Genetic and clinical pharmacology studies in GBA1-associated Parkinson's disease

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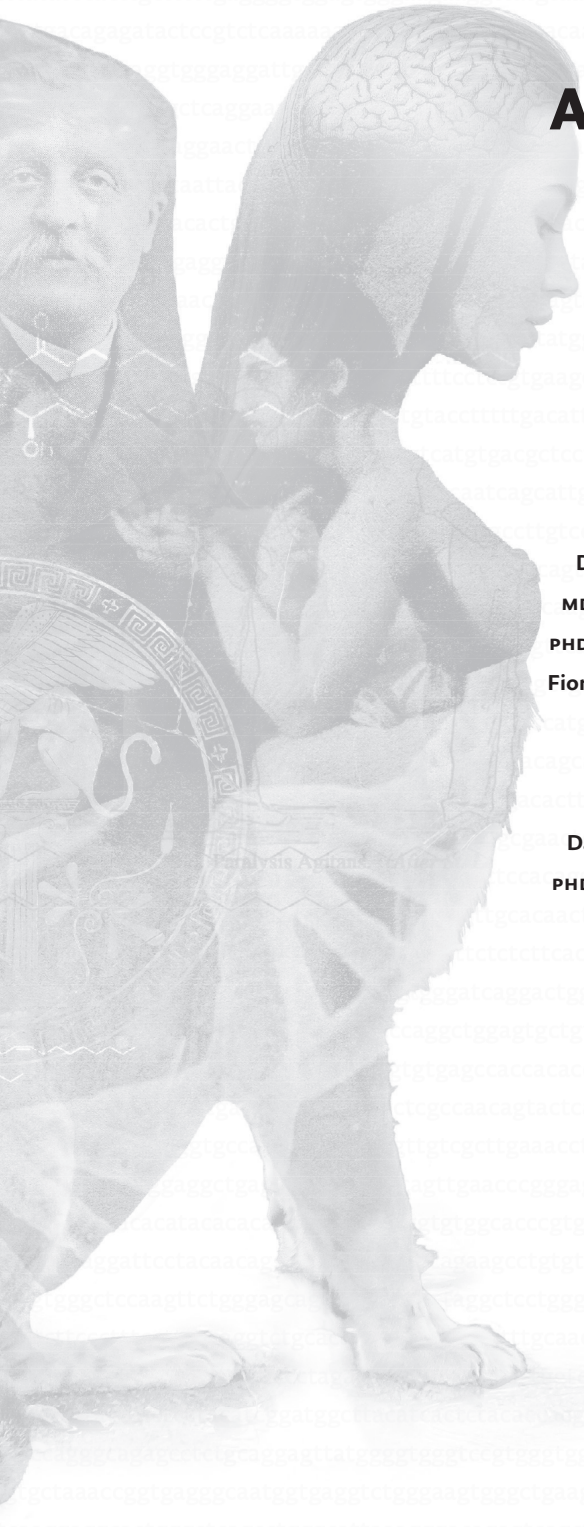

CHAPTER 2

A large-scale full *GBA1* gene screening in Parkinson's disease in the Netherlands

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Abstract

Background The most common genetic risk factor for Parkinson's disease known is a damaging variant in the *GBA1* gene. Rarely the entire *GBA1* gene has been studied in a large cohort from a single population.

Objective Assess the entire *GBA1* gene in Parkinson's disease from a single large population.

Methods The *GBA1* gene was assessed in 3402 Dutch Parkinson's disease patients using next generation sequencing. Frequencies were compared to Dutch controls (n=655). Family history of Parkinson's disease was compared in carriers and non-carriers.

Results 15. 0% of patients had a *GBA1* non-synonymous variant (including missense, frameshift and recombinant alleles), compared to 6. 4% of controls (OR 2. 6;p<0. 001). 18 novel variants were detected. Variants previously associated with Gaucher's disease were identified in 5. 0% of patients compared to 1. 5% of controls (OR 3. 4;p<0. 001). The rarely reported complex allele p. D140H+p. E326K appears to likely be a Dutch founder variant, found in 2. 4% of patients and 0. 9% of controls (OR 2. 7;p=0. 012). The number of first-degree relatives (excluding children) with Parkinson's disease was higher in p. D140H+p. E326K carriers (5. 6%, 21/376) compared to p. E326K carriers (2. 9%, 29/1014) (OR 2. 0;p=0. 022), suggestive of a 'dose-effect' for different *GBA1* variants.

Conclusion Dutch Parkinson's disease patients display one of the largest frequencies of *GBA1* variants reported so far, consisting for a large part of the mild p. E326K variant and the more severe Dutch p. D140H+p. E326K founder allele.

Introduction

The most common genetic risk factor known to date for Parkinson's disease (PD) is a damaging variant in the *GBA* (*GBA1*) gene, encoding the lysosomal glucocerebrosidase enzyme.[1] To avoid confusion with the non-lysosomal genes *GBA2* and *GBA3*, the *GBA* gene is also referred to as *GBA1*. In most populations, 4-12% of PD patients carry a heterozygous *GBA1* variant and in Ashkenazi Jewish PD patients this is approximately 20%.[2,3] The risk of PD in *GBA1* variant carriers is increased by an estimated overall 2-7 fold (Odds Ratios, OR).[2–5] Rare homozygous or compound heterozygous *GBA1* variants can cause the autosomal recessive lysosomal storage disorder Gaucher's disease (GD). Over 400 variants have been reported associated with GD[6,7] and all of these alleles are potential risk factors for developing PD.

Full *GBA1* gene sequencing is essential to unambiguously identify gene variants, considering a long tail of rare variants or even population-specific variants.[3,4,8] Nevertheless, rarely the entire *GBA1* gene has been sequenced in a large cohort from a single population. Here, we report such a large-scale *GBA1* screening performed in the Netherlands, in the framework of a large program aimed to identify patients with *GBA1* variants for a clinical trial targeting the *GBA1* mechanism. We sequenced the *GBA1* entire open reading frame (ORF) in 3402 people with PD living in the Netherlands. Variant frequency was compared to an existing Dutch control cohort (n=655). Family history of PD was assessed in a subset of patients with the most common variants, to compare familial aggregation.

Material and Methods

Participants

PD patients were included in the Netherlands between April-2017 and March-2018, see supplementary data for details. Age at diagnosis of ≤50 years was considered early onset and >50 years was considered late onset PD.

This study was approved by an Independent Ethics Committee. Written informed consent was obtained from all participants according to the Declaration of Helsinki.

An independent Dutch study of 655 patients with abdominal aortic aneurysms was used for comparison (see supplementary), using WES data (average *GBA1* coverage was 101 times). Data regarding the presence of neurological disease were unavailable.

Genotyping

Saliva was obtained from patients using Oragene DNA OG-500 tubes (DNA Genotek). DNA isolation, next generation sequencing (NGS) and data analysis was performed by GenomeScan BV, Leiden, the Netherlands. Primers were selected to unambiguously sequence the functional *GBA1* gene and not the pseudogene, using long-range PCR. In a post-hoc experimental setup using long-read sequencing with the PacBio Sequel system, phasing was assessed in three samples. See supplementary material for methodological details, including validation of a subset using Sanger sequencing.

Historically, *GBA1* variants have been described based on the amino acid position excluding the 39-residue signal sequence at the start (also known as 'allelic nomenclature'). Both the Human Genome Variation Society (HGVS) recommended nomenclature and the allelic nomenclature are given (NCBI Reference Sequence: NM_000157. 3). If an allele contained more than one exonic variant, this is referred to as a complex allele.

Genotypes were classified into four categories, based on clinical associations, using the Human Gene Mutation Database:[7]

- Gaucher disease associated 'GD'
- Parkinson's disease associated 'PD'
- Synonymous
- Novel

If a subject had both a known and a novel variant, the genotype was considered novel. See supplementary data for details.

All variants that were 6 nucleotides or closer to a splice site, were assessed with four in silico splicing programs implemented in Alamut (Alamut Visual version 2. 13; see supplementary data).

A two-step cross-validation was performed to assess risk of both false positive and false negative results when using WES (see supplementary).

Family History

All patients with the *GBA1* p. D140H+p. E326K, p. E326K, p. N370S or p. L444P variants and a random subset of patients who did not carry *GBA1* variants as per our methods and variant selection criteria (henceforth referred to as *GBA1* 'wildtype') were given a questionnaire to assess familial aggregation of PD and to assess a possible founder location of the p. D140H+p. E326K complex allele. See supplementary material for details.

Statistical analysis

Fisher's exact test was used for categorical variables and the Mann-Whitney U-test for continuous variables. Significance was flagged at p<0. 05. ORs were calculated with a 95% CI. IMB SPSS Statistics 25 software was used.

Results

In total, 3638 PD patients were included, of which 3402 could be genotyped. Of the remaining 236 samples, no DNA could be extracted or PCR failed. Demographics can be found in Supplementary Table 1. 81% of patients was recruited through referral by a neurologist.

Sequencing

Average coverage was 2703 times (Supplementary figure 1). The subset of samples used in the Sanger sequencing validation were all confirmed (see supplementary data).

GBA1 variants

All *GBA1* exonic and splice site variants are listed in Table 1, including frequency comparison between PD and controls. In short, the total PD cohort had 15. 0% non-synonymous variants (including missense, frameshift and recombinant alleles) versus 6. 4% in controls (OR 2. 6, 95% CI: 1. 9-3. 6, p<0. 001).

For 'GD'-variants observed in patients (5. 0%) versus controls (1. 5%), the OR is 3. 4 (95% CI: 1. 8-6. 5, p<0.001) and for the 'PD'-variants observed in patients (9. 3%) versus controls (4.4%), the OR is 2. 2 (95% CI: 1. 5-3. 3, p<0. 001).

In total, 19 'GD'-variants, 5 'PD'-variants, 12 synonymous variants and 18 novel variants were identified. In one sample with p. D140H+p. E326K, phasing was confirmed using PacBio sequencing. See supplementary data for a further description of variants found. Supplementary Table 3 contains a variant frequency comparison with data from GoNL[9] and GnomAD[10,11] for reference, however methodology in these cohorts was not dedicated to *GBA1* sequencing.

No intronic variants were assessed to have a possible effect on splicing (Supplementary Table 4).

Control cohorts cross-validation

In the control cohort, 42 samples had a non-synonymous *GBA1* variant detected using WES that could be tested with our NGS protocol. Using NGS, four control samples were detected to be false-positive and three samples were partially false-negative (for p. D140H in a p. D140H+E326K complex allele). Conversely, after rerunning 48 GBA-PD samples with WES, one false-negative was detected. See supplementary data for details.

Demographics based on *GBA1* status

Demographics are given in Supplementary Table 1, divided over carriers of a non-synonymous variant or not. A larger portion of carriers had early onset PD (27. 2%) compared to non-carriers (18.2%) (p<0. 001). Conversely, of all subjects with early onset, 20. 1% had a *GBA1* variant, compared to 13. 1% in those with late onset (p<0. 001).

GBA1 variants and familial aggregation of PD

A questionnaire was completed by 180 carriers of p. E326K, 24 carriers of p. N370S, 28 carriers of p. L444P (including 4 complex and 3 recombinant

alleles), 73 carriers of p. D140H+p. E326K and 135 *GBA1* wildtypes. Combining all carriers, 3. 6% of all siblings and parents combined had PD, compared to 2. 0% in siblings and parents of non-carriers (OR 1. 8, 95% CI: 1.0-3. 2; p=0.043). None of the children developed PD, probably due to the present younger age, so these were excluded from analysis of first-degree relatives (Supplementary Table 2). Supplementary figure 2 depicts the total number of first-degree relatives (excluding children) per variant type and the percentage of these relatives with PD. A variant 'dose-effect' was seen, see supplementary data for details.

Founder location p. D140H+p. E326K

Supplementary data and figure 3 shows a heat map of descent of grandparents of p. D140H+p. E326K carriers, visually suggesting (no formal statistical testing) the northern Netherlands as a possible founder location for this complex allele.

Discussion

To our knowledge, this study is the largest cohort known to date from a single country that has had full gene *GBA1* sequencing in PD patients. A total of 15. 0% of all patients had non-synonymous *GBA1* variants, which is the highest prevalence reported to date in a non-Ashkenazi Jewish population. The relatively high prevalence of the population specific p. D140H+p. E326K complex allele and the long tale of rare variants, including 18 novel variants, highlight the importance of sequencing the full *GBA1* ORF. Identifying all these variants will strengthen our understanding of the effect of *GBA1* variants and it facilitates recruitment for the upcoming *GBA1*-targeted trials, hopefully resulting in a first disease-modifying drug for PD.[12]

Comparing different countries,[3,4,8,13–26] the p. E326K variant is reported most frequently in the Netherlands (present study) and Scandinavian countries.[20,24] Table 2 compares the most common *GBA1* variants and the p. D140H+p. E326K complex allele in large PD cohorts from single countries that performed full *GBA1* ORF sequencing. Swedish[24] and Russian[15] cohorts

were included despite selective sequencing, because of their size, in order to compare the p. E326K variant. This overview shows the near-exclusive appearance of p. D140H+p. E326K in the Netherlands. The p. D140H+p. E326K complex allele has only sporadically been reported, once in GD,[27,28] sporadically in PD[4,29] and once in Lewy Body Dementia.[30]

Intronic splice site variants have rarely been systematically assessed previously,[17,23] however these do not seem to play a role in GBA-PD pathology in our Dutch cohort.

The importance of adequate genotyping methodology when sequencing *GBA1* was once more confirmed. In the control cohort, the *GBA1* variants were reassessed with NGS, which identified four false-positive p. L444P variants in WES. Also, three p. D140H variants were falsely not identified in three samples that also carried the p. E326K variant. The performance of the hybridization capture panel was lower over the p. D140H region, reflected in a local lower coverage. Combined with a possible allelic imbalance for this specific variant, where the amplification prefers the wildtype allele over the p. D140H allele, this could explain the false-negative output. Therefore, caution is advised when using *GBA1* data generated using a methodology not specifically designed for *GBA1* sequencing (including databases like ExAC or gnomAD).

Because the p. E326K and p. T369M variants do not cause Gaucher's disease, these have long been termed polymorphisms. However, it has been shown in meta-analyses that these variants do confer an increased risk of developing PD (OR 1.99 for p. E326K and OR 1.74 for p. T369M)[31–33] and therefore, despite not causing GD, should not be considered neutral polymorphisms.

Of all participants diagnosed with PD at 50 years of age or earlier, 20. 1% had a *GBA1* variant. When genetic testing is performed in early-onset PD, *GBA1* is not always included. Because of the high prevalence of *GBA1* variants in early-onset PD, it deserves consideration to include this in the screening, although the predictive value of a *GBA1* variant for offspring is still limited.

GBA1 variant carriers have a larger frequency of a positive family history for Parkinson´s disease[4,5,34] compared to non-carriers. In the current study, carriers of p. D140H+p. E326K had significantly more first-degree relatives

with PD compared to p. E326K carriers. This implies a 'dose-effect' of variant severity in familial aggregation. However, it did not reach statistical significance for other variant types, likely due to the rarity of these variants.

The current study has some limitations. Since our NGS method used short-read sequencing, phasing of multiple variants could not be determined, unless these were within approximately 500 base pairs of each other. However, for a single p. D140H+p. E326K sample phasing was confirmed using PacBio and p. D140H was never seen without p. E326K. A recombinant gene could be identified if the long-range PCR resulted in two distinct peaks on the Fragment Analyzer. See supplementary data for a further discussion of possible limitations.

In conclusion, this study is a successful example of how to ascertain and genotype a large cohort of patients with PD, within a short timeframe, which is relevant for progressing clinical trials aimed at developing personalized treatments.

The Dutch PD population appears to have a relatively large number of *GBA1* variant carriers, consisting mostly of the mild p. E326K variant and the likely more severe Dutch p. D140H+p. E326K complex allele, with a possible founder-effect in the northern part of the Netherlands. In total, 18 novel *GBA1* variants were detected. *GBA1* variant carriers had a younger age at onset and a higher chance of a positive family history for PD, with a trend towards a 'dose-effect' based on clinical association of the variant.

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Table 1 Listing of all found exonic and splice site variants, including specifications. The sixth column 'allelic name' contains the annotation historically used in Gaucher's disease literature, excluding the 39-amino acid signaling peptide. All genotype frequencies are compared to the AAA control cohort, ors are given with the 95% CI and a p-value. A p-value of <0.05 is given in bold and the rows of these genotypes are filled grey. or could not be calculated if frequency was 0 in either group. If six cases or less were affected in patients and zero in controls, p-value is set to na. The coding (or sense) strand for *GBA1* is the reverse strand of the dna (as opposed to the forward strand). The chromosome position and nucleotide reflect the forward strand, whereas the cdna annotation indicates the variant on the coding strand, which is in this case the reverse strand, and therefore these are complementary. Both intronic splice site variants were predicted not to affect splicing (see supplementary material) and were therefore not included in the overall analysis.

GENOTYPE INFORMATION						COHORTS				
Position Chr 1 (GRCh37/hg19)	cDNA NM_000157.3	rsID	Exon	Protein NP_000148.2	Allelic name	Clinical Association	PD patients % (n) (n=3402)	Control % (n)(n=655)	OR (95% CI)	P-value
HETEROZYGOUS (simple and complex)										
155210876:C	c.26_27del	-	1	p.(Glu9GlyfsTer8)	E-30GFS*8	Novel	0.0 (1)	0 (0)	NA	NA
155210492:G	c.44T>C	-	2	p.(Leu15Ser)	L-24S	Novel	0.0 (1)	0 (0)	NA	NA
155210492:G 155210490:C	c.44T>C c.46A>G	-	2 2	p.[(Leu15Ser;Ser16Gly)]	L-24S+S-23G	Novel Novel	0.0 (1)	0 (0)	NA	NA
155210441:C	c.95A>G	-	2	p.(Gln32Arg)	Q-7R	Novel	0.0 (1)	0 (0)	NA	NA
155209813:T	c.171C>A	-	3	p.(Cys57Ter)	C18*	Novel	0.0 (1)	0 (0)	NA	NA
155209752:A	c.232C>T	rs146774384	3	p.(Arg78Cys)	R39C	Novel	0.0 (1)	0 (0)	NA	NA
155209732:AC	c.251_252insC	-	3	p.(Ser84ArgfsTer15)	S45RFS*15	Novel	0.0 (1)	0 (0)	NA	NA
155208421:A	c.475C>T	rs397515515	5	p.(Arg159Trp)	R120W	GD	0.1 (5)	0 (0)	NA	NA
155208361:G 155206167:T	c.535G>C c.1093G>A	rs147138516 rs2230288	5 8	p.[(Asp179His;Glu365Lys)]	D140H+E326K	GD	2.4 (82)	0.9 (6)	2.7 (1.2-6.1)	**0.012**
155208060:T	c.626G>A	-	6	p.(Arg209His)	R170H	Novel	0.0 (1)	0 (0)	NA	NA

GENOTYPE INFORMATION						COHORTS				
Position Chr 1 (GRCh37/hg19)	cDNA NM_000157.3	rsID	Exon	Protein NP_000148.2	Allelic name	Clinical Association	PD patients % (n) (n=3402)	Control % (n)(n=655)	OR (95% CI)	P-value
155208001:T	c.685G>A	-	6	p.(Ala229Thr)	A190T	GD	0.0 (1)	0 (0)	NA	NA
155207965:T	c.721G>A	rs398123534	6	p.(Gly241Arg)	G202R	GD	0.0 (1)	0 (0)	NA	NA
155207367:T	c.764T>A	rs74500255	7	p.(Phe255Tyr)	F216Y	GD	0.0 (1)	0 (0)	NA	NA
155207266:T	c.865G>A	-	7	p.(Gly289Ser)	G250S	Novel	0.0 (1)	0 (0)	NA	NA
155207249:C	c.882T>G	rs367968666	7	p.(His294Gln)	H255Q	GD	0.1 (2)	0 (0)	NA	NA
155207235:G	c.896T>C	-	7	p.(Ile299Thr)	I260T	GD	0.1 (2)	0 (0)	NA	NA
155206172:G	c.1088T>C	-	8	p.(Leu363Pro)	L324P	GD	0.0 (1)	0.2 (1)	0.2 (0.0-3.1)	0.297
155206170:T	c.1090G>A	rs121908305	8	p.(Gly364Arg)	G325R	GD	0.0 (1)	0 (0)	NA	NA
155206167:T	c.1093G>A	rs2230288	8	p.(Glu365Lys)	E326K	GD	6.3 (213)	2.6 (17)	2.5 (1.5-4.1)	**<.001**
155206158:A	c.1102C>T	rs374306700	8	p.(Arg368Cys)	R329C	GD	0.1 (2)	0 (0)	NA	NA
155206101:C	c.1159T>G	-	8	p.(Trp387Gly)	W348G	GD	0.0 (1)	0 (0)	NA	NA
155206093:G	c.1167G>C	-	8	p.(Gln389His)	Q350H	Novel	0.0 (1)	0.2 (1)	0.2 (0.0-3.1)	0.297
155206037:A	c.1223C>T	rs386626586	8	p.(Thr408Met)	T369M	GD	2.5 (86)	1.8 (12)	1.4 (0.8-2.6)	0.332
155205634:C	c.1226A>G	rs76763715	9	p.(Asn409Ser)	N370S	GD	0.9 (30)	0.3 (2)	2.9 (0.7-12.2)	0.151
155205619:C	c.1241T>G	-	9	p.(Val414Gly)	V375G	Novel	0.0 (1)	0 (0)	NA	NA

GENOTYPE INFORMATION						COHORTS				
Position Chr 1 (GRCh37/hg19)	cDNA NM_000157.3	rsID	Exon	Protein NP_000148.2	Allelic name	Clinical Association	PD patients % (n) (n=3402)	Control % (n)(n=655)	OR (95% CI)	P-value
155205605:A	c.1255G>T	-	9	p.(Asp419Tyr)	**D380Y**	GD	0.0 (1)	0 (0)	NA	NA
155205581:T	c.1279G>A	rs149171124	9	p.(Glu427Lys)	**E388K**	GD	0.1 (3)	0 (0)	NA	NA
155205568:C	c.1292A>G	-	9	p.(Asn431Ser)	**N392S**	GD	0.0 (1)	0 (0)	NA	NA
155205518:G	c.1342G>C	rs1064651	9	p.(Asp448His)	**D409H**	GD	0.0 (1)	0 (0)	NA	NA
155205043:G	c.1448T>C	rs421016	10	p.(Leu483Pro)	**L444P**	GD	0.6 (21)	0 (0)	NA	-
155205016:A 155205017:G	c.[1475A>T; 1474G>C]	-	10 10	p.(Asp492Leu)	**D453L** **(D453V+D453H)**	Novel	0.1 (4)	0 (0)	NA	NA
155204996:T	c.1495G>A	-	10	p.(Val499Met)	**V460M**	GD	0.0 (1)	0 (0)	NA	NA
155204986:G	c.1505G>C	-	10	p.(Arg502Pro)	**R463P**	GD	0.1 (2)	0.2 (1)	0.4 (0.0-4.2)	0.410
155204829:A	c.1568C>T	-	11	p.(Ser523Leu)	**S484L**	Novel	0.0 (1)	0 (0)	NA	NA
155204818:T	c.1579T>A	-	11	p.(Ser527Thr)	**S488T**	GD	0.0 (1)	0 (0)	NA	NA
155204811:C	c.1586A>G	-	11	p.(His529Arg)	**H490R**	Novel	0.0 (1)	0 (0)	NA	NA
LIKELY RECOMBINANT ALLELES										
155207210:A, 155207203:C, 155205008:G,	c.924C>T, c.931A>G, c.1483G>C,	- - -	7 7 9	p.(Leu307=), p.(Ser310Gly), p.(Ala495Pro),	**L268=,** **S271G,** **D409H**	Novel	0.0 (1)	0 (0)	NA	NA
			9 10 10 10		**D409H,** **L444P,** **A456P,** **V460=** **(A.K.A. Rec*TL*)**	GD	0.0 (1)	0 (0)	NA	NA

GENOTYPE INFORMATION							COHORTS			
Position Chr 1 (GRCh37/hg19)	cDNA NM_000157.3	rsID	Exon	Protein NP_000148.2	Allelic name	Clinical Association	PD patients % (n) (n=3402)	Control % (n)(n=655)	OR (95% CI)	P-value
			10 10 10		L444P, A456P, V460= (a.k.a. Rec*Nci*I)	GD	0.1 (4)	0 (0)	NA	NA
HOMOZYGOUS OR COMPOUND HETEROZYGOUS(variant details in listing above)										
				p.[(Leu363Pro)];[(Thr408Met)]	L324P/T369M	GD/GD	0.0 (1)	0 (0)	NA	NA
				p.[(Asp179His;Glu365Lys)]; [(Thr408Met)]	D140H+E326K/ T369M	GD/GD	0.0 (1)	0 (0)	NA	NA
				p.[(Asp179His;Glu365Lys)]; [(Glu365Lys)]	D140H+E326K/ E326K	GD/GD	0.0 (1)	0 (0)	NA	NA
				p.[(Glu365Lys)];[(Thr408Met)]	E326K/T369M	GD/GD	0.1 (4)	0 (0)	NA	NA
				p.[(Glu365Lys)];[(Glu365Lys)]	E326K/E326K	GD/GD	0.2 (6)	0 (0)	NA	NA
				p.[(Thr408Met)];[(Thr408Met)]	T369M/T369M	GD/GD	0.0 (1)	0 (0)	NA	NA
UNCERTAIN PHASING(variant details in listing above)										
155210424:T, ...	c.112T>A, ...	-, ...	2, ...	p.(Ser38Thr)(;)(Thr408Met)	S-1T, T369M	Novel, GD	0.0 (1)	0 (0)	NA	NA
				p.(Gln32Arg)(;)(Asn409Ser)	Q-7R, N370S	Novel, GD	0.0 (1)	0 (0)	NA	NA
				p.[(Asp179His;Glu365Lys)](;) (Val498=)	D140H+E326K, V459=	GD, Syn	0.0 (1)	0 (0)	NA	NA
..., 155204793:T	..., c.1604G>A	..., rs80356773	..., 11	p.[(Asp179His;Glu365Lys)](;) (Arg535His)	D140H+E326K, R496H	GD, GD	0.0 (1)	0 (0)	NA	NA
				p.(Arg209His)(;)(Glu365Lys)	R170H, E326K	Novel, GD	0.0 (1)	0 (0)	NA	NA
				p.[(Glu365Lys)];[(Thr408Met)] (;)(Leu483Pro)	E326K/T369M, L444P	GD/GD, GD	0.0 (1)	0 (0)	NA	NA

GENOTYPE INFORMATION						COHORTS				
Position Chr 1 (GRCh37/hg19)	cDNA NM_000157.3	rsID	Exon	Protein NP_000148.2	Allelic name	Clinical Association	PD patients % (n) (n=3402)	Control % (n)(n=655)	OR (95% CI)	P-value
..., 155205574:T	..., c.1286G>A	..., -	..., 9	p.(Glu365Lys)(;)(Gly429Glu)	**E326K, G390E**	GD, Novel	0.0 (1)	0.2 (1)	0.2 (0.0-3.1)	0.297
				p.(Glu365Lys)(;)(Val498=)	**E326K, V459=**	GD, Syn	0.0 (1)	0 (0)	NA	NA
				p.(Glu365Lys)(;)(Val499=)	**E326K, V460=**	GD, Syn	0.0 (1)	0 (0)	NA	NA
				p.(Thr408Met)(;)(Asp492Leu)	**T369M, D453L**	GD, Novel	0.0 (1)	0 (0)	NA	NA
				p.(Thr408Met)(;)(Leu483Pro)	**T369M, L444P**	GD, GD	0.1 (3)	0 (0)	NA	NA
				p.(Asn409Ser)(;)(Leu483Pro)	**N370S, L444P**	GD, GD	0.0 (1)	0 (0)	NA	NA
SYNONYMOUS										
155209816:A	c.168C>T	rs145773486	3	p.(Val56=)	**V17=**	Syn	0 (0)	0.2 (1)	NA	0.161
155209684:T	c.300G>A	-	3	p.(Thr100=)	**T61=**	Syn	0.0 (1)	0 (0)	NA	NA
155208422:A	c.474C>T	rs147411159	5	p.(Ile158=)	**I119=**	Syn	0.1 (5)	0 (0)	NA	NA
155208389:T	c.507C>A	-	5	p.(Ile169=)	**I130=**	Syn	0.0 (1)	0 (0)	NA	NA
155208350:T	c.546G>A	-	5	p.(Gln182=)	**Q143=**	Syn	0.0 (1)	0 (0)	NA	NA
155207990:T	c.696G>A	rs375731497	6	p.(Gly232=)	**G193=**	Syn	0.0 (1)	0.2 (1)	0.2 (0.0-3.1)	0.297
155207984:A	c.702G>T	-	6	p.(Gly234=)	**G195=**	Syn	0.0 (1)	0 (0)	NA	NA
155206111:A	c.1149C>T	-	8	p.(Gly383=)	**G344=**	Syn	0.0 (1)	0 (0)	NA	NA
155206036:T	c.1224G>A	rs138498426	8	p.(Thr408=)	**T369=**	Syn	0.1 (2)	0 (0)	NA	NA
155205018:A	c.1473C>T	rs149257166	10	p.(Pro491=)	**P452=**	Syn	0.0 (1)	0 (0)	NA	NA

GENOTYPE INFORMATION							COHORTS			
Position Chr 1 (GRCh37/hg19)	cDNA NM_000157.3	rsID	Exon	Protein NP_000148.2	Allelic name	Clinical Association	PD patients % (n) (n=3402)	Control % (n)(n=655)	OR (95% CI)	P-value
155204997:A	c.1494C>T	rs371779859	10	p.(Val498=)	V459=	Syn	0.1 (3)	0 (0)	NA	NA
155204994:G	c.1497G>C	rs1135675	10	p.(Val499=)	V460=	Syn	0.0 (1)	0 (0)	NA	NA
SPLICE SITE (distance of 6 nucleotides or less)										
155207374:T	c.762-5G>A	-	Intr.	-	-	Novel	0.0 (1)	0 (0)	NA	NA
155206264:A	c.1000-4G>T	-	Intr.	-	-	Novel	0 (0)	0.2 (1)	NA	0.161
Exonic variants (details above) fulfilling splice site criteria (variant (distance)) (see supplementary Table 4 for splicing prediction): p.E30Gfs*8 (1), p.S1T (4), p.F216Y (3), p.T369= (1), p.T369M (2), p.N370S (2), p.R463P (1)										
GROUPED COMPARISONS										
All Novel genotypes							0.7 (23)	0.3 (2)	1.5 (0.4-4.9)	0.788
All PD genotypes (p.E326K, p.T369M, p.E388K, p.S488T, p.N392S)							9.3 (317)	4.4 (29)	2.2 (1.5-3.3)	**<.001**
All GD genotypes							5.0 (170)	1.5 (10)	3.4 (1.8-6.5)	**<.001**
Total non-synonymous							15.0 (510)	6.4 (42)	2.6 (1.9-3.6)	**<.001**

GD=Gaucher's disease, PD=Parkinson's disease, syn=synonymous, NA=not applicable, Intr.=intronic.

Table 2 International comparison of Parkinson's disease cohorts that performed full *GBA1* gene sequencing, sorted based on total % of *GBA1* variant carriers. All variant frequencies are given in percentages. Sweden and Russia performed selective sequencing. France is a European study, with 89% of subjects from France. North Africa is primarily Algeria, but also Morocco, Tunisia and Libya.

	PD N=	*GBA1* %	E326K	T369M	N370S	L444P	D140H + E326K	Other
Ashkenazi Jewish (3)	735	18.0	1.6	0	11.8	0.3	0	4.2
This cohort (NL)	**3402**	**15.0**	**6.7**	**2.5**	**0.9**	**0.6**	**2.5**	**1.8**
France (4)	1130	12.5	4.2	1.5	2.9	1	0.1	2.7
Colombia (8)	131	12.2	1.5	0	2.3	2.3	0	6.1
Norway (20)	442	12.0	6.6	3.6	0.2	1.4	0	0.5
Spain (17)	532	11.7	3	0.9	0.9	2.4	0	4.3
US (22)	1369	11.6	5	2.2	1.3	1.2	0.1	1.9
UK (21)	1893	11.1	4.5	1.8	0.6	1.6	0.1	2.4
Eastern Canada (16)	225	11.1	1.8	4.9	0.9	1.8	0	1.8
Belgium (26)	266	9.8	4.1	1.1	1.1	1.5	0.4	1.5
Japan (23)	534	9.4	0	0	0	4.1	0	5.2
New Zealand (25)	229	9.2	4.8	3.1	0.4	0	0.4	0.9
Sweden (24)	1625	8.3	5.8	N/A	0.4	2.2	N/A	N/A
Peru (8)	471	7.2	1.1	0.6	0.2	2.8	0	1.8
Russia (15)	762	6.6	2.4	2.5	0.5	1.1	N/A	N/A
Greece (18)	172	6.4	0.6	0	0	1.2	0	4.7
Portugal (13)	230	6.1	0.9	0.9	2.2	1.3	0	0.9
Korea (14)	277	6.1	0	0	0	0.7	0	5.4
North Africa (19)	194	4.6	0.5	1.0	1.0	1.5	0	0.5

SCAN ME

SUPPLEMENTARY MATERIAL

H2ST1/ H2ST2 / H2ST3 / H2ST4 / H2SF1 / H2SF2 / H2SF3 / H2SF4