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Genetic risk factors in Finnish patients with Parkinson's disease

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Abstract

Introduction—Variation contributing to the risk of Parkinson's disease (PD) has been identified in several genes and at several loci including *GBA*, *SMPD1*, *LRRK2*, *POLG1*, *CHCHD10* and *MAPT*, but the frequencies of risk variants seem to vary according to ethnic background. Our aim was to analyze how variation in these genes contributes to PD in the Finnish population.

Methods—The subjects consisted of 527 Finnish patients with early-onset PD, 325 patients with late-onset PD and 403 population controls. We screened for known genetic risk variants in *GBA*, *SMPD1*, *LRRK2*, *POLG1*, *CHCHD10* and *MAPT*. In addition, DNA from 225 patients with early-onset Parkinson's disease was subjected to whole exome sequencing (WES).

Results—We detected a significant difference in the length variation of the CAG repeat in *POLG1* between patients with early-onset PD compared to controls. The p.N370S and p.L444P variants in *GBA* contributed to a relative risk of 3.8 in early-onset PD and 2.5 in late-onset PD. WES revealed five variants in *LRRK2* and *SMPD1* that were found in the patients but not in the

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Author contributions

S. Ylönen, study design, genetic analysis, writing the manuscript; A. Siitonen, whole exome sequencing; M.A. Nalls, whole exome sequencing; P. Ylikotila, sample collection, clinical analysis; J. Autere, sample collection; J. Eerola-Rautio, sample collection; R. Gibbs, whole exome sequencing; M. Hiltunen, sample collection; P.J. Tienari, sample collection; H. Soininen, sample collection; A.B. Singleton, whole exome sequencing; and K. Majamaa, study design, data analysis, statistical analysis, writing the manuscript, study supervision. All authors contributed to the conception of the study, revising the manuscript for intellectual content and approved the final version for publication.

Conflicts of interest

None.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.parkreldis.2017.09.021.

Finnish ExAC sequences. These are possible risk variants that require further confirmation. The p.G2019S variant in *LRRK2*, common in North African Arabs and Ashkenazi Jews, was not detected in any of the 849 PD patients.

Conclusions—The *POLG1* CAG repeat length variation and the *GBA* p.L444P variant are associated with PD in the Finnish population.

Keywords

Molecular epidemiology; Neurodegenerative diseases; Mutation; Mitochondrial; Gene

1. Introduction

Genetic variation contributing to the risk of Parkinson's disease (PD) has been identified. One of the genes involved is glucosylceramidase beta (*GBA*) that codes for a lysosomal enzyme. It is traditionally associated with Gaucher's disease, but heterozygous mutations are an established risk factor for Parkinson's disease. The two most common mutations in *GBA* include p.L444P and p.N370S. *SMPD1* encodes another gene involved in lysosomal function. It codes for the protein sphingomyelin phosphodiesterase 1 that generates ceramide by cleaving the phosphocholine group of sphingomyelin. Mutations in *SMPD1* are found in Niemann-Pick disease and the variant p.L302P has been suggested to be associated with PD [1].

The *LRRK2* (leucine-rich repeat kinase 2) gene encodes a 2527-amino acid protein with several structural and functional domains such as leucine-rich repeat and kinase. The common p.G2019S mutation is located in the kinase domain and it has been shown to increase the kinase activity [2]. The frequency of the p.G2019S mutation varies greatly in different populations being most common in North African Arabs, where it is found in 39% of sporadic and 36% of familial cases, and Ashkenazi Jews, where it is found in 10% of sporadic and 28% of familial cases. Among Europeans, the frequency is 4% in sporadic cases in Portugal and 14% in familial cases, whereas in Spain, Italy and France it is 2–4%, and in Sweden and Norway the frequency is 1%. In Asian countries the mutation is very rare being found in <0.1% of patients [3]. The penetrance of p.G2019S is high, but not complete, so that the risk of PD is 74% at the age of 79 years [3].

Other genes, where variation may contribute to the risk of PD, include *POLG1*, *CHCHD10* and *MAPT*. Polymerase γ (*POLG1*) is responsible for the replication of the mitochondrial genome. The second exon of the gene harbors a microsatellite consisting of ten CAG trinucleotide repeats that is translated into a tract of ten glutamine moieties (10Q). Genotypes different from the common 10Q variant have been associated with a higher risk of PD [4]. *CHCHD10* encodes a coiled-coil helix coiled-coil helix protein that is involved in the maintenance of mitochondrial cristae integrity [5]. The variant p.S59L in *CHCHD10* has been associated with a complex phenotype consisting of amyotrophic lateral sclerosis, frontotemporal lobar degeneration, cerebellar ataxia, parkinsonism, and myopathy [5] and, subsequently, another variant p.G66V has been identified [6]. *MAPT* (microtubule-associated protein tau) is involved in the assembly and stabilization of microtubules. Of the

two haplotypes H1 and H2, haplotype H1 has been reported to be slightly more common in PD patients than in population controls [7].

Previous data have shown that the frequencies of these mutations in patients with PD vary according to ethnic background [8]. Therefore, we screened for the most common known mutations in the genes *GBA*, *SMPD1*, *LRRK2* and *CHCHD10*, determined the length variation in *POLG1* and determined the *MAPT* haplotype in Finnish patients with Parkinson's disease and population controls.

2. Subjects and methods

2.1. Patients and controls

The study group consisted of a national cohort of 441 patients with EOPD defined by age of onset <55 years [9]. In addition, a case series collected at the Kuopio University Hospital during one year [10] comprised of 209 patients with late-onset Parkinson's disease (LOPD) and 55 patients with EOPD, and a case series collected at the Helsinki University Hospital [4] comprised 116 patients with LOPD and 31 patients with EOPD. Previous analyses on these cohorts have not revealed causative mutations in the common PD genes. Controls consisted of 403 healthy blood donors from three different regions of Finland. Part of the samples in the national cohort of EOPD (N = 225) were subjected to whole exome sequencing (WES). The study has been approved by the Ethics Committee of Turku University Hospital, the Ethics Committee of the Medical Faculty of the University of Kuopio, the Ethics Committee of Helsinki University Hospital and the Ethics committee of the Finnish Red Cross. Written informed consent was obtained from all patients prior to participating the study.

2.2. Molecular methods

Genomic DNA was extracted from peripheral blood using standard protocols. The variations in *GBA* (p.N370S, p.L444P), *SMPD1* (p.L302P), *LRRK2* (p.R1441C/G/H, p.G2019S), *CHCHD10* (p.S59L, p.G66V) and *MAPT* (haplotype H1) were detected using a PCR-restriction digestion protocol. The primers and restriction enzymes used are presented in supplementary table. The detected variations were confirmed by sequencing.

The CAG repeat in exon 2 of the *POLG1* gene was analyzed by fragment length analysis. A fragment containing the CAG repeat was amplified using a FAM-labeled forward primer CTCCGAGGATAGCACTTGC and a reverse primer CTGGGTCTCCAGCTCCGT (CHLC.GCT14A01.P16693.1 and CHLC.GCT14A01.P16693.2, respectively; GenBank accession number G16014). The fragment length was 124 bp in the presence of the most common allele containing ten CAG repeats. The fluorescent-labeled PCR products in the EOPD group were separated on an *ABI PRISM*® 3100 Genetic Analyzer (Perkin Elmer, Foster City, CA, U.S.A.). GeneScan™ −500 LIZ® Size Standard (Applied Biosystems, Foster City, CA, U.S.A.) was used as an internal standard and was run along with each sample. The alleles were identified using the Peak Scanner™Software Version 1.0 (Applied Biosystems). In the LOPD group and the controls an ABI PRISM™ 377 DNA Sequencer (Perkin Elmer) was used with Genescan version 2.1 fragment analysis software (Perkin

Elmer). GENESCAN-500™ TAMRA (Applied Biosystems) was used as an internal standard and the Genotyper 2.0 program (Perkin Elmer) was used for identifying the alleles.

WES was carried out as described previously [11]. Annovar [12], SNPEff [13] and SNPSift [14] were used for functional annotations. The data were unfiltered so that even the unlikely SNPs could be detected. As controls we used 563 STAMPEED population controls from the North Finland Birth Cohort 1966. WES has recently revealed several mutations, but no splice mutations, in established EOPD genes among the 225 EOPD patients [11]. Clinical significance of the mutations is, however, unknown. Nonsynonymous mutations in *LRRK2* and *SMPD1* that were more frequent among the patients than among the controls were verified by direct sequencing and were analysed for their pathogenic potential by using PredictSNP [15].

2.3. Statistical analysis

Fisher exact test was used to compare allele frequencies between patients and controls. The frequency distribution of the CAG repeat alleles in the *POLG1* gene was analyzed with the exact test of population differentiation using the Arlequin software [16].

3. Results

3.1. Genetic variants contributing to the risk of EOPD

None of the EOPD patients harbored *SMPD1* p.L302P, *LRRK2* p.R1441C/G/H, *LRRK2* p.G2019S, *CHCHD10* p.S59L or *CHCHD10* p.G66V. The frequency of haplotype H1 in *MAPT* was 92.6% in the patients and 95.0% in the 292 controls (p = 0.068 for difference, Table 1). The length of the CAG repeat in the *POLG1* gene varied between 8Q and 13Q in the EOPD patients and between 8Q and 12Q in the controls. The frequency distribution of the CAG repeat alleles in *POLG1* differed between the EOPD patients and the controls (p = 0.041, exact test of population differentiation). The frequency of non-10Q alleles was 10.7% in the EOPD patients (Table 2) and 6.8% in the controls (p = 0.0056 for difference). Seven EOPD patients harbored a non-10Q/non-10Q genotype, while none of the controls had such a genotype. The clinical features, age of PD onset and the frequency of family history did not differ between EOPD patients harboring a non-10Q/non-10Q genotype (n = 7) and EOPD patients with 10Q/10Q genotype (n = 397). However, postural instability and on-off variation was less common in patients with non-10Q/non-10Q genotype.

Two EOPD patients and one control had the heterozygous p.N370S mutation in *GBA* and 13 patients and two controls had the heterozygous p.L444P mutation (p = 0.032, Fisher's exact test, Table 3). One of the patients with p.N370S also had the *POLG1* variants p.N468D and p.A1105T that has been reported previously [17]. Three of the patients with p.L444P in *GBA* also had the nonsynonymous p.A456P variant and the synonymous rs1135675 variant at codon 460, which in combination with p.L444P form the RecNciI allele.

3.2. Genetic variants contributing to the risk of late-onset PD

The five mutations in *SMPD1*, *LRRK2* or *CHCHD10* were not found in 323 LOPD patients. The frequency of haplotype H1 in *MAPT* was 94.4% in LOPD patients (p = 0.73 for

difference, Table 1). Two LOPD patients had the heterozygous p.N370S mutation and four patients had the heterozygous p.L444P mutation in *GBA* (Table 3). Interestingly, one patient had both p.L444P and p.N370S mutations. The frequency distribution of the CAG repeat alleles in the *POLG1* gene did not differ between the LOPD patients and the controls (p = 0.13, exact test of population differentiation, Table 2). The frequency of the *POLG1* non-10Q alleles was 7.9% in LOPD and three LOPD patients harbored a non-10Q/non-10Q genotype.

3.3. Variation in WES in GBA, MAPT, SMPD1, LRRK2

As the well-known variants in *LRRK2* and *SMPD1* were not detected in the Finnish patients with EOPD, we then selected randomly 225 out of the 441 samples to WES. Rare variation was detected in *LRRK2* and *SMPD1* (Table 4), where we found five nonsynonymous variants that were present in patients but not in Finnish ExAC controls. Two variants were deemed to be deleterious (Table 4) and, interestingly, the p.R542Q variant in *SMPD1* was homozygous and the p.R1628P in *LRRK2* has previously been reported in association with PD [18].

4. Discussion

We detected a significant difference in *POLG1* CAG repeat length variation between the patients with EOPD and the controls and in the frequency of the *GBA* variant p.L444P. The p.G2019S variant in *LRRK2* was not found among Finnish PD patients, although it has previously been found in European PD patients and is especially common among North African Arab and Ashkenazi Jewish PD patients [3]. WES revealed several variants in *LRRK2* and *SMPD1* that were found only in PD patients and not in the controls. These are putative risk variants, but require further confirmation.

The two most common mutations p.L444P and p.N370S comprise more than 50% of the pathogenic mutations in *GBA*. The allele frequency of the two mutations is high among Ashkenazi Jewish PD patients with a total frequency of 15.3%. The combined frequency of the two mutations is approximately 3.2% in non-Ashkenazi Jewish PD patients [8], 2.3% in Norwegian patients [8] and 2.8% in Swedish patients [19], whereas among non-Finnish Europeans in the ExAC database the frequency is 0.8%. We found these *GBA* mutations in 2.8% of the EOPD patients giving a relative risk of 3.8 for PD and in 1.9% of the LOPD patients giving a relative risk of 2.5. Patients with a *GBA* mutation appear to have an earlier onset of PD compared to that in patients without mutations [8]. In Finnish EOPD patients with a *GBA* mutation the mean age of onset was 44.6 years and at least five out of the 15 mutation carriers reported PD in a first-degree relative.

The variants p.G2019S in *LRRK2* and p.L302P in *SMPD1* are associated with increased risk of PD [1,20]. We did not find these variants in our samples, but WES revealed five other variants that were not found in the controls. PredictSNP suggested that the *LRRK2* variant p.R1628P is deleterious. This variant has been previously associated with PD in Chinese and Thai populations [18]. The p.R1628P variant acts by turning its adjacent amino acid residue S1627 to a new phosphorylation site of Cdk5. Cdk5 phosphorylation of p.S1627 in the presence of p.R1628P increases the LRRK2 kinase activity. Interestingly, cultured mouse

cortical neurons transfected with p.R1628P *LRRK2* plasmids show a higher sensitivity of to MPP+ [21]. Our two patients with p.R1628P had an age of onset of 44 and 49 years and had no family history of PD.

The homozygous *SMPD1* p.L302P mutation causes a fatal infantile type A Niemann-Pick disease. This mutation in heterozygous state has previously been shown to be associated with Parkinson's disease [1].We did not find p.L302P among Finnish PD patients, but WES revealed two other variants that were not found in controls. PredictSNP suggested that the *SMPD1* variant p.R542Q was deleterious, while p.E358K was neutral. The frequency of p.R542Q is 6.0/100,000 among non-Finnish Europeans in the ExAC database and, interestingly, the patient was homozygous. Clinically the patient with p.R542Q was unremarkable with an age of onset of 54 years and with no family history of PD. Homozygous p.R608del mutation has been described previously in a patient with PD and type B Niemann-Pick disease [22].

The length of the CAG repeat in *POLG1* varies between 5 and 16 trinucleotides [23,24].We detected a difference in the repeat allele distribution between EOPD patients and controls and a difference in the frequency of non-10Q alleles. A difference in the frequency of non-10/11Q alleles has previously been obtained in Finnish [4], Swedish [23], Norwegian [24] and Chinese populations [25], but not among North American Caucasians [26]. The latter study, however, reported a higher frequency of non-10Q alleles among PD patients, whereas no difference has been found in this frequency between Australian PD patients and controls [27]. These associations suggest that polymerase γ protein harboring the non-10Q stretch contributes directly to the risk of PD or that the non-10 CAG repeat alleles represent markers of harmful variants elsewhere in the gene. The protein harboring a non-10Q polyglutamine tract could be less optimal in protein-protein interactions [28]. Furthermore, estimation of mRNA folding energies *in silico* has shown that the folding energy is lower in the shorter *POLG* variants and higher in the longer variants [23].

We found that there are common alleles in the Finnish population that increase the risk of PD. The *POLG1* non-10Q variants were associated with an increased risk of PD and the *GBA* p.L444P variant contributed to a relative risk of 4.9 with respect to EOPD. Interestingly, we did not detect the p.G2019S variant in *LRRK2* that is common in other populations, but WES revealed other variants in this gene and in *SMPD1* that were found only in PD patients suggesting that they are putative risk variants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

MAPT H1/H2 genotype and allele distribution.

(A) Genotypes	**H1/H1 N (%)**	**H1/H2 N (%)**	**H2/H2 N (%)**	**Total N**
Controls	263 (90.1)	29 (9.9)	0	292
EOPD	452 (86.1)	68 (13.0)	5 (1.0)	525
LOPD	290 (89.8)	30 (9.3)	3 (0.9)	323
(B) Alleles	H1 N (%)		H2 N (%)	
Controls	555 (95.0)		29 (5.0)	584
EOPD	972 (92.6)		78 (7.4)	1050
LOPD	610 (94.4)		36 (5.6)	646

EOPD, early-onset Parkinson's disease; LOPD, late-onset Parkinson's disease.

Table 2

Frequencies of *POLG1* CAG repeat length genotypes and alleles in patients with PD and controls.

Genotype	EOPD		LOPD		Controls	
	N	%	N	%	N	%
8/10	3	0.6	2	1.0	3	0.7
8/11	1	0.2	1	0.5	0	
9/9	1	0.2	0		0	
9/10	22	4.4	10	4.8	11	2.7
9/11	1	0.2	1	0.5	0	
9/12	1	0.2	0		0	
10/10	397	80.0	179	85.6	348	86.4
10/11	56	11.3	11	5.3	31	7.7
10/12	10	2.0	2	1.0	10	2.5
10/13	1	0.2	2	1.0	0	
11/11	2	0.4	1	0.5	0	
11/13	1	0.2	0		0	
All	496		209		403	
non-10/non-10	7	1.4	3	1.4	0	
Allele						
8	4	0.4	3	0.7	3	0.4
9	26	2.6	11	2.6	11	1.4
10	886	89.3	385	92.1	751	93.2
11	63	6.4	15	3.6	31	3.9
12	11	1.1	2	0.5	10	1.2
13	2	0.2	2	0.5	0	
All	992		418		806	
non-10	106	10.7	33	7.9	55	6.8

EOPD, early-onset Parkinson's disease; LOPD, late-onset Parkinson's disease.

Table 3

Frequencies of GBA mutations in patients with PD and controls.

Mutation	EOPD N (%)	LOPD N (%)	Controls N (%)
p.N370S	2 (0.4)	2 (0.6)	1 (0.3)
p.L444P	13 (2.5)	4 (1.2)	2 (0.5)

EOPD, early-onset Parkinson's disease; LOPD, late-onset Parkinson's disease.

Table 4

Rare variants in exome sequences of *LRRK2* and *SMPD1*.

Gene	Position	Ref	Alt	dbSNP	Amino acid change	PredictSNP		Patients with variant (n)	Finnish alleles in ExAC (n)	
						Effect	Accuracy (%)		All	With variant
Variants not present in controls										
SMPD1	6413367	G	A	n.a.	p.E358K	N	63	1	6558	0
SMPD1	6415566	G	A	rs113467489	p.R542Q	D	65	1	6614	0
LRRK2	40693018	G	T	n.a.	p.C1152F	N	75	1	n.d.	n.d.
LRRK2	40713842	C	T	rs201637880	p.S1627L	N	83	1	n.d.	n.d.
LRRK2	40713845	G	C	rs33949390	p.R1628P	D	72	2	6610	0

Ref, reference variant; Alt, alternative variant; dbSNP, reference SNP ID; n.a., not available; N, neutral; D, deleterious; n.d., not detected; ExAC, Exome Aggregation Consortium.