DJ-1 variants in Indian Parkinson’s disease patients

Tamal Sadhukhan\textsuperscript{a}, Arindam Biswas\textsuperscript{a}, Shyamal K Das\textsuperscript{b}, Kunal Ray\textsuperscript{c,\#} and Jharna Ray\textsuperscript{a,\#}

\textsuperscript{a}S. N. Pradhan Centre for Neurosciences, University of Calcutta, Kolkata, India
\textsuperscript{b}Movement Disorders Clinic, Bangur Institute of Neurosciences, Kolkata, India
\textsuperscript{c}Molecular and Human Genetics Division, CSIR-Indian Institute of Chemical Biology, Kolkata, India

Abstract. Parkinson’s disease (PD) is a common neurodegenerative movement disorder. Among the candidate genes, DJ-1 accounts for about 1% of the cases in different populations. We aim to find the contribution of the gene towards PD among Indians. By screening DJ-1 in 308 PD patients of eastern India and 248 ethnically matched controls, a total of 21 nucleotide variants – including two nonsynonymous changes – were detected. p.Arg98Gln was identified in 6 unrelated patients and 2 controls while p.Val35Ile, a novel change, was found only in 2 unrelated patients. A SNP (rs7517357) was observed to be moderately associated with increased risk of PD (\(p < 0.05\)). The deletion allele (g.168\textsubscript{185}del) of a known 18 bp del/ins/dup polymorphism was found to be over represented (\(p < 0.05\)) among older patients (>40 years) compared to the controls (>45 years). Two of the patients, also heterozygotes for PINK1 mutation, had more severe disease phenotypes, consistent with the reported interaction between PINK1 and DJ-1 gene products [19]. Our results demonstrate that up to 3.9% (12/308) of PD patients of eastern India harbor DJ-1 variants that should be explored further for any causal relationship with PD.

Keywords: DJ-1, Parkinson’s disease, PINK1, SNP, mutation

1. Introduction

Parkinson’s disease (PD) is a common progressive neurodegenerative movement disorder, which affects about 2% of people over the age of 65 [9]. In India, the prevalence rate of PD has been reported to be 53 per 100,000 [6]. Among the causal genes, Parkin harbors the highest number of mutations. Defects reported in other genes, including PINK1 and DJ-1, are less frequent. A large number of studies have been reported to understand the functional role of DJ-1 in PD pathogenesis.

DJ-1 (PARK7) has been reported to be linked to the early onset autosomal recessive form of familial PD. DJ-1 encodes a highly conserved protein consisting of 189 amino acid residues and belongs to the DJ-1/ThiJ/Pfp1 superfamily. Multiple studies show that DJ-1 scavenges free radicals and protects cells from oxidative stress [19], thereby maintaining normal mitochondrial function. Loss of DJ-1 function has been reported to be associated with PD.

A total of 26 mutations (HGMD; http://www.hgmd.cf.ac.uk/ac/all.php), and a number of polymorphisms have been reported in different populations [5,11,20]. Also, a recent study from eastern India reported [18] intronic variation in DJ-1 in PD patients, with no apparent consequence on its pathogenesis. In this study we have screened the DJ-1 gene in an eastern Indian cohort of PD patients and identified potential mutations and variants associated with the disease.

2. Materials and methods

2.1. Patients and controls

A total of 308 clinically diagnosed PD patients having at least two Parkinsonian symptoms (rest tremor,
bradykinesia, rigidity and/or postural instability) with a mean age of onset of 48.12 ± 12.97 years (age range, 7 to 77) and 248 ethnically- and age-matched control subjects (mean age, 48.89 ± 7.73 years), having no personal or family history of parkinsonism or any other neurological problem, were recruited in the present study. Females represented 22% and 25% of the patients and controls, respectively. PD patients were examined in the Movement Disorder Clinic, Bangur Institute of Neurosciences, Kolkata, India. In the patient cohort, 23 represented confirmed familial cases, 181 were sporadic cases, 67 had a history of other neurological problems including dystonia, tremor, etc., and the family history of the remaining 37 cases was not known.

2.2. Screening of the DJ-1 gene

Approximately 10 ml of peripheral blood samples were collected in tubes containing anticoagulant (EDTA) with the informed consent of the patients and their family members. The experiments were conducted in accordance with the Declaration of Helsinki. The internal review committee on research using human subjects cleared the project as per the regulations established by the Indian Council of Medical Research. Genomic DNA was prepared from fresh whole blood using the conventional salting out method, followed by isopropanol precipitation [14]. The DNA precipitate was dissolved in TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) and stored at 4°C.

PCR was carried out to amplify the exons and their flanking regions of all the 308 patient samples. For quick screening of nucleotide variants, PCR products were subjected to Single Stranded Conformation Polymorphism (SSCP) analysis, as described previously [2, 16]. The DNA fragments showing band shifts were subjected to bi-directional DNA sequencing to identify nucleotide variants as compared to the wild-type DJ-1 gene sequence (GenBank ID: AB015652). The variants identified in patients were examined for its occurrence in the controls either by PCR-RFLP or direct sequencing.

To type 18 bp del/ins/dup polymorphisms, the DJ-1 intronic region encompassing the polymorphic site was amplified by PCR using primer pair 5'-GGGT GAGTGCTACCCAAGC-3' and 5'-CTGTCGCTTGGC GTTGGATT-3'. The insertion, deletion, and duplication alleles are expected to yield 238, 220, and 256 bp amplicons, respectively. The band pattern generated in heterozygous condition was monitored by polyacrylamide gel (7%) electrophoresis. The genotype was further confirmed by DNA sequencing. Statistical analysis was performed using Java Stat (http://www.son.wisc.edu/rdsu/stat_routines/ctab2x2.html) employing Fisher’s exact probability Chi-square test. The identified nonsynonymous changes (Val35Ile and Arg98Gln) were screened in the control group by RFLP analysis. A Bcc I (New England Biolabs, UK) site was created by site directed mutagenesis of the Val35Ile (c.103 G > A) variant using the primer pair 5’-TGATTGTCATGCCCCTCT-3’ and 5’-GGTC TTTCCAGCCAGCCTGCAG-3’ for PCR. On digestion, the PCR product with the G-allele generated two fragments (117 bp and 52 bp), while the product for the A-allele resulted in three fragments (99 bp, 52 bp and 18 bp). To score allelic variants for Arg98Gln, a 420 bp PCR product was generated by PCR primers 5’-ATGAGAAATGCCTTGCTTG-3’ and 5’-AACTTCTGCCACCCCAACT-3’, and digested with Msp I (New England Biolabs, UK), which yielded fragments of 243 and 177 bp when G was present and remained undigested when A was present. 94 randomly selected patients and 39 controls from our cohort were analyzed by direct sequencing of all seven exons and exon-intron boundaries. Linkage disequilibrium (LD) was calculated between SNPs identified in both groups that could be used as markers to test DJ-1 as a candidate gene in familial cases of PD.

2.3. DNA dosage analysis by MLPA

The Multiplex Ligation-dependent Probe Amplification (MLPA) assay was done according to the manufacturer’s instructions using 108 of PD samples from a total of 308 cases in our cohort. These samples were selected based on the following criteria: (a) samples heterozygous for missense mutations; (b) familial cases of PD; and (c) samples homozygous for 18-bp duplication or compound heterozygous with a deletion of the repeat element. The rationale for using this assay on this subset of patients was to find whether there were second mutant alleles in DJ-1 gene consistent with its known recessive mode of inheritance in familial cases, and in a genetic background with respect to the association of the 18 bp repeat elements with PD locus (as described in the Results section). The commercially available kit, SALSA MLPA P051-C1 Parkinson-1 probemix (MRCL Holland, Amsterdam, The Netherlands; http://www.mlpa.com), was used to detect the DNA dosage of DJ-1. This (MLPA kit consisted of probes for exons 1 to 12 of Parkin, exons 1 to 8 of PINK1, and 4 exons (1a, 3, 5 and 7) of DJ-1 – where most of the deletion mutations are reported.
A larger number of samples were screened for two nonsynonymous changes (Val35Ileu and Arg98Gln) and the 18 bp Ins/Del/Dup polymorphism to evaluate their potential association with PD, if any. Other nucleotide variants, unlikely to be causal to the disease due to their location in the gene, were screened in relatively smaller number of samples.

† The coordinate of the SNP is given as per the NCBI reference sequence NM_007262.4.

∗ T allele is found to be over represented in patient group than control (8.6% vs. 4.5%, \(p = 0.04\), OR = 1.97, 95% CI value = 1.0–3.97).

3. Results

3.1. DJ-1 mutation screening

A total of 308 patients recruited in this study included 67 (22%) female (mean age at onset, 48.23 ± 13.73 years) and 241 (78%) male (mean age at onset, 49.42 ± 12.46 years) subjects. The cohort has previously been screened for Parkin [2], PINK1 [3] and prevalent/common mutations of LRRK2 (p.Arg1441Cys, p.Arg1441Gly, p.Arg1441His, p.Tyr1699Cys, p.Ile2012Thr, p.Gly2019Ser and p.Ile2020Thr) [17]. No suspect variant was identified in LRRK2 causal to PD in our cohort. Screening of DJ-1 in this cohort identified 21 variants including 2 nonsynonymous coding changes (p. Val35IIe and p. Arg98Gln) (Table 1). p. Val35IIe (c.103 G > A) was found in 2, and p. Arg98Gln (c.293 G > A) in 6 unrelated PD patients – all in heterozygous condition. p. Val35IIe represents a novel change while p. Arg98Gln is a SNP
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(rs71653619) also reported as a mutation [11,13]. It has been reported that the allele frequency of the Gln-variant ranges from 0.004 to 0.027 in different populations [12]. *In silico* analyses of these variants by SIFT and PolyPhen2 did not indicate any damaging effects on the protein. However, Arg98 is evolutionarily conserved in six mammalian species, chicken and fish while Val35 is conserved in all of the above mentioned species except in chimpanzees (Fig. 1). Ishikawa et al. have proposed that DJ-1 directly binds to tyrosine hydroxylase (TH) and 4-dihydroxy-L-phenylalanine decarboxylase (DDC) and positively regulates their activities in human dopaminergic cells and thus influences dopamine synthesis [13]. In addition, they reported that the p. Arg98Gln change of DJ-1 alters expression of tyrosine hydroxylase [13]. Ishikawa et al. [13] also argued that the dominant-negative effect of heterozygous mutants (Arg98Gln and Asp149Ala) against wildtype DJ-1 on TH and DDC activities suggests that heterozygous mutation of the *DJ-1* gene affects onset of PD, although *PARK7/DJ-1* mutations are usually transmitted in a recessive mode in familial PD cases.

Among the noncoding changes, the T allele of SNP IVS2-109 C > T (rs7517357) is over represented in the patients relative to the controls (p = 0.04, OR = 1.97, 95% CI = 1.0–3.97), and an 18 bp change, g.168_185 ins/del/dup, was identified in intron 1 of the gene (Fig. 2). A case-control study using the g.168_185 ins/del/dup variant suggests that the distribution of alleles and genotypes are similar in both groups (Table 2). However, the deletion allele is over represented in older patients compared to age-matched controls (p = 0.038, OR = 1.768, 95% CI = 1.032–3.032) (Table 2). The 18 bp homoyzgyous duplication was found in two unrelated PD patients (PR354 and PR916) and the 18 bp deletion/duplication compound heterozygous genotype was observed in two patients (PR39 and PR286). Neither of these two genotypes was found in the 248 control subjects. Interestingly, among these four PD patients, PR39 and PR 916 also harbored mutations in *PINK1* (p.Arg246Gln and p.Arg276Gln, respectively) (Table 3), and had much worse disease outcome overall, compared to subjects carrying only *DJ-1* mutations. The patients with different alleles for the 18 bp repeat region, or variant alleles for the two nonsynonymous changes were analyzed for gene dosage using the MLPA kit. This assay is performed to identify deletions in *Parkin* (exon 1-12), *PINK1* (exon 1-8) and *DJ-1* (exons 1a, 3, 5 and 7), where most of the deletion mutations have been reported. However, no exon deletion/duplication was detected in any of the target genes in the 108 PD samples analyzed.

![Fig. 2. Analysis of 18-bp repeat polymorphism.](image)

**3.2. DJ-1 association study**

During screening of the patients it was observed that some of the variants, mostly in the 14 kb 5’-region of the gene, were highly polymorphic. To cover the remaining 10 kb region of the gene, two SNPs (rs161807 and rs225119) were selected from the SNP database, reported to be highly polymorphic in the eastern Indian population (Table 1). Thus, a total of 13 SNPs were analyzed in our patients and controls to look for a pairwise LD profile (Fig. 3). Six SNPs (e.g. SNP1, SNP4, SNP5, SNP6, SNP12 and SNP13) with high informativeness and low LD values (r² < 0.8) were identified in the cohort, making them amenable for use as markers for *DJ-1*. These markers could then be used to test for segregation with the familial form of PD and for planning any association study.

**3.3. Clinical features of the patients harboring DJ-1 variants**

In an attempt to develop genotype-phenotype correlation, we compared the clinical features of 12 pa-
Table 2

Distribution of g.168_185 18 bp Ins/Del/Dup polymorphism of DJ-I between cases and controls

| Genotype/Allele | Total case n = 308 (%) | Total control n = 248 (%) | Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases Older cases 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Table 3
Clinical features of patients having DJ-1 variations

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<th>PR301</th>
<th>PR641</th>
<th>PR730</th>
<th>PR881</th>
<th>PR241</th>
<th>PR847</th>
<th>PR354</th>
<th>PR916</th>
<th>PR286</th>
<th>PR39</th>
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<tr>
<td>Variation in <strong>DJ 1</strong></td>
<td>R98Q (h)</td>
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<td>V35I (h)</td>
<td>V35I (h)</td>
<td>g.168,185</td>
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<tr>
<td>Variation in <strong>PINK1</strong> gene</td>
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<tr>
<td>Age at onset (yrs)/Sex</td>
<td>30/M</td>
<td>36/M</td>
<td>46/M</td>
<td>60/M</td>
<td>57/F</td>
<td>51/M</td>
<td>64/M</td>
<td>51/F</td>
<td>64/M</td>
<td>38/M</td>
<td>60/M</td>
<td>43/M</td>
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<tr>
<td>Disease duration (yrs)</td>
<td>8</td>
<td>4</td>
<td>12</td>
<td>8</td>
<td>2</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>14</td>
<td></td>
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<tr>
<td>Family history</td>
<td>Grand mother had PD</td>
<td>Maternal uncle had PD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other tremors</td>
<td>Action</td>
<td>Action</td>
<td>No</td>
<td>Postural</td>
<td>Action</td>
<td>Action</td>
<td>–</td>
<td>Action</td>
<td>No</td>
<td>–</td>
<td>Action</td>
<td>Action</td>
</tr>
<tr>
<td>Dystonia</td>
<td>BS</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>LC</td>
<td>BS</td>
<td>Leg</td>
<td>Foot</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pain</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>Speech</td>
<td>Affected</td>
<td>Normal</td>
<td>Affected</td>
<td>Affected</td>
<td>Normal</td>
<td>Normal</td>
<td>Affected</td>
<td>Normal</td>
<td>Affected</td>
<td>Normal</td>
<td>Normal</td>
<td>Affected</td>
</tr>
<tr>
<td>Ocular movement</td>
<td>Slow saccade</td>
<td>Slow saccade</td>
<td>Normal</td>
<td>Jerky pursuit</td>
<td>Restricted saccade &amp; pursuit</td>
<td>Normal</td>
<td>Normal</td>
<td>Restricted saccade &amp; pursuit</td>
<td>Normal</td>
<td>Restricted</td>
<td>Slow</td>
<td>Restricted</td>
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<tr>
<td>Psychiatric symptoms</td>
<td>Depression</td>
<td>Absent</td>
<td>Depression</td>
<td>Absent</td>
<td>Depression</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Hallucination</td>
<td>Irritable, suicidal</td>
<td>Absent</td>
</tr>
<tr>
<td>Memory problem</td>
<td>Mild</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>NK</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Mild</td>
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<tr>
<td>L-DOPA response</td>
<td>Good</td>
<td>Not used</td>
<td>Good</td>
<td>NK</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Poor</td>
<td>Not used</td>
<td>Poor</td>
</tr>
<tr>
<td>Toxic exposure</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>25 yrs</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>2 yrs</td>
<td>No</td>
<td>Life long</td>
</tr>
<tr>
<td>Drinking water</td>
<td>Tube/ground well</td>
<td>Municipal water</td>
<td>NK</td>
<td>Municipal water</td>
<td>Tube well</td>
<td>NK</td>
<td>Tube well</td>
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<td>Tube well</td>
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<tr>
<td>Rural living</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>NK</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>NK</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

€ – Only daughter harbored the same change but is asymptomatic; £ – Second daughter harbored the same change but is asymptomatic; " – Slow finger tapping; § – stooped posture; ¶ – Drug induced; H – Homozygous; h – Heterozygous; NK – Not Known; T – Tremor; ET – Essential tremor; RT – Rest tremor; R – Rigidity; B – Bradykinesia; PI – Postural instability; BS – Blepharospasm; LC – Laterocollis.
important enzymes in the dopamine biosynthesis pathway, and regulates their activities. The p.Arg98Gln variant shows a dominant negative effect of DJ-1 on TH and DDC function, suggesting that the heterozygous mutations of DJ-1 identified in all our patients, plays a significant role in disease pathogenesis [13]. We also observed p.Arg98Gln in heterozygous state in two control individuals, which indicates that if the variant allele has any role in PD pathogenesis, such function might be modulated by a modifier locus in the control individuals.

Whether the novel missense change found in our patients, p.Val35Ile has any effect on DJ-1 function remains to be elucidated. In silico analysis did not predict any alteration in protein function in case of the p.Val35Ile mutation. However, Val35 is conserved in different species through evolution, which indicates the importance of this residue. This suggests that the p.Val35Ile change may affect DJ-1 protein function.

An 18 bp insertion/deletion polymorphism (g.168_185 ins/del) at intron 1 of DJ-1 was reported previously in different populations, and association studies reported mixed results. In an Italian case-control study, the deletion allele (g.168_185 del) was found to be a risk factor for developing PD. Another Italian study reported that the duplication allele in homozygous state co-segregated with the disease in a family, whereas studies in Finland and UK did not reveal any association [1,8,10,15]. A report from Italy claimed that a nucleotide variation, g.159 C > G, reduces DJ-1 gene expression by 12–13% [21]. The nucleotide g.159 C is located near the region of the g.168_185 ins/del/dup polymorphism, suggesting that this polymorphism might have an effect on gene expression. However, the association of this change with disease pathogenesis needs to be revalidated in a different, larger cohort, and its effect on gene expression needs to be resolved by functional analysis. It had been reported that 30% reduction of DJ-1 expression will reduce tyrosine hydroxylase gene expression by up to 50% [13]. Therefore, reduced expression of DJ-1 might affect dopamine biosynthesis. DJ-1 linked PD cases are reported to show some associated clinical features, including psychiatric symptoms (anxiety/depression), dystonic features (blepharospasm) and good, prolonged response to levodopa therapy [4]. Similar characteristic symptoms were observed in our DJ-1 affected patients (Table 3).

We had reported earlier that patients with PINK1 mutations show poor response to levodopa therapy [3]. In this study we found that patients harboring mutations in both PINK1 and DJ-1 showed rapid disease progression and early disabilities (bed-ridden condition) in comparison to either DJ-1 or PINK1 mutation carriers. It has been reported that DJ-1 forms a complex with PINK1 and makes PINK1 more stable, thereby potentiating its anti-stress activity [20]. PINK1 physically interacts with DJ-1 via amino acid residues 253–334 of its N-terminal kinase domain. Mutation within this region may lead to severe pathogenesis. Between two PINK1 mutations (p.Arg246Gln and p.Arg276Gln), the latter falls within this region and is therefore expected to affect complex formation, leading to cells more vulnerable to oxidative stress and, hence, increased disease severity. It was reported that PINK1 stability is decreased by mutations in DJ-1 [20]. Therefore, reduced expression of DJ-1 may also reduce the stability of mutant PINK1.

In conclusion, we have identified two nonsynonymous changes, one novel and another reported, in 8 pa-
tients. An 18 bp deletion allele at position g.168_185 appears to be a risk factor for late onset PD cases. Also, the intronic SNP (rs7517357) has been found to be a risk factor for PD cases. Two cases harboring variants in both genes DJ-1 and PINK1, with severe phenotypes, suggest a potential digenic effect in disease progression. Our results demonstrate 3.9% (12/308) occurrence of potentially pathogenic variants in DJ-1 among PD patients of eastern India.

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References

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