ORIGINAL ARTICLE
J Clin Neurol 2009;5:29-32

Print ISSN 1738-6586 / On-line ISSN 2005-5013
10.3988/jcn.2009.5.1.29

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

Jin-Whan Cho, MD, PhD[a]; Sung-Yeon Kim[b]; Sung-Sup Park, MD, PhD[b]; Beom S. Jeon, MD, PhD[c]

Department of [a]Neurology, College of Medicine, Seoul National University, Metropolitan Boramae Hospital, Seoul, Korea
Department of [b]Laboratory Medicine, College of Medicine, Seoul National University, Seoul National University Hospital, Seoul, Korea
Department of [c]Neurology, Neuroscience Research Institute and CRI, BK21 Program, College of Medicine, Seoul National University, Seoul, Korea

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

Correspondence
Beom S. Jeon, MD, PhD
Department of Neurology,
College of Medicine,
Seoul National University,
101 Daehang-ro, Jongno-gu,
Seoul 110-799, Korea
Tel +82-2-2072-2876
Fax +82-2-3672-7553
E-mail brain@snu.ac.kr

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 for 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.

J Clin Neurol 2009;5:29-32

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 family[3] and subsequently in two Caucasian families.[1,2] 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 populations[10] (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

1993 and 2006. Blood samples were collected from 2003 with written consents from the patients. DNA was extracted and stored in a deep freezer until analysis. The genetic study was approved by the Institutional Review Board (IRB) of Seoul National University Hospital. PD was diagnosed according to the criteria of the United Kingdom Parkinson's Disease Society Brain Bank,[23] with the exception of the criterion of a positive family history. All MSA subjects were diagnosed as having probable MSA according to the Consensus Criteria.[24]

A total of 1,076 parkinsonian subjects were included in this study, of which 877 patients had PD (including the 453 subjects previously reported[14]) and 199 had MSA. The age of the PD patients at onset was 55.7±11.2 years (mean±SD) and ranged from 12 to 81 years. Twenty-seven of the 877 PD patients (3.1%) had at least one first-degree relative (parent or sibling) with parkinsonism. There were 265 patients with PD who were younger than 50 years at the onset of PD, and 389 of the PD patients were men (age at onset=55.2±12.2 years) and 488 were women (age at onset=56.0±10.4 years). The age of the MSA patients at onset was 60.6±8.1 years and ranged from 36 to 83 years. Ninety-four of the MSA patients were men (age at onset=62.1±8.0 years) and 105 were women (age at onset=59.4±8.0 years).

Genetic analysis was performed as described previously.[14] Briefly, DNA was extracted from peripheral blood using stand-ard 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 CTTTTGACTCTTCTGA-3'. The PCR conditions were an initial denaturation at 95℃ for 10 minutes, followed by 35 cycles of 95℃ for 30 seconds, 60℃ for 30 seconds, and 72℃ for 1 minute, and a final extension at 72℃ for 7 minutes. The PCR products (3 μL) were digested with *Sfc*I (New England BioLabs, Beverly, MA, USA) at 37℃.

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 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.[4,5,25,26] 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.[27] 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.[4,5,25] 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.[4,5,25]

Patients with LRRK2 mutations usually show late-onset typical PD features,[6] and PD associated with mutation of the LRRK2 gene can have a diverse clinical spectrum. Some mutation carriers exhibit autonomic and cognitive dysfunctions.[22,28,29] More than 20 putative pathogenic mutations of the LRRK2 gene have been identified,[7,30] 6 of which (R1441C, R1441G, R1441H, Y1699C, G2019S, and I2020T) have been reported in more than two unrelated families.[6,7,30] The clinical manifestation does not differ according to the type of substitution in the LRRK2 gene.[6] 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.[1-3,5,31,32] 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.[5-7] 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.[5-10] Several studies designed to screen for the G2019S substitution in Asian populations produced negative results.[11-13] We previously reported that the G2019S mutation was not found in a sample of 453 Korean PD patients,[14] 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,[6,33,34] with other types of substitutions being more frequent.[15,20,35] 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.[22] 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.[2,15-18] LRRK2 mutations have recently or been studied in patients with MSA. Tan et al.[20] 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[21] and Ross et al.[22] 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 reportedly more frequent in Asian populations.

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.

REFERENCES

1. Paisán-Ruíz C, Jain S, Evans EW, Gilks WP, Simón J, van der Brug M, et al. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron* 2004;44:595-600.
2. Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, et al. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron* 2004;44:601-607.
3. Funayama M, Hasegawa K, Kowa H, Saito M, Tsuji S, Obata F. A new locus for Parkinson's disease (PARK8) maps to chromosome 12 p11.2-q13.1. *Ann Neurol* 2002;51:296-301.
4. Gasser T. Genetics of Parkinson's disease. *Curr Opin Neurol* 2005;18:363-369.
5. Hardy J, Cai H, Cookson MR, Gwinn-Hardy K, Singleton A. Genetics of Parkinson's disease and parkinsonism. *Ann Neurol* 2006;60:389-398.
6. Healy DG, Falchi M, O'Sullivan SS, Bonifati V, Durr A, Bressman S, et al. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. *Lancet Neurol* 2008;7:583-590.
7. Taylor JP, Mata IF, Farrer MJ. LRRK2: a common pathway for parkinsonism, pathogenesis and prevention? *Trends Mol Med* 2006;12:76-82.
8. Lesage S, Dürr A, Tazir M, Lohmann E, Leutenegger AL, Janin S, et al. LRRK2 G2019S as a cause of Parkinson's disease in North African Arabs. *N Engl J Med* 2006;354:422-423.
9. Ozelius LJ, Senthil G,Saunder-Pullman R, Ohmann E, Deligtisch A, Tagliati M, et al. LRRK2 G2019S as a cause of Parkinson's disease in Ashkenazi Jews. *N Engl J Med* 2006;354:424-425.
10. Infante J, Rodríguez E, Combarros O, Mateo I, Fontalba A, Pascual J, et al. LRRK2 G2019S is a common mutation in Spanish patients with late-onset Parkinson's disease. *Neurosci Lett* 2006;395:224-226.
11. Fung HC, Chen CM, Hardy J, Hernandez D, Singleton A, Wu YR. Lack of G2019S LRRK2 mutation in a cohort of Taiwanese with sporadic Parkinson's disease. *Mov Disord* 2006;21:880-801.
12. Lu CS, Simons EJ, Wu-Chou YH, Fonzo AD, Chang HC, Chen RS, et al. The LRRK2 I2012T, G2019S, and I2020T mutations are rare in Taiwanese patients with sporadic Parkinson's disease. *Parkinsonism Relat Disord* 2005;11:521-522.
13. Tan EK, Shen H, Tan LC, Farrer M, Yew K, Chua E, et al. The G2019S LRRK2 mutation is uncommon in an Asian cohort of Parkinson's disease patients. *Neurosci Lett* 2005;384:327-329.
14. Cho JW, Kim SY, Park SS, Kim HJ, Ahn TB, Kim JM, et al. The G2019S LRRK2 mutation is rare in Korean patients with Parkinson's disease. *Can J Neurol Sci* 2007;34:53-55.
15. Funayama M, Hasegawa K, Ohta E, Kawashima N, Komiyama M, Kowa H, et al. An LRRK2 mutation as a cause for the Parkinsonism in the original PARK8 family. *Ann Neurol* 2005;57:918-921.
16. Wszolek ZK, Pfeiffer RF, Tsuboi Y, Uitti RJ, McComb RD, Stoessl AJ, et al. Autosomal dominant parkinsonism associated with variable synuclein and tau pathology. *Neurology* 2004;62:1619-1622.
17. Gilks WP, Abou-Sleiman PM, Gandhi S, Jain S, Singleton A, Lees AJ, et al. A common LRRK2 mutation in idiopathic Parkinson's disease. *Lancet* 2005;365:415-416.
18. Giasson BI, Covy JP, Bonini NM, Hurtig HI, Farrer MJ, Trojanowski JQ, et al. Biochemical and pathological characterization of Lrrk2. *Ann Neurol* 2006;59:315-322.
19. Hernandez D, Paisan Ruiz C, Crawley A, Malkani R, Werner J, Gwinn-Hardy K, et al. The dardarin G 2019 S mutation is a common cause of Parkinson's disease but not other neurodegenerative diseases. *Neurosci Lett* 2005;389:137-139.
20. Tan EK, Skipper L, Chua E, Wong MC, Pavanni R, Bonnard C, et al. Analysis of 14 LRRK2 mutations in Parkinson's plus syndromes and late-onset Parkinson's disease. *Mov Disord* 2006;21:997-1001.
21. Ozelius LJ, Foroud T, May S, Senthil G, Sandroni P, Low PA, et al. G2019S mutation in the leucine-rich repeat kinase 2 gene is not associated with multiple system atrophy. *Mov Disord* 2007;22:546-549.
22. Ross OA, Toft M, Whittle AJ, Johnson JL, Papapetropoulos S, Mash DC, et al. Lrrk2 and Lewy body disease. *Ann Neurol* 2006;59:388-393.
23. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry* 1992;55:181-184.
24. Gilman S, Low PA, Quinn N, Albanese A, Ben-Shlomo Y, Fowler CJ, et al. Consensus statement on the diagnosis of multiple system atrophy. *J Neurol Sci* 1999;163:94-98.
25. Schapira AH. Etiology of Parkinson's disease. *Neurology* 2006;66:S10-S23.
26. Cho JW, Jeon BS, Jeong D, Choi YJ, Lee JY, Lee HS, et al. Association Between Parkinsonism and Participation in Agriculture in Korea. *J Clin Neurol* 2008;4:23-28.
27. Autere JM, Moilanen JS, Myllylä VV, Majamaa K. Familial aggregation of Parkinson's disease in a Finnish population. *J Neurol Neurosurg Psychiatry* 2000;69:107-109.
28. Goldwurm S, Zini M, Di Fonzo A, De Gaspari D, Siri C, Simons EJ, et al. LRRK2 G2019S mutation and Parkinson's disease: a clinical, neuropsychological and neuropsychiatric study in a large Italian sample. *Parkinsonism Relat Disord* 2006;12:410-419.
29. Tomiyama H, Li Y, Funayama M, Hasegawa K, Yoshino H, Kubo S, et al. Clinicogenetic study of mutations in LRRK2 exon 41 in Parkinson's disease patients from 18 countries. *Mov Disord* 2006;21:1102-1108.

30. Farrer M, Stone J, Mata IF, Lincoln S, Kachergus J, Hulihan M, et al. LRRK2 mutations in Parkinson disease. *Neurology* 2005;65:738-740.
31. Nichols WC, Pankratz N, Hernandez D, Paisán-Ruíz C, Jain S, Halter CA, et al. Genetic screening for a single common LRRK2 mutation in familial Parkinson's disease. *Lancet* 2005;365:410-412.
32. Kachergus J, Mata IF, Hulihan M, Taylor JP, Lincoln S, Aasly J, et al. Identification of a novel LRRK2 mutation linked to autosomal dominant parkinsonism: evidence of a common founder across European populations. *Am J Hum Genet* 2005;76:672-680.
33. Punia S, Behari M, Govindappa ST, Swaminath PV, Jayaram S, Goyal V, et al. Absence/rarity of commonly reported LRRK2 mutations in Indian Parkinson's disease patients. *Neurosci Lett* 2006;409:83-88.
34. Zabetian CP, Morino H, Ujike H, Yamamoto M, Oda M, Maruyama H, et al. Identification and haplotype analysis of LRRK2 G2019S in Japanese patients with Parkinson disease. *Neurology* 2006;67:697-699.
35. Lin CH, Tzen KY, Yu CY, Tai CH, Farrer MJ, Wu RM. LRRK2 mutation in familial Parkinson's disease in a Taiwanese population: clinical, PET, and functional studies. *J Biomed Sci* 2008;15:661-667.