Mutations in *DJ-1* are rare in familial Parkinson disease

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Abstract

Mutations in *DJ-1* (PARK7) are one cause of early-onset autosomal-recessive parkinsonism. We screened for *DJ-1* mutations in 93 affected individuals from the 64 multiplex Parkinson disease (PD) families in our sample that had the highest family-specific multipoint LOD scores at the *DJ-1* locus. In addition to sequencing all coding exons for alterations, we used multiplex ligation-dependent probe amplification (MLPA) to examine the genomic copy number of *DJ-1* exons. A known polymorphism (R98Q) was found in five PD subjects, once as a homozygote and in the other four cases as heterozygotes. No additional missense mutations and no exon deletions or duplications were detected. Our results, in combination with those of previous studies, suggest that alterations in *DJ-1* are not a common cause of familial PD.

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Parkinson disease (PD) is the second most common neurodegenerative disorder. It is characterized by tremor, rigidity, postural instability, and bradykinesia as well as a good response to levodopa therapy [5]. In the past decade, mutations in several genes, including *SNCA*, *PRKN*, *DJ-1*, *PINK1*, and *LRRK2*, have been shown to cause these clinical symptoms [8].

Each of the known PD genes was identified using a positional cloning approach. Linkage to chromosome 1p36 (PARK7 locus) was initially reported in a large consanguineous Dutch kindred [25]. Subsequently, deletion of exons 1–5 of *DJ-1* was reported in this family [3]. Despite screening at least one individual from nearly 1200 families [1–4,6,9–14,17–19,21,22,24], only six families have been reported to harbor disease producing mutations in *DJ-1*. Affected individuals in five of the six families were homozygous for their particular mutation, with two of these families known to be consanguineous. Each of these six families had a unique *DJ-1* mutation. A total of four point mutations and three genomic rearrangements, including partial or entire exon duplication or deletion, have been reported. The clinical presentation of individuals with *DJ-1* mutations is similar to that of patients with homozygous *PRKN* mutations. Patients with mutations in either gene typically have an age of onset under 45 years and a slower disease progression.

In the current study, we have ascertained a large cohort of multiplex PD families and have used an approach based on the linkage evidence to chromosome 1p36 to prioritize families for *DJ-1* screening, an approach we have previously used to prioritize families for *PRKN* mutation screening [7]. We have performed extensive screening of *DJ-1*, testing for coding sequence point mutations as well as gene dosage alterations (whole exon deletions or duplications).
A sample of 287 multiplex PD families was recruited through 59 Parkinson Study Group sites located throughout North America. Families were ascertained through an affected sibling pair, and each participating affected family member underwent a standardized neurological evaluation after appropriate written informed consent approved by each individual institution’s institutional review board was completed. The evaluation included the Unified Parkinson’s Disease Rating Scale (UPDRS) Parts II and III [20] and a diagnostic checklist which was used to provide both inclusion criteria highly associated with autopsy-confirmed PD and exclusion criteria associated with non-PD pathology [15,16]. Peripheral blood for DNA extraction was obtained from all individuals.

Data from a previously completed genome screen [23] was used to prioritize families based on their linkage to the region containing DJ-1. The 93 PD individuals from the 64 families with the highest family-specific multipoint LOD scores (range: 0.1–1.2) at this locus were then screened for with the highest family-specific multipoint LOD scores (range: 0.1–1.2) at this locus were then screened for DJ-1 mutations. The markers used to calculate the family-specific multipoint LOD scores were those closest to the PARK7 locus (D1S468, D1S214, D1S450, and D1S2667) from the 10 cM genome screen (ABI Prism Linkage Mapping Set, Applied Biosystems, Foster City, CA, USA).

A control sample of 96 subjects was also available. All individuals were clinically evaluated and did not have a diagnosis or symptoms of PD or any other neurological disorder. Fifty-two controls were recruited in Indiana and had an average age at exam of 68.8 years (range 55–82 years). The remaining 44 controls were obtained through the National Cell Repository for Alzheimer’s Disease (NCRAD) and had an average age at exam of 76.7 years (range 58–91 years). All control subjects were Caucasian (95%) or Hispanic (5%). The average age of onset of these individuals was 58.0 years (median: 59 years, range: 18–80 years), which is slightly younger than the average age of onset in the full sample (61.2 years). The 93 individuals sequenced represented 64 different families. One affected individual was sequenced from 45 of the families while two affected individuals were sequenced from 11 families and three affected individuals were sequenced from eight families. Some families had more than one individual sequenced due to family properties and genotypes of the markers around the DJ-1 locus. Three different sequence alterations were identified in seven of the 93 individuals screened for mutations. Two of the alterations were synonymous substitutions (480C > A and 501A > G), and the remaining alteration (293G > A) changed an arginine at amino acid 98 to a glutamine. This amino acid substitution (R98Q) was the most common sequence alteration found in our sample. We identified four PD patients who were heterozygous R98Q and one patient who was homozygous R98Q. Three of the four R98Q heterozygotes were affected members of the same family. The fourth heterozygote was from an unrelated family. The two synonymous substitutions (480C > A and 501A > G) were each found in the heterozygous state in a single individual with PD. No change in exon copy number, either as a whole exon deletion or duplication, was detected for exons 1, 3, 5, 6, or 7 in any of the 93 PD samples screened.

The panel of 96 control subjects was screened for the R98Q amino acid substitution as well. Three of the controls were found to carry this polymorphism in the heterozygous state; none of the controls were homozygous for R98Q. Therefore, the frequency of the R98Q polymorphism in our control sample is 1.6%. The controls were not screened for the two synonymous substitutions since neither is predicted to result in an amino acid change.

We have performed thorough screening of DJ-1 to detect mutations which may cause PD. Rather than screening our entire sample of multiplex PD families, we have employed a more efficient approach that utilized data from our previous genome screen to prioritize families for testing based upon their family-specific LOD score at the DJ-1 locus. We did not identify

<table>
<thead>
<tr>
<th>Exon</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>TCAGTGTGCTATGAAACCG</td>
<td>GAGCAATGGGTCACCATGAGGTGA</td>
</tr>
<tr>
<td>3</td>
<td>AGGATCATTTGAGGCCCAGGA</td>
<td>TCCCTACCCCTTCTTTATCTGTT</td>
</tr>
<tr>
<td>4</td>
<td>GTTCTATTGGCAGATAGGCT</td>
<td>ACAGGCTCCTCCTGGCAATAT</td>
</tr>
<tr>
<td>5</td>
<td>ATAGTGTCAAGAGGCTTTTGG</td>
<td>CAGATGGCGCTCGATGTTT</td>
</tr>
<tr>
<td>6</td>
<td>GCCAGGCATCTTGGGATT</td>
<td>AGAAGATCGGTTGAAACCCG</td>
</tr>
<tr>
<td>7</td>
<td>CTTGGAGTGCTTGAACATG</td>
<td>TTCACTGCGCATATGGGACAGT</td>
</tr>
</tbody>
</table>

Multiplex ligation-dependent probe amplification (MLPA) was performed with 100 ng of genomic DNA according to manufacturer’s instructions using the P051 Salsa MLPA Parkinson probe set (MRG-Holland, Amsterdam, The Netherlands). This probe set includes probes for all DJ-1 exons except 2 and 4. Probe amplification products were run on an ABI 3730xl DNA Analyzer using GS500 size standard (Applied Biosystems, Foster City, CA). MLPA peak plots were visualized using Genemapper Software version 3.7 (Applied Biosystems, Foster City, CA). Non-normalized values for peak height and peak area were then exported from Genemapper Software version 3.7 to an Excel template. Normalization of data and calculation of dosage ratios was performed as described at www.mrc-holland.com/MLPA%20analysis.html. The control value for each probe was an average of three control samples run with the PD samples. Due to variation in assay performance, we used Dosage ratio values of ≤0.7 and ≥1.35 as our boundaries for deletions and duplications, respectively.

The 93 individuals screened for DJ-1 mutations were either Caucasian (95%) or Hispanic (5%). The average age of onset of these individuals was 58.0 years (median: 59 years, range: 18–80 years), which is slightly younger than the average age of onset in the full sample (61.2 years). The 93 individuals sequenced represented 64 different families. One affected individual was sequenced from 45 of the families while two affected individuals were sequenced from 11 families and three affected individuals were sequenced from eight families. Some families had more than one individual sequenced due to family properties and genotypes of the markers around the DJ-1 locus. Three different sequence alterations were identified in seven of the 93 individuals screened for mutations. Two of the alterations were synonymous substitutions (480C > A and 501A > G), and the remaining alteration (293G > A) changed an arginine at amino acid 98 to a glutamine. This amino acid substitution (R98Q) was the most common sequence alteration found in our sample. We identified four PD patients who were heterozygous R98Q and one patient who was homozygous R98Q. Three of the four R98Q heterozygotes were affected members of the same family. The fourth heterozygote was from an unrelated family. The two synonymous substitutions (480C > A and 501A > G) were each found in the heterozygous state in a single individual with PD. No change in exon copy number, either as a whole exon deletion or duplication, was detected for exons 1, 3, 5, 6, or 7 in any of the 93 PD samples screened.

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any gene dosage alterations in our 93 PD cases. We identified only one missense mutation, which has been shown in previous studies to be a benign polymorphism [4,13,22], even in the homozygous state [13]. We found the R98Q substitution at similar frequencies in our cases and controls, further confirming that this polymorphism is unlikely to be disease producing.

Previous studies of DJ-1 have often limited the scope of screening to detect only coding sequence changes. Only one-third of the previously completed studies [6,12,19,21], which represent less than one quarter of the 1200 total patients screened to date, employed methods to detect any alterations in gene dosage. Therefore, it is possible that the frequency of DJ-1 mutations might have been underestimated in these studies. However, our analyses would suggest that even when a cohort is enriched for families more likely to carry a DJ-1 mutation and methods include screening for gene dosage alterations, mutations in this gene are rare in familial PD.

There are several notable strengths of our study. First, thorough screening of the DJ-1 gene was performed, including sequencing of all translated exons as well as testing for genomic rearrangements by MLPA analysis. Second, families were selected for testing based on their evidence of linkage to the DJ-1 region on chromosome 1p36. We have previously successfully utilized this approach to prioritize screening of our patient cohort for mutations at the PRKN locus [7]. Thus, we have augmented our tested sample for families most likely to have a mutation in DJ-1. Conversely, our study also has several limitations. First, due to the likely paucity of DJ-1 mutations, we did not screen our entire familial PD sample. However, we did screen more than 15% of our sample which included those most likely to harbor a DJ-1 mutation. Second, we did not screen our entire sample of early-onset PD cases, which is typically defined as having an age of onset of 45 or earlier. Rather we used a more cost effective approach to only screen families with evidence of linkage to this genomic region. Finally, the dosage methods did not interrogate either exon 2 or 4. In addition, positive controls carrying DJ-1 exon deletions were not available to us. While false negatives cannot be ruled out, MLPA analysis was performed at least twice for each sample minimizing this risk. However, based on the results of the screening in our sample as well as the results from other studies, mutations in DJ-1 do not appear to be a common cause of PD, and are much rarer than mutations in the PRKN or LRRK2 genes.

Acknowledgements

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Appendix A

Parkinson Study Group Investigators

PROGENI Steering Committee

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