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Variants in the Niemann-Pick type C gene *NPC1* are not associated with Parkinson's disease

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Abstract

Biallelic variants in *NPC1*, a gene coding for a lysosomal transmembrane protein involved in cholesterol trafficking, may cause Niemann-Pick disease type C (NPC). A few cases of *NPC1* variant carriers with Parkinson's disease (PD) have been reported. In addition, pathological studies have demonstrated phosphorylated alpha-synuclein and Lewy pathology in brains of NPC patients. Therefore, we aimed to examine whether *NPC1* genetic variants may be associated with PD. Full sequencing of *NPC1* was performed in 2,657 PD patients and 3,647 controls from three cohorts, using targeted sequencing with molecular inversion probes. A total of 9 common variants and 126 rare variants were identified across the three cohorts. To examine their association with PD, regression models adjusted for age, sex and origin were performed for common variants, and optimal sequence Kernel association test (SKAT-O) was performed for rare variants. After correction for multiple comparisons, common and rare *NPC1* variants were not associated with PD. Our results do not support a link between heterozygous variants in *NPC1* and PD.

Keywords

Parkinson's disease; *NPC1*; Lysosomal genes; Niemann-Pick disease type C

1. Introduction:

Variants in several genes that are associated with lysosomal storage disorders have been implicated in PD (Alcalay et al., 2018; Nalls et al., 2019; Robak et al., 2017). Homozygous or compound heterozygous (biallelic) mutations in the glucocerebrosidase (*GBA*) gene result in Gaucher disease. Patients with Gaucher disease as well as heterozygous carriers of *GBA* variants are at increased risk for PD (Sidransky et al., 2009). Similarly, biallelic mutations in the lysosomal enzyme gene *SMPD1* may cause Niemann-Pick disease types A and B, and heterozygous variants are associated with risk for PD (Alcalay et al., 2019; Gan-Or et al., 2013). *NPC1* is a lysosomal gene that codes a transmembrane protein involved in

cholesterol trafficking (Cruz et al., 2000). Biallelic mutations in *NPC1* may cause Niemann-Pick disease type C, a lysosomal storage disorder (Chiba et al., 2014). Several reported PD cases carrying a mutation in *NPC1* have suggested that *NPC1* mutations could be a risk factor for developing PD (Josephs et al., 2004; Kluenemann et al., 2013; Schneider et al., 2019). The presence of alpha-synuclein in Lewy bodies, the pathological hallmark of PD, have also been documented in post-mortem brain studies of several Niemann-Pick type C patients (Chiba et al., 2014); (Saito et al., 2004). To study the association between heterozygous variants in the *NPC1* gene and risk of PD, we fully sequenced *NPC1* in a total of 2,657 PD patients and 3,647 controls, and examined the association of both common and rare variants with PD. Full version of the introduction with references can be found in the supplementary full version of the paper.

2. Methods

The study population included 2,657 patients with PD and 3,647 controls from three cohorts (Table 1), collected at McGill University (Quebec, Canada and Montpellier, France), Columbia University (New York, NY), and Sheba Medical Center (Israel). The entire coding sequence of the *NPC1* gene, including exon-intron boundaries and the 5' and 3' untranslated regions, was targeted using molecular inversion probes (MIPs, Supplementary Table 1). The association between common *NPC1* variants and PD was examined by using logistic regression adjusted for age and sex as covariates in all cohorts, and AJ ancestry as an additional covariate in the NY cohort. To analyze rare variants (minor allele frequency, MAF) < 0.01), an optimized sequence Kernel association test (SKAT-O) was performed (Lee et al., 2012). SKAT-O was performed for the whole gene, coding and non-coding variants and by comparing synonymous, nonsynonymous, stop frameshift and splicing variants. SKAT-O was also done separately on rare variants with Combined Annotation Dependent Depletion (CADD) score of ≥ 12.37 representing the top 2% of potentially deleterious variants (Amendola et al., 2015). All local IRBs approved the protocols and informed consent was obtained from all individual participants before entering the study. A more detailed methods section be found in the supplementary full version of the paper.

3. Results

The average coverage of the *NPC1* gene was 880X, with 100% of nucleotides covered at >15X, and 95.7% at >50X in the McGill cohort, 832X, with 100% at >15X and 95,7% at >50X in the Columbia cohort and 1108X, with 100% at >15X and 95.7% at 50X in the Sheba cohort. None of the common variants was associated with PD in all 3 cohorts after Bonferroni correction (Supplementary Table 2). All rare nonsynonymous, loss-of-function and variants affecting splice sites from the three cohorts are detailed in Supplementary Table 3. In the McGill cohort we found 3 variants that are known as pathogenic for NPC, and 4 variants with conflicting data but likely pathogenic. All these variants except one intronic splice variant were found only in controls. In the Sheba cohort we found 1 likely pathogenic variant for NPC in a control subject. SKAT-O revealed a nominal association between all rare variants and PD (p=0.04) in the McGill cohort, which was mainly driven by synonymous intronic variants. This association was not statistically significant after correction for multiple comparisons. When including only nonsynonymous, splice and loss-

of-function variants, or variants with high CADD score, no association was found in any of the cohorts (Supplementary Table 4). The association between two *NPC1* variants that have previously been suggested to be involved in PD, p.Asn222Ser and p.Ser1004Leu, was not replicated in our cohorts (Table 2). A full version of the Results can be found in the supplementary full version of the paper.

4. Discussion

In the current study, we did not find any associations between common and rare variants in *NPC1* and risk of PD, suggesting that *NPC1* does not have a major role in PD in our cohorts. Other lysosomal genes involved in lysosomal storage disorders and in PD, such as *SMPD1*, *ASAH1*, *GALC* and potentially *GLA*, are all directly involved in the *GBA* glycosphingolipid metabolism pathway within the lysosome (Senkevich and Gan-Or, 2019). The lack of association with PD of *NPC1*, involved in cholesterol trafficking and metabolism, and other genes outside of the *GBA* pathway (Robak et al., 2017) may suggest that PD may be specifically associated with the *GBA* glycosphingolipid metabolism pathway.

A recent review (Schneider et al., 2019) summarized cases of patients with neurodegenerative diseases including PD who carried *NPC1* variants. We found in our cohorts a total of 66 carriers of p.Asn222Ser (rs55680026) and p.Ser1004Leu (rs150334966), that have been previously suggested to be associated with PD. Both variants are not very rare and were either equally common or more common in our controls (Table 2). This likely rules out a role for these variants in PD and suggests that their presence in previously reported PD patients may represent chance findings.

Our study has several limitations. In our cohorts, differences between PD patients and controls in sex and age are significant. To address this limitation, we adjusted the regression model with age and sex as covariates. In addition, the cohorts studied here consist of relatively homogeneous populations such as Ashkenazi Jews and French Canadians. Thus, additional studies in other populations are required.

In our study, all but one of the *NPC1* pathogenic mutations known to cause NPC or potentially pathogenic variants were identified only in controls. Nevertheless, we cannot rule out that very rare disease-causing mutations in *NPC1*, may be associated with PD. A full version of the Discussion can be found in the supplementary full version of the paper.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Study population and individuals included in the analysis

Sequenced				Analyzed					
				Cases			Controls		
Cohort	**N (patients)**	**N (controls)**	**Depth of Coverage**	**N**	**Mean age, (SD)**	**Male, %**	**N**	**Mean age, (SD)**	**Male, %**
McGill	1026	2588	>15x	973	65.5 (10.4)	62.8	2473	53.2 (14.22)	47.2
			>50x	886	65.4 (10.3)	62.2	2335	53.8 (13.95)	47.1
Columbia	1026	525	>15x	985	64.8 (10.9)	63.9	507	63.5 (10.2)	37.1
			>50x	936	65.5 (10.9)	63.9	479	64.3 (10,0)	36.7
Sheba	605	534	>15x	586	63.8 (11.0)	61.4	498	33.9 (7.2)	56.8
			>50x	582	63.3 (11.3)	61.5	472	33.9 (7.3)	58.2

N, number; SD, standard deviation

Table 2.

Variants suggested to be involved in PD from previously published studies.

SNP	nt change	AA change	Distribution in our cohorts	Data from the literature
rs55680026	c.A665G	p.Asn222Ser	McGill 11 (1.24%) PD / 28 (1.1%) controls	1 PD patient male 55 age at onset, 65 age at sampling (classical PD) CBD-like phenotype AAO- 32, age at sampling 62, main symptoms: depression, later dementia, epilepsy, slight hepatomegaly
			Columbia 7 (0.75%) PD / 8 (1.67%) controls	
			Sheba 0 PD / 0 controls	
rs150334966	c.C3011T	p.Ser1004Leu	McGill 3 (0.34%) PD / 7 (0.29%) controls	2 PD patients with late onset (Age at onset 79 and 87)
			Columbia 0 PD / 2 (0.41%) controls	
			Sheba 0 PD / 0 controls	

nt, nucleotide; AA, amino acid