Some genetic factors of Parkinson’s disease in the Hungarian population

Summary of Ph.D. thesis

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**IF: 2.180**
Publication not related to the subject of the dissertation

**IF:** 2.746

**IF:** 1.207

**IF:** 3.002

**IF:** 1.568

**IF:** 2.40

**IF:** 2.098

**IF:** 2.820

**IF:** 2.201

**IF:** 3.961
INTRODUCTION

1. Parkinson’s disease
Parkinson’s disease (PD) is the second most common neurodegenerative disorder after Alzheimer’s disease. The most prominent neuropathological features of PD are the loss of dopaminergic neurons in the substantia nigra (SN) pars compacta and the presence of Lewy bodies, which are accumulated and aggregated alpha-synuclein inclusions in the cytoplasm of the surviving neurons. Although the precise pathomechanism of PD is still not fully understood, several molecular mechanisms of neuronal death in PD pathogenesis have been described including mitochondrial dysfunction, oxidative stress, microglia activation and inflammation. With regard to aetiological background, the most common concepts is that PD may result from complex interaction between environmental factors, genetic background, and aging [1]. The majority of PD cases are sporadic; only 15-20% of the cases are identified as familial. Nevertheless, in most populations up to 5%-10% of patients carry mutations in monogenic forms. Apart from monogenic forms of PD, genetic risk factors would stand out as the cause of disease as well, affecting the age at onset and progression, possibly in combination with environmental factors.

2. Monogenic forms of PD
So far, 21 loci have been identified in familial PD. The PARK designations refer to monogenic forms of autosomal dominant PD, and autosomal recessive PD [2]. Several studies suppose that these genetic factors may play a role in the development of sporadic PD as well.

2.1 PARK17: vacuolar protein sorting associated protein 35 (VPS35)
The VPS35 gene is involved in the development of many neurodegenerative diseases, including Alzheimer’s disease and PD [3, 4] The gene is localized to 16q11.2, and various mutations have been reported in it [5]. The gene encodes the vacuolar sorting protein homolog, which is a key component of the retromer complex and is involved in the retrograde transport of proteins from endosomes to the trans-Golgi network [6]. Amongst the mutations of the VPS35 gene, the p.D620N missense mutation has been reported to be pathogenic for PD, mainly in the autosomal dominantly inherited cases, but it has additionally been detected in some sporadic PD cases [4].
3. Genetic risk factor of PD

Besides monogenic forms of PD, genetic risk factors have been identified in PD. Among the identified risk factors for PD, consistent associations have been demonstrated for SNCA, LRRK2 and MAPT [7]. In addition, heterozygous mutations in the GBA gene have been validated as a genetic susceptibility factor for PD [8].

3.1 GBA: glucocerebrosidase

The lysosomal enzyme glucocerebrosidase (GCase) is encoded by the GBA gene on chromosome 1q21. So far, more than 300 mutations of this gene have been identified [9]. Homozygous mutations of GBA gene cause an autosomal recessively inherited glycolipid storage disorder, Gaucher’s disease (GD). Heterozygous mutations carriers are recognised to be at risk of PD development. Numerous genotyping studies have demonstrated associations between several GBA mutations and PD in different ethnic groups. A recent meta-analyses have revealed that GBA variants are the most common genetic risk factors associated with PD, increasing the risk of PD ~5 fold, and the three most frequent mutations in non-Ashkenazi Jewish PD patients are p.N370S, p.L444P and p.R120W [10].

4. Other PD-associated genes

Besides the causative genes and risk factors, several polymorphisms in various genes have been described which associated with PD, like BACE1 and PITX3 [11]. Furthermore, several research has focused on VDR gene and identified some polymorphic variants which may be associated with PD [12].

4.1 VDR: vitamin D receptor

Vitamin D, as an environmental factor, has been the subject of various studies on different neurological disorders, from which it has emerged that a vitamin D deficiency is associated with an increased risk of many diseases, Alzheimer’s disease and PD [13, 14]. The VDR gene encodes a nuclear transcription factor. The human gene is localized to 12q12 and various polymorphisms have been reported in it [15]. Among the polymorphisms, BsmI, ApaI, TaqI and FokI are the most studied polymorphisms. However, only limited data are available regarding the association between VDR polymorphisms and PD, and the results have been contradictory. The difference between the results of the various studies might stem from the different study populations.
AIMS OF THE WORK

The PD associated genes show a geographic variability and ethnic variations. Several mutations were investigated in Caucasian population, mainly in Western and Northern Europe, but the results are controversial. Moreover, there are only limited data from the Middle and Eastern Europe, and no study has been conducted previously to assess the frequency of VPS35, GBA mutations or VDR gene polymorphisms in Hungarian PD patients.

The general aim of our study was to determine the frequency of some PD associated mutations in the Hungarian population.

The specific aims of the study were:

- To investigate whether the p.D620N mutation of the VPS35 gene is present in SPD in the Hungarian population.
- To examine the genotype distribution of VDR ApaI, FokI, TaqI or BsmI polymorphisms in Hungarian PD patients and controls. Moreover, we want to analyse the possible associations between the age at onset, the male-female ratio and the VDR polymorphisms in the PD group.
MATERIAL AND METHODS

1. Subjects
124 SPD patients (mean age: 66.5±9.5) and 122 healthy control (mean age: 64.3±8.2) subjects were enrolled in the VPS35 and GBA mutations analysis. The study group were age-matched. 100 SPD patients (mean age: 66.4±9.3) and 109 age-matched healthy controls (mean age: 64.0±8.2) were enrolled the analysis of VDR polymorphisms. All of the patients were examined by movement disorder specialists, who confirmed the diagnosis of SPD. Early-onset PD (EOPD) was defined as an age at onset ≤ 60 years and late-onset PD (LOPD) as an age at onset > 60 years. The control group individuals had no history of neurological or psychiatric disorders. The study protocol was approved by the Medical Research Council Scientific and Research Ethics Committee (47066-3/2013/EKU (556/2013)) and Ethics Committee of the Faculty of Medicine (22/2012), University of Szeged. All study participants gave their written informed consent in accordance with the Helsinki Declaration.

2. DNA isolation
Genomic DNA was extracted from peripheral blood by a standard desalting method, and stored at –20 °C until further use.

3. Genotyping
All polymorphisms were determined by polymerase chain reaction techniques and restriction fragment-length polymorphism.

4. Statistical analysis
SPSS software version 22.0 was applied for the evaluation of the data populations. The genotype frequencies in the patients and the controls were analysed by using the Fisher exact test or the χ² test. The normality of the data was checked with the Kolmogorov-Smirnov test. Since the data exhibited Gaussian distribution, and the Levene test did not reveal significant differences in the homogeneity of variances, we applied the independent t test for the comparison of the difference in age between the PD groups and the controls. The associations between the genotypes and the PD were estimated via the odds ratio (OR), with a 95% confidence interval (CI) (95% CI). A p value of less than 0.05 was considered statistically significant. The observed FokI, BsmI, ApaI and TaqI genotype frequencies were in accordance with the Hardy–Weinberg equilibrium in both the patients and the controls.
RESULTS

1. VPS35 gene mutation
The common VPS35 p.D620N mutation was not detected either in the PD patients or in the controls in the assessed population (Table 1).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>GG (%)</th>
<th>GA (%)</th>
<th>AA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD patients</td>
<td>124 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Control</td>
<td>122 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Table 1. Distribution of p.D620N mutation in the PD patients and the control group

2. GBA gene mutations
Among PD patients, 3 individuals (2.4%) carried a heterozygous mutant GBA allele: in all 3 cases the p.L444P substitution. Moreover, all the patients who carried the mutant allele were in the EOPD group. In contrast, no mutations were detected in the control group. The difference in mutation frequencies between the patients (2.4%) and controls (0%) was not statistically significant (p = 0.247) (Table 2). However, the carriers of the GBA mutation were at an increased risk of developing PD (OR = 6.05, 95% CI 0.300 to 122.06).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>AA (%)</th>
<th>AG (%)</th>
<th>GG (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD patients ≤60 yr</td>
<td>64 (97.6)</td>
<td>3 (2.4)</td>
<td>0 (0)</td>
<td>0.247</td>
</tr>
<tr>
<td>PD patients &gt;60 yr</td>
<td>57 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>122 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. GBA p.L444P genotypes distributions in patients with PD and controls

Neither the p.R120W nor the p.N370S variant of the GBA gene was identified among the assessed PD cases and controls.
3. VDR polymorphisms

There was a significant difference in FokI genotypes between the PD patients and the healthy controls ($\chi^2 = 6.7; p = 0.035$). The frequency of genotype with C (CC+CT) was significantly higher among the patients with PD relative to the controls: OR = 2.677 and 95% CI = 1.214-5.91, $p = 0.015$ for CC+CT vs. TT. Moreover, the C allele showed a significant association with PD group (OR = 1.615, 95% CI = 1.087-2.399, $p = 0.017$) (Table 3). No significant association was found between this polymorphism and the age at onset.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC (%)</td>
</tr>
<tr>
<td>PD patients</td>
<td>42 (42)</td>
</tr>
<tr>
<td>Control</td>
<td>35 (32.1)</td>
</tr>
</tbody>
</table>

Table 3. VDR FokI genotypes and allele frequencies in the PD patients and the controls

There was no significant difference in the BsmI, TaqI and ApaI genotypic distributions and allele frequencies between the PD patients and the healthy controls. Moreover, there was no statistically significant association between the BsmI, TaqI and ApaI polymorphisms and the age at onset in PD patients, and no significant difference was found between this polymorphism and gender in the PD group.
DISCUSSION

PD is a frequent and heterogeneous disorder. The majority of PD cases (75-80%) are sporadic; the remaining 15-20% of the patients have a familial history. The precise patomechanism of PD is not fully understood, therefore the treatment poses a great challenge for clinical investigation. For this reason, it would be important to determine genetic alterations may promote the selection of homogenous subpopulations, hereby a target specific therapy would be enable in the different subgroups. It is well known, that the environmental factors influence the development of PD, but the sensibility to environmental factors is mostly determined by genetic and epigenetic background.

Therefore, we investigated some mutations of three genes in PD patients and healthy controls.

p.D620N mutation of VPS35 gene in PD

VPS35 is a subunit of retromer complex and is involved in endosomal-lysosomal trafficking. Recently studies focused on the link between the retromer complex and PD and have been investigated in different model organisms. Decreased VPS35 levels in Drosophila dopaminergic neurons were shown to lead locomotor defects and shortened lifespan [16]. In addition, the expression of the VPS35 with p.D620N mutation in the rat brain was shown to cause a dopaminergic neuron degeneration [17]. Furthermore, the p.D620N mutation in VPS35 was recently discovered as a new cause of PD, mainly in the autosomal dominantly inherited cases, although it may additionally have a role in SPD, but the results are inconsistent [18, 19]. The frequency of the mutation carriers have been estimated ranging from 0.1% to 1% of the PD population [20]. The distribution of the VPS35 mutation shows ethnic differences; the p.D620N mutation is more frequent in Yemenite Jews (1.67%), in France (1.2%), in Tunisia (0.5%), and in Austria (0.4%), however this mutation has not been found in other ethnicities, like in Canada, Norway, Ireland, Taiwan, Chinese and Greeks [4, 21]. Noticeably, this mutation has been examined in a number of Caucasian populations with different results. Therefore, we investigated the presence of the p.D620N mutation of the VPS35 gene in Hungarian SPD patients, but we were unable to identify this mutation in any of the investigated patients or controls. This suggests that the p.D620N mutation of the VPS35 gene is a rare cause of SPD.
**Association between GBA mutations and PD**

Besides the Mendelian genes, several studies have focused on genetic variability conferring susceptibility to sporadic PD. Multiple studies in PD patients and controls have revealed numerous loci, including GBA and MAPT, as risk factors for sporadic PD [7].

GBA gene encode a lysosomal enzyme and several mutation have been reported in it. The precise mechanisms underlying the relation between the mutations of GBA and the development of PD is still indefinable. Recent studies provide some perspectives; the link among GBA mutations, GCase activity, and SNCA has made an important contribution in the pathogenesis of PD [22].

Several studies have reported that the frequencies of GBA mutations are higher in PD patients than in controls, but the range varies in different ethnic groups. The three most common variant among non-Ashkenazi Jewish patients are p.L444P, p.N370S and p.R120W.

Our results indicated that the PD patients demonstrated a higher frequency (2.4%) of the p.L444P mutation of the GBA gene as compared with the controls (0%), although the difference was not statistically significant. This finding is similar to those of previous studies that the p.L444P mutation was shown to occur at incidences of 3.1- 1.1% among Caucasian population [23, 24]. Moreover, our study revealed that all the patients who carried the mutant allele were in the EOPD group. In accordance with our data higher p.L444P frequency was observed in some other European EOPD population, like Greece (3.3%), Spain (2.66%), and United Kingdom (1.15%) [24-26]. These data emphasized the significance of the GBA mutation, particularly in EOPD cases.

The p.N370S has been demonstrated to be the most frequent mutation in several European (e.g. Serbian (1.9%) and French (2.9%)) population [27, 28]. A significant association between the p.R120W mutation and PD have been detected in a Japanese study [29]. In contrast, we did not detect either the p.R120W or the p.N370S variant of the GBA gene among the PD cases and the controls. These diverse data suggest that the Caucasian population is not homogeneous in this respect.

**Association between VDR mutations and PD**

Earlier reports revealed that 25OHD levels were decreased in PD patients [30, 31]. Overall, it has been clearly shown that the vitamin D metabolism is affected in PD patients. Additionally, differences have also been demonstrated in the VDR polymorphisms in PD in various populations [32-35], but there are only limited data in Caucasian population.
Our results have indicated a significant difference in the FokI genotype distribution between PD and controls in Hungarian population; the frequency of the C allele was significantly higher in PD patients than in the healthy control group, suggesting that the C allele may have a role in the development of PD. Previously, a Japanese and a Chinese study detected difference in this polymorphism between healthy subjects and PD patients. In Japan, FokI CC genotype was associated with milder forms of PD [34]. Han et al. [35] suggested that FokI C allele might be a risk factor for sporadic PD development.

FokI polymorphism is located in 5′ coding region of the gene. This polymorphism results in different translation initiation sites: if the VDR gene contains C allele, the protein will be three amino acids shorter. Difference in length may result in altered VDR function [15, 36].

BsmI, ApaI and TaqI polymorphisms are located in the 3′-end region of the VDR gene, which do not result in changes in the amino acid sequence of the VDR [15]. We did not identify significant associations with these VDR polymorphisms. Although, the BsmI and the ApaI polymorphisms were associated with PD in previous studies [32, 33].

These diverse data suggest that the Caucasian population is not homogeneous in this respect. As far as we are aware, this is the first report on the potential correlation between a VDR polymorphism and PD from a European country.

The differences between the results of the various studies might stem from the different sample size and the different study populations with the possibility of certain ethnic variations.

**CONCLUSION**

The fact that the genetic analysis of SPD patients is important, because it could help to understand the development of PD. Our results suggest that the p.L44P mutation of GBA and the FokI polymorphism of VDR gene is associated with PD in Hungarian population.

The detection of the different genetic factors in the various PD group is important, because it may permit the development of new therapeutic targets. Furthermore, the identification of novel genetic risk factors may facilitate a better selection of homogeneous subpopulations for therapeutic studies.
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