ORIGINAL ARTICLE

Multicenter Analysis of Glucocerebrosidase Mutations in Parkinson's Disease

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ABSTRACT

BACKGROUND

Recent studies indicate an increased frequency of mutations in the gene encoding glucocerebrosidase (*GBA*), a deficiency of which causes Gaucher's disease, among patients with Parkinson's disease. We aimed to ascertain the frequency of *GBA* mutations in an ethnically diverse group of patients with Parkinson's disease.

METHODS

Sixteen centers participated in our international, collaborative study: five from the Americas, six from Europe, two from Israel, and three from Asia. Each center genotyped a standard DNA panel to permit comparison of the genotyping results across centers. Genotypes and phenotypic data from a total of 5691 patients with Parkinson's disease (780 Ashkenazi Jews) and 4898 controls (387 Ashkenazi Jews) were analyzed, with multivariate logistic-regression models and the Mantel–Haenszel procedure used to estimate odds ratios across centers.

RESULTS

All 16 centers could detect two *GBA* mutations, L444P and N370S. Among Ashkenazi Jewish subjects, either mutation was found in 15% of patients and 3% of controls, and among non–Ashkenazi Jewish subjects, either mutation was found in 3% of patients and less than 1% of controls. *GBA* was fully sequenced for 1883 non–Ashkenazi Jewish patients, and mutations were identified in 7%, showing that limited mutation screening can miss half the mutant alleles. The odds ratio for any *GBA* mutation in patients versus controls was 5.43 across centers. As compared with patients who did not carry a *GBA* mutation, those with a *GBA* mutation presented earlier with the disease, were more likely to have affected relatives, and were more likely to have atypical clinical manifestations.

CONCLUSIONS

Data collected from 16 centers demonstrate that there is a strong association between *GBA* mutations and Parkinson's disease.

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N Engl J Med 2009;361:1651-61.

Several lines of evidence suggest an association between parkinsonism and mutations in the gene encoding the lysosomal enzyme glucocerebrosidase (*GBA*), which is deficient in patients with Gaucher's disease. In this rare mendelian disorder, lysosomal accumulation of glucocerebroside results in a broad spectrum of disease manifestations including hepatosplenomegaly, anemia, thrombocytopenia, bone disease, and, at times, neurologic involvement.[1,2] Multiple independent studies have reported an increased frequency of *GBA* mutations in various cohorts of patients with parkinsonism.[3-21] However, several genomewide association studies have not identified this locus, and the degree of association has remained somewhat unclear, as many studies were not large enough to unequivocally label *GBA* mutations as risk alleles associated with Parkinson's disease.

Recognition of the association between *GBA* mutations and parkinsonism began in the clinic, with the identification of rare patients with Gaucher's disease who also had parkinsonian symptoms. Clinical descriptions appeared as case reports,[22,23] larger series of patients[24,25] and prospective studies.[26] Pedigree analyses revealed that relatives of patients with Gaucher's disease, many of whom were obligate heterozygotes, had an increased incidence of Parkinson's disease.[27,28]

Almost 300 *GBA* mutations have been identified in patients with Gaucher's disease, including missense, nonsense, and frameshift mutations as well as insertions, deletions, and complex alleles.[29] Various expression studies have shown that many of these mutations result in a significant loss of glucocerebrosidase activity.[30] The *GBA* gene, located on 1q21–22, includes 11 exons and has a similar pseudogene 16 kb downstream. The 85-kb region surrounding *GBA* is particularly gene-rich, encompassing seven genes and two pseudogenes.[31] Recombination within and around the *GBA* locus occurs relatively frequently,[32] complicating genotype analyses.

The frequency and distribution of *GBA* mutations vary among populations. Among Ashkenazi Jews, for whom the carrier frequency is between 1 person in 12 and 1 in 16,[1,6] one common mutation, N370S, accounts for approximately 70% of mutant alleles.[1] In other ethnic groups, the carrier frequency is usually less than 1%, and the associated mutations are more diverse. Although N370S is also common in European populations and is exclusively associated with non-neuronopathic Gaucher's disease, it has not been encountered among Asians.[33] A second relatively frequent and panethnic mutation associated with neuronopathic Gaucher's disease is L444P, which can be a point mutation or part of a complex allele encompassing a portion of the pseudogene sequence.[32]

This international collaborative study of *GBA* mutations in patients with Parkinson's disease was undertaken to better ascertain the frequency of *GBA* mutations by pooling data for individual persons from 16 centers, representing 4 continents, including 5691 patients and 4898 controls. Findings based on the data from 13 of the 16 centers (4185 of the case patients and 3597 of the controls) have been published previously.[5-16,19] Our goals were to establish the combined frequency of *GBA* mutations among sites, to explore the range of *GBA* mutations encountered, and to identify clinical features shared among *GBA* mutation carriers.

METHODS

STUDY SUBJECTS AND PROCEDURES

Researchers known to be genotyping cohorts of patients with Parkinson's disease for *GBA* mutations were solicited for this collaboration. Sixteen centers participated, and data were collected and analyzed at the National Human Genome Research Institute (NHGRI), Bethesda, Maryland (Table 1). The subjects were from a total of 16 centers: 4 in North America, 1 in South America, 3 in Asia, 2 in Israel, and 6 in Europe. Ethnicity was self-reported. Ashkenazi Jews provided the country of origin of their grandparents. Written informed consent was obtained from all subjects under the supervision of each local ethics committee. All patients fulfilled the clinical diagnostic criteria for Parkinson's disease of the U.K. Parkinson's Brain Bank.[34]

Because the detection methods and number of mutations that could be identified varied greatly from center to center, 12 standard DNA samples from patients with Gaucher's disease were genotyped by each center, and the results were analyzed at the NHGRI. All centers could reliably detect mutations N370S and L444P, unless the mutant allele included large stretches of the *GBA* pseudogene sequence. Some centers could identify three to eight specific selected point mutations (Table 1). In addition, five centers sequenced all the exons of *GBA*, and a sixth sequenced exons for a sub-

group of subjects. Thus, results evaluating two mutations (N370S and L444P), six to eight mutations, and the entire coding sequence were analyzed separately (see Table 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). Two frequent *GBA* variants, E326K and T369M, were evaluated but not included as mutant alleles.

The extent of clinical data collected from each site varied (Table 1). Some study centers provided only the age, family history, sex, ethnic group, and diagnosis of each subject, whereas others reported more complete data, including symptoms at presentation, age at disease onset, specific clinical manifestations, response to medications, and standardized scores on the Hoehn and Yahr staging system, the Unified Parkinson's Disease Rating Scale, or both. Only one proband was studied per family, and patients with diagnoses other than Parkinson's disease were excluded.

Controls were screened for signs or symptoms of parkinsonism, and centers attempted to match controls and patients with regard to age, sex, and ethnic group. Controls with a family history of Parkinson's disease were removed because of their increased risk of having parkinsonism ultimately develop.

STUDY DESIGN

The a priori aim in this study was to conduct a combined analysis of risk associated with *GBA* mutations from various centers. Our analyses used patient-level data from both published and unpublished studies. We assessed associations between Parkinson's disease and *GBA* mutations in not only all available genotyped samples, but also in distinct subgroups of the total population (see the Supplementary Appendix for detailed analyses of subgroups).

STATISTICAL ANALYSIS

Descriptive statistics were calculated for each study center. Available data on family history of Parkinson's disease and seven clinical features of Parkinson's disease (asymmetric onset, bradykinesia, cognitive changes, orthostatic hypotension, postural instability, resting tremor, and rigidity) were analyzed. Clinical features were compared between case patients carrying *GBA* mutations and those without mutations, means were compared with the use of a two-tailed Student's t-test, and frequency differences were compared between patients with and those without *GBA* mutations by means of the chi-square test.

We first estimated the odds ratios for mutations in *GBA* across cohorts. To accomplish this, we conducted fixed-effects Mantel–Haenszel analyses using all available case patients and controls from each study center. These models calculate crude odds ratios and confidence intervals from counts of mutations in case patients and controls in each study and then combine the odds ratios from study-specific two-by-two tables into a summary measure. Three Mantel–Haenszel analyses were performed, using any mutation, N370S, and L444P as the separate independent variables. No multiple-test correction was used in our analyses.

The heterogeneity of effects in the Mantel–Haenszel analyses were evaluated using Woolf's test for heterogeneity.[35] Analyses of the possible interactions contributing to the heterogeneity of odds ratios were limited by the data available for analysis, so several additional Mantel–Haenszel analyses were carried out. First, we excluded data from Japan and Norway, both together and independently, on the basis of the assumption that general genetic homogeneity in these samples could be compounded by the active recruitment of family members of case patients, influencing the independence (or in the Norwegian cohort, the nonindependence) of differences in mutation frequency between case patients and controls. An additional analysis, performed by omitting data from Tel Aviv, Israel — the center with the most robust odds ratio with respect to the standard error of the estimate — confirmed that these data were not inflating the combined odds ratio.

To follow up on the results from the Mantel–Haenszel analyses, multivariate logistic-regression models were used to examine the association between *GBA* mutations and Parkinson's disease in subgroups stratified on the basis of the level of sequencing coverage and Ashkenazi Jewish or non–Ashkenazi Jewish ethnicity. These stratified models combined data for subjects across studies, including all subjects with complete data for covariates of self-reported ethnic group, age at the time of sampling, and sex. For regression modeling and covariate descriptions, data for 702 patients and 570 controls were excluded because age, sex, or ethnic group was unknown. Identical parameters were used in logistic-regression models testing the association of Parkinson's disease with variants E326K and T369M. Chi-square tests of

heterogeneity were used to compare effect-size differences between models stratifying Ashkenazi Jewish and non–Ashkenazi Jewish ethnicity assessing the risks attributable to all *GBA* mutations and N370S and L444P only, across all levels of sequencing coverage. Detailed results and a description of these regression models are given in the Supplementary Appendix.

All data analyses were conducted with the use of R software (version 2.8.0).[36] Source code for plotting of the meta-analysis results is available in the software package "r.meta" (available from http://cran.cnr.berkeley.edu).

RESULTS

Genotyping was performed for a total of 5691 patients with Parkinson's disease: 780 Ashkenazi Jewish patients and 4911 patients with no known Ashkenazi Jewish ancestry. The 4898 controls for whom genotyping was performed included 387 Ashkenazi Jewish persons and 4511 from other ethnic groups. Table 1 lists the frequency of mutations and baseline characteristics of cohorts at each center. Full sequencing was performed on DNA samples obtained from 2060 patients and 1677 controls.

Table 1. Baseline Data for the Study Groups, According to Center.*

Center	No. of Subjects (No. Who Are Ashkenazi Jewish)†	Sex Ratio	Mean Age of Subjects at Time of Collection	Mutations	Mutations Screened	Clinical Data Provided‡
		M:F (unknown)	*yr*	*no. of subjects (%)*		
Brazil					N370S, L444P, G377S	Limited
Patients	65 (0)	42:23	54.1	4 (6)		
Controls	264 (0)	169:95	54.4	0		
New York					Full sequencing	Full
Patients	275 (177)	171:104	65.6	34 (12)		
Controls	140 (65)	73:67	62.8	3 (2)		
France					N370S, L444P, D409H	Full
Patients	297 (0)	185:112	57.8	12 (4)		
Controls	251 (0)	142:109	57.8	1 (<1)		
Haifa, Israel					N370S, L444P, c.84dupG, V394L, IVS2+1G→A, R496H	Partial
Patients	162 (162)	94:65 (3)	68.5	40 (25)		
Controls	NP	NP	NP	NP		
Italy					L444P, N370S	Full
Patients	395 (0)	244:151	66.5	11 (3)		
Controls	483 (0)	180:303	56.9	1 (<1)		
Norway					L444P, N370S	Limited
Patients	311 (0)	186:123 (2)	NP	7 (2)		
Controls	473 (0)	267:206	64.1	8 (2)		
NHGRI					Full sequencing	Partial
Patients	539 (0)	275:166 (98)	73.5	29 (5)		
Controls	209 (1)	100:109	67.6	6 (3)		
Portugal					Full sequencing	Limited
Patients	231 (0)	110:121	65.3	15 (6)		
Controls	482 (0)	Unknown	65.5	6 (1)		
Rostock, Germany					Full sequencing	Partial
Patients	298 (0)	190:108	64.3	18 (6)		
Controls	212 (0)	105:107	74.5	5 (2)		

Table 1. (Continued.)

Center	No. of Subjects (No. Who Are Ashkenazi Jewish)†	Sex Ratio	Mean Age of Subjects at Time of Collection	Mutations	Mutations Screened	Clinical Data Provided‡
		M:F (unknown)	*yr*	*no. of subjects (%)*		
Singapore					L444P, N370S	Partial
Patients	329 (0)	170:158 (1)	70.3	8 (2)		
Controls	201 (0)	99:102	64.2	0		
Taiwan					N370S, L444P, Rec*Nci*I, R120W, some full sequencing (183 patients and 88 controls)	Full
Patients	559 (0)	304:255	69.1	22 (4)		
Controls	377 (0)	198:179	60.5	4 (1)		
Tel Aviv, Israel					c.84dupG, IVS2+1G→A, N370S, V394L, D409H, L444P, R496H, RecTL	Limited
Patients	420 (419)	262:158	68.0	81 (19)		
Controls	321 (321)	159:162	65.3	13 (4)		
Japan					Full sequencing	Limited
Patients	534 (0)	282:252	65.3	50 (9)		
Controls	546 (0)	294:252	44.8	2 (<1)		
Tübingen, Germany					L444P, N370S	Partial
Patients	377 (0)	222:155	64.8	12 (3)		
Controls	325 (0)	192:132 (1)	58.3	0		
Toronto					N370S, K178T, L444P, c.84dupG, R329C, IVS2+1G→A, Rec*Nci*I	Partial
Patients	88 (2)	51:37	55.0	5 (6)		
Controls	96 (0)	27:69	69.6	1 (1)		
Seattle					N370S, L444P, Rec*Nci*I, L444R	Full
Patients	811 (20)	607:204	66.9	24 (3)		
Controls	518 (0)	193:324 (1)	65.2	2 (<1)		

* NHGRI denotes National Human Genome Research Institute, and NP not provided.
† Ashkenazi Jewish ancestry was self-reported.
‡ "Limited" clinical data were age, family history, sex, ethnic group, and diagnosis of each subject; "full" were the limited clinical data plus symptoms at presentation, age at disease onset, specific clinical manifestations, response to medications, and standardized scores on the Hoehn and Yahr staging system, the Unified Parkinson's Disease Rating Scale, or both; and "partial" were the limited clinical data plus some but not all of the full clinical data.

Figure 1 shows the odds ratios for mutations in patients versus controls, standard errors, and confidence limits from the Mantel–Haenszel procedure performed on data from each independent study center; the odds were also combined across the centers. Data from four centers were excluded in this analysis, because individual controls were not provided (Haifa, Israel) or mutations were not found among the controls (Brazil, Singapore, and Tübingen, Germany).

Results from the analysis of any *GBA* mutation as an independent variable (Fig. 1A) show that each center had an overrepresentation of mutations among patients as compared with controls, although the confidence intervals varied considerably. Eight centers had odds ratios greater than 5. The overall combined odds ratio shows how greatly the confidence interval is reduced when data from the individual centers are combined. Individual odds ratios for the *GBA* point mutations L444P and N370S are also shown (Fig. 1B and 1C, respectively). Although the odds ratios for each mutation are overwhelmingly positive, they were higher for L444P.

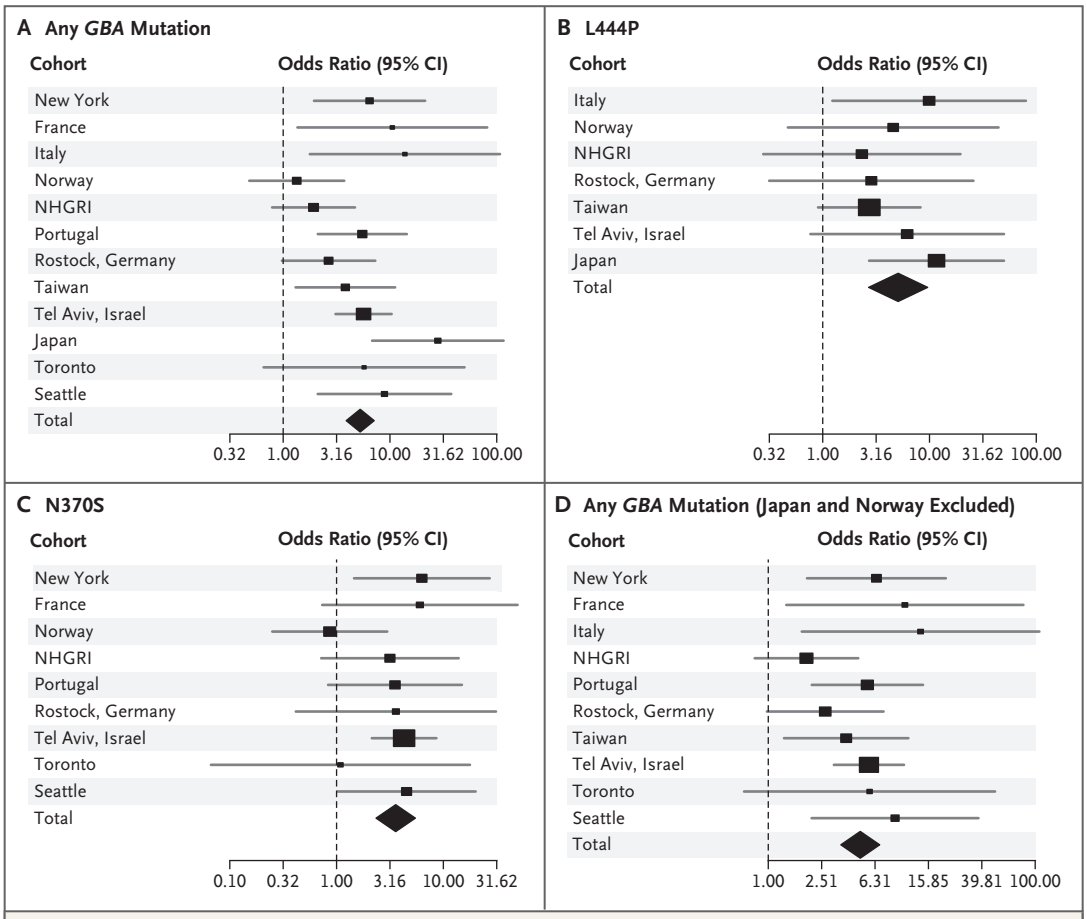

Figure 1. Odds Ratios for *GBA* Mutations among Patients, as Compared with Controls, at Each Study Center and Overall.

Shown are the combined estimates (on a $\log_{10}$ scale) of the risk of possessing any *GBA* mutation (Panel A), the L444P mutation (Panel B), the N370S mutation (Panel C), and any *GBA* mutation when all data from the Japan and Norway study centers are excluded (Panel D). The squares represent point estimates, with the height of the square inversely proportional to the standard error of the estimates. The horizontal lines indicate the 95% confidence intervals of the estimates. The diamonds represent the summary odds ratio, with the width indicating the 95% confidence interval. Data from four centers were excluded, because individual controls were not provided (Haifa, Israel) or mutations were not found among the controls (Brazil, Singapore, and Tübingen, Germany). NHGRI denotes National Human Genome Research Institute.

Combined odds ratios from Mantel–Haenszel analyses were homogeneous for L444P and N370S (P=0.35 and P=0.50, respectively, by Woolf's test for heterogeneity). This finding allows for confidence in reporting the Mantel–Haenszel combined odds ratios for N370S (odds ratio, 3.96; 95% confidence interval [CI], 2.60 to 6.02) and L444P (odds ratio, 6.73; 95% CI, 4.50 to 15.42). However, the Mantel–Haenszel odds ratio for any *GBA* mutation was significantly heterogeneous (P=0.02 by Woolf's test for heterogeneity). After excluding data from Japan and Norway, the odds ratio was slightly attenuated (5.43; 95% CI, 3.89 to 7.57), but the heterogeneity was no longer significant (P=0.41 by Woolf's test for heterogeneity), and further excluding the Tel Aviv data resulted in a homogeneous odds ratio (P=0.34 by Woolf's test for heterogeneity) of 5.30 (95% CI, 3.59 to 7.94). Independent exclusions of data from either Norway or Japan resulted in homogeneous odds ratios (Japan data excluded: odds ratio, 4.91; 95% CI, 3.60 to 6.70; P=0.14 by Woolf's test for heterogeneity; Norway data excluded: odds ratio, 6.35, 95% CI, 4.60 to 8.75; P=0.10 by Woolf's test for heterogeneity) (Fig. 1D). Thus, we can confidently report a Mantel–Haenszel odds ratio of 5.43 for

GBA mutations, in patients versus controls, even when these centers are excluded.

Overall, when screening solely for N370S and L444P, one of these two mutations was found in 15% of Ashkenazi Jewish patients as compared with 3% of Ashkenazi Jewish controls and in 3% of non–Ashkenazi Jewish patients as compared with less than 1% of non–Ashkenazi Jewish controls. However, the frequency of N370S in Ashkenazi Jewish patients differed among the centers. N370S was not seen in any Asian patients or controls.

All Ashkenazi Jewish subjects were screened for the presence or absence of six to eight different *GBA* mutations (Table 1). Including this larger number of screened mutations (rather than just L444P and N370S) increased the odds ratio for any mutation, among patients as compared with controls, to 6.48 (95% CI, 3.78 to 11.09). The distribution of specific *GBA* mutations among Ashkenazi Jewish patients with Parkinson's disease, of whom approximately 20% carried a *GBA* mutation, is shown in Figure 2A. Approximately 20% of the identified mutant alleles were neither L444P nor N370S. Among the non–Ashkenazi Jewish subjects, the entire *GBA* coding region was sequenced in 1883 patients and 1611 controls; the odds ratio for any *GBA* mutation was 6.51 (95% CI, 3.62 to 11.74). The distribution of mutations identified among non–Ashkenazi Jewish patients (Fig. 2B) indicates that as many as 45% of mutant alleles could be missed if focusing solely on N370S and L444P. Moreover, 20 of 43 non–Ashkenazi Jewish patients who had complete sequence data and had L444P carried other pseudogene changes and hence had recombinant alleles.

The full-sequencing data identified two common *GBA* variants, E326K and T369M, which are not pathogenic in patients with Gaucher's disease.[37] Neither mutation had a significant association with Parkinson's disease (see the Supplementary Appendix).

Seventeen patients (13 of whom were Ashkenazi Jews) carried two *GBA* mutations. Thirteen of these patients had the N370S/N370S genotype, two had the N370S/R496H genotype, one had the N370S/V394L genotype, and one had the N370S/L444P genotype.

Age at onset was found to be significantly lower among patients with *GBA* mutations as compared with those without mutations (P<0.001), with a mean age of 54.9 years and 58.8 years, respectively. The mean period from diagnosis to evaluation was 7.8 years in both groups.

Information about a family history of parkinsonism was available for 4401 of the patients studied. In all, 18% of patients without *GBA* mutations reported having a first- or second-degree relative with Parkinson's disease, as compared with 24% of patients with a *GBA* mutation (P=0.006).

The frequencies of seven clinical findings associated with Parkinson's disease in patients with *GBA* mutations and those without mutations are shown in Figure 3. In general, the symptom profile for the two groups was similar, although mutations were associated with a significantly lower frequency of asymmetric onset (P<0.001), bradykinesia (P<0.001), resting tremor (P=0.03), and rigidity (P<0.001). There was no significant difference between the two groups in the frequency of orthostatic hypotension (P=0.26) or postural instability (P=0.12). Among the 1948 patients for whom the presence or absence of cognitive changes was recorded, changes were reported for 26% of patients with mutations and 19% of those without (P=0.007).

DISCUSSION

The results of our analysis indicate that there is an association between *GBA* mutations and Parkinson's disease. The combined data show that this finding is not exclusive to a specific ethnic group or a specific *GBA* mutation. Although mutations in *GBA* are most likely a susceptibility factor rather than a mendelian cause of Parkinson's disease, the high frequency of mutations among ethnically diverse, heterogeneous cohorts of patients with Parkinson's disease makes the mutations in this gene the most common genetic risk factor for Parkinson's disease that have been identified to date.

The major limitation of this study was the unavoidable differences in data-ascertainment methods among the study centers. Moreover, some sites were more successful than others in matching case patients and controls with regard to age and sex. To ensure that the analysis was not driven by a small subgroup of centers, we evaluated the variance in odds ratios across centers. Excluding the center with the most precise estimate (Tel Aviv) and the centers with the most extreme odds ratios (Norway and Japan) resulted in only a slight at-

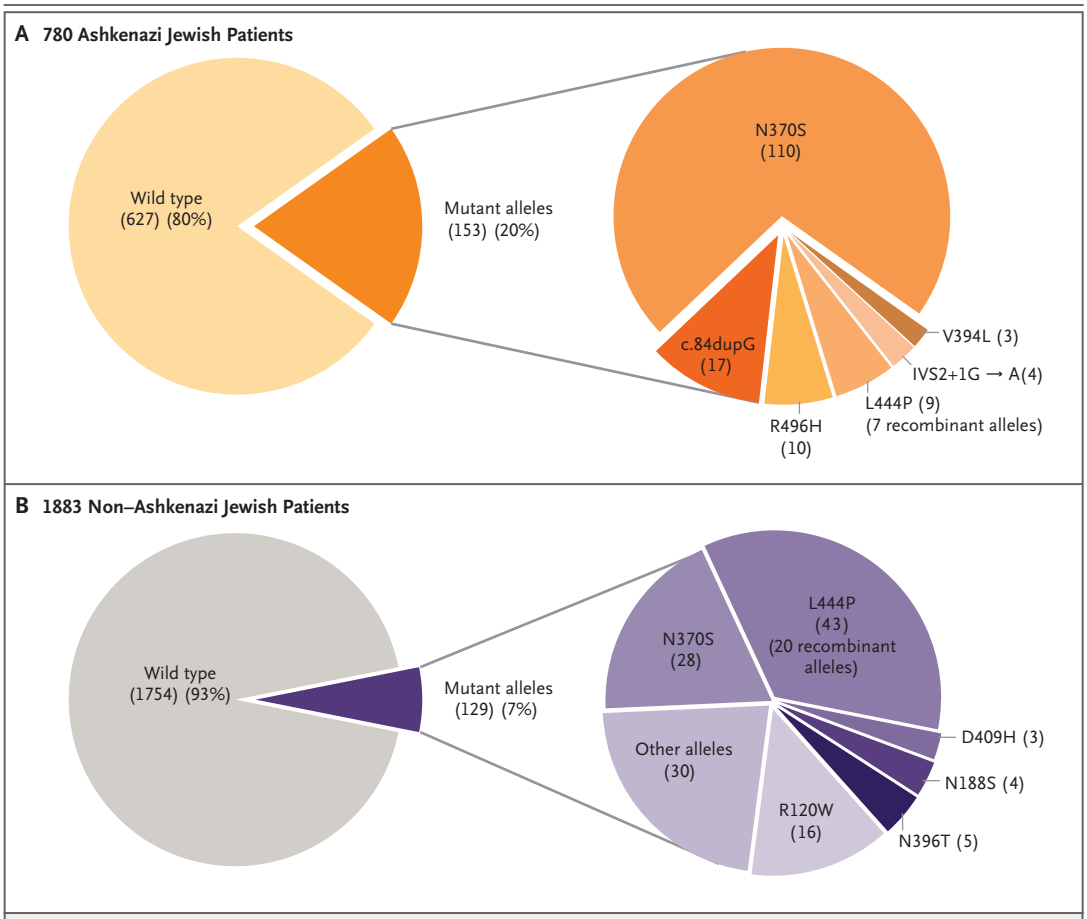

Figure 2. Distributions of Mutations among Study Patients of Ashkenazi Jewish Ancestry and Those of Non–Ashkenazi Jewish Ancestry.

Data are shown for patients with Parkinson's disease, with the numbers (and percentages) of patients in parentheses. A total of 20% of Ashkenazi Jewish patients were found to carry a *GBA* mutation (Panel A). Among non–Ashkenazi Jewish patients for whom full sequencing was performed, a total of 7% carried *GBA* mutations (Panel B). "Other alleles" are D140H, F216Y, N370K, D380N, F213I, G193W, G344S, K198T, K27R, L336P, L371I, Q497R, R131C, R131S, R163Q, R163X, R239C, R262H, R39H, R44C, S271G, T323I, R329C, R496C, and V460M.

tenuation of the combined odds ratio for any *GBA* mutation among patients as compared with controls, which in all analyses remained 5.43 or higher.

This study confirms the need to sequence all exons to accurately ascertain the frequency of *GBA* mutations among both patients and controls. The combined odds ratios may be underestimated, because so many of the centers performed limited mutation analyses. Our data show that among non–Ashkenazi Jewish patients, as many as 45% of mutant alleles can be missed when screening for only the N370S and L444P mutations. Furthermore, analysis of specific mutations may produce a serious bias. Among the 1883 non–Ashkenazi Jewish patients who had sequenced samples, *GBA* mutations were found in 7% (odds ratio vs. controls, 6.51), although only 35% of the samples included in our entire analysis were fully sequenced. An adequate sample size and accurate genotyping of control samples are imperative to avoid underestimating the frequencies of rare variants.

Despite the difficulty in determining the phenotypic profile associated with the *GBA* mutations found in this study, which intentionally included only patients that met diagnostic criteria for Parkinson's disease, some trends are apparent. As compared with patients who did not have a *GBA* mutation, those with a mutation presented on av-

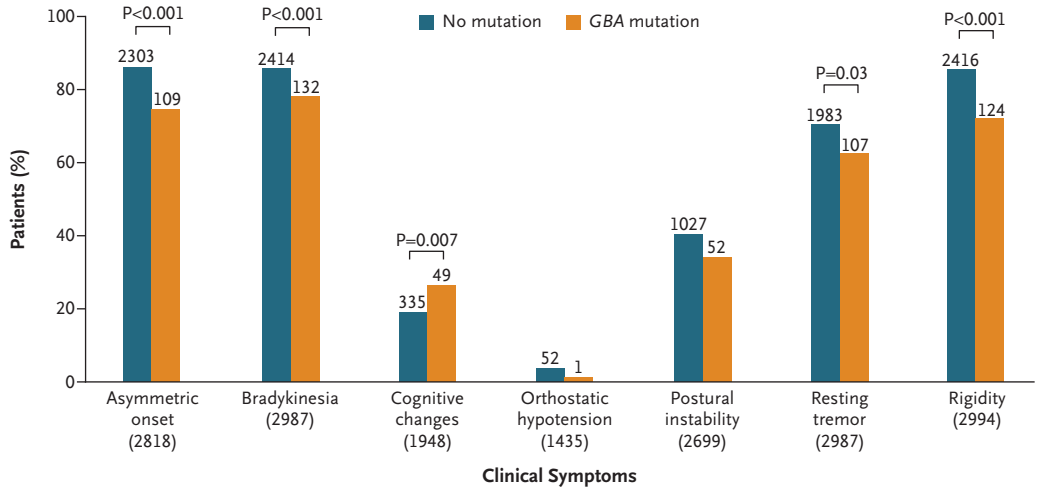

Figure 3. Clinical Manifestations of Parkinson's Disease in Study Patients with a *GBA* Mutation and Those without a Mutation.

The numbers of patients for whom the presence or absence of each symptom was recorded are indicated below the pairs of bars. The number of patients with the symptom is given at the top of each bar.

erage 4 years earlier, were more likely to have a family history of Parkinson's disease, and had a lower incidence of bradykinesia and resting tremor and a higher incidence of cognitive changes, a trend supporting earlier reports.[3,5,6,18,19,24,25] However, because of the exclusion criteria used in the analysis, our findings do not accurately reflect the full spectrum of parkinsonian symptoms associated with *GBA* mutations. An increased frequency of *GBA* mutations has also been described in cohorts with Lewy-body disorders,[38-40] although not in multiple-system atrophy,[41] and further studies are warranted.

Even though our data provide validation of *GBA* as a risk factor associated with Parkinson's disease, the ultimate challenge is to establish the mechanisms contributing to this association. Both a gain-of-function mechanism due to enhanced protein aggregation or lysosomal dysfunction[42] and a loss of function related to fluctuations in levels of ceramide[43] have been postulated. Further research to elucidate the pathophysiology of both Gaucher's disease and Parkinson's disease will facilitate more accurate genetic counseling and lead to the development of new therapeutic strategies.

Supported in part by the Intramural Research Programs of NHGRI and the National Institute on Aging (project Z01AG000957-05), National Institutes of Health; grants from the National Institute of Neurological Disorders and Stroke and the National Center for Research Resources (NS050487, NS060113, and NS40256); grants from the Parkinson's Disease Foundation; a Merit Review Award from the Department of Veterans Affairs, Seattle; a grant from the German National Genome Network, German Ministry for Education and Research (01GS08134); a grant from the French Parkinson's Disease Genetics Study Group; a grant from the Duke–National University of Singapore (NUS) Graduate Medical School, National Medical Research Council, Biomedical Research Council, Singapore Millennium Foundation; a grant from SingHealth, Singapore General Hospital, National Neuroscience Institute; the Tel Aviv Sourasky Medical Center Grant of Excellence; grants from the Wolfson and Kahn Foundations; a grant from the Portuguese Fundação para a Ciência e a Tecnologia; a grant from the National Science Council, Executive Yuan, Taiwan (NSC-95-2314-B-182A-061); and a Grant-in-Aid for Scientific Research on Priority Areas, Applied Genomics, and Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Dr. Berg reports receiving lecture fees from UCB, GlaxoSmithKline, Teva, and Lundbeck, advisory fees from Novartis, UCB, GlaxoSmithKline, and Teva, and grant support from Janssen Pharmaceuticals and Teva Pharma; Dr. Fahn, receiving lecture fees from Boehringer Ingelheim and consulting fees from Anteres Pharma, Boehringer Ingelheim, Elsai, EMD Serono, Genzyme, Impax Pharmaceuticals, Intec Pharma, Kyowa, Novartis, Teva, Valeant, and Vernalis and serving on the Merz Pharma Data and Safety Monitoring Board; Dr. Farrer, holding a provisional patent for RNA interference as a treatment for neurodegenerative disease and a patent for LRRK2 mutations and receiving research support from Lundbeck and Isis Pharmaceuticals and lecture fees from Genzyme and GlaxoSmithKline; Dr. Gasser, receiving research support from Novartis, consulting fees from Cephalon Pharma and Merck Serono, and lecture fees from Boehringer Ingelheim, Merck Serono, Novartis, and Schwarz Pharma and holding a patent for the detection of LRRK2 mutations; Dr. Griffith, receiving consulting fees from Teva and lecture fees from Teva and Novartis; Dr. Lang, receiving advisory fees from Biovail, Boehringer Ingelheim, Cephalon, Ceregene,

Eisai, Medtronic, Lundbeck, NeuroMolecular, Novartis, Solvay, Taro, and Teva, speaker's fees or other support from GlaxoSmithKline and UCB, and grant support from Taro; Dr. Ottman, receiving consulting fees from Ortho-McNeil Janssen Scientific Affairs and holding equity in Trigeminal Solutions; Dr. Perera, receiving grant support from Genzyme of Brazil; and Dr. Rozenberg, receiving grant support form Genzyme of Brazil. No other potential conflict of interest relevant to this article was reported.

We thank John Hardy, Ph.D. (from the Institute of Neurology, Queen's Square, London) for helpful discussion; Dora Yearout for technical assistance; John W. Roberts, M.D., for recruitment of subjects at the University of Washington, Seattle; Jae Choi and Julia Fekecs for preparation of drafts of the figures and Chunhui Yu for developing the online survey (all from NHGRI); Neil Oden (from Emmes, Rockville, MD) for statistical assistance; and T. Lumley for maintenance of source codes in R software.

APPENDIX

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