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Exploring a Gene Panel for Parkinson's Disease in an Egyptian Cohort

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Graduate Studies

Exploring a Gene Panel for Parkinson's Disease in an Egyptian Cohort

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Declaration of Authorship

I, Asmaa Saeed, declare that this thesis titled, “Exploring a Gene Panel for Parkinson’s Disease in an Egyptian Cohort” and the work presented in it are my own. I confirm that:

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- Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated.
- Where I have consulted the published work of others, this is always clearly attributed.
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Abstract

Parkinson’s disease is a highly heterogeneous disorder characterized by diverse neuropathological features, clinical presentations and progression patterns. In Egypt, Parkinson’s disease incidence rates lie outside the range reported elsewhere. The genetic background to the pathogenesis of Parkinson's disease has been postulated for a long time. However, Parkinson’s disease has never been systematically investigated in Egypt. This study aimed to explore genetic variants and interactions that are associated with the familial and sporadic forms of Parkinson's disease in an Egyptian cohort. This includes examining variants in PD-related genes, exploring the role of specific genes like *MAPT* and adjacent genomic regions, and investigating the combined effects between genes such as *APOE* and *BCHE*. Whole exome sequencing was conducted for individuals with a family history of PD. Identified variants were filtered for rarity and potential pathogenicity. Notable findings included variants in *LRRK2, GBA, DNAJC6, DLG2, SYNJ1*, *PITRM1* and others. The *PITRM1* R892K variant, identified in multiple family members, showed incomplete and age-related penetrance. Furthermore, investigating the association between genetic variants in *MAPT, KANSL1 and SPPL2C* genes and the risk of developing PD did not find statistically significant associations between the studied variants and PD risk. Similarly, the *APOE* and *BCHE* variants did not show a strong association with PD risk, and the association was statistically non-significant. The studied variants showcased varying correlations with the clinical outcomes, and the *BCHE* K-variant demonstrated a statistically significant correlation with the severity of Parkinson's disease symptoms as measured by the UPDRS. Additionally, the analysis revealed a series of SNP-SNP interactions that were significantly associated with a lower risk of PD. These findings are a piece of the larger puzzle in understanding the genetics of PD and underscore the importance of considering multiple genetic factors rather than single genetic variants.

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List of Abbreviations

AD	Autosomal Dominant
ALP	Autophagy-Lysosome Pathway
ANADs	Atypical Neuroaxonal Dystrophies
APOE	Apolipoprotein E
AR	Autosomal Recessive
ASAP	Aligning Science Across Parkinson's
ASH1L	Achaete-scute homolog 1
ATX2	Ataxin-2
BCHE	Butyrylcholinesterase
BH4	Tetrahydrobiopterin
BPAN	Beta-Propeller Protein-Associated Neurodegeneration
BST1	Bone marrow stromal cell antigen-1
Ca^{2+}	Calcium
CADD	Combined Annotation Dependent Depletion
CBD	Corticobasal Degeneration
CHCHD2	Coiled-Coil-Helix-Coiled-Coil-Helix Domain Containing 2
CNS	Central Nervous Systems
DA	Dopamine
DaT	Dopamine Transporter
DLB	Dementia With Lewy Bodies
DLG2	Discs-large membrane-associated guanylate kinase scaffolding protein 2
DNAJC6	Dnaj Heat Shock Protein Family Member C6

DRD	DOPA-Responsive Dystonia
EAPDGC	East Asian Parkinson Disease Genomics Consortium
ENS	Enteric Nervous System
ERBIN	Erbb2 Interacting Protein
ETC	Electron Transport Chain
FBXO7	F-Box Protein 7
FTD	Frontotemporal dementia
GBA	Glucocerebrosidase
GBD	Global Burden of Diseases
GCase	Glucocerebrosidase
GCH1	GTP Cyclohydrolase 1
GD	Gaucher's Disease
GIGYF2	GRB10-Interacting GYF 2
GP2	Global Parkinson's Genetics Program
GWAS	Genome-Wide Association Study
H&Y	Hoehn & Yahr
HGMD	Human Gene Variant Database
HRQoL	Health-Related Quality of Life
INAD	Infantile Neuroaxonal Dystrophy
IPDBGC	International Parkinson Disease Genomics Consortium
KANSL1	KAT8 regulatory NSL complex subunit 1
KASP	Kompetitive Allele Specific PCR

KRS	Kafer-Rakeb Syndrome
L-dopa	Levo-Dopa
LARGE-PD	Latin American Research Consortium On The Genetics Of PD
LD	Linkage disequilibrium
LoF	Loss of function
LRP10	Low-Density Lipoprotein Receptor-Related Protein 10
LRRK2	Leucine-Rich Repeat Kinase 2
MAF	Minor Allele Frequency
MAPT	Microtubule-associated protein tau
MDS	Movement Disorder Society
MeCP2	Methyl CpG-Binding Protein 2
MENA	Middle East and North Africa Region
MoCA	Montreal Cognitive Assessment
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	Magnetic Resonance Imaging
MSA	Multiple System Atrophy
mtDNA	Mitochondrial DNA
NGS	Next-Generation Sequencing
NSL	Non-Specific Lethal
OR	Odds Ratio
PD	Parkinson's Disease
PDD	PD Dementia
PGK1	Phosphoglycerate Kinase-1 Deficiency Syndrome

PINK1	PTEN-Induced Kinase 1
PITRM1	Pitrilysin Metallopeptidase 1
PLA2G6	Phospholipase A2 Group VI
PNS	Peripheral Nervous System
PODXL	Podocalyxin-Like
POLG	Polymerase Gamma
PPS	Parkinsonian-Pyramidal Syndrome
PSP	Progressive Supranuclear Palsy
PTRHD1	Peptidyl-Trna Hydrolase Domain Containing 1
QC	Quality Control
RBD	Rapid eye movement sleep behaviour disorder
REM	Rapid Eye Movement
ROS	Reactive Oxygen Species
SCA2	Spinocerebellar ataxia type 2
SCF	SKP1-Cullin-F-Box
SIFT	Sorting Intolerant from Tolerant
SLC6A3	Solute Carrier Family 6 Member 3
SNARE	Soluble N- Ethylmaleimide-Sensitive Factor Attachment Protein Receptor
SNCA	Alpha-Synuclein
SNpc	Substantia Nigra Pars Compacta

SNPs	Single Nucleotide Polymorphisms
SPPL2C	Signal peptide peptidase like 2C
STK39	Serine/threonine kinase 39
STN-DBS	Subthalamic Nucleus Deep Brain Stimulation
SYNJ1	Synaptojanin 1
TFAM	Transcription Factor of a Mitochondria
TMEM230	Transmembrane Protein 230
TMPRSS9	Transmembrane Serine Protease 9
UCHL1	Ubiquitin C-Terminal Hydrolase L1
UKPDSBB	United Kingdom Parkinson's Disease Society Brain Bank
UPDRS	Unified Parkinson's Disease Rating Scale
UPS	Ubiquitin-Proteasome System
UQCRC1	Ubiquinol-Cytochrome C Reductase Core Protein 1
VaP	Vascular Parkinsonism
VP35	Vacuolar Protein Sorting-Associated Protein 35
VPS13C	Vacuola Protein Sorting 13
XDP	X-Linked Dystonia-Parkinsonism
XPDS	X-Linked Parkinsonism and Spasticity

List of Symbols

β	Regression Coefficient
ρ	Correlation Coefficient

Chapter 1

Introduction

Parkinson's disease (PD) was clinically characterized as a movement disorder since its discovery by James Parkinson, who described this disorder as "paralysis agitans''. He provided an extraordinarily detailed account of this condition's clinical features and its debilitating progressive nature in "An Essay on the Shaking Palsy" (Parkinson, 2002). This essay conveyed the primary manifestations of PD that are still recognized today, such as resting tremors, decreased muscle strength, and abnormal posture and gait. A significant turning point in the history of PD treatment was the discovery of the neurotransmitter dopamine and its role in the disease. In the 1960s, Arvid Carlsson demonstrated that dopamine was a neurotransmitter in the brain and that PD symptoms were related to dopamine deficiency (Yeragani et al., 2010). This led to the development of Levodopa (L-DOPA), which remains a cornerstone of PD treatment. L-DOPA, a precursor to dopamine, can cross the blood-brain barrier and then turn into dopamine, replenishing the depleted levels and alleviating symptoms. Later in the 20th century, research advances in the understanding of the genetic and environmental factors behind PD pathogenesis opened the door for investigating the impact of neurotoxins like 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in inducing parkinsonism in a group of young drug addicts (William Langston et al., 1983). This was followed by the discovery of variants in the α-synuclein gene (SNCA) in families with hereditary PD highlighting the disease's genetic component (Polymeropoulos et al., 1997). Subsequent research has identified multiple genetic loci and variants associated with PD, offering insights into its pathophysiology.

Despite the significant strides made in Parkinson's disease genetics research over the past few decades, notable disparities and gaps remain, particularly regarding the underrepresentation of certain populations, like those in Egypt and other regions outside of Western countries. The majority of molecular genetic research of various diseases was historically focused on Caucasians for a variety of geopolitical, cultural, linguistic, and economic reasons (Sirugo et al., 2019). As for Parkinson's disease, people with European ancestry make up a disproportionate amount of the

research used to understand PD. This focus has led to a considerable knowledge gap and a skewed understanding of the genetic landscape of PD, potentially missing key genetic factors prevalent in other ethnicities or regions. That being said, genetic regional and racial differences in PD are observed for many genes. Certain genetic variants may be unique or have different frequencies and effects in populations that are not well-studied, which could lead to missed opportunities for understanding the disease fully. For instance, the LRRK2 G2019S variant. While it is a common cause of PD among North African Arabs, with frequencies up to 30-40% in PD patients, it is much less common in Asian and European populations (Lesage et al., 2006).

Epidemiological figures of prevalence and incidence rates of PD in Africa, including Egypt are under-reported and mostly outdated in comparison with Europe and North America. According to a recent systematic review, the scarcity of data on different aspects of PD in Africa may be explained by geographic and environmental variations in risk factor exposure and genetic diversity in addition to the widespread under-diagnosis and under-reporting resulting from the relatively small numbers of neurological professionals with knowledge of PD research, diagnosis, and management (Okunoye, Zewde, et al., 2023). There is a scarcity of large-scale, comprehensive genetics PD studies that tackled the populations living in Central, Eastern and the French-speaking West Coast of Africa whereas around 77% of the PD genetics studies conducted in Northern (mainly Tunisia) and Southern Africa (mainly South Africa) (Okunoye, Zewde, et al., 2023). Several initiatives have been undertaken to address the disparities in Parkinson's Disease research, particularly those related to genetic understanding and the inclusion of diverse populations. Aligning Science Across Parkinson's (ASAP) established the Global Parkinson's Genetics Programme (“GP2: The Global Parkinson’s Genetics Program,” 2021) that aims to expand and diversify genetic research in PD. It focuses on increasing the representation of under-studied populations from Africa, Asia, and Latin America, thus providing a more comprehensive understanding of the genetic architecture of PD globally. The Genomics Consortium Africa (IPDGC Africa), the Latin American Research Consortium on the Genetics of PD (LARGE-PD) in Latin America, and the East Asian Parkinson Disease Genomics Consortium (EAPDGC) in East Asia, which includes Japan are just a few examples of the hubs that make up GP2 (Mok et al., 2021; Rizig et al., 2021; Zabetian & Mata, 2017). Through such open science initiatives, GP2 is extending its reach beyond Caucasians to incorporate various ethnic and racial groups that have been underrepresented in PD studies.

This thesis focuses on the genetics of Parkinson's disease in an Egyptian cohort and aims to conduct an in-depth genetic analysis of both familial and sporadic Parkinson's disease in Egypt, to identify unique and common genetic variants associated with the disease, and to explore the interaction of these variants within the global context of PD genetics. This study seeks to contribute to the broader goals of diversifying PD genetic research, enhancing the understanding of PD in underrepresented populations, and fostering international collaborative efforts in PD research.

Thereafter this thesis has three main objectives:

1- To identify and characterize genetic variants within known PD-associated genes, as well as novel loci, in an Egyptian population, with a focus on familial cases.
2- To investigate the contribution of the Microtubule-Associated Protein Tau (MAPT) gene and its surrounding genomic regions to the risk of PD in an Egyptian cohort.
3- To explore the potential combined effects of variants in the Apolipoprotein E (APOE) and Butyrylcholinesterase (BCHE) genes on the risk and clinical course of PD in an Egyptian cohort.

Chapter 2

Literature Review

1.1. Parkinson's Disease Etiopathogenesis

The etiology of Parkinson's disease is multifaceted, with genetic and environmental exposure factors contributing to disease progression. The very intensive research in the etiopathogenesis of Parkinson's disease resulted in significant strides in understanding this disease. From the identification of intraneuronal α-synuclein protein inclusions known as Lewy bodies reported by Frederick Lewy in 1912 as a pathological hallmark and establishing the link between dopamine deficiency and PD by Carlsson and Oleh Hornykiewicz in 1957 till the discovery of the genetic and modifiable environmental factors underlying PD pathogenesis. With the ageing and increasing life span of the global population, age is the most known etiology of PD, with the median age of onset being 60 years of age, and it is considered to be the major contributing factor to the development of this disease (Lees et al., 2009). Sex also significantly contributes to Parkinson's disease development, which is 1.5–2 times more frequent in males than in females (Haaxma et al., 2007). Researchers have pinpointed several genetic variants that can cause Parkinson's disease. A fraction of PD cases is due to Parkinsonian conditions with familial inheritance. Studying the genetic determinants of these phenotypes has provided insights on the common pathogenetic mechanisms underlying Parkinsonian conditions. However, these are less likely common, except for a few cases where numerous family members are affected by Parkinson's disease. However, certain gene variants appear to slightly increase the risk of Parkinson's disease but with a relative impact for each of these genetic markers (will be discussed in detail in section 1.5). A higher chance of developing Parkinson's disease later in life may result from exposure to specific toxins or environmental factors. Theoretically, prolonged neurotoxic exposure or a short dose that starts a chain reaction of harmful events could cause the progressive neurodegeneration associated with PD. However, the implications of these interactions with PD pathology and progression are still poorly understood. Numerous environmental risk factors have been identified, including exposure to pesticides and heavy metals, rural living, working in an

agricultural setting, air pollution, traumatic head injuries, consuming dairy products, and type 2 diabetes mellitus; however, PD risk is minimized using antidiabetic drugs (Jankovic & Tan, 2020). However, the association of these environmental risk factors with PD is relatively inconsistent due to the underlying biological plausibility and inherent limitations in some studies.

PD is thought to arise from a deficiency of dopamine (DA) in the substantia nigra pars compacta (SNpc), an area of the midbrain concerned with regulation of movement. Since the SNpc is the origin of nigrostriatal dopaminergic system, gradual degeneration of dopamine neurons in this brain region will perturb dopaminergic neuronal projection to the striatum and result in subsequent loss of dopamine input. Primary motor symptoms of Parkinson's disease are brought on by abnormal brain activity resulting from a drop in dopamine levels. James Parkinson originally reported these symptoms as a heterogeneous manifestation of PD (Parkinson, 2002). Understanding the underlying mechanisms causing the selective loss of dopamine-producing neurons in the substantia nigra or the build-up of aggregated α-synuclein in PD is challenging. These pathways mainly involve the maintenance of cell homeostasis, which has been shown to deteriorate with normal ageing. The most prominent molecular pathogenic mechanisms which can cause DA neurons to die and degenerate include α-synuclein misfolding and aggregation, oxidative stress, mitochondrial dysfunction, neuroinflammation, and protein clearance impairment (Dong-Chen et al., 2023).

There is a crosstalk between oxidative stress, mitochondrial dysfunction and neuroinflammation. Mitochondrial dysfunction is caused by oxidative stress, and neuroinflammation is induced by mitochondrial dysfunction. Reactive Oxygen Species (ROS) is mainly derived from mitochondria and considered to be natural byproduct of the electron transport chain (ETC) activity (Thannickal & Fanburg, 2000). Usually, mitochondrial anti-oxidative systems detoxify ROS. However, under different stimuli, over-production of ROS in the brain results in impaired mitochondrial quality control and oxidative stress rises (Dias et al., 2013). Besides increased reactive oxygen species generation, mitochondrial function is impaired in PD patients as a result of other various causes, including defects in mitochondrial biogenesis, impaired mitophagy, compromised trafficking, calcium (Ca^{2+}) imbalance, and electron transport chain (ETC) dysfunction (Moradi Vastegani et al., 2023). Macromolecules in the mitochondrial structure

are susceptible to oxidative damage, which creates a vicious cycle that ultimately leads to complete organelle dysfunction, contributing to a cascade of cellular structure disruption, including proteins, lipids, and DNA. By binding to danger-signal receptors, mitochondrial DNA (mtDNA) can function as a damage-associated molecular pattern (DAMP) and start an innate immune inflammatory response (Picca et al., 2020). Glia can easily recognize mitochondrial-derived molecules such as mtDNA, a transcription factor of A mitochondria (TFAM) and cytochrome c as harmful substances, thereby activating neuroinflammation (Lin et al., 2022). Additionally, some studies have linked neuroinflammation with synucleopathies (Varanita & Bubacco, 2020; Vieira et al., 2015). α-syn secreted by neurons directly acts on the astrocytes and induces inflammation by producing inflammatory cytokines and chemokines, including inflammatory regulatory factors such as IFN-γ and TNF-α (S. He et al., 2020). Furthermore, among the pathogenic mechanisms of neurodegeneration in PD is the disruption of protein clearance pathways that lead to the accumulation of α-synuclein protein. In neurons, the two main mechanisms for the degradation of misfolded or aggregated proteins within the cell are the ubiquitin-proteasome system (UPS) and the autophagy-lysosome pathway (ALP). An increasing number of studies in both disease models and patients have linked dysfunction of the UPS and ALP with Parkinson's disease pathogenesis (Ebrahimi-Fakhari et al., 2012; Lehtonen et al., 2019).

Not only is PD neuropathology limited to dopaminergic cells loss but also neurodegeneration and Lewy bodies formation were found in noradrenergic (locus coeruleus), serotonergic (raphe), and cholinergic (nucleus basalis of Meynert, dorsal motor nucleus of vagus) systems, as well as in the cerebral cortex (especially cingulate and entorhinal cortices), olfactory bulb, and autonomic nervous system (Dauer & Przedborski, 2003). According to Braak et al., α-synuclein aggregation may initiate in the olfactory bulb and the dorsal nucleus of the vagus nerve in the lower brain stem, and it may then gradually move to the interconnected brain regions that lead to the cerebral cortex (Braak, Rüb, et al., 2003). However, some studies do not agree with the Braak hypothesis since PD neuropathology is pleomorphic and not all PD patients exhibit Lewy body pathology, it was found that Lewy bodies are absent in some familial forms of PD (Johansen et al., 2018). Another study proposed the Threshold theory that better accounts for the current neurobiology of PD symptom progression compared to Braak's hypothesis that the disease ascends from the peripheral nervous system (PNS) to the central nervous system (CNS) and shows that PD is a systemic disease

and Lewy body pathology starts simultaneously in all affected regions (Engelender & Isacson, 2017). The symptoms of Parkinson's disease may, however, be correlated with a varying threshold that cells in various parts of the central nervous system (CNS) and the enteric nervous system (ENS) have to the impact of neuronal loss. For instance, PD symptoms appear early in the gut due to the gut's lower neuronal density than the SNpc, whereas the latter requires at least 60% neuronal loss to occur before PD symptoms are noticed. (Engelender & Isacson, 2017). The involvement of these various pathways provides insights into the rich and variable clinical phenomenology associated with PD.

1.2. Clinical features of Parkinson's disease

The classical cardinal symptoms of resting tremor, rigidity, bradykinesia, and postural instability are the most commonly identified motor symptoms of PD. It is now recognized that Parkinson's disease is not exclusively a movement disorder. There are additional non-motor clinical characteristics, such as mental symptoms, gastrointestinal abnormalities, and cognitive impairments. The spreading pathology of Parkinson's disease suggests that it is a multisystem disorder that affects both the peripheral and central nervous systems and manifests motor symptoms long after the onset of neurodegeneration (Braak, Del Tredici, et al., 2003). In terms of the appearance of symptoms, PD can be separated into preclinical, prodromal and clinical stages (Hawkes et al., 2010). A continuous neurodegeneration begins in the substantia nigra in the initial, preclinical stage, with no visible clinical symptoms. Then comes a prodromal stage that lasts for more than 10 years, during which there is ongoing neuronal death along with some non-motor symptoms (Váradi, 2020). Motor symptoms are often preceded by a range of non-motor symptoms, such as depression, loss of smell, constipation and rapid eye movement (REM) sleep behavior disorder, and these symptoms can occur decades before the onset of cardinal motor symptoms (Hawkes et al., 2010). These prodromal symptoms are not specific to Parkinson's disease, but their co-occurrence is associated with a raised risk of Parkinson's disease. Over time, the first motor symptoms (bradykinesia, rigidity, and tremors) appear after this stage, when 40%–60% of dopaminergic cells are destroyed, and the patients enter the early stage of Parkinson's disease (Váradi, 2020). The signs and symptoms of Parkinson's disease are progressive, meaning they emerge gradually and worsen over time. Eventually, patients experience disabling autonomic

failure, cognitive decline and swallowing difficulties that make them unable to continue with their daily activities unassisted.

1.2.1. Cardinal Motor Symptoms

Tremor

Tremor is one of the most commonly recognized features of PD and is reported to affect up to 75% of patients during their disease course (Abusrair et al., 2022; Poewe et al., 2017). It is an involuntary shaking motion resulting from rhythmic muscle contraction and relaxation that the majority of PD patients report initially on the extremities but can expand to lips, chin and jaw. It was also the initial symptom James Parkinson highlighted, by titling his essay “The Shaking Palsy”, which refers to tremor (Parkinson, 2002). In most cases, once the tremor is noticed, Parkinson's disease becomes typically diagnosed. While PD patients can develop a variety of tremor types, the usual pill-rolling resting tremor is the most prevalent. It is also possible for kinetic and re-emergent postural forms to coexist, which could seriously impede functional ability (Jankovic et al., 1999). When a patient with Parkinson's disease experiences a tremor, it often affects only one side or area of the body and is most noticeable at the distal part of the extremities. Depending on where it started, it ultimately spreads to the arms and legs and frequently occurs when the limbs are at rest (Abusrair et al., 2022).

Rigidity

Rigidity (stiffness) is described as an increased resistance to passive movement and is considered the second-most common primary motor symptom of PD (Váradi, 2020). Even while some PD patients may have stiffness or even functional limitations (such as a "frozen" shoulder), rigidity is typically a sign detected by the physician rather than a symptom reported by the patient. Rigidity in PD patients is demonstrated by passive flexing, extension, and rotation of their bodies (Baradaran et al., 2013). It may occur in the lower part of the body, upper body, or the limbs. When it is present along with an underlying tremor, it is frequently referred to as a cogwheel phenomenon and may also be accompanied by pain and discomfort.

Bradykinesia

In PD patients, bradykinesia, slowness of movement, results in reduced reaction times and difficulty performing daily activities. PD patients exhibit difficulty in quick, alternating movements like tying shoelaces and writing, indicating a deficit in fine motor movement. Bradykinesia might also occur in conjunction with a reduction in the amount of spontaneous movement (hypokinesia or akinesia). Additional signs of bradykinesia include reduced facial expressions, swallowing issues, troubled speech, and reduced walking upper limb swing. Research suggests that the motor wiring and programming in PD patients' brains remain intact, but patients may have difficulty accessing and applying them (Berardelli et al., 2001). This could also explain why people with Parkinson's disease can use prior knowledge to carry out an exercise regimen but cannot initiate or choose a movement when they are not performing the routine.

Postural Instability

Postural instability manifests in PD patients as a result of the loss of balance and postural abnormalities in the form of a bent posture, trunk, and neck (Palakurthi & Burugupally, 2019). The majority of falls among PD patients and subsequent hip fractures are caused by this primary symptom, together with freezing (unexpected inability to move) and shuffling gait (Williams et al., 2006). In contrast to bradykinesia, stiffness, and tremor, postural instability develops later in the course of the disease progression. Postural instability among PD patients is unresponsive to all kinds of treatments including dopaminergic therapy, however deep brain stimulation has been found to be promising (Shivitz et al., 2006).

1.2.2. Non-motor Features

Studies previously mainly focused on PD's four cardinal motor symptoms as they are easily visible upon physical examination. However, the involvement of peripheral and enteric nervous systems through dysfunctionality in glutamatergic, GABAergic, noradrenergic, serotonergic, histaminergic and cholinergic nerves resulted in an extremely wide range of non-motor symptoms such as sleep disturbance, sensory abnormalities, autonomic dysfunction, psychiatric disorders, and cognitive impairment (Olanow et al., 2011). PD patients at an earlier stage have co-existing mild cognitive impairment, boosting the risk of converting to cognitive

deterioration and dementia; about 80% of PD cases report late-onset dementia (Hely et al., 2008). An autonomic nervous system dysfunction can manifest approximately 5–20 years prior to the initiation of typical Parkinson's disease motor symptoms (Postuma & Berg, 2016). It is a crucial factor that affects daily living and health-related quality of life (HRQoL) of PD subjects. Autonomic dysfunction symptoms include, but are not limited to, constipation, urinary urgency, orthostatic hypotension, sweating impairment, and erectile dysfunction (Poewe, 2008). Psychiatric disorders reported include depression, anxiety, hallucinations, impulsive behavioral symptoms and apathy. Some PD patients also exhibit abnormal sensory symptoms such as loss of smell and visual problems (Schapira et al., 2017). Some non-movement symptoms that have been observed in patients with Parkinson's, like REM sleep behaviour disturbance, appear to develop before the onset of movement symptoms and may serve as a sign of the initiation of the disease's progression (Postuma & Berg, 2016).

1.3. Parkinson's Disease Epidemiology in Egypt

Globally, PD prevalence and incidence are dramatically increasing with the advancement of the ageing trend. Research has shown that the global burden of neurodegenerative disorders, which drastically affects the elderly, such as PD, has increased significantly as populations are ageing. A large-scale worldwide systematic analysis of the global prevalence, mortality and disability rates for PD between 1990 and 2016 was performed by the Global Burden of Diseases (GBD) 2016 Parkinson’s Disease Collaborators. They reported in their study that the number of patients with Parkinson's disease doubled to over 6 million (Ray Dorsey et al., 2018). They are also expecting this number to more than double, reaching about 12 million by 2040, mostly as a result of ageing (Dorsey et al., 2018). Although the worldwide burden of PD has been previously documented using data from the GBD study 2016 (Ray Dorsey et al., 2018) and GBD study 2019 (Ou et al., 2021), regional-specific patterns, which may differ significantly from global patterns, are typically not taken into account in the global-level articles. A recent study by Safiri et al. utilized GBD 2019 data to investigate the trends and burden associated with PD in the Middle East and North Africa region (MENA) across the period 1990-2019 (Safiri et al., 2023). They reported that a clear rising trend was observed in the point prevalence of PD over the last 30 years in the MENA region, indicating that the disease will keep becoming a bigger public health concern

(Safiri et al., 2023). The incidence and prevalence of PD in Egypt have been investigated in a number of epidemiological studies, yet published data vary considerably, and direct comparisons are often impeded by differences and limitations in the methodology and reporting.

1.3.1. Prevalence in Egypt

The prevalence data for Parkinson's disease in Egypt is outdated. The most recent study on the prevalence of PD in Egypt was conducted in 2015. Since then, there have been a number of changes in the country's population, including an increase in the elderly population and an increase in exposure to environmental toxins. It is likely that the prevalence of PD in Egypt has increased since 2015. Prevalence rates of PD are low in Arab countries; on the other hand, Egypt shows an exceptionally high prevalence (Khedr et al., 2012). Although an increase in PD cases and risk is observed across all populations, the burden of PD decreases with increasing socio-economic development (Safiri et al., 2023), which isn't the case with Egypt. Like many Arab countries, a large proportion of the population in Egypt lives in rural regions where farming is widespread, resulting in a higher prevalence of PD among rural dwellers. One assumption was that a significant proportion of the population chews on the leaves of Catha edulis ("Khat") which have been linked to the development of parkinsonism through dopaminergic cell toxicity (Khalil et al., 2020; Mereu et al., 1983). The prevalence of Parkinson's disease (PD) is generally reported to range from 1 to 2 per 1000 people and to impact 1% of the population over the age of 60 (Tysnes & Storstein, 2017). However, the study by Khedr and colleagues in the Assiut Governorate in Upper Egypt, shows the highest PD prevalence: 557 per 100,000 persons (age-adjusted: 562) (Khedr et al., 2012). They suggested that environmental and genetic factors were responsible for this high prevalence in addition to their extensive case-finding strategy (door-to-door survey) and the evaluation by a neurologist, which contributed to increasing the diagnostic precision, all played a role in the high rate that they observed (Khedr et al. 2012). Additionally, most of the study subjects were from rural areas, and 15 % had a positive family history of PD (Khedr et al., 2012). However, another study by the same group in the Assiut and Qena governates of Egypt found a higher prevalence in urban areas where industrial pollutants from manufacturers may discharge into the Nile River (Khedr et al., 2015). This finding agrees with large population-based studies in the USA and Taiwan that demonstrated a substantially higher prevalence of PD in urban regions than in rural ones (C. C. Chen et al., 2009; Wright Willis et al., 2010). Another study from Egypt that

focused on older populations: ≥40 years found a high prevalence of 213−1,440 per 100,000 persons (El-Tallawy et al., 2013). Therefore, their results are not surprising. Although El-Tallawy et al. studied a population aged ≥40 years, these results are in contrast with Khedr et al. who reported a very high prevalence of 2,748 per 100,000 persons for people aged 50 years or more (El-Tallawy et al., 2013; Khedr et al., 2012). The differences between El Tallawy's and Khedr's reported prevalence are difficult to explain, especially since El-Tallawy and colleagues did not provide any prevalence (crude or age-adjusted) for the total study population (El-Tallawy et al., 2013). These findings illustrate the challenges in comparing prevalence among research, even within the same country. A well-designed multi-centric study in Egypt is therefore needed to explain these rather contradictory results.

1.3.2. Incidence in Egypt

Few PD incidence studies have been undertaken to date in Egypt. Although the generally reported PD incidence rates vary between 8 to 18 per 100,000 patient-years (de Lau & Breteler, 2006), the incidence rates of Parkinson's disease in Egypt are extremely high in comparison with that reported elsewhere. It is important to note that the incidence rates of PD in Egypt are based on a single study. The study is cross-sectional and community-based, done in Assiut Governorate; the crude incidence of PD reported was 84 per 100,000 person-years (95 % CI 10−158) (Khedr et al. 2012). The study used a screening questionnaire consisting of eight questions about tremors, bradykinesia, rigidity, balance, and changes in the speech and writing fields (Khedr et al., 2012). The UK Parkinson's Disease Society Brain Bank Criteria were used to confirm the diagnosis of PD (Khedr et al. 2012).

Although the reason for the surprisingly high incidence in the Egyptian study is not clear, it should be kept in mind that incidence studies require large cohorts, and the Egyptian study used a small sample (Khedr et al. 2012). However, the door-to-door approach adopted in this community-based study is more applicable to the Egyptian population, as fewer individuals have access to healthcare systems. Additionally, age distribution greatly influences crude and age-standardized incidence rates. Study design and diagnostic standards (but not survival) may also have an impact on the observed PD incidence. Environmental factors and genetics might also be important. Although the causes for this are not entirely evident, it is obvious that the PD incidence statistics provided from

Egypt are beyond the range recorded from other regions of the world. More epidemiological research in Egypt is required to verify and better understand the current findings.

1.4. Diagnosis

Parkinson's disease is a "clinical" diagnosis. This implies that the patient's medical history, physical examination, family history and symptoms altogether play a role in the diagnosis. Neither is there a definitive sign that could allow direct disease identification nor a specific lab or imaging test that can diagnose PD. Due to this, there have been several approaches towards developing guidelines that are primarily based on the existence or absence of certain symptoms and treatment responses. The developed criteria sought to identify and classify affected patients in order to reduce their disabilities and enhance their quality of life by offering a potential route through the complex set of symptoms. Furthermore, certain procedures can be used to confirm the diagnosis of PD or rule out other disorders that can resemble PD. These procedures include magnetic resonance imaging of the brain, genetic testing and dopamine transporter scan (DaT scan).

The initial set of clinical diagnostic criteria for PD was developed by the United Kingdom Parkinson's Disease Society Brain Bank (UKPDSBB) (Gibb & Lees, 1988). Three levels of the classification procedure have been implemented by this guideline, including diagnostic (presence of a Parkinsonian motor syndrome), exclusion of possible causes of secondary Parkinsonism, such as the use of antipsychotic drugs or cerebrovascular disease affecting the basal ganglia and supportive criteria (Gibb & Lees, 1988). In most cases, it has been demonstrated to yield a 90% diagnosis accuracy (Hughes et al., 2001) however, it should be emphasized that the presence of some similar phenotypic traits with other Parkinsonian diseases makes the diagnosis of PD challenging. Thus, the only way to prove the presence of PD is to confirm the presence of Lewy bodies which is done to the post-mortem brains of affected people. Multiple system atrophy (MSA), progressive supranuclear palsy (PSP), vascular Parkinsonism (VaP), and corticobasal degeneration (CBD) are among the Parkinsonian syndromes that have clinical and physiological similarities with Parkinson's disease (Williams & Litvan, 2013). In the early stages of PD consultations, misdiagnosis is more common and becomes less frequent with regular follow-ups. Furthermore, various disease progression rating scales have been developed to describe how

Parkinson's disease has progressed. The initial PD progression scale developed by Hoehn and Yahr's 1967 described the disease's progression by classifying it into several stages according to the intensity of the symptoms, and improvements to this scale took place since then (Hoehn & Yahr, 1967). The Hoehn and Yahr scale (Hoehn & Yahr, 1967), which has five stages, was developed to represent the level of disability in PD patients in relation to the course of the disease. Patients can be categorized along the course of the disease according to the motor impairment and disability that are specific to each stage. Even though this original scale was later modified by the Movement Disorder Society (MDS), it is still one of the most widely used rating scales in PD progression (Goetz et al., 2004). The Unified Parkinson's Disease Rating Scale (UPDRS) is another disease progression rating scale (Fahn & Elton, 1987). The four sections of the UPDRS scale are evaluated separately, with zero denoting normal and 199 denoting a severe condition (Fahn & Elton, 1987). The Movement Disorder Society has also modified this scale, which is known now as MDS-UPDRS. Part 1 of the MDS-UPDRS is dedicated to intellectual function, mood, and behaviour symptoms. Part 2 is for symptoms that impair daily activities, whereas part 3 is devoted to some motor complications, and part 4 is titled "Severe motor complications." These four components further divide the scale into a broad range of symptoms (Goetz et al., 2008).

1.5. Parkinson's Disease Genetics

Parkinson's disease has a complex genetic makeup that includes rare, high-penetrance pathogenic variants that cause familial disease and high-frequency, low-penetrance variants that only slightly increase the risk in a significant minority of sporadic PD (Day & Mullin, 2021). The majority of PD cases are sporadic, having no known cause; about 10-15% of the cases are identified as familial and are linked to the Mendelian kind of inheritance, which is the result of variants in inherited genes. Despite advances in identifying these genetic components, not every PD gene is linked to a clear pattern of disease inheritance and complete penetrance due to regional variations in ethno-social variables, which added to the complexity of understanding these genetic factors' contribution to PD pathogenesis and their molecular pathways. Genetic risk factors may be widespread in the population and may have a negligible impact on PD risk or modify the disease pattern by interacting closely with other environmental and genetic risk factors. So far, genetic studies have identified over 200 PD-related genes (Buniello et al., 2019), with 21 loci have been identified in familial PD. The discovery of these genes has shed light on the pathways implicated

in PD, including oxidative stress, mitochondrial dysfunction and toxic protein accumulation. The first gene linked to the familial form of PD was the α-synuclein, discovered in 1980 in a large Italian family (Polymeropoulos et al., 1996). After that, additional genes have been uncovered as studies focused on identifying genetic components contributing to both the hereditary and sporadic forms of PD. Progress in the study of the complex genetics of Parkinson's disease has followed a similar course for many common, non-communicable diseases over the last decades, led on by advances in technology and collaborative efforts to develop crucial database resources, such as The International Parkinson Disease Genomics Consortium (IPDGC) and IPDGC Africa (Rizig et al., 2021; Singleton, 2020) and Global Parkinson's Genetics Program ("GP2: The Global Parkinson's Genetics Program," 2021). The genetic study of complex diseases like PD has several aims. Firstly, the identification of genetic risk loci may pave the way for an improved understanding of pathogenic molecular mechanisms. Genes implicated in disease susceptibility are likely to encode proteins that participate in relevant cellular pathways. Mapping of disease-related genes, pathways and networks generates hypotheses for further functional research, and may eventually open new possibilities for therapeutic developments. A second type of aim concerns the utility of genetic information in the assessment of individual patients. Comprehensive knowledge about the genetic architecture of common diseases could conceivably allow for some degree of prediction or identification of individuals at risk. In combination with clinical outcomes, an individual genetic profile could also be useful in distinguishing subgroups of patients with similar characteristics. This strategy could have implications for the selection of patients for future clinical trials and would also be in line with the proposed idea of tailored personalized medicine.

1.5.1. Monogenic forms of PD

Monogenic PD genes can be inherited via the autosomal dominant (AD), autosomal recessive (AR), or X-linked patterns of inheritance. While some of these genes cause diseases that mimic sporadic PD, others are characterized by a phenotype with a broader range of symptoms, with Parkinsonism being just one of the multiple features that make up a genetic syndrome. When a single aberrant copy of the gene results in the disease, an AD inheritance pattern develops. However, if the two copies of the gene carry the variant, this is considered an AR inheritance pattern. The presence of the inherited gene variant on the X chromosome results in an X-linked inheritance pattern. Typically, late-onset PD symptoms are linked to the

monogenic PD-associated genes that exhibit an autosomal dominant inheritance pattern. On the other hand, monogenic PD genes with an autosomal recessive inheritance pattern are primarily found in cases that have an early or juvenile PD onset and unusual clinical features such as significant cognitive decline, mental disorders, or other Parkinsonian comorbidities. The acute form of early-onset Parkinson's disease is the autosomal recessive juvenile-onset PD that usually manifests before the age of twenty years old.

1.5.1.1. Confirmed Autosomal Dominant PD Genes

SNCA

SNCA is the first gene found to cause autosomal dominant PD. A large family of Italian ancestry had the first PD-causing *SNCA* variant (p.A53T), which was later found in five unrelated Greek families (Polymeropoulos et al., 1996, 1997). α-synuclein protein encoded by *SNCA* is known to take role in the assembly of the SNARE (soluble N- ethylmaleimide-sensitive factor attachment protein receptor) complex and trafficking of the synaptic vesicles (Burré et al., 2010) in addition to multiple cellular functions such as the induction of inflammatory responses, regulation of apoptosis, control of glucose levels, differentiation of neurons and dopamine synthesis (Bendor et al., 2013). According to Deng et al. (2018), *SNCA* variants are linked to a wide range of disease age of onset, from 20 to 85 years of age (Deng et al., 2018). The most prevalent SNCA variant, p.A53T, is linked to an earlier onset PD (less than 50 years) (Magistrelli et al., 2021). One year following the first *SNCA* variant discovery, the p.A30P variant was reported to co-segregate in five affected cases of one German family (Krüger et al., 1998). The p.H50Q variant was reported in one sporadic case (Appel-Cresswell et al., 2013) and one dominant family (Proukakis et al., 2013). Because there was no obvious enrichment in PD cases compared to healthy individuals in large population datasets, it was eventually determined that it was probably not pathogenic (Blauwendraat et al., 2018). The p.A53V variant was discovered in one dominant Japanese family in a homozygous form (Yoshino et al., 2017) and in one dominant family and two sporadic PD cases from China in a heterozygous form (Y. Chen et al., 2020). However, further functional confirmatory research is required to determine whether the p.A53V variant can predispose carriers to PD in either a homozygous or heterozygous state. Other additional *SNCA* missense variants, including p.E46K, p.G51D, and p.A53E have been found in several families and/or cases (Lesage et al., 2013; Pasanen et al., 2014; Zarranz et al., 2004). Recently, a novel

SNCA p.A30G variant was found to co-segregate with the disease in three Greek families (H. Liu et al., 2021). The clinical manifestations observed in *SNCA* variant carriers might be different from one patient to another, depending on the variant type (Deng et al., 2018). Carriers of the p.A30P variant have a less severe phenotype, while those with the p.E46K variant experience an early-onset disease with severe parkinsonism and dementia (Kasten & Klein, 2013). Dementia is also prominent in p.A53T variant carriers in addition to a higher capacity to initiate and maintain inflammation by producing pro-inflammatory cytokine IL1α (Alvarez-Erviti et al., 2011). Furthermore, the multiplication of *SNCA*, including duplications and triplications, can also cause PD with evidence for a 'dosage effect', where greater expression of α-synuclein leads to more severe clinical features (Ross et al., 2008). Overall, all patients with the SNCA variants exhibit the same neuropathology, which is characterized by the presence of Lewy bodies, the loss of neurons in the substantia nigra, and interconnected brain areas. Additionally, all patients exhibit cardinal motor symptoms and a good response to levodopa.

LRRK2

The leucine-rich repeat kinase 2 protein, which is encoded by the LRRK2 gene, is involved in several processes, such as lysosomal pathways and autophagy regulation (Alessi & Sammler, 2018).The *LRRK2* gene locus (12p11.23–q13.11) was mapped for the first time within a large Japanese family exhibiting autosomal dominant PD (Funayama et al., 2002). After that, it was reported in 2004 as the causative gene of the autosomal forms of PD (Zimprich et al., 2004). In contrast to Caucasians, the frequency of the LRRK2 gene in PD patients in Japan is extremely low, despite the gene's historical association with Japan. The most frequent pathogenic variant worldwide has been found as the G2019S. It is particularly common among Ashkenazi Jewish and North African Arab groups, with prevalence rates of up to 26% and 41% in some cohorts (Healy et al., 2008a; A. J. Lee et al., 2017). Since then, a population-specific prevalence was identified for a number of pathogenic *LRRK2* variants including p.N1437H, p.R1441G, p.R1441C, p.R1441H, p.Y1699C, p.G2019S, and p.I2020T. The mode of inheritance in the families of the majority of PD patients with *LRRK2* variants was found to be autosomal dominant. However, some *LRRK2*-carrier patients appeared to have sporadic PD without a family history of the disease, indicating incomplete penetrance, suggesting that genetic and environmental factors may have different effects on *LRRK2*-PD penetrance (Hentati et al., 2014). According to studies, pathogenic

variants in the *LRRK2* gene may cause the death of dopaminergic neurons by interfering with a number of cellular processes, including protein synthesis, mitochondrial function, and vesicle clearing (Alessi & Sammler, 2018). Although *LRRK2* variants are typically linked to late-onset PD (> 50 years), age of onset can also vary between patients. The clinical presentation of people with *LRRK2* variants frequently resembles that of sporadic Parkinson's disease with a good response to levodopa (Healy et al., 2008a). Clinical features such as tremors, postural instability, gait with small steps, constipation, and olfactory disturbances were common among *LRRK2* variants carriers, and there was no significant difference in clinical signs among pathogenic variants (Y. Li et al., 2020).

VPS35

VPS35 encodes vacuolar protein sorting-associated protein 35. Based on whole-exome analysis employing next-generation sequencing, *VPS35* was identified as the first causal gene for autosomal dominant Parkinson's disease in 2011 (Vilariño-Güell et al., 2011). The missense variant p.D620N in the *VPS35* gene was identified in Swiss and Austrian families with an autosomal dominant pattern of Parkinson's disease, which co-segregated within the families but was absent in healthy individuals (Vilariño-Güell et al., 2011; Zimprich et al., 2011). The *VPS35* gene encodes a key element of the multimeric retromer complex which mediates trafficking of endosomes, facilitating the regeneration of synaptic vesicles to regulate synaptic endocytosis (Choy et al., 2014). The p.D620N variant exhibits aberrant α-synuclein and reactive oxygen species accumulation as well as impaired endosomal trafficking (Bono et al., 2020; Hanss et al., 2021). It also has an average age of onset of 50 years and a clinical presentation similar to sporadic Parkinson's disease with typical cardinal PD symptoms and good response to L-DOPA, while cognitive and psychiatric symptoms are rare (Lunati et al., 2018). A slower disease progression was also reported in p.D620N variant carriers compared to *SNCA* and LRRK2-carrying PD cases.

1.5.1.2. Unconfirmed Autosomal Dominant PD Genes

Numerous genes associated with autosomal dominant PD that have not been replicated or validated were reported and included in PD screening gene panels (Lunati et al., 2018). There is a suggestion that the populations that have been extensively examined may not share these genes. It

is referred to these genes as ‘unconfirmed’ autosomal dominant PD genes. Variants in these genes are regarded as rare causative variants and may occur in varying frequencies among various populations. Additionally, some of the variants within these genes have been identified in healthy individuals showing incomplete penetrance of PD pattern. The unconfirmed AD PD genes include *GCH1, EIF4G1, HTRA2, GIGYF2, DNAJC13, UCHL1,* and newly discovered *RIC3*, *CHCHD2, POLG, TMEM230, LRP10, UQCRC1,* and *DCTN1*. Before being regarded as PD genes, these genes have not been independently replicated and may yet need additional confirmation.

GCH1

The GTP cyclohydrolase 1 protein is a cofactor that is encoded by the *GCH1* gene, which is an essential enzyme for controlling the first and rate-limiting step of the biosynthesis of tetrahydrobiopterin (BH4) required for dopamine production in nigrostriatal cells (Kurian et al., 2011). Loss-of-function variants in the *GCH1* gene, such as p.DYT5, result in severe reduction of dopamine synthesis in nigrostriatal cells and are known to cause a rare disease that classically presents in childhood called DOPA-responsive dystonia (DRD) (Clot et al., 2009). One neurodegenerative condition that has been identified among carriers of *GCH1* variants is Parkinson's disease. PD early disease onset is linked to *GCH1*-related variants, with a mean age of onset of 43 years. The cardinal motor symptoms and non-motor characteristics including autonomic dysfunction, cognitive decline, sleep disturbances, and hyposmia are all part of the reported clinical profile for GCH1 variant carriers (Mencacci et al., 2014). The neuropathology of *GCH1* variants also includes neuronal loss and Lewy bodies (Gibb et al., 1991).

EIF4G1

The *EIF4G1* gene encodes the translation initiation factor 4-gamma 1 protein, which regulates the translation initiation of mRNAs that encode mitochondrial genes. It is also involved in cell survival and regulating growth proteins released in response to stress. *EIF4G1* variants (p.R1205H and p.A502V) were initially implicated in autosomal dominant PD in a multi-incident French family that is characterized by late-onset PD (Chartier-Harlin et al., 2011). Additionally, several subsequent studies from diverse ethnicities could not conclude that *EIF4G1* variants are associated with or cause PD (Nishioka et al., 2014; Nuytemans et al., 2013; Siitonen et al., 2013). Interestingly, the p.R1205H variant was also reported in healthy individuals of Caucasian cohorts

in two studies (Saini et al., 2021a; E. C. Schulte et al., 2012). Therefore, *EIF4G1* is unlikely to be involved in PD given the numerous negative results and the detection of the p.R1205H in numerous healthy individuals.

HTRA2

The *HTRA2* gene encodes Htra serine peptidase 2, found to be positioned on the mitochondrial intermembrane space (Martins et al., 2004). To maintain regular mitochondrial activity, the *HTRA2* serine protease localize itself in the intermembrane gap of the mitochondria to couple with *PINK1*. *HTRA2* two missense variants (p.G399S and p.A141S) were reported to be associated with familial and idiopathic PD (Deng et al., 2018; Strauss et al., 2005), yet without evidence for pathogenicity. However, several other investigations in PD populations from China, Belgium, and Taiwan discovered non-overlapping variants in the HTRA2 gene, suggesting their rarity and specificity in PD (Bogaerts et al., 2008; C. H. Lin et al., 2011; C. Y. Wang et al., 2011). On the other hand, a recent study identified a novel rare heterozygous novel pathogenic variant exhibiting PD phenotype in an Indian family (Bose et al., 2021). The relevance of *HtrA2* as a PD-associated gene is being questioned more and more due to discrepancies in the data from later clinical and functional investigations for p.G399S and other HtrA2 variations, as well as the exclusion of *HTRA2* in a recent genome-wide association study meta-analysis of PD. This challenge may be caused by the fact that previous studies have primarily been concerned with the heredity of the condition, omitting the close connection between *HtrA2* variants, mitochondrial dysfunction, neurodegeneration, and PD.

GIGYF2

The *GIGYF2* gene encodes the GRB10-interacting GYF protein which was originally discovered by multipoint nonparametric linkage analysis of many cases with a family history of PD (Pankratz et al., 2003). Later, in 12 unrelated French and Italian PD patients, seven variants (p.V1242I, p.N56S, p.N457T, p.I278V, p.S335T, p.T112A, and p.D606E) with unclear pathogenicity were detected (Lautier et al., 2008). However subsequent association studies and meta-analyses in different Italian cohorts and other ethnicities did not find evidence of association of GIGYF2 variants with PD (Bartoníková et al., 2018; Bonetti et al., 2009; Huo et al., 2017; Saini et al., 2021a; Vilariño-Güell et al., 2009; Y. Zhang et al., 2015). Thus, *GIGYF2's* role in PD

etiology is therefore extremely unlikely. The clinical phenotype reported in patients with *GIGYF2* variants is mild motor parkinsonism with essential tremor and cognitive impairment.

DNAJC13

The *DNAJC13* gene encodes for DnaJ heat shock protein family (Hsp40) member C13, which plays a role in clathrin-mediated endocytosis. It stimulates ATP production by functioning as a co-chaperone of the 'stress-activated' heat-shock proteins (Vilariño-Güell et al., 2014). Patients with *PARK21* have late-onset, dopa-responsive parkinsonism with Lewy bodies pathology and neuronal cell death in the substantia nigra, which is similar to sporadic Parkinson's disease. *DNAJC13* variants (p.L1207W, p.N855S, p.R903K, p.G394V, p.R1382H, p.R1516H and p.L2170W) were identified in a large multi-incidental Canadian family of Dutch-German–Russian descent and in other additional families with PD (Saini et al., 2021b; Vilariño-Güell et al., 2014). These variants showed variable frequencies in healthy individuals compared to PD cases in addition to varying co-segregation patterns with the disease status (Gagliardi et al., 2018; C. H. Lin et al., 2019). Moreover, none of these variants' pathogenicity has yet been confirmed.

POLG

POLG is one of the few known mammalian polymerases existing in mitochondria, and it functions to integrate the molecular complex that polymerizes mtDNA (Graziewicz et al., 2006). In addition to its polymerase activity, *POLG* also performs the functions of an exonuclease (which ensures the accuracy of mtDNA replication) and a 5' deoxyribose phosphate lyase (Chan & Copeland, 2009). Together, these three enzymatic domains: exonuclease, a linker region, and polymerase, position *POLG* as a significant contributor to the preservation of mtDNA homeostasis. Therefore, it is not surprising that variants that impair the function of POLG can cause mitochondria-associated disorders, such as parkinsonism. Parkinsonism may present in case of a pol domain variant. However, there were few gene variants in other regions (Luoma et al., 2007). Individuals harboring alterations in mitochondrial polymerase gamma (POLG) often exhibit signs of parkinsonism as it aggravates oxidative stress in the dopaminergic neurons, albeit a clinically more atypical form with good response to levodopa. More than 300 pathogenic variants were reported for *POLG*, resulting in mtDNA deletions or depletions with no direct genotype-phenotype correlation (Borsche et al., 2021). The missense *POLG* variant R964C has been

suggested to be linked to diverse phenotypic spectrum with onset from early infancy to late adulthood (Hsieh et al., 2019). Patients with the typical form of Parkinson's disease rarely have variants of this gene. Also, both autosomal-dominant and -recessive inheritance were reported (Luoma et al., 2007). Variable clinical manifestations, such as parkinsonism, epilepsy, cerebellar ataxia, neuropathy, and progressive external ophthalmoplegia, may result from *POLG* variants.

UCHL1

The Ubiquitin C-terminal hydrolase L1 (*UCHL1*) gene encodes a member of the deubiquitinase family that is known to be highly expressed in neurons and plays a role in α-synuclein degradation and Parkinson's disease susceptibility (Maraganore et al., 2004a). The association of *UCHL1* with Parkinson's disease was initially reported in a German family with familial Parkinson's disease was found to have the *UCHL1* variant I93M, which provides the first evidence to categorize *UCHL1* as a PD-susceptible gene (Leroy et al., 1998) but has still not been replicated 25 years later. Then, Some evidence exists for an association between a relatively common p.S18Y variant (Lincoln et al., 1999). This finding was supported by two large meta-analyses (Maraganore et al., 2004b; Ragland et al., 2009) however, other studies neither identified this so-called common variant in their GWAS studies nor demonstrated any association with PD (Foo et al., 2020; Nalls et al., 2019; Saini et al., 2021a)

CHCHD2

The protein product of *CHCHD2* gene (Coiled-coil-helix-coiled-coil-helix domain containing 2) has recently been linked to PD. It encodes a transcription factor that supports in binding to and activating a respiratory chain protein that has a role in apoptosis mediated by the mitochondria, oxidative phosphorylation, and neuronal relocation (Aras et al., 2015; Funayama et al., 2015). Variants of *CHCHD2* (TG1I) were reported for the first time in a large Japanese family, as well as unrelated PD cases with the autosomal dominant form of late-onset PD (Funayama et al., 2015). Another single nucleotide variant, P2L, was also discovered by Funayama et al. (2015) in their Japanese study cohort of sporadic PD patients (Funayama et al., 2015). P2L has also been reported to be associated with sporadic form of PD in additional studies in mainland China (Shi et al., 2016). Nine rare exonic *CHCHD2* variants were found in a large multicenter sample of

Caucasian Americans from the United States (878 PD and 717 healthy), Ireland (355 PD, 365 healthy), and Poland (394 PD, 350 healthy) (Ogaki et al., 2015). These variants are P2L, G4R, P14S, A16A, V31V, A49V, A37V, A93V, and P34L (Ogaki et al., 2015). Despite finding rare pathogenic variants in *CHCHD2* gene that was suggested to be associated with early-onset PD and other neurodegenerative diseases, several studies couldn't replicate these results and link the genetic association of *CHCHD2* to PD (Tejera-Parrado et al., 2017; Voigt et al., 2019). At the global level, it seems that various populations have distinct frequencies and distributions of *CHCHD2* variants. Rare potential harmful *CHCHD2* variations classified to specific populations do exist; nevertheless, in both Asian and Caucasian populations, PD patients had significantly higher rates of these variants than healthy individuals (Kee et al., 2021). The prevalence of motor and non-motor symptoms in their patients has been shown in studies of both Caucasian and Asian populations. The following are examples of motor symptoms reported: bradykinesia, stiffness, hyperreflexia, postural instability, restless leg syndrome, and resting tremor. Non-motor symptoms involved impairments with taste or smell, hallucinations, dementia, depression, orthostatic hypotension, constipation, and urine urgencies. In addition, a brain autopsy revealed widespread α-synuclein pathology with Lewy bodies present in the brainstem, neocortex, and limbic regions (Ikeda et al., 2019).

TMEM230

Another study of other members of the multi-incident Dutch-German-Russian family identified variants in one of the recent PD-associated genes, *TMEM230* (H. X. Deng et al., 2016a). The function of TMEM230 is not clear, it encodes a transmembrane protein 230 suggested to play a part in vesicle trafficking and recycling, autophagy, protein aggregation, and cell toxicity. Variants in *TMEM230* were first identified in cases of familial PD (H. X. Deng et al., 2016b). Similar to DNAJC13, *TMEM230* demonstrated imperfect disease segregation within the family. However, variants in both genes have been identified in a few unrelated PD cases (Gagliardi et al., 2018), which calls into question the role of *TMEM230* in PD. Recent studies by other groups showed that TMEM230 is probably associated with PD risk (Lunati et al., 2018). Deng et al. reported four PD-linked *TMEM230* variants (p.Y92C, p.*184PGext*5 p.R141L, p.*184Wext*5) in a large North American family with PD for two decades (H. X. Deng et al., 2016b). Numerous follow-up genetic analyses with other cohorts of cases conducted by other

groups have revealed no obvious PD-linked variants in TMEM230 (Buongarzone et al., 2017; Quadri et al., 2017; Yan et al., 2017). According to these findings, *TMEM230* variants could act as a risk gene for PD or might result in a rare frequency of familial PD. Additionally, one report even states that ‘‘*TMEM230* is not a gene for Parkinson’s disease‘‘ (M. J. Farrer et al., 2017) which suggests that future studies are required to validate whether *TMEM230* is a PD-causative gene or not. Patients with TMEM230-linked Parkinson's disease presented with a late onset, a fair response to levodopa, and typical sporadic PD clinical characteristics with dopaminergic neuronal loss in substantia nigra besides Lewy body pathology (X. Wang et al., 2021).

RIC3

The *RIC3* gene is known to encode Acetylcholine receptor chaperone protein with neuronal nicotinic acetylcholine receptor subunit alpha 7 implicated in dopaminergic, glutamatergic and cholinergic pathways that are associated with PD (Sudhaman, Muthane, et al., 2016). Two rare missense variants, p.P57T and p.V168L were identified in a large Indian PD family with an autosomal-dominant pattern of inheritance (Sudhaman, Muthane, et al., 2016). Nevertheless, *RIC3* variants were not found to be associated with PD in several large cohorts of different ethnicities including European, Latin American, or East Asian cohorts (Brolin et al., 2022). Further research on rare *RIC3* variants in non-European groups, particularly South Asian populations, is encouraged in light of the variable frequency of *RIC3* rare variants among geographic populations in order to completely assess the hypothesized role for *RIC3* in PD pathogenesis.

LRP10

LRP10 (low-density lipoprotein receptor-related protein 10) is a single-pass transmembrane protein that belongs to the LDL receptor subfamily. *LRP10* expression was found to be high in astrocytes and to be present in the trans-Golgi network, plasma membrane, retromer, and early endosomes in non-neuronal cells but not in neurons (Grochowska et al., 2021). It was proposed that disrupted vesicle trafficking and loss of *LRP10* activity are essential in the development of neurodegenerative disorders. *LRP10*-mediated pathogenicity included the interaction of *LRP10* and *SORL1* in vesicle tracking pathways (Grochowska et al., 2021). LRP-10-associated PD phenotype is often preceded by dementia which is frequently associated with

this gene (Guadagnolo et al., 2021). Using genome-wide linkage analysis, Quadri and colleagues identified the *LRP10* gene on chromosome 14 as a potential disease gene in an Italian family with autosomal dominant PD (Quadri et al., 2018). This was confirmed through the study of a broader cohort of individuals, where it was discovered that rare, possible variants in *LRP10* are linked to the development of familial forms of α-synucleinopathies, including PD, PD dementia (PDD), or dementia with Lewy bodies (DLB). These findings were unable to be replicated in other studies where the results for co-segregation analysis did not support a causal role for *LRP10* in PD (Tesson et al., 2018). These results suggest the association between *LRP10* and α-synucleinopathies and possibly also with amyloidopathy, even if specifics of *LRP10*'s function are still unknown. Recently, two studies in Asian population also reported no sufficient evidence to support the role of *LRP10.* The study in a large Han Chinese familial PD cohort identified 1 variant (p.R661H) cosegregating with PD, however, the burden test could not support the causality of *LRP10* in PD (C. Y. Li et al., 2021). The other did not find any significant associations between the five new and possibly pathogenic variants identified initially and the risk of PD in their Taiwanese cohort (Liao et al., 2021).

UQCRC1

Ubiquinol-cytochrome c reductase core protein 1 (*UQCRC1)* is a core component of complex III in the respiratory chain. In 2020, Lin et al. discovered a new heterozygous variant (p.Y314S) in the *UQCRC1* gene that co-segregated with disease in a Taiwanese family with autosomal dominant parkinsonism with polyneuropathy (C. H. Lin et al., 2020). This study was the first to link *UQCRC1* variants to familial PD. *UQCRC1* p.Y314S may interfere with the function of mitochondrial respiratory chain complex III in neurons and be linked to decreased mobility, loss of dopaminergic neurons, and deterioration of peripheral nerves. They also reported another variant in *UQCRC1* (p.I311L) that was co-segregated with PD (C. H. Lin et al., 2020). However, further studies in the European and Taiwanese cohorts reported no common variants in *UQCRC1* were significantly associated with Parkinson's disease (Liao et al., 2022; Senkevich et al., 2021).

DCTN1

DCTN1 encodes p150^{glued}, the large subunit of the dynactin complex that binds to the motor protein dynein, which in turn binds to microtubules and other dynactin subunits. Intracytoplasmic inclusions and reduced microtubule binding are caused by *DCTN1* variants (M. J. Farrer et al., 2009). A rare autosomal dominant disorder known as Perry syndrome, which is also known as *DCTN1*-associated Parkinson-plus disorder, is characterized by quickly progressing parkinsonism, mood swings, weight loss, and progressive respiratory abnormalities, most notably tachypnoea and nocturnal hypoventilation (Richardson et al., 2020). Exon 2 of the *DCTN1* gene variants is related to the disease. The median age of onset of the disease is 48 years (range: 35–61), and the mean time from diagnosis to death is 5 years (Wider et al., 2010).

1.5.1.3. Autosomal Recessive Genes with Typical PD Features

PRKN/ PINK1/ DJ-1

These genes will be reported collectively since biallelic variants of these genes cause autosomal recessive forms of PD and their protein products are linked to mitochondrial function. After 1998, biallelic loss of function variants in *PRKN* (Kitada et al., 1998), *PINK-1* (Valente et al., 2004) and *PARK7* (Bonifati et al., 2003) were discovered in studies of families, frequently consanguineous, affected by early-onset forms of Parkinson's disease (PD) with a possible autosomal recessive inheritance pattern. Parkin, the E3-ubiquitin ligase protein, is encoded by the PRKN gene, which is regarded as one of the largest genes in the human genome(Y. Zhang et al., 2000). *Parkin* has a role in preserving mitochondrial homeostasis and DNA integrity by degrading damaged proteins via the proteasome degradation system. *PRKN* variants are the largest contributor to early-onset PD (Ferreira & Massano, 2017). They account for approximately 77% of all early-onset (with an age of onset <30 yr) familial PD cases with and 18% of young-onset idiopathic PD (Klein & Westen berger, 2012). PTEN-induced kinase, which is a mitochondrially associated kinase, is a product of PINK1 and has anti-inflammatory properties besides its involvement in the phosphorylation of parkin to degrade damaged mitochondria selectively (Sliter et al., 2018). Taken together, they jointly mediate selective autophagy of damaged mitochondria (mitophagy). Two-thirds of the reported variants in *PINK1* are loss-of-function variants affecting the kinase domain, demonstrating the importance of *PINK1*'s enzymatic activity in the

pathogenesis of PD (Klein & Westenberger, 2012). *PARK7* (previously known as *DJ-1*) is the third gene associated with autosomal recessive PD (Pankratz et al., 2006). Similar to *Parkin* and *PINK1* variants, *DJ-1* homozygosity is accompanied by a complete disease penetrance and early onse phenotype, and it is mutated in about 1%–2% of early-onset PD cases (C. Schulte & Gasser, 2011). Encoding for the parkinsonism-associated deglycase protein, *DJ-1* is widely expressed and acts as a cellular oxidative stress sensor. It is essential for *PINK1*/*parkin*-mediated mitophagy. Parkin, *DJ-1* and *PINK1* bind together to form a ubiquitin ligase complex that maintains mitochondrial structure, and this function is impaired by pathogenic variants (Xiong et al., 2009). The clinical phenotype of *Parkin*-, *PINK1*-, and *DJ-1*-linked PD is indistinguishable, including slow disease progression, typical PD motor symptoms with dystonia, and positive response to levodopa. However, non-motor symptoms such as cognitive decline, psychosis, and mood disorders are more common in *DJ-1* carriers (Repici & Giorgini, 2019).

1.5.1.4. Autosomal recessive genes with early-onset atypical parkinsonism

VPS13C

VPS13C is part of the family of conserved *VPS13* (Vacuola protein sorting 13) proteins and is considered a phospholipid transporter protein localized at contact sites between the ER and late endosomes/lysosomes and on the outer mitochondrial membrane (S. Chen et al., 2020). It is implicated in the regulation of lysosomal homeostasis and mitophagy, besides modulating the *Pink1/Parkin* pathway in cellular models. The neurodegeneration associated with the loss of VPS13C function is attributable to the alteration of lysosomal homeostasis and reduced mitochondrial membrane potential or mitophagy activity induced by the *PINK1/PRKN* pathway (Hancock-Cerutti et al., 2022; Lesage et al., 2016). Patients were presented with early-onset PD (37.5 ± 10.5 years of age) and a severe disease progression compared to idiopathic PD but with a good response to levodopa therapy (Darvish et al., 2018; Rudakou et al., 2020). However, treatment-induced dystonia, pyramidal tracts with brisk reflexes progressing to spastic quadriplegia later occurred, as well as non-motor features, including dysautonomia, hyposmia, early cognitive decline, and visual hallucinations are presented (Gu et al., 2020; Hopfner et al., 2020).

SYNJ1

Synaptojanin-1, the protein encoded by *SYNJ1*, plays a crucial role in dephosphorylation of phosphoinositide which has an essential role in cell signaling, membrane trafficking and maintaining synaptic vesicle dynamics, including endocytosis at nerve terminals and autophagy. Loss of *SYNJ1* impairs transferrin receptor recycling to the plasma membrane causing synaptic autophagy, which highlights the critical function of the autophagy-lysosome pathway in the pathogenesis of Parkinson's disease (Fasano et al., 2018). Around 20 families with biallelic *SYNJ1* missense, nonsense, frameshift, or splicing variants have been reported (Yahya et al., 2023). *SYNJ1* variant carriers present with autosomal recessive, early-onset or atypical parkinsonism in the third decade of life. Those patients show a heterogeneous multitude of phenotypes ranging from parkinsonism (tremor, bradykinesia, postural instability) with a variable response to levodopa treatment, as well as additional atypical signs such as dystonia, dyskinesia, juvenile-onset seizures, cognitive impairment, dementia, eyelid apraxia and developmental delay (Lesage et al., 2021a).

PLA2G6

Variants in the *PLA2G6* gene, which encodes calcium-independent Phospholipase A2 group VI protein, were linked to autosomal recessive L-dopa responsive dystonia-parkinsonism with onset in the second to third decades of life in 2009 (Paisan-Ruiz et al., 2009). Since then, further phenotypes that emerge later than infantile neuroaxonal dystrophy (INAD) and atypical neuroaxonal dystrophies (ANADs) have been identified (Magrinelli et al., 2022). These include parkinsonism, either alone or in combination with other neurological or psychiatric symptoms, ataxia, ophthalmic abnormalities and spastic paraplegia (Chu et al., 2020). On a molecular level, the *PLA2G6* gene has been implicated in cell homeostasis, cell signaling and mitochondrial function (Malley et al., 2018). There are still controversial questions about why the phenotypic spectrum of *PLA2G6* variants is so broad and why multiple abnormalities might result from the same variant, even in the same pedigree.

ATP13A2

A transmembrane endo-lysosomal-associated protein P5 type transport ATPase is encoded by the *ATP13A2* gene (Fujii et al., 2023). *ATP13A2* variants were first identified in 2006 in a Chilean family and are a known cause of Kafer-Rakeb Syndrome (KRS) which is an autosomal

recessive form of Parkinsonism characterized by a phenotype that includes cognitive dysfunction, dystonia, levodopa-responsiveness, juvenile-onset, dementia, and supranuclear palsy (Ramirez et al., 2006; Yang & Xu, 2014). In the human brain, *ATP13A2* is mainly localized in lysosomes of the neuronal cells reticulum and is found to be significantly expressed in dopaminergic neurons of the substantia nigra, while the mutated gene was found to localize to the endoplasmic (Ramonet et al., 2012). It also has a protective function against the aggregation of α-synuclein by preserving the integrity of the lysosomal membrane and promoting the ATPase-independent secretion of α-synuclein through nanovesicles (Si et al., 2021). Therefore, PD-associated variants and knockdown of *ATP13A2* cause lysosomal dysfunction and α-synuclein aggregation, which are pathological hallmarks of PD. *ATP13A2* symptoms are characterized by significant clinical heterogeneity, including but not limited to kineto-rigid syndrome, learning incapacity, motor skills impairment, in addition to behavioural abnormalities observed in some affected patients (J. S. Park et al., 2015)

FBXO7

FBXO7 (F-box protein 7) is an adapter protein that forms part of the SKP1-cullin-F-box (SCF) interacting with the E3-ubiquitin complex facilitating the degradative or non-degradative ubiquitination of substrates in the SCFFBXO7 ubiquitin E3 ligase complex to maintain mitochondrial health with the help of *PRKN* and *PINK1* (Burchell et al., 2013). A family from Iran was found to have the first FBXO7 genetic variations linked to PD when they presented with parkinsonian-pyramidal syndrome (PPS), a kind of parkinsonism marked by pyramidal symptoms.(Shojaee et al., 2008). Later, a few homozygous and compound heterozygous variants were identified in multiple populations with PPS (Lohmann et al., 2015; Zhou et al., 2018). The phenotype associated with the FBXO7 variants was reported as early-onset and akinetic-rigidity dominant parkinsonism (Yalcin-Cakmakli et al., 2014). As with *ATP13A2* variant carriers, long-term levodopa use results in dyskinesia in FBXO7 carriers in addition to some psychiatric signs (Fonzo et al., 2009). Apart from pyramidal signs, atypical features have also been reported, including mental retardation, eyelid apraxia, supranuclear gaze palsy, and chorea (Yoo et al., 2020). On the other hand, carriers of one *FBXO7* variant showed typical idiopathic PD with some of its common nonmotor features, such as rapid eye movement sleep behavior disorder, depression, and anxiety (Lohmann et al., 2015).

DNAJC6

Dnaj heat shock protein family member C6 (*DNAJC6)* encodes for the co-chaperone putative tyrosine-protein phosphatase auxilin, known as auxilin 1, a brain-specific form of auxilin involved in the clathrin-mediated synaptic vesicle endocytosis which is necessary for integrating clathrin-coated vesicles with dopaminergic receptors in the cells (Milosevic, 2018). Auxilin deficiency results in impaired synaptic vesicle endocytosis, and thus negatively impacts synaptic neurotransmission, homeostasis, and developmental and synaptic signaling (Kaksonen & Roux, 2018; Yim et al., 2010). A homozygous *DNAJC6* variant was first reported in two siblings presented with juvenile-onset autosomal recessive PD (Edvardson et al., 2012). Later, additional variants in of *DNAJC6*-related parkinsonism have been reported in 21 cases, carrying biallelic missense, nonsense, frameshift, or splicing variants (Jesús et al., 2014; Yahya et al., 2023). Juvenile-onset, atypical parkinsonism with onset during childhood and a very rapid disease progression with loss of ambulation within 10 years of onset was demonstrated in DNAJC6 variant carriers (Jia et al., 2022). Patients are poorly responsive to levodopa therapy and have additional manifestations such as developmental delay, intellectual disability, seizures, and other movement disorders (e.g., dystonia, spasticity, myoclonus) (Mittal, 2020). Although classic PD is a possible form of *DNAJC6*-related parkinsonism with tremors and bradykinesia, others report late-onset postural instability. Some patients also experience cognitive deterioration and seizures, while others experience epilepsy, psychosis, hallucinations, pyramidal symptoms, gaze paresis, or cerebellar indications. (Wittke et al., 2021).

SLC6A3

The *SLC6A3* (Solute carrier family 6 member 3) gene is established to encode DAT, a dopamine transporter expressed exclusively in dopamine neurons and plays an essential role in sustaining the integrity of dopamine neurons by taking up dopamine from the synaptic cleft into the presynaptic neurons, thus terminating dopamine transmission. Pathogenic loss-of-function *SLC6A3* variants (p.L368Q and p.P395L) were reported in two unrelated consanguineous families with infantile parkinsonism-dystonia who demonstrated motor response to L-dopa treatment (Kurian et al., 2009). However, recent studies couldn’t confirm the motor response to L-dopa treatment observation (Soraya et al., 2022). On the other hand, *SLC6C3* variants were also shown to be a major risk factor for PD in a meta-analysis study of individuals with PD (Zhai et al., 2014).

Zhai et al., suggested that *SLC6A3* increases the risk of Parkinson's disease in different populations by a small but significant amount and that the 10-repeat allele of 3′ variable number tandem repeat is protective against PD through lowering DAT availability that prevents dopamine from entering the dopamine neurons, resulting in low cytosolic concentration of toxic dopamine (Zhai et al., 2014). However, a recent study by Li and his colleagues reported that this neurodegeneration-related gene is not related to PD risk (J. Li et al., 2022).

PTRHD1

Peptidyl-tRNA hydrolase domain containing 1 (*PTRHD1*) perform the essential function of recycling peptidyl-tRNAs. The *PTRHD1* protein contains ubiquitin-like domain inhibits ubiquitin-mediated protein degradation and is known to be involved in the ubiquitin-proteasome pathway which is one of the key mechanisms contributing to PD. The pathogenicity of *PTRHD1* in causing disease is not supported by functional evidence, and it is uncertain whether *PTRHD1* variants exist in other groups with young onset of PD. Homozygous missense variants (p.C52Y and p.H53Y) and a homozygous deletion with frameshift variant (c.169_196del, p.A57Rfs∗26) in *PTRHD1* can cause autosomal recessive early-onset parkinsonism with intellectual impairment in 2 consanguineous Iranian families and a South African pedigree (Elahi, 2018; Khodadadi et al., 2017; Kuipers et al., 2018). A recent study aimed to investigate *PTRHD1* variants in a Taiwanese cohort with young-onset and familial PD could not find any pathogenic coding variants or previously reported variants, suggesting that *PTRHD1* variants are rare in young-onset and familial PD patients in Taiwan (S. J. Chen et al., 2021).

PODXL

The podocalyxin-like gene (*PODXL*) encodes a highly glycosylated neural adhesion molecule, a member of the sialomucin protein family and abundantly expressed in the brain. *PODXL* is associated with neuronal development and synaptogenesis. In a consanguineous Indian family, a homozygous frameshift variant (exon 1 c.89_90insGTCGCCCC in the podocalyxin-like gene, or *PODXL*) was recently discovered to be connected to and co-segregated with autosomal-recessive juvenile-onset (22 to 71 years) Parkinson's disease (Sudhaman, Prasad, et al., 2016).

This variant was suggested to cause neurite degeneration. However, uncertainty persists regarding the pathogenicity of *PODXL* variants and their function in PD. A recent study aimed to investigate the genetic contribution of *PODXL* in patients with familial and early-onset PD in the Taiwanese population reported that they did not find any pathogenic coding variants or previously reported variants, indicating that *PODXL* variants may not have a typical role in early-onset or familial PD in this Taiwanese population (S. J. Chen et al., 2019).

1.5.2. X-linked PD genes

Despite the relative risk for PD being more prevalent in males than in females, most GWAS studies focused solely on autosomes (Nalls et al., 2019). A few studies have identified variants or loci on the X-chromosome linked to PD (Di Lazzaro et al., 2021; Le Guen et al., 2021). The genetics of X-linked PD are also heterogeneous, with numerous causative genes and various variant types ranging from "classical" coding variants to intronic repeat expansions. The pathogenesis of these x-linked PD genes is implicated with various disorders ranging from well-known metabolic alterations to non-specific lysosomal dysfunctions and vesicular trafficking alterations. The most significant X-linked parkinsonian syndromes that have different clinical and genetic profiles include X-linked dystonia-parkinsonism (XDP, Lubag disease), methyl CpG-binding protein 2 (MeCP2) spectrum disorder, fragile X-associated tremor/ataxia syndrome (FXTAS), beta-propeller protein-associated neurodegeneration (BPAN, NBIA/PARK-WDR45), Fabry disease, phosphoglycerate kinase-1 deficiency syndrome (PGK1), Waisman syndrome, and X-linked parkinsonism and spasticity (XPDS) (Di Lazzaro et al., 2021).

1.5.3. Genetic risk factor of PD

GBA

GBA constitutes the most common genetic risk factor for PD, where the presence of certain variants confers an increased risk of developing PD. It rarely exhibits a Mendelian inheritance pattern. The *GBA* gene encodes the lysosomal enzyme beta glucocerebrosidase (GCase), that hydrolyzes glycosphingolipids into ceramide and glucose. Biallelic pathogenic variants in the GBA gene are a known cause of Gaucher's disease (GD), reduced activity of the GBA-encoded enzyme glucocerebrosidase results in this most common recessively inherited sphingolipidosis

lysosomal storage disorder. Its significance for Parkinson's disease was originally recognized when it was discovered that patients with Gaucher disease disproportionately developed Parkinsonism-like symptoms (Neudorfer et al., 1996). Further large cohort studies investigated the link between GBA variants and the increased PD risk (Anheim et al., 2012; Sidransky et al., 2009; Skrahina et al., 2021; Tayebi et al., 2001). The studies have identified over 300 pathogenic *GBA* variants, which are associated with loss- and gain-of-function mechanisms (Smith & Schapira, 2022). *GBA1* variants reported to contribute to the development of PD include p.E326K, p.L444P, p.N370S and p.T369M, which decrease the activity of glucocerebrosidase enzyme and consequently reduce the ability to degrade alpha-synuclein in lysosomes (Blauwendraat et al., 2020). Multiple studies also highlighted that PD is more common in heterozygous carriers of *GBA* variants, and the frequency of these variants is population-specific and varies among different ethnic groups. For instance, p.N370S variant is particularly seen in Ashkenazi Jewish populations, while p.E326K is extremely uncommon in the Asian population but occurs in the European population at a 1-5% frequency. GBA variants increase the risk of PD by 5–20 folds, depending on the variants, age, and ethnicity (Smith & Schapira, 2022).

The clinical phenotype of *GBA*-PD is observed to resemble that of idiopathic PD. However, GBA carriers typically show an earlier age of onset (around 40 years) and faster progression of motor symptoms (Day & Mullin, 2021). Penetrance, age of onset, and clinical progression of PD are all influenced differently by the "severe" or "mild" classification of GBA1 variants (Petrucci et al., 2020). Despite the relatively minor increase in PD risk, both p.E326K and p.T369M are linked to a higher risk of cognitive impairment and a faster rate of disease progression (Gan-Or et al., 2017; Iwaki et al., 2019). Hyposmia, constipation, urinary dysautonomia, orthostatic hypotension, sleep problems, anxiety, hallucinations, delusions, and especially cognitive impairment (with a deterioration in working memory, visuospatial function, and executive function) are more prevalent and significant non-motor symptoms (Yahya et al., 2023). Neuropathological features of *GBA1* carriers precisely resemble those of sporadic PD, with classic PD Lewy body aggregations in the brainstem and cortex and nigrostriatal dopaminergic neuron loss (Velayati et al., 2010).

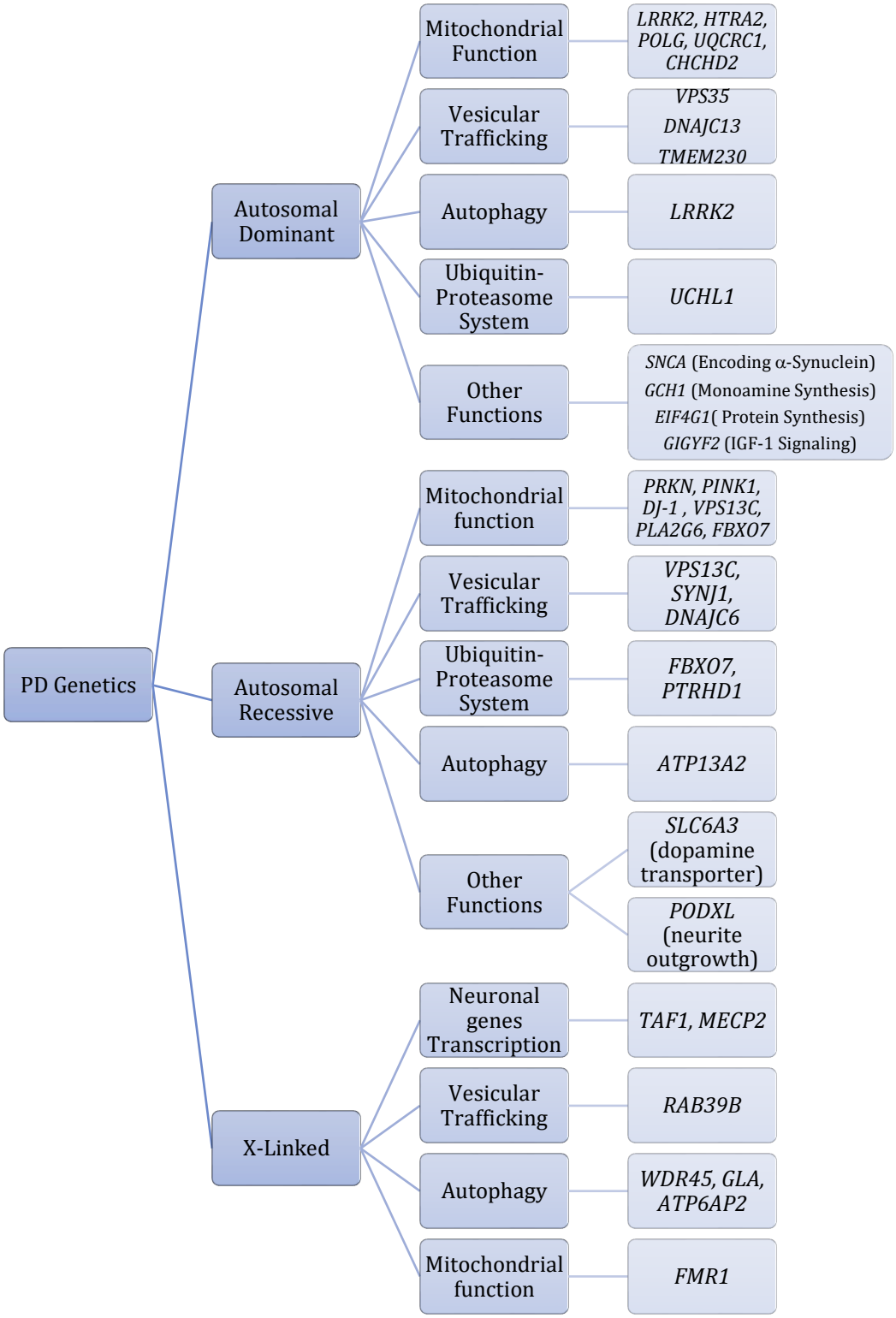

Figure 1. Summary of Mendelian PD genes and their cellular function

1.6. Genome-wide Association Studies in Parkinson's Disease

The genetic factors that play a role in the pathogenesis of sporadic PD have mainly advanced through case-control genome-wide association studies (GWAS) of common genetic variants, in contrast to familial PD studies, which initially found monogenic associations through studying the affected pedigrees. Large-scale GWAS have represented a breakthrough in PD genetics by linking a range of previously unidentified loci across the genome to disease risk by means of powerful statistical association. GWAS are effective methods for finding genetic loci that contribute significantly to traits of interest and can pinpoint the biological processes involved in that trait. In GWAS, microarray technology, which enabled large-scale parallelization of genotyping, is used to genotype relatively frequent single nucleotide polymorphisms (SNPs) where a vast number of SNP markers, ranging from 500 000 up to several million SNPs, are genotyped on the same chip across the genome in each subject. Based on the understanding of frequent haplotype structures, these markers are chosen to maximize the genetic data obtained. SNPs with significant differences in allele frequencies between the patient and healthy groups are considered. A widely accepted threshold for genome-wide significance is $5x10^{-8}$. Below this level, sample size is critical to obtaining results that are statistically reliable. Therefore, the establishment of large, cooperative, international consortia has been crucial for the success of GWAS. Even after GWAS discovered risk loci, identifying the particular causative variant(s) and gene(s) at each locus remains a substantial challenge.

The initial GWAS in PD lacked sufficient power to identify signals that exceeded a strict significance threshold. In 2009, Caucasian and Japanese populations revealed the first genome-wide significant hits in sporadic PD (Satake et al., 2009a; Simón-Sánchez et al., 2009a). Among these, *SNCA* and *LRRK2* were identified as Mendelian forms of PD causal genes; however, GWAS demonstrated that they are also involved in the onset of sporadic PD. The results also validated *MAPT* in Caucasians and *SNCA* as risk loci in both populations. The Japanese study also discovered three more loci, including *BST1, PARK16*, and *LRRK2*. These results were partially replicated in subsequent GWAS investigations, and new loci like *GBA*, *GAK* and *HLA* were also added to the list (Nalls et al., 2014). About 200 PD-related genes have so far been reported, according to the GWAS catalog (Buniello et al., 2019). The variability in disease risk accounted for by significant GWAS loci constitutes only a small fraction of the total estimated heritability of

PD. This phenomenon is commonly known as "missing heritability", has been a general feature of the GWAS era in complex genetics. In addition, a recent meta-analysis of GWAS found 90 distinct genome-wide risk alleles in 78 loci linked to PD European population, accounting for 16–22% of PD heritability (Nalls et al., 2019). In the Asian population, there are nine replicated loci, two new population-specific signals, and eleven newly nominated unique loci from multi-ancestry GWAS. Before the results of large-scale genetic studies can be more useful to related fields of study and ultimately inspire translational studies aiming for improved therapy, it will be crucial to improve our understanding of implicated genes and causal mechanisms behind GWAS signals. However, significant knowledge gaps must be filled to understand how these variants alter pathogenesis before translational research and medicine can meaningfully benefit from such a list of genetic loci. A few unanswered questions call for focused follow-up research that builds on the GWAS results.

1.7. Genetic Testing in Parkinson's Disease

The field of genetic testing in PD is rapidly evolving during recent years, due to the better availability of next-generation sequencing (NGS)-based molecular tests and the initiation of genetic diagnosis-based interventional clinical trials. Prior to that, movement disorder specialists very seldom recommended genetic testing to PD patients, and genetic workups were not routinely conducted as part of PD examination. This was a result of a number of issues, including expense, lack of perceived impact on patient management, and discomfort with test selection, test results, or their effects on patients and their families on the part of the clinician (Alcalay et al., 2020). In the past, patients with early-onset Parkinson's disease before age 50—and especially before age 40—would most likely be suspected of having a monogenic etiology, and a genetic test would therefore be considered. The decision to conduct genetic testing may also be influenced by a patient's ethnic background, as in the case of Ashkenazi Jews or African Berbers. Additionally, a striking familial history, either of an autosomal dominant or autosomal recessive pattern, is yet another clue for a possible monogenic cause that may suggest that a genetic test should be considered, even though polygenic risk and multifactorial inheritance would likely explain the majority of cases with familial clustering of PD (Jia et al., 2022). A recently proposed permissive approach, in contrast to the traditional case-by-case approach, supports a more widespread use of genetic testing in PD to enhance patient care, allow patients to participate in clinical trials based

on molecular diagnosis, and benefit therapeutic insights and strategies for the larger PD population, including patients with sporadic disease (Cook, Schulze, Kopil, et al., 2021). Both individually and collectively, PD patients can greatly benefit from this idea. However, it should be supported by thorough knowledge of the various evolving aspects of genetic testing in PD and by a personally tailored explanation to patients and potential family members carriers regarding the test and the potential implications of its results for them and for their family members, both prior to testing and when returning them the test results (Jia et al., 2022). The projected course of the disease and the responsiveness to therapeutic interventions for PD patients may both be significantly affected by a genetic diagnosis. As previously described, some monogenic variants are anticipated to respond favourably to levodopa therapy (e.g., *PRKN*), but others are not (e.g., *DNAJC6*). Additionally, a recent study discovered that GBA mutant carriers have more cognitive decline following bilateral subthalamic nucleus deep brain stimulation (STN-DBS) than do *PRKN* and *LRRK2* variant carriers or people without known disease-associated pathogenic variations (Mangone et al., 2020). A recent study that suggests that STN-DBS is connected to a higher incidence of cognitive deterioration in GBA variant carriers supports these findings further (Pal et al., 2022). This study also called for checking for GBA pathogenic variants in PD patients before having DBS surgery, and those who carry these variants should be informed of the increased risk of cognitive decline. According to the type of variant, the response of DBS for *SNCA*-PD may also vary (Youn et al., 2022). The growing significance of a genetic diagnosis in PD also opened the door for gene-based targeted approaches that have been developed recently (Senkevich et al., 2022). This is because a particular molecular genetic testing may permit inclusion in interventional clinical trials that focus on a genetically determined subgroup of PD patients. Additionally, a genetic diagnosis for other family members who are at risk of developing PD enables a more precise assessment of the risk of recurrence and supports genetic counselling and family planning. A genetic diagnosis may also provide them with a great deal of relief because some patients are already very concerned by the fact that they are unsure of what is causing their condition.

1.7.1. Challenges in Genetic Testing

The patient, the genetic test of choice, and the test results all play a role in the challenges of genetic testing in PD. Patients may be hesitant to undergo genetic testing for a variety of reasons, such as a lack of perceived benefit, worry about how the results may affect them or their family

members, or cost. Genetic counselling prior to performing a genetic test is non-directive, meaning that patients or their relatives cannot be persuaded to undergo a genetic test. Nonetheless, it should include a thorough, individually tailored explanation of the rationale for offering a genetic test, the test itself, its benefits and drawbacks, and the potential implications of the test results for the patient and their family members. This sort of pre-test conversation with the patient is essential to soothe their worries and ensure they have all the information they need to decide whether to proceed with genetic testing.

In clinical and research settings, various genetic tests are available, from specialized testing for a single gene or a single variant through variant panels and gene panels to exome or genome sequencing. Due to the increased availability and decreased cost of NGS-based tests, the traditional method of testing one gene at a time has been largely replaced in recent years by broader tests, such as gene panels and exome or genome sequencing, with the exception of rare situations where a very high suspicion has been raised for a particular gene due to a pathogenic variant that runs in the patient's family history. One should take into account the substantial variation in gene content of different panels while deciding whether to use one. A recent study compared the several clinical genetic tests used to diagnose Parkinson's disease and found significant variations in the size of the gene panels, which ranged from 5 to 62 genes. That study revealed that only five genes—*SNCA, PRKN, PINK1, PARK7* (*DJ1*), and *LRRK2*—were consistently included in all panels, while VPS35 and GBA were only variably included. It also revealed that the differences between panels were primarily caused by the variable inclusion of genes linked to atypical parkinsonism and dystonia disorders or genes with an ambiguous association with Parkinson's disease (Cook, Schulze, Verbrugge, et al., 2021). Ideally, the chosen gene panel should contain all recognized PD genes with sequence and deletion/duplication analyses. When a patient exhibits a mixed or unusual phenotype, a broader approach should be taken. Depending on the individual clinical symptoms, this may include utilizing a more extensive gene panel or a genomic investigation with exome or genome sequencing. The *GBA* gene's notable limitations should be taken into account, as they may make it more difficult to identify pathogenic variants due to a similar pseudogene and structural changes. Long-read sequencing is a novel strategy to evaluate this gene, and a New Zealand cohort of patients recently used the GridION nanopore sequencing platform for this purpose. The price of genetic

tests, which might not be covered by the patient's insurance and could consequently influence choices made during the molecular workup in some circumstances, is another aspect to take into account. In conclusion, the choice of which genetic test to use depends on case-specific circumstances and must take into account the various types of tests that are available, their benefits and drawbacks, and their suitability for each unique patient.

1.8. Challenges in Identifying new PD-causing Genes

It has been widely recognized for more than 20 years of research that Parkinson's disease is genetically heterogeneous and that a small proportion of cases can be attributed to genetic variants which show small to modest effects. Roughly 20% of Mendelian-inherited disorders have an identifiable etiology through whole-exome research (H. Lee et al., 2014). This reduces the hope for the use of broad genetic testing in the near future, beyond the few Parkinson’s disease genes that unequivocally have a causal role when mutated. Utilizing the heterogeneity of Parkinson's disease, researchers can look at subgroups based on variables including age at onset (AAO), motor function, and other clinical features, as various phenotypic groups may have different genetic causes. In light of that, focusing the target population to less studied ethnic groups is not only ethically required but also a potential way to find more underlying disease variants. The lack of research targets is one of the challenges with Mendelian PD research. Gathering more genetic samples over several generations in case of apparent Parkinson's disease offers a higher possibility of finding the origin of the disease. One strategy to circumvent this constraint is to gather as many samples in the same family with Mendelian PD as possible. However, we need to keep in mind that sometimes the parents or grandparents with PD might already die by the time the proband develops the disease. Another challenge is using the findings from GWAS to explain heredity and guide genetic testing, as in the case of Nalls et al., study where they found 90 variants with interesting functional associations; however, a large proportion of the heritability is still unknown (Nalls et al., 2019). Even though direct practical implications from their findings cannot yet be inferred, they have laid the groundwork for further research to gain insights into the functionality of the identified variants by using tissue-specific expression, methylation, rare variants, and nongenetic risk factors.

Chapter 3

Uncovering Familial Parkinson's Disease Genetic Variants in an Egyptian Cohort

Introduction

Parkinson's disease is a progressive neurodegenerative disorder characterized by motor symptoms such as tremor, rigidity, bradykinesia, and postural instability, as well as non-motor symptoms including cognitive impairment and mood disorders. While the etiology of PD is multifactorial, involving both environmental and genetic factors, familial forms of the disease provide critical insights into its pathogenesis. Recent advancements in genomics have underscored the significance of genetic contributions to PD. Although the majority of PD cases are sporadic, approximately 10-15% are familial, stemming from the Mendelian inheritance of genetic variants (Pang et al., 2019). Identifying these genetic variants is crucial for understanding the molecular mechanisms underlying PD and for developing targeted therapies. The genetic landscape of PD exhibits considerable heterogeneity, with variations across different populations. Studies have identified several genes associated with familial PD, including *SNCA, LRRK2, VPS35*, and *GBA*, among others (Funayama et al., 2022). Many genetic studies of PD have been conducted primarily on populations of European and Asian descent. This lack of diversity has led to conclusions that may not be applicable to other ethnic groups, which might have different genetic variants associated with PD. The genetic determinants in the Egyptian population, which may have unique genetic predispositions, are not thoroughly explored. This study aimed to bridge this gap by investigating the genetic variants associated with familial PD in an Egyptian cohort. This approach not only enhanced our understanding of the genetic underpinnings of PD but also paved the way to shed light on the genetic variants associated with PD in the Egyptian context. Understanding the genetic basis of PD in different populations is pivotal for global health, as it informs both diagnostic and therapeutic strategies.

Methods

Ethics approval

The present study was approved by the local Institutional Review Board of The American University in Cairo, Egypt, and all study participants provided written informed consent. The study was conducted in compliance with the 1975 Declaration of Helsinki and all relevant guidelines and regulations.

Study population

In this study, stage one investigated a discovery cohort comprising 16 Egyptian patients, originating from 13 distinct families, all presented with a consultation of PD symptoms. A family history of PD, particularly focusing on first-degree relatives, was obtained from the index patient. For this purpose, family pedigrees were constructed for each patient (Annex 3). The majority of the families contributed solely the index patient to the study cohort. However, one family (designated as Family 9) also included 3 healthy individuals acting as a healthy group. An independent cohort of 72 participants from 15 families, including PD patients and healthy individuals, were further recruited for a validation study (stage two).

Whole exome sequencing, data processing and primary analysis

Whole exome sequencing was performed for the 16 PD cases. Genomic DNA libraries were captured using the Nextera Rapid Capture Expanded Exome Kit (Illumina, San Diego, CA), and DNA fragments were sequenced on an Illumina HiSeq2000 system with an average coverage of 80×. First, reads were subjected to quality control (QC) checks to eliminate any low-quality reads, then were mapped and aligned to the UCSC Human reference genome (hg19) (http://genome.ucsc.edu/). Paired-end sequence reads (2 × 100 bp paired-end read cycles) were aligned using the Burrows-Wheeler aligner (H. Li & Durbin, 2009). Format conversion and indexing was performed with Picard (www.picard.sourceforge.net/index.shtml). The Genome Analysis Toolkit was used to recalibrate base quality scores, perform local re-alignments around indels, and call and filter the variants (McKenna et al., 2010). All variants were identified and annotated using ANNOVAR (K. Wang et al., 2010) (http://annovar.openbioinformatics.org). Variants were checked against established databases (1000 Genomes Project and dbSNP). The

pathogenicity, protein-coding effects of variants and their disease-causing potential were predicted using SIFT, Polyphen-2 and MutationTaster.

Filtering and variant prioritization

Of the selected genes, only functional variants (LoF and missense) were retained. Sex chromosomes and variants with a minor allele frequency (MAF) > 0.01 in gnomAD (Genome Aggregation Database) "all" (https://gnomad.broadinstitute.org) exome as well as genome data were discarded. Variants with a CADD score below 20 (not strongly deleterious) were also discarded. Candidate variants were further prioritized on the bases of meeting at least 1 of 4 strict criteria that were previously adopted (Yemni et al., 2019): (1) presence in a gene that was observed in 2 or more cases, (2) presence in a gene previously associated with PD, (3) the gene harbouring the variant has a mouse model with documented neurological or behavioural deficits, (4) same variant was found in additional affected family members (when available).

Sanger sequencing

Rare variants identified by whole-exome sequencing in the candidate genes from the discovery cohort were validated by Sanger sequencing in the validation cohort.

Results

Discovery Cohort

After whole exome sequencing of the 16 participants who presented with familial Parkinson's disease, a total of 18 rare variants were initially identified (Annex 2). Filtering for non-functional variants and those with CADD <20, yielded 16 variants, which appeared to be all of them are missense (Table 1). SIFT, PolyPhen-2, and MutationTaster were utilized to predict the impact of these variants on protein function and potential disease association. The analysis revealed several genetic variants of interest across 10 genes, including *LRRK2, DLG2, ATXN2, GBA, STK39, TMPRSS9, SYNJ1, DNAJC6, APOE* and *PITRM1*.

Table 1. *Variants identified by Whole Exome Sequencing*

gnomAD_exome_ALL	MutationTa	Polyphen	SIFT_pre	CADD_ph	AA	Base	Gene	rsID
0.000004	D	B	T	21.1	p.Q1231H	c.G3693T	*LRRK2*	rs200221850
0.00000407	D	P	T	29.5	p.R1538L	c.G4613T	*LRRK2*	rs200631999
0.000004	D	B	T	21.3	p.T261S	c.C782G	*DLG2*	rs749483521
-	D	D	D	27.1	p.H922R	c.A2765G	*ATXN2*	not reported
0.0013	A	B	D	24	L444P	c.T1187C	*GBA*	rs421016
0.000008	D	B	D	20.2	p.P19L	c.C56T	*STK39*	rs1690960365
0.00000814	D	P	D	23	p.V311L	c.G931T	*TMPRSS9*	rs137891765
0.000021	D	P	D	23.7	p.Y10C	c.A29G	*SYNJ1*	rs967674859
0.000007	D	D	D	31	p.D169Y	c.G505T	*BST1*	rs553990087
-	D	P	T	23.4	p.G778R	c.G2332A	*DNAJC6*	rs550296923
0.000004	D	D	D	28	p.S2562G	c.A7684G	*ASH1L*	rs1415924589
0.000004	N	B	D	22.3	p.S1747F	c.C5240T	*ASH1L*	rs1430305901
0.000526	A	D	D	31	p.G2019S	c.G6055A	*LRRK2*	rs34637584
0	N	D	D	25.1	p.R242W	c.C724T	*APOE*	rs387906568
0.0000084	N	P	T	27.3	p.F1288L	c.C3864G	*ERBIN*	rs754881985
-	D	D	D	25	p.R892K	c.G2675A	*PITRM1*	not reported

SIFT: Deleterious(D), Tolerated(T). **PolyPhen-2**: Damaging(D), Possibly damaging(P), Benign(B). **MutationTaster**: Disease-causing(D), Polymorphism(N), Automatic(A)

LRRK2 is a potentially causative variant. The patient carries two heterozygous presumably pathogenic variants in *LRRK2*. Both variants are rare (~0.000004), have a high predicted pathogenicity and are not listed in Human Gene Variant Database (HGMD). In addition, the p.G2019S variant in *LRRK2*, which is a well-known autosomal dominant PD and has reduced penetrance, was found to be heterozygous and predicted to be highly deleterious in the pedigree studied. Another potentially causative variant is the GBA, rs421016. The *GBA* gene, known for its role in Gaucher's disease and association with PD, presented a variant, which is not a monogenic cause, but increases the risk of PD by ~5-20 fold which would fit well with the pedigree. *Synaptojanin 1* (*SYNJ1*), discs-large membrane-associated guanylate kinase scaffolding protein 2 (*DLG2*), serine-threonine kinase 39, transmembrane protease serine 9 (*TMPRSS9*), and the DnaJ heat shock protein family (*Hsp40*) member C (*DNAJC6*) has been debated as PD-genes. The heterozygous variants reported in these genes are extremely rare and not listed in HGMD. They were all predicted to be pathogenic therefore, it can't be excluded that these variants are likely to be the cause of the disease. On the other hand, *ASH1L* variants have been associated with Schizophrenia, intellectual disability and autism spectrum disorder but not for PD. There are two heterozygous variants reported in the same patient. Both are extremely rare, predicted to be deleterious and not listed in HGMD. The *APOE* variant reported in this cohort is predicted as deleterious by SIFT and PolyPhen-2 but neutral by MutationTaster. Despite the conflicting results, the variant's presence in a gene associated with neurodegenerative diseases warrants further investigation into its potential role in PD.

The *BST1* and *ERBIN* variants were found heterozygous in the PD patients as well as in the healthy individuals of the same family and, therefore, most likely the variants aren’t segregating with the disease and are not disease-causing. *Ataxin 2* (*ATXN2*) is known to cause spinocerebellar ataxia type 2 (*SCA2*) and CAA repeats were recently reported to cause autosomal dominant Parkinson's disease. *ATXN2* variant was found in two PD patients of the same pedigree. Although no single nucleotide variants were reported in the ATXN2 gene associated with PD, this heterozygous variant is predicted to be pathogenic, and therefore, it can't be excluded that this variant might be the cause of the disease. The *PITRM1* variant segregates with PD and might be causative. It has a very high CAAD score (25.0), is predicted to be deleterious by all prediction softwares and has never been observed in any of the current genotype repositories. The *PITRM1*

variant was of high interest for the study because it is reported for the first time in this study to be associated with PD. The heterozygous variant (R892K) was found in PD patients of Family 9 and was absent in the healthy individuals during the discovery phase (Table 2).

Table 2. *Clinical characteristics of family 9 and PITRM1 variant results*

Family 9	Age	AOO	Duration	Clinical features	PITRM1 variant
Patient 1 (Mother)	50	30	20	Akinetic rigid syndrome, asymmetrical, Respond to levodopa with severe levodopa-induced dyskinesia. Dementia (MoCA 11) Exaggerated DTRs Extensor planter responses Mild LL dystonia Normal MRI Postural instability H&Y 3	R892K
Patient 2 (Daughter)	35	29	6	Asymmetrical (Rt>Lt) Akinetic rigid Syndrome mild tremor L-dopa-induced dyskinesia. FOG, LL dystonia (toes) Shuffling gait Mild postural instability Abnormal saccades Cognitive impairment (MoCA 21)	R892K
Patient 3 (Daughter)	23	16	7	Akinetic rigid Syndrome LD induced Dyskinesia. Square wave jerks, postural instability, FOG, falling. Dysarthria, depression, anxiety, abnormal behaviour (impulsivity) LL dystonia Abnormal saccades Cognitive impairment (MoCA 23) Normal MRI brain	R892K
Reference 1 (Father)	54			Healthy	R892R
Reference 2 (Daughter)	29			Healthy	R892R
Reference 3 (Son)	28			Healthy	R892R

Validation Cohort

The pedigree (Figure 1) in which the *PITRM1* variant was found is composed of two closely related Egyptian families with Parkinsonism. Family 9 that was studied in the discovery phase and the second family (family 9.2) whose members underwent Sanger sequencing for the

PITRM1 variant in the validation phase. Results of Sanger sequencing showed that The R892K variant is present in both PD patients and some healthy individuals (Table 3), which may suggest incomplete penetrance or that additional factors are needed for the manifestation of the disease. The mother (reference 2) carries the R892K variant and exhibits mild rigidity and bradykinesia, which could be early signs of PD or related movement disorder. This could indicate a possible age-related penetrance, where symptoms appear or worsen with age. Reference individuals 4 and 5, who are healthy despite carrying the R892K variant, may still develop symptoms later, considering the early-onset nature of PD in this family. The broader genetic screening for the R892K variant in familial PD patients and healthy individuals in the other 14 families showed that the variant was absent in both groups of the other families.

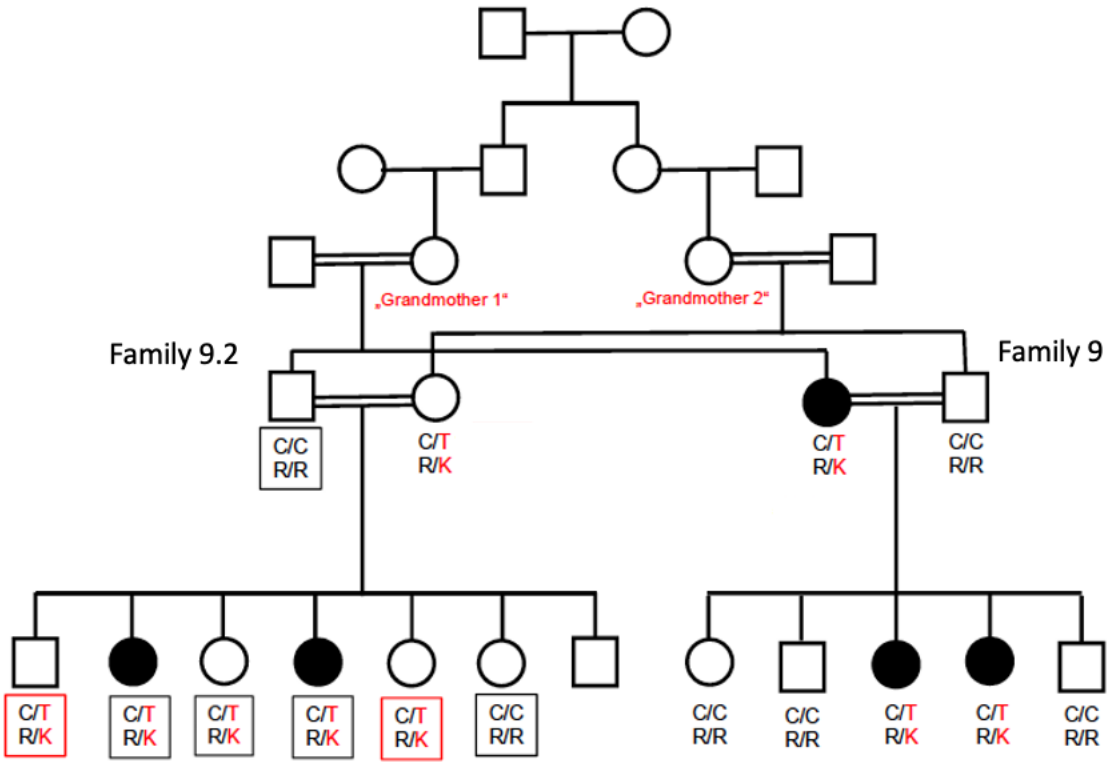

Figure 2. Pedigree of Families 9 and 9.2

Table 3. *Clinical characteristics of family 9.2 and PITRM1 variant results*

Family 9.2	Gender	Age	AOO	Clinical features	PITRM1 variant
Reference 1 (Father)	M	52		Healthy	R892R
Reference 2 (Mother)	F	47		Mild rigidity and bradykinesia, more on left side	R892K
Patient 1	F	28	20	Bilateral rigidity and bradykinesia, postural UL tremor, shuffling gait, FOG, and falling (walk supported) Respond to levodopa. No ophthalmoplegia LL dystonia, anxiety, depressive symptoms, insomnia, constipation Breast Cancer in 2020	R892K
Reference 3	F	26		Mild right UL rigidity (Foment sign)	R892R
Patient 2	F	23	19	Bilateral rigidity and bradykinesia, postural UL tremor, shuffling gait Respond to levodopa. No ophthalmoplegia LL dystonia, anxiety, depressive symptoms	R892K
Reference 4	F	21		Mild rigidity of upper limbs (lt > Rt), Mild postural tremor	R892K
Reference 5	M	16		Healthy	R892K
Reference 6	F	13		Healthy	R892K

Discussion

The current study, focusing on familial Parkinson's disease in an Egyptian cohort, has uncovered several genetic variants with potential implications for the pathogenesis of PD. The identification of 14 variants in genes such as *LRRK2, GBA*, *DLG2*, *SYNJ1*, *DNAJC6*, and notably, *PITRM1*, represents a significant contribution to our understanding of PD's genetic underpinnings in this population. Variants in *LRRK2* are the most common monogenic cause of PD and have been linked to late-onset Parkinson's disease. The patient was presented with PD-FTD and carried two heterozygous, presumably pathogenic variants in *LRRK2*. Even though *LRRK2* is not a Frontotemporal dementia gene, there are suggestions in the literature that severe variants in *LRRK2* could give rise to a "PD-FTD" like phenotype (Srivatsal et al., 2015). LRRK2 G2019S variant has a global frequency of 1% in sporadic PD and 4% in familial PD (Healy et al., 2008b) and cosegregates with PD in large families. The identification of the *LRRK2* G2019S variant in PD patients in our cohort aligns with previous findings where the LRRK2 G2019S variant is prevalent in North African and Arabic populations (Bouhouche et al., 2017;EL Desoky et al., 2019; Hashad

et al., 2011; Simpson et al., 2022). The variant also showed a consistent prediction as deleterious and disease-causing across the used bioinformatics tools, aligning with its documented role in PD. The *GBA* L444P variant is associated with lysosomal function, highlighting the importance of lysosomal pathways in PD pathogenesis. The identification of the *GBA* L444P variant is significant, given its established role in increasing PD risk by 5-20-fold depending on the population (Gan-Or, Amshalom, et al., 2015). The *GBA* L444P variant was previously studied in an Egyptian cohort from the region of Assiut, showing insignificant frequencies of 5.1% (3/59) PD cases and 0.46% (1/217) healthy group, which is probably due to a small sample size (L. R'Bibo et al., 2017). The identification of these variants, particularly in *LRRK2* and *GBA*, aligns with global trends in PD genetics, suggesting common pathways in the disease's development across different ethnicities.

This study also identified a variant in the *STK39* gene, which has been associated with PD in other studies. *STK39* variants were reported to pose a risk factor for PD in the Han-Chinese population (Chang et al., 2015; L. Wang et al., 2016) and in the Ashkenazi Jews (X. Liu et al., 2011). The variant identified in our study, rs1690960365, is different than the one reported in the Han-Chinese and Ashkenazi Jewish populations, underscoring the genetic heterogeneity of PD across different ethnicities. Although we found variants in the *ATXN2* gene, its specific roles in PD are less clear based on the available literature. *Ataxin 2* (*ATXN2*) is known to cause spinocerebellar ataxia type 2 (*SCA2*) and CAA repeats were recently reported in families with predominant parkinsonian symptoms and in some cases with typical autosomal dominant PD (Casse et al., 2023). Our studied pedigree had two siblings with the recurrent heterozygous variant predicted to be pathogenic, and therefore, further research is needed to determine how this gene might contribute to PD pathogenesis. Furthermore, The *ASH1L* gene presented with two heterozygous variants, rs1415924589 and rs1430305901, within our cohort. *ASH1L* has been associated with various neurodevelopmental disorders, including schizophrenia, intellectual disability, and autism spectrum disorder. However, its direct involvement in PD has not been established. Both variants were present in the same PD patient and were predicted to be deleterious. The gene's role in neural development and function suggests that disruptions in its activity could potentially contribute to neurodegenerative processes suggesting that these specific variants are likely to play a causative role in PD within our study population.

The identification of variants in *SYNJ1, DNAJC6* and *TMPRSS9*, while previously debated, adds to the growing evidence that PD's genetic basis is highly heterogeneous. Our findings contribute to this understanding, indicating that these genes might play more substantial roles in PD than previously recognized. Variants in SYNJ1 are linked to autosomal recessive early-onset parkinsonism, characterized by a poor response to levodopa and additional atypical features and often lead to more severe diseases compared to variants in genes like *PRKN*, *PINK1*, and *DJ-1*, which usually cause typical PD phenotypes (Quadri et al., 2013). Previous variants reported in the SNJ1 gene were p.D791fs, p.Y232H, p. Y832C and p.R258Q, were absent or rare in the Genome Aggregation Database and were predicted to be deleterious on in silico analysis (Lesage et al., 2021b) similar to the variant reported in this study. The *SYNJ1* rs967674859 variant found in the Egyptian cohort expands the variants spectrum of *SYNJ1*-related parkinsonism. The DNAJC protein family, including *DNAJC6*, has been associated with Parkinsonism. Variants in *DNAJC6* and other *DNAJC* proteins are linked with synaptic trafficking and clathrin dynamics, suggesting a role in the pathogenesis of autosomal recessive juvenile parkinsonism (Kurian & Abela, 2021). While previous studies have identified several *DNAJC6* variants in various populations (Köroğlu et al., 2013; Olgiati et al., 2016), our finding of the G778R variant in an Egyptian cohort adds to the understanding that different populations may harbour distinct pathogenic variants in the same PD-related genes. Similar to *DNAJC6*, the TMPRSS9 variant is rare and predicted to be pathogenic. *TMPRSS*9's role in PD is not well-documented. Its primary function is related to protease activity, which can impact various cellular processes. The rs137891765 variant was found to be present in two unrelated pedigrees within our studied cohort, raising questions about its direct involvement in PD and highlighting the importance of exploring this variant in larger studies.

The analysis of *DLG2* and *APOE* genes in this Egyptian cohort provides some noteworthy insights, though these findings require careful interpretation within the broader context of PD genetics. *DLG2* is known for its role in the synaptic organization and neuronal signalling. While not traditionally linked to PD, several genome-wide association studies in Asian cohorts have reported that *DLG2* SNPs are associated with Parkinson's disease risk (Foo et al., 2017; Wu et al., 2018; Zhao et al., 2020) however, the *DLG2* variant found in our study exhibited mixed predictions across the tools to be causative for PD but its role in disease could still be relevant and

should be investigated further. Furthermore, the rare *APOE* rs387906568 variant's presence in our PD cohort also showed intriguing predictions that suggest that some aspects of the variant may indicate a potential impact on the protein function while other aspects might not strongly support a disease-causing role. We assume that this *APOE* variant may play a modifying role in the disease, possibly influencing its progression or the risk of developing PD-related dementia. However, this hypothesis requires further validation.

On the other hand, the findings from our cohort suggest that the variants identified in *BST1* and *ERBIN* are likely not causative for PD, or these variants have reduced penetrance. Their presence in healthy individuals of the same family indicates a lack of a clear pathogenic relationship with the disease in this specific population. The results of *BST1* rs553990087 in our study are consistent with findings from another study in the Taiwanese population where their results showed that the *BST1* rs11724635 polymorphism alone is not associated with the development of PD, but it can interact with well water drinking to increase the risk of PD in this Taiwanese population (M. L. Chen et al., 2014) however a large meta-analysis suggested that the rs11931532 and rs4698412 in *BST1* might be risk factors for PD in Asian populations (J. Li et al., 2019). The *ERBIN* gene was never reported to be associated with PD in any previous studies. It's known to be involved in signal transduction and cellular communication, which are critical in neuronal function. However, its direct role in PD remains unclear, and our findings do not strongly implicate the rs754881985 variant in PD within the Egyptian population.

PITRM1 has not been previously reported to be implicated in PD. The gene is known for its role in mitochondrial function, particularly in the processing of mitochondrial precursor proteins, which is crucial for maintaining mitochondrial health. Mounting evidence draws a connection between mitochondrial dysfunction and neurodegenerative disorders, including Alzheimer Disease. The phenotypic profile reported for PITRM1 loss of function variants includes a slow-progressing syndrome characterized by obsessive behavior, cerebellar ataxia, cognitive decline as well as psychotic episodes (Pérez et al., 2021). Some of these phenotypes, especially cognitive decline, are consistent with the clinical signs reported in the affected patients of the Egyptian cohort. Mitochondrial dysfunction is a well-established factor in PD pathogenesis. The R892K variant's potential impact on mitochondrial processing aligns with this broader context

presenting a unique case for exploration in PD genetics. It was identified as potentially significant in the discovery phase of this study since it was consistently present in PD-affected members (mother and two daughters), suggesting a possible link to the disease. The variant segregated with the disease in this family, as all affected members carried the R892K variant, while it was absent in healthy family members. It also has a very high CAAD score (25.0) and is predicted to be pathogenic by in-sillico analysis. Unlike other variants we identified, the R892K variant in *PITRM1* was subject to additional validation steps. Intriguingly, the R892K variant was present in both PD patients and some healthy individuals in the second branch of the pedigree. The presence of variants like *PITRM1* in both affected and healthy individuals suggests a possible incomplete penetrance. This could mean that while the variant contributes to PD risk, it alone might not be sufficient to cause the disease. Observations in family members, particularly the mother showing mild symptoms, could indicate age-related penetrance, where the variant's effects manifest or intensify with age which is a common theme in many neurodegenerative diseases, including PD. The absence of the variant in a broader set of families reinforces the idea that this variant is not a common or definitive cause of PD across populations. It is possibly restricted to specific populations or even specific families. A key limitation of this study is the relatively small sample size, which may impact the generalizability of these findings. Another limitation is that not all the recruited families in the discovery phase included healthy individuals as a reference group. Additionally, the presence of the *PITRM1* variant in both PD patients and the healthy group in the validation cohort suggests incomplete penetrance and indicates the potential influence of other genetic or environmental factors. Future research should focus on larger, more diverse cohorts and explore the functional implications of these novel variants.

Chapter 4

Exploring the Association of MAPT and Adjacent Genomic Regions on Parkinson's Disease: An Extensive Genetic Analysis

Introduction

The Microtubule-associated protein tau (MAPT) gene is involved in the assembly and stabilization of microtubules. Abnormalities in tau, including hyperphosphorylation, have been implicated in a range of neurodegenerative diseases, collectively known as tauopathies Frontotemporal dementia (FTD) and Alzheimer's disease (M. K. Lin & Farrer, 2014). This gene is located within an inversion region on chromosome 17q21, known for high linkage disequilibrium, and is associated with the H1 and H2 haplotypes. The H1 haplotype of MAPT, in particular, has been consistently associated with PD, whereas H2, correlating with reduced tau protein expression, may be protective. The *MAPT* H1 haplotype is part of an extended haplotype in a complex genetic region that includes an inversion. One of the first phenotypes that the major haplotype, H1 has been genetically associated with is the increased risk of several neurodegenerative disorders, including Parkinson's disease, Alzheimer's disease, progressive supranuclear palsy and corticobasal degeneration (Bowles et al., 2022). In PD, however, the role of *MAPT* is particularly intriguing due to its association with both the risk and progression of the disease. This suggests that other genes or variants that are in complete linkage disequilibrium with *MAPT* could also be implicated in PD. Strong, Independent signals from MAPT neighboring genes *KANSL1, SPPL2C, NSF, CRHR1, LRR37A* and *WNT3* have also been noted in some of these diseases (Berwick & Harvey, 2014; Bowles et al., 2022; L. He et al., 2021; Soto-Beasley et al., 2020). Thus, genetic variability in neighbouring genes, within or flanking the MAPT inversion, may contribute to PD development.

KANSL1, in particular, is a part of the chromatin modifier gene that has been associated with neurodegeneration and is encompassed within the 17q21.31 microdeletion syndrome region,

a region where the risk-associated gene was initially thought to be *MAPT*. Variants in the *KANSL1* gene are known to be associated with Koolen-De Vries syndrome (T. Li et al., 2022), which is characterized by developmental delay, intellectual disability, and a range of physical features. Although the *KANSL1* gene is not classically associated with Parkinson's disease, it has been identified through Genome-wide association studies as one of the genetic variants associated with PD (Soto-Beasley et al., 2020; Soutar et al., 2022). Therefore, the strong evidence now suggests that *KANSL1*, not *MAPT*, may be the critical gene at this locus associated with PD. There is ongoing research into how changes in gene regulation, possibly influenced by factors like histone acetylation, could contribute to Parkinson's disease (G. Park et al., 2016; Toker et al., 2021). It's possible that proteins involved in chromatin remodelling, such as those associated with the *KANSL1* gene, might indirectly influence the risk or progression of Parkinson's disease through broad effects on gene expression. *KANSL1*, as a part of the Non-Specific Lethal (NSL) complex, along with KAT8, has been identified as a new regulator of *PINK1*-dependent mitophagy initiation, a process which has been linked to PD. *PINK1*-mitophagy is seen as a contributing factor to sporadic Parkinson's disease. Depletion of *KANSL1* impairs downstream parkin activation, its mitochondrial recruitment, and mitophagy in response to depolarization. This further supports the link between *KANSL1* and PD, where impaired mitophagy is a key causative pathway.

SPPL2C, a relatively less explored gene in the context of neurodegeneration, is located on chromosome 17q25.3, and encodes an intramembrane-cleaving aspartic protease involved in various cellular processes, including immune regulation and apoptosis. While *SPPL2C* has been relatively underexplored compared to other genes associated with PD, emerging evidence suggests its involvement in neuroinflammatory pathways that are increasingly recognized as critical in PD pathogenesis. Understanding the mechanisms by which these genes influence neurodegeneration could lead to significant advances in the management and treatment of PD and related neurodegenerative diseases. The aim of this paper is to investigate the roles of *MAPT*, *KANSL1*, and *SPPL2C* in Egyptian PD patients.

Methods

Ethics approval

The present study was approved by the local Institutional Review Board of The American University in Cairo, Egypt, and all study participants provided written informed consent. The study was conducted in compliance with the 1975 Declaration of Helsinki and all relevant guidelines and regulations.

Study population

A total of 910 unrelated Egyptian participants were enrolled in the study. Among them, 433 patients were diagnosed with PD and a healthy group of 477 healthy volunteers without neurodegenerative diseases. PD patients and healthy subjects were recruited from the collaborating Cairo, Mansoura, Ain Shams, Assiut, Sohag and Kafr Elshiekh universities. Participants underwent a standardized clinical assessment by consultant neurologists specialized in movement disorders. Patients with PD were diagnosed using the UK PD Brain Bank Criteria (Annex 4) (Clarke et al., 2016) and PD severity was evaluated with the Unified Parkinson's Disease Rating Scale (UPDRS) (Nakatsuka et al., 1987). The modified Hoehn & Yahr stage (Goetz et al., 2004; Hoehn & Yahr, 1967) was ascertained in the on-medication cases. Patients with atypical or secondary forms of Parkinsonism or other neurodegenerative diseases were excluded from the study. Healthy visitors or patients without neurodegenerative disorders were recruited from the affiliated hospitals to serve as reference group, who were determined to be free of neurodegenerative diseases based on a neurological examination and medical history.

Sample preparation and genotyping

Genomic DNA was extracted from peripheral blood leukocytes using QIAamp DNA Blood Mini Kit (Qiagen, Germany) following the manufacturer's instructions. The genotyping of the common polymorphisms of *MAPT/SPPL2C* (rs12185268), *KANSL1* (rs17585974, rs34043286, rs34579536), and *MAPT* (rs807072) was performed in LGC genomics (LGC Genomics, UK) using Kompetitive Allele Specific PCR (KASP) chemistry coupled with a Förster resonance energy transfer-based genotyping system (http://www.kbioscience.co.uk/reagents/KASP/KASP.html). *MAPT* locus haplotypes were investigated by tagging the H1/H2 haplotypes with the major allele and minor allele of SNP

rs12185268. For rs12185268, allele A and G correspond to H1 and H2 haplotypes, respectively (Pankratz et al., 2009).

Statistical analysis

Statistical analyses were performed with the statistics software R version 4.3.1 (www.r-project.org). Alleles and genotype frequencies were estimated, and Hardy-Weinberg equilibrium (HWE) was assessed for the five polymorphisms. A logistic regression model was employed to examine the association between Parkinson's disease onset and several predictors, including specific SNPs, age, and sex. A p-value of less than 0.05 was considered statistically significant for all tests. Combined annotation-dependent depletion scores were determined using the online CADD single nucleotide variant lookup tool (https://cadd.gs.washington.edu/snv); a CADD score >20 indicates a variant is among the 1% most deleterious for the gene. To evaluate the linear relationship between different clinical variables and SNPs, Spearman's rank correlation was used. The correlation coefficient (ρ) and associated p-values were computed.

Results

Population Characteristics

The study sample consisted of $n = 433$ unrelated PD patients and $n = 477$ unrelated healthy group of Egyptian ancestry. The sex distribution differed between the groups, and PD patients were older than the reference group. Age at onset, age at diagnosis, PD dementia, family history and history of consanguinity are shown in Table 4.

Table 4. *Sociodemographic characteristics of study subjects*

Characteristic	**PD Cases (N=433)**	**Reference group (N=477)**
Males [*n* (%)]	278 (64.2%)	177 (37.1%)
Females	155 (35.8%)	300 (62.9%)
Age (y) [*mean* (SD)]	61.4 (10.3)	44.8 (14.0)
Age (male) [*mean* (SD)]	61.4 (9.95)	49.9 (13.7)
Age (Females) [*mean* (SD)]	61.3 (11.0)	41.8 (13.2)
Age at onset (y) [*mean* (SD)]	54.0 (9.21)	NA
EOPD [*n* (%)]	106 (24.5%)	NA

LOPD [*n* (%)]	327 (75.5%)	NA
Age at diagnosis (y) [*mean* (SD)]	54.7 (9.38)	NA
PD Dementia [*n* (%)]	13 (3.1%)	NA
Family History of PD [*n* (%)]	57 (13.8%)	NA
History of Consanguinity [*n* (%)]	17 (4.1%)	NA

NA: Not Applicable

Association between the KANSL 1, MAPT/SPPL2C, MAPT and PD risk

The results in Table 5. suggest there are no statistically significant associations between these SNPs and the risk of PD development, either before or after adjusting for age and sex. However, the direction of the estimates (negative for *KANSL1* and *MAPT/SPPL2C*, positive for *MAPT*) can provide hypotheses for further investigation.

Table 5. *Association between MAPT, KANSL1 and MAPT/SPPL2C SNPs and PD onset*

Gene	**Predictor**	**Estimate**	**p-value**	**Crude Odds ratio (95% CI)**	**Estimate**	**p-value**	**Adjusted Odds ratio (95% CI)**
KANSL1	rs17585974	-0.253	0.122	0.776 (0.563-1.07)	-0.273	0.164	0.761 (0.518-1.117)
KANSL2	rs34043286	-0.2097	0.127	0.811 (0.619-1.06)	-0.165	0.315	0.847 (0.615-1.169)
KANSL3	rs34579536	-0.2122	0.124	0.809 (0.617-1.06)	-0.157	0.345	0.855 (0.618-1.183)
MAPT	rs807072	0.107	0.269	1.113 (0.921-1.35)	0.143	0.227	1.154 (0.915-1.456)
MAPT/ SPPL2C	rs12185268	-0.2103	0.124	0.81 (0.620-1.06)	-0.17	0.296	0.843 (0.613-1.161)

Genotyping results

Within the studied Egyptian population, the nonsynonymous SNP in *MAPT/SPPL2C* (rs12185268) exhibited a lower MAF in PD cases (12.587%) compared to references (15.094%). The major allele A (H1) haplotype frequency was 87.413% in PD and 84.906% references (p = 0.111). The most frequent genotype was H1/H1 in both PD (332, 76.7%)

and controls (343, 71.9%) (p = 0.265). *MAPT* H2 was present in 25.8% (235/910) of the entire cohort, 23.3% in PD and 28.1% in the healthy subjects. However, with a CADD score of 2.193, this variant is not classified among the most deleterious variants for the gene, suggesting a limited functional impact. In contrast, the *MAPT* variant (rs807072) was observed to have a higher MAF in PD cases (34.841%) versus reference group (32.796%), potentially implicating it as a risk factor for PD. Similarly, its CADD score of 0.716 indicates that this SNP is not among the most deleterious variants within the *MAPT* gene. Variants within the *KANSL1* gene presented a more notable potential functional significance. Specifically, rs17585974 displayed a lower MAF in PD cases (9.145%) relative to references (11.311%) and a CADD score of 26.0, placing it within the 1% most deleterious variants for the gene (Table 6). Similarly, rs34043286 also showed a lower MAF in PD cases (12.679%) than references (15.116%) and had a CADD score of 18.93, again indicating a variant with high deleterious potential. Conversely, the *KANSL1* variant rs34579536, while also demonstrating a lower MAF in PD cases (12.381%) compared to references (14.850%), had a CADD score of 8.103, suggesting it is less likely to exert a major deleterious effect on the gene's function. The five variants showed no significant departure from the Hardy-Weinberg Equilibrium.

Table 6. *Minor allele frequencies distribution for MAPT, KANSL1 and MAPT/SPPL2C SNPs*

rsID	Gene	Exon	Genotypes	Variant type	MAF	MAF (% cases)	MAF (% references)	CADD
rs12185268	MAPT/ SPPL2C	1	A/G	Nonsynonymous	G	12.587	15.094	2.193
rs17585974	KANSL 1	1	T/G	Nonsynonymous	G	9.145	11.311	26.0
rs34043286	KANSL 1	7	A/G	Nonsynonymous	G	12.679	15.116	18.93
rs34579536	KANSL 1	14	A/G	Nonsynonymous	G	12.381	14.850	8.103
rs807072	MAPT		G/A		A	34.841	32.796	0.716

MAF: Minor Allele Frequency
CADD: Combined Annotation Dependent Depletion

Association between MAPT *HI haplotype and PD risk*

The odds ratio of 1.23 with a 95% confidence interval for the H1/H1 genotype indicates a slightly higher occurrence in the PD group compared to references (Table 7), but this is not statistically significant even after adjusting for age and sex.

Table 7. *MAPT/SPPL2C rs12185268 genotype and risk of Parkinson's disease*

Genotype	**References n = 477 (%)**	**PD n = 433 (%)**	**Odds ratio (95%CI)**	[a]**p-value**
H1/H1	343 (71.7 %)	332 (76.7 %)	1.23 (0.851-1.768)	0.273
H1/H2 or H2/H2	134 (28.1 %)	101 (23.3 %)		

[a] adjusted for sex and age

Correlation of SNPs with clinical outcomes

Correlations with age of PD onset for the SNPs in addition to other clinical outcomes like the severity of the disease using the UPDRS scores and the cognitive impairment (MoCA) were examined. According to the results (Table 8), there are no statistically significant correlations between the SNPs tested and the clinical outcomes as age at onset, UPDRS scores, and MoCA scores for the given sample sizes (n values). This suggests that these particular SNPs might not be strong predictors or related to the clinical variables measured in this sample, or that the sample size may not be large enough to detect a significant effect.

Table 8. *Correlation between MAPT, KANSL1 and MAPT/SPPL2C variants and clinical variables*

Variable	rs12185268	rs17585974	rs34043286	rs34579536	rs807072
Age at Onset (n=433)	0.006 (P= 0.907)	0.014 (P= 0.778)	-0.005 (P = 0.921)	0.014 (P= 0.775)	-0.002 (P= 0.963)
UPDRS scores (n=413)	-0.088 (P=0.075)	-0.092 (P=0.065)	-0.065 (P=0.191)	-0.088 (P=0.075)	-0.023 (P=0.647)
MoCA score (n=88)	0.080 (p=0.459)	0.038 (P=0.729)	0.038 (P=0.729)	0.082 (P=0.455)	0.192 (P=0.079)

Discussion

This study embarked on a comprehensive exploration of the association of the *MAPT* gene and adjacent genomic regions with Parkinson's Disease within the Egyptian population. The aim was to understand the genetic landscape surrounding *MAPT* gene, with a particular focus on understanding how variations in this region, including the *KANSL1* and *SPPL2C* genes, contribute

to the risk of PD. The study focused on genotyping common polymorphisms of *MAPT/SPPL2C* (rs12185268), *KANSL1* (rs17585974, rs34043286, rs34579536), and *MAPT* (rs807072).

The *MAPT* gene plays a pivotal role in neurodegenerative diseases, not only in PD but also in other tauopathies like Alzheimer's disease and Frontotemporal dementia. According to a large meta-analysis that investigated the association between variants in *MAPT* and neurodegenerative diseases, a robust association between *MAPT* and PD was found between rs242557, rs7521 and H2 haplotype with PD in Caucasians with the latter having a protective role from PD (C. C. Zhang et al., 2017). Previous studies investigating the *MAPT*-PD connection have been conducted in populations of predominantly Caucasian ancestry. The rs12185268 is a missense variant located in the *MAPT/SPPL2C* gene; it was found to be statistically significantly associated with PD risk in a recent study on the Cypriot population (Georgiou et al., 2019). These results are in accordance with GWAS results that identified common coding variants in strong linkage disequilibrium (LD) within the associated loci on chr17q21 harbouring *MAPT in PD patients* and reported Strong association with PD spans the entire region around *MAPT*, including SNP rs12185268 ($p = 3.6\times10{-}6$ OR = 0.76; 95% CI [0.67–0.85]) in Caucasian population (Edwards et al., 2010). Soto-Beasley et al. reported that rs12185268 was in complete LD with the *MAPT* H1/H2-tagging SNP rs8070723 ($r^2 = 1.00$) (Soto-Beasley et al., 2020). Additionally, the rs12185268 SNP was also reported to be significantly associated with Rapid eye movement sleep behaviour disorder (RBD) in candidate gene studies (Gan-Or, Girardet al., 2015; M. Li et al., 2018). RBD is known to be present in 25–58% of patients with Parkinson's disease and up to 90% of those with Dementia with Lewy Bodies (DLB) or multiple system atrophy (Hu, 2020). It is associated with increased severity and frequency of non-motor features, poorer subjective motor performance and a greater impact on health-related quality of life in early-onset Parkinson's disease patients (Rolinski et al., 2014). Furthermore, the rs12185268 was reported previously to be a *MAPT* H1/H2 haplotype tagging variant (Pankratz et al., 2009). The *MAPT* H2 haplotype, known to be protective for PD (Elbaz et al., 2011) has a low distribution in Africa (less than 6%), while having its highest frequencies in Mediterranean regions of southwest Asia and Europe (20–37%), and much lower frequency in the Arabian Peninsula (5–10%) (Alves et al., 2015; Steinberg et al., 2012). Few studies previously described the distribution of the inverted (H2) haplotype in in west Africans population utilizing the *MAPT* rs1052553 variant (Donnelly et al., 2010; Okunoye, Ojo, et al., 2023). They concluded that the H2 inverted haplotype occurs at low frequencies in Africa (0.75-

6.3%) with the highest frequency in North Africa (Donnelly et al., 2010). The results of this study are in accordance with the assumption of Donnely et al., with H2 occurring at 25.8% in our study participants, whereas another study showed contradicting low frequencies (2.1%) in the Nigerian population (Okunoye, Ojo, et al., 2023).

Regarding the risk of PD conferred by the *MAPT* H1 haplotype, some previous reports indicated an increased risk and increased transcriptional activity of H1 in comparison to H2 implying a possible role in the development of PD (Edwards et al., 2010; Elbaz et al., 2011; Okunoye, Ojo, et al., 2023; Soto-Beasley et al., 2020; Vandrovcova et al., 2009; C. C. Zhang et al., 2017). In contrast to other studies on predominantly Caucasian European and North American groups (Bowles et al., 2022; Davis et al., 2016; Winkler et al., 2007), we did not observe significant association between H1 and PD risk, a finding similar to the Nigerian study (Okunoye, Ojo, et al., 2023). This supports the assumption that the role of H1 haplotypes in PD etiology may be ethnically dependent (Winkler et al., 2007).

On the other hand, the rs807072 was identified definitively as *MAPT* H2 variants from prior *MAPT* genomic sequencing studies that identified it in progressive supranuclear palsy patients and were reported to have a low-rank score with the disease (Melquist et al., 2007; Rademakers et al., 2005). The rs807072 was found in this study to be associated with PD risk, though the results did not find it statistically significant. This study investigated the *MAPT* rs807072 SNP for the first time in PD patients which underscores the necessity of considering a wide array of genetic variants, including less-studied SNPs like rs807072, to fully understand the genetic landscape of PD.

The *KANSL1* gene, along with *MAPT*, has been identified as playing a significant role in the genetic risk for neurodegenerative diseases such as Parkinson's disease and Alzheimer's Disease. Variants in these genes, particularly those with high CADD scores (>20), are considered to be among the most deleterious substitutions in the human genome, indicating their potential contribution to the increased genetic risk of these disorders. The three studied SNPs (rs17585974, rs34043286, and rs34579536) exhibited slightly weaker associations, indicating a potential reduction in the odds of PD onset. Notably, the SNP rs17585974 revealed a CADD score of 26.0, situating it within the strongly deleterious variants. These results align with findings from Soto-

Beasley et al. (2020), who identified common coding variants within the chr17q21 locus, known to harbour *MAPT*, which were in strong linkage disequilibrium with PD-associated loci (Soto-Beasley et al., 2020). In the Caucasian cohort of the Soto-Beasley et al. study, the non-synonymous KANSL1 rs17585974 variant demonstrated a weak association with PD (OR = 0.61, P < 0.001) and was found to be in very strong LD with the *MAPT* H1/H2 haplotypes. Notably, the variant had a high CADD score of 24.7, indicative of its substantial deleterious potential. In our population, the CADD score for this variant was slightly higher, augmenting the variant's significance as a genetic marker for PD susceptibility. The elevated CADD score in the present study suggests a possible population-specific genetic susceptibility that could be influenced by ethnic diversity. The rs34043286 variant, also located within this gene-rich region, exhibited a CADD score of 18.93 in our study, which falls slightly below the benchmark CADD score of 20 and still represents a variant with considerable deleterious potential. This is an increase from the score of 15.71 reported in the study by Soto-Beasley et al., suggesting that our population may harbour a variant with a potentially higher functional impact on PD. The increased CADD score warrants further investigation into the biological implications of this variant and its contribution to PD pathology. Further supporting this notion, Soto-Beasley et al. reported that rs34043286 and the other non-synonymous SNP, rs34579536, were in complete LD with the *MAPT* H1/H2-tagging SNP rs8070723 (r^2 = 1.00). The identification of these high CADD scores in our study, coupled with the lack of statistically significant associations, points to the possibility of these SNPs exerting functional effects that were not detected within the study scope.

Chapter 5

Exploring the Combined Effects of APOE and BCHE Genetic Variants in Egyptian Parkinson's Disease Patients

Introduction

Parkinson's disease is a highly heterogeneous disorder characterized by diverse causes, neuropathological features, clinical presentations and progression patterns. A recent study employed data from GBD 2019 to showcase the escalating issues and patterns related to PD in the Middle East and North Africa (MENA) from 1990 to 2019 highlighted a significant rise in the PD prevalence in MENA over the past 30 years, suggesting that the disease is increasingly becoming a public health concern in the region (Safiri et al., 2023). In Egypt, Parkinson's disease prevalence and incidence rates lie outside the range reported elsewhere (El-Tallawy et al., 2013; Khalil et al., 2020; Khedr et al., 2012, 2015) the incidence in Egypt is exceptionally high. A genetic background to the pathogenesis of Parkinson's disease has been postulated for a long time, with specific gene polymorphisms emerging as potential risk factors. Among these genes, Apolipoprotein E (*APOE*) has garnered attention due to its pivotal role in lipid metabolism and neurodegeneration. The APOE gene encodes for a serum glycoprotein involved in the transport of lipids to neurons and has been previously implicated in Alzheimer's disease. Two prevalent SNPs are found in exon 4 of this gene: rs429358 and rs7412, which can lead to three distinct alleles (ε2, ε3, ε4) (Seripa et al., 2011) with APOE-ε4 (often referred to as *APOE4*) being the most well-known for its strong correlation with an increased risk of late-onset Alzheimer's disease (Seshadri et al., 2010). However, its relationship with Parkinson's disease is controversial. Previous studies have reported that *APOE4* contributes to α-Syn pathology in PD (Dickson et al., 2018) and might be a risk factor for PD in the Asian population (J. Li et al., 2018). Recent studies also indicate that *APOE* alleles may differently influence PD course progression, with *APOE*4 having a particularly detrimental impact (Pu et al., 2022; Real et al., 2023; Tunold et al., 2021). Meanwhile, Butyrylcholinesterase (*BCHE*) is an enzyme that hydrolyzes acetylcholine in the synaptic cleft and has been studied for

its role in neurotransmission and potential implications in PD's cholinergic deficit. Genetic studies have examined the *BCHE* gene for variants that might be associated with an increased risk of Parkinson's disease or its particular phenotypes. *BCHE*-K allele (Ala539Thr) lowers the serum activity of butyrylcholinesterase, increasing the risk for PD in individuals exposed to pesticides (Rösler et al., 2018). It is also suggested that the *BCHE*-K allele works in conjunction with the APOE-ε4 allele to promote risk for Alzheimer's disease (Jasiecki et al., 2019), raising questions about its role in other neurodegenerative disorders like PD. Although individual studies have explored the associations of *APOE* and *BCHE* with PD, there is a paucity of research investigating their combined or synergistic effect. Understanding this synergy could unveil intricate genetic interactions that underpin the disease's complexity and guide targeted therapeutic interventions. In this paper, the main aim is to explore the combined relationship between *APOE* and *BCHE* in the context of Parkinson's disease, in order to shed light on the interplay between these genetic factors and their potential combined influence on disease risk and progression.

Methods

Ethics approval

The present study was approved by the local Institutional Review Board of The American University in Cairo, Egypt, and all study participants provided written informed consent. The study was conducted in compliance with the 1975 Declaration of Helsinki and all relevant guidelines and regulations.

Study population

A total of 878 unrelated Egyptian participants were enrolled in the study. Among them, 412 patients were diagnosed with PD and a reference group of 466 healthy volunteers without neurodegenerative diseases. PD patients and reference subjects were recruited from the collaborating Cairo, Mansoura, Ain Shams, Assiut, Sohag and Kafr Elshiekh universities. Participants underwent a standardized clinical assessment by consultant neurologists specialized in movement disorders. Patients with PD were diagnosed using the UK PD Brain Bank Criteria (Clarke et al., 2016), and PD severity was evaluated with the Unified Parkinson's Disease Rating Scale (UPDRS) (Nakatsuka et al., 1987). The modified Hoehn & Yahr stage (Goetz et al., 2004; Hoehn & Yahr, 1967) was ascertained in the on-medication cases. Patients with atypical or

secondary forms of Parkinsonism or other neurodegenerative diseases were excluded from the study. Healthy visitors or patients without neurodegenerative disorders were recruited from the affiliated hospitals to serve as reference group, who were determined to be free of neurodegenerative diseases based on a neurological examination and medical history.

Sample preparation and genotyping

Genomic DNA was extracted from peripheral blood leukocytes using QIAamp DNA Blood Mini Kit (Qiagen, Germany) following the manufacturer's instructions. The genotyping of *APOE* common polymorphisms rs429358 and rs7412 was performed in LGC genomics (LGC Genomics, UK) using Kompetitive Allele Specific PCR chemistry coupled with a Förster resonance energy transfer-based genotyping system (http://www.kbioscience.co.uk/reagents/KASP/KASP.html). *BCHE* polymorphism (rs1803274) was genotyped as previously described.

Statistical analysis

Statistical analyses were performed with the statistics software R version 4.3.1 (www.r-project.org). Alleles and genotype frequencies were estimated, and Hardy-Weinberg equilibrium was assessed for the three variants. A logistic regression model was employed to examine the association between Parkinson's disease onset and several predictors, including specific variants, age, and sex. A p-value of less than 0.05 was considered statistically significant for all tests. To evaluate the linear relationship between different clinical variables and studied variants, Spearman's rank-order correlation was used. The correlation coefficient (ρ) and associated p-values were computed. Model-based multifactor dimensionality reduction, an R package (Calle et al., 2008), was used for gene interaction analyses. Age and sex were used as control variables in the analyses to account for their potential confounding effects.

Results

Population Characteristics

The study sample consisted of $n = 412$ unrelated PD patients and $n = 466$ unrelated healthy group of Egyptian ancestry. The sex distribution differed between the groups, and PD patients

were older than the healthy group. Age at onset, age at diagnosis, PD dementia, family history and history of consanguinity are shown in Table 9.

Table 9. *Sociodemographic characteristics of study subjects*

Characteristic	**PD Cases (N=412)**	**References (N=466)**
Males [*n* (%)]	271 (65.8%)	173 (37.1%)
Females	141 (34.2%)	293 (62.9%)
Age (y) [*mean* (SD)]	61.3 (10.28)	44.5 (13.86)
Age (male) [*mean* (SD)]	61.4 (9.98)	49.6 (13.64)
Age (Females) [*mean* (SD)]	61.1 (10.86)	41.6 (13.13)
Age at onset (y) [*mean* (SD)]	54.2 (9.24)	NA
EOPD [*n* (%)]	99 (24%)	NA
LOPD [*n* (%)]	313 (76%)	NA
Age at diagnosis (y) [*mean* (SD)]	54.7 (9.33)	NA
PD Dementia [*n* (%)]	13 (3.2%)	NA
Family History of PD [*n* (%)]	55 (13.3%)	NA
History of Consanguinity [*n* (%)]	17 (4.1%)	NA

EOPD: Early-Onset Parkinson's Disease. LOPD: Late-Onset Parkinson's Disease

Logistic regression analysis of the risk of PD in Egyptian population

The results in Table 10. suggest that in the Egyptian population, sex and age are significant predictors of PD risk, with sex being a stronger predictor. The adjustments made in the analysis seem to reduce the effect size of sex on PD, indicating that other factors in the model might contribute to the risk associated with sex. The logistic regression results show that the genetic variants for *APOE* and *BCHE* genes are not significant predictors for PD, as shown by the high p-value (>0.05) and an odds ratio near 1, indicating very little or no effect on the likelihood of PD. After adjusting for other factors, the genetic variants remain non-significant. The lack of association in both crude and adjusted models, implies that these genetic factors might be less relevant for PD pathogenesis in this specific population.

Table 10. *Logistic regression analysis of the risk of PD in Egyptian population*

Variable	Crude values			Adjusted values		
	β - Coefficient	***P*-Value**	**OR (95% CI)**	**β - Coefficient**	***P*-Value**	**OR (95% CI)**
Sex	1.18	<0.001*	3.255 (2.47-4.3)	0.722	< .001*	2.06 (1.48-2.87)
Age	0.11	<0.001*	1.116 (1.1-1.13)	0.106	< .001*	1.11 (1.09-1.13)
rs7412	-0.03	0.865	0.970 (0.68-1.38)	0.203	0.365	1.23 (0.79-1.9)
rs429358	0.185	0.922	1.018 (0.71-1.46)	0.016	0.945	1.02 (0.65-1.59)
rs1803274	0.118	0.347	1.125 (0.88-1.44)	-0.207	0.185	0.812 (0.6-1.1)

*statistically significant (P <0.05)

Genotyping results

The *APOE* rs429358, rs7412 and BCHE rs1803274 polymorphisms were genotyped in the 878 study subjects of the GP2 cohort. Allele frequencies for rs429358 were 0.93 and 0.07 for T and C alleles, respectively. For rs7412, allele frequencies for C and T alleles were 0.925 and 0.075, respectively. Allele frequencies for rs1803274 were 0.81 and 0.19 for C and T alleles, respectively. The three SNPs showed no significant departure from the Hardy-Weinberg Equilibrium (P=0.298, P= 0.486 and P=0.833 for rs1803274, rs429358 and rs7412, respectively).

Genotype and Allele Frequencies of APOE and their Association with PD

Both the *APOE* gene variants (rs429358 and rs7412) were checked for their association with PD by using logistic regression analysis. The genotypic and allelic distributions of both variants are displayed in Table 11. In our study population, the ε3 allele is the most common. On the other hand, the ε4 allele was absent in PD cases and only observed in the healthy group (n=3). All *APOE* genotypes (ε2/ε2, ε2/ε3, ε2/ε4, ε2/ε2, ε3/ε3, ε3/ε4, and ε4/ε4) showed no statistically significant difference (all $p > 0.05$).

Table 11. *Genotypes distribution of APOE gene in PD patients and controls*

Genotypes	PD patients (n=412)	References (n=466)	OR (95% CI)	*P*-value
ε3/ε3	298 (72.34%)	335 (71.89%)	ref	-
ε2/ε2	3 (0.73%)	2 (0.43%)	1.686 (0.280-10.1)	0.569
ε2/ε3	49 (11.89%)	63 (13.52%)	0.874 (0.583-1.31)	0.515
ε2/ε4	6 (1.47%)	4 (0.86%)	1.686 (0.471-6.03)	0.422
ε3/ε4	56 (13.57%)	59 (12.7%)	1.067 (0.717-1.59)	0.749
ε4/ε4	0 (0%)	3 (0.6%)	NA	NA
rs429358				
TT	350 (84.95%)	400 (85.84%)	ref	-
CT	62 (15.05%)	63 (13.52%)	1.125	0.543
CC	0 (0%)	3 (0.64%)	NA	NA
rs7412				
CC	354 (85.92%)	397 (85.19%)	ref	-
CT	55 (13.35%)	67 (14.38%)	0.921 (0.627-1.35)	0.673
TT	3 (0.73%)	2 (0.43%)	1.682 (0.280-10.12)	0.570

NA: Not Applicable

Association of rs1803274 SNP of BCHE with PD

Gene SNP *(rs1803274)* of BCHE was also checked for its association with PD by using logistic regression analysis. The genotypic distributions of the variant is displayed in Table 12. The genotype distribution of *BCHE rs1803274* was not found to be significantly different between the PD cases and healthy subjects. The results showed no significant association of the *BCHE rs1803274* genotypes with PD ($p > 0.05$).

Table 12*. Genotypes distribution of BCHE gene in PD cases and controls*

Genotypes	PD patients (n=412)	References (n=466)	OR (95% CI)	*P*-value
CC	264 (64.08%)	314 (67.38%)	ref	-
CT	136 (33.01%)	139 (29.83%)	1.164 (0.873-1.551)	0.301
TT	12 (2.91%)	13 (2.79%)	1.098 (0.493-2.447)	0.819

Epistatic interactions between APOE and BCHE and PD risk

Using the model-based multifactor dimensionality reduction method, epistatic interactions were analyzed between the K-variant *BCHE* SNP (rs1803274) and *APOE* common polymorphisms rs429358 and rs7412. Table 13 indicates that the analyzed SNP pairs show a combined interaction towards the outcome, but the effects, although suggestive, are not statistically significant. The categorization as "H" suggests these genotype combinations pose a high PD risk. The adjustment for age and sex for one of the interactions helps provide a clearer picture of the SNP interaction's effect by removing potential confounding factors. We also examined the epistatic interactions between *APOE, BCHE* and the other previously studied genes (*MAPT, SPPL2C and KANSL1*) in relation to Parkinson's disease risk (Table 14). The analysis revealed a series of SNP-SNP interactions that were significantly associated with a lower risk of PD. The interaction between rs429358, rs807072 (MAPT), and rs17585974 (KANSL1) exhibited the most significant association with a reduced PD risk. Similarly, the interaction of rs1803274, rs807072, and rs17585974 showed a substantial decrease in PD risk. Further, the combinations of rs429358 with rs17585974 and either rs12185268 or rs34579536/rs34043286 also demonstrated a lower risk association, however with smaller effect sizes and P-values nearing the significance cutoff.

Table 13. *Epistatic interactions between APOE and BCHE*

SNP1 x SNP2	Genotypes	Cases	References	β	P-value	Category
rs1803274 x rs429358	C/T x C/T	29	20	0.5238	0.07965	H
rs1803274 x rs7412	C/C x C/T	37	40	[a]0.496	0.098	H

[a] adjusted for age and sex

Category: Predicted risk category for the genotype

H: High risk for PD

Table 14*. Epistatic interactions between APOE, BCHE, MAPT, SPPL2C and KANSL1*

SNP1	SNP2	SNP3	β	P-value	Category
rs429358	rs807072	rs17585974	-1.4729	0.02064	L
rs1803274	rs807072	rs17585974	-1.4016	0.02853	L
rs429358	rs17585974	rs12185268	-0.4312	0.0348	L
rs429358	rs34579536	rs17585974	-0.4058	0.04572	L
rs429358	rs34043286	rs17585974	-0.4058	0.04572	L

L: Low risk for PD

Correlations between SNPs and clinical outcomes

rs1803274 shows a weak correlation with age at onset (P=0.039) and UPDRS scores (P=0.009) in the crude analysis (Table 15). However, after adjusting for age and sex, the significance for age at onset variable drops, whereas UPDRS scores became more significant in both crude and adjusted models. The significant correlation between the rs1803274 variant and UPDRS scores implies that there's a relationship between the presence (or absence) of this particular genetic variant and the severity of Parkinson's disease symptoms as measured by the UPDRS.

Table 15*. Correlations between APOE and BCHE variants and clinical variables*

Variable	Crude values			Adjusted values		
	rs1803274	rs7412	rs429358	rs1803274	rs7412	rs429358
Age at Onset (n=412)	0.102 (P= 0.039)*	-0.036 (P= 0.460)	-0.044 (P= 0.369)	-0.003 (P= 0.946)	0.026 (P=0.598)	-0.009 (P=0.856)
UPDRS scores (n=408)	0.129 (P=0.009)*	0.008 (0.870)	0.027 (P=0.585)	0.107 (P=0.032)*	0.022 (P=0.663)	0.036 (P=0.470)
H&Y scores (n=6)	0.495 (P=0.318)	-0.399 (P=0.434)	0.315 (P=0.543)	0.850 (P= 0.150)	-0.257 (P=0.743)	0.058 (P=0.942)
MoCA score (n=87)	0.013 (p=0.904)	-0.139 (P=0.198)	-0.033 (0.761)	0.058 (P=0.598)	-0.164 (P=0.133)	-0.030 (0.784)

*statistically significant (P <0.05)

Correlations between variants and clinical variables with PD Dementia onset

For all three variants, none show a statistically significant association with dementia onset in Parkinson's disease patients (Table 16). Also, neither age nor sex show a statistically significant association with dementia onset in Parkinson's disease. However, age appears to be borderline significant. The wide confidence intervals for the odds ratios, especially for the variants and sex, indicate a high degree of uncertainty around these estimates, possibly due to a limited sample size or other factors.

Table 16. *Association between adjusted variants and clinical variables and the onset of dementia in PD patients*

Variable	β - Coefficient	p-value	Odds ratio (95% CI)
rs7412	0.11665	0.874	1.12 (0.265-4.766)
rs429358	0.58527	0.393	1.80 (0.469-6.875)
rs1803274	0.00717	0.989	1.01 (0.369-2.746)
Age	0.05810	0.054	1.06 (0.999-1.124)
sex	0.57232	0.395	1.77 (0.474-6.630)

Discussion

The intricate relationship between genetics and neurodegenerative disorders has long been a focal point of research. In the current study, the main aim was to elucidate the potential combined interaction between the *APOE* and *BCHE* genes in the context of Parkinson's Disease and whether the association between the *APOE-ε4* allele and the K-variant *BCHE* reinforces the intricate web of genetic and clinical factors that could influence PD development and progression. Among the present study observations was the significant association of both sex and age with the risk of PD development. The crude values showcased that the odds of PD were more than three times higher in males compared to females, and this effect remained significant even after adjusting for other variables. Age demonstrated a consistent increase in PD risk, evident from both the crude and adjusted odds ratios. This reinforces the globally recognized understanding of age as a significant risk factor for PD (Reeve et al., 2014) and stresses the importance of early detection and management strategies for the aging Egyptian population.

The *APOE* gene has been extensively studied for its role in Alzheimer's disease. However, its involvement in PD, particularly in conjunction with the *BCHE* gene, remains elusive. Among the three alleles (ε2, ε3, and ε4), the *APOE* ε4 was shown to be significantly associated with neurodegenerative diseases. The *APOE* ε4 is the "risk" variant for several phenotypes compared with the other two alleles. *APOE* ε3 was considered to be neutral, and *APOE* ε2 was considered to be a protective factor in neurodegenerative diseases. The most common genotype observed in this study was ε3/ε3, which is in broad agreement with data on the frequency ε3 allele globally (L. A. Farrer et al., 1997). The *APOE*-ε4 allele, which has been previously associated with a higher risk of neurodegenerative diseases, was not present in the studied PD group, and the odds ratios for the six *APOE* genotypes did not suggest a robust association with PD risk. Moreover, the p-values for these associations remained above the standard significance threshold, further supporting the notion that *APOE* might not play a decisive role in PD susceptibility for the Egyptian population. These results are in agreement with the findings of another recent study that examined the association between the *APOE* gene polymorphisms and Parkinson's Disease in African populations, particularly in Nigerians (Okubadejo et al., 2022). Their results showed that there was no significant association between *APOE* genotype and allele frequencies and PD. Also, findings from a large study done by Fedroff et. al., (Federoff et al., 2012) on subjects with European ancestry suggested that there is no genetic association between common variants in *APOE* and Parkinson's disease. On the other hand, a meta-analysis of 47 studies provided support for the risk effect of *APOE* ε4 allele only in the Asian population (J. Li et al., 2018). Furthermore, it also suggested that the genotype ε2ε4 may be a susceptible factor for PD in the Asian population, and the genotype ε3ε4 may be a susceptible factor for PD in both Caucasian and Latin-American populations (J. Li et al., 2018).

Complementing our analyses on the *APOE* gene, the study also examined the genotypic distribution of the *BCHE* gene in both PD patients and healthy individuals. The *BCHE* gene has garnered attention in various studies due to its potential involvement in Parkinson's disease. The rs1803274 SNP defines the K-variant and is associated with reduced serum activity of butyrylcholinesterase when exposed to pesticides. In this study, the majority of the PD patients and references exhibited the CC genotype, establishing it as the predominant genotype in this

population and thus, acts as the reference category. The CT and TT genotypes showed a slight increase in its occurrence in PD patients relative to references; however did not show a strong association with PD risk, and the association was statistically non-significant. The slight deviations in genotype frequencies between PD patients and references, while intriguing, do not provide compelling evidence of a deterministic role of the *BCHE* gene in PD risk. These findings are in line with another study that investigated the gene-environment interaction between *BCHE* K-variant and pesticide exposure in the Egyptian population and suggested that the K-variant *BCHE* was not associated with an increased risk for PD by itself; however, individuals with the K-variant *BCHE* exposed to pesticides appeared to exhibit an increased risk for PD (Rösler et al., 2018). It is well-documented that exposure to certain environmental toxins, including pesticides, is a significant risk factor for PD. When combined with a genetic predisposition, like the one potentially provided by the K-variant *BCHE*, the risk might be further accentuated.

Beyond examining individual genes, understanding the combined effects of multiple genes is critical. Epistasis refers to the interaction between two or more genetic loci to affect a particular phenotype, a phenomenon where the effect of one gene is masked or enhanced by the presence of another gene. There's an epistatic interaction between *APOE* and *BCHE* genes, which has been associated with an increased risk of Alzheimer's Disease. Specifically, the interaction between the ε4 allele of the *APOE* gene (APOEε4) and the K-variant of the *BCHE* gene (*BCHE-K*) has been reported to augment the risk of AD (Chuang et al., 2020; Jasiecki et al., 2019). In the present study, two interactions were found between rs1803274 (C/T) and rs429358 (C/T), and between rs1803274 (C/C) and rs7412 (C/T). Both interactions are suggestive of a potential relationship with PD susceptibility and are categorized as 'H', indicating a possible high-risk association with PD. Furthermore, the epistatic interactions between the *APOE* or *BCHE* genes and the *MAPT*, *SPPL2C* and *KANSL1* genes were examined. Despite the variation in the effect sizes, the results suggest that while the presence of these SNP combinations is associated with a reduced risk of PD, the extent of risk reduction may be influenced by the specific genes. These findings support the hypothesis that *APOE's* role in neurodegenerative disease risk extends beyond a simple additive genetic model and involves complex interactions with other loci, such as those in *BCHE*, *MAPT*, and *KANSL1*.

The study also examined correlations between various variants and clinical outcomes. The results shed light on the potential genetic factors associated with Parkinson's disease and how they might be linked to clinical presentations. The findings suggest that among the variants studied, rs1803274 shows potential associations with the age at onset and severity of PD symptoms, as determined by UPDRS scores. However, the relationships are mild, and further research with larger cohorts might be needed to validate these findings. The other SNPs, rs7412 and rs429358, did not exhibit significant correlations with the clinical variables. This supports the findings from the Nigerian cohort that there is no significant association between *APOE* genotypes and age at onset of PD (Okubadejo et al., 2022). The sample sizes for some correlations, especially the H&Y scores (n=6), are very small, making these results less reliable and harder to interpret in a broader context.

Furthermore, among the variables analyzed, none showed a definitive association with the onset of dementia in PD patients, as indicated by their p-values. However, age demonstrated borderline significance, suggesting it may play a role in dementia risk among PD patients, albeit subtly. The genetic factors analyzed, rs7412, rs429358, and rs1803274, did not provide strong evidence for their association with dementia onset in this cohort. While this data offers valuable insights, further research with larger sample sizes and more comprehensive genetic analyses might be needed to unearth more definitive associations between these variables and dementia onset in PD patients.

Chapter 6

Conclusion and Future Recommendations

The study builds on the existing understanding that PD is highly heterogeneous, not just in its clinical manifestations but also in its genetic underpinnings. The genetic exploration of Parkinson's disease within the Egyptian cohort presents a multifaceted picture of the disease's etiology, defined by both global trends and unique population-specific findings. This study confirms the significant role of well-characterized genes such as *LRRK2* and *GBA* that are globally recognized to play a role in PD. The identification of variants in *SYNJ1, DNAJC6* and *TMPRSS9, ATXN2* while previously debated, contributes significantly to the broader understanding of PD. Noteworthy is the discovery of variants in genes not traditionally associated with PD, such as *PITRM1*, which was found to have partial and age-related penetrance. This is a significant contribution, as *PITRM1* is not traditionally associated with PD, suggesting that there may be novel pathways involved in the disease that have yet to be fully explored. These findings contribute to the understanding that PD's genetic basis is highly heterogeneous and may involve a broader array of genetic variants than previously recognized. Additionally, the investigation into *MAPT* and the *APOE* gene suggests their influence on PD may vary by ethnicity. For the Egyptian cohort, these genes appear to play a lesser role than they might in other populations, suggesting the possibility of ethnic-specific genetic risk profiles. While the associations between *KANSL1* variants and PD risk were slightly weaker, the elevated CADD scores, particularly for rs17585974, highlight a population-specific genetic marker for PD susceptibility that requires further exploration. Furthermore, the significant correlation between the *BCHE* gene variant and Parkinson's disease clinical outcomes implies that there's a relationship between the presence of this particular genetic variant and the severity of Parkinson's disease symptoms. Taken together, the identification of epistatic interactions between the studies *APOE, BCHE, MAPT,* and *KANSL1* genes revealed a series of SNP-SNP interactions that were significantly associated with a lower risk of PD. These findings lay the groundwork for further research to gain insights into the functionality of these genes and the identified variants within the Egyptian context.

Future studies should include larger sample sizes to validate the findings and increase the generalizability of the results. Given the variations in *APOE* and *MAPT* gene influence by ethnicity, further research into genetic risk profiles for PD in diverse ethnic groups would be valuable. This could help in understanding the global heterogeneity of PD and tailoring interventions across populations. Additionally, longitudinal studies are needed to observe the progression of PD in relation to genetic factors, especially for variants with potential modifying effects on disease progression or incomplete penetrance, like those found in *PITRM1*. Due to the familial nature of the genetic variants identified in *PITRM1*, community education about genetic risk factors and the availability of genetic counselling should be part of comprehensive PD management.

There is also a need for replication studies to confirm the novel genetic associations found along with functional analyses to elucidate the biological significance of the identified variants, especially those in genes like *STK39, ATXN2, ASH1L, SYNJ1, DNAJC6*, and *TMPRSS9*, to understand their exact role in PD pathogenesis. The findings highlight the need for public health strategies that incorporate genetic risk factors into PD screening and management programs, particularly in ageing populations like in Egypt. Given the complexity of PD, which includes both genetic predispositions and potential environmental triggers, a comprehensive public health strategy would involve the development of integrated genetic and environmental risk assessment programs. By assessing both genetic risk factors, such as those studied in the *LRRK2* and *GBA, MAPT, KANSL1, SPPL2C, APOE and BCHE* genes, and environmental exposures, such as pesticides, these programs can identify at-risk populations more accurately. This can go in parallel with advocating for policies that mitigate environmental risks, such as stricter regulations on pesticide use, and promoting healthy urban and rural environments.

Additionally, public education campaigns could be designed to inform the public about the risks associated with certain genetic profiles and environmental exposures. This would also involve promoting understanding of PD symptoms for earlier recognition and diagnosis. On a research level, encouraging international collaboration to validate genetic findings across different ethnicities and to explore the global implications of these findings. Also, researchers, policymakers, and public health professionals shall collaborate closely to translate findings from genetic research into public health policies.

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Annexes

Annex 1: IRB Approval Letter

THE AMERICAN UNIVERSITY IN CAIRO
INSTITUTIONAL REVIEW BOARD

Case# 2021-2022-203

To: Asmaa Saeed Gabr
Mohamed Salama
Sherihan Hassan

From: Heba Kotb Chair of the IRB
Date 17th August 2022

Re: IRB approval

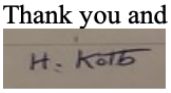
This is to inform you that I reviewed your revised research proposal entitled

"dentifying an Egyptian gene panel for Parkinson's Disease in Egypt"

It required consultation with the IRB under the "expedited" category. As you are aware, there were minor revisions to the original proposal, but your new version addresses these concerns successfully. Your proposal used appropriate procedures to minimize risks to human subjects and that adequate provision was made for confidentiality and data anonymity of participants in any published record. I believe you will also make adequate provision for obtaining informed consent of the participants.
This approval letter was issued under the assumption that you have not started data collection for your research project. Any data collected before receiving this letter could not be used since this is a violation of the IRB policy.

Please note that IRB approval does not automatically ensure approval by CAPMAS, an Egyptian government agency responsible for approving some types of off-campus research. CAPMAS issues are handled at AUC by the office of the University Counsellor. The IRB is not in a position to offer any opinion on CAPMAS issues, and takes no responsibility for obtaining CAPMAS approval.
This approval is valid for only one year. In case you have not finished data collection within a year, you need to apply for an extension.

Thank you and good luck.



Heba Kotb
IRB chair, The American University in Cairo
2078 HUSS Building
T: 02-26151857
Email: hebakotb@aucegypt.edu

Annex 2: Exome Sequencing Results

Family 5	Family 4	Family 4	Family 3	Family 2	Family 1	Family 1	Family 1	Pedigree designation
F09540	F09539	F09538	F09536	F09530	F15694	F15694	F15694	sample
F	M	M	M	M	M	M	M	Sex
GBA	ATXN2	ATXN2	UBOX5	DLG2	TMPRSS9	LRRK2	LRRK2	Gene.refGene
nonsynonymous SNV	nonsynonymous SNV	nonsynonymous SNV	nonsynonymous SNV	nonsynonymous SNV	nonsynonymous SNV	nonsynonymous SNV	nonsynonymous SNV	ExonicFunc.refGene
GBA:NM_001171811:exon9:c.T1187C:p.L396P,GBA	ATXN2:NM_001310123:exon20:c.A2765G:p.H922	ATXN2:NM_001310123:exon20:c.A2765G:p.H922	UBOX5:NM_001267584:exon3:c.A1180G:p.T394A,	DLG2:NM_001142700:exon7:c.C782G:p.T261S,DLG	TMPRSS9:NM_182973:exon7:c.G931T:p.V311L	LRRK2:NM_198578:exon32:c.G4613T:p.R1538L	LRRK2:NM_198578:exon27:c.G3693T:p.Q1231H	AAChange.refGene
D	D	D	T	T	D	T	T	SIFT_pred
B	D	D	B	B	P	P	B	Polyphen2_HVAR_pred
A	D	D	N	D	D	D	D	Mutationaster_pred
24.8	23.5	23.5	1.18	21.3	23	29.5	21.1	CADD_phred
0.0013	NA	NA	0.00006091	NA	0.00000814	0.00000472	0.000004069	gnomAD_exome_ALL
0.0161	0.0323	0.0323	0.0161	0.0161	0.0323	0.0161	0.0161	AF_all_sample
1	2	2	1	1	2	1	1	AC
het	het	het	het	het	het	het	het	zygosity
chr1:155205043,	chr12:111893891,	chr12:111893891,	chr20:3102105,	chr11:83674018,	chr19:2408544,	chr12:40707850,	chr12:40697852,	HGBrowser
clearly heterozygo	clearly heterozygo	clearly heterozygo	clearly heterozygo	clearly heterozygo	clearly heterozygo	clearly heterozygo	clearly heterozygyo	inBAM
			Excluded: CADD <20					Comment

Family 9	**Family 12**	**Family 11**	**Family 11**	**Family 10**	**Family 9**	**Family 9**	**Family 9**	**Family 8**	**Family 7**	**Family 6**
F15684	F15683	F09537	F09537	F15692	F15693	F15685	F15684	F15682	F15681	F15680
M	M	F	F	F	F	F	M	M	M	F
ATXN2	LRRK2	ASH1L	ASH1L	DNAJC6	BST1	BST1	BST1	SYNJ1	TMPRSS9	STK39
nonsynonymous SNV	nonsynonymous SNV	nonsynonymous SNV	nonsynonymous SNV	nonsynonymous SNV	nonsynonymous SNV	nonsynonymous SNV	nonsynonymous SNV	nonsynonymous SNV	nonsynonymous SNV	nonsynonymous SNV
ATXN2:NM_002973:exon1:c.C340T:p.P114S	LRRK2:NM_198578:exon41:c.G6055A:p.G2019S	ASH1L:NM_018489:exon5:c.C5240T:p.S1747F	ASH1L:NM_018489:exon20:c.A7684G:p.S2562G	DNAJC6:NM_001256864:exon16:c.G2332A:p.G778R,DNAJ	BST1:NM_004334:exon4:c.G505T:p.D169Y	BST1:NM_004334:exon4:c.G505T:p.D169Y	BST1:NM_004334:exon4:c.G505T:p.D169Y	SYNJ1:NM_001160302:exon2:c.A29G:p.Y10C,SYNJ1:NM_0	TMPRSS9:NM_182973:exon7:c.G931T:p.V311L	STK39:NM_013233:exon1:c.C56T:p.P19L
T	D	D	D	T	D	D	D	D	D	D
B	D	B	D	P	D	D	D	P	P	B
D	A	N	D	D	D	D	D	D	D	D
13.98	31	22.3	28	23.4	31	31	31	23.7	23	22.5
0	0.0005	NA	NA	NA	0.00000406	0.00000406	0.00000406	NA	0.00000814	NA
0.129	0.0161	0.0161	0.0161	0.0161	0.0484	0.0484	0.0484	0.0161	0.0323	0.0161
8	1	1	1	1	3	3	3	1	2	1
hom	het	het	het	het	het	het	het	het	het	het
chr12:112036979-	chr12:40734202-	chr1:155408706-	chr1:155317566-	chr1:65871657-	chr4:157133483-	chr4:157133483-	chr4:157133483-	chr21:34099178-	chr19:2408544-	chr2:169103890-
clearly homozygot	clearly heterozygo	clearly heterozygo	clearly heterozygo	clearly heterozygo	clearly heterozygo	clearly heterozygo	clearly heterozygo	clearly heterozygo	clearly heterozygo	clearly heterozygo
Excluded: CADD <20					Same variant in both	Same variant in both	Same variant in both			

Family 9	Family 9	Family 13
		F15687
		M
PITRM1	ERBIN	APOE
nonsynonymous SNV	nonsynonymous SNV	nonsynonymous SNV
PITRM1:c.G2675A,p.R892K	ERBIN:c.C3864G,p.F1288L	APOE:NM_000041:exon4:c.C724T:p.R242W,APOE
D	T	D
D	P	D
D	N	N
25	27.3	25.1
NA	0.0000084	0
		0.0161
1	1	1
het	het	het
chr10:31804478-	chr5:65374313-	chr19:45412277-
clearly heterozygo	clearly heterozygo	clearly heterozygo
	Same variant in both	

Annex 3: Discovery Cohort Pedigrees

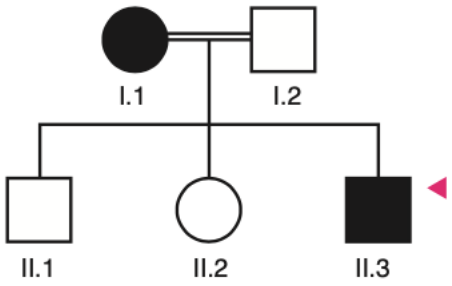


Figure 3. Family 1 Pedigree

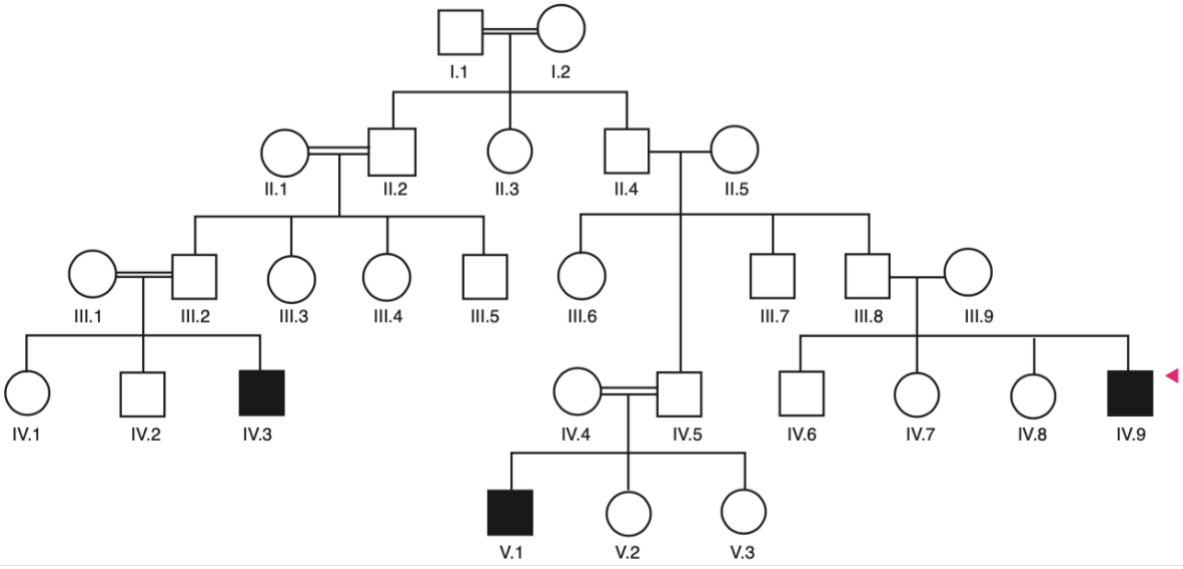


Figure 4. Family 2 Pedigree

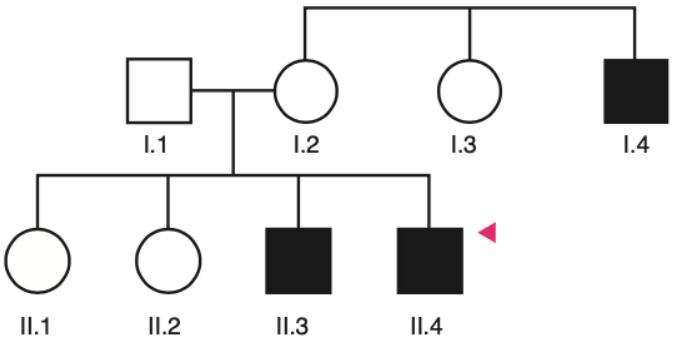


Figure 5. Family 3 Pedigree

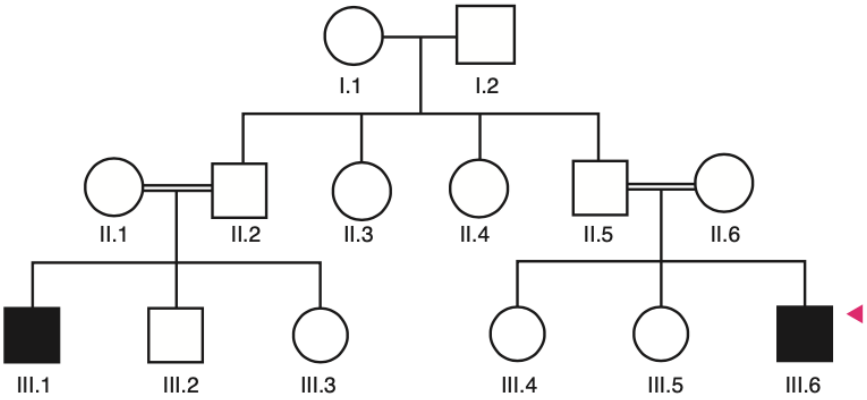


Figure 6. Family 4 Pedigree

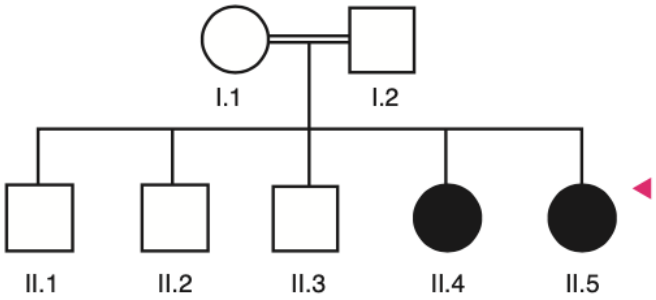


Figure 7. Family 5 Pedigree

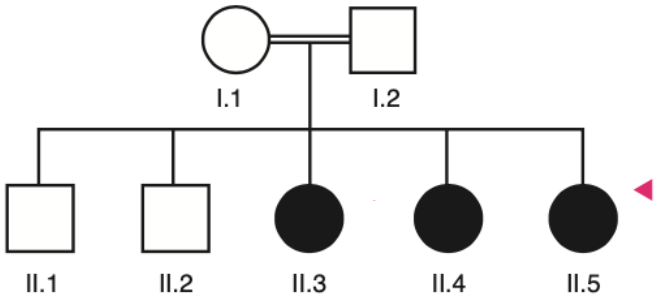


Figure 8. Family 6 Pedigree

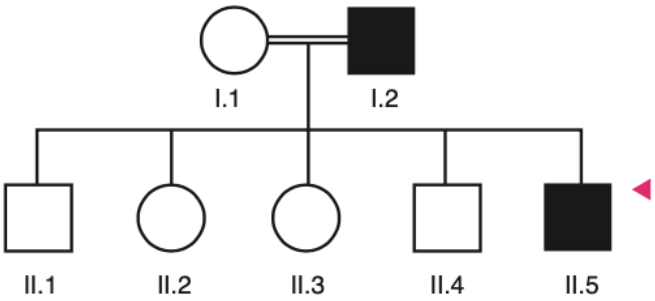


Figure 9. Family 8 Pedigree

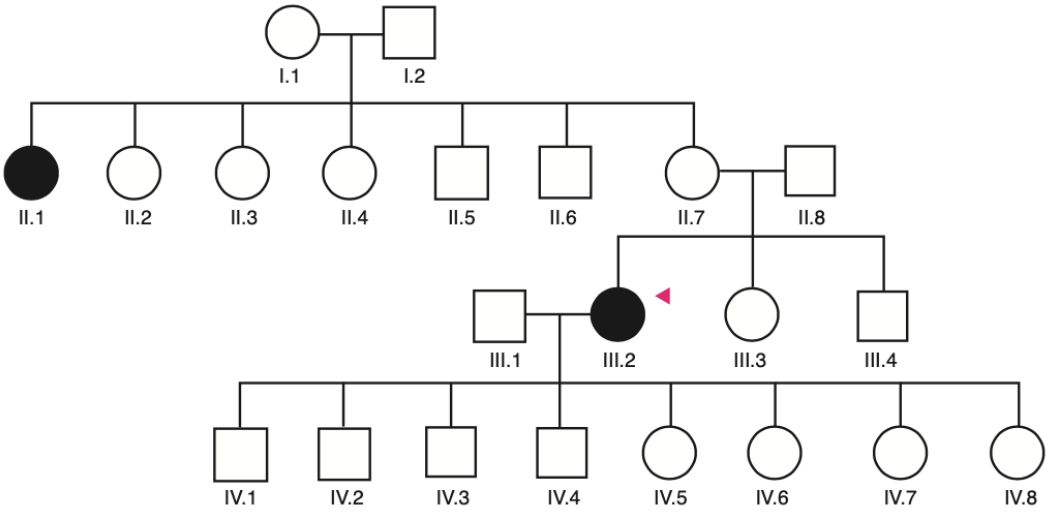


Figure 10. Family 10 Pedigree

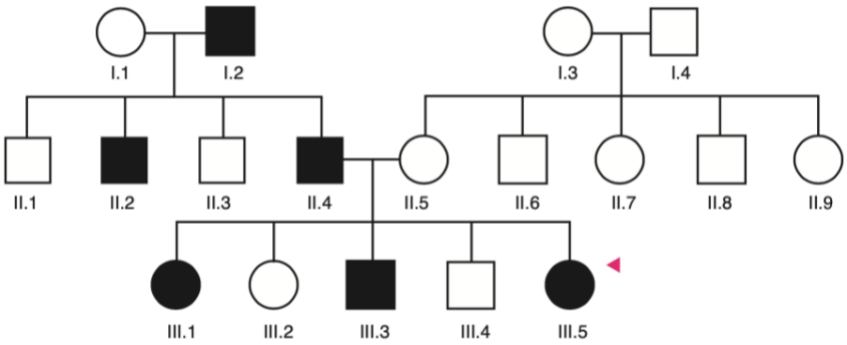


Figure 11. Family 11 Pedigree

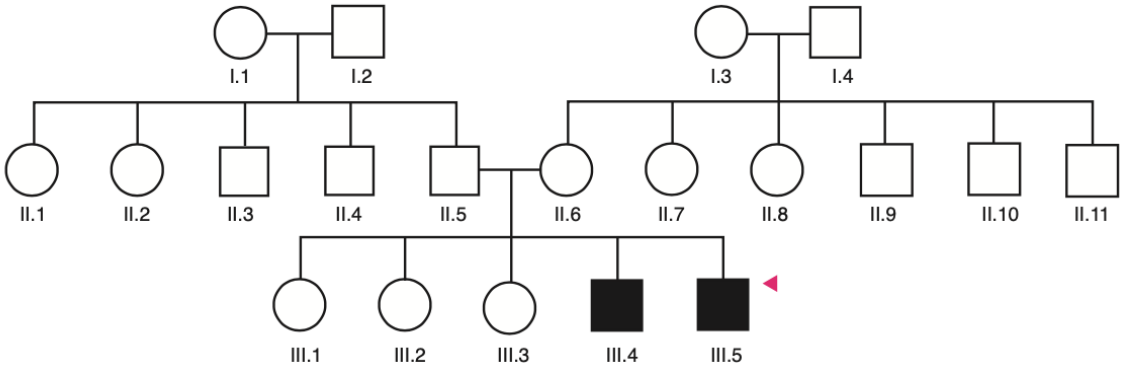


Figure 12. Family 12 Pedigree

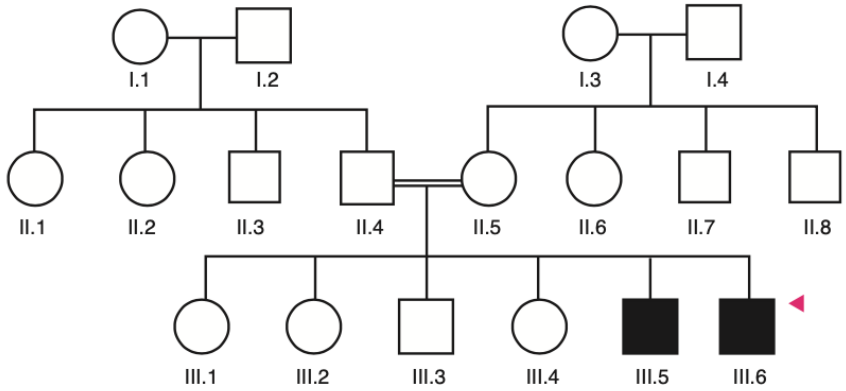


Figure 13. Family 13 Pedigree

Annex 4: UK PD Brain Bank Criteria

Step 1: Diagnosis of Parkinsonian syndrome

- Bradykinesia and
- At least 1 of the following:
- Rigidity
- 4-6 Hz rest tremor
- Postural instability not caused by primary visual, cerebellar, vestibular or proprioceptive dysfunction

Step 2: Exclude other causes of parkinsonism

- History of repeated strokes or head injuries or encephalitis
- Cerebellar signs
- Early severe autonomic dysfunction
- Supranuclear gaze palsy
- Early severe dementia with disturbances of language, praxis and memory
- Oculogyric crises
- Neuroleptic treatment at onset of symptoms
- More than one affected relative
- Sustained remission
- Strictly unilateral features after three years
- Babinski sign
- Cerebral tumor or communicating hydrocephalus on CT scan
- Negative response to large doses of levodopa (if malabsorption excluded)
- MPTP exposure

Step 3: At least 3 of the following supportive criteria

- Unilateral onset
- Rest tremor
- Progressive disorder
- Persistent asymmetry primarily affecting side of onset
- Excellent response (70-100%) to levodopa
- Severe levodopa-induced chorea (dyskinesia)
- Levodopa response for 5 years or more
- Clinical course of 10 years or more