

Homozygous Exon 4 Deletion in *Parkin* Gene in a Korean Family with Autosomal Recessive Early Onset Parkinsonism

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The gene responsible for autosomal recessive parkinsonism, *parkin*, has recently been identified on chromosome 6q. It has been shown to be mutated in Japanese and European families, most of whom had early-onset parkinsonism. Here, we present a family with young-onset parkinsonism of an autosomal recessive inheritance. A homozygous exon 4 deletion in the *parkin* gene was found in 3 family members. To the best of the authors' knowledge, this is the first report in Korea of familial parkinsonism with the *parkin* gene mutation.

Key Words: Autosomal recessive juvenile parkinsonism, *parkin*, familial, mutation, exon

INTRODUCTION

Autosomal recessive juvenile parkinsonism (ARJP) is a distinct clinical and genetic entity that is characterized by the selective degeneration of nigral dopaminergic neurons associated with early-onset parkinsonism and by a remarkable response to levodopa.¹ Recently a gene locus for ARJP was mapped to chromosome 6q25.2-q27 by linkage analysis, and a novel, large gene (*parkin*) was identified.² Mutations in the *parkin* gene were originally described in Japan. Since then, several families throughout the world have been shown to carry similar mutations.³⁻⁵ Recently, a sporadic case of juvenile parkinsonism with the *parkin* gene mutation in a Korean woman was reported.⁶

However, the mutation in this gene in familial cases of early-onset parkinsonism has not yet been established in any Korean family members.

Here, we report the molecular and clinical findings from a Korean family with early onset parkinsonism due to a *parkin* gene mutation.

CASE REPORT

Family report

There is no known consanguinity between patients who reside in different regions of Korea (Fig. 1A).

The index case (II-3, female, born 1950) first noticed a gait disturbance at age 44. The clinical course was very slow. She was diagnosed with idiopathic Parkinson's disease and treated with levodopa from 1994, with marked response. Owing to motor fluctuations, since 1998 oral dopamine agonists were used with a satisfactory response for years. Our examination in 2001 (at age 51, after 7 years of disease course) showed bradykinesia and postural instability. Tremor and rigidity were absent and the tendon reflexes were normal. Cognition was normal and there were no severe autonomic disturbances. Her on state Hoehn and Yahr stage was I. Brain MRI was normal.

The younger sister (II-4, born 1954) noticed a bilateral resting tremor at age 38. When bradykinesia and gait disturbance developed later, she was diagnosed with Parkinson's disease. The disease progressed slowly, affecting both sides of the body. She was treated with levodopa from

Received June 20, 2002

Accepted July 29, 2002

This work was supported by a grant from the Korean Ministry of Health and Welfare (HMP-00-GN-01-0002).

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1992, with a good response. She showed only mild dysarthria on an examination in 2001 (at age 47) and brain MRI was normal.

The younger brother (II-5, born 1958) presented with paresthesia of the lower extremities at the age of 34. In 1996, he complained of a twisting sense in the left toe. A neurologic examination at that time showed no abnormalities. During a 10-year follow up period, he did not exhibit any tremors, bradykinesia or gait disturbance. Brain MRI was normal.

Another brother (II-1, born 1939) was normal neurologically. Limited information was available regarding their parents. The father and mother died at age 61 and 71, respectively, with no reported evidence of parkinsonism.

Molecular genetic studies

DNA was extracted using the standard technique from peripheral blood leukocytes after

obtaining informed written consent. The 12 exons, including the flanking intronic sequences, were amplified individually using the same primer pairs, as described by Kitada et al.² The PCR products were run through a 2% agarose gel in 1x TAE buffer to determine the presence of the exonic deletions.

The PCR amplification of the family members revealed a homozygous deletion of exon 4 in the *parkin* gene (II-3, II-4, and II-5; Fig 1B).

DISCUSSION

Although most patients with Parkinson's disease are considered to have a sporadic form of the disease, 16% have an affected first-degree relative.⁷ The molecular pathogenesis of Parkinson's disease remains unclear, but genetic factors play a role in some cases. The gene encoding *parkin*, α -synuclein, and ubiquitin carboxyl-terminal hydro-

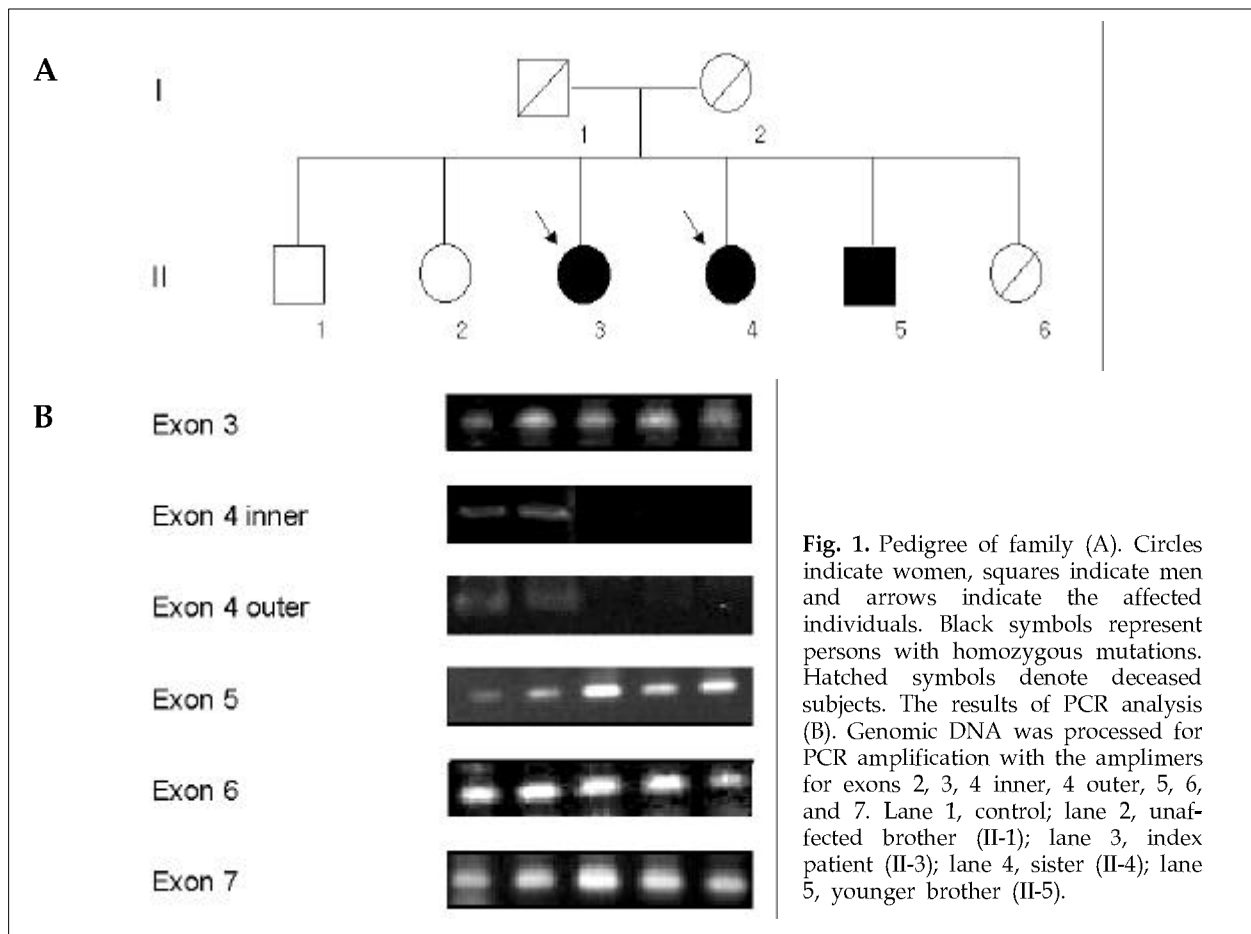


Fig. 1. Pedigree of family (A). Circles indicate women, squares indicate men and arrows indicate the affected individuals. Black symbols represent persons with homozygous mutations. Hatched symbols denote deceased subjects. The results of PCR analysis (B). Genomic DNA was processed for PCR amplification with the amplimers for exons 2, 3, 4 inner, 4 outer, 5, 6, and 7. Lane 1, control; lane 2, unaffected brother (II-1); lane 3, index patient (II-3); lane 4, sister (II-4); lane 5, younger brother (II-5).

lase L1 have been linked to the familial form of Parkinson's disease.^{2,8,9} These three specific genes are either closely involved in the proper functioning of the ubiquitin-proteasome pathway or are degraded by this protein-clearing machinery of cells.¹⁰

Exonic deletions of the *parkin* gene have been associated with ARJP, which is a distinct clinical and genetic entity causing the typical signs of parkinsonism (rigidity, tremor and akinesia), with an onset before the age of 40, and with an extremely good response to L-dopa. Postmortem examinations revealed a loss of neurons in the pars compacta of the substantia nigra without Lewy bodies.^{11,12}

The *parkin* gene is composed of 12 exons encoding a 52 kDa protein of an unknown function, termed Parkin.² In a molecular analysis of 34 ARJP patients from 18 unrelated Japanese families, homozygous deletions were found in exon 4 in 9 patients, exons 3-4 in 6 patients, exons 3-7 in 1 patient, and in other exons in 10 patients. This suggests that deletion mutations are clustered around exons 3-7, which encode the central part of *parkin* where multiple phosphorylation sites for various protein kinases are located.^{2,13} From this evidence, various other mutations and deletions of the *parkin* gene have been reported in other ARJP cases.¹³⁻¹⁵

The domain structure of Parkin includes a ubiquitin homologous domain in its N-terminus, and two RING finger domains in its C-terminus.¹⁶ Similar to many other proteins with a RING finger domain,¹⁷ Parkin has an E3 ubiquitin ligase function,^{16,18} linking Parkin-associated parkinsonism to the ubiquitin-proteasome system as well. Because mutations in *parkin* are associated with recessive inherited parkinsonism, they result in loss or diminished E3 ligase function in the nigra and striatum of individuals with these mutations,¹⁶ leading to abnormal accumulation of substrate proteins such as 22-kDa glycosylated form of α -synuclein,¹⁹ Parkin-associated endothelin receptor-like receptor (Pael-R),²⁰ and CDCrel-1.¹⁸ The search for these substrates has provided important information elucidating the biochemical consequences of *parkin* mutations.

Our genetic study provides evidence that the ARJP locus also exists in the Korean population.

The phenotype is characterized by early-onset parkinsonism with a slow progression, a good response to levodopa and levodopa-induced motor fluctuations. Other signs often reported in ARJP, such as dystonia at onset and sleep benefit, were absent. The clinical pictures in this family suggested the involvement of *parkin* gene mutations. Patients II-3 and II-4 clearly suffered from parkinsonism associated with a homozygous exon 4 deletion in the *parkin* gene. Interestingly, patient II-5 who had a homozygous exon 4 deletion in the *parkin* gene did not have any symptoms of parkinsonism during the 10-year follow-up period. It will of course be necessary to follow this patient's symptoms because *parkin*-related disease displays wide intrafamilial variability in the age of onset,^{4,5,21} and the latest age at onset reported thus far in patients with proven *parkin* mutations is 64 years.²² A possible explanation is that some *parkin* gene mutations might increase the susceptibility for parkinsonism, possibly in association with additional risk factors (i.e., environmental insults or other genetic factors).²²

ARJP is inherited in an autosomal recessive fashion and frequently occurs in the offspring of consanguineous marriages.² However, there was no evidence of consanguinity in this family, and neither parent was known to be affected when they died. The presence of a homozygous deletion indicates that the both parents were also a heterozygous carriers of the *parkin* gene mutation.

To our knowledge, this is the first report of familial parkinsonism with a *parkin* gene mutation in Korea. ARJP is probably a universal entity where the genetic basis of the phenotypic heterogeneity and the correlation between clinical features and specific mutations remain to be determined.

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