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The Glucocerobrosidase E326K Variant Predisposes to Parkinson's Disease, But Does Not Cause Gaucher's Disease

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Abstract

Background—Heterozygous loss-of-function mutations in the acid beta-glucocerebrosidase (*GBA1*) gene, responsible for the recessive lysosomal storage disorder, Gaucher's disease (GD), are the strongest known risk factor for Parkinson's disease (PD). Our aim was to assess the contribution of *GBA1* mutations in a series of early-onset PD.

Methods—One hundred and eighty-five PD patients (with an onset age of ≤50) and 283 age-matched controls were screened for *GBA1* mutations by Sanger sequencing.

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Results—We show that the frequency of *GBA1* mutations is much higher in this patient series than in typical late-onset patient cohorts. Furthermore, our results reveal that the most prevalent PD-associated *GBA1* mutation is E326K, a variant that does not, when homozygous, cause GD.

Conclusions—Our results confirm recent reports that the mutation, E326K, predisposes to PD and suggest that, in addition to reduced GBA1 activity, other molecular mechanisms may contribute to the development of the disease.

Keywords

GBA; E326K; Parkinson's disease; Gaucher's disease; early onset

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A large number of Mendelian genetic loci have been described for Parkinson's diseases (PD; OMIM#168600)[1] and genome wide association studies (GWAS) have also identified many common, low-risk variants.[2] However, the most prevalent genetic risk factor identified to date is heterozygous loss-of-function variants in the acid beta-glucocerebrosidase gene (*GBA1*).[3] Homozygous or compound heterozygous loss-of-function mutations in *GBA1* cause Gaucher's disease (GD) (OMIM 606463), a recessive lysosomal storage disorder with multisystem involvement.[4]

The association between PD and *GBA1* was first identified through the observation of parkinsonism and Lewy body (LB) pathology among GD cases and their first- and second-degree relatives.[5]

So far, genetic analysis has suggested that all pathogenic *GBA1* mutations, causing GD, can equally predispose to PD. Therefore, the assumption has been that variants that, when homozygous, did not lead to GD would not predispose to PD.[3]

We have embarked on a systematic analysis of the relationship between GD, PD, and other LB disorders and the *GBA1* gene.[6-8] As part of this study, we performed a complete sequence analysis of the open reading frame (ORF) of the gene by Sanger sequencing in a cohort of 185 PD cases with an age at onset of ≤50 years. We compared these data with those obtained from 283 controls (these latter overlapped with our recent report[6]) and with a similar analysis of 73 patients with type 1 GD from the same geographic region.[9]

Patients and Methods

Subjects

The PD patients were a group of 185 unrelated individuals with an onset age of ≤50 years who came in to the movement disorders clinics at the National Hospital for Neorology and Neurosurgery (London, UK). They all met the UK Brain Bank Clinical Criteria for PD.[10] Average age at onset was 40 ± 7.2 years (range, 16–50).

A total of 27.5% of patients (51 of 185) reported a family history compatible with PD in at least a first- or second-degree relative. All the patients in this series were previously screened for mutations in *parkin* and genotyped for the common *LRRK2* mutation, G2019S.

Controls were age-matched subjects. This series of controls has been previously reported,[6] with the addition of 26 age-matched spouse controls. Controls were examined and interviewed by experienced neurologists to rule out signs and symptoms suggestive of PD. All controls were of UK Caucasian origin, and no individual reported an Ashkenazi background.

GD patients were 73 unrelated subjects, affected with the type 1 form of the disease (non-neuronopathic), attending the national GD clinics at the Royal Free and Addenbrookes hospitals. 16 were Ashkenazi Jewish (22%) and 4 had Eastern European ancestry (5.7%); the remainder were white UK citizens without Ashkenazi ancestry.

Written informed consent was taken from each participant; this study was approved by the North West London and Cambridge University hospitals ethics committees.

DNA Sequencing

The ORF (11 exons) of the *GBA1* gene was entirely sequenced in cases and controls, as we have previously described.[6,7] The sequence reference and exon numbering were those of GenBank accession number NM_000157.3.

Results

The results of this analysis are summarized in Tables 1 and 2.

They are surprising in two ways. The first is that, in this population of early-onset PD, a very high proportion of cases carried a *GBA1* variant (25.94%; 48 of 185 individuals; odds ratio [OR] for carriers of any variant in cases versus controls: 7.9; 95% confidence interval [CI]: 4.1–15.4; $P < 0.0001$).

Among those, 22 cases had a known GD-causing pathogenic mutation (19 with one mutation and 3 with two mutations), a proportion far higher than in late-onset PD from the same population we have recently reported (11.9%; 22 of 185 individuals versus 3.8%; 30 of 790; $P = 0.0001$).[6] Notably, 2 PD patients were homozygous, respectively, for N370S and R463C, and despite both genotypes being known to cause GD, they did not display any GD features.

Nevertheless, the clinical spectrum in GD is wide, and some studies suggest that patients homozygous for mild mutations, such as N370S or R463C, may never develop the disease.[11,12] Moreover, subjects with two pathogenic *GBA1* mutations, affected with typical PD but showing no signs of GD, have already been described.[13]

The second surprising discovery is that, by far, the most common variant in the PD cases was E326K, which had a frequency of 7.57% in cases (14 of 185 individuals; 12 cases were heterozygote, including 1 in combination with L444P and 2 homozygote) and 2.47% in controls (7 of 283 individuals). To further confirm this observation, an additional 202 controls were screened for just this variant and the frequency in these samples was 2.97% (6 of 202). The total frequency in controls, taking into account both sets, was 2.68% (13 of 485 individuals), indicating a significant association of this variant with PD ($P = 0.0059$; OR,

2.97; 95% CI: 1.3–6.4). In contrast, this variant did not occur in the GD cohort from the same population,[9] consistent with previous reports that this variant does not segregate with GD.[14]

Indeed, the 2 PD cases homozygous for the E326K variant did not have any of the clinical or hematological features of GD, and their white blood cell GBA1 enzymatic activity was at the lower limit of normal range.

It should be noted that we found other variants in our PD series that have not been found in GD cases and that, collectively, these were more common in cases than in controls. Although further population studies and functional analyses are needed, our results suggest that there may be other mutations that predispose to PD, but are not associated with GD (Table 1). The possible effect of these variants on protein function was evaluated by *in silico* prediction tools (Supporting Materials).

Among the PD carriers of the E326K, all of which were British with no reported Ashkenazi Jewish origin, none had *parkin* mutations, whereas 1 carried also the common *LRRK2* mutation, G2019S. This patient developed PD symptoms when he was 35, much earlier than the average age at onset of the LRRK2 mutation carriers,[15] hinting at a possible synergistic effect of the two variants on the onset anticipation.

Discussion

Herein, we report the largest series of early-onset PD cases in which *GBA1* was entirely sequenced, and we show that the frequency of *GBA1* mutations is much higher than in late-onset patients from the same geographic area. Interestingly, the *GBA1* variant, E326K, is, by far, the most common variant found in this cohort.

The possibility that the E326K may be associated with PD has been raised before, but dismissed, because in previous studies, it was considered a benign polymorphic variant and the association with PD was not as strong as we report in this study.[13,16] Our results put this uncertainty beyond doubt and show that indeed this variant does predispose to PD. Consistent with this finding, a similar result was recently published by Pankratz et al. as part of a large meta-analysis of the existing available PD GWAS datasets, in which the E326K reached genome-wide significance (OR, 1.71; $P = 5 \times 10^{-8}$) and hence was indicated as a susceptibility allele for PD.[17]

These positive results are in contrast with the large multicenter analysis of *GBA1* in PD from Sidransky et al., which did not show this association.[3] This discrepancy could be explained by the heterogeneity of populations included in that study, because it was enriched with individuals from populations (Asian, Jewish, and Portuguese) in which the E326K is absent or very rare.[18-20]

Although biochemical observations have suggested that the E326K is a subtle loss-of-function variant,[21,22] this reduction of the activity is clearly not severe enough to lead to disease in the tissues where GD is associated with lysosomal storage dysfunction. Nevertheless, when E326K is found in the same allele with another mutant variant, such as

L444P, N188S, or N370S, it does not act as a neutral allele and instead contributes to GD severity by further reducing the residual enzymatic activity.[23-26]

We propose that the association of the E326K with PD and its absence in the GD cohort, together with the presence of many other novel, rare *GBA1* variants in the PD group, which are not associated with GD, might suggest a dissociation of the pathogenic molecular mechanisms that underlie these two diseases.

However, it is not clear whether the mild loss of function of the allele, E326K, is the cause of the increased risk for PD or whether other, still unknown, effects of the mutation on the lysosomal machinery are contributing to neuron degeneration. Recent studies from our laboratories suggest that heterozygous *GBA1* mutations may accelerate the pathogenesis of PD through a gain-of-function effect that may include endoplasmic reticular stress.[27]

We propose to investigate the molecular mechanisms potentially involved in the pathogenesis of PD of this interesting GBA mutation in cultured skin fibroblasts derived from patients. We suggest it could be a suitable cell model to characterize the molecular bases of GBA contribution to PD.

Conclusion

In conclusion, our study strong links the allele E326K with early-onset PD in a sample of UK individuals and reflects the need to accurately explore the contribution of this and other *GBA* rare variants to the increased risk of PD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Proportion of cases and controls with *GBA* mutations and OR

***GBA* Variants**	**% in PD Cases (n = 185)**	**% in Controls (n = 283)**	**% in Type 1 GD Cases (n = 73)**	**OR (95% CI)**
All GBA variants	25.94 (48)	4.24 (12)	100 (73)[f]	7.9 (4.1–15.4)
All pathogenic GBA variants	20 (37)	3.18 (9)	100 (73)	7.6 (3.57–16.20)
N370S	2.70 (5)	0.35 (1)	86.30 (63)	7.8 (0.9–67.6)
L444P	1.08 (2)	0(0)	24.66 (18)	ø
Rec*Nci*I (L444P+A456P+V460V)	1.6(3)	0 (0)	10.96 (8)	ø
R463C	1.62 (3)	0 (0)	5.48 (4)	ø
E326K	7.57 (14)	2.47 (7)[a]	0(0)	3.2 (1.2–8.1)
Other pathogenic mutations	5.4 (10)[b]	0.35 (1)[c]	45.2 (33)	16.1 (2.04–126.97)
Other variants with unproven pathogenicity	7.03 (13)[d]	1.06 (3)[e]	0 (0)	7.0 (1.9–25.1)

Allele names refer to the processed protein, excluding the 39-residue signal peptide. The number of carriers for each *GBA* variant is in brackets. ø means that OR cannot be calculated because all carriers were cases (divide by zero).

[a] Additionally, a further 202 individuals were screened for just this variant and the frequency in these additional samples was 2.97% (6 of 202). The total frequency, taking into account both sets of controls, was 2.68% (13 of 485 individuals). Furthermore, the frequency in Caucasians of this variant, as reported by the Exome Variant Server database (http://evs.gs.washington.edu/EVS/), is 2.36% (82 of 3,469 individuals).

[b] IVS2+1 (1), R131C (2), W184R (1), N188S (1), H255Q (1), R257Q (1), D409H (2), and RecTL (1).

[c] R257Q (1).

[d] E388K (1), G113A (2), T369M (1), and S465P (1) have been previously reported in both cases and controls; L(−14)V (1), V172L (2), S177T (1), L217P (1), L317L (1), L354P (1), V375G (1), IVS10-4 C>T(1), and IVS10-12 C>T (1) and are novel variants.

[e] E340A (1), T369M (1), and V458L (1).

[f] In 2 patients, a second mutation could not be identified. Both showed a clear enzymatic deficiency and a clinical diagnosis fully consistent with type 1 GD.

Table 2

Genetic, demographic, and clinical information of patients with GBA variants

Patient	Gender	Genotype	Age at Onset	Familial History	Parkin	LRRK2
1	M	R463C/wt	45	Yes	–	–
2	M	N370S/wt	46	Yes	–	–
3	F	E326K, L444P[a]	34	No	–	–
4	F	E326K/wt	36	NA	–	–
5	F	R257Q/wt	42	No	–	–
6	F	RecTL/wt	44	No	–	–
7	M	E388K/wt	37	NA	–	–
8	M	E326K/E326K	42	Yes	–	–
9	M	RecNcil/wt	41	No	–	–
10	F	D409H/wt	40	No	–	–
11	F	T369M, IVS10 −12 C>T[a]	39	NA	p.P437L/wt	–
12	M	E326K/E326K	38	Yes	–	–
13	F	IVS2+1 G>A/wt	42	No	–	–
14	M	R463C/R463C	45	Yes	–	–
15	M	L217P/wt	42	No	–	–
16	F	N188S/wt	49	NA	–	–
17	M	IVS10 −4C>T/wt	29	No	Deletion of exons 4,5, and 6	–
18	M	E326K/wt	50	Yes	–	–
19	M	L444P/wt	49	No	–	–
20	M	E326K/wt	35	No	–	p.G2019S/wt
21	M	N370S/wt	48	Yes	–	p.G2019S/wt
22	F	E326K/wt	44	No	–	–
23	F	E326K/wt	49	Yes	–	–
24	F	E326K/wt	44	No	–	–
25	F	G113A/wt	37	Yes	–	–
26	M	S465P/wt	45	No	–	–
27	M	E326K/wt	42	Yes	–	–
28	F	V375G/wt	50	No	–	–
29	M	R463C/wt	42	NA	–	–
30	F	RecNcil/wt	31	NA	p.P437L/wt	–
31	F	L354P/wt	38	NA	–	–
32	F	L317L/wt	40	NA	–	–
33	M	RecNcil/wt	39	NA	–	–
34	M	S177T, V172L[a]	38	Yes	–	–
35	M	E326K/wt	21	NA	–	–
37	M	L(−14)V/wt	30	No	–	–
38	F	V172L/wt	45	Yes	–	–
39	M	N370S/N370S	48	No	–	–

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Patient	Gender	Genotype	Age at Onset	Familial History	Parkin	LRRK2
40	F	H255Q, D409H[a]	38	Yes	–	–
41	F	R131C/wt	43	No	–	–
42	M	W184R/wt	36	Yes	p.Pro113fs/p.Gly430Asp	–
43	M	E326K/wt	40	No	–	–
44	M	E326K/wt	45	No	–	–
45	M	N370S/wt	35	No	–	–
46	M	R131C/wt	50	NA	–	–
47	F	E326K/wt	29	No	–	–
48	M	N370S/wt	27	NA	c.535-9T>A	–

Age at onset is expressed in years. Dashes represent negative. Patients 14 and 39 did not have GD symptoms.

Abbreviations: M, male; F, female; NA, data not available; ND, not done; wt, wild type.

[a]Unknown phase.