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Specifically Neuropathic Gaucher’s Mutations Accelerate Cognitive Decline in Parkinson’s

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Objective: We hypothesized that specific mutations in the β-glucocerebrosidase gene (GBA) causing neuropathic Gaucher’s disease (GD) in homozygotes lead to aggressive cognitive decline in heterozygous Parkinson’s disease (PD) patients, whereas non-neuropathic GD mutations confer intermediate progression rates.

Methods: A total of 2,304 patients with PD and 20,868 longitudinal visits for up to 12.8 years (median, 4.1) from seven cohorts were analyzed. Differential effects of four types of genetic variation in GBA on longitudinal cognitive decline were evaluated using mixed random and fixed effects and Cox proportional hazards models.

Results: Overall, 10.3% of patients with PD and GBA sequencing carried a mutation. Carriers of neuropathic GD mutations (1.4% of patients) had hazard ratios (HRs) for global cognitive impairment of 3.17 (95% confidence interval [CI], 1.60–6.25) and a hastened decline in Mini–Mental State Exam scores compared to noncarriers (p = 0.0009). Carriers of complex GBA alleles (0.7%) had an HR of 3.22 (95% CI, 1.18–8.73; p = 0.022). By contrast, the common, non-
neuropathic N370S mutation (1.5% of patients; HR, 1.96; 95% CI, 0.92–4.18) or nonpathogenic risk variants (6.6% of patients; HR, 1.36; 95% CI, 0.89–2.05) did not reach significance. **Interpretation:** Mutations in the GBA gene pathogenic for neuropathic GD and complex alleles shift longitudinal cognitive decline in PD into “high gear.” These findings suggest a relationship between specific types of GBA mutations and aggressive cognitive decline and have direct implications for improving the design of clinical trials.

**Subjects and Methods**

**Study Participants and Procedures**

Seven longitudinal cohorts from North America and Europe representing 2,304 patients with PD (and available DNA) were analyzed (Table 1). The analysis included two population-based, incident cohort studies (Cambridgeshire Parkinson’s Incidence from GP to Neurologist [CamPaIGN], Parkinsonism: Incidence, Cognition and Non-motor heterogeneity in Cambridgeshire [PICNICS])14,18; five purpose-built biomarkers and clinical observational studies from academic centers (Harvard Biomarker Study [HBS], PROfiling Parkinson’s disease [PROPARK], and the French Drug Interaction with Genes in PD [DIGPD]); as well as two well-phenotyped, failed phase III clinical trials with longitudinal, observational extension studies (Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism [DATATOP])15; Parkinson Research Examination of CEP-1347 Trial/A Longitudinal Follow-up of the PRECEPT Study Cohort [PreCEPT/PostCEPT])13. Six cohorts enrolled patients with a diagnosis of PD established according to modified UK PD Society Brain Bank diagnostic criteria. In DATATOP, the eligibility criteria required a diagnosis of early, idiopathic PD (Hoen & Yahr [HY] stages 1 or 2) not on antiparkinsonian medications.23 Detailed eligibility criteria for the cohorts have been previously reported.10,13,14,16,22–25 For all cohorts, diagnostic certainty was increased by confirming the clinical diagnosis of PD during longitudinal follow-up visits.26 In PRECEPT, the diagnosis was supported by neuroimaging. In the DATATOP, the diagnosis was further informed by record reviews and autopsies. Patients identified during follow-up whose evaluations were not consistent with a diagnosis of PD were excluded from analysis. Written informed consent was obtained from all subjects under the supervision of each local ethics committee. Patients with a known LRRK2 G2019S mutation were excluded.
Mutations were identified through targeted next-generation sequencing of the entire \textit{GBA} coding sequence and flanking intronic regions in four data sets (Table 1). For 173 PD samples in HBS, 332 PD samples in PreCEPT/PostCEPT and 437 PD samples in DATATOP, \textit{GBA} mutations were systematically identified through full sequencing of the exons and flanking intronic regions of \textit{GBA} in RefSeq (NM_001005741.2). To avoid sequencing its neighboring pseudogene, the entire locus was amplified in a single long-range polymerase chain reaction (PCR) reaction using the LA PCR Kit (version 2.1; Takara Bio Inc., Otsu, Japan). Template DNA (100ng) was added to a 50-\textmu{l} reaction along with primers (final concentration, 0.4 \textmu{M}) with the following sequences: forward primer (5'-CGACTTTACAAACCTCCCTG-3') and reverse primer (5'-CCAGATCCTATCTGTGCTGG-3'), and cycling conditions were 94°C for 1 minute (one cycle), 98°C for

### Table 1. Overview of Study Cohorts

| Study (Country) | N (% male) | Age at Enrollment (years, SD) | Years of Education (years, SD) | Study Years (years, range) | Mutation No. of Subjects (%) | Mutations Screened |
|-----------------|------------|-------------------------------|-------------------------------|---------------------------|----------------------------|-------------------|
| HBS (USA)       |            |                               |                               |                           | 42 (7.6)                  | Targeted sequencing or N370S, E326K, T369M genotyping |
| Carriers        | 42 (59.5)  | 65.2 (10.2)                   | 15.0 (1.7)                    | 1.7 (0.0–5.2)             |                           |                   |
| Noncarriers     | 514 (64.6) | 66.1 (9.8)                    | 15.1 (1.9)                    | 1.8 (0.0–8.0)             |                           |                   |
| DATATOP (USA, Canada) | 38 (8.7)                    | Targeted sequencing           |                               |                           |                           |                   |
| Carriers        | 39 (51.3)  | 61.1 (8.1)                    | 13.6 (3.2)                    | 6.6 (0.0–7.6)             |                           |                   |
| Noncarriers     | 398 (68.1)| 60.0 (9.1)                    | 14.3 (3.4)                    | 6.3 (0.0–7.8)             |                           |                   |
| DIGPD (France)  |            |                               |                               |                           | 32 (7.8)                  | Sanger sequencing    |
| Carriers        | 32 (50.0)  | 60.9 (8.9)                    | 11.3 (3.3)                    | 2.5 (0.0–5.0)             |                           |                   |
| Noncarriers     | 377 (59.4)| 62.5 (9.8)                    | 12.1 (3.3)                    | 2.2 (0.0–5.0)             |                           |                   |
| CamPaIGN (UK)   |            |                               |                               |                           | 15 (13.2)                 | Sanger sequencing    |
| Carriers        | 15 (73.3)  | 67.1 (9.4)                    | 10.5 (2.6)                    | 6.3 (0.0–11.8)            |                           |                   |
| Noncarriers     | 99 (54.5)  | 69.8 (9.9)                    | 11.7 (3.4)                    | 7.0 (0.0–12.8)            |                           |                   |
| PICNICS (UK)    |            |                               |                               |                           | 8 (6.2)                   | Sanger sequencing    |
| Carriers        | 8 (62.5)   | 63.8 (7.8)                    | 12.5 (2)                      | 3.7 (0.0–4.7)             |                           |                   |
| Noncarriers     | 121 (66.1)| 69.2 (9.2)                    | 12.1 (2.9)                    | 3.0 (0.0–6.7)             |                           |                   |
| PROPARK (Netherlands) | 53 (16.2) | Targeted sequencing or whole-exome sequencing |
| Carriers        | 53 (69.8)  | 58.9 (10.1)                   | 12.2 (4.4)                    | 4.5 (0.0–5.4)             |                           |                   |
| Noncarriers     | 274 (65.3) | 59.7 (10.9)                   | 12.0 (4.1)                    | 4.7 (0.0–6.3)             |                           |                   |
| PreCEPT (USA, Canada) | 32 (9.6) | Targeted sequencing          |
| Carriers        | 32 (56.3)  | 58.5 (7)                      | 15.8 (3.4)                    | 6.7 (0.0–8.2)             |                           |                   |
| Noncarriers     | 300 (67.7)| 60.7 (9.6)                    | 16.1 (3.1)                    | 6.7 (0.0–8.6)             |                           |                   |

The study names are Harvard Biomarkers Study (HBS)\textsuperscript{10,21,22}; Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism (DATATOP)\textsuperscript{15,25}; Parkinson Research Examination of CEP-1347 Trial/A Longitudinal Follow-up of the PRECEPT Study Cohort (PreCEPT/PostCEPT)\textsuperscript{13}; Cambridgeshire Parkinson’s Incidence from GP to Neurologist (CamPaIGN)\textsuperscript{6,11,24}; Parkinsonism: Incidence, Cognition and Non-motor heterogeneity in Cambridgeshire (PICNICS)\textsuperscript{14}; Drug Interaction with Genes in PD (DIGPD)\textsuperscript{25}; and PROfiling PARKinson’s disease (PROPARK) study.\textsuperscript{16} HBS was examined in two parts: 383 participants for whom targeted genotyping of three \textit{GBA} mutations was performed; 173 individuals for whom full sequencing of the \textit{GBA} locus was performed (targeted sequencing).
10 seconds followed by 68°C for 15 minutes (30 cycles), and 72°C for 10 minutes (one cycle). This long-range PCR assay uses primers that target sequences that uniquely flank GBA and produces a single 7,755-base-pair (bp) PCR product. PCR products were visualized on an 0.8% ethidium bromide gel to confirm successful amplification, which were then used to construct Illumina ready sequencing libraries using the NexteraXT kit (Illumina Inc., San Diego, CA), following the manufacturer’s instructions. Uniquely indexed samples were pooled (up to 384 samples/pool) and run on the Illumina MiSeq instrument to generate 150-bp paired-end reads. Sequencing reads were aligned to the human assembly genome (GRCh37/hg19) using BWA27 (version 0.6.1). GBA mutations were called by the GATK28 (version 1.6) toolkit. A genotype quality of at least 50 and at least 10× coverage was achieved for all samples. As a quality control, reproducibility of mutation detection was assessed by sequencing 57 samples across different batches in replicates, and the concordance rate was 100% across the GBA mutation locus for all replicates. For 383 PD sample in HBS, E326K, N370S, and T369M were previously genotyped.21 Genotypes for 339 of the 383 samples were confirmed on the Illumina NeuroX chip with a genotyping concordance rate of 100%; for 114 PD samples in CamPaIGN and 129 PD samples in PICNICS, mutations and common genetic variants were aligned to the human reference genome (hg19) with BWA.27

Analysis (version 5.1) and Seqscape (version 2.6) software (Applied Biosystems), then electrophoresed on an 0.8% agarose gel with ethidium bromide. Sequencing products were purified using the Big Dye XT erminator Reaction kit (Applied Biosystems, Foster City, CA), as prescribed. PCR products were visualized on an 0.8% agarose gel with ethidium bromide. Sequencing reads were aligned to the human reference genome (hg19) with BWA.27 Single-nucleotide variants and small insertions/deletions (indels) were called and filtered using the GATK (version 3.x).28 For 91 of the 163 samples, the BAM files were available and calculation of GBA coverage was performed. On average, 99.5% of the exonic GBA regions were covered for at least 15×. For the remaining samples, the overall targeted exome coverage (10×) is 90.8%.

Study Design and Statistical Analysis

Differences in continuous and categorical baseline characteristics were compared between noncarriers and carriers of genetic variation in GBA (all carriers, carriers of the non-neuropathic N370S mutation, and carriers of neuropathic GD mutations, respectively), with Student t tests, and distribution-free Mann–Whitney U or Fisher’s exact tests, as appropriate.

The primary aim of this study was to analyze the effect of four types of GBA mutations on cognitive progression in PD. To characterize how distinct types of GBA mutations are associated with longitudinal disease progression in PD, we compared four operationally defined groups of PD patients with distinct types of GBA mutations to patients with PD not carrying a GBA mutation. Patients with PD carrying one of four types of GBA mutations or variant were considered and compared to patients with PD not carrying a GBA mutation (non-carriers), respectively. 1. Carriers of a GBA risk variant. The E326K, T369M, and E388K variants are associated with risk of PD21,30 and are linked to GD when occurring in conjunction with other GBA mutations, but it is controversial whether they are per se pathogenic for GD.31 2. Carriers of the common, non-neuropathic N370S mutation. The pathogenic N370S mutation is classically associated with mild, non-neuropathic phenotypes of GD type 1. 3. Carriers of a neuropathic GD mutation. For the purpose of this study, patients with PD who were heterozygous carriers of a pathogenic GBA mutation associated with neuropathic GD subtypes 2 or 3 in one or more published reports (found on PubMed searches in September 2015) were operationally defined as “carriers of a neuropathic GD mutation” (Supplementary Table 1). This included carriers of the L444P mutation, the prototypical mutation associated with neuropathic GD types 2 and 3. 4. Carriers of complex GBA alleles. Patients carrying complex GBA alleles (eg, more than one GBA mutation/variant) were grouped separately. We also explored longitudinal cognitive decline in patients with PD carrying any pathogenic GBA mutation or risk variant taken together ("all GBA carriers") compared to patients with PD not carrying a GBA mutation.

Generalized longitudinal mixed fixed- and random-effects analysis of cognitive decline was performed32 using serial Mini–Mental State Exam (MMSE) scores longitudinally observed in all cohorts starting from study enrollment. Ninety-five percent of visits (19,801 of 20,868 study visits) were conducted within 6.5 years from the enrollment visit (median follow-up period, 4.1 years; maximum follow-up period, 12.8 years). The MMSE score was the dependent variable and the primary predictors were carrier status, time in the study (years), and their interaction. Fixed covariates were sex, age, and disease duration upon enrollment and years of education. An intercept term and linear rate of change across time per subject were the random terms (permitted to be correlated). A study term was also included as a random effect. To avoid problems with somewhat non-normal residuals, p values were obtained by penalized quasi-likelihood ratio tests of the full model with the effect in question contrasted with the model without the effect in question. These analyses were implemented in R33 (version 3.1.2; R Foundation for Statistical Sciences) using the glmmPQL function in the MASS package (version 7.3-40). p values less than or equal to 0.05 were considered statistically significant.
Cox proportional hazards models were used to estimate the influence of carrier status on the hazard ratio of time to the endpoint reaching global cognitive impairment as indicated by a MMSE ≤25 according to the recommendation the International Parkinson and Movement Disorder Society (MDS) Task Force.34 Age at onset was defined as the patient’s age at the time of PD diagnosis for six studies. PROPARK defined age at onset as the patient’s age at the time of first patient-reported motor symptoms. A total of 63.7% of patients (1,467 of 2,304) were enrolled into a study cohort within 2 years from their onset age, and 95% of study visits fell within 10 years since onset (19,840 of 20,868 visits). For exploratory analysis of motor progression, we evaluated the HR of time to reaching advanced PD operationally defined as reaching an HY stage 3 (bilateral disease with loss of postural balance). One hundred eighty-eight patients with global cognitive impairment at enrollment and 244 with HY stage 3 already reached at enrollment were removed from the Cox proportional hazards analyses, respectively. Multivariate Cox regression analysis was performed for each study separately and then across studies with carrier status, age at onset of PD, sex, and years of education as predictors of global cognitive impairment and the same except for education as predictors of HY stage 3. For the metanalysis across studies, a “study” term was included as a random effect (a “frailty” model). The proportional hazards assumption of the Cox regression model was tested and not violated by any predictor in any analysis. Cochran’s Q-test was used to test for heterogeneity of effects across studies.

**Hypothetical Power Analysis for a Personalized Clinical Trial Targeting Neuropathic GBA-PD**

To estimate sample-size requirements for a personalized, 3-year clinical trial of a hypothetical drug designed to halt cognitive decline (as measured by the MMSE) specifically in patients carrying a neuropathic GBA mutation, we ran a power analysis. We used a repeated-measures analysis of variance design of two groups versus four time points (enrollment, 1 year, 2 years, and 3 years in study). One hypothetical group was assigned to placebo and therefore stipulated to have MMSE means across time predicted by our mixed-effects model, and the second group was assigned to treatment with an experimental drug, which has the hypothetical ability to halt decline in MMSE scores (all scores set equal to the predicted MMSE scores at enrollment). We ran analyses assuming a two-tailed \( \alpha = 0.05 \) to detect the difference in trajectories across time for the two groups (group \( \times \) time interaction), and assuming a within group/time-point standard deviation (SD) of 2 and a 1-year test-retest correlation of 0.7 as approximate to those values found empirically, with a first-order autoregressive decay across longer periods. The conservative Greenhouse-Geisser correction for degrees of freedom for correlated error was also used. For comparison, analogous computations were performed for a hypothetical clinical trial scenario with “all comer” PD patients (not carrying a GBA mutation).

We found required sample sizes to be 36 per placebo and 36 per drug group in order to achieve 80% power. By contrast, if instead all-comer patients with PD (not carrying a GBA mutation) were enrolled to test the same experimental drug (over the same time period, assuming same \( \alpha \), same SD, and same test-retest correlations), 893 patients would be required per placebo and 893 per drug group to achieve 80% power (Fig 3). Thus, a trial targeted to neuropathic GBA-PD could shrink sample-size requirements by as much as 25-fold compared to an equally powered trial of all-comer PD patients without a GBA mutation.

**Results**

**Clinical Cohort Characteristics at Enrollment**

Mean ages at enrollment across the seven cohorts ranged from 59.6 to 69.4, and mean MMSE scores from 27.1 to 29.3. Mean HY stages at enrollment ranged from 1.0 to 2.6.

**GBA Mutations**

Overall, 10.3% of the 1,921 patients with PD analyzed by sequencing were carriers of a GBA mutation (Fig 1). A total of 1.4% (26) of patients were heterozygous carriers of a neuropathic GD mutation (Fig 1). These included the L444P mutation (found in 12 heterozygous carriers), as well as the 84GG, G195E, H255Q, R257Q, P266L, R359X, G377S, D409H, L444R, A456P, N462K, R120W, and R463C mutations (Supplementary Table 1). A total of 1.5% (28) were carriers of the non-neuropathic N370S mutation. A total of 6.6% (127) were heterozygous carriers of a risk variant (79 were carriers of the E326K, 46 with T369M, and 2 with E388K risk variants). A total of 0.7% (14) were carriers of a complex GBA allele. Three additional rare mutations (D140H, K(-27)R, and R463P) were detected. The R463P and K(-27)R mutations have not been conclusively associated with a GD subtype and were thus excluded from the mutation-type specific analyses. D140H mutations identified were part of complex alleles (Fig 1; Supplementary Table 1).

**Patients With PD Carrying GBA Mutations Enriched in Neuropathic GD Had an Aggressive, Accelerated Longitudinal Cognitive Decline**

At enrollment, disease duration, age at onset, and levodopa equivalent drug dose were not materially different in carriers of a neuropathic GBA mutation compared to noncarriers included in the proportional hazards model analysis (Table 2). Importantly, for this analysis, at enrollment, MMSE scores were identical for carriers of a neuropathic GD mutation compared to noncarriers; their motor scores (HY stage and MDS Unified Parkinson’s Disease Rating Scale Part III) at enrollment were slightly elevated (Table 2).
We performed a generalized mixed random- and fixed-effects longitudinal meta-analysis for MMSE scores, adjusting for the covariates of age at enrollment, sex, duration of PD at enrollment, and years of education. Neuropathic GD mutations predicted a significant decline in MMSE scores over time with $p < 0.0001$ (Fig 2A).

Carriers of a neuropathic GBA mutation had a Cox proportional HR for global cognitive impairment of 3.17 (95% confidence interval [CI], 1.60–6.25) compared to noncarriers ($p = 0.0022$), adjusting for age of onset, sex, years of education, and study (Fig 2C,E). At 10 years from diagnosis, 79.5% (95% CI, 76.4–82.8) of noncarriers were free of global cognitive impairment compared to 52.2% (95% CI, 33.9–80.5) of neuropathic GD mutation carriers (a 27.3% difference).

Patients with PD Carrying Complex GBA Alleles Also Had a More Rapid Longitudinal Cognitive Decline

Carriers of complex GBA alleles had a Cox proportional HR for global cognitive impairment of 3.22 (95% CI, 1.18–8.73) compared to noncarriers ($p = 0.022$), adjusting for age of onset, sex, years of education, and study (Fig 2C,E). In the mixed random- and fixed-effects model analysis, the decline in MMSE scores was somewhat less pronounced, likely due to lower MMSE scores at enrollment recorded for this group of patients (Fig 2A).

Cognitive Decline in Patients With PD Carrying the Non-Neuropathic N370S Mutation Was Not Materially Faster Than in Noncarriers

Carriers of the non-neuropathic N370S mutation alleles had a Cox proportional HR for global cognitive impairment of 3.22 (95% CI, 1.18–8.73) compared to noncarriers ($p = 0.022$), adjusting for age of onset, sex, years of education, and study (Fig 2C,E). In the mixed random- and fixed-effects model analysis, the decline in MMSE scores was somewhat less pronounced, likely due to lower MMSE scores at enrollment recorded for this group of patients (Fig 2A).
impaired of 1.96 (95% CI, 0.92–4.18) compared to noncarriers, adjusting for age of onset, sex, years of education, and study (Fig 2C,E).

Patients with PD carrying GBA risk variants had a Cox proportional HR for global cognitive impairment of 1.36 (95% CI, 0.89–2.05) compared to noncarriers, adjusting for age of onset, sex, years of education, and study (Fig 2E).

GBA mutations predicted a decline in MMSE scores over time (Fig 2B) in all carriers compared to noncarriers with \( p = 0.0001 \) in the generalized mixed random- and fixed-effects model analysis, adjusting for covariates and study. Multiple lines of evidence, including sensitivity analyses, \(^{35}\) indicated that dropout bias did not unduly influence this analysis. All carriers of a GBA mutation, taken together, had a Cox proportional HR for global cognitive impairment of 1.75 (95% CI, 1.28–2.40) compared to noncarriers (\( p = 0.0005 \)), adjusting for age of onset, sex, years of education, and study (Fig 2D). The HRs for global cognitive impairment in mutation carriers versus noncarriers from the Mantel-Haenszel procedure were examined for each of the seven independent cohorts. Proportional HRs across studies were homogeneous with \( I^2 = 40.5\% \), \( p = 0.21 \) by Cochran’s Q-test for heterogeneity, suggesting it was not imperative, though permissible, to analyze “studies” as a random term in order to allow a more-universal inference.

We then explored the effect of GBA mutations on motor disease progression. HY stage 3 marks the transition from mild to moderate disease with impaired balance. It is meaningful for patients because of fall risk and impact on quality of life.\(^ {36}\) In this population, none of the four types of GBA mutations investigated was statistically significantly associated with progression to HY stage 3 compared to noncarriers, respectively, in the Cox proportional hazards model adjusted for sex and age at onset. For all carriers of a GBA mutation, taken together, the HR for progressing to HY stage 3 was increased by a factor of 1.26 compared to noncarriers. However, this missed the threshold for statistical significance (95% CI, 0.98–1.61; \( p = 0.068 \)).

**Discussion**

Seven deeply phenotyped, longitudinal cohorts from North America and Europe representing 2,304 patients with PD were followed longitudinally for up to 12.8 years (median, 4.1) with a total of 20,868 in-person study visits. This makes the current analysis one of the largest longitudinal observational studies reported for PD. Much progress has been made in delineating genome variation associated with susceptibility for

| TABLE 2. Clinical Characteristics of Participants With PD at Enrollment |
|---------------------------------------------------------------|
| **N** = 2,116 | Noncarriers | All GBA Carriers | \( p^* \) | Carriers of Non-neuropathic N370S | \( p^{**} \) | Carriers of Neuropathic GD Mutations | \( p^{***} \) |
|---------------------------------------------------------------|
| Total No. (N) | 1,918 | 198 | 38 | 24 |
| No. of men (N, %) | 1,242 (64.6) | 115 (58.1) | 0.07 | 24 (63.2) | 0.86 | 9 (37.5) | 0.01 |
| No. of Europeans (N, %)* | 1,602 (83.5) | 150 (75.8) | 0.01 | 32 (84.2) | 1.00 | 19 (79.2) | 0.58 |
| Age of onset (years, SD) | 60.1 (10.6) | 58.4 (9.7) | 0.02 | 59.3 (8.9) | 0.56 | 59.7 (10.9) | 0.83 |
| Disease duration at enrollment (years, SD) | 2.6 (3.4) | 2.6 (3.7) | 0.96 | 2.0 (2.3) | 0.13 | 2.0 (3.3) | 0.36 |
| Years of education (years, SD) | 14.1 (3.4) | 13.6 (3.6) | 0.07 | 13.7 (3.2) | 0.49 | 13.8 (3.9) | 0.77 |
| MMSE (mean, SD) | 28.8 (1.2) | 28.8 (1.2) | 0.97 | 28.8 (1.1) | 0.89 | 29.2 (1.0) | 0.17 |
| MDS-UPDRS III (mean, SD) | 27.7 (13.1) | 29.4 (12.9) | 0.06 | 23.9 (12.3) | 0.06 | 32.2 (10.5) | 0.04 |
| Hoehn & Yahr stage (mean, SD) | 1.8 (0.7) | 1.9 (0.7) | 0.03 | 1.7 (0.7) | 0.49 | 2.1 (0.6) | 0.02 |
| Levodopa equivalent drug dose\(^b\) (mean, SD) | 330.5 (393.3) | 375.5 (430.9) | 0.27 | 403.2 (377.1) | 0.09 | 481.2 (612.8) | 0.28 |

Group comparisons were performed using Student t test for age at enrollment, age at onset, and disease duration at enrollment; Mann-Whitney-Wilcoxon test for MDS-UPDRS, Hoehn & Yahr, MMSE, and levodopa equivalent drug dose; and the Fisher’s exact test for sex and ancestry. Note that at enrollment, mean MMSE scores for carriers and non-carriers were virtually identical (bold).

* All carriers compared to noncarriers; \(^{**}\) carriers of the non-neuropathic N370S mutation compared to noncarriers; \(^{***}\) neuropsych GD mutation carriers compared to noncarriers.

\(^b\)Ancestry information was not available for PROPARF.

\(^a\)Levodopa equivalent drug dose was not available for the PreCEPT/PostCEPT cohort.
developing PD,25,37 but little is known about the genetic architecture controlling disease progression.11,24,38 In a surprising analogy to GD, GBA mutations enriched in neuropathic GD in homozygotes (leading to death at age 2 or severe neurological complications) were associated with aggressive cognitive decline in PD. Patients carrying these mutations had an HR of 3.17 (95% CI, 1.60–6.25) for developing global cognitive impairment compared to patients without a mutation—a 217% risk increase. By contrast, patients carrying the common N370S mutation linked to non-neuropathic GD showed a trend toward intermediate rates of longitudinal cognitive decline. This did not reach statistical significance, likely due to insufficient power. This report is the first to evaluate the differential effects of distinct types of neuropathic, complex alleles, non-neuropathic, and risk variation in GBA on the longitudinal trajectory of cognitive decline in PD. It suggests the clinical-genetic concept of aggressive, neuropathic GBA-PD. The cross-sectional observation that PD is more frequent among carriers of a
neuropathic GD mutations, compared to carriers of non-neuropathic GD mutations in an Ashkenazi-Jewish population, is fully consistent with the new longitudinal trajectories here discovered.

This concept has implications for trial design. Enrolling patients with neuropathic GBA-PD into genetics-guided, proof-of-concept trials for disease-modifying precision therapeutics may increase power, shorten trial duration, shrink sample sizes, and, possibly, cut costs. Our hypothetical power analysis estimates that a trial targeted to neuropathic GBA-PD could shrink the sample size required by as much as 25-fold compared to an equally powered trial of PD patients without a GBA mutation (Fig 3). Furthermore, the MMSE used in the current analysis has limited sensitivity. More-sensitive instruments, such as the Montreal Cognitive Assessment scale, might detect cognitive changes in carriers of a neuropathic GD mutation earlier and further improved statistical power and shorten trial duration. Importantly, prospective interventions (eg, designed to prevent dementia in patients with PD) or therapies with significant side effects require accurate risk-benefit analyses based on the prognosis for individual patients. Can enough patients with PD and a neuropathic GD mutation be recruited for clinical trials? Neuropathic GD mutations are as common as sporadic PD and

**FIGURE 2:** Specifically neuropathic GD mutations accelerate cognitive decline in patients with PD. (A,C,E) Neuropathic GD mutations carriers were linked to a more-rapid cognitive decline in PD (in heterozygotes). (A) Neuropathic GD mutations predicted decline in Mini–Mental State Exam scores over time in the generalized longitudinal mixed model meta-analysis in heterozygous patients. Illustrative mean scores on the MMSE across time predicted from the estimated fixed-effect parameters in the mixed random- and fixed-effects model analysis are shown for Parkinson’s patients carrying specific types of GBA mutations and those without a GBA mutation. Carriers of a neuropathic GD mutation showed accelerated longitudinal cognitive decline compared to noncarriers with \( p < 0.0001 \), adjusting for the covariates of age at enrollment, sex, duration of PD upon enrollment, and years of education. Illustrative mean scores on the MMSE across time for PD patients with a neuropathic GD mutation are shown as magenta triangles; values for PD patients without a GBA mutation are represented as blue squares. Illustrative mean scores on the MMSE across time for carriers of a risk variant (heterozygous carriers of E326K, T369M, and E388K; green triangles), the non-neuropathic N370S mutation (brown rhombi), or complex GBA alleles (orange circles) are also shown. (B) Illustrative mean MMSE scores across time predicted from the estimated fixed-effect parameters in the mixed random- and fixed-effects model analysis are shown for Parkinson’s patients without a GBA mutation (noncarriers) and those carrying any of the GBA mutations (all carriers). Carriers had overall a more-rapid decline in cognitive function (as measured by serial MMSE) compared to noncarriers with \( p = 0.0001 \), after adjusting covariates (solid lines indicate mean value of disease duration at enrollment; dotted-dashed lines indicate 1 SD longer disease duration at enrollment; and dashed lines indicate 1 SD shorter disease duration at enrollment). (C) Covariate adjusted survival curves for Parkinson’s patients without a GBA mutation (noncarriers; blue line) and those carrying specific types of GBA mutations: risk variants (green, interrupted line), the common, non-neuropathic N370S mutation (brown, dotted line), neuropathic GD mutations (magenta line), or complex GBA alleles (orange, dotted-dashed line). (D) All carriers of a GBA mutation, taken together, had an overall hazard ratio for global cognitive impairment of 1.75 (95% CI, 1.28–2.40) compared to noncarriers with \( p = 0.0005 \), adjusting for age of onset, sex, years of education, and study. The covariate adjusted survival curves are shown (carriers, red interrupted line; noncarriers, blue line). The means of covariate-adjusted predicted values are visualized. (E) The forest plot shows hazard ratios for global cognitive impairment in carriers of one of these specific types of GBA mutations. The hazard ratio for global cognitive impairment in carriers of neuropathic GD mutation was 3.17 (95% CI, 1.60–6.25; magenta). The squares represent point estimates, with the height of the square inversely proportional to the standard error of the estimates. The horizontal lines indicate 95% confidence intervals of the estimates. In (A), the group of patients with neuropathic GD mutations includes 26 heterozygous carriers with the following mutations: 12 with L444P, 2 with R463C, and 1 each of R257Q, 8dupG, R120W, D409H, R359X, P266L, N462K, A456P, L444R, G377S, H255Q, and G195E. The 14 carriers of complex alleles shown in (A) included 8 patients with E326K and D140H, 1 with E326K and T369M, 1 with E326K and R463C, and 1 with E326K and R257Q; and homozygous carriers with E326K/E326K, T369M/T369M, and E326/E326K/L444P/L444P genotypes, respectively. In (C), the group of patients with neuropathic GD mutations includes 24 heterozygous carriers with neuropathic GD mutations: 12 with L444P, 2 with R463C, and 1 each of R257Q, 8dupG, R120W, D409H, R359X, P266L, N462K, A456P, L444R, G377S, H255Q, and G195E. The 14 carriers of complex alleles shown in (A) included 8 patients with E326K and D140H, 1 with E326K plus D140H mutations, 1 with E326K plus T369M, 1 with E326K plus R463C, and 1 with E326K plus R257Q; and homozygous carriers with E326K/E326K, T369M/T369M, and E326/E326K/L444P/L444P genotypes, respectively. It should be noted that in the Cox proportional hazards analyses, the number of mutation carriers differs from that in the mixed fixed- and random-effects analysis, attributed to the removal of subjects, who had already reached the endpoint at enrollment (left censored). The number of mutation carriers available for this analysis also differs from the number of carriers shown in Figure 1 because partially genotyped samples were here included, whereas data only for fully sequenced samples are shown in Figure 1. CI = confidence interval; GD = Gaucher’s disease; SD = standard deviation.
This study has considerable strengths. Genetic analyses of the GBA locus were performed at the end of the clinical longitudinal follow-up period. Physicians therefore recruited and longitudinally assessed the participants without knowledge of their GBA genotype. This design should be less vulnerable to recruitment and ascertainment bias than previous case-control studies given that patients were assigned to one of two groups simply based on the presence or absence of mutated GBA alleles in a form of “double-blinded Mendelian randomization.” The meta-analysis included two community-based cohorts6,14 and six from academic centers with cohort-specific eligibility criteria, differences in enrollment ages, and recruitment from distinct source populations.

Confirmation across multiple heterogeneous populations likely adds to the validity of the results. Although time-static “cross-sectional” studies certainly have their value, longitudinal studies are generally considered more informative and powerful and provide information about individual change.32 Most important, of course, is the fact that longitudinal designs examine the critical time dimension, which could be entirely hidden in a cross-sectional study.

A constraint of this meta-analysis is that in DATA-TOP and PreCEPT, DNA was collected several years after enrollment for a subset of participants.13 Thus, they may under-represent patients with more rapidly progressive disease, but it is unlikely that this would yield a spurious association between GBA mutations and rapid cognitive decline.

The mechanism through which GBA mutations modulate the PD process is controversial.45 Autosomal-recessive mutations in GBA cause GD through a decrease or loss in GCase function and replacing GCase enzyme in patients with GD corrects hepatosplenomegaly and hematological abnormalities. L444P, the prototypical mutation of neuropathic GD, leads to reduced β-glucocerebrosidase (GCase) enzymatic activity.37,46 N370S appears to confer a milder reduction in GCase activity.46 In PD, however, both GBA loss-of-function and toxic gain-of-function hypotheses have been proposed.5,47 In cultured cells, mutant GBA promoted α-synuclein accumulation in a dose-dependent manner without observed loss of GCase function.47 However, in PD patients heterozygous for a GBA mutation, GCase activity is reduced in induced pluripotent stem-cell–derived neurons,48 brain,45 cerebrospinal fluid,48 and blood.37 Reduced GCase activity causes accumulation of glucosyleramide,48 lysosomal dysfunction,49 and accumulation of α-synuclein protein,47–50 a neuropathological hallmark of PD. Furthermore, α-synuclein, in turn, may further reduce the availability of functional glucocerebrosidase,45,49 and GCase activity is low even in PD patients without a GBA mutation, although to a lesser extent than those with a mutation.45 Thus, it is possible that therapeutics effective in GBA-PD could also be of benefit for PD patients without a GBA mutation.

In GD, enzyme replacement therapy is effective in reversing peripheral disease manifestations, but does not penetrate the blood–brain barrier. In an encouraging development for both neuropathic GD and PD, small
molecules designed to correct the GCase pathway in the brain are in development.

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Potential Conflicts of Interest
C.R.S. has a scientific collaboration with Genzyme.

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