DJ-1 mutations in Parkinson’s disease

D G Healy, P M Abou-Sleiman, E M Valente, W P Gilks, K Bhattacharyya, N Quinn, A J Lees, N W Wood

Mutations in the DJ-1 gene have recently been shown to cause autosomal recessive Parkinson’s disease. To estimate the prevalence of this mutation, an analysis was undertaken of 39 index cases of Parkinson’s disease in whom a family history suggested autosomal recessive inheritance. No DJ-1 mutations were found in these patients, indicating that this gene is unlikely to be of numerical significance in clinical practice. The hypothesis was also tested that young onset Parkinson’s disease patients in whom, despite extensive analysis, only a single heterozygous parkin mutation was found, might harbour a second mutation in the DJ-1 gene—that is, digenic inheritance. No patient was found with a single mutation in both DJ-1 and parkin genes, making this mode of inheritance unlikely. Finally it was confirmed that PARK6 and PARK7 (DJ-1), despite being phenotypically similar and mapping to the same small chromosomal region of 1p36, are caused by mutations in separate genes.

Following the recent publication of a fifth Parkinson’s disease gene, DJ-1, by Bonifati et al., there has been much interest in ascertaining its biological and clinical importance. To date, however, there has been no published data estimating DJ-1 prevalence in Parkinson’s disease.

Mutations in DJ-1 were discovered in two consanguineous families from geographically isolated regions in Europe. This brings to five the number of genes reported that are directly implicated in Mendelian Parkinson’s disease. While the function of the protein remains to be elucidated, it has been proposed that it may be involved in the cellular response to oxidative stress. The gene maps to chromosome 1p36 (PARK7 locus) and comprises eight exons, the first of which is alternatively spliced and non-coding. Three other recessive Parkinson’s disease loci map to this chromosomal region, including PARK6 (1p36-p35), which appears to have a broadly similar phenotype. This has led to suggestions that PARK6 and PARK7 are allelic, either because of chromosomal rearrangements or because of erroneous linkage mapping. We therefore sought to confirm that PARK6 was not caused by DJ-1 mutations.

There has also been considerable recent interest in the increasingly large number of young onset or familial cases of Parkinson’s disease, in whom only one pathogenic parkin mutation has been found despite extensive analysis. This has led to suggestions that a single mutation in these patients may be sufficient to cause the phenotype, either by haploinsufficiency or by a dominant negative effect. An alternative hypothesis that we chose to test is that these patients may also harbour pathogenic mutations in the DJ-1 gene and that only the combination of a heterozygous mutation in each of these genes resulted in Parkinson’s disease. This digenic mode of inheritance has been described in other autosomal recessive disorders such as human insulin resistance, which is bi-allelic digenic, and Bardet-Biedl syndrome, where triallelic inheritance—that is, three mutations in two genes—is necessary for disease. This hypothesis would be supported if Parkinson’s disease pathogenesis turned out to involve a pathway where interaction between DJ-1 and parkin occurred. It may also explain why affected parkin heterozygotes usually have clinically unaffected parents.

Methods
This study was approved by the joint research ethics committee of the Institute of Neurology and the National Hospital for Neurology and Neurosurgery. Informed consent was obtained from all patients.

We selected for study 39 index cases of Parkinson’s disease with probable autosomal recessive inheritance, on the basis that they had at least one other sibling similarly affected with the disease, without evidence of disease in any other generation. Thirty-two of these families were of European origin and seven were of Asian origin. We excluded cases known to have even a single parkin gene mutation, though seven of these patients had not had parkin gene analysis. Thirteen patients had the additional clinical finding of focal dystonia, but no patient had blepharospasm, a possible marker for DJ-1. Two patients reported depression, but none suffered anxiety disorder, which has also been noted in DJ-1.

Methods

At the time of collection, some of these patients may not have had a full psychiatric history recorded.

We also selected four index Parkinson’s disease patients of European ancestry with heterozygous parkin gene missense point mutations. These heterozygous mutations were determined by directly sequencing the entire opening reading frame of the gene, as well as the intron–exon boundaries and 1 kb upstream of the start of gene transcription, including the parkin promoter. A semiquantitative multiplex polymerase chain reaction (PCR) was used to detect exon rearrangements (deletions and duplications). Only patients with a single heterozygous mutation were included in this heterozygous parkin subgroup.

The original PARK6 family (the Marsala kindred) and two other families, which this group has previously mapped to 1p36-35 by linkage analysis, were chosen for analysis by direct sequencing, to determine whether PARK6 was caused by D-1 mutations.

Genetic analysis
DNA was extracted from peripheral lymphocytes according to standard protocols. For sequence analysis, coding exons were amplified by PCR under the following conditions: denaturation at 94°C for 15 minutes, followed by 35 cycles of denaturation at 94°C, annealing at 58–60°C and elongation at 72°C for 30 seconds each, and a final elongation step of 10 minutes at 72°C. PCR was carried out in a final volume of 50 μl including 2.5 mM MgCl₂, 0.2 mM deoxynucleotide triphosphate, 0.5 μM forward and reverse primer (as published), 1 U Taq polymerase, and 10 ng of DNA. Bidirectional deoxyxynucleotide chain terminator sequencing was carried out according to the manufacturers’ instructions (BigDye, Applied Biosystems, Warrington, UK) and the products were electrophoresed on an ABI 3100 automated DNA sequencer (Applied Biosystems).
Long range PCR for the detection of the exon 1–5 deletion described by Bonifati et al was done as previously reported."

**RESULTS**

In a cohort of 39 index Parkinson’s disease patients with probable autosomal recessive inheritance, we did not find any pathogenic mutation in the DJ-1 gene.

We also did not find pathogenic DJ-1 mutations in a smaller cohort of four patients in whom, despite extensive genetic analysis, we could only determine a single heterozygous parkin gene mutation.

Finally no DJ-1 mutations were found in three PARK6 linked families.

**DISCUSSION**

The large cohort of autosomal recessive patients studied indicates that the frequency of pathogenic DJ-1 mutations is low in Parkinson’s disease, especially given that we excluded from analysis any known parkin gene mutations. We also screened all our samples for the 14 kbp deletion reported by Bonifati et al; and did not find it. This indicates that the deletion may be confined to the genetically isolated population in the Netherlands, where it was originally found.

At present the role of DNA diagnostic testing for mutations in DJ-1 is unclear, but these data suggest that such testing will not yield numerically significant results and therefore, unlike the parkin gene, DJ-1 is unlikely to be of clinical importance in neurological practice. More work will also be needed to elucidate the possible role of large genomic rearrangements.

We were unable to prove our hypothesis of digenic inheritance between parkin and DJ-1 in Parkinson’s disease. This does not exclude other recessive genes being involved in this mode of inheritance of Parkinson’s disease. Indeed our group also has evidence of young disease onset patients with non-synonymous heterozygous DJ-1 mutations in whom a second pathogenic mutation has not been found (unpublished data). Large scale rearrangements, however, have not as yet been excluded in these patients. Further work is needed to establish the role of digenic or oligogenic inheritance in Parkinson’s disease.

Finally, we have confirmed that PARK6 and PARK7 are not allelic and that at least two recessive Parkinson’s disease loci lie within a very small chromosomal region of 1 p.

**REFERENCES**