Clinical Findings in a Large Family With a Parkin Ex3Δ40 Mutation

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Objective: To describe a large consanguineous family in which inheritance of a 438– to 477–base pair deletion in exon 3 (Ex3Δ40) in the parkin gene resulted in parkinsonism (age range at onset, 24-32 years).

Design: Fifty-two family members underwent genetic analysis.

Main Outcome Measure: Two clinical examiners blinded to genetic status evaluated 21 family members, including all mutation carriers (4 homozygous and 12 heterozygous individuals; 5 family members did not have the mutation).

Results: In this family, the parkin Ex3Δ40 mutation is recessive; only homozygotes manifest symptoms of early-onset levodopa-responsive parkinsonism, including resting tremor, dystonia, and slow progression, with the caveat that presymptomatic signs of dopaminergic loss in heterozygotes must be excluded by fluorodopa F 18 with positron emission tomography. This contrasts with the autosomal dominant pattern of inheritance of parkinsonism described in families with the same mutation.

Conclusion: In families with a dominant inheritance, an additional genetic or environmental cause must coexist with the Ex3Δ40 mutation.

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Mounting evidence suggests that Parkinson disease (PD) has a substantial genetic component that is more pronounced in earlier-onset cases (age, <50 years).1 About 15% of patients with PD have a positive family history; however, the genetic contribution is predicted to be much higher. Recessive disease in small families can present in the absence of family history, and longitudinal assessment of twins with late-onset PD using fluorodopa F 18 with positron emission tomography suggests that cross-sectional studies may be overlooking more subtle early disease.1,2 Ten loci responsible for inherited parkinsonism have been reported, and in 4 of these the genes have been identified, including α-synuclein,3,4 parkin,5 and DJ16.7

Mutations in the parkin gene are the most common cause of early-onset familial PD (18%-50%)7 and explain at least 15% of sporadic cases with onset younger than 45 years.8 Most of these patients with young-onset PD have homozygous deletions or compound point mutations and deletions, which cause the loss of parkin function, leading to a failure of ubiquitin-mediated protein degradation. In addition, recent studies have suggested that inheritance of a single parkin mutation may be associated with subclinical abnormalities of the nigrostriatal dopamine system9 or typical late-onset PD.10

Several families have been described in which a 438– to 477–base pair (bp) deletion in exon 3 (Ex3Δ40) in the parkin gene segregated with a parkinsonian syndrome,11-13 including a family with autosomal dominant inheritance of a single Ex3Δ40 mutation.12,13 If this mutation is capable of causing dominant inheritance, one might expect that the additional effect of a second identical mutation could result in a clinically more aggressive or “malignant” presentation. Herein, we report the complete pedigree and detailed clinical and molecular genetic data of one large family in which all affected individuals segregated with homozygous parkin Ex3Δ40 mutations. Partial details of the proposal and genetic research have been reported with a series of other patients with Ex3Δ40 mutation.11

METHODS

SUBJECTS

We studied a family from a circumscribed community (831 habitants) in northeastern Canada.
Among 72 living family members, 52 consenting individuals had blood samples collected for parkin gene analysis (Figure). The proband (patient 130) and 2 affected brothers (patients 131 and 132) were initially assessed at the Movement Disorders Centre, Toronto Western Hospital. Two neurologists trained in movement disorders (R.P.M. and D.S.S.) and blinded to genetic status evaluated 21 family members in a randomized fashion following a standardized protocol. The assessments included clinical history, neurological examination, Unified Parkinson's Disease Rating Scale, Hoehn and Yahr scale, and Mini-Mental State Examination. All assessments were videotaped and reviewed by one of us (A.E.L.) who was also blinded to the genetic status.

GENETIC ANALYSIS

Genomic DNA was isolated from the white blood cells. For the proband, exonic duplication and deletion mutations within the 12 exons of the parkin gene were examined by semiquantitative polymerase chain reaction using established methods.11-13 Sequencing was performed on DNA strands and the chromatographs assessed using SeqScape software version 1.0 (Applied Biosystems, Foster City, Calif). Chromosome 6q segregation was examined using microsatellite markers D6S1277, D6S1581, D6S1599, D6S411, and D6S980 in all family members from whom DNA was available (n=52). In addition, the Ex3/H900440 mutation was assayed in all DNA samples, as previously described.11-13

RESULTS

The sequencing of the entire open reading frame of the parkin gene from the proband (patient 130, Figure) revealed a homozygous deletion in exon 3 (438-477 bp), which is predicted to cause a frameshift and the production of a truncated parkin protein. Mutation analysis of the 52 family members revealed that 16 individuals tested positively for the Ex3/H900440 mutation and were included in the subsequent clinical study. Of 21 clinically evaluated subjects, 4 were homozygous (age range, 30-48 years; mean age, 41.0 years), 12 were heterozygous (age range, 28-88 years; mean age, 48.5 years), and 5 did not have the mutation (age range, 21-53 years; mean age, 42.2 years). All homozygous patients manifest symptoms of early-onset levodopa-responsive parkinsonism, with resting tremor, dystonia, and slow progression (Table).

PROBAND (PATIENT 130)

This 40-year-old man developed bilateral leg dragging 10 years before evaluation. He had a history of leg dystonia and bilateral leg tremor beginning at age 16 years. At 30 years, he noticed slight upper and lower limb stiffness and slowness. There was no evident progression for the first 7 to 8 years, after which he noticed that his legs were
PATIENT 131

This 28-year-old brother of patient 130 had a 1-year history of left leg resting tremor. During the last few months before he was first seen by us, he had noticed the same kind of tremor in his left upper extremity and mild stiffness in the left upper and lower extremities. All symptoms worsened with anxiety. There was no history of dystonia, diurnal fluctuations, or autonomic disturbances. On examination, mental status was normal. There was mild hypomimia and hypophonia. Resting tremor, bradykinesia, and rigidity were evident in the left side, all rated as mild. There were no cerebellar signs, deep tendon reflexes were normal and symmetric, and no atypical signs were noticed. \(^{18}F\)fluorom-tyrosine positron emission tomography demonstrated severe and symmetric reduction in radionucleotide activity affecting the striatum bilaterally.

PATIENT 132

This 42-year-old brother of patient 130 had a history of abnormal posturing of the feet and lower extremity resting tremor starting at the age of 17 years. These symptoms were asymmetrical, worse on the right, and remained unchanged until age 32, when he noticed leg dragging and rigidity. A few months later, lack of dexterity and intermittent resting tremor became evident in the right upper extremity. There was marked diurnal fluctuation, with deterioration toward the end of the day. As in the case of the proband, he had a previous diagnosis of dopa-responsive dystonia, established 2 years before his first visit to our unit, after a levodopa trial showed good benefit. When first seen by us, he was taking 250 mg of levodopa and 25 mg of carbidopa twice daily, with stable benefit and occasional peak-dose dyskinesias. There were no symptoms of autonomic failure. Examination off levodopa revealed a Mini-Mental State Examination score of 28 of 30 points, with the patient being unable to identify the date and recall 1 of 3 items. There was minimal hypomimia, hypophonia, and decreased blinking. Resting tremor was rated as mild in the upper extremities and moderate in the lower extremities. Rigidity was mild in the right upper extremity and moderate in the left upper extremity. Bradykinesia was also asymmetrically worse on the left, where it was rated as moderate, in comparison with mild on the right. No cerebellar signs or dystonic postures were evident. Deep tendon reflexes were normal and symmetric. Glabellar tap response was present. His gait was slightly wide-based, shuffling, and slow, with normal tandem. On postural stability testing, there was retropulsion with unaided recovery. Improvement after commencing levodopa was pronounced, with the Unified Parkinson’s Disease Rating Scale motor score falling from 29 to 14. \(^{18}F\)fluoro-m-tyrosine emission tomography revealed similar severe and symmetric reduction in radionucleotide activity in the striatum bilaterally.

PATIENT 138

This 48-year-old cousin of patients 130, 131, and 132 had a history of left hand resting tremor starting at the age of 24 years. Shortly afterward, he noticed some degree of loss of dexterity and stiffness in the upper left extremity and, to a lesser degree, in the lower left extremity. After about 2 years, similar symptoms appeared in the right side, and his voice started to become softer. No diurnal fluctuation or additional signs such as abnormal posturing were noticed. After 3 more years, he began receiving levodopa, with good response and no adverse effects or motor fluctuations until about 5 years before he was seen by us, when wearing-off phenomenon, peak-dose dyskinesias, and off-period left foot dystonia became evident. His symptoms slowly progressed over the years, and most recently he was taking 100 mg of levodopa, 25 mg of carbidopa, and 2 mg of trihexyphenidyl hydrochloride 4 times daily. He had no symptoms of autonomic failure. Examination while not receiving levodopa revealed a Mini-Mental State Examination score of 30. There was minimal hypomimia and hypophonia. Bilateral resting tremor was rated as moderate in the upper extremities, worse on the left. In the lower extremities, tremor was minimal. Rigidity and bradykinesia were mild and asymmetrically worse on the left. No cerebellar signs or dystonic postures were noticed during the examination. Deep tendon reflexes were normal and symmetric. Glabellar tap response was present. Gait was slow, with reduced arm swing on the left. Postural stability was normal.

OTHER AFFECTED SUBJECTS

Among the 12 Ex3Δ40 carriers with 1 normal parkin allele, the only person with abnormal movement was the 63-year-old mother of the proband, with isolated minimal multivectorial head tremor. None of the other het-
homozygous carriers had any features of a movement disorder or other neurological abnormalities, and the blinded evaluators were unable to clinically distinguish heterozygous carriers from noncarriers. All heterozygous carriers were older at the time of evaluation (age range, 28-88 years) than the age at onset of the affected homozygous individuals (ages 16, 27, 17, and 24 years). The oldest carrier, examined at age 88 years, had sequelae of multiple strokes but no evidence of parkinsonism. In conclusion, this family demonstrates a clear recessive inheritance, with no signs of parkinsonism in the heterozygous Ex3Δ40 mutation carriers.

The Ex3Δ40 mutation is frequent in parkin-positive patients; a recent comprehensive study focused on the probands of 6 families, including partial details of the propositus presented herein. This mutation gained particular attention because it was identified in an unusual family with Lewy body pathology and dominant inheritance of PD,12 which demonstrated that the relationship between parkin function and the clinical and pathological features of parkinsonism is more complex than previously thought.

The family presented herein represents an unusual situation of extensive consanguinity, explaining the high prevalence of homozygotes, including both parents of affected patients. This demonstrates a definite recessive pattern of inheritance, which contrasts with the dominant inheritance of PD described in 2 other families with the same mutation.13 Our results fail to confirm the more severe phenotype that might have been expected in homozygous carriers if a single mutation were sufficient to cause PD by a presumed loss-of-function mechanism. However, presymptomatic dopaminergic neuronal loss in the Ex3Δ40 mutation carriers has yet to be excluded by functional imaging.

Compared with an earlier study of Japanese patients with compound heterozygous Ex3Δ40 mutation, the homozygous patients described herein are clinically indistinguishable from the Japanese families, with early onset of parkinsonism, diurnal fluctuations, slow progression, dystonia, and a good sustained response to low doses of levodopa. Tan et al3 described a 3-generation family with apparent heterozygous dominant inheritance of the Ex3Δ40 mutation. Carriers varied from having no signs of parkinsonism at age 93 years (generation I); to having later adult-onset parkinsonism, preceded in 1 patient by lifelong writer's cramp (generation II); to having early-onset dystonia and parkinsonism similar to our patients (generation III). Our data suggest that in the previously published cases with dominant inheritance an additional causative factor contributing to the parkinsonian syndrome must coexist with the Ex3Δ40 mutation.

The consequence of different parkin missense mutations on clinical and pathological outcomes is complex and may depend on background genetic, environmental, and stochastic factors. For most missense coding mutations, genetic epidemiologic data on population frequencies or functional analyses are limited. However, community-based research indicates that carrier status (inheritance of a single parkin mutation) is not a significant risk factor for disease.15 In contrast, in cell culture models, parkin mutations may confer recessive loss of function or dominant gain of function, depending on the protein domain affected.16,17 Given this background, commercial parkin testing may be presently ill-advised; further research studies are needed to interpret the results.

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