühlacker Enzkreiskliniken Innere, München Diabetes-Zentrum Süd, München Schwerpunktpraxis, Münster Clemens-Hospital Innere, Münster Herz Jesu Innere, Neumarkt Innere, Neunkirchen Innere Medizin, Neuwied Marienhaus Klinikum St. Elisabeth Innere, Nidda Bad Salzhausen Klinik Rabenstein/Innere-1 Reha, Nidda Bad Salzhausen Klinik Rabenstein/Innere-2 Reha, Nürnberg Med. Klinik 4, Oberhausen Innere, Oberndorf Gastroenterologische Praxis Schwerpunkt Diabetologie, Offenbach/Main Innere Medizin, Oschersleben MEDIGREIF Bördekrankenhaus, Osterkappeln Innere, Ottobeuren Kreiskrankenhaus, Pfullendorf Innere Medizin, Pirmasens Städtisches Krankenhaus Innere, Plauen Vogtlandklinikum, Prenzlau Krankenhaus Innere, Rastatt Kreiskrankenhaus Innere, Recklinghausen Dialysezentrum Innere, Reutlingen Klinikum Steinenberg Innere, Rodalben St. Elisabeth, Rosenheim Innere Medizin, Rosenheim Schwerpunktpraxis, Rostock Universität Innere Medizin, Saaldorf-Surheim Diabetespraxis, Scheibbs Landesklinikum, Schwerin Innere Medizin, Sinsheim Innere, Spaichingen Innere, St. Pölten Universitätsklinik Innere, Stockerau Landeskrankenhaus, Stolberg Kinderklinik, Stuttgart Bethesda Agaplesion, Tettnang Innere Medizin, Timmendorfer Strand, Traunstein diabetol. Schwerpunktpraxis, Trostberg Innere, Ulm Schwerpunktpraxis Bahnhofsplatz, Ulm Uni Innere Medizin, Villingen-Schwenningen Schwarzwald-Baar-Klinikum Innere, Wangen Oberschwabenklinik Innere Medizin, Weisswasser Kreiskrankenhaus, Wels Innere, Wernberg-Köblitz SPP, Wetzlar Schwerpunkt-Praxis, Wien 3. Med. Hietzing Innere, Wien Uni Innere Med III, Wien Wilhelminenspital 5. Med. Abteilung, Wiesbaden Helios Horst-Schmidt-Kinderkliniken, Wilhelmshaven St. Willehad Innere, Wittenberg Innere Medizin, Wolgast Innere Medizin, Zweibrücken Ev. KH. Innere.

Chapter 3 Discussion

Over the past decades, significant progress has been made on discovering the genetic origin of disease (Lill, 2016). In particular, advances have been made in understanding the genetic provenance of rare diseases with highly penetrant traits that follow Mendelian inheritance patterns. However, much remains to be done in understanding the origins of complex diseases such as PD. Media portrayal of famous patients such as Muhammad Ali or Michael J Fox have increased the disease visibility, with traditional portrayal of the disease as neurodegenerative characterized by motor symptoms. PD, however, is a degenerative disease that affects much more than just the motor systems. Sensory, digestive, psychiatric, and urinary systems are also involved, and more recent research has implicated oxidative stress and mitochondrial dysfunction not simply in the brain but also other regions of the body as a part of disease pathogenesis. As our understanding of PD evolves, it is more accurate to consider PD as a degenerative disease affecting multiple systems, with a strong neurological component. A complex disease such PD has also a complex and large web of genetic risk-affecting mutations that act both singularly and in combination. However, much remains to be done to elucidate the genetic basis of PD.

One of the first methods used to determine the heritability of PD was twin studies. Given the shared childhood environment and the genetic overlap differences between monozygotic versus dizygotic twins, it is possible to study causality of genetic factors while adjusting for the possible mediating effects of environmental factors. While results from the twin studies have been inconsistent, nevertheless the evidence, though not statistically significant, pointed towards an underlying genetic etiology for PD (Wirdefeldt et al., 2011a). Linkage studies using large multi-generational families were also one of the early drivers of PD genetics. The identification of the first PD-associated mutations were through large familial kindreds. Examples include the autosomal genes SNCA, LRRK2, DJ-1, PINK1, ATP13A2 and PARKIN discovered through familial studies. While the linkage approach was relatively successful for identifying highly penetrant but rare mutations, the majority of PD cases are sporadic, with only 10% of patients reporting a positive family history for PD

(Thomas and Beal 2007). As study sizes increased, GWAS studies became sufficiently powered and thus were able to identify 26 independent loci that modify disease risk, including genes in SNCA, MAPT, GBA, HLA, and GAK.

Next-generation sequencing (NGS) methods such as exome sequencing identified novel mutations in VPS35, VPS13C, CHCHD2, and DNAJC6 genes. However, the vast majority of PD cases is still sporadic with unclear etiology, and "missing heritability" continues to be a strong theme in PD genetics even through the many advancements over the years. With the advances in research, our understanding of PD has evolved from a single system neurodegenerative disease to a syndrome encompassing mutiple systems as well (Titova et al., 2017a). Figure 1 illustrates our currently knowledge of penetrance versus frequency of genetic risk variants.

Figure 7: Penetrance versus Frequency. X-axis indicates frequency of genetic variants, Y-axis indicates penetrance or likelihood of disease given existence of mutation. (Adapted from Lill C, 2016. Mol Cell Probes).
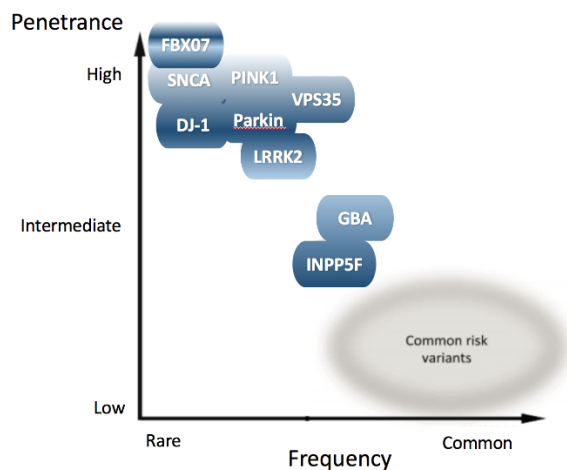
Due to the complexity of the genetic architecture of PD, it is hypothesized that the resulting observed PD phenotype comes from a complicated interplay between different genes with each other and with the environment and life-style of the person. This has led to research focusing on not just simple Mendelian inheritance patterns, but also rare variants, gene-gene interaction, and gene-

environment interactions. The repeatedly observed incomplete penetrance of identified genetic risk variants only reinforce the theory of PD as a multifaceted and multi-origin neurodegenerative disease.

One of the common threads running within neurodegenerative diseases is the aggregation of proteins and its toxicity towards neural tissue (Goedert et al., 2017). In 1907, Alois Alzheimer already noted the presence of neuritic plaques and neurofibrillary lesions in what would become known as Alzheimer's disease. Subsequently, Alzheimer also described the protein aggregations known as "Pick bodies" that are characteristic of Pick's disease. Lewy later described in 1912 the "Lewy bodies" that are characteristic of PD.

Aggregration of amyloidogenic proteins has been observed in molecular pathways associated with a wide spectrum of neurodegenerative diseases including AD, PD, and dementia (Ciryam et al., 2013). AD is defined by deposits of tau proteins, and deposits of fibrous beta-amyloid deposits (Goedert et al., 2001). It is the most famous diseases in the diverse group of diseases known as tauopathies, linked by the degenerative aggregation of tau protein inclusions. Lewy bodies, protein deposits strongly immunoreactive for SNCA, are also found in an entire class of neurodegenerative diseases known as synucleinopathies (Goedert, 2001). The discoveries cemented the importance of protein aggregation in neurodegenerative diseases.

One subset of neurodegenerative diseases with protein accumulation are the polyglutamine (polyQ) diseases. Early studies showed that isolated PolyQ peptides quickly form insoluble amyloid aggregates in solution and linked polyQ repeated expansions to neurodegeneration (Chen et al., 2002). Over the last two decades, scientists discovered that polyglutamine (polyQ) repeat-containing proteins are common within the human proteome. These include huntingtin in Huntington's disease, ataxin-1, ataxin-2, ataxin-3, voltage-dependent calcium channel alpha-1A subunit, ataxin-7, TATA-binding protein, and spinocerebellar ataxia 1, 2, 3, 6, 7, and 17. In particular, spinocerebellar ataxia 3

(Machado-Joseph Disease) is the second most common PolyQ disease after Huntington's disease (Riess et al., 2008).

Autosomal dominant cerebellar ataxias (ADCA) is a group of rare neurodegenerative diseases that primary affect the motor system, though mild intellectual deficits are also observed (Durr, 2010). Motor degeneration is characterized by cerebellar atrophy and neuronal loss in the spinocerebellar tracts, with coordination problems, loss of balance, slurred speech, and gait disorders commonly observed (Schöls et al., 2004). The diseases, more than 40 different types have been identified, are heteregenous both clinically and genetically, with mutations in 32 identified genes. While the majority of mutations are repeat expansions (65%), a minority is also caused by missense, nonsense, and truncations (Durr, 2010). Recently, studies have linked SCA to changes in the synaptic transmission and transcription regulation, showing these mechanisms to be commonly attacked between the genetically distinct spinocerebellar ataxias (Nibbeling et al., 2017). Breakdown in the natural autophagy process leading to pathological protein aggregation was also observed (Nibbeling et al., 2017), a common trend within neurodegenerative diseases.

Emerging evidence in neurodegeneration has provided support that late-onset neurodegenerative diseases with biologic overlap may have common genetic risk factors disease even though they present with clinically heterogeneous symptoms (Ross et al., 2011, Simon-Sanchez et al., 2005). In particular, evidence of SCA-2 expansion was found in a family with autosomal dominant Parkinson's disease and families with SCA2 and SCA3 expansions presenting with typical Parkinson's disease symptoms (Furtado et al., 2002, Simon-Sanchez et al., 2005, Kim et al., 2017). SCA2 is characterized by repeated CAG triplets in the N-terminal region of the protein ataxin 2, with the presence of more than 31 repeats possibly causing clinical neurodegeneration (Ross et al., 2011). The parkinsonism phenotype has also been observed in single cases in patients with ATXN3 expansions (Bettencourt et al., 2011). Similarly, atypical Parkinsonism and PD-like phenotypes have also been observed in SCA6

and SCA17, with a particular number of studies performed within the Korean ataxia and Parkinson's scientific community.

A recent study found 40 CAG repeats with 4 interruptions in the PD patients of a Korean family presenting with typical physical phenotype of autosomal dominant PD without cerebellar ataxia (Kim et al., 2017). The 40 repeat length is the longest observed in families exhibiting Parkinsonism without ataxia. There is considerable debate in the choice of cut-off for defining "pathogenicity" of repeats. Using a multi-ethnic cohort as discussed in Part 1 of Chapter 2, we ascertained our cut-off threshold. Our own allelic density plots showed comparable distribution of repeat length between our cohort and previously published studies (Figure e1, pg. 43), but our SCA2 repeat length ranged from 24 to 32, much shorter than the 40 observed in the South Korea family. On the other hand, we did not investigate the role of interrupted repeats on PD, thus our results cannot comment on the South Korean family with Parkinsonism. However, our results do suggest a trend effect for SCA2 in the Asian population. In conjunction with the recent familial evidence from the longitudinal Korean study, our trend results suggest a connection between SCA2 and PD in the Asian cohort is an important topic to further study.

Synuclein aggregation, one of the hallmarks of PD, has been linked to mutations in the cell transport systems, and the retromer pathway has been specifically implicated in PD pathogenesis (Mohan and Mellick, 2017). VPS35 codes a part of the retromer complex that is responsible for protein sorting between the endosome-lysosome degradation pathways and the Golgi apparatus (Bonifacino and Hurley, 2008). Mutations in the VPS35 gene, a protein integral to intracellular retrieval of membrane proteins in the retromer pathway has been linked to patients with autosomal dominant PD (Zimprich et al., 2011, Tang et al., 2015b). Furthermore, mutations in VPS35 has also been demonstrated to result in alpha-synuclein aggregation in animal models (Tang et al., 2015a).

A series of studies in the previous years demonstrated functional interaction between two distinct loci that both affect the functionality of the VPS35 component of the retromer complex (Chuang and

Gitler, 2013, MacLeod et al., 2013). From there, evidence mounted that faulty protein sorting within the vesicle components was tied to PD pathogenesis (Bonifacino and Hurley, 2008). LRRK2 has been implicated repeatedly as a PD risk gene in genetic studies; the gene codes a large multi-domain protein that is tied to GTPase and kinases activities (Webber and West, 2009). Mutations in LRRK2 damage lysosomal protein degradation and autophagy, thus hindering the cellular process of delivering protein aggregates and cytosolic proteins to the lysosome, the structure responsible for breaking down biological polymers (Kuwahara et al., 2016). Thus taken together, the evidence suggests a strong connection between defective protein sorting and vesicle transport and PD pathogenesis.

Interaction studies between PD risk-modifying variants has been of particular interest in PD genetics, as newly discovered genes had small effects on the total "missing heritability" seen in PD. Thus, an open question is the degree to which PD genes modulate each other and influence the effect of the singular mutations. A previous study found connections and synergistic effects between VPS35 and EIF4G1 mutations and SNCA toxicity (Dhungel et al., 2015). Recent research has already demonstrated LRRK2 and PARK16 functional interaction. Wildtype VPS35 expression has been demonstrated to rescue defects in the endolysosomal and Golgi apparatus sorting complex caused by RAB7L1 or LRRK2 mutations (MacLeod et al., 2013). In the same study, Macleod et al also demonstrated functionally that deficiency of the PARK16 locus gene RAB7L1 synergistically affected neurodegeneration in rodent models of familial PD due to mutant LRRK2 expression, while RAB7L1 over-expression rescued the LRRK2 mutant phenotype. RAB7L1 suppressed LRRK2 mutant pathology in both in vitro and in vivo screenings.

The functional studies preceding our investigation set up the foundation for our gene-gene interaction study between PARK16 and LRRK2. While there is a consensus that genetic interaction studies are generally underpowered, that does not indicate that they are not of value. Working with the constraints of availability, we conducted the largest interaction study to date on an international

multi-ethnic cohort. The functional studies on the interaction between PARK16 and LRRK2 in the retromer pathway argue convincingly of a biological effect (MacLeod et al., 2013). As expected with gene interaction studies, our study failed to show statistical significance of interaction. Nevertheless, we observed a trend for interaction, which did not reach significance due to sample size. The trend effect is observed in OR's between concomitant carriers of both LRRK2 and PARK16 versus carriers of only LRRK2; with carriers of both mutant genotypes at significantly lower risk of PD versus LRRK2 carriers (Chapter 2 Part 3, Supplementary Table 2). We found results that suggest an effect in the same direction as previous literature, but our study does not support the presence of a strong interaction effect.

In a post-publication analysis of the data, we considered the possibility that certain sites with particularly large variance covered a true effect of interaction, and thus performed leave-one out analysis across all sites. However, the results did not change, and no significant interaction effect was observed (Table 1). This, in turn, is consistent with our data quality screening where we did not observe any statistically significant heterogeneity in the data.

Table 15: Post-hoc Leave-one-out analysis, Site column indicates dataset that was withheld

Sites	OR Coefficient	95% CI	P-Value
Aasly	0.87	(0.75, 1.02)	0.085
Annesi	0.86	(0.74, 1.01)	0.064
Bozi	0.87	(0.74, 1.01)	0.071
Belgium	0.88	(0.75, 1.03)	0.125
Brice	0.87	(0.74, 1.01)	0.075
Hadjigeorgiou	0.87	0.74, 1.02)	0.087
Lynch/Ross	0.88	(0.75, 1.03)	0.105
Maraganore	0.87	(0.74, 1.01)	0.069
Mellick	0.87	(0.75, 1.03)	0.100
Morrison	0.85	(0.72, 0.99)	0.036
Opala/Ross	0.86	(0.74, 1.01)	0.066
Wirdefeldt	0.87	(0.74, 1.01)	0.071
Wszolek/Ross	0.86	(0.74, 1.01)	0.065
Chung	0.89	(0.75, 1.05)	0.171
Lin	0.88	(0.85, 1.04)	0.125

Japan	0.76	(0.62, 0.93)	0.008
Tan	0.88	(0.75, 1.04)	0.128

OR: Odds Ratio, CI: Confidence Interval

The etiology of idiopathic PD has proven to be a complex and difficult to untangle interconnected web of aging, environmental, and genetic factors. In addition to protein aggregation and defects in the transport pathways, mitochondrial dysfunction, oxidative stress and neuroinflammation have also come under consideration as etiological factors for PD (Lee et al., 2017). Pathology in brains of PD and DLB patients versus healthy controls found that brain cortex activity and mitochondrial O_2 uptake were significantly lower in diseased patients, and signs oxidative stress and mitochondrial dysfunction were significantly higher in diseased patients (Navarro and Boveris, 2009). Within PD neuropathogenesis, research has also found evidence that microglial activation causes increased activity in the cyclooxygenase (COX) pathway (Tansey et al., 2007). Furthermore, anti-inflammatory treatment protected against dopaminergic cell death and blocked activation of microglial cells in the MPTP-induced mouse model of PD (Kim et al., 2012).

Inflammation is a shared manifestation of pathogenesis observed in both PD and auto-immune diseases. Genetic pleiotropy, the idea that a single gene has multiple different manifestations through its functionality within separate biological pathways, has also been a growing theme in genetic research. A study from 2016 used GWAS data to identify a common mutation in the extended MAPT region that is associated with both AD and PD (Desikan et al., 2015). Outside of pleiotropy between neurodegenerative diseases, common genetic variants have also been established between PD and auto-immune diseases. Crohn's disease has also identified LRRK2 as a susceptibility loci uses GWAS (Franke et al., 2010). Additionally, SNCA has been identified in the enteric neurons in the myenteric plexus situated within the gastrointestinal tract (Sharrad et al., 2013). Research has found that mitochondrial dysfunction and inflammation, a common theme between PD and autoimmune diseases, is a potential shared byproduct of immune dysfunction (McGeer and McGeer, 2011). Pleiotropic genes have also been identified between AD and T1D

(Christensen et al., 2016). On the other side of this growing body of evidence, human leukocyte antigen (HLA), a major histocompatibility complex tied to immune dysfunction and autoimmune diseases, has also emerged as an important risk loci for PD (Witoelar et al., 2017, Wissemann et al., 2013). Associations between AD and immune-mediated diseases has also been demonstrated (Yokoyama et al., 2016). Candidate gene studies assessing genetic variable have found that neuroinflammatory genes such as TNF, IFN-gamma, and IL also increase risk of PD.

Given the emerging evidence of immune dysfunction and neuroinflammation in neurodegenerative diseases, and in particular PD, we looked at connections between PD and Type 1 Diabetes, a disease with a very strong auto-immune component. Connections between PD and T1D goes beyond the HLA connection. Alpha-synuclein deposits have been discovered in the pancreas of PD patients (Titova et al., 2017a). Autoimmune diseases such as T1D are also tied to many of the same inflammatory mediators, and it has been suggested that the immune response from mediators such as cytokines not also contribute to cell death and disease pathogenesis of the autoimmune disease, but also increases the risk as well of neurodegenerative diseases. With the development of evidence on not only genetic risk factors but also disease pathogenesis, we performed a comparison study between the prevalence and diabetic end-points of neurologically normal T1D patients and patients with PD.

We found evidence that supports the emerging relationship between PD and T1D. Our findings on the differences in renal function are very interesting in light of the discovery of alpha-synulcein deposits outside of the brain in PD patients. With the discovery of alpha-synuclein deposits in the pancreas of PD patients, the increased risk of renal failure in PD patients is even more interesting given the knowledge that PD does affect organs outside of the brain. We also observed differences in rates of stroke and dementia between PD patients and neurologically normal patients. Stroke has been tied to inflammation and hypertension, two symptoms also related to PD pathogenesis. Diabetes and PD both see increased rates of dementia within its patient population, and the

increased rate at which PD T1D patients presented with dementia raises the question of whether it is a manifestation resulting from common genetic etiology between two diseases. Thus, it warrants further study to understand the genetic etiology of dementia and its ties to both PD and T1D.

<u>Future perspectives and directions</u>

PD affects the quality of life of patients, caregivers, and consequently the national health economies through lost productivity. As the world population ages, its impact will only increase. One of the keys to treating and targeting PD is through a better understanding of its risk architecture. With that in mind, the underlying aim in this thesis is to advance the understanding of risk factors of PD.

With the research presented here, we have not only contributed to the current body of knowledge, but also have highlighted important avenues of future research. In particular, while the ataxia study and the interaction study were negative studies, they nonetheless contributed substantially to the general conversation. Clinicians now know that it is not necessary to screen for PD in patients with the aforementioned ataxia types. In the case of the LRRK2 PARK16 interaction study, we demonstrated that it is unlikely that a strong synergistic or negating effect exists between the two genes, but also highlighted the need for substantially larger studies, particularly in the context of gene-gene statistical interaction.

PD is a complicated illness, with many environmental and genetic risk factors and a diverse web of non-motor and motor symptoms. It shares pathology with numerous other neurodegenerative diseases. Furthermore, the role of the auto-immune system also bears future exploration, as demonstrated by our diabetes study, among others. Science rarely comes from large leaps, instead one can only reach the next set of stars by first standing on the shoulders of giants, of those who have gone before us. The epidemiology of Parkinson's disease is no exception to this old adage by Newton. Our ultimate goal, as researchers, is to fully understand the genetic landscape of PD. However, one can only poke holes into the darkness, chipping away at this complex task, one study at a time, one piece of research at a time.

Chapter 4 Summary

English Summary

PD is the second most common neurodegenerative disease in the world, with the disease burden only expected to rise as the world population ages. The discovery of new genetic risk factors through a range of methods over the years has resulted in a long list of genetic variants that influence PD risk. However, our understanding of the genetic variants and the mechanisms through which they influence PD risk still leave much room for future discovery. The incompleteness of the inheritance patterns and the limited ability (up to 10%) of the genetic variants to explain PD cases suggests complex and multifactorial origins for PD. Hence, this thesis seeks to fill some of the open questions.

The first part of the thesis addresses the etiological factor of protein aggregation through the question of cross-disease effect of ataxia genes on PD risk. We did not find any increase in in PD risk in relation to the existence of intermediate repeat expansions in SCA2, 3, 6, and 17 in a large scale consortium study, and thus no evidence that the familial affect seen extrapolates to sporadic PD.

 The second part of the thesis takes the theme of aggregration but approaches it from the transportation pathways. Given the known functional interaction between two genes in the retromer pathway, we also investigate a possible genetic interaction between the two previously identified risk modulating loci, PARK16 and LRRK2. Using a large scale multi-site study, we did not find any conclusive evidence of strong genetic interaction between the two loci. However, our results suggest that further research is needed with larger sample sizes, due to our trend findings within subgroups of our population.

Immune response and mitochondrial dysfunction drive the third part of the thesis; we investigate the differences in clinical outcomes between type 1 diabetic patients with PD and neurologically normal patients. Recent studies have demonstrated the sharing of pathways and commonalities in pathogenesis between the two diseases due to shared auto-immune dysfunction, along with the discovery of HLA as a risk loci for both diseases. Due to the interconnectedness of the two disease,

we felt it was important to also study the clinical pathogenesis of PD patients within a T1D

population. We found several significant differences in T1D patients with and without PD, mainly

renal failure, stroke, and hospitalizations, suggesting that a correlation between PD and certain

diabetic outcomes.

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Parkinson-Krankheit ist die zweithäufigste neurodegenerative Krankheit in der Welt, wobei die Krankheitslastmit steigender Weltbevölkerung nur steigen wird. Die Entdeckung neuer genetischer Risikofaktoren durch eine Reihe von Methoden im Laufe der Jahre, hat zu einer langen Liste von genetischen Varianten geführt, die das PD-Risiko beeinflussen. Unser Verständnis der genetischen Varianten und der Mechanismen, durch die sie das PD-Risiko beeinflussen, lässt jedoch noch viel Raum für zukünftige Entdeckungen. Die Unvollständigkeit der Vererbungsmuster und die begrenzte Fähigkeit (bis zu 10%) der genetischen Varianten, PD-Fälle zu erklären, deuten auf komplexe und multifaktorielle Ursachen für PD hin. Daher versucht diese Arbeit einige der offenen Fragen zu beantworten.

Der erste Teil der Arbeit befasst sich mit dem ätiologischen Faktor der Proteinaggregation durch die Frage der Kreuz-Krankheit-Wirkung von Ataxie-Genen auf PD-Risiko. In einer groß angelegten Konsortium Studie fanden wir keinen Anstieg des PD-Risikos in Bezug auf die Existenz von repetitiven Zwischenexpansionen in SCA2, 3, 6 und 17, und somit keinen Hinweis darauf, dass der beobachtete familiäre Affekt sich auf die sporadische Parkinson-Krankheit ableiten lässt.

Der zweite Teil der Arbeit behandelt das Thema Aggregation, nähert sich aber von seiten der Transportpfade. Angesichts der bekannten funktionellen Interaktion zwischen zwei Genen im Retromer-Signalweg untersuchen wir auch eine mögliche genetische Interaktion zwischen den beiden zuvor identifizierten Risiko-modulierenden Loci, PARK16 und LRRK2. In einer großen multizentrischen Studie fanden wir keine schlüssigen Beweise für eine starke genetische Interaktion zwischen den beiden Loci. Unsere Ergebnisse deuten jedoch darauf hin, dass aufgrund unserer positiven Trends in Subgruppen unserer Population weitere Untersuchungen mit größeren Stichproben erforderlich sind.

Immunantwort und mitochondriale Dysfunktion bestimmen den dritten Teil der These; Wir untersuchen die Unterschiede in den klinischen Ergebnissen zwischen Typ-1-Diabetikern mit PD und neurologisch normalen Patienten. Jüngste Studien haben geteilte Stoffwechselwege und

übereinstimmende Pathologien zwischen beiden Krankheiten, die auf eine gemeinsame Autoimmunfunktionsstörung zurückzuführen sind, gezeigt und gleichtzeitg HLA als Risiko-Loci für beide Erkrankungen entdeckt. Aufgrund der Vernetzung der beiden Erkrankungen war es uns wichtig, auch die klinische Pathogenese von PD-Patienten innerhalb einer typ 1 Diabetes Population zu untersuchen. Wir fanden mehrere signifikante Unterschiede bei T1D-Patienten mit und ohne PD, hauptsächlich Nierenversagen, Schlaganfall und Krankenhauseinweisungen, was auf eine Korrelation zwischen PD und bestimmten diabetischen Ergebnissen hindeutet.

Chapter 7 Declaration of contribution of others

The dissertation work was carried out at the Institute of Clinical Epidemiology and Medical Biometry, as a part of the Genetic Epidemiology group led by Dr. Manu Sharma, under the joint supervision of Dr. Sharma and Professor Dr. Martus.

The studies were designed in collaboration with Dr. Sharma. Data was contributed through the GEOPD consortium from member sites for the PolyQ and LRRK2/PARK16 interaction study. The individual sites collected and sequenced patient level case-control data. I planned, programmed, and carried out the statistical analysis of the two projects myself, with support from Manu Sharma, and Michael Heckman in the LRRK2/PARK16 study.

The DPV registry supplied the diabetes patient-level data. I co-wrote the manuscript with Nicole Prinz, co-first author of the study.

I confirm that I wrote the cumulative thesis myself under the supervision of Dr. Sharma and Professor Dr. Martus and that any additional sources have been cited.

Signed _____

On the 16th of January 2018 in Tuebingen

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List of Abbreviations

1-methyl-4-phenyl-1,2,3,6-tetra hydropyridine (MPTP)

1-methyl-4-phenyl pyridnium (MPP$^+$)

alpha-synuclein (SNCA)

alpha-synucleinopathies (AS)

Alzheimer's disease (AD)

Amyotrophic lateral sclerosis (AML)

corticobasal degeneration (CBD)

Eukaryotic translation initiation factor 4-gammon (EIF4G1)

F-box only protein 7 (FBXO7)

Frontotemporal dementia-17 (FTD-17)

Glucocerebrosidase (GBA)

Genome wide association studies (GWAS)

GTP cyclohydrolase (GCH1)

Guanosine triphosphatase (GTPase)

Human leukocyte antigen (HLA)

Leucine-rich repeat kinase 2 (LRRK2)

MAPT (microtubule-associated protein tau)

Minor allele frequency (MAF)

Multiple system atrophy (MSA

N-methyl-D-aspartate receptor (NMDA)

Parkinson's disease (PD)

Pick's disease (PiD)

Progressive surpranuclear palsy (PSP)

PTEN-induced kinase 1 (PINK1)

Single nucleotide polymorphisms (SNPs)

Substantia nigra pars compacta (SNPc)

Type 1 Diabetes Mellitus (T1DM)

Vacuolar protein sorting 35 (VPS35)

Whole-exome sequencing (WES)

Whole-genome sequencing (WGS)

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