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Multi-center analysis of glucocerebrosidase mutations in Parkinson disease

Ellen Sidransky, M.D.^{1,+}, Michael A. Nalls, Ph.D.⁸, Jan O. Aasly, M.D., Ph.D.², Judith Aharon-Peretz, M.D.³, Grazia Annesi, Ph.D.⁴, Egberto Reis Barbosa, M.D.⁵, Anat Bar-Shira, Ph.D.⁶, Daniela Berg, M.D.⁷, Jose Bras, M.S.^{8,9}, Alexis Brice, M.D.^{10,11,12}, Chiung-Mei Chen, M.D., Ph.D.¹⁴, Lorraine N. Clark, Ph.D.^{15,16,17}, Christel Condroyer^{10,11}, Elvira Valeria De Marco, Ph.D.⁴, Alexandra Dürr, M.D., Ph.D.^{10,11,12}, Michael J. Eblan, B.A.¹, Stanley Fahn, M.D.¹⁸, Matthew Farrer¹⁹, Hon-Chung Fung, M.D., Ph.D.¹⁴, Ziv Gan-Or^{6,20}, Thomas Gasser, M.D.⁷, Ruth Gershoni-Baruch, M.D.^{21,22}, Nir Giladi, M.D.^{20,23}, Alida Griffith, M.D.²⁴, Tanya Gurevich, M.D.^{20,23}, Cristina Januario, M.D.²⁵, Peter Kropp, M.D.²⁶, Anthony E. Lang, M.D., F.R.C.P.C.²⁷, Guey-Jen Lee-Chen, Ph.D.²⁸, Suzanne Lesage, Ph.D.^{10,11}, Karen Marder, M.D. M.P.H.^{15,18,29,30}, Ignacio F. Mata, Ph.D.³¹, Anat Mirelman, Ph.D.²³, Jun Mitsui, M.D.³², Ikuko Mizuta, M.D., Ph.D.³³, Giuseppe Nicoletti, M.D.^{4,34}, Catarina Oliveira, M.D., Ph.D.^{9,25}, Ruth Ottman, Ph.D.^{29,35}, Avi Orr-Urtreger, M.D., Ph.D.^{6,20}, Lygia V. Pereira, Ph.D.³⁶, Aldo Quattrone, M.D.^{4,34}, Ekaterina Rogaeva, Ph.D.³⁷, Arndt Rolfs, M.D.³⁸, Hanna Rosenbaum, M.D.³⁹, Roberto Rozenberg, Ph.D.³⁶, Ali Samii, M.D.³¹, Ted Samadder, B.S.¹, Claudia Schulte⁷, Manu Sharma⁷, Andrew Singleton, Ph.D.⁸, Mariana Spitz, M.D.⁵, Eng-King Tan, M.D.^{40,41}, Nahid Tayebi, Ph.D.¹, Tatsushi Toda, M.D., Ph.D.³³, André Troiano, M.D.^{10,11}, Shoji Tsuji, M.D., Ph.D.³², Matthias Wittstock, M.D.⁴², Tyra G. Wolfsberg, Ph.D.⁴³, Yih-Ru Wu, M.D.¹⁴, Cyrus P. Zabetian, M.D., M.S.³¹, Yi Zhao, M.D., Ph.D.⁴⁰, and Shira G. Ziegler, B.A.^{1,*}

¹Section on Molecular Neurogenetics, Medical Genetics Branch, National Institutes of Health, Bethesda, Maryland, USA

²Department of Neurology, St. Olav's Hospital and Department of Neuroscience, Norwegian University of Science and Technology, Trondheim, Norway

³Cognitive Neurology and Movement Disorder Unit, Technion, Israel Institute of Technology, Haifa, Israel

⁴Institute of Neurological Sciences, National Research Council, Cosenza, Italy

⁵Movement Disorders Unit, Neurology Department, University of São Paulo Medical School, São Paulo, Brazil

+Corresponding author: Ellen Sidransky M.D. sidranse@mail.nih.gov, Chief, Section on Molecular Neurogenetics, NHGRI, Room 1A213, 35 Convent Drive, MSC 3708, Bethesda, MD 20892-3708†, Phone: 301-451-0901, Fax: 301-402-6438.

*Authors are listed in alphabetical order

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⁶The Genetic Institute, Department of Neurology, Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel

⁷Hertie-Institute for Clinical Brain Research, Department of Neurodegenerative Diseases, University of Tuebingen, Tuebingen, Germany

⁸Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, Maryland, USA

⁹Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal

¹⁰INSERM, CRicm UMR_S975 (Formerly UMR_S679), Department of Genetics and Cytogenetics, Paris, France

¹¹Pierre et Marie Curie University, CRicmUMR_S975 CRicm, Department of Genetics and Cytogenetics, Paris, France

¹²AP-HP, Pitié-Salpêtrière Hospital, Department of Genetics and Cytogenetics, Paris, France

¹⁴Department of Neurology, Chang Gung Memorial Hospital, Chang-Gung University College of Medicine, Taipei, Taiwan

¹⁵Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University College of Physicians and Surgeons, New York, NY

¹⁶Center for Human Genetics, Columbia University College of Physicians and Surgeons, New York, NY

¹⁷Department of Pathology, Columbia University College of Physicians and Surgeons, New York, NY

¹⁸Department of Neurology, Columbia University College of Physicians and Surgeons, New York, NY

¹⁹Laboratory of Neurogenetics, Department of Neuroscience, Mayo Clinic College of Medicine, Jacksonville, Florida

²⁰The Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv, Israel

²¹Baruch Human Genetics Rambam Medical Center, Technion, Israel Institute of Technology, Haifa, Israel

²²Bruce Rappaport Faculty of Medicine, Technion, Israel Institute of Technology, Haifa, Israel

²³Movement Disorders Unit, Parkinson Center, Department of Neurology, Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel

²⁴Booth Gardner Parkinson's Care Center, Evergreen Hospital Medical Center, Kirkland, WA

²⁵Neurology Service, University of Coimbra Hospital, Coimbra, Portugal

²⁶Institute for Medical Psychology, University of Rostock, Rostock, Germany

²⁷Movement Disorders Centre, Toronto Western Hospital, Department of Medicine, Division of Neurology, University of Toronto, Toronto, Canada

²⁸Department of Life Science, National Taiwan Normal University, Taipei, Taiwan

²⁹Gertrude H. Sergievsky Center, Columbia University College of Physicians and Surgeons, New York, NY

³⁰Department of Psychiatry, Columbia University College of Physicians and Surgeons, New York, NY

³¹Parkinson's Disease and Geriatric Research Education and Clinical Centers, VA Puget Sound Health Care System, and Department of Neurology, University of Washington, Seattle, WA

³²Department of Neurology, University of Tokyo Graduate School of Medicine, Tokyo, Japan

³³Division of Neurology and Molecular Brain Science, Kobe University Graduate School of Medicine, Kobe, Japan

³⁴Institute of Neurology, Department of Medical Sciences, University Magna Graecia, Catanzaro, Italy

³⁵Department of Epidemiology, Mailman School of Public Health, Columbia University and New York State Psychiatric Institute, New York, NY

³⁶Molecular Genetics Lab, Department of Genetics and Evolutionary Biology, Biosciences Institute, University of São Paulo, São Paulo, Brazil

³⁷Centre for Research in Neurodegenerative Diseases, Tanz Neuroscience Building, University of Toronto, Toronto, Canada

³⁸Albrecht-Kossel-Institute for Neuroregeneration, University of Rostock, Rostock, Germany

³⁹Hematology and Bone Marrow Transplantation Departments, Technion, Israel Institute of Technology, Haifa, Israel

⁴⁰Singapore General Hospital, National Neuroscience Institute, Singapore

⁴¹Duke-NUS Graduate Medical School, Singapore

⁴²Department of Neurology, University of Rostock, Rostock, Germany

⁴³Genome Technology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, USA

Abstract

Background—Recent studies indicate an increased frequency of mutations in the gene for Gaucher disease, glucocerebrosidase (*GBA*), among patients with Parkinson disease. An international collaborative study was conducted to ascertain the frequency of *GBA* mutations in ethnically diverse patients with Parkinson disease.

Methods—Sixteen centers participated, including five from the Americas, six from Europe, two from Israel and three from Asia. Each received a standard DNA panel to compare genotyping results. Genotypes and phenotypic data from patients and controls were analyzed using multivariate logistic regression models and the Mantel Haenszel procedure to estimate odds ratios (ORs) across studies. The sample included 5691 patients (780 Ashkenazi Jews) and 4898 controls (387 Ashkenazi Jews).

Results—All 16 centers could detect *GBA* mutations, L444P and N370S, and the two were found in 15.3% of Ashkenazi patients with Parkinson disease (ORs = 4.95 for L444P and 5.62 for N370S), and in 3.2% of non-Ashkenazi patients (ORs = 9.68 for L444P and 3.30 for N370S). *GBA* was sequenced in 1642 non-Ashkenazi subjects, yielding a frequency of 6.9% for all mutations, demonstrate that limited mutation screens miss half the mutant alleles. The presence of any *GBA* mutation was associated with an OR of 5.43 across studies. Clinically, although phenotypes varied, subjects with a *GBA* mutation presented earlier, and were more likely to have affected relatives and atypical manifestations.

Conclusion—Data collected from sixteen centers demonstrate that there is a strong association between *GBA* mutations and Parkinson disease.

INTRODUCTION

Several lines of evidence suggest an association between parkinsonism and mutations in the gene encoding the lysosomal enzyme glucocerebrosidase (*GBA*), which is deficient in Gaucher 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 different cohorts with parkinsonism^{3–21} However, different genome wide association studies have not identified this locus, and the picture of association has remained somewhat unclear, as many studies were not large enough to unequivocally label *GBA* mutations as risk alleles for typical Parkinson disease.

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

Almost 300 different *GBA* mutations have been identified in patients with Gaucher disease including missense, nonsense and frame shift mutations as well as insertions, deletions and complex alleles.²⁹ Different expression studies have demonstrated that many of these mutations result in a significant loss of glucocerebrosidase activity³⁰. The *GBA* gene, located on 1q21–22, includes 11 exons, and has a highly similar pseudogene 16kb 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 is relatively frequent,³² complicating genotypic analyses.

The frequency and distribution of *GBA* mutations varies among different populations. Among Ashkenazi Jews, where the carrier frequency is between 1 in 12 and 1 in 16^{1, 6}, one common mutation, N370S, accounts for approximately 70% of mutant alleles.¹ In other ethnicities, the carrier frequency is usually less than 1%, and the associated mutations are more diverse. While N370S is also common in European populations, and is exclusively associated with non-neuronopathic Gaucher disease, it has not been encountered among Asians.³³ A second relatively frequent and pan-ethnic mutation, associated with neuronopathic Gaucher disease, is L444P, which can be a point mutation or part of a complex allele encompassing additional pseudogene sequence.³²

This international collaborative study of *GBA* mutations in Parkinson disease was undertaken to better ascertain the frequency of *GBA* mutations by pooling individual data from sixteen centers, representing four continents, including 5691 cases and 4898 controls. Findings based on the data from 13 of the 16 centers (4185 of the cases and 3597 controls) have been previously published^{5–16, 19}. The goals were to establish the frequency of *GBA* mutations among different sites, to explore the range of *GBA* mutations encountered and to identify clinical features shared by *GBA* mutation carriers.

Methods

Patients and procedures

Researchers known to be genotyping Parkinson disease cohorts for *GBA* mutations were solicited for this collaboration. Sixteen centers participated and data were collected and analyzed at the National Human Genome Research Institute (NGHRI) Bethesda, Maryland

(Table 1). The centers included subjects from North America (four groups), South America (one group), Asia (three groups), Israel (two groups) and Europe (six groups). Ethnicity was by self-report. Ashkenazi Jews provided the origin of grandparents. Informed consent was obtained under the supervision of each local ethics committee. All subjects fulfilled the UK Parkinson Disease Brain Bank Clinical Diagnostic Criteria for Parkinson disease.³⁴

As 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 disease were genotyped by each center and the results were analyzed at the NHGRI. All participating groups could reliably detect mutations N370S and L444P, unless the mutant allele included large stretches of *GBA* pseudogene sequence. Four centers could identify 4–9 specific selected point mutations (Table 1). In addition, five participating centers sequenced all exons of *GBA*, and in a sixth, a subgroup was sequenced. Thus, results evaluating two mutations, 4–9 mutations, and the entire coding sequence were analyzed separately (see supplementary table 1). 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 age, family history, sex, ethnicity and diagnosis, while others reported more complete data, including presenting symptoms, age at disease onset, specific clinical manifestations, response to medications and standardized Hoehn and Yahr and/or Unified Parkinson's Disease Rating Scale scores. Only one proband was collected per family and subjects with diagnoses other than Parkinson disease were excluded.

Controls were screened for signs or symptoms of parkinsonism and centers attempted to match for age, sex and ethnicity. Controls with a family history of Parkinson disease were removed due to their increased risk for ultimately developing parkinsonism.

Study Design

The a priori aim in this study was to conduct a combined analysis of risk associated with *GBA* mutations from different centers including both published and unpublished data. Our analyses utilized patient level data from both published and unpublished studies. We assessed associations between Parkinson disease and *GBA* mutations not only in all available genotyped samples, but also in distinct subgroups of the total population (for detailed analyses of subgroups please refer to supplemental text).

Statistical Analyses

Descriptive statistics were calculated and stratified by study center. Available data on family history of Parkinson disease and seven clinical features of Parkinson disease (asymmetric onset, bradykinesia, cognitive changes, orthostatic hypotension, postural instability, rest tremor and rigidity) were analyzed. Clinical features were compared between cases carrying *GBA* mutations and cases without mutations, means were compared using two-tailed Student's T-tests and frequency differences between patients with and without *GBA* mutations were assessed using chi-squared tests.

The primary aim was to estimate the odds ratios (ORs) associated with mutations in *GBA* across cohorts. To accomplish this we conducted fixed effects Mantel-Haenszel analyses using all available cases and controls from each study center. These models calculate crude ORs and confidence intervals from counts of mutations in cases and controls in each study, then combining the ORs from study specific 2×2 tables into a summary measure. Three separate Mantel-Haenszel analyses were performed, using any mutation, N370S and L444P as the 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 Possible analyses of interactions contributing to heterogeneity of ORs 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, based on the assumption that general genetic homogeneity in these samples could be compounded by active recruitment of family members of cases, influencing the independence (or in the Norwegian cohort, the non-independence) of mutation frequency differences between cases and controls. An additional analysis performed omitting data from Tel Aviv, the center with the most robust OR with respect to the standard error of the estimate, confirmed that it was not inflating the combined OR.

To follow up the results from the Mantel-Haenszel analyses, multivariate logistic regression models were used to examine the association between *GBA* mutations and Parkinson disease in subgroups stratified by level of sequencing coverage and Ashkenazi/ non-Ashkenazi status. These stratified models combined data for participants across studies, including all participants with complete data for covariates of self-reported ethnicity, age at sampling and gender. Identical parameters were used in logistic regression models testing the association with variants E326K and T369M. Chi-square tests of heterogeneity were used to compare effect size differences between Ashkenazi and non-Ashkenazi stratified models assessing risk attributable to all *GBA* mutations, and N370S and L444P separately across all sequencing depths. Detailed results and description of these regressions can be found in supplementary materials.

All data analyses were conducted using R 2.8.0.³⁶ Source code for plotting of meta-analysis results is available in the package *r.meta* maintained by Thomas Lumley (available from <http://cran.cnr.berkeley.edu/>).

RESULTS

A total of 5691 genotyped patients with Parkinson disease were evaluated, including 780 Ashkenazi Jewish subjects and 4911 patients with no known Ashkenazi ancestry. The 4898 controls genotyped included 387 Ashkenazi Jewish individuals and 4511 with other ethnicities. Table 1 lists the frequency of mutations and demographics for each individual study. Full sequencing was performed on 1859 patient and 1674 control samples

In Figure 1, the odds ratios, standard errors and confidence limits from the Mantel-Haenszel procedure for each independent study are shown graphically, where the precision of the effect estimate is reflected in the size of the squares, then combining odds across study sites. Panel 1A summarizes the results using any mutation as an independent variable. Each center had an over-representation of mutations among patients compared to controls, although confidence intervals varied considerably. Eight centers had an OR greater than 5. Because one center did not provide individual controls (Haifa, Israel) and three centers did not find mutations among their controls (Brazil, Singapore and Tubingen, Germany), they do not appear in the forest plots. The overall combined OR denoted by the diamond symbol demonstrates how greatly the confidence interval is reduced when the individual studies are combined. Panels 1B and 1C show the individual ORs for *GBA* mutations L444P and N370S respectively. While the results are overwhelmingly positive for each mutation, they were higher for L444P.

Combined ORs for Mantel-Haenszel analyses were homogenous for L444P and N370S (Woolf's test for heterogeneity, p-values of 0.347 and 0.499 respectively). This allows for confidence in reporting the Mantel-Haenszel combined ORs attributable to N370S (OR = 3.96, 95% CI 2.6–6.02) and L444P (OR = 6.73, 95% CI 4.5–15.42). However, the Mantel-

Haenszel OR in the model for risk attributable to all *GBA* mutations was significantly heterogeneous (Woolf's test for heterogeneity, p -value = 0.0207). After excluding Tokyo and Norway, the OR was slightly attenuated (OR = 5.43, 95% CI 3.89–7.57), but heterogeneity was no longer significant (Woolf's test, p -value = 0.414), and further excluding Tel Aviv resulted in a homogenous (Woolf's test, p -value = 0.3386) OR of 5.3, (95% CI 3.59–7.94). Independent exclusions of data from either Norway or Japan showed homogenous ORs (Japan excluded: OR = 4.91, 95% CI 3.6–6.7, Woolf's test p -value = 0.1447; Norway excluded: OR = 6.35, 95% CI 4.6–8.75, Woolf's test p -value = 0.0991) (Panel 1D). Thus, we can confidently report a Mantel-Haenszel OR of 5.43 associated with *GBA* mutations even when these centers are excluded.

Overall, when screening solely for N370S and L444P, one of these two mutations were found in 15.3% of Ashkenazi Jewish patients versus 3.4% of controls, and in 3.2% of non-Ashkenazi patients versus 0.6% of controls. As expected, N370S was particularly prevalent among Ashkenazi Jews (OR 5.62, 95%CL 3.04–10.39) and was not seen among any of the Asian patients or controls (supplementary Table 1).

All Ashkenazi Jewish subjects were screened for the presence or absence of 6–8 different *GBA* mutations (Table 1). Including this larger number of screened mutations increased the OR for any mutation to 6.48 (95%CL 3.78–11.09). The distribution of specific *GBA* mutations among Ashkenazi Jewish patients with Parkinson disease, where ~20% of patients carried a *GBA* mutation, is shown in Figure 2A, demonstrating that 20% of the identified mutant alleles were not L444P or N370S. Among non-Ashkenazi Jewish subjects the entire *GBA* coding region was sequenced in 1682 patients and 1609 controls. In patients where full sequencing was performed, the OR for any *GBA* mutation was 6.51 (95%CL 3.62–11.7). Figure 2B illustrates the distribution of mutations identified among non-Ashkenazi patients. The mutations identified indicate that as many as 46% of mutant alleles could be missed when focusing solely on N370S and L444P. Moreover, 22 of 43 subjects with L444P carried other pseudogene sequence and hence had recombinant alleles.

Full sequencing data identified two common *GBA* variants, E326K and T369M which are not pathogenic in subjects with Gaucher disease³⁷. Neither demonstrated a significant association with Parkinson disease.

Seventeen patients (15 Ashkenazi) carried two *GBA* mutations. Genotype N370S/N370S was observed in fourteen, N370S/ R496M in two and N370S/V394L in one.

Age at onset was found to be significantly lower among subjects with *GBA* mutations (p -value <0.001), with a mean age of 54.9 in subjects with *GBA* mutations as compared to 58.8 in subjects without. The mean length of disease duration from diagnosis to evaluation was 7.8 years both groups.

Information about family history of parkinsonism was available on 4401 of the patients studied. 17.8% of participants without *GBA* mutations reported a first or second degree relative with Parkinson disease, as compared to 24.0% of subjects with a *GBA* mutation (p -value =0.0057).

The frequency of seven clinical findings associated with Parkinson disease in subjects with and without *GBA* mutations is shown in Figure 3. Generally, the symptom profile for the two groups was similar, although mutations were associated with a significantly lower frequency of asymmetric onset (p -value<0.0001), bradykinesia (p -value=0.0001), resting tremor (p -value=0.0298), and rigidity (p -value<0.0001). There were no significant differences between orthostatic blood pressure changes (p -value=0.2591) or postural instability (p -value=0.1194) among subjects with and without *GBA* mutations. The presence

or absence of cognitive changes was recorded for 1948 patients, reported as present in 26.3% of patients with mutations and 19.0% without (p-value= 0.0071).

DISCUSSION

The results of this analysis overwhelmingly support the association between *GBA* mutations and Parkinson disease. The combined study demonstrates that this finding is not exclusive to a specific ethnicity, nor associated with any specific *GBA* mutation. While mutations in *GBA* are likely to be a susceptibility factor rather than a Mendelian cause of Parkinson disease, the high frequency of mutations among ethnically diverse heterogeneous cohorts of subjects with Parkinson disease render mutations in this gene among the most common and universally found risk factors for Parkinson disease identified to date.

The major limitation of this study was the unavoidable differences in data ascertainment among the different sites. Moreover, some sites were more successful in matching cases and controls with regard to age and gender. To ensure that the analysis was not driven by a small subset of centers, we evaluated the variance in ORs across centers. Excluding the center with the most precise estimate (Tel Aviv), and the centers with the most extreme ORs (Norway and Japan) only resulted in a slight attenuation of the combined OR, which in all analyses remained 5.4 or higher.

This study confirms the need to sequence all exons to accurately ascertain the frequency of *GBA* mutations in both patients and controls. The combined ORs may be underestimated because so many of the centers performed limited mutation analyses. Our data demonstrate that among non-Ashkenazi cases as many as 46% of mutant alleles can be missed when screening for only two mutations. Furthermore, analysis of specific mutations may produce a serious bias. While in sequenced samples the frequency of *GBA* mutations was 6.9% among 1642 patients (OR 6.51), only 36% of the samples included in our entire analysis were fully sequenced. Adequate sample size and accurate genotyping in controls are imperative to avoid underestimating rare variants.

Despite the difficulty in determining the phenotypic profile associated with *GBA* mutations from this study, which intentionally only included subjects that met diagnostic criteria for Parkinson disease, some trends are apparent. Subjects carrying mutations presented on average 4 years earlier, were more likely to have a family history of Parkinson disease, and had less bradykinesia and rest tremor and more cognitive changes reported, a trend supporting other reports.^{3, 5, 6, 17, 19, 24, 25} However because of the exclusion criteria used in this 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 atrophy⁴¹, and further studies are warranted.

Now that *GBA* is a well-validated risk factor for Parkinson 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 dysfunction⁴² or a loss of function related to fluctuations in levels of ceramide⁴³ have been postulated. Further research is in progress to elucidate the pathophysiology of both disorders, facilitate more accurate genetic counseling and develop new therapeutic strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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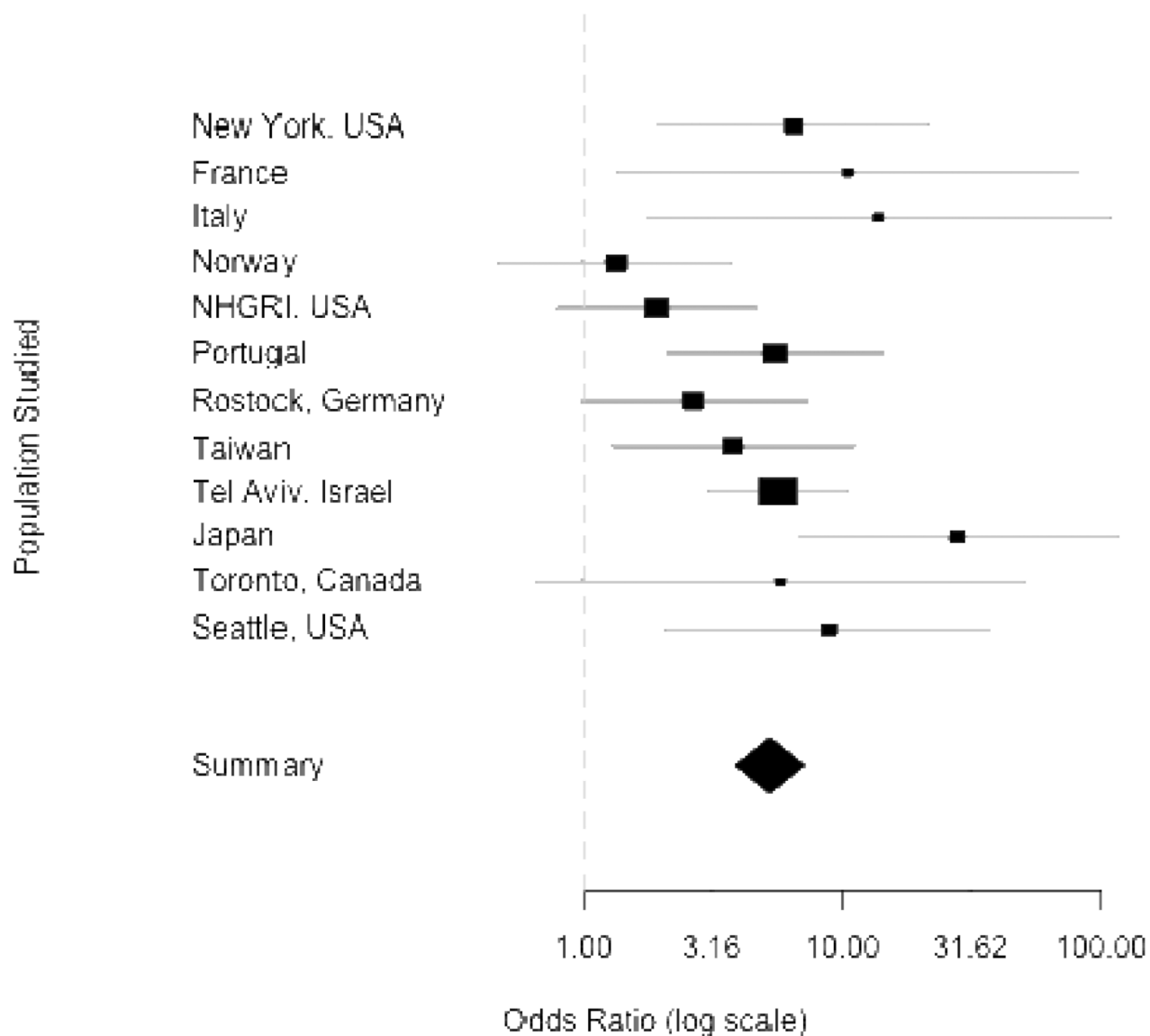
Figure 1A

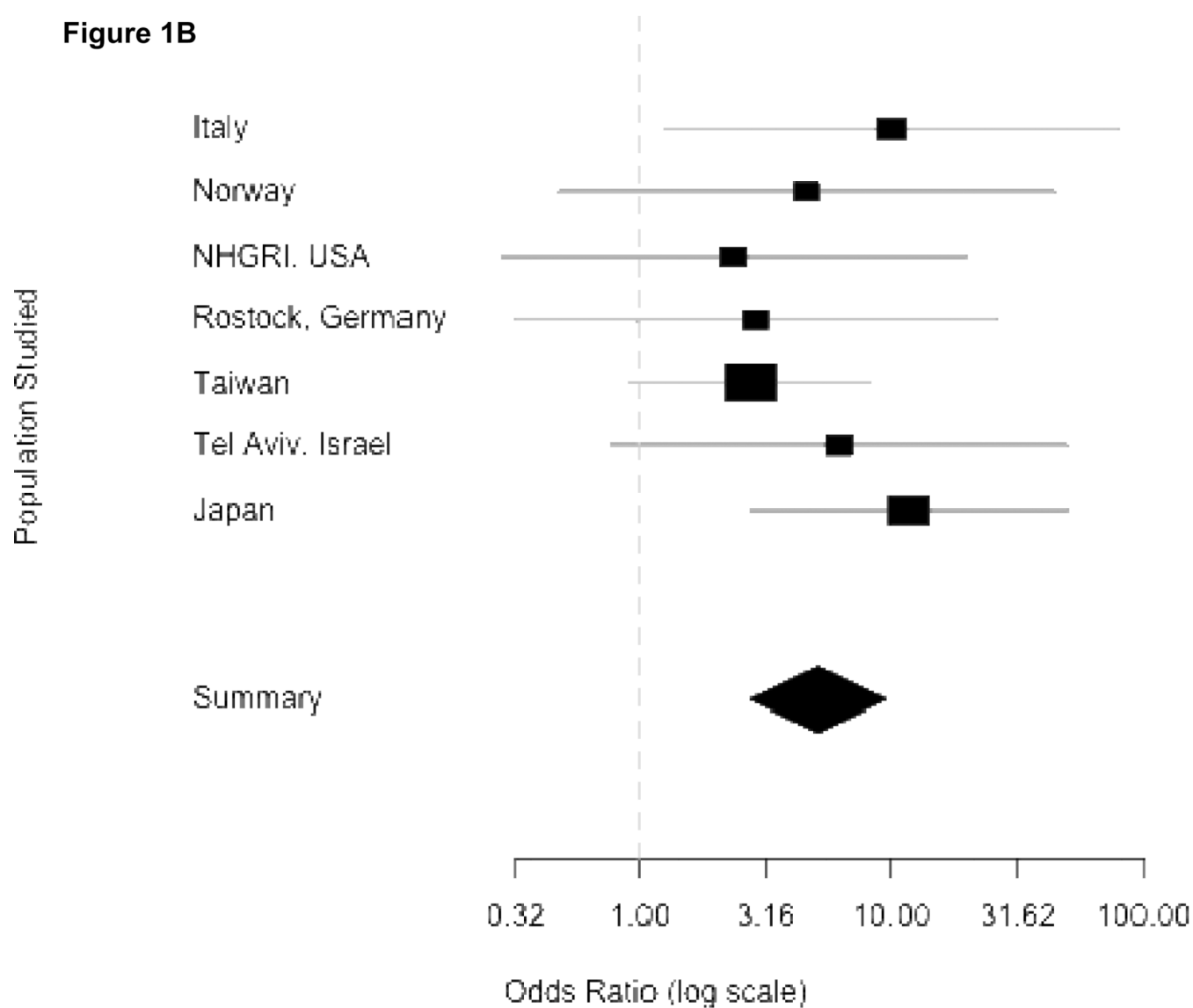
Figure 1B

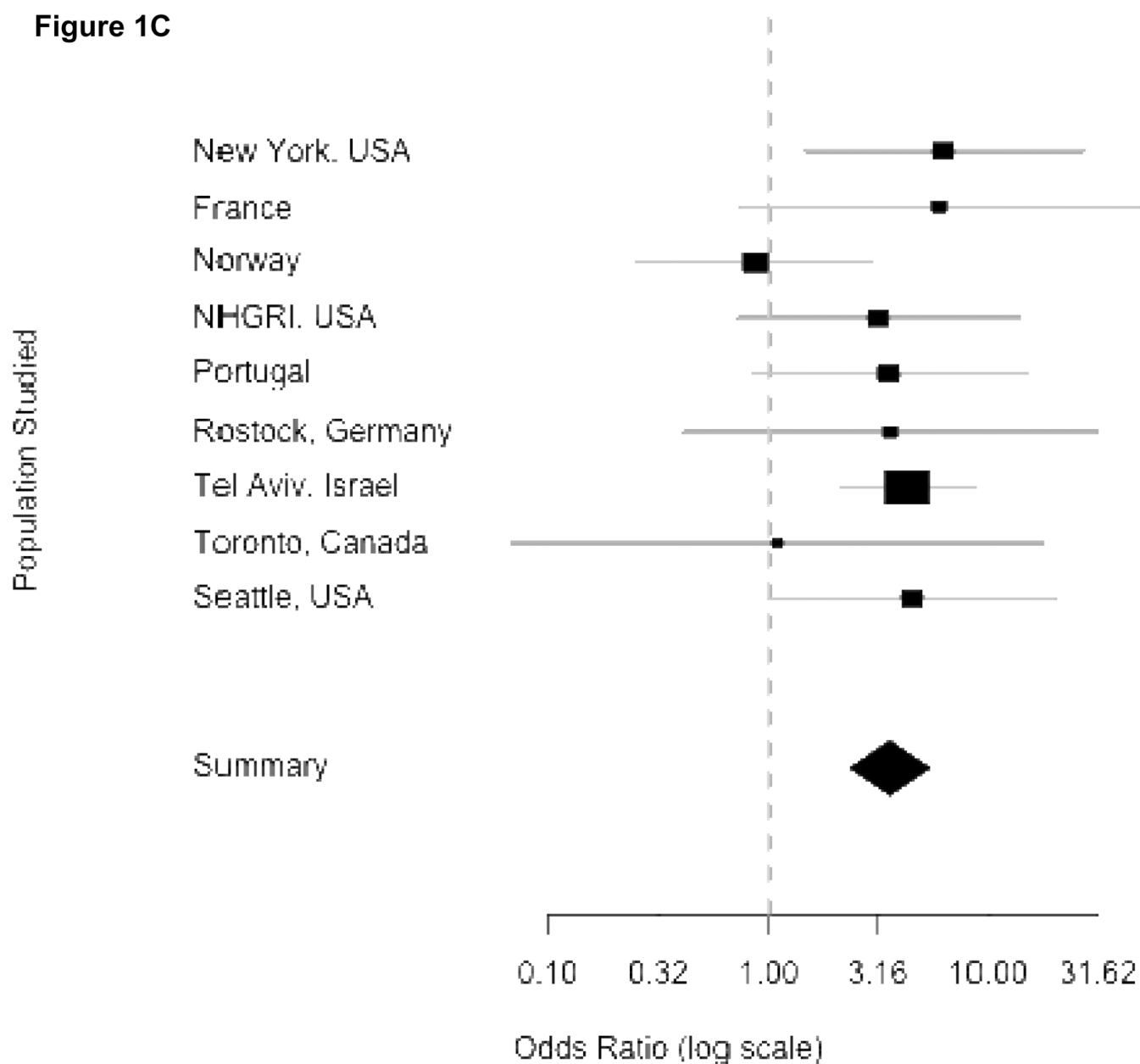
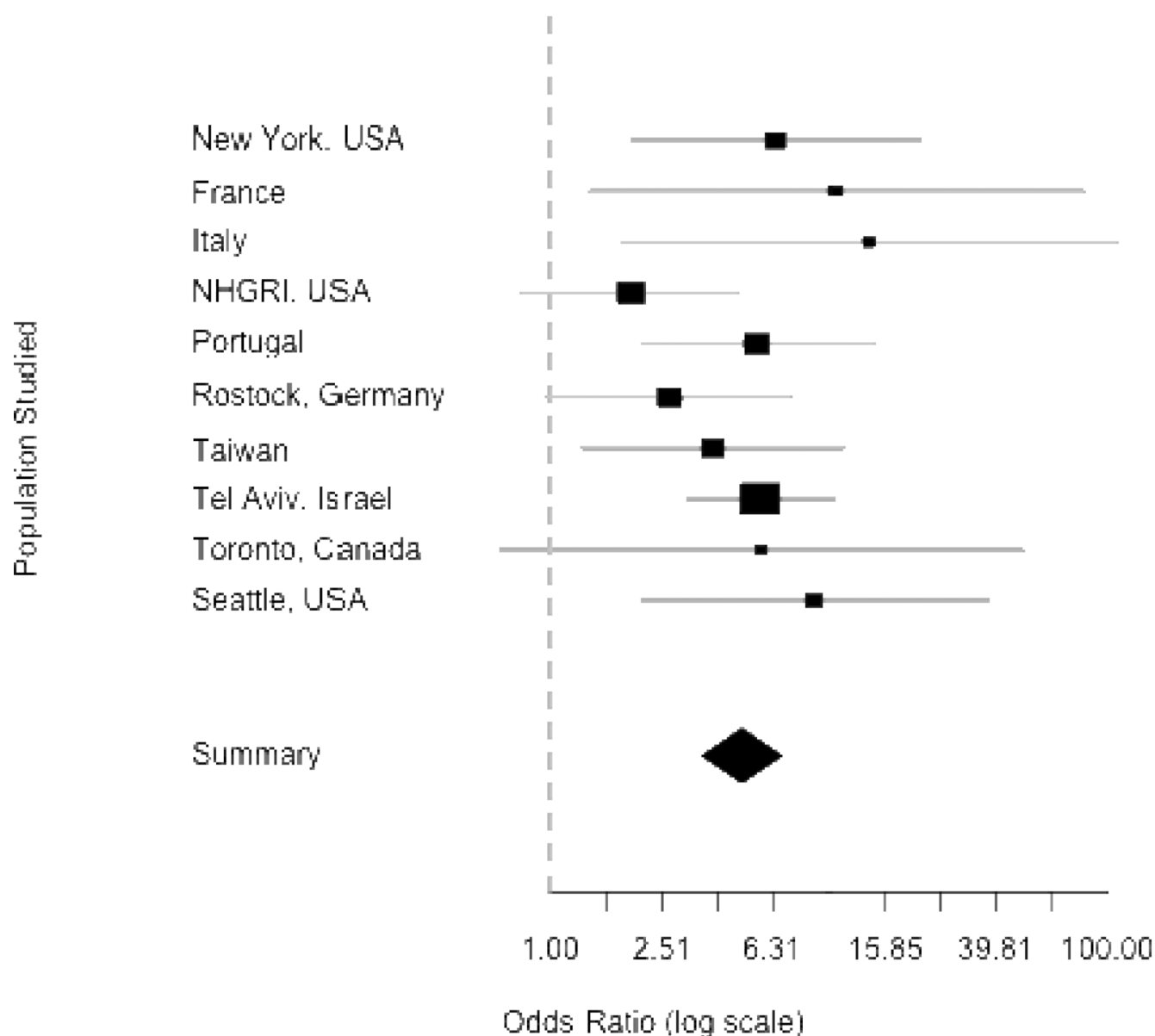
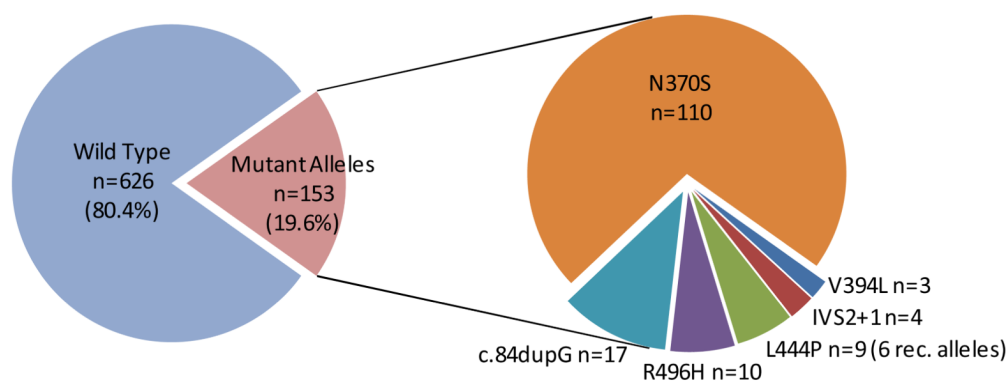
Figure 1C

Figure 1D

**Figure 1.**

Odds ratios from meta-analyses. Panels correspond to combined risk estimates associated with possessing (A) any *GBA* mutation, (B) mutation L444P (C) mutation N370S and (D) any *GBA* mutation excluding Japan and Norway study centers. Horizontal grey lines indicate 95% confidence intervals of estimates. Point estimates per study population are indicated by squares where height is inversely proportional to the standard error of the estimates. Diamonds represent the summary odds ratio whose width indicates the 95% confidence intervals.

Ashkenazi Jewish Patients (n=779)



Non-Ashkenazi Patients (n=1682)

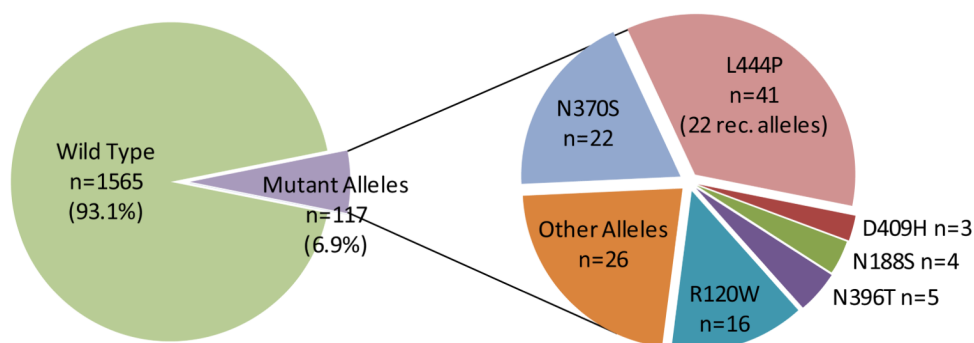


Figure 2.

The distribution of mutations identified. (A) Ashkenazi Jewish patients with Parkinson disease. 19.6% carried a *GBA* mutation. Insert demonstrates the distribution of the different mutations identified. (B) Non-Ashkenazi patients with Parkinson disease with full sequencing. 6.9% carried a *GBA* mutation. The insert shows that among the 117 mutations identified, 54% were not N370S or L444P.

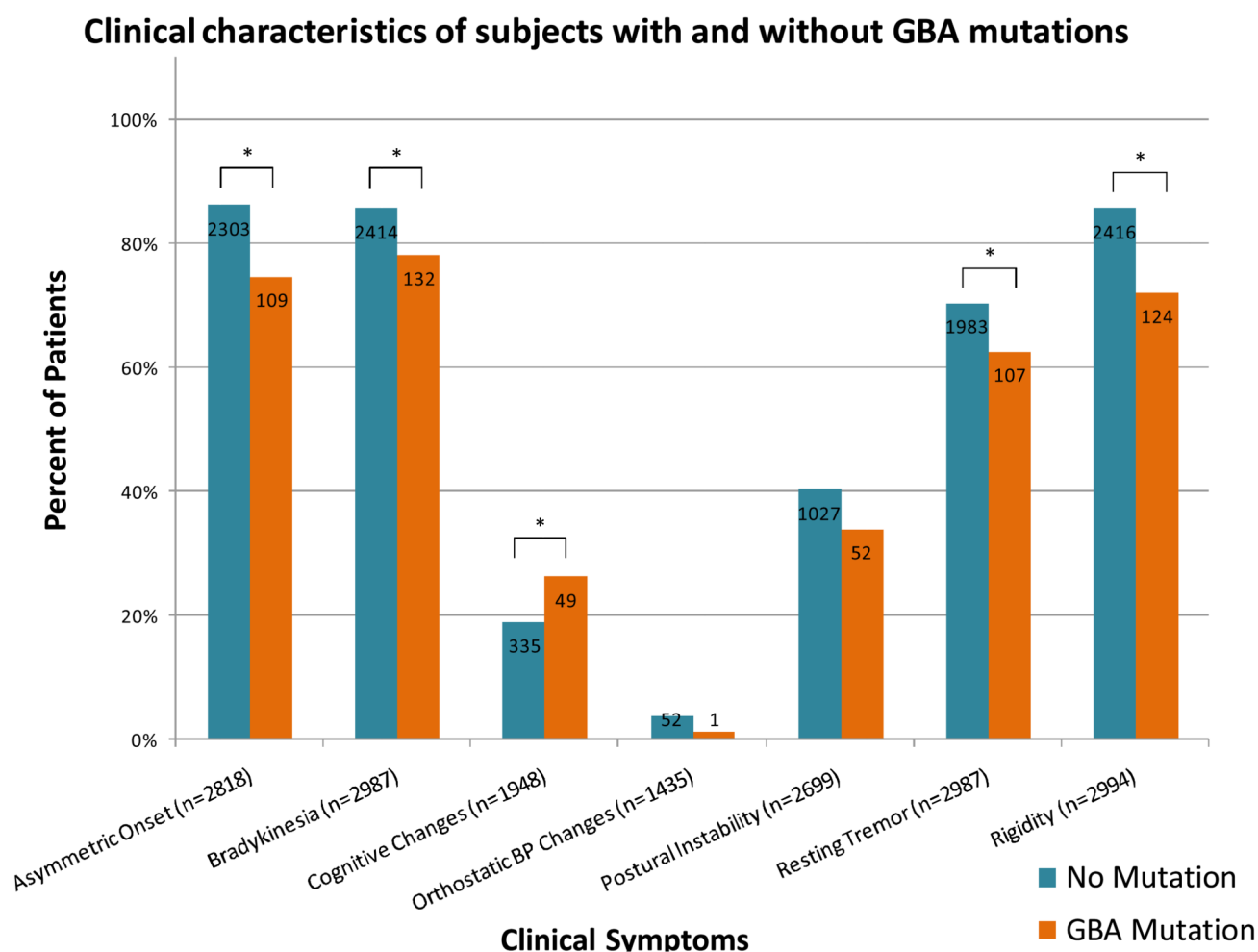


Figure 3. Clinical manifestations of Parkinson disease reported in subjects without (teal) and with (orange) *GBA* mutations. The number of subjects with data recorded for each symptom is indicated in each respective bar. * = p -value < 0.05; significant difference between clinical symptom of patients with and without a *GBA* mutation.

Table 1

Demographic data from the different centers

Center Name	Sample Size (Number of AJ)	Male: Female Ratio (unknown)	Mean Age of Collection	Number of Mutations	Percent Mutations	Mutations Screened	Clinical Data Provided*	Percent Ashkenazi#
Brazil						N370S, L444P G377S	Limited	0
Patients	65	42:23	54.1	4	6.2%			
Controls	264	169:95	54.4	0	0%			
NYC, USA						Full sequencing	Full	58.3
Patients	275 (177)	171:104	65.6	34	12.4%			
Controls	140 (65)	73:67	62.8	3	2.14%			
France						N370S, L444P, D409H	Full	0
Patients	297	185:112	57.8	12	4.0%			
Controls	251	142:109	57.8	1	0.39%			
Haifa, IL						D409H, 84GG, V394L, IVS2+1, R496H	Partial	100.0
Patients	162 (162)	94:65 (3)	68.5	40	24.7%			
Controls	NP	NP	NP	NP	NP			
Italy						L444P, N370S	Full	0
Patients	395	244:151	66.5	11	2.8%			
Controls	483	180:303	56.9	1	0.21%			
Norway						L444P, N370S	Limited	0
Patients	311	186:123 (2)	NP	7	2.3%			
Controls	473	267:206	64.1	8	1.69%			
NHGRI, USA						Full sequencing	Partial	0.2
Patients	539	275:166 (98)	73.5	29	5.4%			
Controls	209 (1)	100:109	67.6	6	2.87%			

Center Name	Sample Size (Number of AJ)	Male: Female Ratio (unknown)	Mean Age of Collection	Number of Mutations	Percent Mutations	Mutations Screened	Clinical Data Provided*	Percent Ashkenazi#
Portugal						Full sequencing	Limited	0
Patients	231	110:121	65.3.0	15	6.5%			
Controls	482	Unknown	65.5	6	1.24%			
Rostock, DE						Full sequencing	Partial	0
Patients	298	190:108	64.3	18	6.0%			
Controls	212	105:107	74.5	5	2.4%			
Singapore						L444P, N370S	Partial	0
Patients	329	170:158 (1)	70.3	8	2.4%			
Controls	201	99:102	64.2	0	0%			
Taiwan						L444, recNciI, R120W, some full sequencing	Full	0
Patients	559	304:255	69.1	22	3.9%			
Controls	377	198:179	60.5	4	1.06%			
Tel Aviv, IL						84GG, IVS2+1, N370S, V394L, D409H, L444P, R496H, RecTL	Limited	99.9
Patients	420 (419)	262:158	68.0	81	19.3%			
Controls	321 (321)	159:162	65.3	13	4.05%			
Japan						full sequencing	Limited	0
Patients	534	282:252	65.3	50	9.4%			
Controls	546	294:252	44.8	2	0.37%			
Tubingen, DE						L444P, N370S	Partial	0
Patients	377	222:155	64.8	12	3.2%			
Controls	325	192:132	58.3	0	0%			

Center Name	Sample Size (Number of AJ)	Male: Female Ratio (unknown)	Mean Age of Collection	Number of Mutations	Percent Mutations	Mutations Screened	Clinical Data Provided*	Percent Ashkenazi#
Toronto, CA								
						N370S, K178T, L444P, 84GG, R329C, IVS2+1,recNcii I	Partial	1.1
Patients	88 (2)	51:37	55.0	5	5.7%			
Controls	96	27:69	69.6	1	1.0%			
Seattle, USA								
						N370S, L444P, recNciiI	Full	1.5
Patients	811 (20)	607:204	66.9	24	2.9%			
Controls	518	193:324	65.2	2	0.39%			

• Clinical data: Limited-age, gender, ethnicity, Partial-age, gender, ethnicity, family history and some information on symptoms, Complete- data provided includes age, gender, ethnicity, duration, family history, presenting symptoms, prominent clinical findings, cognitive changes, and scores on Hoehn and Yahr and/or Unified Parkinson Disease Rating scales

total percent Ashkenazi across all sites, 11.3%.