Homozygous deletion mutation of the parkin gene in patients with atypical parkinsonism

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Abstract

Autosomal recessive juvenile parkinsonism (AR-JP) is characterised by homogenous clinical features and selective degeneration of nigral neurons. Recent progress in molecular genetic analyses of AR-JP has led to the identification of a novel ubiquitin-like protein, parkin. The responsible gene for AR-JP has been well characterized. Antioxidant drugs, including coenzyme Q10, and levodopa therapy, have been effective in patients with AR-JP. We performed genetic analysis of the parkin gene in both families and identified a deletion mutation, indicating the extensive involvement of parkin protein in the extranigral nervous system.

Keywords: AR-JP, parkin; multiple system degeneration; deletion mutation

Autosomal recessive juvenile parkinsonism (AR-JP) is characterised by several homogenous features such as early onset, typical signs of parkinsonism, and a good response to levodopa therapy. The responsible gene for AR-JP was recently identified and designated parkin. The clinical features elicited by selective degeneration in the substantia nigra and locus ceruleus suggest that the parkin protein functions selectively in the substantia nigra compacta. However, it is also possible that the parkin protein functions not only in the substantia nigra but also in extranigral regions of the brain.

We previously reported on two Japanese siblings with hereditary parkinsonism complicated with cerebellar and pyramidal tract dysfunction. Recently, we treated another patient with similar clinical features. These three patients showed very good responses to treatment with anticholinergic drugs, but not with levodopa, unlike most patients with AR-JP.

Materials and methods

POLYMERASE CHAIN REACTION (PCR) ANALYSIS OF THE PARKIN GENE

After obtaining informed consent, genomic DNA was isolated from peripheral blood leukocytes from the patients, members of their families, and 20 control subjects. All of the 12 exons of the parkin gene were amplified individually according to the reaction conditions described by Kitada et al.
Figure 1  (A) Genetic studies of family N and family Y. In the pedigrees (upper panel), closed squares (men) and closed circles (women) represent affected members. The results of polymerase chain reaction (PCR) analysis (lower left of panels A and B) disclosed deletions of exons 3 and 4 in the parkin gene in affected members (II-1 and II-2 in family N, III-1 in family Y). Southern blot analysis (lower right of panels A and B) showed five EcoRI fragments. (Arrows indicate 16 kb, 11 kb, 4.5 kb, 1.5 kb, and 0.6 kb bands) in a control subject (C), but no 1.5 kb fragment was found (asterisks) and the sizes of 16 kb, 4.5 kb, 1.5 kb, and 0.6 kb fragments were slightly smaller than those of the control subjects. In parents of family N, a 1.5 kb fragment was detected and one extra fragment with a size of 4.0 kb was found. (B) RT-PCR amplification products were obtained using total RNA derived from peripheral blood leukocytes in control subjects (control) and patient II-2 in family N (patient). As expected, RT-PCR products of exons 1–6, 2–9, and 9–12 in the control subjects showed 711-bp, 821-bp, and 500-bp fragments, respectively. RT-PCR products of exons 1–6, 2–9, and 9–12 in the patient showed 348 bp, 458 bp, and 466 bp fragments, respectively. (C) Sequencing analyses of RT-PCR products in control subjects disclosed a normal sequence of exons 2, 3, 4, and 5, whereas sequencing analysis of RT-PCR products in the patient showed that the 3′ end of exon 2 was connected directly to the 5′ initial base of exon 5.
Southern Blotting Analysis

Ten micrograms of genomic DNA from each of the patients and their family members were digested with EcoRI, subjected to electrophoresis, and blotted onto a nylon membrane. For preparation of a probe, human brain total RNA (Clontech, CA, USA) was reverse transcribed using a Super Script™RT kit (Life Technologies Inc, MD, USA) and then subjected to PCR using both the forward primer 5'-GACCATGATAGTGTTTG-3' (nt 98–114) and the reverse primer 5'-GATGTTCGCCGATTTTGCGGATCGGT-3' (nt 1034–1056). The amplified product was ligated into the pCR™2.1 vector (Invitrogen, CA, USA) and sequenced. Southern blotting was performed using a Gene Images CDP-Star detection module (Amersham Pharmacia Biotech, UK) and a Luminoimage analyzer LAS-1000 (Fujifilm Co, Tokyo, Japan).

Reverse transcription (RT)-PCR and Sequencing Analyses of the Parkin mRNA

Total RNA was isolated from peripheral blood leucocytes from patients II-2, III-1 and 15 control subjects as previously described. One microgram of total RNA was subjected to PCR to amplify a full region of parkin cDNA using three sets of oligonucleotide primers: (1) exons 1–6: forward, 5'-GACCATGATAGTGTTTG-3' (nt 98–114); reverse 5'-GATGTTCGCCGATTTTGCGGATCGGT-3' (nt 1034–1056). The amplified product was ligated into the pCR™2.1 vector (Invitrogen, CA, USA) and sequenced. Southern blotting was performed using a Gene Images CDP-Star detection module (Amersham Pharmacia Biotech, UK) and a Luminoimage analyzer LAS-1000 (Fujifilm Co, Tokyo, Japan).

Results

PCR and Southern Blot Analyses of the Parkin Gene

As shown in figure 1, PCR analysis of each of the 12 exons in the parkin gene demonstrated that all of the exons except for exons 3 and 4 were amplified in the three patients (results of exons 7 to 12 not shown for patients: II-1 and II-2 in family N and III-1 in family Y). When the parents (I-1 and I-2) and sons (III-1 and III-2) of the patients as well as the 20 control subjects were examined, all of the 12 exons were successfully amplified. In Southern blot analysis, five fragments (16 kb, 11 kb, 4.5 kb, 1.5 kb, and 0.6 kb) were detected (lane C in the lower right panels of fig 1 A). However, the analysis of the parents’ genomic DNA disclosed that a 1.5 kb fragment was not detected but one extra fragment of 4.0 kb in size was detected (lanes I-1 and I-2 in the lower right panels of fig 1 A). These results indicate that the three patients possess identical homozygous deletion mutations in the parkin gene and that the parents in family N are heterozygous for the mutation.

RT-PCR and Sequencing Analyses

We attempted to amplify parkin mRNA derived from peripheral blood leucocytes obtained from 15 control subjects, and we successfully obtained the expected sizes of RT-PCR products (fig 1 B; a 711 bp product for exons 1–6, an 821 bp product for exons 2–9, and a 466 bp product for exons 9–12). The amplified products were confirmed to be parkin cDNA by sequencing analysis. When three regions encoding exons 1–6, exons 2–9, and exons 9–12 in the three patients were amplified, the sizes of RT-PCR products obtained were 348 bp, 458 bp, and 466 bp, respectively (fig 1 B). The difference between the product sizes for the control subjects and patients was 363 bp in both the regions encoding exons 1–6 and 2–9. This size seems to correspond with the total length of exons 3 (241 bp) and 4 (122 bp) in the control subjects. Sequencing analysis of the patients’ cDNA clearly showed that the 3’ end of exon 2 was connected directly to the 5’ initial base of exon 5 (fig 1 C). These results, together with the results of PCR analysis, suggest that the patients’ parkin mRNA lacks both exons 3 and 4.

Discussion

In this study, we identified an abnormal parkin mRNA with a homozygous deletion mutation spanning exons 3 to 4 in three affected patients from two unrelated families. We simultaneously detected not only mutant parkin mRNA, but also normal mRNA, the full length parkin mRNA in peripheral blood leucocytes. These findings do not agree with the results of a previous study in which only alternatively spliced parkin mRNA lacking exons 3 and 4 was detected in normal peripheral leucocytes by a nested PCR method. Our finding of peripheral blood leucocytes containing full length parkin mRNA would allow an easy and convenient method of finding abnormalities of the parkin gene in patients with AR-JP.

Various deletions and several point mutations in the parkin gene have been reported in patients with AR-JP and similar deletion mutations of exons 3 and 4 have also been reported. However, the clinical features of the present patients, which are different from previously reported clinical features, indicate dysfunctions of the corticospinal tract, cerebellum, and striatum as well as nigral dopaminergic neurons. Although the present cases indicate multiple system dysfunction, they do not fulfill the criteria of multiple system atrophy because of the presence of family history and the absence of autonomic and urinary disturbances.

The parkin gene transcript is extensively expressed in the brain, including the cerebral cortex, brainstem, putamen, cerebellum, and the substantia nigra, and the protein has been detected in the substantia nigra, putamen, and cerebral cortex. Parkin protein, localised in...
the Golgi complex, functions as a ubiquitin-protein ligase.\textsuperscript{11, 14} Therefore parkin gene mutation may induce accumulation of unubiquitinated substrates in neural cells, resulting in cell death.\textsuperscript{14} However, it is not known at present whether abnormal protein ubiquitination induces neural cell death only in the nigral dopaminergic neurons. The present results suggest that the parkin protein has crucial roles in the striatum, corticospinal tract, and cerebellar system as well as in the substantia nigra, where the parkin protein and its mRNA are abundantly expressed. It is not clear why our patients with deletions in the parkin gene exhibited different clinical features from those of patients with typical AR-JP. However, these clinical and genetic findings should contribute to the elucidation of the inherent functions of the parkin protein.

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