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Increased yield of full *GBA* sequencing in Ashkenazi Jews with Parkinson's disease

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Abstract

Background—Variants in *GBA* are the most common genetic risk factor for Parkinson's disease (PD), and are especially prevalent in the Ashkenazi Jewish (AJ) population. However, most studies on *GBA* in AJ genotype only seven selected Gaucher-associated pathogenic variants rather than sequencing the whole gene, which may leave carriers of PD-associated *GBA* variants undiscovered.

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Methods—*GBA* was fully sequenced using molecular inversion probes (MIPs) and Sanger sequencing in 735 AJ PD patients and 662 AJ controls, from Israel and New York. Additional AJ control data (n=3044) from the Inflammatory Bowel Disease Exome Portal was used.

Results—Full *GBA* sequencing increased the number of variants discovered by 17.4%, compared to targeted genotyping. An additional 17 PD patients were identified with *GBA*-associated PD. The p.E326K variant was found in 1.6% of AJ PD patients, making it the second most common PD-associated *GBA* variant in AJ. *GBA* variants were found in 18% of PD patients and 7.5% of controls (OR=2.7, 95%CI=1.9-3.8, p<0.0001).

Conclusion—Without full sequencing of *GBA*, or at minimum including p.E326K in the genotyping panel, a significant proportion of variant carriers go undiscovered and may be incorrectly assigned as non-carriers in studies or clinical trials.

INTRODUCTION

GBA variants are the most common genetic factors which lead to PD, and in different populations they are found in 5-20% of PD patients.[1] The association between *GBA* variants and PD was initially described in a study on brains of PD patients,[2] and later in the Ashkenazi-Jewish (AJ) population,[3, 4] but they are important in numerous other populations.[5] *GBA* pathogenic variants can be classified as severe or mild based on their association with Gaucher's disease (GD); severe variants are those that in homozygous carriers lead to the severe forms of GD, type II and type III, while mild variants lead to the mild type I GD.[1] Severe and mild *GBA* variants have different effects on risk for PD, age at onset,[1] and disease progression.[6, 7] In the AJ population, the most common *GBA* variant is p.N370S, but in other populations the p.E326K substitution is the most common PD-associated variant.[1] Interestingly, the p.E326K variant does not cause GD in homozygous carriers.[8]

Thus far, genetic and genotype-phenotype studies in the AJ population were based on genotyping of specific *GBA* variants – p.N370S, p.L444P, p.V394L, p.R496H, IVS 2+1 G>A, dup84G and RecTL (also termed Rec370), all linked to GD.[1–4] Therefore, the contribution of the p.E326K variant and other *GBA* variants to PD in the AJ population is not known, and it is possible that by genotyping only specific variants, a substantial portion of *GBA* variant carriers remains genetically undiagnosed. As *GBA*-specific PD therapies are in development, it will be crucial to identify all PD patients with *GBA* variants. In addition, full *GBA* sequencing will allow for more accurate genotype-phenotype studies, as it will better discriminate between carriers and non-carriers.

In the current study, we sequenced the entire *GBA* gene in two cohorts of AJ patients and controls, and examined the role of additional *GBA* variants in this population.

METHODS

Study population

A total of 735 PD patients and 662 controls, all unrelated and of full AJ ancestry, were recruited in two centers, Sheba Medical Center in Tel-Hashomer, Israel and Columbia

University Medical Center in New York, NY. The Sheba Medical Center cohort included 517 patients (62.1% men, average age at enrollment of 61.72 ± 11.89 years) and 563 young controls (57.1% men, 33.68 ± 7.92 years) that participated in routine genetic screening or were counseled at the Genetic institute at the Sheba Medical Center for other purposes. The NY cohort included 517 patients and 252 controls that were previously published,[9] of which there were 218 patients (61.9% men, 68.94 ± 10.49 years) and 99 controls (40.4% men, 68.35 ± 8.77 years) of full AJ origin. In addition, to examine whether these control populations are representative of the general AJ population, we extracted data from the Inflammatory Bowel Disease (IBD) Exome Portal (http://ibd.broadinstitute.org),[10] which includes whole-exome sequencing data for 3044 non-IBD samples of AJ origin. Patients were diagnosed by movement disorder specialists according to the UK brain bank criteria (without excluding patients with family history of PD). Informed consent was obtained from all participants at enrollment, and the study protocol was approved by institutional ethics review boards.

GBA sequencing

DNA was extracted using a standard salting out protocol. For the Sheba Medical Center cohort, targeted next generation sequencing was performed, complemented by Sanger sequencing of exons 10 and 11. For the targeted sequencing, a total of 91 molecular inversion probes (MIPs) were designed as previously described.[11] The probes used for targeting *GBA* are detailed in supplementary table 1. The MIPs capture was performed as described by O'Roak et al.;[12] briefly, MIPs were hybridized to 100 ng of genomic DNA, circular DNA including the target regions was generated, linear DNA was degraded, and captured DNA was amplified by PCR. Captures were then pooled and sequenced using the Illumina HiSeq 2500 platform at the McGill University and Genome Quebec Innovation Centre. The full protocol is available upon request. To account for the reduced coverage of exons 10 and 11, these exons were amplified using Sanger sequencing as previously described.[13] In the NY cohort, *GBA* was sequenced as previously described,[9] and also went through the MIP sequencing for quality control purposes. Raw sequence data was processed using the Burrows-Wheeler aligner, the Genome Analysis Toolkit (GATK, v. 2.6.4), and ANNOVAR (annotation variant software), as previously described.[11] Variants from the targeted MIP sequencing were visualized using the Integrative Genomics Viewer (http://software.broadinstitute.org/software/igv/).[14] The Sanger sequencing chromatograms were analyzed using the Genalys 3.3b software.

Statistical analysis

As both cohorts consisted of individuals with 100% Ashkenazi Jewish origin, we pooled them for all statistical analyses. Fisher's exact test was used for comparison of carrier frequencies, and odds ratios and 95% confidence intervals were calculated. All statistical analyses were conducted in R.[15]

RESULTS

Sequencing coverage and quality control

The average coverage of *GBA* was >400× across all samples of the Sheba Medical Center cohort. Exons 10 and 11 had reduced coverage, so these exons were sequenced in all samples using Sanger sequencing, with 100% success rate. The NY cohort, for which *GBA* had been previously fully sequenced using Sanger sequencing,[9] also underwent the targeted MIPs sequencing. All variants that were previously found using Sanger sequencing on exons 1-9 were also identified using MIP sequencing, validating the method.

Increased yield of full *GBA* sequencing, and an important role for the p.E326K variant in the AJ population

Table 1 details the *GBA* variants found in the current study, their location and their nomenclature. Since in the mature glucocerebrosidase enzyme, encoded by *GBA*, a leader peptide of 39 amino acid was removed, there are different ways in which the variants are named in the literature. Table 1 details these names, and throughout the paper we use the most conventionally used nomenclature, of the mature enzyme without the 39 amino acids leader peptide.

Table 2 details the frequency of *GBA* variants found in patients (17.96%) and controls (7.54%, OR=2.7, 95% CI=1.9-3.8, p <0.0001) from both cohorts. Full sequencing of *GBA* increased the yield of detection of *GBA* variants by 17.4% (27 new variant carriers in PD patients and controls) compared to genotyping using the targeted kit; in the Sheba Medical Center cohort there were an additional 19 variant carriers on top of the 118 variant carriers with variants that are being regularly screened for in AJ (yield increased by 16.1%), and in the NY cohort, there were 8 additional variant carriers on top of 37 variant carriers (yield increased by 21.6%). The p.E326K variant, which is not regularly screened for in AJ PD patients, is the second most common *GBA* variant in the AJ PD patient population, found in 1.63% of patients. The p.E326K variant was found in 0.3% of our in-house controls (OR 5.5, 95%CI 1.2-24.6, p=0.01), and in about 0.9% of the IBD controls. Similar to previous reports,[1] the OR for PD for mild variant carriers was 2.4 (95% CI=1.6-3.5), compared to an OR of 12.9 (95% CI=1.7-98.0) for severe variant carriers. Interestingly, we found a surprisingly high frequency of the p.R44C variant in our control population (1.06%), however, in the IBD Exomes Portal AJ population it was only 0.18%, lower than the 0.27% in our PD cohort. Of the homozygous and compound heterozygous carriers of *GBA* variants, only one patient, with the p.N370S/p.L444P genotype, was previously diagnosed with type I GD.

DISCUSSION

Our results demonstrate that by sequencing the entire *GBA* gene in AJs, there is a substantial increase (17.4%) in the *GBA* variant detection rate. Most importantly, these results highlight the role of the p.E326K substitution in PD in the AJ population. Lastly, we identified a surprisingly high percentage of the p.R44C variant in our in-house controls, yet much lower frequency in the IBD exomes portal.

In recent years, numerous genotype-phenotype studies comparing PD patients with and without *GBA* variants were performed. Our results demonstrate that in the AJ population, in order to avoid a substantial proportion of *GBA* variant carriers erroneously classified as non-carriers, the entire coding region of *GBA* should be sequenced. Based on both our control population and the IBD Exomes Portal AJ population, up to 1.5% of the general AJ population may carry *GBA* variants that affect the risk for PD and are not screened for in the routinely used panels for GD. Without sequencing, these panels identify only about 80-85% of variant carriers in AJ. As the first clinical trials aimed specifically at PD patients with *GBA* variants are enrolling patients, full sequencing of *GBA* – or, at minimum, genotyping for p.E326K – will be crucial for identification of those who can benefit from participating in trials and from future treatment. This will be even more relevant in populations that are less homogeneous than the AJ populations, where the yield increase by full sequencing is expected to be even higher.

Of particular importance is the p.E326K substitution. This variant does not cause GD, but its association with PD is very clear, and it has odds ratios of 1.60-3.34 in different studies.[16–18] In these studies, the p.E326K variant was identified in 3-5% of PD patients,[16-18] and in the largest genome-wide association study performed to date,[19] the tagging variant rs35749011, which is in linkage disequilibrium with p.E326K, was found in about 4% of the patients.[19] Our study demonstrates that one out of 111-331 individuals in the general AJ population is a carrier of this variant, and that it is present in 1.6% of AJ PD patients. Thus, it is crucial to include this variant in any study performed on PD in the AJ population. This variant is important not only because of its high frequency in AJ PD patients, but also because of the genotype-phenotype correlations already demonstrated between the p.E326K substitution and disease progression, and its reduced enzymatic activity.[6, 7, 9, 17] It has been demonstrated that carrying *GBA* p.E326K is associated with more rapid progression of both motor and cognitive symptoms. Specifically, the p.E326K variant is associated with faster progression of UPDRS III and PIGD (postural instability and gait difficulty) scores over almost 3 years follow up, compared to non-carriers.[17] Despite having shorter disease duration, more p.E326K carriers progressed to mild cognitive impairment or dementia as compared to non-carriers.[17] Other studies also showed faster cognitive decline among carriers of the p.E326K variant.[6, 7]

The p.R44C substitution is of particular interest, as in our in-house controls the frequency of this variant was about 4 times higher than in PD patients, yet in the IBD exomes project AJ controls it was lower than in PD patients. Given the much larger population in the IBD exomes project, it is possible that the high frequency in our control population is due to chance. Of note, the carriers of this variant among controls are from different countries of origin and are not known to be related. Therefore, it will be important to examine the effect of the p.R44C variant in additional populations of AJ and other ancestries.

Out of the seven carriers of homozygous or compound heterozygous carriers of *GBA* variants, four PD patients had genotypes that may lead to type I GD – p.N370S/p.R496H (two PD patients), p.N370S/p.N370S (one PD patient) and p.N370S/p.L444P (one PD patient). Of those, only one patient, with the p.N370S/p.L444P genotype, was diagnosed with type I GD. This is consistent with previous reports in which PD patients with

homozygous or compound heterozygous *GBA* variants did not develop GD in most cases.[1, 5] This may suggest that in order to develop GD in carriers of these variants, other genetic or environmental modifiers are required.

Our study has several limitations; first, the control population from Sheba Medical Center is significantly younger than the patient population. However, this control population was selected to represent the general AJ population, and the purpose of the analysis was not to validate the well-established differences between patients and controls in *GBA* variant frequencies. Rather, the purpose of this study – for which this control population is adequate – was to examine how full sequencing of *GBA* increases the yield of variant detection even in a homogeneous population such as the AJ population. Another limitation is that the frequency of *GBA* variants in our PD patients and controls seems slightly different (albeit not significantly) than previously published. In our control population the carrier frequency was 7.5%, compared to 6.4% in a previous, large study that included 3,805 AJ controls.[1] This 1.1% difference can be entirely explained by the p.R44C substitution, which was not tested in the previous study. Furthermore, it is possible that some of the controls (recruited at early age without any clinical data), will develop PD in the future. In our patient population, the frequency of *GBA* variants was 18% compared to 19.2% in the previous study, which is not a statistically significant difference.[1] In part, this difference can be explained by not detecting the RecTL (or Rec370) allele in the current study that was reported in the previous study in 1% of PD patients.[1] The RecTL/Rec370 allele was suggested to be a recombinant allele that is in cis with p.N370S, and include the pseudogene sequence starting in exon 9. However, it was demonstrated that this specific allele is likely a false-positive read,[20] possibly due to amplification of the pseudogene in specific carriers of the p.N370S variant. Therefore, it is likely that the reported carriers of RecTL/Rec370 in previous studies carry only the p.N370S variant or no variant at all, and this needs to be further examined in future studies. Additional causes for this difference may be different structures of sub-populations within the different studies, or chance.

To conclude, our study highlights the importance of fully sequencing *GBA* in future studies of *GBA* in the AJ population, as well as the importance of the p.E326K variant in this population, and highlights the p.R44C variant, which needs to be further studied.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

GBA variants identified in the current study and their nomenclature.

Genomic Position	cDNA nucleotide substitution	Full length Protein	GBA variant
1:155209737	c.247C>T	p.Arg83Cys	p.R44C
1:155210451_155210452	c.84dupG	p.Leu29Alafs*18	84GG
1:155208006	c.680A>G	p.Asn227Ser	p.N188S
1:155206167	c.1093G>A	p.Glu365Lys	p.E326K
1:155206037	c.1223C>T	p.Thr408Met	p.T369M
1:155205634	c.1226A>G	p.Asn409Ser	p.N370S
1:155205592	c.C1268A	p.Ala423Asp	p.A384D
1:155205563	c.1297G>T	p.Val433Leu	p.V394L
1:155205514	c.1346C>T	p.Thr449Met	p.T410M
1:155205043	c.1448T>C	p.Leu483Pro	p.L444P
1:155204992	c.1499T>C	p.Leu500Pro	p.L461P
1:155204793	c.1604G>A	p.Arg535His	p.R496H

Table 2

Frequency of GBA variants by Parkinson's disease status

Variant	Severity[a]	PD patients (n=735), % (n)	Controls (n=662), % (n)	Odds Ratio (95% CI)	p-value	IBD Exomes Portal Controls, %
Heterozygous carriers						
GBA						
c.84dupG[b]	severe	1.5 (11)	0.15 (1)	10.1 (1.3-78.1)	<0.01	0.05
p.R44C	unknown	0.27 (2)	1.06 (7)	0.3 (0.1-1.2)	0.07	0.18
p.N188S	severe	0.14 (1)	0 (0)	NA	NA	0
p.E326K	benign[c]	1.63 (12)	0.3 (2)	5.5 (1.2-24.6)	0.01	0.9
p.T369M	benign[c]	0 (0)	0 (0)	NA	NA	0.6
p.N370S[b]	mild	11.84 (87)	5.58 (37)	2.3 (1.5-3.4)	<0.0001	5.9
p.A384D	unknown	0.14 (1)	0 (0)	NA	NA	0
p.V394L[b]	severe	0.14 (1)	0 (0)	NA	NA	0.05
p.T410M	unknown	0 (0)	0.15 (1)	NA	NA	0
p.L444P[b]	severe	0.27 (2)	0 (0)	NA	NA	NC
p.L461P	unknown	0.14 (1)	0 (0)	NA	NA	0
p.R496H[b]	mild	0.95 (7)	0.3 (2)	3.2 (0.7-15.4)	0.1	NC
Total mild *GBA* variant carriers		12.93 (95)	5.88 (39)	2.4 (1.6-3.5)	<0.0001	
Total severe *GBA* variant carriers		1.9 (14)	0.15 (1)	12.9 (1.7-98)	0.001	
Total heterozygous *GBA* variant carriers		17.01 (125)	7.54 (50)	2.5 (1.8-3.6)	<0.0001	
Homozygous/compound heterozygous						
p.N370S/p.R496H		0.27 (2)	0 (0)	NA	NA	
p.E326K/p.N370S		0.14 (1)	0 (0)	NA	NA	
p.T369M/84GG		0.27 (2)	0 (0)	NA	NA	
p.N370S/p.N370S		0.14 (1)	0 (0)	NA	NA	
p.N370S/p.L444P		0.14 (1)	0 (0)	NA	NA	
Total homozygous and compound heterozygous		0.95 (7)	0 (0)	NA	NA	
Total heterozygous, homozygous, and compound heterozygous carriers		17.96 (132)	7.54 (50)	2.7 (1.9-3.8)	<0.0001	

PD, Parkinson's disease; n, number; CI, confidence interval; IBD, inflammatory Bowel disease; NA, not applicable (OR and p values cannot be calculated if there is a value of 0 in one of the groups); NC, not called (due to the homology with pseudo-*GBA*, some variants cannot be called properly)

[a]The severity was determined by the effect of the variant in Gaucher's disease. Variants that in homozygous form cause the mild type I of Gaucher's disease are considered as "mild", and those that cause the severe forms of Gaucher's disease are considered as "severe".

[b]Included in 7-variant genotyping panel.

[c]These variants do not cause Gaucher's disease, therefore defined as benign. However, they may still be risk factors for PD.