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Community-based genetic study of Parkinson´s disease in Estonia

A running title: Estonian PD genetic study

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ABSTRACT

Objective: To examine the genetic variability of Estonian Parkinson´s disease (PD) patients using an ongoing epidemiological study in combination with a genetic analysis.

Methods: This study was a community-based genetic screening study of 189 PD patients and 158 age and sex matched controls screened for potential mutations in 9 PD genes using next-generation sequencing and multiplex ligation-dependent probe amplification method. Different clinimetric scales and questionnaires were used to examine PD patients and assess clinical characteristics and severity of the disease.

Results: The overall frequency of pathogenic PD-causing variants was 1.1% (2/189), any rare genetic variant was present in 21.2% (40/189) of the patients and in 8.2% (13/158) of the controls ($p<0.05$). Variants of unknown significance accounted for 10.6% (20/189). Frequency of any *GBA* variant among PD patients was 10.1% (19/189) and in controls 3.8% (6/158). The frequency of any *GBA* variant in PD compared to controls was significantly higher ($p = 0.035$; OR 2.82; CI 95% 1.05-8.87). Burden of rare variants was not different between patients and controls. Also, a novel *GBA* pathogenic variant p.E10X was detected.

Conclusion: Among different genetic variants identified in Estonian PD patients, *GBA* variants are the most common while an overall pathogenic variant frequency was 1.1%.

Key words: Parkinson´s disease, genetics, next generation sequencing, multiplex ligation-dependent probe amplification

Introduction

Parkinson´s disease (PD) is the second most common neurodegenerative disease with a global crude prevalence of 2-3% in people ≥65 years of age.[1] Although mostly thought to be caused by a combination of environmental and genetic risk factors, about 15% of the patients have positive family history and 5-10% have a specific monogenetic form with Mendelian inheritance.[2]

The first PD related gene, *α-synuclein*, was discovered in 1997[3] and ever since at least 19 disease causing genes and 23 loci have been reported. In addition, genome wide associations studies have detected multiple genes and loci that increase the risk of PD.[4] Estonia lies in the North-Eastern part of Europe and has a population of about 1.3 million. The age-adjusted prevalence of PD is 314/100 000[5] so there are about 4000 PD patients in Estonia.

Native Europeans, in general, are genetically relatively homogenous, but still have a northwest to southeast gradient in genetic diversity that correlates with geography.[6]

The genetic structure of North-Eastern Europeans has been well described and reveals that Estonians cluster together with their neighbours Latvians and Lithuanians, as well as with Western Russians and Poles, leaving the Finnish population to a separate cluster.[7] At the same time, there are intra-population differences, with South-Eastern Estonians being genetically very close to Latvians, whereas Northern Estonians share more with Southern Finns.[8]

This study implemented a community-based approach to examine the genetic variability of Estonian PD patients, gathering data from an ongoing PD epidemiological study combined with genetic analysis. A gene panel was designed in the University College London´s Institute of Neurology, and included already established PD-causing genes as well as more recently PD-linked risk variants (Box 1).[9,10]

Methods

Subjects

The current study was a community-based genetic screening study. Cases consisted of 189 PD patients of Estonian descent who had been previously diagnosed according to the Queen Square Brain Bank (QSBB) criteria[11] and treated in the Tartu University Hospital´s Neurology Clinic. The patients enrolled in this study were also a part of the larger cross-sectional study that aimed to evaluate the prevalence and incidence of PD in Estonia.[5] 22 patients had a positive family history for PD in a first degree relative, and 9 patients in a distant relative. Early onset PD was defined by the age of onset below 50 years.

Comparison was made with 158 age and sex matched controls of the Estonian descent. The controls were recruited from the Tartu University Hospital´s Neurology Outpatient Clinic, and comprised of patients without any known central nervous system disease. All participants reported “white” ethnicity.

The following clinimetric scales were used to assess PD patients: the Movement Disorders Society’s Unified Parkinson’s Disease Rating Scale (MDS-UPDRS)[12], the Hoehn and Yahr Scale (HY)[13], the Schwab and England Activities of Daily Living Scale (SE-ADL)[14], the Beck Depression Inventory (BDI)[15] and the Mini Mental State Examination (MMSE).[16] Disease subtypes were based upon the most prevalent symptom during a standard neurological examination: tremor; bradykinesia-hypokinesia; or postural instability gait disorder (PIGD).

The study was approved by the Research Ethics Committee of the University of Tartu. A written informed consent was obtained from all patients and controls.

Genetic analysis

A pathogenic variant was defined as a variant that has been previously shown to be causative for autosomal dominant or recessive PD or to be an established risk allele for PD. A novel pathogenic variant was defined as a previously unreported loss-of-function variant in a gene for which loss-of-function is an established mechanism.

Peripheral blood was obtained from PD patients and controls. Genomic DNA (gDNA) was extracted from the whole blood by the salting out protocol.[17]

Both PD patients and controls were screened for potential mutations across 117 exons in 9 PD genes using next-generation sequencing (NGS) with Illumina´s MiSeq sequencer and polymerase chain reaction (PCR) amplicon-based (TruSeq custom amplicon) target enrichment. TruSeq custom amplicon assay Design Studio v1.6 online was used to design the probes. The assay was performed following the manufacturer´s recommended protocol. Exons not covered at all by at least 10 reads (*PRK7/DJ1*, *PINK1*, *GBA*, *PRKN/PARK2*, *LRRK2* and *GCH1*) were subsequently Sanger sequenced. Variants with minor allele frequency (MAF) ≥ 1% in general population according to 1000 Genome Project (www.1000genomes.org) were excluded from the analysis. PCR primers and conditions are available upon request.

All familial and early onset PD cases were screened by multiplex ligation-dependent probe amplification (MLPA) method looking for duplication and triplication of *SNCA*, copy number variations (CNVs) in *PRKN*, *PRK7*, *PINK1* and point mutation in *LRRK2* G2019S and *SNCA* A30P. Copy number variations were also looked for in carriers of heterozygous missense mutations in recessive genes. The P051-C3 Salsa MLPA Parkinson probe set was used (MRC Holland, Amsterdam, the Netherlands). Data were analyzed using Genemarker, version 2.6.2, software.

91.3% of the exons were completely covered by at least 10 reads. 1.7% of the exons were covered for the 50-99% by at least 10 reads. 7% of the exons failed (not covered at all by at least 10 reads): *PRK7*x4, *PINK1*x6, *GBA*x3, *PRKN*x8, *LRRK2*x41-x45-x49 and *GCH1*x1 and were Sanger sequenced.

Statistical analysis

Statistical analysis was conducted using R software (version 1.1.383). The level of significance was set to 0.05. Fisher exact test was used for comparing variation frequencies between PD patients and controls and calculating odds ratios.

Results

A total of 189 patients with PD (113 women, 76 men) and 158 controls (96 women, 62 men) comprised the study group. The descriptive statistics of the clinimetric scales, the data on the clinical subtypes and the family history of PD patients are shown in Table 1.

The mean age of PD patients and controls at examination were 72.5±8.4 years (range 47-87) and 72.0±9.1 years (range 47-95), respectively. The mean age at PD onset was 65.2 ±10.1 years (range 35-83), and the duration of PD 7.3 ±5.6 years (range 0.3-35). 17 early onset PD cases were identified including 3 cases with PD onset before 40 years. Any rare genetic variant was present in 21.2% (40/189) of the patients and in 8.2% (13/158) of the controls. Of all the rare variants detected in PD patients, the frequency of pathogenic PD-causing variants was 1.1% (2/189), both patients were carriers of single mutations in a recessive gene and had negative MLPA. The frequency of *GBA*-related risk variants was 9.5% (18/189) and variants on unknown significance accounted for 10.6% (20/189).

Three patients carried more than one variant: first patient carried three different variants in *GBA*, *PRK7* and *PINK1*, another patient had two different variants in *GBA* and *GHC1*, and one patient had also two different variants in *GBA* and *PRK7*.

Three different pathogenic variants were identified in four PD patients: two patients carried a single heterozygous pathogenic variant in *PRKN*, one patient was a carrier of Gaucher´s disease causing variant in *GBA*, and another patient carried a novel pathogenic variant in *GBA*.

Out of all patients with a positive family history (PD in 22 first degree relatives and in 9 distant relatives), only five patients carried rare risk variants or variants with unknown significance, and no one carried pathogenic variants.

No duplication or triplication of *SNCA*, copy number variations in *PRKN*, *PRK7*, *PINK1* and point mutation in *LRRK2* G2019S and *SNCA* A30P were detected. No variants in *DCTN1* were found. No cases with homozygous or compound heterozygous mutations in recessive genes (*PRKN*, *PINK1*, *PRK7*) were identified.

Summary of the genetic findings is presented in Table 2 and the frequencies of rare variants are shown in Table 3.

GBA

Five different variants were identified in the *GBA* gene. Frequency of any *GBA* variant among PD patients was 10.1% (19/189, MAF 0.05) and in controls 3.8% (6/158, MAF 0.018). The frequency of any *GBA* variant in PD compared to controls was significantly higher than in controls (p = 0.035; OR 2.82; CI 95% 1.05-8.87). Novel or pathogenic mutations were found in three patients and in none of the controls. A novel *GBA* pathogenic variant p.E10X is likely to result in haploinsufficiency. Also, a novel variant of unknown significance p.L276I was detected in one patient and one patient carried a pathogenic variant L444P that in homozygous state causes severe form of Gaucher´s disease (types 2 and 3).

The difference between PD and controls was not significant for *GBA* common risk factors (T369M and E326K) for PD, however compared to other Caucasian controls there is an excess of T369M in PD cases (MAF=0.0265 in the Estonian cohort versus MAF=0.011 in a recent meta-analysis[18]).

GCH1

Two different variants were identified in PD patients only (p.V204I in two and p.Q110E in one PD patient), reported in detail elsewhere.[19]

LRRK2

Three different variants were found in the *LRRK2* gene. The frequency of any *LRRK2* variant was 3.7% (7/189) among PD patients and 2.5% (4/158) in controls. No p.G2019S was detected in early onset or familial PD. Two patients and one control carried the variant p.E334K, four patients and three controls carried the variant p.R1514Q and the variant p.R767H was found in one patient only. 23 PD patients had the variant p.P1542S (MAF 0.06) but it was present at similar frequency in controls. However, for Estonian PD patients vs Caucasian controls the OR is 1.8. Overall, burden of rare variants did not differ between PD patients and controls.

VPS35

Only one variant of unknown significance (p.G51S) was found in one PD patient and one control.

Recessive genes

Burden of rare variants was not different in PD patients versus controls. One *PRK7* variant (p.R98Q) was found in four PD patients and in none of the controls. Considering *PRKN*, two different variants were identified in PD patients: a pathogenic heterozygous variant (p.R275W) was detected in two PD patients, MLPA excluded the presence of a second mutation. An additional variant of unknown significance (p.V109M) was identified in another patient. Also, a heterozygous variant p.A82E was identified in three controls but in none of the PD patients.

Regarding *PINK1*, three different variants were present in three patients and two controls. One patient carried the variant p.K186N, one patient and two controls had the variant p.G411S and one patient carried the variant p.P209L.

Discussion

The aim of this study was to explore the genetic variability of Estonian PD patients using a community-based approach. To our knowledge, this is the first report of its kind in the Baltic region, as there are no similar genetic studies available for Latvian and Lithuanian PD populations.

The present study identified a pathogenic variant frequency of 1.1% that is comparable to the UK´s Tracking Parkinson´s study, the largest multicentre clinico-genetic incidence study in the world, where a pathogenic variant frequency of 1.4% was reported.[20] These low frequencies are interesting when considering that the general opinion is that 5-10% of PD patients have a genetic cause. It might be that previously described PD-linked variants do not contribute to PD in the Estonian cohort in the level as they contribute in other cohorts. Also, a relatively small sample size may set a limit to the generalizability of these results.

The most common PD associated pathogenic variant *LRRK2* p.G2019S was not found in this study. Similarly, a recent Finnish study reported no p.G2019S point mutation in their cohort.[21] It is known that the frequency of p.G2019S has a geographical north to south gradient and can range from 0% to up to 35.7% in sporadic and 42% in familial North-African Arab patients.[22] In the Scandinavian-Baltic region, *LRKK2* p.G2019S is most frequent in Norwegian PD population, where its overall frequency is 2.1% which rises up to 9.2% in familial cases.[23] Haplotype analysis has revealed common founders for p.G2019S in different ethnic groups[24] and a Norwegian study suggests that the variant was imported to Norway before the 17th century through tradesman from Europe.[25] *LRRK2* p.G2019S is also relatively common in North-Western Russian PD cohort, where it is present in 1.6% of all patients and in 5.3% of familial cases.[26]

LRRK2 p.E334K detected in two patients and in one control has been previously reported as a possible rare risk variant, however it has been thought that due to its overall low frequency more studies are needed to draw conclusions.[27]

The *LRRK2* variant p.1514Q was detected in 2.1% in Estonian PD patients and 1.9% in Estonian controls. An earlier analysis of p.1514Q in three different European case-control series reported

the total frequency of 2.2% in patients and 1.6% in controls, concluding that it is not linked to increased risk of PD.[28]

The *LRRK2* variant p.P1542S was present at similar frequency in PD patients and controls, although the odds ratio compared to other Caucasian controls is slightly elevated for Estonian PD patients. The variant has previously been reported not to be associated with susceptibility to PD[27], so it is possible that this variant is just more frequent in the Estonian population.

The largest number of different variants was encountered in the *GBA* gene. It is expected, as in general *GBA* mutations are the most common genetic risk factors for PD.[29] In our study, the frequency of any *GBA* variant was 10.1% which is similar to the findings of a recent study in the UK.[30] Interestingly, the odds ratio for any *GBA* variant among Estonian PD patients versus controls was about twice lower than in a large multinational multicentre study (OR 2.82 versus 5.43 respectively).[31] However, there are known ethnic differences also shown by the aforementioned study with similar odds ratios reported for example in the cohorts of National Human Genome Research Institute and Rostock, Germany.

The most common PD-linked *GBA* variants world-wide are L444P and N370S, which are present in approximately 15% of Ashkenazi Jewish and in 3% of non-Ashkenazi Jewish patients.[31] In European PD patients these variants account for 70% of all mutant *GBA* alleles.[32]

Only one case of L444P (frequency of 0.5%) was found in the Estonian PD cohort and no N370S was detected. As *GBA* N370S is known to be very common among Ashkenazi Jewish patients[33] and less so in other populations, it was surprising to find just one Estonian carrier of L444P. When looking at the Scandinavian-Baltic region, *GBA* L444P appears to be the most frequent in Northern Sweden. A large Swedish case-control study revealed that the overall frequency of L444P was 2.2%, but there were significant geographical differences.[34] Namely, L444P was more common in Northern Sweden, where it was detected in 4.11% of PD patients. Northern Sweden also has a higher prevalence for Gaucher´s disease.[34] L444P is present in 2% of Finnish PD patients[21] and in 1.1% of North-Western Russian PD patients.[26] It remains unclear whether our finding is an underestimation due to a smaller cohort size or not.

8.5% of the *GBA* variance reported in the Estonian study is made up of *GBA* frequent variants T369M and E326K. Both are considered to act as PD risk variants with mild effect.[18,35]

This study also reported a novel *GBA* stopgain variant p.E10x which causes haploinsufficiency that has recently been shown to accelerate α-synuclein related pathology by altering lipid metabolism in a mouse model.[36]

The only generally accepted *VPS35* pathogenic variant to date is the point mutation p.D620N with a frequency of 0.1%-1% in autosomal-dominant familial PD.[37,38] Therefore, it is not surprising that the variant was not found in the Estonian cohort. The only variant reported herein, p.G51S, has so far not been associated with PD.[39,40]

The only known PD causing variant detected in the current study was *PRKN* p.R275W in a heterozygous state in two patients. Both patients were late-onset cases and had no positive family history. Indeed, it is debated whether heterozygous variants in recessive genes alter the risk for developing PD or not.[41,42,43] Furthermore, the heritability of PD is probably more complex than generally thought.[44,45,46]

An interesting finding to illustrate that is the possible autosomal-dominant behaviour with complete or incomplete penetrance of *PINK1* variant p.G411S,[47,48] A recent thorough multicentre case-control study provided evidence for a partial dominant-negative function of heterozygous p.G411S and established it as a rare genetic risk factor. The same study also speculated that other variants in recessive genes might increase the risk for late-onset PD over time and that the level of disease penetrance might be dependent on the level of mitochondrial stress.[49] However, the role of this variant is still controversial with other reports indicating that p.G411S is likely not pathogenic.[50]

At present, other variants found in the Estonian cohort in recessive genes (*PINK*, *PRKN*, *PRK7*) are all of unknown significance.

Variants with unknown significance were reported with a frequency of 10.6% in our study. The frequency and role of such variants have recently been analyzed in two large independent cohorts.[51] The analysis concluded that PD patients with a known pathogenic variant have an excess of additional rare variants with unknown significance compared to PD patients without a known pathogenic variant and controls showing that the burden of such variants might influence the onset and presentation of PD.

Unfortunately, this study failed to show the same results as the burden of rare variants was not different between Estonian PD patients and controls and the two patients who were carrying a known pathogenic variant did not carry any other variant.

A low number of early onset PD cases in the current cohort explains the fact that no homozygous or compound heterozygous cases were found.

The strength of this study is definitely the inclusion of a broad range of patients in the population, regardless of their age at PD onset, family history or genetic profile. In addition, community-based approach can be considered as a major strength, because it helps to ensure genetic homogeneity among the study cohort and by that limits possible confounding (population stratification).

A minor limitation of the study is not having assessed systematically CNVs in recessive genes in all samples. Indeed, CNVs in *SNCA*, *PRKN*, *PINK1*, *PRK7* were screened by MLPA only in early onset PD, PD with positive family history and carriers of heterozygous missense mutations in recessive genes. It is still debated whether single heterozygous CNVs are risk factors for late onset PD.[52]

While we used clear and concise definitions for pathogenic variants in present paper, the definition of pathogenicity remains a matter of debate. Current definitions are based on gene-centric annotations where the causative gene or mutation is either already defined or predictable. However, considering the missing heritability problem, some of the pathogenic variants are possibly hidden in the non-coding regions of the genome where the pathogenic mechanisms are often elusive. These mechanisms can involve altered splicing or changed transcriptional efficiency and therefore are not discoverable by the gene-centric approach. We are still on our way to discover the functional impact of the non-coding variants. That was out of the scope of the present manuscript, but certainly deserves attention in our future studies.

In conclusion, this is the first PD genetic screening study in the Baltics. We have identified the frequencies of different genetic variants for Estonian PD patients and found an overall pathogenic variant frequency of 1.1%. Knowing the country-specific genetic profile of PD patients will be of great help in the coming future when treatments aimed at genetic targets, namely *LRRK2* and *GBA*, will become available.

Conflict of Interest

The authors have no conflict of interest to report.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Box 1. Design of the next-generation sequencing panel.

HGNC gene symbol	Inheritance	Disease onset	Role
PINK1	AR	Early-onset	Mitochondrial function/mitophagy
PRKN	AR	Early-onset	Mitochondrial function/mitophagy; ubiquitination; synaptic function
PARK7	AR	Early-onset	Infl ammation/immune system; mitochondrial function
SNCA	AD	Early-onset	Synaptic function; autophagy/lysosomal degradation; mitochondrial function
LRRK2	AD	Late-onset	Synaptic function; inflammation/immune system; autophagy/lysosomal degradation
VPS35	AD	Late-onset	Autophagy/lysosomal degradation; endocytosis
DCTN1	AD	NA	Cell division; axonal transport (including autophagosomes)
GCH1†	NA	NA	GTP binding; calcium ion binding; BH4 metabolism; metabolic pathways
GBA†	NA	NA	Inflammation/ immune system; autophagy/lysosomal degradation; metabolic pathways

Abbreviations: HGNC, HUGO Gene Nomenclature Committee; AR, autosomal recessive; AD, autosomal dominant; †, PD risk variant; NA, not applicable

Table 1. Characteristics of the PD patients.

Variable	PD (n=189)
Clinical subtype	
• Tremor-dominant (n)	43.6% (83)
• Hypokinetic-rigid dominant (n)	5.3% (10)
• PIGD (n)	50.8% (96)
No family history of PD (n)	83.6 % (158)
1st degree relative with PD (n)	11.6% (22)
2nd degree relative with PD (n)	4.8% (9)
MDS-UPDRS total score, mean (±SD)	72.8 (28.8)
HY, median (range)	3 (1-5)
SE-ADL, median (range)	80 (30-100)
BDI, mean (±SD)	14.8 (7.4)
MMSE, mean (±SD)	26.8 (3.5)

Abbrevations: BDI, Beck Depression Inventory; *HY*, Hoehn and Yahr stage; *MDS-UPDRS*, Movement Disorders Society Unified Parkinson´s Disease Rating Scale; *MMSE*, Mini Mental State Examination; *PD*, Parkinson´s disease; *PIGD*, Postural instability and gait disturbance dominant; *SE-ADL*, Schwab and England Activities of Daily Living Scale.

Table 2. Summary of genetic findings.

Gene	Chromosome	AA change	Interpretation	Count in PD	Count in controls
GBA	1	p.L396P (allele name L444P)	Pathogenic variant causing Gaucher disease	1	0
GBA	1	pT321M (allele name T369M)	Risk variant	10	3
GBA	1	p.E278K (allele name E326K)	Risk variant	6	3
GBA	1	p.L276I	Novel variant of unknown relevance	1	0
GBA	1	p.E10X	Novel pathogenic variant	1	0
GCH1	14	p.V204I	Likely pathogenic/Unknown relevance	2	0
GCH1	14	p.Q110E	Unknown relevance	1	0
LRRK2	12	p.E334K	Unknown relevance	2	1
LRRK2	12	p.R767H	Unknown relevance	1	0

LRRK2	12	p.R1514Q	Unknown relevance	4	3
PRKN	6	p.V109M	Unknown relevance	1	0
PRKN	6	p.R126W	Single heterozygous pathogenic variant	2	0
PRKN		p.A82E	Unknown relevance	0	3
PARK7	1	p.R98Q	Unknown relevance	4	0
PINK1	1	p.K186N	Unknown relevance	1	0
PINK1	1	p.G411S	Unknown relevance	1	2
PINK1	1	p.P209L	Unknown relevance	1	0
VPS35	16	p.G51S	Unknown relevance	1	1

Table 3. Summary of frequency of any rare variant found.

Gene	Prevalence in PD % (n)	Prevalence in controls % (n)	p-value
GBA	10.1 (19)	3.8 (6)	0.035*
GCH1	1.6 (3)	0 (0)	0.254
LRRK2	3.7 (7)	2.5 (4)	0.750
PRKN	1.6 (3)	1.9 (3)	1
PARK7	*2.1 (4)*	*0 (0)*	*0.128*
PINK1	1.6 (3)	1.3 (2)	1
VPS35	0.5 (1)	0.6 (1)	1

*Statistically significant difference