Mutations for Gaucher Disease Confer High Susceptibility to Parkinson Disease

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Background: Increased frequency of pathogenic variants in GBA, the causative gene for Gaucher disease, has been suggested to be associated with Parkinson disease (PD).

Objectives: To conduct comprehensive resequencing of GBA to identify all sequence variants and to investigate the association of these variants with PD.

Design: Case-control study.

Setting: Multicenter university-based study.

Participants: Five hundred thirty-four patients with PD, 34 families in which multiple patients with PD are present, and 544 control subjects.

Main Outcome Measures: Disease status and GBA variations.

Results: Comprehensive resequencing of GBA in 534 patients with PD and 544 controls revealed 27 sequence variants: 11 pathogenic variants associated with Gaucher disease, 11 nonsynonymous variants not associated with Gaucher disease, and 5 synonymous variants. Fifty patients with PD (9.4%) had 1 of the 11 pathogenic variants in the heterozygous state, whereas only 2 controls (0.37%) had such variants (odds ratio, 28.0). Among the pathogenic variants, R120W and L444P/RecNci were highly prevalent, and each showed a significant association with PD. Furthermore, other rare pathogenic variants were found in 13 patients with PD but not in the controls, further confirming the role of these rare variants in the susceptibility to PD. Patients with PD carrying pathogenic variants were significantly younger than those not carrying them. In addition, concordance of PD states and pathogenic variants was observed in 8 multiplex families with PD.

Conclusion: Heterozygous pathogenic variants in GBA confer a high risk for sporadic PD, even for familial clustering, and are associated with significantly earlier age at onset of disease.

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Parkinson disease (PD), characterized by tremor, rigidity, bradykinesia, and postural instability, is the second most common neurodegenerative disease after Alzheimer disease, with usual onset in late adulthood, that is, after age 50 years. The prevalence of PD is estimated to be 0.3% in the general population and 1% in individuals older than 60 years. Although SNCA, LRRK2, UCHL-1, PARK2, PINK1, and DJ-1 have been identified as the causative genes for familial PD, patients with PD with pathogenic mutations in these genes are rare. Most cases of PD are sporadic and the etiologies poorly understood. A population-based study coupled with genealogic information demonstrated that the estimated risk ratio for PD in siblings of patients with PD was significantly high (As=6.7), which suggests that genetic factors substantially contribute to the development of sporadic PD. To elucidate susceptibility genes for sporadic PD, numerous case-control association studies using the analyses of single nucleotide polymorphisms have been conducted under the common disease–common variants hypothesis; however, only a few consistent findings have been observed. Recently, polymorphisms of SNCA, a major component of Lewy bodies, a pathologic hallmark of PD, have been reported to be associated with sporadic PD (odds ratio [OR], 1.4-2.0).

Several articles have suggested the association of sporadic PD with heterozygous variants in the glucocerebrosidase gene (GBA) (OMIM 606463) encoding the enzyme that is deficient in patients with Gaucher disease, an autosomal recessive lysosomal storage disease. Although GBA

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variants associated with Gaucher disease are diverse and each carrier frequency is rare, most of the previous studies analyzed only specific variants7-16 and sample sizes were small.17-21 Therefore, ORs assessed for the GBA variants have been highly variable in the subsequent studies, making the medical implications of GBA variants associated with PD inconclusive. We conducted extensive resequencing analysis of GBA in patients with PD and in control subjects and found that GBA variants that are pathogenic for Gaucher disease confer high susceptibility to sporadic PD and, furthermore, familial clustering of PD.

METHODS

SUBJECTS

We conducted a resequencing of GBA in patients with PD and control subjects using a microarray-based, high-throughput resequencing system (first tier). As an independent data set, resequencing of GBA was conducted on large-scale samples (second tier) using direct nucleotide sequence analysis. The first tier comprised 61 unrelated patients with PD at the University of Tokyo Hospital and 47 controls provided by the Japan Multiple System Atrophy Research Consortium. The second tier comprised 473 unrelated patients with PD and 497 controls provided by the Japanese Parkinson Disease Susceptibility Gene Consortium (Table 1). In addition, 34 families in which multiple patients with PD are present (hereafter referred to as “multiplex families”) independent of participants in tiers 1 and 2, having more than 1 patient with PD in the second degree, were provided by the Japanese Parkinson Disease Susceptibility Gene Consortium. The diagnosis of PD was based on diagnostic criteria for PD.22 This study was approved by the institutional review boards of the participating institutions.

GENOMIC DNA AND AMPLIFICATION OF GBA

Genomic DNA was extracted from peripheral blood leukocytes using standard procedures. Three primer pairs were designed to selectively amplify GBA but not its pseudogene, as previously described (Table 2).23

RESEQUENCING OF TIER 1

Resequencing of GBA was conducted using newly designed resequencing microarrays TKYPD02 and TKYPD03, both of which were composed of tiled sequences of all 11 exons of GBA and the flanking 12 base pairs of the splicing junctions.24 The analysis was conducted according to the manufacturer’s instructions (Affymetrix Inc, Santa Clara, California). All variants were further confirmed at direct nucleotide sequence analysis using a genetic analyzer (ABI PRISM 3100; Applied Biosystems Inc, Foster City, California).

RESEQUENCING OF TIER 2

The polymerase chain reaction products were subjected to direct nucleotide sequence analysis for the coding sequences and the flanking splice sites of GBA using DNA analyzers (ABI3730xl; Applied Biosystems Inc). The primers for sequence analysis are given in Table 2.
STATISTICAL ANALYSIS

Standard statistical methods were used to test the difference in carrier frequency (Fisher exact test), to compute ORs and corresponding 95% confidence intervals, and to compare mean age at onset of PD (t test). For a meta-analysis, a pooled OR was calculated using a fixed-effects model (Mantel-Haenszel method). \( P < .05 \) was considered statistically significant. Data were analyzed using commercially available statistical software (StatsDirect version 2.6.3; StatsDirect Ltd, Cheshire, England).

RESULTS

Resequencing of tier 1 (61 patients with PD and 47 controls) revealed that 6 patients with PD carried the variants (1 R120W, 1 R329C, 3 RecNciI, and 1 R496C) that are pathogenic for Gaucher disease, whereas none of these variants were present in the controls. Given this result, we further expanded the comprehensive resequencing analysis to tier 2 (473 patients with PD and 497 controls) and identified 44 patients with PD carrying the variants that have been reported to be pathogenic for Gaucher disease, whereas these variants were present in only 2 controls.

Pathogenic variants were either single-base substitutions (R120W, R131C, N188S, G193W, F213I, R329C, L444P, and R496C) or complex multiple substitutions (R120W-N188R-V191G-S196P-F213I, L444P-A456P-V460V, and A456P-V460V). The precise structures of the complex alleles were confirmed at nucleotide sequence analysis of the subcloned mutant alleles. Among the complex mutant alleles, L444P-A456P-V460V is a RecNciI allele, a recombination allele that consists of 3 single-base substitutions of the pseudogene origin in exon 10.25 In summary, we found that 50 of 534 patients with PD (9.4%) had these pathogenic variants in the heterozygous state, whereas only 2 of 544 controls (0.37%) had such variants in the heterozygous state (OR [95% confidence interval] for patients with PD compared with controls, 28.0 [7.3-238.3], which was highly significant \( (P = 6.9 \times 10^{-14}) \) \textbf{Table 3}. When individual variants were analyzed, the frequency of the R120W, L444P, and RecNciI carriers was significantly higher in patients with PD than in controls \( (P < .001, .004, \) and \( .002, \) respectively). In addition, we identified 11 nonsynonymous variants and 5 synonymous variants in tiers 1 and 2, and none of these has been shown to be causative for Gaucher disease. When these variants were analyzed individually and in combination, the frequency of patients with PD was not significantly different from that of the controls \( \textbf{Table 4} \).

We analyzed the clinical manifestations in the 50 patients with PD carrying pathogenic variants in \( GBA \). The age at disease onset in the patients with PD who were carriers of such variants was significantly younger than in those who were not carriers \( \textbf{Table 5} \). Detailed clinical data were available for 49 of 50 patients with PD carrying pathogenic variants. Forty-one of 49 patients with PD (83.7%) showed good responsiveness to antiparkinsonian drug treatment. Iodine 123-labeled metaiodobenzylguanidine cardiac scintigraphy26 was carried out in 33 patients with PD, revealing that 29 of 33 patients with PD (87.9%) had reduced cardiac uptake, consistent with a diagnosis of PD. In the 49 patients with PD, 13 (26.5%) manifested overt dementia (clinical dementia rating27 \( \geq 1 \) and 17 (34.7%) developed visual hallucinations during the course of the disease (mean [SD] interval between onset of PD and evaluation of dementia or visual hallucinations, 9.1 [4.1] and 7.9 [5.0] years, respectively). N-isopropyl-p-[\(^{123}\)I]iodoamphetamine single-photon emission computed tomography was performed in 15 patients with PD, of whom 8 had dementia. All 8 patients with dementia exhibited hypoperfusion in the occipital areas. In the 7 patients without dementia, 5 exhibited hypoperfusion in the occipital areas and 2 had normal findings.

Detailed inquiry into the family history of the 50 patients with PD carrying pathogenic variants in \( GBA \) revealed that 11 patients (22.0%) had parents or siblings with PD. Genomic DNA was available for 3 affected siblings. All 3 affected siblings had the same \( GBA \) variants (2 R120W and 1 RecNciI) as did their probands. Given the concordant \( GBA \) variants in the 3 affected siblings, we analyzed probands of an additional 34 multiplex families independent of those in tiers 1 and 2 with more than 1 patient (parent or sibling) with PD. We found that 5
of 34 probands (14.7%) had pathogenic variants in GBA (1 each, R120W, N188S, IVS6+1g>a, L444P, and RecNciI), and all 5 affected relatives also concordantly had the same GBA variants as did their probands. The splice junction mutation IVS6+1g>a is a novel variant that has not been reported even in patients with Gaucher disease; however, it is likely the pathogenic variant because it would affect splicing of intron 6. In total, 8 multiplex families with patients with PD concordantly carrying the pathogenic variants were identified (Figure).

We compared the distributions of pathogenic variants in GBA in the 534 Japanese patients with PD (50 alleles) with those of the mutations that have been previously described in the 50 Japanese patients with Gaucher disease; however, it is likely the pathogenic variant because it would affect splicing of intron 6. In total, 8 multiplex families with patients with PD concordantly carrying the pathogenic variants were identified (Figure).

Multiple rare GBA variants that are responsible for Gaucher disease confer high risk for PD on the basis of the extensive resequencing of GBA of large data sets of Japanese patients with PD and controls. The combined carrier frequency of the pathogenic variants was as high as 9.4% in patients with PD and highly significantly more frequent than in controls (0.37%) with a markedly high OR (95% confidence interval) for patients with PD compared with controls (28.0 [7.3-238.3]). The frequency of nonneuronopathic and neuronopathic Gaucher disease in Japan is estimated to be 1 in 500,000 and 1 in 1,200,000 live births, respectively, which is in accord with the frequency of pathogenic variants in the controls (2 carriers per 544 individuals) in this study.
Among the pathogenic variants identified in the patients with PD, R120W and L444P/RecNci were highly prevalent. The identification of multiple rare variants that are pathogenic for Gaucher disease was achieved only by extensive resequencing of large data sets, as clearly demonstrated in the present study. For these pathogenic variants except R120W and L444P/RecNci, the frequency of the individual variants was low in patients with PD, and the association with PD should be confirmed in much larger association studies. However, we observed these various rare pathogenic variants in 13 patients with PD, whereas such variants were not observed in the controls. These findings further strengthen the role of these rare GBA variants in susceptibility to PD as well.

In contrast to the present findings, previous association studies demonstrated substantially variable ORs. In the studies that demonstrated a significant association of GBA variants with PD, 7,11,13-17,20,21 N370S is the variant accounting for most of the significant association. N370S is highly prevalent in the Jewish population, with a carrier frequency of 4% to 6%, 7,8,16,20 and that significant association of N370S with PD has not been demonstrated in other ethnic populations. In contrast to N370S, L444P/RecNci has been found regardless of ethnic background. When previous studies that analyzed L444P/RecNci 7,9-21 and the present study were subjected to meta-analysis (4181 patients with PD and 9587 controls), a high pooled OR (95% confidence interval) of 6.8 (4.0-11.8) for L444P/RecNci was obtained without evidence of significant heterogeneity (Cochran Q = 7.3; P = .88), further confirming the role of GBA variants in PD. However, previous studies with small sample sizes failed to detect controls carrying L444P/RecNci 9,11,14,15,17-21 although L444P/RecNci was detected in patients with PD. Thus, it is crucially important to determine the frequency of the GBA variants in the controls for accurate evaluation of ORs conferred by rare variants, necessitating the analysis of large data sets with at least several hundred patients and controls. Furthermore, there seems to be a bias in the distribution of sequence variants in GBA associated with PD compared with that observed in Gaucher disease. In most of the previous studies, 7,10 however, only specific variants considered common in patients with Gaucher disease have been analyzed, which may have led to the underestimation of mutant GBA carrier frequency.

Clinically, patients with PD with heterozygous pathogenic variants in GBA were significantly younger at disease onset than those without such variants, which confirms findings of previous studies. 7,11,12,16,20 To further determine the exact effects of heterozygous GBA variants on PD phenotypes, extensive clinical and epidemiologic analyses should be conducted in large cohorts.

In the present study, we identified 8 multiplex families with patients with PD concordantly having heterozygous pathogenic variants in GBA. Given the markedly high ORs caused by heterozygous pathogenic variants in GBA, it is conceivable that such variants underlie not only sporadic PD but also familial PD.

The roles of the pathogenic variants in the pathogenesis of PD still needed to be elucidated. Gain of toxic functions of mutant glucocerebrosidase proteins independently of enzyme activities might be involved in the pathogenesis. However, all variants associated with PD are pathogenic variants for Gaucher disease, which raises the possibility that decrease in glucocerebrosidase activities has a role in the pathogenesis of PD. Identification of the splice junction mutation IVS6+1g>a in the present study may further support this notion.

We should emphasize a paradigm shift from the common disease–common variants hypothesis to the common disease–multiple rare variants hypothesis in our search for disease susceptibility genes in sporadic PD, which may be applicable to studies of other diseases. The multiple rare variants can be identified only by extensive resequencing and are difficult to detect in association studies using common single nucleotide polymorphisms. Such multiple rare variants confer strong genetic risks, as demonstrated in the present study, which is also in striking contrast to the low ORs of those identified in genomewide association studies using common single nucleotide polymorphisms. Our results strongly emphasize the importance of conducting a comprehensive resequencing analysis of disease susceptibility genes in detecting even the rarest variants.

In conclusion, we have established GBA as a robust and relatively prevalent genetic risk factor for sporadic PD. Further studies of the biological implications of mutant glucocerebrosidase in the pathophysiological processes of PD are expected to provide new avenues for developing therapeutic measures for PD.

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