Clinicogenetic study of *GBA* mutations in patients with familial Parkinson disease

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Abstract

GBA is a known risk factor of Parkinson disease (PD). We sequenced entire coding exons and exon/intron boundaries of *GBA* in 147 Japanese FPD patients from 144 families and 100 unrelated controls. In total, 27/144 (18.8%) of index patients were heterozygous for known GD mutations, suggesting that *GBA* heterozygous mutations are strongly associated with FPD (odds ratio=22.9, 95% confidence interval=3.1-171.2). The frequency was significantly higher in autosomal dominant PD (ADPD) compared to autosomal recessive PD (ARPD). According to clinical assessments, PD patients with *GBA* mutations exhibited typical manifestations of PD or dementia with Lewy bodies (DLB), such as L-dopa responsive parkinsonism with psychiatric problems and/or cognitive decline. Interestingly, they also presented with reduced myocardial ¹²³I-metaiodobenzylguanidine (MIBG) uptake. Our findings suggest that heterozygous *GBA* mutations are strong risk factors in FPD, especially for ADPD. Some patients with *GBA* heterozygous mutations develop clinical features of DLB. We speculate that *GBA* dysfunction may promote Lewy body formation, resulting in more severe PD or DLB phenotypes that are inherited in families.

Keywords: Parkinson's disease/Parkinsonism; GBA; Dementia; Gaucher disease.

1. Introduction

Parkinson disease (PD) is one of the most common neurodegenerative disorders. Patients develop disabled movement and complicating non-motor symptoms, such as psychiatric disorders, cognitive dysfunction, olfactory nerve dysfunction, and sleep disorders.(Weintraub and Burn, 2011) Cardinal features of PD are caused by marked loss of dopaminergic neurons in the substantia nigra, which is evidenced by the pathological hallmark of Lewy bodies (LBs), however, PD is more complicated and systemic disease. PD etiology was thought to be influenced mainly by the interactions between genetic and environmental factors. Nevertheless, recent developments in genetics have revealed that causative genes are involved in Mendelian-inherited parkinsonism.(Hatano, et al., 2009) Moreover, recent genome-wide association studies have also identified several common loci as genetic risk factors for PD.(Hamza, et al., 2010,Satake, et al., 2009,Simon-Sanchez, et al., 2009) During these studies and a subsequent meta-analysis, rare variants of the glucocerebrosidase gene (*GBA*; MIM#606463) have been found as risk factors of sporadic PD (SPD).(Aharon-Peretz, et al., 2004,Sidransky, et al., 2009) *GBA* is also known as the causative gene of Gaucher disease (GD), which is due to a loss of function of hydrolytic enzyme activity and is inherited in an autosomal recessive pattern.(Hruska, et al., 2008,Tsuji, et al., 1987)

Some genetic mutations in *GBA* were characterized as strong risk factors for SPD; however, there are few large studies of *GBA* mutations in familial PD (FPD).(Mitsui, et al., 2009,Nishioka, et al., 2010,Sidransky,

et al., 2009) Co-segregation was previously reported in a small number of families.(Mitsui, et al., 2009) One recent study emphasized an association between *GBA* mutations and cognitive impairment in PD.(Alcalay, et al., 2012)

Here, we aimed to clarify the role of *GBA* mutations in PD, especially in FPD, by sequencing *GBA* in 147 FPD patients from 144 families and performing a comparative analysis of the clinical phenotype and severity to evaluate the association between *GBA* mutations and FPD.

2. Materials and Methods

2.1 Subjects

The study subjects comprised 147 FPD patients from 144 Japanese families and 100 Japanese controls (**Table 1**).

The cohort of Japanese patients consisted of 85 autosomal dominant PD (ADPD) and 59 autosomal recessive PD (ARPD). For the mode of inheritance to be considered autosomal dominant, there had to be affected family members in at least two consecutive generations; for autosomal recessive, we looked for affected siblings in the same generation. After detecting mutations, family members were analyzed to assess co-segregation. DNA samples were provided from various hospitals. Each patient submitted to a neurological examination performed by expert neurologists and was given a diagnosis of PD based on established criteria.(Hughes, et al., 1992)

Diagnosis of dementia was given by the each clinician based on MMSE score.(Folstein, et al., 1975) All

patients had good response to L-dopa. This study was approved by the ethics review committee of Juntendo University School of Medicine. All subjects provided informed and written consent prior to participation.

2.2 *GBA* mutation analysis

Genomic DNA was extracted from peripheral blood lymphocytes using standard protocols. PCR was performed using previously reported primers to avoid amplifying the pseudogene.(Mitsui, et al., 2009) The purified PCR product obtained by ExoSAP IT (GE Healthcare, Salt Lake City, UT) was subsequently used for dideoxy sequencing with BigDye Terminator Chemistry (Applied Biosystems, Foster City, CA). The resulting products were loaded on ABI 310 or 3130 automated DNA sequence analyzers (Applied Biosystems) and analyzed with Sequencing Analysis Software v5.1 (Applied Biosystems). All exons and exon-intron boundaries were analyzed by direct sequencing.

To confirm *RecNcII* allele, we used TOPO TA cloning kit (Invitrogen, Carlsbad, CA) for TA cloning and separating alleles. After separating each allele, direct sequencing was performed as described above.

2.3 Clinical data analysis

To clarify the clinical features of patients with *GBA* mutations, 19 items (**Table 3**) concerning prominent PD symptoms were statistically compared between *GBA* mutation-positive and *GBA* mutation-negative groups.

Other affected members with *GBA* mutations were also included.

2.4 Statistical analysis

Statistical analysis included the t-test, Fisher's exact test, odds ratio and its confidence interval (95% CI), using GraphPad Prism version 5.0d (GraphPad Prism Software). Hardy-Weinberg equilibrium test was performed using SNPalyze v5.1 software (Dynacom, Chiba, Japan). In all statistical analyses, *P*-values of 0.05 or less were considered statistically significant.

3. Results

3.1 *GBA* mutations observed in FPD

In this study, we only observed heterozygous mutations; no individual had mutations in both alleles. We detected six nonsynonymous mutations: p.I(-20)V, p.G64V, p.R120W, p.D409H, p.L444P, and p.I489V; one nonsense mutation: p.W393X; one synonymous mutation: p.K466K; one frame-shift mutation: c.1447-1466delTGins; and one recombinant allele (Rec*NciI*) in the Japanese population (**Table 2**). Among them, five mutations, p.R120W, p.D409H, p.L444P, c.1447-1466delTGins, and Rec*NciI*, have been reported as causative mutations in GD patients.⁹ Patients with *GBA* mutation did not have causative *PARK2*, *PINK1* and common *LRRK2* mutations.

p.I(-20)V is an amino acid change in the signal peptide region and was considered to be a single nucleotide polymorphism (SNP). The frequency of p.I(-20)V in patients with FPD (13/144=9.0%) was not significantly different from the controls (10/100=10.0%, $P=0.83$), and its genotype distribution was in Hardy-Weinberg equilibrium (HWE) in both populations. Although p.K466K mutation was not detected in any controls, this synonymous mutation was also excluded for later clinical analyses because it is in a less important region of the final protein.

Two mutations, p.G64V and p.W393X (Figure 1), were novel, and p.I489V was previously reported in SPD patients.(Mitsui, et al., 2009) The p.G64V mutation was caused by the substitution of the first amino acid of exon 4, and it seems to be segregated with PD (Figures 1 and 2). In the family with p.W393X, no affected family member was confirmed for the mutation because of absent genomic DNA. The sequences around the region of mutation were interspecifically conserved in both mutations (Figure 1).

The recombinant allele *RecNciI* was found in one patient and one control. p.L444P is more common, and p.R120W is less common in Japanese patients with GD, although p.R120W was frequently seen in Japanese FPD patients in the present study (9/144=6.3%, **Table 2**). However, p.L444P was the most frequent mutation seen in FPD patients (12/144=8.3%, **Table 2**).

In total, we found 31 FPD patients with heterozygous mutations that were reported in GD (p.R120W, p.D409H, p.L444P, c.1447-1466delTGins, and *RecNciI*) or unreported in GD (p.G64V, p.W393X, and p.I489V)

(**Table 2**). Interestingly, the frequency of *GBA* mutations among FPD patients was significantly higher than in the controls (31/144=21.5% vs. 1/100=1.0%, $P<0.0001$, odds ratio (OR)=27.2, 95% confidence interval (CI)=3.6-202.7) (**Table 2**). This cohort included 23 ADPD and 8 ARPD out of a total of 31 FPD patients. When individual mutations were analyzed, the frequency of the p.R120W and p.L444P carriers was significantly higher in index PD patients than in the controls ($P=0.01$, and $P=0.002$, respectively).

3.2 Mode of inheritance

We compared the differences between groups with AD and AR modes of inheritance and found that the frequency of mutations reported in GD was significantly higher in the AD group than in the AR group (22/85=25.9% vs. 5/59=8.5%, $P=0.009$). Four known mutations reported in GD (p.R120W, p.D409H, c.1447-1466delTGins, and RecNciI) were all found in the AD group. In one AR family (p.L444P) with two brothers affected with PD/dementia with Lewy bodies (DLB), the parents had been recorded as asymptomatic, but they died at relatively young ages during the war. Thus, the possibility that this family also should be included in AD family with co-segregation remains (Family 28; Figure 2).

In two families with p.L444P and one family with p.G64V, both affected siblings had the same mutations, suggesting co-segregation (Family 25, 28, and 31; Figure 2). The present study indicates the incomplete penetrance of p.R120W (Family 7 and 9; Figure 2), which is consistent with previous reports suggesting co-segregation.(Mitsui, et al., 2009) p.I489V was not considered to show co-segregation (Family 35;

Figure 2).

3.3 Clinical symptoms

In this study, we performed a comparative analysis between two groups: *GBA* mutation-positive (n=34) and *GBA* mutation-negative patients (n=113). Clinical and demographic data are listed in **Table 3** and **Supplementary Table 1**. The mean age at onset (AAO) was not significantly different ($P=0.437$). Statistical analysis of 19 symptoms in PD revealed significant differences in four indexes of hallucination, delirium, dementia, and other psychosis (**Table 3**). Notably, psychiatric symptoms and/or cognitive decline were more common in *GBA* mutation-positive patients (see **Supplementary Table 2**). Nine patients with *GBA* mutations underwent cardiac ^{123}I -metaiodobenzylguanidine (MIBG) scintigraphy (see **Supplementary Table 3**), and all patients showed marked reduction of myocardial MIBG uptake.

4. Discussion

Recently, the largest multicenter analysis of *GBA* mutations has proved an association between *GBA* mutations and PD.(Sidransky, et al., 2009) To disclose the role of *GBA* in FPD, we performed a *GBA* mutational analysis for in 147 FPD patients from 144 families. The frequency of *GBA* mutations was 21.5% and 25.9% in FPD and ADPD, respectively, which are higher values than reported previously, even compared to SPD patients(Lesage,

et al., 2011, Mitsui, et al., 2009, Sidransky, et al., 2009) Therefore, our data suggest that *GBA* mutations play an important role in not only SPD but also FPD, and especially in ADPD. This finding implies that *GBA* could play a major role in FPD and ADPD.

Rare *GBA* mutations have been reported to be a strong risk factor of PD, with a robust odds ratio of 28.0.(Mitsui, et al., 2009) Thus, an analysis for *GBA* mutations in FPD was essential to clarify the role of each *GBA* mutation in PD patients. Indeed, p.L444P and p.G64V co-segregated with PD in some families. However, compared with a previous report,(Mitsui, et al., 2009) p.R120W did not co-segregate in this study. Regarding an association with PD, some mutations remained controversial because of the small number of patients with each mutation and restricted genetic testing in the families. Considering allele frequency, it might be reasonable that *GBA* mutations are incompletely inherited with PD. The carrier frequency of *GBA* mutations was extremely low among the normal Japanese population in this study and in a previous report,(Mitsui, et al., 2009) whereas many controls among the Jewish population have p.N370S, which is associated with type I GD(Aharon-Peretz, et al., 2004) and many controls among the UK population have p.E326K, which is a risk factor for PD.(Duran, et al., 2013) These racial differences might be derived from the differences of effects of each mutation and other genetic risk factors, which have different distributions due to founder effects.(Lesage, et al., 2011, Nishioka, et al., 2010, Sidransky, et al., 2009) Although racial or regional differences exist for each *GBA* mutation, our data emphasize that *GBA* mutations are more strongly associated with FPD, rather than SPD.

A recent study reported a relatively high estimated penetrance ratio in *GBA* carriers, depending on age (7.6%, 13.7%, 21.4%, and 29.7% at 50, 60, 70, and 80 years, respectively). This result should lead to the consideration of *GBA* as a dominant causal gene with reduced penetrance.(Anheim, et al., 2012) Supporting this consideration, we detected heterozygous *GBA* mutations most frequently in ADPD patients. On the basis of our findings, we conclude that *GBA* is a strong and common risk factor but not a definite causal gene for ADPD and FPD.

In hereditary forms of PD, the frequency of heterozygous *GBA* mutations in ADPD (25.9%) was higher than that observed for ARPD (8.5%). Even allowing for classification bias by definition of ADPD and ARPD, the data imply that *GBA* mutations are strongly associated with ADPD, suggesting heterozygous *GBA* mutations have a role in familial aggregation, especially in ADPD. In addition, heterozygous mutations have been identified among ARPD patients, suggesting the incomplete penetrance of forms even through the AR mode of inheritance.

Our PD patients with *GBA* mutations frequently developed psychiatric symptoms and/or cognitive decline. Our data support previous results in which PD patients with *GBA* mutations manifest exacerbated psychiatric symptoms and/or cognitive decline compared to those without *GBA* mutations.(Alcalay, et al., 2012,Sidransky, et al., 2009,Winder-Rhodes, et al., 2013) Some previous studies have reported the association of *GBA* mutations and DLB.(Clark, et al., 2009,Nalls, et al., 2013,Tsuang, et al., 2012) Our data further suggest

that *GBA* heterozygous mutation carriers can develop clinical symptoms of PD and DLB. Accordingly, we found decreased cardiac MIBG uptake associated with *GBA* mutations. The H/M ratio correlated with PD/DLB clinical severity; a decreasing ratio corresponded to an ascending Hoehn and Yahr stage.(Nagayama, et al., 2005) Thus, MIBG-scintigraphy could be a useful biomarker for PD/Lewy body diseases in patients with *GBA* mutations.

Lewy body diseases are alpha-synucleinopathies characterized by abnormal accumulation of alpha-synuclein in neuronal cytoplasm. Recently, biochemical analysis using cell and animal models demonstrated that some *GBA* mutations lead to increased alpha-synuclein concentration.(Cullen, et al., 2011) *GBA* mutation may promote alpha-synuclein accumulation and Lewy body development via the aberrant lysosomal function, resulting in severe parkinsonism and cognitive decline associated with DLB.(Tsuang, et al., 2012) (Mazzulli, et al., 2011) (Yap, et al., 2013)

Collectively, our findings have major implications for the genetic and pathogenic mechanisms of *GBA*. GD is an autosomal recessive disorder caused by mutations in both *GBA* alleles, which leads to a loss of function or reduced enzyme activity. Contrary to GD, our FPD and ADPD patients carried one mutant allele and one wild-type *GBA* allele. Therefore, the pathogenic mechanism may be due to haploinsufficiency, dominant-negative effect, or toxic gain of function rather than a loss of function.

In conclusion, heterozygous *GBA* mutations play a greater role in FPD, especially in ADPD, and are

likely to facilitate the development of PD and Lewy body diseases via different genetic and pathogenic mechanisms. Our findings suggest that further functional analyses for *GBA* should elucidate the pathogenesis of PD and Lewy body diseases.

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Table 1. Patient information

	n	Male/Female	AAS (range)	AAO (range)
Patients	147	59/88	60.0±12.8 (21-84)	50.8±13.9 (13-81)
AD	85	35/50	57.7±13.7 (21-84)	48.3±14.2 (13-77)
AR	62	24/38	63.1±10.8 (37-84)	54.3±12.7 (25-81)
Controls	100	47/53	56.8±16.3 (29-87)	

AAS = age at sampling; AAO = age at onset; AD = autosomal dominant; AR = autosomal recessive

Table 2. Frequency of each *GBA* mutation found in this study

Mutations	FPD (n=144)	AD (n=85)	AR (n=59)	Control (n=100)	<i>P</i> Value ^a (FPD vs. Control)	OR (95% CI) (FPD vs. Control)
Reported in GD						
p.R120W (%)	9 (6.3)	9 (10.6)	0 (0)	0 (0)	0.01	NA
p.D409H (%)	4 (2.8)	4 (4.7)	0 (0)	0 (0)	0.14	NA
p.L444P (%)	12 (8.3)	7 (8.2)	5 (8.5)	0 (0)	0.002	NA
indel (%)	1 (0.7)	1 (1.2)	0 (0)	0 (0)	NA	NA
RecNciI (%)	1 (0.7)	1 (1.2)	0 (0)	1 (1)	NA	NA
Unreported in GD						
<i>p.G64V</i> (%)	1 (0.7)	0 (0)	1 (1.7)	0 (0)	NA	NA
<i>p.W393X</i> (%)	1 (0.7)	0 (0)	1 (1.7)	0 (0)	NA	NA
p.I489V (%)	2 (1.4)	1 (1.2)	1 (1.7)	0 (0)	0.51	NA
Total (%)	31 (21.5)	23 (27.1)	8 (13.6)	1 (1)	<0.0001	27.2 (3.6-202.7)

FPD = familial PD; AD, autosomal dominant; AR = autosomal recessive; OR = odds ratio; CI = confidence interval; GD = Gaucher disease; NA = not applicable; indel = c.1447-1466delTGins.

^aFisher's exact test. Italic characters denote novel mutations found in this study.

Table 3. Comparison of clinical symptoms between *GBA*-positive and *GBA*-negative patients

Symptom	<i>GBA</i> mut (+)	<i>GBA</i> mut (-)	<i>P</i> Value ^a
n	34	113	
Age at onset (mean±SD)	49.1±11.7	51.3±14.5	
Resting tremor (%)	18 (52.9)	77 (68.1)	0.151
Bradykinesia (%)	27 (79.4)	99 (87.6)	0.265
Rigidity (%)	29 (85.3)	99 (87.6)	0.772
Gait disturbance (%)	28 (82.4)	95 (84.1)	0.795
Postural instability (%)	20 (58.8)	72 (63.7)	0.687
Wearing off (%)	20 (58.8)	49 (43.4)	0.112
Asymmetry at onset (%)	25 (73.5)	75 (66.4)	0.531
Orthostatic hypotension (%)	5 (14.7)	21 (18.6)	0.798
Incontinence (%)	7 (20.6)	11 (9.7)	0.131
Urinary urgency (%)	10 (29.4)	20 (17.7)	0.150
L-dopa-induced dyskinesia (%)	10 (29.4)	37 (32.7)	0.834
Sleep benefit (%)	3 (8.8)	21 (18.6)	0.288
Dystonia at onset (%)	4 (11.8)	11 (9.7)	0.750
Hyperreflexia (%)	5 (14.7)	17 (15.0)	1.000
Hallucination (%)	14 (41.2)	20 (17.7)	0.009**
Delusion (%)	8 (23.5)	6 (5.3)	0.004**
Other psychosis (%)	13 (38.2)	11 (9.7)	0.0003***
Dementia (%)	12 (35.3)	18 (15.9)	0.027*
Gaze palsy (%)	3 (8.8)	2 (1.8)	0.081

P*<0.05, *P*<0.01, and ****P*<0.001^aFisher's exact test.

Figure legends

Figure 1. *Novel GBA mutations.*

Genomic sequence chromatogram and comparison of interspecific amino acids around the mutated site.

Figure 2. *Co-segregation study of GBA-positive families.*

Pedigrees of families with *GBA* mutations are shown. Affected PD patients are represented with a black symbol.

The number of each family member represent their patient ID in Supplementary Table 1.

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Figure(1)

	p.G64V	p.W393X
Mutant	NHTGT V LLLT	EGGPN X -----
H.sapiens	NHTGT G LLLT	EGGPN W VRNFV
C.lupus	NHTGT G LLLT	EGGPN W VRNFV
B.taurus	NRTGT G LLLT	EGGPN W VRNFV
M.musculus	NRTGT G LLLT	EGGPN W VRNFV
D.rerio	NSTGA A LRITL	DGGPN W VKNFV

A T G T C C T C A G G C C T G C T A C T G

p.G64V

GG A C C C A A T T G G G T G C G T A A C

p.W393X

Figure(2)