Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site.

A Large-Scale Full GBA1 Gene Screening in Parkinson’s Disease in the Netherlands

Jonas M. den Heijer, MD,1,2 Valerie C. Cullen, PhD,3 Marialuisa Quadri, PhD,4,5 Arnoud Schmitz, MSc,6 Dana C. Hilt, MD,3 Peter Lansbury, PhD,3 Henk W. Berendse, MD, PhD,7 Wilma D.J. van de Berg, PhD,7 Rob M.A. de Bie, MD, PhD,7 Jeffrey M. Boertien, MD,8 Agnita J.W. Boon, MD, PhD,4 Henk W. Berendse, MD, PhD,7 M. Fiorella Contarino, MD, PhD,7,9 Jacobsus J. van Hiltien, MD, PhD,2 Jorrit I. Hoff, MD, PhD,10 Tom van Mierlo, MD, PhD,11 Alex G. Munts, MD, PhD,11 Anne A. van der Plas, MD, PhD,12 Mirthe M. Ponsen, MD, PhD,13 Frank Baas, MD, PhD,2 Danielle Majoor-Krakauer, MD, PhD,4 Vincenzo Bonifati, MD, PhD,4 Teus van Laar, MD, PhD,8 and Geert J. Groeneveld, MD, PhD1,2*

1Centre for Human Drug Research, Leiden, The Netherlands 2Leiden University Medical Center, Leiden, The Netherlands

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*Correspondence to: Dr. Geert Jan Groeneveld, Zernikedreef 8, 2333 CL, Leiden, The Netherlands; E-mail: ggroeneveld@chdr.nl

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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. The entire GBA1 gene has rarely been studied in a large cohort from a single population. The objective of this study was to 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 with Dutch controls (n = 655). Family history of Parkinson’s disease was compared in carriers and noncarriers.

Results: Fifteen percent of patients had a GBA1 non-synonymous variant (including missense, frameshift, and recombinant alleles), compared with 6.4% of controls (OR, 2.6; P < 0.001). Eighteen novel variants were detected. Variants previously associated with Gaucher’s disease were identified in 5.0% of patients compared with 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 of 376) compared with p.E326K carriers (2.9%, 29 of 1014); OR, 2.0; P = 0.022, suggestive of a dose effect for different GBA1 variants.

Conclusions: Dutch Parkinson’s disease patients display one of the largest frequencies of GBA1 variants reported so far, consisting in large part of the mild p.E326K variant and the more severe Dutch p.D140H + p.E326K founder allele. © 2020 The Authors. Movement Disorders published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: familial aggregation; GBA sequencing; genetic risk factor; glucocerebrosidase; heredity

The most common genetic risk factor known to date for Parkinson’s disease (PD) is a damaging variant in the GBA gene (GBA1), encoding the lysosomal glucocerebrosidase enzyme.1 To avoid confusion with the nonlysosomal 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- to 7-fold (odds ratios [ORs]).\(^2-5\) Rare homozygous or compound heterozygous GBA1 variants can cause the autosomal-recessive lysosomal storage disorder Gaucher’s disease (GD). More than 400 variants have been reported to be associated with GD,\(^6,7\) and all 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 at identifying 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 with 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.

**Materials 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 data), using whole-exome sequencing (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 B.V., Leiden, the Netherlands. Primers were selected to unambiguously sequence the functional GBA1 gene and not the pseudogene, using long-range polymerase chain reaction (PCR). In a post hoc experimental setup using long-read sequencing with the PacBio Sequel system, phasing was assessed in 3 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 recommended nomenclature, and the allelic nomenclature is given (NCBI Reference Sequence: NM_000157.3). If an allele contained more than 1 exonic variant, this is referred to as a complex allele.

Genotypes were classified into 4 categories based on clinical associations using the Human Gene Mutation Database\(^7\): (1) Gaucher’s disease associated (GD), (2) Parkinson’s disease associated (PD), (3) synonymous, or (4) 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 4 in silico splicing programs implemented in Alamut (Alamut Visual version 2.13; see supplementary data).

A 2-step cross-validation was performed to assess risk of both false-positive and false-negative results when using WES (see supplementary data).

**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 wild type) 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. IBM SPSS Statistics 25 software was used.

**Results**

In total, 3638 PD patient samples 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. Eighty-one percent of patients were recruited through referral by a neurologist.
Sequencing

Average coverage was 2703 times (Supplementary Fig. 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 patients and controls. In short, the total PD cohort had 15.0% nonsynonymous 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 was 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 was 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 1 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 GoNL9 and GnomAD10,11 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 nonsynonymous GBA1 variant detected using WES that could be tested with our NGS protocol. Using NGS, 4 control samples were detected to be false-positive, and 3 samples were partially false-negative (for p.D140H in a p.D140H + E326K complex allele). Conversely, after rerunning 48 GBA-PD samples with WES, 1 false-negative was detected. See supplementary data for details.

Demographics Based on GBA1 Status

Demographics are given in Supplementary Table 1, divided over whether subjects carried a nonsynonymous variant. A larger portion of carriers had early-onset PD (27.2%) compared with noncarriers (18.2%), \( P < 0.001 \). Conversely, of all subjects with early onset, 20.1% had a GBA1 variant, compared with 13.1% in those with late onset (\( P < 0.001 \)).

GBA 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 wild types. Combining all carriers, 3.6% of all siblings and parents combined had PD compared with 2.0% in siblings and parents of noncarriers (OR, 1.8; 95% CI, 1.0–3.2; \( P = 0.043 \)). None of the children developed PD, probably because of 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 Supplementary Figure 3 show 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 nonsynonymous 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 tail 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. Swedish24 and Russian15 cohorts were included despite selective sequencing because of their size 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 PD4,29 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 4 false-positive p.L444P variants in WES. Also, 3 p.D140H variants were falsely
### TABLE 1. Listing of all found exonic and splice-site variants, including specifications [Color table can be viewed at wileyonlinelibrary.com]

| Position Chr 1 | cDNA     | rsID    | Exon | Protein | Allelic name | Clinical | PD patients | Control | OR     | P     |
|---------------|----------|---------|------|---------|--------------|----------|-------------|---------|--------|-------|
| (GRCh37/hg19) | NM_000157.3 | c.26_27del | 1 | p.(Glu9GlyfsTer8) | E-30Gfs*8 | Novel | 0.0 (1) | 0 (0) | NA | NA |
| 155210490:C   | c.46A > G | -       | 2   | p.(Leu15Ser) | L-24S     | Novel | 0.0 (1) | 0 (0) | NA | NA |
| 155210411:C   | c.95A > G | -       | 2   | p.(Gln32Arg) | Q-7R      | Novel | 0.0 (1) | 0 (0) | NA | NA |
| 155208421:A   | c.475C > T | rs397515515 | 3 | p.(Arg159Trp) | E326K     | Novel | 0.1 (1) | 0 (0) | NA | NA |
| 155206167:T   | c.1088T > C | rs121908305 | 8 | p.(Glu365Lys) | G325R     | PD      | 0.1 (2) | 0 (0) | NA | NA |
| 155206178:C   | c.1202G > A | rs374306700 | 8 | p.(Val408Met) | T369M     | PD      | 2.5 (86) | 1.8 (12) | 1.4 | 0.332 |
| 155205619:C   | c.1241T > G | -       | 9   | p.(Asn414Gly) | V375G     | Novel | 0.0 (1) | 0 (0) | NA | NA |
| 155205643:C   | c.1226A > G | rs76763715 | 9 | p.(Asn409Ser) | N370S     | PD      | 0.9 (30) | 0.3 (2) | 2.9 | 0.151 |
| 155205581:T   | c.1279G > A | rs14971124 | 9 | p.(Asp427Gly) | D380Y     | PD      | 0.0 (1) | 0 (0) | NA | NA |
| 155205591:C   | c.1342G > C | rs1064651 | 10  | p.(Asp442His) | D409H     | GD      | 0.6 (21) | 0 (0) | NA | 0.037 |

(Continues)
| Position Chr 1 | cDNA          | rsID | Exon | Protein       | Allelic name | Clinical | PD patients | Control | OR | P   |
|---------------|---------------|------|------|---------------|--------------|----------|-------------|---------|----|-----|
| 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 | 0.410 (0.0–4.2) |
| 155204829:A   | c.1568C > T   | -    | 11   | p.(Ser237Leu) | S484L        | Novel    | 0.0 (1)     | 0 (0)   | NA | NA  |
| 155204818:T   | c.1579C > G   | -    | 11   | p.(Ser277Thr) | S488T        | PD       | 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

| Position Chr 1 | cDNA          | rsID | Exon | Protein       | Allelic name | Clinical | PD patients | Control | OR | P   |
|---------------|---------------|------|------|---------------|--------------|----------|-------------|---------|----|-----|
| 155207210:A,  | c.924C > T    | —    | 7    | p.(Leu307=), | L268=, S271G, D409H | Novel    | 0.0 (1)     | 0 (0)   | NA | NA  |
| 155207203:C,  | c.931A > G    | —    | 7    | p.(Ser310Gly), | 9, D409H, L444P, A456P, V460=(a.k.a. Rec71) | GD       | 0.0 (1)     | 0 (0)   | NA | NA  |
| 155205006:G,  | c.1483G > C   | —    | 10   | p.(Asn495Pro), | 10, 10 | GD / PD | 0.0 (1)     | 0 (0)   | NA | NA  |

Homozygous or compound heterozygous (variant details in listing above)

| Position Chr 1 | cDNA          | rsID | Exon | Protein       | Allelic name | Clinical | PD patients | Control | OR | P   |
|---------------|---------------|------|------|---------------|--------------|----------|-------------|---------|----|-----|
| 155204793:T   | c.1604G > A   | rs80356773 | 11   | p.(Glu365Lys);(Gly429Glu) | E326K / T369M, L444P | PD / PD | 0.0 (1)     | 0 (0)   | NA | NA  |
| 155205574:T   | c.1286G > A   | —    | 9    | p.(Glu365Lys);(Val498=) | E326K, V459= | PD, Syn  | 0.0 (1)     | 0 (0)   | NA | NA  |

Uncertain phasing (variant details in listing above)

| Position Chr 1 | cDNA          | rsID | Exon | Protein       | Allelic name | Clinical | PD patients | Control | OR | P   |
|---------------|---------------|------|------|---------------|--------------|----------|-------------|---------|----|-----|
| 155210424:T,  | ... c.112T > A | ... | 2    | p.(Ser38Thr);(Thr408Met) | S-1T, T369M | GD / PD | 0.0 (1)     | 0 (0)   | NA | NA  |

(Continues)
## TABLE 1. Continued

| Position Chr 1 | cDNA | rsID | Exon | Protein | Allelic name | Clinical | PD patients | Control | OR | P value |
|----------------|------|------|------|---------|--------------|----------|-------------|---------|----|---------|
| **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 | —    | —    |         | —    | —    | —    | 0.0 (1) | 0 (0) | NA | NA |
| 155208350:T    | c.546G > A | —    | —    |         | —    | —    | —    | 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 | 0.297 |
|                |      |      |      |         |      |      |      |         |      |    |         |
| **Splice site (distance of 6 nucleotides or less)** |      |      |      |         |      |      |      |         |      |    |         |
| 155207984:A    | c.702G > T | —    | —    |         | —    | —    | —    | 0.0 (1) | 0 (0) | NA | NA |
| 155206111:A    | c.1149C > T | —    | —    |         | —    | —    | —    | 0.0 (1) | 0 (0) | NA | NA |
| 155206386:T    | c.1224G > A | rs138498426 | 8 | p.(Gly383=) | G344= | Syn | 0.0 (1) | 0 (0) | NA | NA |
| 155205018:A    | c.1473C > T | rs149257166 | 10 | p.(Pro491=) | P452= | Syn | 0.0 (1) | 0 (0) | NA | NA |
| 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 |

**Exonic variants (details above) fulfilling splice-site criteria (variant [distance])** — see Supplementary Table 4 for splicing prediction: p.E-30S*8 (1), p.S-1T (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 | 0.788 |
|---------------------|---------|--------|-----|------|
| PD genotypes (p.E326K, p.T369M, p.E388K, p.S488T, p.N370S) | 9.3 (317) | 4.4 (29) | 2.2 | <0.001 |
| GD genotypes | 5.0 (170) | 1.5 (10) | 3.4 | <0.001 |

**Total non-synonymous**

| 15.0 (510) | 6.4 (42) | 2.6 | <0.001 |

GD, Gaucher’s disease; PD, Parkinson’s disease; syn, synonymous; NA, not applicable; Intr., intronic.

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 with the abdominal aortic aneurysm control cohort, ORs are given with the 95% CIs and a P value. A P < 0.05 is given in boldface, and the rows of these genotypes are filled gray. OR could not be calculated if frequency was 0 in either group. If 6 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 reflects 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.
not identified in 3 samples that also carried the p.E326K variant. The performance of the hybridization capture panel was lower over the p.D140H region, reflected in local lower coverage. Combined with a possible allelic imbalance for this specific variant, in which the amplification prefers the wild-type 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 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 younger, 20.1% had a GBA1 variant. In clinical practice, 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 compared with noncarriers. In the current study, carriers of p.D140H + p.E326K had significantly more first-degree relatives with PD compared with 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 because of the rarity of these variants.

The current study has some limitations. Because 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 2 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 time frame, 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

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**TABLE 2.** International comparison of Parkinson’s disease cohorts that performed full GBA1 gene sequencing, sorted based on total percent of GBA1 variant carriers [Color table can be viewed at wileyonlinelibrary.com]

| Country            | PD (n) | GBA1 (%) | E326K | T369M | N370S | L444P | D140H + E326K | Other |
|--------------------|--------|----------|-------|-------|-------|-------|----------------|-------|
| Ashkenazi Jewish   | 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             | 1130   | 12.5     | 4.2   | 1.5   | 2.9   | 1     | 0.1            | 2.7   |
| Colombia           | 131    | 12.2     | 1.5   | 0     | 2.3   | 2.3   | 0              | 6.1   |
| Norway             | 442    | 12.0     | 6.6   | 3.6   | 0.2   | 1.4   | 0              | 0.5   |
| Spain              | 532    | 11.7     | 3     | 0.9   | 0.9   | 2.4   | 0              | 4.3   |
| United States      | 1369   | 11.6     | 5     | 2.2   | 1.3   | 1.2   | 0              | 1.9   |
| United Kingdom     | 1893   | 11.1     | 4.5   | 1.8   | 0.6   | 1.6   | 0.1            | 2.4   |
| Eastern Canada     | 225    | 11.1     | 1.8   | 4.9   | 0.9   | 1.8   | 0              | 1.8   |
| Belgium            | 266    | 9.8      | 4.1   | 1.1   | 1.1   | 1.5   | 0.4            | 1.5   |
| Japan              | 534    | 9.4      | 0     | 0     | 0     | 4.1   | 0              | 5.2   |
| New Zealand        | 229    | 9.2      | 4.8   | 3.1   | 0.4   | 0     | 0.4            | 0.9   |
| Sweden             | 1625   | 8.3      | 5.8   | N/A   | 0.4   | 2.2   | N/A            | N/A   |
| Peru               | 471    | 7.2      | 1.1   | 0.6   | 0.2   | 2.8   | 0              | 1.8   |
| Russia             | 762    | 6.6      | 2.4   | 2.5   | 0.5   | 1.1   | N/A            | N/A   |
| Greece             | 172    | 6.4      | 0.6   | 0     | 0     | 1.2   | 0              | 4.7   |
| Portugal           | 230    | 6.1      | 0.9   | 0.9   | 2.2   | 1.3   | 0              | 0.9   |
| Korea              | 277    | 6.1      | 0     | 0     | 0     | 0.7   | 0              | 5.4   |
| North Africa       | 194    | 4.6      | 0.5   | 1.0   | 1.0   | 1.5   | 0              | 0.5   |

PD, Parkinson’s disease; NL, the Netherlands; N/A, not applicable.

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. References: Ashkenazi Jewish (1), Netherlands (current study), France (2), Colombia (3), Norway (4), Spain (5), United States (6), United Kingdom (7), eastern Canada (8), Belgium (9), Japan (10), New Zealand (11), Sweden (12), Peru (3), Russia (13), Greece (14), Portugal (15), Korea (16), and north Africa (17).
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 toward a dose effect based on clinical association of the variant.

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Supporting Data

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