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Strong association between glucocerebrosidase mutations and Parkinson's disease in Sweden

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ABSTRACT

Several genetic studies have demonstrated an association between mutations in glucocerebrosidase (*GBA*), originally implicated in Gaucher's disease, and an increased risk of Parkinson's disease (PD). We have investigated the possible involvement of genetic *GBA* variations in PD in the Swedish population. Three *GBA* variants, E326K, N370S, and L444P were screened in the largest Swedish Parkinson cohort reported to date; 1625 cases and 2025 control individuals. We found a significant association with high effect size of the rare variant L444P with PD (odds ratio 8.17; 95% confidence interval: 2.51−26.23; *p*-value = 0.0020) and a significant association of the common variant E326K (odds ratio 1.60; 95% confidence interval: 1.16−2.22; *p*-value = 0.026). The rare variant N370S showed a trend for association. Most L444P carriers (68%) were found to reside in northern Sweden, which is consistent with a higher prevalence of Gaucher's disease in this part of the country. Our findings support the role of *GBA* mutations as risk factors for PD and point to lysosomal dysfunction as a mechanism contributing to PD etiology.

1. Introduction

The human glucocerebrosidase gene (*GBA*) is located on chromosome 1q21 and encodes a lysosomal protein, which cleaves the beta-glycosidic bond of glucosylceramide, an intermediate formed during glycolipid metabolism (Brady et al., 1965). Mutations in *GBA*, originally implicated in Gaucher's disease (GD), have been identified both in familial and sporadic Parkinson's disease (PD; Balicki and Beutler, 1995; Lwin et al., 2004). These mutations also seem to influence the risk of developing Lewy body dementia and Lewy body dementia with Alzheimer-like neuropathologic changes, but do not associate with Alzheimer's disease (Clark et al., 2009; Tsuang et al., 2012). Genetic alterations of *GBA*, α-synuclein (*SNCA*), and leucine-rich repeat kinase 2 (*LRRK2*) together constitute the most common known genetic risk-factors for sporadic PD today (Ran and Belin, 2014).

Genetic variations in *GBA* are known to alter glucocerebrosidase (GCase) activity and decreased GCase activity, and protein levels have repeatedly been reported in brain tissue from PD patients with *GBA* mutations (Gegg et al., 2012; Lwin et al., 2004; Mazzulli et al., 2011). Interestingly, PD patients without *GBA* mutations were recently reported to have lower GCase activity as compared with healthy controls, suggesting *GBA* is involved in the pathologic mechanisms of PD even in the absence of known *GBA* gene

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mutations (Alcalay et al., 2015; Chiasserini et al., 2015; Murphy et al., 2014). The most frequent mutations in *GBA* reported to be associated with increased risk of PD are L444P and N370S (Sidransky et al., 2009). The associations of these 2 mutations with PD have been observed in populations with diverse genetic backgrounds and represent over 50% of the pathogenic *GBA* mutations found in patients globally (Sidransky et al., 2009). These findings were verified in a large European study comprising almost 1400 sporadic and familial PD patients, where L444P or N370S were found in 70% of the *GBA* mutation carriers (Lesage et al., 2011). Many other *GBA* mutations have been reported to influence the risk of developing PD, and the frequency of each variant differs globally according to genetic background (Gan-Or et al., 2015; Sidransky et al., 2009). In particular, *GBA* mutations are more common in subjects with Ashkenazi Jewish ancestry (Aharon-Peretz et al., 2004; Gan-Or et al., 2008; Sidransky et al., 2009).

Not only rare mutations but also single nucleotide polymorphisms (SNPs) such as E326K have been suggested to affect the risk of developing PD (Clark et al., 2007; Lwin et al., 2004). E326K has been reported to associate with PD in European populations, although there are conflicting results (Lesage et al., 2011; Sidransky et al., 2009). The association with E326K is particularly strong in PD patients with early disease onset and suggested to result in an aggravated phenotype with cognitive decline (Duran et al., 2013; Mata et al., 2015). GCase activity measured in post-mortem brains of PD patients heterozygous for E326K ranged between 90% and 100% of the activity in wild-type carriers (Lwin et al., 2004), but in combination with other *GBA* mutations, for example L444P, the E326K mutation leads to further deterioration of enzymatic activity (Chabas et al., 2005; Montfort et al., 2004).

GBA mutations have not frequently reached significance in genome-wide association studies (GWAS), probably because of the low minor allele frequencies of PD associated *GBA* mutations and the methodological difficulties in avoiding contaminating signals from the pseudogene *GBAP1* (glucosidase, beta, acid pseudogene 1). Other factors that might influence the outcome of a GWAS are which genetic markers are included and how these are linked to disease associated variants. Nevertheless, 2 GWAS studies published in 2011 and 2012 reported association between PD and N370S, and one of them further supports the E326K association (Do et al., 2011; Pankratz et al., 2012). These data have also been confirmed in large meta-analyses (Lill et al., 2012; Nalls et al., 2014).

Parkinson patients carrying heterozygous *GBA* mutations are characterized by relatively early disease onset, but are otherwise clinically similar to patients with sporadic PD and respond well to L-DOPA (Clark et al., 2007). However, patients with *GBA* mutations have been reported to be more likely to suffer from nonmotor symptoms, in particular cognitive impairment and dementia (Alcalay et al., 2012; Mata et al., 2015; Seto-Salvia et al., 2012). To gain insight into the role of *GBA* in the etiology of PD in the relatively homogenous Swedish population, we investigated the presence of 3 most reported nonsynonymous *GBA* variants in PD, N370S, L444P, and E326K, among Swedish Parkinson patients and controls. In the light of the particularly elevated prevalence of *GBA* mutations in individuals with Ashkenazi Jewish descent, the Swedish population, which has not previously been screened for GBA mutations, is especially interesting to study as there is a known occurrence of GD type III (Norrbottnian type) in northern Sweden (Dahl et al., 1990).

2. Materials and methods

2.1. Human DNA and tissue

A total of 1625 Swedish PD patients were recruited from the neurology clinics at Karolinska University Hospital, Stockholm, Sahlgrenska University Hospital, Gothenburg, Skåne University Hospital, Lund, Umeå University Hospital, Umeå and Linköping University Hospital, Linköping. Patient material was obtained after informed consent and approval of the local ethics committees in Stockholm, Gothenburg, Lund, Umeå, and Linköping, respectively (http://www.epn.se). Information on cognitive decline was only available for patients from Lund and was reported for 33 of the 122 patients (27.9%) which were also included in the genetic screening. The PD patients were diagnosed according to the United Kingdom Parkinson's Disease Society Brain Bank criteria for idiopathic PD, including patients who declared having one or more first, second, or third degree relatives with PD to get a large material (Gibb and Lees, 1988). The 2025 Swedish control subjects were recruited from the corresponding catchment areas as the patient material and consisted of spouses of PD patients, individuals visiting the neurology clinic, blood donors, and subjects recruited from an ongoing longitudinal study, SNAC-K (The Swedish National Study on Aging and Care in Kungsholmen, http://www.snac-k.se/), see Table 1 for further site specific demographic information. Screenings of known PD mutations in *LRRK2* and *SNCA* have been performed previously on a subset of the joint case-control material (Carmine Belin et al., 2006; Puschmann, 2011; Westerlund et al., 2008). All subjects were unrelated and a majority of Caucasian origin (>95%). DNA was extracted from whole blood according to standard protocols.

2.2. Genotyping

2.2.1. Pyrosequencing

The 3 genetic *GBA* variants were genotyped by pyrosequencing (Ronaghi et al., 1998), except for a fraction of samples which were genotyped for N370S with TaqMan (see paragraph 2.2.2 on TaqMan SNP genotyping for details). Primer sequences are available on request (Thermo Scientific, MA, USA). Primers were designed using free online software (Primer 3 v4.0.0 and mFold v3.6; Koressaar and

Table 1
Demographic data for the Parkinson patients and control populations

Variable	Gothenburg		Linköping		Lund		Stockholm		Umeå	
	PD	CTRL	PD	CTRL	PD	CTRL	PD	CTRL	PD	CTRL
Individuals, *n*	171	190	195	366	122	43	528	1271	609	155
Mean age at enrollment, y	68.2	69.1	71.4	70.1	69.9	67.9	67.4	71.6[b]	68.9	64.1
Mean age at diagnosis, y	59.0	NA	63.6	NA	61.9	NA	58.8[a]	NA	62.9	NA
Females, *n* (%)	74 (43.3)	120 (63.2)	74 (38.0)	185 (50.5)	48 (37.7)	27 (62.8)	193 (36.55)	631 (49.9)	225 (36.9)	77 (49.6)
Heredity, *n* (%)	41 (24.0)	NA	42 (21.5)	NA	68 (54.8)	NA	125 (36.8)[a]	NA	121 (19.9)	NA

Heredity was defined as having one or more first, second, or third degree relatives with PD.
Key: CTRL, control subjects; NA, data not applicable; PD, Parkinson's disease.
[a] Calculation based on 355 individuals for whom this information was available.
[b] Calculation based on the 416 individuals for whom this information was available, the remaining 855 controls were blood donors.