Genetic factors influencing the development and treatment of cognitive impairment and psychosis in Parkinson's Disease

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Abstract

Parkinson's Disease (PD) is a neurodegenerative disease in which both genetic and environmental factors play significant roles. In addition to increasing the risk of developing PD, gene mutations might also influence the phenotypical characteristics of the disease, including the development of cognitive impairment and psychosis. For instance, mutations of the *GBA* gene, which encodes the enzyme γ-glucocerebrosidase, have been related to cognitive impairment or dementia and visual hallucinations. Interest in the *APOE* gene, which encodes the apolipoprotein E, stems from the finding of increased Alzheimer's Disease risk in carriers of *APOE ε4* alleles. In a cohort of 390 PD patients, *APOE ε4* allele carriers showed significantly increased cognitive decline during the 2-year follow-up period. Mutations of the *LRRK2* gene, which encodes the leucine-rich repeat kinase 2, have been related to a lower risk of cognitive impairment and dementia and lower scores for apathy and hallucinations. PD patients with mutations in the *BDNF, COMT, PARP4 and MTCL1 genes* showed increased risk cognitive impairment, dementia and visual hallucinations, but these results have not been replicated yet. Hallucinations have also been related to mutations in the cholecystokinin *CCK* gene. These findings suggest that gene mutations may be important determinants of cognitive impairment and psychosis in PD and highlight promising targets for new therapeutic approaches.

Keywords: Parkinson's Disease, genetics, gene mutations, cognitive impairment, hallucinations, neuropsychiatric symptoms

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting about 1 person out of every 1,000 in their fifth decade and 19 out of every 1,000 in their eighth decade or older [1]. The most characteristic motor symptoms are bradykinesia, rigidity and tremor. Postural abnormalities and gait disorders are also frequent. Patients also suffer from non-motor symptoms, including cognitive impairment, mood disorders, sleep alterations, dysautonomia and hallucinations, among others [2].

Progressive loss of the nigrostriatal dopaminergic pathway is the most characteristic, though not the only, histopathologic change in PD [3]. L-DOPA, which was introduced in the 60s', is still the most effective treatment for motor symptoms [4].

Neuronal death may be preceded by a series of dysfunctional states, including loss of redox control, alteration of lysosomal activity, abnormal protein control mechanisms in the endoplasmic reticulum (ER) and in the ER-Golgi trafficking mechanisms, as well as synaptopathies. It is believed that these pathologies ultimately lead to abnormal accumulation of misfolded protein aggregates [5], which constitute the Lewy bodies, characteristic intracellular protein aggregates found in nerve cells in PD.

In this chapter we will discuss the basis of PD genetics and the relationships between gene mutations and cognitive impairment and psychosis in the disease, beginning in the following paragraphs with a brief review of the main characteristics of these symptoms. For in-depth reviews the reader is referred to refs. [6] and [7].

Cognitive impairment and psychosis are common in Parkinson's disease, even at the earliest stages, and have important consequences for quality of life and daily functioning [7]. Patients with PD have a greater risk of developing dementia compared with age-matched individuals without PD [6]. In a 12-year population study of patients with PD, the cumulative incidence of dementia increased steadily with age and disease duration reaching 80–90% by age 90 years (conditional on survival) [8]. The diagnosis of PD Dementia (PDD) is now made on the basis of a predefined set of criteria proposed by the International Parkinson's Disease and Movement Disorders Society [6]. The primary defining feature of PDD is dementia that develops in the setting of established PD. In this context, a 'dementia' syndrome is defined as (i) impairment in at least two cognitive domains and (ii) cognitive deficiency severe enough to impair daily life (social, occupational or personal care) that must be independent of impairment owing to PD motor symptoms.

Visual hallucinations (VH), illusions, passage and presence phenomena are common psychotic symptoms in PD [7, 9]. Thought disorders, such as delusions, can also be observed. These symptoms may or may not be accompanied by awareness of the pathological nature of the symptoms and their severity sometimes requires psychiatric hospitalization. In PD, psychotic symptoms may be observed in up to 50% of patients [7]. They tend to evolve in a persistent and progressive manner [10]. Psychotic symptoms develop from a complex interplay of extrinsic and intrinsic factors. It was previously considered that VH in PD were caused by dopaminergic medications. However, other factors may contribute to their emergence, including cognitive impairment, older age and more advanced disease stage.

2. Overview of PD genetics

Parkinson's disease is a complex disease involving environmental, genetic and epigenetic factors [11]. The past twenty years of genetics research have shown that DNA sequence variants play a substantial role in the development of the disease. Notwithstanding, only 5 to 10% of all patients suffers from monogenic forms of PD caused by highly penetrant mutations, which are rare and tend to segregate with the disease in families. Conversely, the vast majority of PD cases are not related to these mutations, but acquire the disease through the combined action of DNA sequence variants displaying weak effects. In these patients, the development of PD is also related to environmental, lifestyle, and epigenetic factors.

The first mutations in PD were discovered in the 90s' [12]. The analysis of a large multigenerational Italian family (the Contursi Kindred), in which parkinsonism segregated in an autosomal dominant pattern, led to the discovery of the first PD-related mutation, which affected the *SNCA* gene encoding for α-synuclein (PARK1) [13]. In the following twenty years several gene mutations have been discovered by linkage analysis (PARK1-15 locus) or by genome-wide association studies (PARK16-18) [14]. The classification has been recently revised in the light of newer evidence [15].

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The findings that mutations of the *SNCA* gene could cause PD suggested that the protein that it encoded, α-synuclein, could be of importance for the development of PD [11]. Indeed, α-synuclein was described as the major constituent of Lewy bodies in PD shortly after [16]. It has become clear that α-synuclein misfolding and precipitation is one of the most important pathogenic

factors in PD [17]. According to the “selective vulnerability hypothesis”, α-synuclein aggregates first in a subset of neurons that are particularly susceptible to some adverse influence, and subsequently aggregates also in less susceptible neuronal cells [18, 19]. In contrast, the more recent “pathogenic spread hypothesis” postulates that abnormal α-synuclein proteins or aggregates generated in one neuron are trans-synaptically transferred to a neighboring neuron [18, 19].

As opposed to *SNCA* mutations, which are a rare cause of PD, mutations in *LRRK2* (leucine-rich repeat kinase 2), which are also transmitted in an autosomal-dominant manner, are more frequent [20, 21]. A minimum of six highly penetrant, pathogenic mutations have been described in *LRRK2* (Asn1437His, Arg1441Cys/Gly/His, Tyr1699Cys, Gly2019Ser, Ile2020Thr) [22, 23], among which the most common mutation, Gly2019Ser (rs34637584), has an estimated carrier frequency of 4% in “familial” and 1% in “sporadic” PD patients (i.e. in patients who do not have a family history of PD) [22]. *LRRK2* mutations may exert their effect through a toxic gain of function, possibly due to an increase in autophosphorylation/kinase activity [24].

Causative *SNCA* and *LRRK2* mutations have been mapped and identified based on the “traditional” approach of linkage and subsequent positional cloning; however, new techniques have emerged in recent years enabling more extensive searches of new candidate genes [11]. For example, gene mapping in human diseases allows for the localization of genes underlying the clinical phenotypes of the disease on the basis of correlation with DNA variants (polymorphic markers), without prerequisite hypotheses on biological function [12]. Genome-wide association studies (GWAS) are one such technique. In

GWAS, the identification of genetic risk factors for the development of PD is achieved by analyzing as many as 500,000 different single nucleotide polymorphisms (SNPs) in groups of a few thousands of sporadic PD patients and healthy individuals per study, and comparing *SNPs* frequencies in the two groups. If certain variants are more frequent in PD patients, they are considered to be “associated” with the disease.

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Gene mapping techniques can uncover genetic variants with lower penetrance compared to gene mutations transmitting diseases from generation to generation in a monogenic manner [11]. Therefore, as discussed earlier, disease might manifest when environmental and lifestyle factors are combined with these genetic variants.

GWAS results from the US, Germany, Greece, UK and France have been summarized in a recent meta-analysis, which included genome-wide SNP data from 13,708 cases with Parkinson’s disease and 95,282 controls [25]. In an attempt to further identify which of the putatively associated loci were truly disease-related, each locus was replicated in an independent sample series using a semi-custom genotyping array called NeuroX. This array typed the >240,000 exonic variants available on the Illumina Infinium Human Exome Bead Chip and an additional ~24,000 variants proven or hypothesized to be relevant in neurodegenerative disease. The array included the 26 genome-wide significant candidate loci implicated in PD from the primary meta-analysis. Association analysis showed replication of 22 of the 26 loci tested, based on a nominal one-sided P-value threshold of <0.05 and consistent direction of association that incorporated the premise of prior knowledge for most loci based on previous meta-analysis of GWAS data. Six additional loci, previously reported

to be associated with risk for Parkinson's disease that did not show association at $p < 5x10^{-8}$ in the discovery phase, were also explored. Two additional loci were found to be associated with PD. Presence of multiple independent risk alleles at any of the 26 genome-wide significant loci identified in the discovery phase was also investigated, four of these variants showing significant association. In total, Nalls et al. identified 28 independent risk variants for PD were identified: 22 found in the discovery phase and confirmed by replication, 2 previously reported variants confirmed in the replication phase and 4 variants identified by a second risk allele acting independently of the primary risk allele. One major finding of this study was that GBA gene (which encodes for the glucocerebrosidase enzyme) mutations conferred the greatest PD risk among the 28 SNPs identified. The association between *GBA* mutations and parkinsonism was first recognized in the clinic, where it was observed that patients with Gaucher's disease developed parkinsonian symptoms more frequently than expected [26], suggesting a link between mutations in *GBA* and PD. The frequency of *GBA* mutations was then studied in 5691 patients with PD (780 Ashkenazi Jews) and 4898 controls (387 Ashkenazi Jews) [27]. Among Ashkenazi Jewish subjects, L444P and N370S mutations were found in 15% of patients and 3% of controls ($p<0.001$), and among non–Ashkenazi Jewish subjects, in 3% of patients and less than 1% of controls ($p<0.05$). Notwithstanding, when *GBA* was fully sequenced for 1883 non–Ashkenazi Jewish patients, mutations were identified in 7% of the cases, suggesting that sequencing the whole gene can help avoid false-negative results.

Genetic risk profiles (GRS) represent the contribution of the genetic load to subjects' risk of developing a given disease. GRS for PD were generated using

the 28 SNPs previously discussed, by adding the multiplication of SNPs' risk induction (as represented by beta coefficients) by the number of mutated alleles [25]. These authors reported that the predictive power for GRS scores was marginal, with areas under the receiver operator curves of 0.616 without age and sex included as covariates and 0.633 with age and sex included.

Individuals with a genetic risk profile score greater than 1 S.D. from the population mean (indicative of roughly 34% increase in genetic risk score above the mean for controls), had a significantly higher risk of Parkinson's disease (from meta-analysis; OR = 1.51, 95% CI = 1.38–1.66; $p = 2\times10^{-16}$). Patients in the fifth quintile of genetic risk scores had a much higher genetic risk compared to those in the first quintile (OR = 3.31 (95% CI = 2.55–4.30; $p = 2\times10^{-16)}$).

In a further study, data on 6,249 unrelated European ancestry PD cases with onset at the age of at least 18 years old, and stemming from the US, Holland, France, Germany and Greece, were retrieved [28]. Samples including the 28 PD risk variants and a number of proxies from recent GWAS were genotyped on the NeuroX array at the Laboratory of Neurogenetics at the US National Institute on Aging and genetic risk scores (GRS) were generated. Briefly, for each variant of interest, the allele dosage was multiplied by the previously published beta coefficient then summed per sample to create the GRS. Increasing GRS were significantly associated with an overall trend for earlier age at disease onset in pooled analyses (beta=–0.10, p value =$2.92\text{x}10^{-28}$, adjusted r^2=0.27).

GRS were related to PD characteristics in a single-center study of 336 patients [29] and significantly associated with time from diagnosis to Hoehn and Yahr

stage 3, which is a marker of disability in PD, in a Cox regression model ($p<0.010$).

3. Genetic abnormalities in PD patients with neuropsychiatric disturbances

In this section, the relationship between neuropsychiatric symptoms and genetic mutations in PD will be discussed. A double-entry table is provided to summarize the evidence (Table 1).

3.1 APOE and other β-Amyloid-related genes

According to the amyloid hypothesis, formation of β-Amyloid plaques depends on an imbalance between β-Amyloid production and clearance [30]. In sporadic forms of Alzheimer Disease, impaired clearance appears to be the most important factor for plaque formation [31]. β-Amyloid plaques can also be found in PD, and are associated with increased risk of dementia [32].

The influence of genetic variations in the enzymes that metabolize β-Amyloid in PD -mainly *APOE*, membrane metalloendopeptidase (*MME*), and cystatin C (*CST3*) [33]- has been assessed in a recent trial [34]. This study included 353 PD patients without dementia and 103 PDD. The following SNPs were studied: *APOE*: rs429358 (to determine the *APOE ε4* carrier status), and *MME*: rs6776185, *CST3*:rs1064039. Genotyping was performed using either matrix-assisted laser desorption/ionization time of flight mass spectrometry method (MassArray system; Sequenom, San Diego, CA) or SNaPshot single-base extension (Applied Biosystems, Foster City, CA; software: GeneMapper) with capillary electrophoresis. Mutations in the *LRRK2* (G2019S) and in the *GBA*

(L444P, N370S, E326K) were ruled out. Results showed that risk variants in the genes *APOE* and *CST3* were associated with lower cerebrospinal fluid β-Amyloid$_{1-42}$ levels, which are a marker of β-Amyloid plaques. Interestingly, patients with 2 risk alleles in *CST* tended to show a shorter interval from age at onset of PD to age at onset of dementia.

Several pieces of evidence suggest a prominent role of *APOE* mutations in Alzheimer and Parkinson's dementia. The primary function of the apolipoprotein E (APOE) is to maintain the structure of lipoprotein particles and to direct lipoproteins to specific cell surface receptors. APOE influences the rate of β-Amyloid fibrillization and clearance [35]. The transport of β-Amyloid$_{1-42}$ was found to be isoform-dependent, being slowest with *APOE ε4* [36]. Consequently, the ε4 isoform confers a higher risk for AD by increasing the parenchymal β-Amyloid load.

As shown in one study, the frequency of *APOE ε4* was significantly higher in Alzheimer Disease (38.1%), Alzheimer-Lewy Body dementia mix (40.6%), pure Lewy Body dementia (31.9%), and PDD (19.1%) groups compared with the control group (7.2%; overall chi-sq2,4=185.25; P=5.56x10^{-39}) [37].

In another study, cognitive status data from 390 PD patients from the Parkinson's Progression Markers Initiative (PPMI) cohort study at baseline and after a 2-year follow-up period were analyzed [38]. In this study, patients with two mutated ε4 alleles showed a 3.7 higher risk of cognitive deterioration after two years, as assessed by the Montreal Cognitive Assessment (MoCA) tool.

The profile of cognitive impairment has been studied in 1079 PD patients [39]. Patients underwent assessments of memory (Hopkins Verbal Learning Test–Revised [HVLT-R]), attention and executive function (Letter-Number

Sequencing Test and Trail Making Test), language processing (semantic and phonemic verbal fluency tests), visuospatial skills (Benton Judgment of Line Orientation test), and global cognitive function (Montreal Cognitive Assessment). The *APOE ε4* allele was associated with lower performance on the HVLT-R Total Recall ($P = 6.7 \times 10^{-6}$), Delayed Recall (P = 0.001), and Recognition Discrimination Index (P = 0.004); a semantic verbal fluency test (P = .002); the Letter-Number Sequencing Test ($P = 1 \times 10^{-5}$); and Trail Making Test B minus Trail Making Test A (P = 0.002). In non-demented carriers, lower scores on the HVLT-R Total Recall and on the semantic verbal fluency were found. In this study, patients also underwent genotyping for the *MAPT* H1/H2 haplotypes and *SNCA* rs356219. These variants were not associated with scores on any tests.

The association between mutations in *APOE, MAPT and SNCA* with psychosis was studied in 500 PD patients [40]. Presence of psychotic symptoms was assessed by means of the *Unified PD Rating Scale* Item #2. *APOE* and *MAPT* mutations did not show any relationship with psychotic symptoms. One mutation in the *SNCA* gene was associated with increased risk (OR=2.35, 95% CI=0.91–6.05), but was non-significant (p<0.08).

In another case-control study involving 44 PD patients with visual hallucinations and 44 parkinsonian controls that had never hallucinated, *APOE* mutations showed no relationship to hallucinations [41].

3.2 LRRK2

The G2019S mutation (a glycine to serine substitution at amino acid 2019) in the *LRRK2* is one of the most common genetic contributors to PD [42, 43]. The

hypothesis that *LRKK2* mutation carriers could be phenotypically different from non-carries was tested in a study with 50 carriers and 50 age-, disease duration-, and disease severity-matched PD non-carriers [44]. Carriers showed the worst gait function and more frequent falls. Conversely, there were no major differences in cognitive function tests, including MoCA, trail making test, verbal fluency, digit span, and Stroop test.

Further studies were carried out on a cohort of 1,447 PD patients enrolled in the PD Cognitive Genetics Consortium [45]. *LRRK2* mutation carriers (n=29) demonstrated better performance on the Mini Mental State Examination ($p<0.03$) and the Letter-Number Sequencing Test ($p<0.005$). A smaller proportion of *LRRK2* mutation carriers were demented ($p<0.03$). There were no relevant differences in age, sex, education and disease duration between carriers and non-carriers. The authors suggested that these findings may be related to the fact that *LRRK2* mutation carriers had less Lewy body pathology [46]. Nonetheless, this was a cross-sectional study that included a small sample of carriers. A study with a Spanish sample of 27 PD patients with *LRRK2* mutations (12 G2019S and 15 R1441G) and 27 non-carrier PD patients confirmed these results [47]. Interestingly, carriers also showed less hallucinations, as shown by the neuropsychiatric inventory (NPI).

3.3 GBA

The phenotypical characteristics of *GBA* mutation carriers has been widely studied. In one of the first studies, mutations were studied in 790 PD patients and 257 controls [48]. Cycle sequencing was performed for each exon and the flanking intronic sequences using the Dye Terminator Sequencing Kit (Applied

Biosystems) and run on an ABI 3700xl genetic analyzer (Applied Biosystems). Results showed a significantly higher frequency of mutations in PD compared to controls (4.18% vs 1.17%, $p < 0.01$; odds ratio = 3.7; 95% confidence interval = 1.12–12.14). Interestingly, diffuse neocortical Lewy body-type pathology tended to occur more frequently in the group with *GBA* mutations compared to matched Parkinson's disease controls. Fifteen out of the 31 (48.39%) PD patients with *GBA* mutations developed symptoms of cognitive decline during the course of the disease. Furthermore, visual hallucinations were present in 45.16% (14/31) of patients. Cognitive impairment was also found in about 50% of a small sample of US *GBA* mutations carriers [49]. In both studies, patients showed good responsivity to L-DOPA during the course of the disease.

In a later study, the *GBA* coding region was fully sequenced in 225 Parkinson's disease patients, 17 pathologically confirmed Lewy body dementia patients (LBD), and 186 controls from Spain [50]. Mutations were more frequent in PD and LBD as compared to controls (9.8% and 11.8% vs 0.5%, $p<0.01$ and $p<0.02$). Dementia was found in 50% of carriers vs 23.6 of non-carriers ($p<0.01$). Carriers also showed less frequent Rigid-akinetic phenotype and more frequent tremor phenotype. Carriers also showed a good response to L-DOPA.

In another study, DNA from 1,000 patients initially diagnosed with idiopathic PD was subjected to mutational screening for 2 of the most common mutations of the GBA gene (N370S, L444P) by genotyping with restriction enzymes [51]. A total of 33 patients with PD heterozygous for one of the 2 *GBA* mutations from all over Germany were identified, of whom 20 consented to be further evaluated. Patients with *GBA* mutations had lower MoCA values, higher

neuropsychiatric inventory scores for depression, anxiety, and apathy, higher Beck Depression Index II scores. The authors hypothesized that these disturbances could be related to the diffuse neocortical Lewy body-type pathology observed in *GBA* mutation carriers [48].

In a more recent study, time to fully-developed dementia and psychosis was compared between Japanese *GBA* mutation carriers (n=19) and non-carriers (n=167), by means of a retrospective cohort study [52]. Carriers showed a significantly earlier development of dementia (6-y vs >10-y, $p<0.001$) and psychosis (8-y vs 12-y, $p<0.017$), compared with subjects without mutation. After adjusting for sex and age at PD onset, Hazard Ratios were 8.3 for dementia (95% CI, 3.3-20.9; $p < 0.001$) and 3.1 for psychosis (95% CI, 1.5-6.4; $p < 0.002$).

The cognitive profile of *GBA* mutations carriers was further studied in 60 carriers vs. 1055 non-carriers [53]. Participants underwent assessments of learning and memory (Hopkins Verbal Learning Test–Revised), working memory/executive function (Letter-Number Sequencing Test and Trail Making Test A and B), language processing (semantic and phonemic verbal fluency), visuospatial abilities (Benton Judgment of Line Orientation), and global cognitive function (MoCA). Carriers had a higher prevalence of dementia (OR=55.1; $p<0.0001$). Carriers also showed lower performance on Letter-Number Sequencing, Trail Making B-A, and Benton Judgment of Line Orientation. These results suggest that *GBA* mutations are associated with a distinct cognitive profile characterized by greater impairment in working memory/executive function and visuospatial abilities in PD patients that differs from the profiles associated with other mutations. For example, lower

performance on semantic verbal fluency and word-list learning have been found in non-demented *APOE ε4* carriers, as discussed earlier [39].

Follow-up data were available for a subset of these patients [54]. The mean (SD) duration of follow-up was 3.0 (1.7) years. During the study, a higher proportion of *GBA* E326K carriers (10 of 21 [47.6%]; $p < 0.01$), but not other mutation carriers (5 of 18 [27.8%]; $p< 0.69$), progressed to mild cognitive impairment and dementia compared with non-carriers. The association with conversion to MCI and dementia was also significant for the combined GBA variant group (15 of 39 [38.5%]; $p < 0.04$).

Effects of *GBA* mutations were further studied in a cohort of 532 well-characterized PD patients and 542 controls from southern Spain [55]. The potential pathogenicity of the identified variants was assessed using in-silico analysis and subsequently classified as benign or deleterious. This analysis allows for the assessment of the pathogenic effect of the amino acid substitutions on the three-dimensional protein structure and its impact on protein function. Deleterious mutations included N370S, L444P, W312R, V457D, T369M, E326K, c.116-8C>T, and others. Interestingly, the progression of the disease to cognitive impairment was influenced by the presence of deleterious GBA variants (HR = 2.6, 95% CI 1.25-3.88; $p < 0.001$). Visual hallucinations were also influenced by the presence of deleterious GBA variants (HR = 3.15, 95% CI 1.71±5.79; $p<0.001$). Patients with benign mutations also showed visual hallucinations, though the authors apparently did not compare groups of mutations.

In a recent study 2,764 unrelated consecutive PD patients, of whom 123 were *GBA* mutation carriers (67 mild-p.N370S and 56 severe mainly p.L444P), were

followed for two years [56]. Carriers had greater risk for dementia compared to mild mutations (HR= 3.16, 95% CI= 2.3–4.4, p<0.001). Interestingly, carriers showed reduced posterior parietal and occipital cortical synaptic activity and nigrostriatal function than PD non-carriers. The authors highlighted the fact that these results are in line with neuropsychological profiles of *GBA* carriers, which, as discussed earlier, are constituted by disturbances of visuospatial and nonverbal (mainly visual) memory tasks with relatively preserved executive functions and attention [53]. The former are associated with posterior cortical areas, whereas the latter depend on the frontal lobe.

Finally, in a very recent study, 1105 PD patients were genotyped for 249,336 variants using the NeuroX array [57]. Patients also underwent the full set of cognitive assessment tools: learning and memory (Hopkins Verbal Learning Test–Revised [HVLT-R]), working memory/executive function (Letter-Number Sequencing and Trail Making Test [TMT] A and B), language processing (semantic and phonemic verbal fluency), visuospatial abilities (Benton Judgment of Line Orientation [JoLO]), and global cognitive function (MoCA). Eighteen common variants in 13 genomic regions exceeded the significance threshold for one of the cognitive tests. These included *GBA* rs2230288 (E326K; P_{FDR} = 2.7 x 10^{-4}) for JoLO, *PARP4* rs9318600 (P_{FDR} = 0.006) and rs9581094 (P_{FDR} = 0.006) for HVLT-R total recall, and *MTCL1* rs34877994 (PFDR = 0.01) for TMT B-A. These results have not been replicated.

3.4 BDNF and *COMT*

Data from the PPMI study allowed the assessment of the relationship between some SNPs and cognitive impairment. In a sub-analysis of these data 423

newly diagnosed patients with idiopathic PD were followed for 3 years [58]. Genotyping was performed with NeuroX. For this sub-study, SNPs previously associated with cognitive impairment or decline in PD were examined (i.e., *APOE ε4*, *GBA* [N3705], *LRKK2* [G20195], *SNCA* [rs3910105 and rs356181], microtubule-associated protein tau [*MAPT*; rs17649553, which is in linkage disequilibrium with the H1 haplotype], brain-derived neurotrophic factor val66met [*BDNF* val66met], and catechol-O-methyltransferase val158met [*COMT* val158met]). *BDNF* and *COMT* mutations predicted incident cognitive impairment. *BDNF* val66met C/C was associated with higher risk compared to C/T (HR=2.327, 95% CI 1.008-5.373, p<0.05). COMT val158met A/G also increased risk compared to G/G (0.335, 0.138-0.813). These results have not been replicated.

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3.5 CCK

Cholecystokinin, a neuropeptide found in the gut and central nervous system, has been implicated in dopaminergic regulation. The *CCK* gene is active in dopaminergic neurons in the central nervous system. The relationship between polymorphisms in this gene and hallucinations has been explored in a number of trials. One of the first studies involved 116 PD patients [59]. Four polymorphic sites of the CCK gene (-196G:A, -45C:T, 1270C:G, 6662C:T) were found in PD patients. The CT allele was significantly more frequent in patients who displayed hallucinations (65.2% vs 5.2% CC or 8.7% TT, p<0.02). In another study conducted in 45 PD patients with and 45 PD without hallucinations, the CCK CT/TT genotype was associated with a 4.429-fold increased risk for visual hallucinations in ParkPD [60]. Finally, a case-control study involving 28 PD

patients with hallucinations and 35 without, showed the T allele to be more frequent in the former (19% vs 9%) but with the difference being of only borderline significance ($p<0.06$) [61].

4. Conclusions and future perspectives

The genetics of PD are only beginning to be unraveled. The cost of genetic testing has dropped to a few thousand dollars and it is expected that it will continue to fall. Yet, medical understanding of the consequences of having one or more deleterious mutations is still limited, thus imposing barriers on the utilization of genetic results in clinical decision-making.

Genetic information may prove very useful for the management of cognitive disturbances and psychosis in PD. Available evidence suggests that several genetic mutations modify the risk of developing these neuropsychiatric symptoms. For example, patients carrying the *APOE ε4* allele or *GBA* mutations have a well-documented increased risk of cognitive deterioration and dementia [39, 53]. Interestingly, *APOE ε4* allele have been shown to correlate with reduced cerebrospinal fluid $A\beta_{1-42}$ levels, which are a marker of parenchymal amyloid plaque deposition [62]. Mutations in the *CST3* gene have also been correlated with cerebrospinal fluid $A\beta_{1-42}$ levels and with PDD [34]. Conversely, the positive association between cognitive impairment and mutations in the *BDNF*, *COMT*, *PARP4*, and *MTCL1* genes [57, 58], has not been replicated and there is no data on subsequent progression to PDD. Finally, *LRKK2* mutation have been shown to reduce the risk of cognitive impairment [45], but there is no data on dementia. It should be mentioned that definitions of dementia vary from study to study and that the currently most widely accepted definition of PDD,

proposed by the *International Parkinson's Disease and Movement Disorder Society* [63], has been used in a minority of reports.

The effects of *GBA* and *LRRK2* mutations on visual hallucinations followed the same trend as those on cognitive performance, i.e. the former increased risk [52] whereas latter reduced it [47]. It is therefore not clear whether these are real effects on hallucinations or by-products of mutation effects on patients' cognitive status [40]. The case of *CCK* may be different, as cholecystokinin is known to regulate dopamine, which is the main substrate of hallucinations [64].

Genetic information may serve for establishing a prognosis in clinical practice. As mentioned earlier, several pieces of evidence link genetic mutations to the development of cognitive impairment and possibly also psychosis. In the event that a Genetic Risk Score is validated to predict the development of dementia, then high-risk patients can be selected for closer follow-up and early behavioral stimulation or administration of neuroprotective therapies, when they become available. This "personalized" approach can thus help medicine focus on high-risk individuals [65] and achieve higher efficacy with administered treatments. Furthermore, this focalization will help avoiding unnecessary therapeutic interventions in patients who will ultimately not develop cognitive impairments, thus reducing costs and safeguarding patients from exposure to adverse events.

Knowledge of genetic factors may also help direct the course of research. The protective effects of *LRRK2* mutations as well as the deleterious effects of *GBA* mutations have been hypothesized to be related to decreased and increased neocortical Lewy body pathology, respectively [46, 48]. However, the finding that *SNCA* mutations do not affect cognitive status could indicate that these

mutations may not be related to the formation of Lewy bodies. One hypothesis is that such mutations affect the distribution of Lewy bodies rather than their formation. Further research is needed to further clarify this important issue.

As discussed earlier, the domains of cognitive impairment impacted by *APOE, GBA, PARP4*, and *MTCL1* differed [39, 57]. This suggests, in the first place, that PDD may not be a single entity. It seems logical to suggest that a first division be made between mutations affecting β-amyloid metabolization, and thus eventual plaque formation, from those that may affect Lewy bodies' disposition, such as *LRRK2* or *GBA*. Indeed, degeneration appears to follow two different patterns, i.e. frontal-lobe dysfunction if the predominant pathology is related to β-amyloid or a posterior parietal alteration in the case of α-synuclein pathology [56, 66]. This is further supported by the finding that parkinsonian *GBA* mutation carriers with impaired cognition show levels of CSF β-amyloid similar to those of healthy controls, whereas non-carriers with cognitive impairment show reduced CSF β-amyloid levels [67]. Genetic information may therefore be essential for differentiating one type of dementia from another. This may have important implications for the therapeutic strategies to follow. For example, β-amyloid immunization [68] may work in one case but not in the other. Patients with *GBA* mutations, on the other hand, could be treated by drugs targeting the production of glycosphingolipids, such as the molecule named GZ/SAR402671, which is undergoing Phase II clinical trials.

The manner in which the different mutations cause dementia remains to be explored. For example, cholinergic dysfunction in the basal forebrain (i.e. the Nucleus Basalis Magnocellularis) is the hallmark of cognitive impairment in Alzheimer's Disease [69]. Cholinergic disturbances also underlie PDD [70], and

it is tempting to hypothesize that this will further correlate with β-Amyloid pathology and *APOE* mutations. But cholinergic disturbances may not underlie dementias related to other mutations in PD. For example, *LRRK2* mutations are protective from the cognitive standpoint but are also related to gait disturbances. This suggest that *LRRK2* mutations may not affect cholinergic pathways, which are known to regulate gait [70]. There is no data available for the other mutations. A better understanding of the neuropathological processes underlying cognitive disturbances in PD calls for further study of the precise manner in which these mutations induce the malfunction or degeneration of particular nuclei. Such studies will also broaden the scope of potential targets for the development of new therapeutic agents.

5. References

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Table 1. Association between genetic mutations and cognitive disturbances or psychosis in PD.

Genes	CSF $A\beta_{1-42}$ levels	Cognitive deterioration	Risk of dementia	Psychotic symptoms
APOE ε4	↓	↑	↑	0
MME	0	-	-	-
CST3	↓	-	-	-
MAPT	-	0	-	0
SNCA	-	0	-	↓*
LRRK2	-	↓ or 0	-	↓
GBA	-	↑ or 0	↑	↑
PARP4	-	↑	-	-
MTCL1	-	↑	-	-
BDNF	-	↑	-	-
COMT	-	↑	-	-
CCK	-	-	-	↑

APOE ε4: Apolipoprotein E; MME: membrane metalloendopeptidase; CST3: cystatin C ; MAPT: microtubule associated protein tau ; SNCA: α-synuclein ; LRRK2: leucine-rich repeat kinase 2; GBA: γ-glucocerebrosidase; PARP4: Poly [ADP-ribose] polymerase 4; MTCL1: Microtubule Crosslinking Factor 1; BDNF: Brain-derivated Neurotrophic Factor; COMT. Catechol-O-Methyl Transferase; CCK= cholecystokinin; CSF: cerebrospinal flud; $A\beta_{1-42}$: β-amiloid 1-42 fragment. Dashes indicate absence of data about the association between a given feature and a particular gene. * The effect was non-significant ($p<0.08$).