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.

Keywords: early onset parkinsonism; parkin gene; exon rearrangements

Mutations in the parkin gene are a frequent cause of autosomal recessive, early onset parkinsonism. Various mutations, including exon rearrangements (deletions and multiplications) 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 mutations in 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 Owing to motor fluctuations and levodopa induced dyskinesias, since 1994 levodopa has been replaced by oral dopamine agonist monotherapy (0.5 mg lisuride four times/day), with a satisfactory response for years.

In conclusion

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

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. 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
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.](http://jnnp.bmj.com/)

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Wild type (2 copies of each exon)

Heterozygous deletion of exon 5
(1 copy = 1/2 of expected peak height)

Heterozygous duplication of exon 3
(3 copies = 1.5 times the expected peak height)

Homozygous deletion of exon 5
(0 copy = no amplification)

Heterozygous deletion of exon 3, 4, 6, 7, 8, 9
(1 copy = 1/2 of expected peak height)
Haplotypes of the parkin gene region were constructed manually using the following DNA repeat markers: D6S1581, D6S959, D6S1579, D6S1599, D6S305, D6S411, AFMa155td9, AFMb281wf1, D6S1570, 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 TGTGCTTGGATTCCTTCATG-3’ and “reverse” 5’-TAGCATGTGACTCGATAT 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 D6S1035 (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. 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. 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. 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. Patient RM612 is the first described with marked cognitive disturbances, which might be ascribed either to the extremely long course of parkin disease.
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.8 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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