SHORT REPORT

Three parkin gene mutations in a sibship with autosomal recessive early onset parkinsonism

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Abstract

The objective was to describe a family with autosomal recessive, early onset parkinsonism, with affected siblings carrying three different exon rearrangements in the parkin gene.

The living affected siblings were personally examined. Molecular genetic analyses included exon dosage of the parkin gene using a semiquantitative multiplex polymerase chain reaction (PCR) protocol and haplotype analysis.

The index case was a compound heterozygote with a deletion of exon 5 and a duplication of exon 3. His affected sister was a compound heterozygote for a deletion of exon 5 and a deletion of exons 3-9. Haplotype analysis confirmed the presence of three mutant alleles at the parkin locus. The phenotype was early onset parkinsonism with marked response to levodopa, and a very slow, prolonged course.

In conclusion, the frequency of mutations in the parkin gene in certain populations might be high enough to cause allelic heterogeneity in the same sibship.

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become more sensitive. To date, a pseudo-dominant transmission of parkin related disease has been reported in a single Japanese family segregating three mutant alleles in different sibships (two alleles carrying an exon 4 deletion, and the third allele carrying the Cys431Phe point mutation in exon 12).¹⁰

We describe a sibship with early onset, slowly progressive parkinsonism whose members are compound heterozygotes for three different exon rearrangements in the parkin gene.

Family report

There is no known consanguinity between the parents of the patients, who originated from different regions in Italy.

The index case (RM611, born 1922) first noticed slowness of movements in the right leg at the age of 35. The clinical course was very slow. He was diagnosed with Parkinson's disease and treated with levodopa from 1970, with marked response. Owing to motor fluctuations and levodopa induced dyskinesias, since 1994 levodopa has been replaced by oral dopamine agonist monotherapy (0.5 mg lisuride four times/day), with a satisfactory response for years.

Our examination in 1998 (at age 76, after 40 years of disease course) showed a flexed posture, axial akinesia and gait difficulties; moderate generalised rigidity, and mild diffuse bradykinesia in all four limbs, although more severe in the upper limbs and on the left side. Tremor was absent and the tendon reflexes were normal. Dysarthria and hypomimia were mild, and a Meyerson's sign was present. Cognition was normal and there was no severe autonomic disturbance. His on state Hoehn and Yahr stage was III. Brain MRI was normal. He was still treated with oral lisuride (0.5 mg four times/day) and deprenyl (5 mg/day).

The sister (RM612, born 1909) first experienced resting tremor in the upper limbs at the age of 31. Tremor was bilateral, although more severe on the right side. When bradykinesia and rigidity developed later, she was diagnosed with Parkinson's disease. The disease was slowly progressive, affecting both sides of the body, although more severely on the right side. In the late 1960s, after more than 25 years, the symptoms had worsened to the extent that she underwent stereotactic left thalamotomy, which satisfactorily controlled the contralateral

Mutations in the parkin gene are a frequent cause of autosomal recessive, early onset parkinsonism. Various mutations, including exon rearrangements (deletions and duplications) and point mutations (truncating and missense) have been identified.¹⁻¹⁰ The mutational screening is probably still incomplete, as only one mutant allele has so far been detected in 15 out of 54 European families with parkin mutations, even after exon dosage and sequence analysis have been performed.⁷ The parkin protein has recently been shown to possess ubiquitin ligase activity, which is lost when mutated.¹¹

The finding of a pedigree with parkin mutation in affected persons from two different generations raised the question of a possible dominant negative effect of some parkin mutations.⁹ A pseudodominant transmission is an alternative explanation which cannot be ruled out until mutational screenings in parkin

tremor. She was treated with levodopa from 1970, with good response. After several years she developed motor fluctuations and levodopa induced dyskinesias. We were able to examine her at the age of 89, after 58 years of disease course, a few months before her death; she had a severe akinetic rigid syndrome, with Hoehn

and Yahr stage IV in the on, and V in the off states, respectively. Only mild resting tremor was evident in both upper limbs. Tendon reflexes were brisk. She also had a memory deficit, visual hallucinations, and frank confusional episodes, which were reported to have been present during the past 3 years. At the

Figure 1 Exon rearrangements detected in the *parkin* gene. (A) Electropherograms showing fluorescently labelled multiplex PCR products (see methods). The peak height reflects the quantity of template exon DNA. *Het del*=heterozygous exon deletion, indicated by half of the expected peak height; *hom del*=homozygous exon deletion, indicated by no amplification product; *het dupl*=heterozygous duplication, indicated by 1.5 times the expected peak height. (B) Schematic representation of the exon rearrangements at the genomic level. Exons 2–12 within the pair of homologous chromosomes are depicted as boxes, introns as solid lines. As the size of the introns is not known, double slashes separate the exons. Exon deletions are indicated as dotted lines. The in tandem position of the duplicated exon 3 is hypothetical.

Figure 2 Haplotype analysis. Six intragenic (the intronic position is given in parenthesis) and four flanking markers were analyzed. The haplotype associated with the exon 5 deletion is boxed. The alleles for markers D6S1581 and D6S959 were not attributable to either haplotype and are indicated in italics.

time of our examination she was still receiving levodopa (375 mg/day), amantadine (200 mg/day), and biperiden (5 mg/day). Brain imaging was not available, and pathological studies could not be performed.

Another sister (born 1913) was diagnosed with Parkinson's disease with onset at the age of 31. She died in 1969, after 25 years of disease course. Very limited information is available on the parental and previous generations. The father of the patients died at the age of 54, whereas the mother of the patients died at the age of 67, with no reported evidence of parkinsonism.

Molecular genetic studies

DNA was extracted using standard techniques from peripheral blood leucocytes after informed written consent. Exon rearrangements of the parkin gene were detected using a semi-quantitative multiplex polymerase chain reaction (PCR) protocol described elsewhere.⁷ Exons 2–12 were amplified simultaneously in three combinations by multiplex PCR using HEX labelled forward primers. A 328 base pair sequence of the transthyretin gene on chromosome 18 (C328) was also amplified as an internal control.⁷ The PCR products were analyzed on an ABI377 automated sequencer with the Genescan 3.1 and Genotyper 1.1.1 software (Applied Biosystems). All possible peak height ratios were calculated between the peaks in given multiplex reactions. The peak height ratios of the family members were compared with a normal control. Differences of a factor of 0.5 were interpreted as indicating a heterozygous deletion, and those of a factor of 1.5 as indicating a heterozygous exon duplication. All reactions were carried out at least in duplicate. This method has been validated by cosegregation studies in several families.⁷

The consequences of exon rearrangements at the level of parkin protein were predicted according to the published exonic sequences (Genome database accession number AB009973).

Haplotypes of the parkin gene region were constructed manually using the following DNA repeat markers: D6S1581, D6S959, D6S1579, D6S1599, D6S305, D6S411, AFMa155td9, AFMb281wf1, D6S1550, D6S1035 (intragenic markers underlined). They were amplified with the primers specified in the Genome database, except for D6S1599 which was amplified using "forward" 5'-GGG TGTGCTTGGATTCCCTTCATG-3' and "reverse" 5'-TAGCATGTGGACTGCATAT CAAC-3'. PCR conditions were 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 45 seconds in 35 cycles. The PCR products were analysed on an ABI377 automated sequencer (Applied Biosystems), and marker alleles were assigned in increasing order.

Molecular findings

The index case is a carrier of heterozygous deletion of exon 5 and heterozygous duplication of exon 3. His sister carries a heterozygous deletion of exon 5, and a heterozygous deletion of exons 3 to 9 (fig 1).

At the level of the parkin protein, the exon 5 and exon 3–9 deletions lead to in-frame rearrangements. By contrast, assuming that the duplicated exons 3 are in tandem (fig 1 B), the mutation introduces a frameshift rearrangement and a complete loss of function of the parkin protein.

Reconstructed haplotypes using intragenic and flanking polymorphic markers are shown in figure 2. As expected, the two sibs only share one haplotype, the one associated with exon 5 deletion. In addition, for the intragenic markers D6S305, D6S411, and AFMa155td9 patient RM612 was an obligate hemizygote, because the deletion of exons 3–9 encompasses intron 7, where these markers are located (fig 1 B). For the remaining intragenic markers D6S1599 (located in intron 2), and AFMb281wf1 and D6S1550 (both located in intron 9), it was not possible to determine whether patient RM612 was homozygote or hemizygote because parental genotypes were not available.

Discussion

The young onset, very slow disease progression and good response to levodopa in this family suggested the involvement of parkin gene mutations.⁷ In addition, the prolonged satisfactory response to dopamine agonist monotherapy in case RM611 is of interest. A sustained response to combined therapy with bromocriptine and biperiden has been reported in a patient with parkin mutations.⁸ Further studies are warranted to characterise the response to dopaminergic stimulation in patients with parkin mutations.

Dementia is not considered as part of the phenotype in patients with parkin mutations.^{7,12} In the largest series so far analyzed, this may have been due to the young age of patients at the examination or exclusion from screening of cases with early dementia.⁷ Patient RM612 is the first described with marked cognitive disturbances, which might be ascribed either to the extremely long course of parkin

disease, or to a coincidental Alzheimer-type pathology. However, they more likely represent side effects of the antiparkinsonian drugs in an old subject who also underwent stereotactic thalamotomy.

The presence of three different mutations indicate that one of the parents was also a compound heterozygous carrier of parkin mutations. Neither the father nor mother was known to be affected when they died at the ages of 54 and 67, respectively. This finding may represent a case of as yet undescribed non-penetrance of parkin mutations, or more likely reflect the age dependent penetrance of the disease. Parkin related disease displays wide intrafamilial variability of ages at onset,⁵⁻⁷ and the latest age at onset reported so far in patients with proved parkin mutations is 64 years.⁹ Moreover, owing to the mild severity of symptoms in the first years from onset, the disease of one parent might not have been recognised. Mistaken paternity could be another explanation, but is unlikely because one would have to assume that both biological fathers were heterozygous carriers of different parkin mutations.

Both scenarios therefore suggest that mutations in the parkin gene are frequent enough in some populations to lead to allelic heterogeneity in the same sibship, as described here, or to pseudodominant transmission, as recently reported.¹⁰

Owing to the presence of three mutant alleles, linkage and haplotype analyses could have led to an erroneous exclusion in this family of the parkin locus. Therefore, parkin gene mutation in patients with levodopa responsive parkinsonism, onset before the age of 40 and slow disease progression should be tested by direct analyses.

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