Effects of genetic rare variants on Parkinson's disease in the Korean population

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Effects of genetic rare variants

on Parkinson's disease in the Korean population

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ABSTRACT

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Background: The genetic background of a disease is usually explained by either rare mutations in a disease-causing gene or common susceptibility alleles that increase risk for a disease. A number of causative or susceptibility genes have been discovered for familial and idiopathic Parkinson's disease. Although mutations in *PARK2* are known to be the most common autosomal recessive gene for early onset Parkinson's disease, these mutations are rare in Asians. Moreover, there is still much debate about whether a single heterozygous *PARK2* mutation is a risk factor for Parkinson's disease. There are very few patient-control gene-dosage studies for *PARK2*, and notably there

has been only 1 study on the Asian population. Mutation in the glucocerebrosidase (*GBA*) gene is a causal mutation for Gaucher's disease; however, many different ethnic populations have a heterozygous carrier of this mutation, which has a 3-fold higher risk for Parkinson's disease. There have been no studies on the effect of the *GBA* mutation on Parkinson's disease in the Korean population, although given that Gaucher's disease is extremely rare in Korea, a heterozygous mutation of *GBA* might not be a risk allele. The purpose of this study was to investigate whether rare variants of *GBA* or *PARK2* are linked to Parkinson's disease in the Korean population. **Methods**: The frequency of the *GBA* mutation was compared between patient and control populations in order to determine whether a heterozygous mutation in *GBA* is associated with Parkinson's disease in the Korean population. Common *GBA* mutations in the Korean population were also determined. *GBA* mutations were assessed in 277 PD patients and 291 normal control subjects. All exons of the *GBA* gene were analyzed in all patients and in 100 normal control subjects. Exons 2 and 5–11, where mutations were observed in the patient population, were further analyzed in 191 additional normal control subjects. To investigate whether a single heterozygous mutation of *PARK2* is associated with Parkinson's disease, the gene dosage of all exons of *PARK2* was analyzed in patients with early onset and familial Parkinson's disease and

control subjects. Dosage analysis of mutations in the *PARK2* gene was performed in 188 early onset or familial Parkinson's disease patients and 191 normal control subjects by using real-time polymerase chain reaction. In patients who showed a heterozygous gene-dosage mutation, sequence analysis was performed to exclude the possibility of complex heterozygous mutations. **Results:** *GBA* mutation analysis revealed 5 heterozygous mutations, N188S, P201H, R257Q, S271G, and L444P, in 9 (3.2%) Parkinson's disease patients. No *GBA* mutations were detected in the normal control group ($p < 0.01$; odds ratio, 20.6; 95% confidence interval, 1.2–356.4). The patients with a heterozygous *GBA* mutation showed a significantly earlier onset of disease than the patients without the mutation (48.6 ± 11.9 vs. 57.9 ± 13.5; $p < 0.05$; Mann–Whitney test). Analysis of the *PARK2* mutation revealed that 21 (11.2%) patients had a mutation in *PARK2*. Of them, five (23.8%) patients had compound heterozygous mutations and 13 (61.9%) had a heterozygous mutation. Among 29 mutated alleles, one small sequence variation was found (3.4%). Gene-dosage mutation accounted for most of the total mutations found (96.6%), and all of the heterozygous mutations were gene-dosage mutations. The frequency of a heterozygous *PARK2* gene-dosage mutation was higher in PD than in the controls. **Conclusion:** Rare genetic variant mutations of the *GBA* or *PARK2* genes, implicated as a background for Parkinson's disease, are

also risk factors for Parkinson's disease in the Korean population.

Keywords: Parkinson's disease, rare variants, *PARK2*, glucocerebrosidase

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Ⅰ. INTRODUCTION

Parkinson's disease (PD) is second to Alzheimer's disease in being the most common neurodegenerative disease that involves the central nervous system. The overall prevalence of PD is about 0.3% and it appears to increase with age—from about 1% in patients more than 60 years of age to about 4% in patients more than 85 years of age.[1] Since population numbers in these older age groups are rapidly increasing because of increasing life expectancy, neurodegenerative diseases, like PD, will be an increasing economic burden on society. The characteristic features of classical PD are resting tremors, rigidity, bradykinesia, and postural instability. Combined with an asymmetrical onset and a good responsiveness to levodopa treatment, these features are typical in PD patients.[2]

The pathological hallmarks of PD are selective and progressive loss of

nigrostriatal dopaminergic neurons in the substantia nigra pars compacta of the midbrain and α-synuclein-containing Lewy bodies (LB) in surviving neurons. Decreased levels of striatal dopamine lead to functional deterioration of basal ganglia and, as a result, motor deterioration. In spite of recent advances, the pathophysiologic mechanism underlying selective dopaminergic neuronal loss and LB formation has not been clearly defined. Most patients present with sporadic onset PD. PD has been regarded as a prototypic nongenetic disorder. The discovery of methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism in intravenous drug users[3] created excitement about the identification of potential environmental causative agents. Epidemiological studies have identified risk factors (e.g., pesticides) as well as protective factors (e.g., smoking, caffeine) for PD.[4, 5]

Several lines of evidence support the theory that genetic risk factors play a role in PD. PD patients tend to have a positive family history,[6] and first-degree relatives of PD patients are more likely to develop PD than controls.[7] Studies of twins suggest that genetic factors play an important role in the development of early onset PD (EOPD) patients;[8] the results from studies of twins in late onset PD patients are much less consistent. Genetic association studies have not shown consistent results.[9] Except in EOPD patients, the concordance rate of PD in dizygotic twins is similar to that in monozygotic twins, supporting the role of nongenetic risk factors. Because only about 10% of PD patients have a clear family history of PD,[1] it has been widely accepted that the majority of PD patients have a complex etiology that includes both genetic and environmental factors. The relative contribution of genetic and

environmental factors in the pathogenesis of PD has been debated for many years. The discovery of the *SNCA* gene, which codes for α-synuclein, has markedly changed the scientific view about the causes of PD. As the knowledge on the disease has increased, the proportion of risk assigned to the environment has decreased. Since the finding of a missense mutation in the *SNCA* gene that results in a rare familial form of PD,[10] several causative genes for PD have been identified in familial PD patients with an autosomal dominant or autosomal recessive inheritance pattern.[11-16] To date, at least 18 genes or loci have been implicated in familial PD (Table 1)[17]. Out of the 6 genes confirmed to cause monogenic forms of familial PD, mutations in *SNCA* and *LRRK2* are responsible for autosomal dominant familial PD, and mutations in *PARK2*, *PINK1*, *DJ-1*, and *ATP13A2* are responsible for autosomal recessive forms of familial PD. Although these may account for only a small part of PD patients, studies on the proteins encoded by these genes have substantiated many of the functional mechanisms in the pathogenesis of the more common sporadic forms of PD. Several lines of evidence suggest that oxidative stress, mitochondrial dysfunction, impairment of the ubiquitin–proteasome system (UPS), and protein aggregation may contribute to PD pathogenesis.[18] Most of these pathophysiological links with PD are the result of studying the functional consequences of gene mutations implicated in familial PD.

Further evidence for the genetic role of PD has been obtained from the identification of causal mutations of familial PD genes in sporadic PD patients. Subsequent screening studies after the discovery of the *SNCA* gene showed that variability at the *SNCA* locus not only plays a role in this familial form of PD but is

Table 1. PARK-designated PD-related loci[17]

Symbol	Gene locus	Disorder	Inheritance	Gene	Status and remarks	Mode of identification
PARK1	4q21-22	EOPD	AD	SNCA	Confirmed	Linkage analysis
PARK2	6q25.2–q27	EOPD	AR	Parkin	Confirmed	Linkage analysis
PARK3	2p13	Classical PD	AD	Unknown	Unknown Unconfirmed; may represent a risk factor; gene not found since first described in 1998	Linkage analysis
PARK4	4q21–q23	EOPD	AD	SNCA	Erroneous locus (identical to PARK1)	Linkage analysis
PARK5	4p13	Classical PD	AD	UCHL1	Unconfirmed (not replicated since described in 1998)	Functional candidate gene approach
PARK7	1p36	EOPD	AR	DJ-1	Confirmed	Linkage analysis
PARK8	12q12	Classical PD	AD	LRRK2	LRRK2 Confirmed; variations in LRRK2 gene include risk-conferring variants and disease-causing mutations	Linkage analysis
PARK9	1p36	Kufor-Rakeb syndrome; atypical PD with dementia, spasticity, and supranuclear gaze palsy	AR	ATP13A2	Confirmed; but complex phenotype that would not be mistaken for early-onset or classical parkinsonism	Linkage analysis
PARK10	1p32	Classical PD	Risk factor	Unknown	Confirmed susceptibility locus; gene unknown since first described in 2002	Linkage analysis
PARK11	2q36-27	Late-onset PD	AD	Unknown; not GIGYF2	Not independently confirmed; possibly represents a risk factor; gene not found since first described in 2002	Linkage analysis
PARK12	Xq21–q25	Classical PD	Risk factor	Unknown	Unknown Confirmed susceptibility locus; possibly represents a risk factor; gene not found since first described in 2003	Linkage analysis
PARK13	2p12	Classical PD	AD or Risk factor	HTRA2	Unconfirmed	Candidate gene approach
PARK14	22q13.1	Early-onset dystonia-parkinsonism	AR	PLA2G6	Confirmed	Linkage analysis (homozygosity mapping)
PARK15	22q12–q13	Early-onset parkinsonian-pyramidal syndrome	AR	AR FBX07	Confirmed	Linkage analysis
PARK16	1q32	Classical PD	Risk factor	Unknown	Confirmed susceptibility locus	locus Genome-wide ssociation studies
PARK17	16q11.2	Classical PD	AD	VPS35	Confirmed	Exome sequencing
PARK18	3q27.1	Classical PD	AD	EIF4G1	Unconfirmed; recently published	Linkage analysis

AD, autosomal dominant; AR, autosomal recessive.

also associated with disease risk in sporadic cases.[19, 20] Several studies have suggested that heterozygous *PARK2* and *PINK1* mutations are risk factors for the sporadic form of PD without a family history; however, there has been much debate about this hypothesis.[21] Analysis of the *PARK2* gene mutation in EOPD patients (age at onset <50 years) in the Korean population revealed that 12.5% of patients had mutations, regardless of family history.[22]

Gaucher disease (GD; MIM 230800), the most common recessively inherited glycogen storage disease, is caused by a mutation in the lysosomal enzyme glucocerebrosidase (*GBA*). A loss-of-function mutation in β-GBA causes an accumulation of glucocerebroside in multiple organs. Lysosomal accumulation of glucocerebroside results in a broad spectrum of clinical manifestations, including hepatosplenomegaly, bone abnormalities, anemia, and thrombocytopenia. In addition, in approximately 5–10% of the cases, involvement of the central nervous system can occur, resulting in myoclonic epilepsy, oculomotor apraxia, and progressive neurodegeneration.[23] To date, about 300 missense, nonsense, and frame-shift pathologic mutations have been identified. Recent studies have shown an association between PD and GD.[24-28] *GBA* mutations have been found to increase the risk of PD and are also found in 8–14% of the pathologically confirmed PD patients. Parkinsonism and LB have been noted in a subset of patients with GD, and parkinsonism is more frequent among carrier relatives of subjects with GD.[24, 25, 29-31] Since the discovery of an association between *GBA* mutations and PD, a number of studies in diverse ethnic groups have reported an increased frequency of *GBA* gene mutations.[25-46] The

frequency and distribution of *GBA* mutations vary among populations. Among Ashkenazi Jews, the carrier frequency of the *GBA* mutation is between 1 in 12–16 persons, whereas the carrier frequency is less than 1% in other ethnic groups.[23, 27, 30, 36, 47] In Asians, GD is rare, and the exact prevalence of GD or the carrier frequency of *GBA* mutations has not been reported.[23, 48-50] Despite the rarity of GD or *GBA* mutation carriers in Asians, genetic association studies performed on the Japanese and Chinese populations have showed that heterozygous *GBA* mutations confer risk for PD, although there are some limitations to these studies.[28, 35, 36, 40, 43-45] Only 2 studies have performed the full sequencing of *GBA*; one study showed the highest odds ratio (OR) among all ethnic groups, whereas the other study observed a higher frequency trend of *GBA* mutation in PD patients without statistical significance.[36, 40] Other studies have screened only a small number of the mutations in *GBA*, such that the OR may have been underestimated.[28, 35, 43-45] In Korea, the frequency of *GBA* mutations in patients with PD has not been reported. To investigate whether there is an association between PD and mutations in *GBA* in the Korean population, we analyzed mutations of *GBA* and compared mutation frequencies between a Korean PD cohort and a control population.

Among the genes identified as causative for PD, mutations in *PARK2* are the most common genetic risk factor for EOPD.[21, 51] *PARK2* was the first gene unequivocally linked to an autosomal recessive form of familial PD. Clinical features of *PARK2* homozygous mutation carriers are generally indistinguishable from those of sporadic PD patients, with the exception of an earlier age of onset. The frequency of *PARK2*

mutations is as high as 49% in EOPD patients with an autosomal recessive mode of inheritance,[21] whereas this frequency is 14–15% in EOPD patients without a family history of PD.[51, 52] The frequency of *PARK2* mutations decreases as the age of onset increases; therefore, these mutations are uncommon in patients with late-onset PD.

The types of mutations found in *PARK2* are highly variable, such as point mutations, small deletions/insertions, and exonic rearrangement (either deletion or duplication), and have been reported in all exons of the *PARK2* gene.[53] Notably, point mutations or small insertions/deletions, which are found in approximately 50% of Caucasian PD patients with *PARK2* mutations, are infrequent in Asian populations.[21, 22, 51, 53-58] Although *PARK2* mutations were initially found in familial PD patients with an autosomal recessive mode of inheritance, heterozygous mutations of the *PARK2* gene were not rarely found in PD patients with sporadic onset and even in healthy controls.[11, 21, 51, 59, 60] There is much debate on whether a single heterozygous mutation of *PARK2* is a risk factor for PD.[61-67] ^{18}F-dopa positron emission tomography showed striatal dopaminergic dysfunction in asymptomatic heterozygous mutation carriers; however, in the largest case-control study, mutation frequency was similar in both groups.[67] To date, only a few studies have included control populations as well as PD patients for sequencing or gene-dosage analysis. Moreover, only 1 study screened for the *PARK2* gene-dosage mutation in 54 Asian populations included as controls.[68] Recently, Pankratz et al.[65] reported that the *PARK2* dosage mutation rather than a point mutation or small insertion/deletion mutation, was a risk factor for familial PD and may also be associated with earlier age at onset.[66] Here, we assessed the heterozygosity of *PARK2*

mutations in relation to the risk of PD by performing gene-dosage analysis in 188 EOPD or familial PD patients and 191 control individuals.

The purpose of this study was to investigate the effect of rare genetic variants (known genetic backgrounds involved in idiopathic Parkinson's pathogenesis) of *GBA* and *PARK2* on PD in the Korean population by analyzing two rare variants in patient and control populations.

Ⅱ. SUBJECTS & METHODS

1. Subjects

A. *GBA* mutation

Two hundred seventy-seven Korean PD patients (102 men; 175 women) were recruited from 5 movement disorder clinics in Korea. The PD patients were evaluated by movement disorder specialists, according to the United Kingdom (UK) PD Brain Bank criteria.[69] Patients ranged from 18 to 89 years of age at disease onset (mean 57.6 ± 13.5 years). Further, 291 healthy Korean controls (119 men; 172 women), who were asymptomatic upon neurological examination, were recruited from the National Health Examinee in Hallym University Sacred Heart Hospital. The mean age of the control individuals at blood draw was 57.5 ± 13.3 years. The study was approved by the institutional review boards at Hallym University, and informed consent was obtained from all subjects.

B. *PARK2* mutation

Fifty one familial PD (45.1% men) and 137 early onset PD (59.1% men) patients of sporadic onset were recruited from 5 movement disorder clinics in Korea. The PD patients were diagnosed by movement disorders specialists, according to the UK PD Brain Bank criteria.[69] EOPD was defined as the age at onset less than or equal to 55 years. Patients were 6–55 years of age at disease onset (mean, 40.0 ± 9.0 years) and 17–68 years of age at blood draw (mean, 43.4 ± 9.7 years). Patients who had been included in our previous study were excluded from this study.[22] Further, 191 healthy

controls (34.6% men), who were asymptomatic when screened by a neurological examination, were recruited from the National Health Examinee in Hallym University Sacred Heart Hospital. Mean age of the control individuals at blood draw was 51.9 ± 13.0 years. Ethnicity of all the subjects was Korean.

2. Genetic analysis

A. *GBA* mutation analysis

Peripheral blood was collected from each patient and genomic DNA was extracted from peripheral lymphoblasts according to a standard protocol. All exons of the *GBA* gene in 277 PD patients were amplified using the GeneAmp PCR System 2700 (Applied Biosystems, Foster City, CA), and direct sequencing was performed using the ABI PRISM 3730 Genetic Analyzer (Applied Biosystems). All exons of *GBA* were analyzed in 100 control subjects. Exon 2 and exons 5–11, where mutations of *GBA* were found in our PD patients, were analyzed in an additional 191 control subjects. To detect mutations in the *GBA* gene, we directly sequenced all 11 exons, according to previously reported methods, by using 3 sets of primers, which excluded amplification of the *GBA* pseudogene.[70] A fragment encompassing exons 1–5 was amplified by the forward primer 5′-CCTAAAGTTGTCACCCATAC-3′ and the reverse primer 5′-AGCAGACCTACCCTACAGTTT-3′ (annealing temperature, 58 ℃; extension time, 2 min and 30 s). A second fragment encompassing exons 5–7 was amplified using the forward primer 5′-GACCTCAAATGATATACCTG-3′ and the reverse primer 5′-AGTTTGGGAGCCAGTCATTT-3′ (annealing temperature, 58 °C; 2 min and 30 s). Lastly, a fragment extending across exons 8–11 was

amplified by the forward primer 5′-TGTGTGCAAGGTCCAGGATCAG-3′ and the reverse primer 5′-ACCACCTAGAGGGGAAAGTG-3′ (annealing temperature, 61 °C; 2 min). These amplified segments were purified using the QIAquick PCR Purification Kit (Qiagen, Germany). Cycle sequencing was accomplished using the Dye Terminator Cycle Sequencing Kit (Applied Biosystems) with appropriate primers, as reported previously.[40] For genotyping of G2385R of LRRK2, exon 48 of *LRRK2* from each individual was amplified using polymerase chain reaction (PCR) with primers (5′-CACGTAGAAATTTTAAGAAGAAAACA-3′, 5′-TGGGAATAAAATTAAAAACCACAGA-3′) under previously described conditions, and direct sequencing was performed using the ABI PRISM 3730 Genetic Analyzer (Applied Biosystems).[22] Fisher's exact test was used to test the difference in carrier frequency between PD patients and control subjects. A p value of <0.05 was considered statistically significant.

B. *PARK2* mutation analysis

Genomic DNA was extracted from the peripheral lymphoblasts of each subject, according to a standard protocol. Quantitative real-time PCR was performed using the StepOnePlus™ Real-Time PCR System (Applied Biosystems). A typical 20-μl reaction mixture contained 10 μl of 2× TaqMan® Universal PCR Master mix, 300 nM of each primer, 200 nM of probe, RNase P, 1 μL of template, and distilled water. For exons 1, 3, 6, 8, 9, 10, and 12, commercial kits (Taqman Copy Number Assays, Applied Biosystems) were used (see Supplementary Table 1). Primers and probe

sequences of the amplified genes for the other exons are shown in Supplementary Table 2. RNase P was used as the endogenous control. All PCR reactions were performed using the following program: 2 min at 50 ℃, 10 min at 95 ℃, and 40 cycles of 15 s at 95 ℃ and 1 min at 60 ℃. The fold change in *PARK2* expression was calculated using the $2^{-\Delta\Delta CT}$ method. In the case of PD patients with a confirmed *PARK2* gene-dosage mutation, variants were screened in all exons of *PARK2* to detect point mutations or small insertions/deletions by using PCR and direct sequencing with previously described conditions.[22] To test whether a gene dosage mutation in one allele increases the risk for PD, we compared the frequency of a single heterozygous mutation between PD and control individuals by using the Fisher exact test. To compare age at onset between or among the groups, we used the *t* test or analysis of variance.

Ⅲ. RESULTS

1. *GBA* mutation analysis

Sequencing for *GBA* in 277 PD patients revealed 18 heterozygous single nucleotide variants in 17 patients (Table 2). Among these variants, N188S, P201H, R257Q, and L444P are reported to be pathogenic either in the literature or in the Human Genome Mutation Database (HGMD).[40, 48, 49, 71] R257Q was the most common mutation (3 patients), and L444P was the second most common mutation (2 patients) in our PD cohort. There was no N370S mutation carrier. Two mutations (R163Q and F347L) were reported as nonpathogenic in the literature.[40] Two variants, found only in the PD group (S271G and R277C), were not present in the HGMD database.[71] An *in silico* prediction by the HumVar-trained Polyphen-2 model for the effect of a novel mutation (R277C) suggested that the mutation is benign, whereas the HumDiv-trained model predicted that R277C is probably a damaging mutation.[72] S271G is deposited in dbSNP (rs1057942) with minor allele frequencies of 0% in Han Chinese and 5% in African Americans, and prediction by Polyphen-2 was benign. However, a heterozygous S271G mutation was reported in a Greek PD cohort, and a compound heterozygous mutation of S271G/R359X was reported in a Korean GD patient with parkinsonism.[73, 74] Other non-pathogenic *GBA* variants found in both PD (n = 6) and controls (n = 5) included synonymous SNPs that do not result in amino acid changes (L268L and K466K) and a variant in the noncoding region (I-20V). Taken together, we classified 9 PD patients (3.2%) as carriers of pathogenic *GBA* mutations (Table 2). There was no family history of GD or PD in those who carried pathogenic *GBA* mutations. On the

other hand, none of the 291 control subjects had pathogenic *GBA* variants, suggesting that the *GBA* mutations are associated with PD in Koreans ($p < 0.01$; OR, 20.6; 95% confidence interval [CI], 1.2–356.4). The heterozygous *GBA* variant carriers were younger than the noncarriers (48.6 ± 11.9 vs. 57.9 ± 13.5; $p < 0.05$; Mann–Whitney test). None of the PD patients with heterozygous *GBA* mutations carried the G2385R polymorphism of *LRRK2*, an established PD risk allele in Koreans.

Table 2. List of *GBA* variants in patients with Parkinson disease.

Case / control	Case ID	Sex	Age at onset	Family history of Parkinson disease	Exons	Nucleotide change	Amino acid position and change	Previous reports in literatures or SNP ID (rs#)
PD	6140	F	42	–	2	A>G	I-20V	Non-coding SNP
PD	6100	F	89	–	6	G>A	R163Q	Non-pathogenic
PD	7055	M	35	+	6	A>G	N188S	Pathogenic
PD	5480	F	37	–	6	C>A	P201H	Pathogenic
PD	6061	F	46	–	7	G>A	R257Q	Pathogenic
PD	7131	F	73	–	7	G>A	R257Q	Pathogenic
PD	6068	M	58	–	7	G>A	R257Q	Pathogenic
PD	6011	M	49		7	C>T	L268L	Synonymous SNP
PD	6011	M	49	–	7	A>G	S271G	Pathogenic (rs1057942)
PD	4429	M	54	–	7	A>G	S271G	Pathogenic (rs1057942)
PD	7094	M	62	–	7	C>T	R277C	Novel
PD	6084	M	39	–	8	T>C	F347L	Non-pathogenic
PD	5478	M	40	–	10	T>C	L444P	Pathogenic
PD	7028	F	45	+	10	T>C	L444P	Pathogenic
PD	6088	F	39	–	11	G>A	K466K	Synonymous SNP
PD	6096	F	78	–	11	G>A	K466K	Synonymous SNP
PD	7137	F	63	–	11	G>A	K466K	Synonymous SNP
PD	7159	F	52	–	11	G>A	K466K	Synonymous SNP
Control	3010	M	75	–	2	A>G	I-20V	Non-coding SNP
Control	3099	F	39	–	2	A>G	I-20V	Non-coding SNP
Control	4020	M	43	–	2	A>G	I-20V	Non-coding SNP
Control	7207	M	60	–	2	A>G	I-20V	Non-coding SNP
Control	7275	F	54	–	11	G>A	K466K	Synonymous SNP

2. *PARK2* mutation analysis

We identified mutations of *PARK2* in at least one allele in 21 patients (11.2%; see Table 3). Among these patients with *PARK2* mutations, 11 had a family history of PD (52.4%). 8 patients (38.1%) had mutations in both alleles (5 compound heterozygous and 3 homozygous mutations), and 13 patients (61.9%) had heterozygous mutations, all of which were gene-dosage mutations that occurred by exonic rearrangement. Among 29 mutated alleles, 1 small sequence variation (c.101delAinAG) was found (3.4%), with the majority of the mutations being exonic rearrangement (96.6%). In 28 gene-dosage mutations, 25 were deletions and 3 were duplications. Exon 1 and Exon 4 were the most common sites of gene-dosage mutations. All the observed exonic rearrangements occurred in exons 1–11. No mutation was found in Exon 12.

To assess the risk for PD posed by gene-dosage mutation in only 1 allele of the *PARK2* gene, we also studied *PARK2* gene-dosage mutation in 191 control individuals. Among our non-PD control individuals, none had *PARK2* gene-dosage mutation. For an association study, we excluded PD patients with compound heterozygous *PARK2* mutations. Because the frequency of *PARK2* gene-dosage mutations in each exon was so low that it could not provide sufficient power to test for association separately, the frequency of gene-dosage mutation in 1 allele of *PARK2* in any exon, was compared between the PD patients and control individuals. The frequency of a single *PARK2* mutation due to exonic rearrangement was higher among the PD patients than the control individuals (6.9% vs. 0.0%, $p < 0.001$).

Table 3. Demographic characteristics of patients with *PARK2* mutations and the type and location of the mutations.

	Sex	Age at sample	Age at onset	Family history	Variants of *parkin*	Zygosity
1	F	17	17	-	Ex1 del	Heterozygous
2	M	56	35	-	Ex1 del	Heterozygous
3	M	26	26	+	Ex1 del	Heterozygous
4	M	34	28	-	Ex2 del / Ex4 del	Compound heterozygous
5	M	46	42	+	Ex2 del / Ex3 del	Compound heterozygous
6	M	24	16	-	Ex3 del / Ex1-4 del	Compound heterozygous
7	F	38	12	-	Ex7 dupl /c.101delAinsAG[1]	Compound heterozygous
8	F	44	41.5	+	Ex7-10 del / Ex8-10 del	Compound heterozygous
9	M	41	23	+	Ex1 del	Heterozygous
10	F	32	32	+	Ex1 del	Heterozygous
11	M	36	36	-	Ex2 del	Heterozygous
12	F	48	45	+	Ex2 dupl	Heterozygous
13	F	28	28	+	Ex3 del	Heterozygous
14	F	25	25	-	Ex4 del	Heterozygous
15	M	30	29	+	Ex4 del	Heterozygous
16	F	23	15	-	Ex5 dupl	Heterozygous
17	F	36	36	+	Ex6 del	Heterozygous
18	F	42	23	+	Ex7 del	Heterozygous
19	M	43	41	-	Ex10 del	Homozygous
20	F	25	6	-	Ex11 del	Homozygous
21	F	50	30	+	Ex4 del	Homozygous

1. rs55777503

Ⅳ. DISCUSSION

1. *GBA* mutation analysis

Our results indicate that *GBA* mutations represent a genetic risk factor for PD in Koreans, supporting the notion that the association between *GBA* mutations and PD is not exclusive to a specific ethnic group.[28] There are a number of reports that analyze *GBA* mutations in Asian PD patients and control populations.[28, 35, 36, 40, 43-45] However, only 2 groups have performed full sequencing analysis of ethnic Chinese and Japanese PD patients, and their reported *GBA* mutation carrier frequencies (4.3% and 9.4%, respectively) were higher than those calculated from our data.[36, 40] In studies where only the common *GBA* mutations (L444P, N370S, or both with F213I and R353W) were screened in Chinese PD patients and controls, *GBA* mutation carrier frequencies were between 1.8% and 3.2%.[28, 35, 43-45] There are some similarities and differences in terms of types of mutations among Korean, Japanese, and Chinese populations. L444P, which is reported worldwide, is a mutation common to all 3 populations.[28, 35, 40, 44] The most common *GBA* mutations in Asian PD cohorts were R257Q in Koreans, R120W in Japanese, and L444P in ethnic Chinese in Taiwan.[36, 40] The N370S mutation was reported in 1.8% of the Chinese PD patients; however, the N370S mutation has not been reported in Korean or Japanese GD and PD cohorts, including in our subjects.[40, 45, 48, 50] There is much debate about whether S271G mutation is a pathogenic variant because existing data from the literature and dbSNP and bioinformatics prediction are contradictory, as described in Section Ⅲ. Performing segregation analysis in families with S271G mutation might provide an

answer; however, given the rarity of GD in Korea, this type of testing might not be possible. In all 3 populations, the age-at-onset was earlier in PD patients carrying heterozygous *GBA* mutations.[36, 40] The OR in our PD cohort (20.6; 95% CI, 1.2–356.4) was lower than that reported in the Japanese population (28.0; 95% CI, 7.3–238.3); however, the value is higher than that reported in a meta-analysis in which 2 extreme datasets from Japan and Norway were excluded (5.43; 95% CI, 3.89–7.57).[28, 40] We sequenced all the exons of *GBA* in 100 controls and exon 2 and exons 5–11 in the additional 191 controls. Therefore, the *GBA* mutation frequency in the control population might have been underestimated and the OR might have been exaggerated in our study. The higher ORs in the Japanese study[40] and our data are probably attributable to the absence or rarity of *GBA* mutation carriers in the control subjects. GD is a panethnic disorder, and the overall frequency of GD variants is estimated at about 1 in 40,000 to 50,000 live births.[23] Although growing recognition of GD is evident, GD is rare in Asians. There has been no epidemiological study to estimate the exact prevalence of GD or carrier frequency of a *GBA* mutation in Asians.[23] In Japan, the frequency of GD is estimated to be between 1 in 500,000 and 1 in 1,200,000 live births.[48] It will be important to know the prevalence of GD in Korea to accurately estimate the risk of *GBA* mutations for PD.

The mechanism of how rare variants of *GBA* are related to PD or LB pathology is unclear. Possible pathogenesis may include changes in the sphingolipid composition of the membranes, secondary to the loss of *GBA* enzyme activity leading to α-synuclein aggregation, and retarded protein degradation secondary to autophagy–lysosomal

dysfunction.[75] Recently, direct protein interaction between α-synuclein and *GBA* was shown under lysosomal solution conditions (pH 5.5), which supports this possibility.[76] The missense mutation in the *SNCA* gene is reported to cause PD through a toxic gain of function,[77] and LB may represent the attempt to purge the cell of toxic damaged α-synuclein.[78] Wild-type α-synuclein is selectively translocated into lysosomes for degradation,[79] and inhibitors of the lysosomal enzyme β-glucocerebrosidase modulate α-synuclein levels.[80] The bi-directional effect of α-synuclein and β-glucocerebrosidase forms a positive feedback loop that results in the accumulation of α-synuclein.[81] Functional loss of β-glucocerebrosidase causes the accumulation of glucocerebroside, which directly influences the aggregation of α-synuclein by stabilizing oligomeric intermediates.[81] In turn, α-synuclein inhibits the lysosomal activity of β-glucocerebrosidase, as shown in the neurons and the idiopathic PD brain.[81]

In conclusion, the presence of heterozygous mutations in *GBA* is a genetic risk factor for PD in Koreans, similar to that in other ethnic groups.

2. *PARK2* mutation analysis

Mutation in *PARK2* has been reported as the most common genetic cause for familial or EOPD.[82] Although the frequency of *PARK2* mutations in our study was low, the types of mutation, the effect of the number of mutations on the age at onset,[83, 84] and the location of the observed mutations are all consistent with those reported previously in both Asian and other populations.[22, 53-56, 68, 85] Depending on the characteristics of the

study population, such as family history or age at onset, the frequency of *PARK2* mutations, regardless of zygosity, in Asian populations varies between 5.6% and 48.3%.[22, 54-58, 68, 85] The frequency of *PARK2* mutations in our study was 11.2%, which is lower than that in other studies. We think that the low mutation frequency observed in our cohort is attributed to our selection criteria;[86] we defined age at onset for EOPD to be older than that in other studies. The oldest age at onset for *PARK2* mutation in our study was 45 years. Because we did not sequence *PARK2* for all PD patients, the frequency of the *PARK2* mutation might have been underestimated. However, we do not expect that complete sequencing for all PD patients is likely to considerably alter the frequency of the observed *PARK2* mutations. Point mutations in *PARK2* are not common in Asian populations, unlike those in Caucasian populations, and most of the *PARK2* mutations in Asian populations are exonic rearrangements. Point mutations or small insertions/deletions, which have been reported to represent 8.0–16.7% of all mutations in Asian PD patients, were found in only one patient (3.4%) of our cases. The frequency of heterozygous mutations in our PD cohort (61.9%) is within the frequency range described by previous studies in Asian PD patients (16.7–75%).

To exclude compound heterozygous mutations due to sequence variation in patients with heterozygous gene-dosage mutations, we sequenced all the exons of *PARK2* but did not find any sequence variation. Heterozygous *PARK2* gene-dosage mutation in PD has been reported worldwide.[22, 51-54, 56, 62-64, 68, 87] However, there is debate about whether heterozygous mutations of *PARK2* are genetic risk factors for PD, even though some researchers have performed *PARK2* genotyping in control

populations.[61-67] Because most of the subjects who participated in these studies were non-Asian, we cannot directly compare our results with those of these studies. Only one study analyzed gene-dosage mutations of *PARK2* in a small healthy Asian control population (n = 54).[68] A possible explanation for the lack of consensus lies with the selected study populations of PD patients because age-at-onset appears to be an important factor. Studies of EOPD or familial PD have reported a positive association or a trend toward association,[62, 63, 65, 66] whereas those investigating idiopathic PD generally reported a negative association between heterozygous carriers of *PARK2* mutations and PD.[61, 67] In a recent study that comprehensively analyzed *PARK2* mutations in 1,686 controls and 2,091 PD patients, the frequency of *PARK2* mutations among the PD patients was shown to vary depending on age at onset, whereas that among controls remained constant across all age groups.[67] The frequency of *PARK2* mutations is extremely high in EOPD patients and declines sharply with increasing age at onset. By 45 years and thereafter, the mutation frequencies in PD and control subjects are completely superimposed.

The type of mutation is another potentially confounding factor because, as Pankratz et al.[65] have reported, a *PARK2* dosage mutation but not a simple sequence variation may be a risk factor for PD.[66] Reported frequencies of heterozygous mutations in controls vary considerably depending on the type of mutation. Heterozygous point mutations of *PARK2* are found in as many as 3.4% of controls;[66] in contrast, heterozygous gene-dosage mutations are extremely rare in control subjects.[61-63, 65-67, 88] Of 7 studies that performed *PARK2* gene-dosage analysis in

controls, 3 found no *PARK2* gene-dosage mutation in the controls, as was the case in the current investigation.[62, 63, 66] In the other 4 studies, heterozygous gene-dosage mutations, although rare, were reported in the controls, and the average frequency of a heterozygous carrier in the controls was 0.85% (range, 0.52–1.09%).[61, 65, 67, 88] There are caveats in our study. We studied only EOPD or familial PD patients, and therefore our results cannot be generalized to the entire PD population. In addition, our sample sizes are smaller than those of two recent studies in which comprehensive analyses were conducted.[66, 67] However, given that gene-dosage mutation is a common form of *PARK2* mutation in Asian populations, we believe that heterozygous gene-dosage mutation as a risk factor for EOPD or familial PD has important clinical implications.

The *PARK2* protein (parkin) is involved in the cell's response mechanism to cellular and oxidative stress, implying cell dysfunction or increased vulnerability to neurodegeneration in patients carrying mutations in these genes. Mutations in *PARK2* were reported to impair the E3 ubiquitin ligase activity of parkin,[89] which resulted in insufficient protein clearance and the subsequent formation of protein aggregates. In addition, parkin was shown to be recruited for dysfunctional mitochondria, pointing toward a possible role for parkin in the induction of mitophagy.[90, 91] Mutations in *PARK2* might impair this function and eventually result in increased cellular toxicity. This hypothesis was supported by a *PARK2* null *Drosophila* model, which showed mitochondrial defects and elevated oxidative stress rather than UPS impairment;[92, 93] this implied that the involvement of *PARK2* in the mitochondria might be its primary activity.

Ⅴ.CONCLUSION

In conclusion, the heterozygous mutations of *GBA* and the heterozygous gene-dosage mutation of *PARK2* are genetic risk factors for PD in Koreans.

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APPENDICES

Supplementary table 1. TaqMan® Copy Number Assays

EXON	Assay ID	Reporter 1dye	Reporter1 Quencher	Context Sequence
Exon 1	Hs00072707_cn	FAM	NFQ	TCACTGGGTAGGTGGCGGCTGCGGG
Exon 3	Hs00054624_cn	FAM	NFQ	TTCCAGCTGGTGGTGAGTCCTTCCT
Exon 6	Hs00134402_cn	FAM	NFQ	CTGATGTTTCCTTGTCAGAGGTGGG
Exon 8	Hs00014635_cn	FAM	NFQ	TTAATCAAGGAGTTGGGACAGCCAG
Exon 9	Hs00104645_cn	FAM	NFQ	GGTACCGGTTGTACTGCAAAACCCA
Exon 10	Hs00089553_cn	FAM	NFQ	CAGAAGGCAAACTGCAAAAGAACAC
Exon 12	Hs00115097_cn	FAM	NFQ	CCTGTTGGTGGTGTCGCAGATGGCT

Supplementary table 2. Custom primers and probes for TaqMan® Copy Number Assays

EXON	Forward primer(5'-3')	Reverse primer(5'-3')	Probe
Exon 2	TTTTCCCAAAGGGTCCATCTT	GCTTAGCAACCACCTCCTTGA	5'-FAM-CACCAGCATCTTC-MGB-NFQ-3'
Exon 4	AGCCACTTCTTCTGCTTTTTCTTC	TTTGCAATACACATAAAAGCTGTTGT	5'-FAM-CAGCAGGTAGATCAA-MGB-NFQ-3'
Exon 5	TTTTCCCAAAGGGTCCATCTT	CATTCACCACTCATCCGGTTT	5'-FAM-CTGGGATGATGTTTTAAT-MGB-NFQ-3'
Exon 7	CCGCCACGTGATTTGCTTA	CTGCCGATCATTGAGTCTTGTC	5'-FAM-CGTTTCCACTTATACTGTG-MGB-NFQ-3'
Exon11	CAGGCTCGTTGGGAAGCA	5'-GACAGGGCTTGGTGGTTTTC	5'-FAM-CCTCCAAAGAAACCATC-MGB-NFQ-3

ABSTRACT(IN KOREAN)

유전적 희귀변이가
한국인의 파킨슨병 발병에 미치는 영향

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김 원 찬

배경: 가족성은 물론 특발성 파킨슨병 환자로부터 다양한 질환 유전자 및 감수성 유전자가 밝혀져 왔다. 파크2 (*PARK2*) 돌연변이는 조기발병 파킨슨병에서 가장 흔한 위험인자로 알려져 있지만 아시아인에서는 흔치 않은데다 파크2의 단일이형접합변이가 파킨슨병 발병의 위험인자인지에 대하여는 아직 논란이 많다. 파킨 유전자의 빈도분석 (gene dosage analysis)을 시행한 환자-대조군 연구는 매우 드물며 그 중에서도 특히 아시아인을 대상으로 한 연구는 오직 하나에 불과하다. 글루코세레브로시다제 (*GBA*) 돌연변이는 고셔병의 원인 유전자이나, 최근 이 유전자의 이형접합체 돌연변이 보인자는 파킨슨병의 위험도가 3배 이상 증가된다는 사실이 다양한 인종에서 보고되었다. 하지만 아직까지 한국인 파킨슨병 환자를 대상으로 GBA 돌연변이가 어떤 영향을

끼치는 지는 연구된 바가 없다. **방법:** 본 연구는 유전적인 희귀 변이인 GBA 유전자 돌연변이 및 파킨 돌연변이가 한국인에서 파킨슨병의 발병과 상관관계가 있는지 알아보는 것을 목적으로 시행되었다. GBA 유전자의 이형접합 돌연변이가 한국인에서 파킨슨병 발병에 관여하는지를 알아보기 위하여 GBA 돌연변이의 빈도를 파킨슨병 환자와 대조군 간에 비교하고 한국인에서 흔한 GBA 유전자 돌연변이형을 파악하고자 하였다. 또 조기발병 및 가족성 파킨슨병 환자와 대조군에서 파킨 유전자의 돌연변이 빈도를 조사 비교하여 한국인에서 파크2의 이형접합 돌연변이가 파킨슨병 발병의 위험인자로 작용하는지 조사해 보고자 하였다. GBA 돌연변이 조사는 277명의 파킨슨병 환자와 291명의 정상대조군을 대상으로 하였다. 우선 모든 환자군과 100명의 정상 대조군을 대상으로 하여 GBA 유전자의 모든 엑손에 대한 유전자 분석을 시행한 후, 환자군에서 돌연변이가 발견된 엑손 2와 엑손 5-11에 대하여 추가적인 191명의 대조군에서 유전자 분석을 시행하였다. 파크2 유전자의 빈도분석은 188명의 조기발병 혹은 가족성 파킨슨병 환자들과 191명의 정상 대조군을 대상으로 실시간 중합효소연쇄반응을 사용하여 시행하였다. 분석 결과 이형접합

유전자 빈도변이가 관찰된 파킨슨병 환자들에서 복합 이형 접합 돌연변이의 가능성을 배제하기 위하여 유전자 염기서열 분석을 시행하였다. **결과:** GBA 유전자 분석 결과 9명 (3.2%)의 파킨슨병 환자에서 N188S, P201H, R257Q, S271G, L444P 의 5개의 이형접합 돌연변이가 발견되었다. 정상 대조군에서는 GBA 유전자 돌연변이가 발견되지 않았다 ($p < 0.01$, OR 20.6, 95% CI 1.2–356.4). 이형접합GBA 유전자 돌연변이를 갖고 있는 파킨슨병 환자들의 평균 발병 연령은 돌연변이를 갖고 있지 않은 환자들에 비해 통계적으로 유의하게 낮았다 (48.6 ± 11.9 versus 57.9 ± 13.5, $p < 0.05$, Mann-Whitney test). 파크2 유전자 분석 결과 21명 (11.2%)의 파킨슨병 환자가 돌연변이를 갖고 있었다. 이 중 5명 (23.8%)는 복합 이형접합변이를 13명 (61.9%)는 이형접합변이를 갖고 있었다. 돌연변이가 발견된 29개의 대립형질 유전자 중 작은 염기서열 변이가 1개 (3.4%) 였으며 유전자 빈도 변이가 전체 돌연변이의 96.6%를 차지하였고, 발견된 이형접합 돌연변이는 모두 유전자 빈도 변이였다. 파크2 유전자의 유전자 빈도 변이는 파킨슨병 환자들에서 정상 대조군에 비해 더 많이 관찰되었다. **결론:** 파킨슨병의 유전적 배경으로 작용하는 희귀변이 중 GBA와 파크2 유전자 돌연변이는 한국인에서도 파킨슨병의 발병

위험인자로 작용한다.

핵심되는 말 : 파킨슨병, 희귀변이, 파킨, 글루코세레브로시다제