The G2019S LRRK2 Mutation is Rare in Korean Patients with Parkinson’s Disease and Multiple System Atrophy

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Received March 24, 2008
Revised October 23, 2008
Accepted October 23, 2008

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Background and Purpose
The LRRK2 (PARK8; OMIM607060) substitution was recently identified as a causative mutation for Parkinson’s disease (PD). The pathologic heterogeneity of LRRK2-positive patients suggests that mutation of the LRRK2 gene is associated with the pathogenesis of PD and Parkinson-plus disorders, such as multiple system atrophy (MSA). We previously reported that the G2019S LRRK2 mutation—which is the most common LRRK2 mutation—was not found in a sample of 453 Korean PD patients. In the present study, we extended the screening of the G2019S mutation to a larger group of PD and MSA patients.

Methods
We performed a genetic analysis of the G2019S mutation in 877 patients with PD and 199 patients with MSA using a standard PCR and restriction digestion method.

Results
None of the subjects carried the G2019S mutation.

Conclusions
The results of the present study support that the G2019S mutation is extremely rare in PD and is unlikely to be associated with MSA in the Korean population.

Key Words
Parkinson’s disease, multiple system atrophy, LRRK2, G2019S mutation.

Introduction
Several causative mutations of Parkinson’s disease (PD) have been identified, the most recent of which is the pathogenic LRRK2 (PARK8; OMIM607060) substitution.1,2 Funayama and colleagues reported genetic linkage to chromosome 12 in a large Japanese family3 and subsequently in two Caucasian families.4,5 Mutation of LRRK2 is of great clinical importance because the LRRK2 gene has been reported to be present in both familial and sporadic forms of PD.4,5 Several LRRK2 pathogenic mutations have been reported previously, of which the G2019S substitution is the most common.5,7 Mutation of the LRRK2 gene has been reported worldwide, with a wide ethnicity variability, and patients with LRRK2 mutations account for 3-7% of familial PD cases and 0.5-3% of sporadic cases of PD.5,7 LRRK2 mutations are frequent in North African Arabs,8 Jews,9 and some Spanish populations10 (6.1-41% in sporadic PD and 18.7-37% in familial PD), but they are very rare in Asian populations.6,11-14 Clinically, most patients with LRRK2 mutations have late-onset typical idiopathic PD; however, a pleomorphic pathology—including Lewy bodies, tau-positive and/or ubiquitin inclusions—has also been reported.2,15-18 Therefore, investigation of LRRK2 mutations has been extended to other neurodegenerative diseases, such as progressive supranuclear palsy, multiple system atrophy (MSA), and frontotemporal dementia,19-22 and the G2019S mutation was found in a single case of Alzheimer’s disease.22

We previously reported that the G2019S mutation was not found in a sample of 453 Korean PD patients.14 Therefore, in the present study we extended the screening of the G2019S mutation to a larger group of Korean patients with PD and MSA.

Methods
All of the patients included in the study were native Koreans who were personally examined and followed by the senior neurologist at Seoul National University Hospital between
Briefly, DNA was extracted from peripheral blood using standard methodologies. We used 50 ng of DNA template and generated PCR products using the following primer pair based on National Center for Biotechnology Information (NCBI) accession number NC_000012.10: forward, 5'-AA GGGACAAAGTGAGCACAGA-3'; reverse, 5'-TGTTTTC CTTTGTGACTCTTCTGTA-3'. The PCR conditions were an initial denaturation at 95°C for 10 minutes, followed by 35 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 1 minute, and a final extension at 72°C for 7 minutes. The PCR products (3 μL) were digested with SfoI (New England BioLabs, Beverly, MA, USA) at 37°C.

Wild-type PCR products produced fragments of 251 and 127 bp, and the mutant produced fragments of 230, 127, and 21 bp. Since we did not have a positive control, we used the 677C>T mutant DNAs of the methylene tetrahydrofolate reductase (MTHFR) gene with similar fragments sizes (175 and 127 bp). Since we did not have a positive control, we used the 677C>T mutant DNAs of the methylene tetrahydrofolate reductase (MTHFR) gene with similar fragments sizes (175 and 23 bp) for controlling the quality of restriction digestion and electrophoretic separation. Several samples were sequenced to confirm the quality of our methods.

Results

None of the 1,076 study subjects (877 PD and 199 MSA) carried the G2019S mutation.

Discussion

The etiology of PD involves multiple environmental and genetic factors. Until the recent discovery of a causative mutation of PD, environmental factors had been emphasized in the pathogenesis of PD because most cases of PD are sporadic and only 5-10% of patients with PD have one or more affected relatives. The identification of familial PD and the discovery of genetic abnormalities led to genetic factors becoming a primary focus in the field of PD research, and studies on several causative mutations of PD have furthered our understanding of the molecular processes involved in the pathogenesis of PD. Nevertheless, analyses of genetic factors in PD have their own limitations, since these mutations are usually found in familial cases on rare occasions. Recently discovered LRRK2 mutations have received considerable attention due to their prevalence in sporadic PD being higher than those of other causative mutations.

Patients with LRRK2 mutations usually show late-onset typical PD features, and PD associated with mutation of the LRRK2 gene can have a diverse clinical spectrum. Some mutation carriers exhibit autonomic and cognitive dysfunctions. More than 20 putative pathogenic mutations of the LRRK2 gene have been identified, 6 of which (R1441C, R1441G, R1441H, Y1699C, G2019S, and I2020T) have been reported in more than two unrelated families. The clinical manifestation does not differ according to the type of substitution in the LRRK2 gene. The penetrance of LRRK2 mutations appears to differ from that of other causative mutations. LRRK2 mutations have an autosomal dominant pattern of inheritance with incomplete and age-related penetrance. Therefore, these patients could be reported as late onset with sporadic presentation.

It is well known that the G2019S mutation is the most frequent of several amino acid substitutions in the LRRK2 gene. However, the prevalence of the G2019S LRRK2 mutation appears to vary with ethnicity, with it being frequent in Western populations but very rare (<0.01%) in Asian populations. Several studies designed to screen for the G2019S substitution in Asian populations produced negative results. We previously reported that the G2019S mutation was not found in a sample of 453 Korean PD patients, and the present study further confirms the rarity of the G2019S mutation in PD in Korean and Asian populations. Until now, only four cases of LRRK2 G2019S substitution have been reported in Asian populations, with other types of substitutions being more frequent. Therefore, further investigations are necessary to fully screen for other types of substitution in Korean PD patients.

In this study, we extended the G2019S screening to MSA
patients due to the pleomorphic pathology and clinically indistinguishable cases of MSA found in studies of patients with LRRK2 mutations. LRRK2 mutations usually lead to the development of the typical features of late-onset PD; however, some carriers of the G2019S LRRK2 mutation showed severe autonomic dysfunctions that were indistinguishable from those of MSA. In addition, autopsy findings have revealed diverse pathologies. LRRK2 mutations have a pleomorphic pathology, including the classical changes seen in PD such as Lewy bodies, nigral degeneration without Lewy bodies, tau-positive and ubiquitin inclusions. LRRK2 mutations have recently or been studied in patients with MSA. Tan et al. screened for 14 mutations of the LRRK2 gene and did not find any mutations in 15 MSA Singaporean subjects. The North American MSA study group and Ross et al. did not find the G2019S mutation in 136 clinically diagnosed and 43 pathologically confirmed cases of MSA, respectively. Our finding that the G2019S mutation was not present in 199 Korean MSA patients is consistent with previous reports that the G2019S mutation does not cause MSA.

The rarity of LRRK2 mutations in our population needs to be confirmed by screening all 51 exons of the LRRK2 gene, since substitutions other than G2019S in the LRRK2 gene are distinguishable cases of MSA found in studies of patients. The North American MSA study group and Ross et al. did not find the G2019S mutation in 136 clinically diagnosed and 43 pathologically confirmed cases of MSA, respectively. Our finding that the G2019S mutation was not present in 199 Korean MSA patients is consistent with previous reports that the G2019S mutation does not cause MSA.

Acknowledgments

This study was in part supported by a grant of the Korea Health 21 R & D Project. Ministry of Health & Welfare, Republic of Korea (03-PJ10-PG13-GD01-0002). We deeply appreciate a generous donation from Mr. Chung Suk-Gyoo and Shinyang Cultural Foundation.

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