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DJ-1 variants in Indian Parkinson's disease patients

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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_185del) 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,

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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-HCI, 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 GAGTGGTACCCAACG-3' and 5'-CTGTCGCTGGC GTTGGATTT-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'-TGATTGTGTCACTGCCCTCT-3' and 5'-GGTC TTTTCCAGCCAGGCCTGCGA-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'-ATGAGAAATGCCTTGCTTGG-3' and 5'-AACTTCATGCCACCCAAACT-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 (MRC 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.

Table 1
Nucleotide variants identified in *DJ-1* gene among patients and controls

Nucleotide change	Amino acid change	Location in gene	Patient (Chr. no.)	Control (Chr. no.)	Status
g. −21 G > A	NA	Promoter	59/276	8/76	rs17523802
c.-99T > C†	NA	Exon 1a	121/276	25/76	rs226249
g. 168_185 del	NA	Intron 1	68/616	43/496	Novel
g. 168_185 dup	NA	Intron 1	28/616	23/496	Novel
g. 213 G > T	NA	Exon 1b	38/172	4/78	rs35675666
IVS1-22 T del	NA	Intron 1	96/192	39/78	Novel
IVS2-109 C > T*	NA	Intron 2	52/602	12/262	rs7517357
c. 103 G > A	*Val35Ile*	Exon 3	2/602	0/400	Novel
IVS4 + 30 T > G	NA	Intron 4	61/218	9/78	rs2641116
IVS4 + 45 G > A	NA	Intron 4	60/218	9/78	rs2640906
IVS4 + 46 G > A	NA	Intron 4	60/218	9/78	rs56327722
IVS4 + 47 G ins	NA	Intron 4	4/218	1/78	Novel
IVS4 + 47 A ins	NA	Intron 4	2/218	5/78	Novel
IVS4 + 81 C ins	NA	Intron 4	17/218	7/78	Novel
IVS4-98 G > A	NA	Intron 4	13/236	6/118	rs6703670
IVS4-4 C > T	NA	Intron 4	0/236	1/118	Novel
c. 293 G > A	*Arg98Gln*	Exon 5	6/616	2/496	rs71653619
IVS5 + 31 G > A	NA	Intron 5	30/236	23/118	rs389298
IVS5-216 G > A	NA	Intron 5	37/190	16/78	rs161807
IVS6-593 T > C	NA	Intron 6	119/288	86/224	rs225119
Stop + 38 G > A	NA	3' UTR	1/230	0/78	Novel

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).

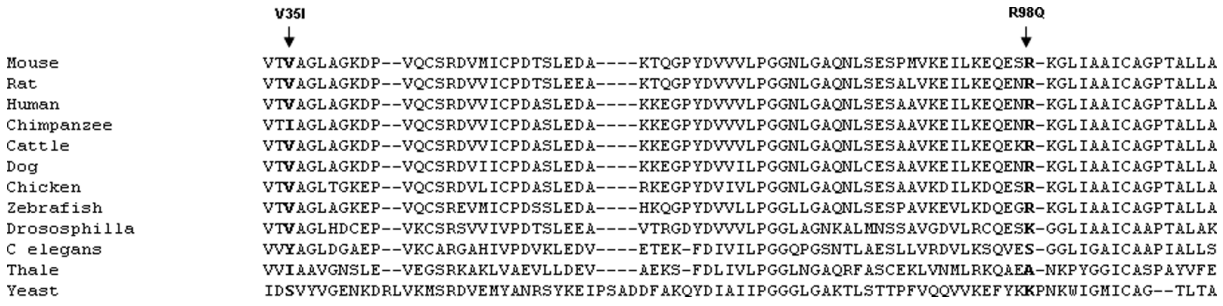

Fig. 1. Conservation of Val35 and Arg98 residues in DJ-1 homologues in different species. Polymorphic residues for codons 35 (Val35Ile) and 98 (Arg98Gln) are shown at the top of the illustration. The residues present in these two positions in the homologs of different species are highlighted by bold faced letters in corresponding positions.

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

(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 homozygous 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.

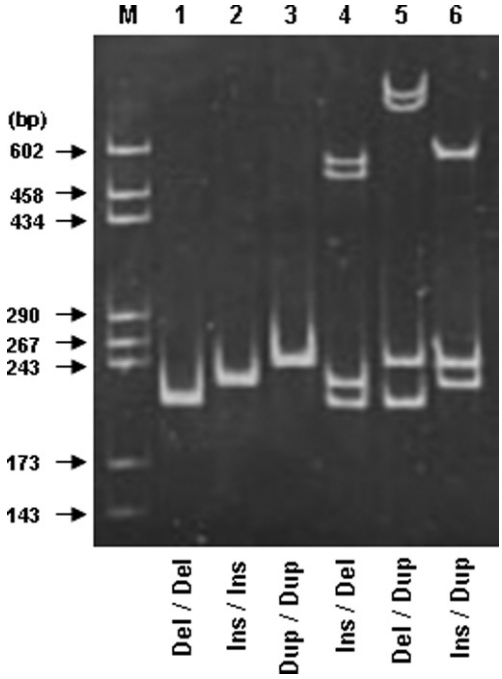

Fig. 2. Analysis of 18-bp repeat polymorphism. PCR products capturing the allelic variants of the g.168_185 polymorphism, containing an 18-bp repeat, were electrophoresed on 7% polyacrylamide gel, and the alleles were scored. Lanes 1 to 6 show band patterns demonstrating genotypes of the DNA samples as shown at the bottom of the gel picture. The upper bands represent heteroduplexes resulting from pairing between two different alleles of different sizes. Lane M represents the pBS x Hae III marker.

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 ($r^2 < 0.8$) 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-1* between cases and controls

Genotype/Allele	Total case $n = 308$ (%)	Total control $n = 248$ (%)	Older cases$^{\Phi}$ $n = 214$ (%)	Aged control§ $n = 150$ (%)
GENOTYPES				
Ins/Ins	218 (70.8)	184 (74)	149 (69.6)	116 (77.3)
Ins/Del	62 (20.1)	39 (16)	42 (19.6)	18 (12)
Ins/Dup	22 (7.1)	23 (9)	18 (8.4)	15 (10)
Del/Del	2 (0.6)	2 (1)	2 (0.9)	1 (0.7)
Del/Dup	2 (0.6)	0	2 (0.9)	0
Dup/Dup	2 (0.6)	0	1 (0.5)	0
ALLELES				
Ins	520 (84.4)	430 (87)	358 (83.6)	265 (88.3)
Del	68 (11)	43 (9)	48 (11.2)	20 (6.6)$^{¥}$
Dup	28 (4.5)	23 (4)	22 (5.1)	15 (5)

$^{\Phi}$, Older cases (age of onset) > 40 yrs; §, Older control (age) > 45 yrs; $^{¥}$, Deletion allele is over represented in older patients compared to aged controls (11.2% vs. 6.6%, $p = 0.038$, OR $= 1.768$, 95% CI value $=$ 1.032–3.032).

tients harboring suspect variants in *DJ-1* (Table 3). Two (PR171 and PR641) of the 6 patients with p.Arg98Gln have a positive family history, whereas tremor and essential tremor have been observed in the families of two other patients (PR354 and PR286). Among these patients the age of onset varied from 30–64 years and most of them developed cardinal Parkinsonian features including bradykinesia, rigidity, rest tremor and postural instability, and 8 out of the 12 cases (66.7%) also developed action and/or postural tremor. In addition, some of these patients developed some characteristic features such as pain, speech problems and slow or restricted ocular movement. Five patients (41.67%) also developed dystonia (especially blepharospasm and laterocolis), which is reported to be common among *DJ-1* linked PD cases [7]. Some patients also developed psychiatric problems (6/12, 50%) and memory problems (6/12, 50%) shown in Table 3.

All patients carrying *DJ-1* suspect variants responded very well to L-DOPA, except two (PR916 and PR 39), who also carried *PINK1* mutations (p.Arg276Gln and p.Arg246Gln, respectively). PR916 showed slowness of daily activities, rest tremor, slurring of speech and some abnormal pen rolling and dancing movements. Disease progression was very fast and he became completely bed-ridden within two years. His voice became completely choked and he often became violent. However, patient PR354 harbored the same homozygous g.168_185 duplication in *DJ-1* as PR916, but lacked any *PINK1* mutation, showed only rest tremor, responded well to L-DOPA therapy. The patient PR39, carrying potential mutations in both *DJ-1* and *PINK1* responded poorly to L-DOPA treatment and had all four Parkinsonian symptoms as well as severe gait and speech impairment, and he gradually became bedridden. The patient carrying only the del/dup mutation in *DJ-1* (PR286), however, only presented with mild bradykinetic symptom, action tremor with some psychiatric problems. From clinical data, it appears that patients PR916 and PR39 are less responsive to L-DOPA and are prone to rapid disease progression.

4. Discussion

In the present study we analyzed 308 PD patients from eastern India for *DJ-1* mutation and identified two nonsynonymous changes in 8 patients in heterozygous state. The p.Arg98Gln variant was reported in a patient of Indian origin whose mother was diagnosed with PD [11]. Later, the variant was reported to be present in the control group in both European and Asian populations [12] suggesting an association of the variant with PD pathogenesis based on the influence of other factors. Subsequent studies, however, suggest a potential role of the variant in altering the biological function of the protein. *DJ-1* containing a p.Arg98Gln substitution has been reported to have reduced antioxidant activity in eliminating exogenously added H_2O_2 *in vitro* [19]. Arg98 is present at the edge of α-helix 4, which is located outside the *DJ-1* protein. Therefore, substitution might affect the interaction between *DJ-1* and *DJ-1*-binding proteins necessary to perform its normal function without altering the protein stability, or the dimer formation necessary for its function [19]. *DJ-1* regulates the transcription of tyrosine hydroxylase (TH) in human. It physically binds to TH and 4-dihydroxy-L-phenylalanine decarboxylase (DDC), two

Table 3

Clinical features of patients having *DJ-1* variations

Clinical features	PR91	PR171	PR301	PR641	PR770	PR881	PR241	PR847	PR354	PR916	PR286	PR39
Variation in *DJ 1*	R98Q (h)	R98Q (h)	R98Q (h)	R98Q (h)	R98Q (h)	R98Q (h)	V35I (h)	V35I (h)	g.168_185 Dup (H)	g.168_185 Dup (H)	g.168_185 Dup/Del	g.168_185 Dup/Del
Variation in *PINK1* gene										Arg276Gln (h)		Arg246Gln (h)
Age at onset (yrs)/ Sex	30/M	36/M	46/M	60/M	57/F	51/M	64/M	51/F	64/M	38/M	60/M	43/M
Disease duration (yrs)	18	8	4	12	8	2	7	4	2	5	3	14
Family history		Grand mother had PD	–	Maternal uncle had PD€	–£	–	NK	-	ET in Mother	NK	T in Mother, Son, two daughters	–
Parkinsonian Symptoms	R, B, PI, RT	R, B, RT	R, B, PI, RT	R, B, PI, RT	R, B, RT	R, B, RT	R, B, PI, RT	R, B, RT	RT	R, B, PI, RT	B⁂, PI§	R, B, PI, RT
Other tremors	Action	Action	No	Postural	Action	Action	–	Action	No	–	Action	Action
Dystonia	BS	–	–	–	LC	BS	Leg	Foot	–	–	–	–
Pain	Yes	Yes	No	Yes	Yes	No	No	No	No	Yes	No	Yes
Speech	Affected	Normal	Affected	Affected	Normal	Normal	Affected	Affected	Normal	Affected	Normal	Affected
Ocular movement	Slow saccade	Slow saccade	Normal	Jerky pursuit	Restricted saccade & pursuit	Normal	Slow saccade & pursuit	Normal	Normal	Restricted	Slow saccade	Restricted
Psychiatric symptoms	Depression	Absent	Depression	Absent	Depression	Depression	Absent	Absent	Absent	Hallucination¶	Irritable, suicidal	Absent
Memory problem	Mild	No	Yes	Yes	No	Yes	NK	No	No	No	Yes	Mild
L-DOPA response	Good	Not used	Good	NK	Good	Good	Good	Good	Good	Poor	Not used	Poor
Toxin exposure	No	No	No	No	No	25 yrs	NK	No	No	2 yrs	No	Life long
Drinking water	Tube/ ground well	NK	Municipality /Tube well	NK	Municipality	Tube well	NK	Tube well	NK	NK	NK	Tube well
Rural living	Yes	No	Yes	NK	No	Yes	Yes	No	NK	Yes	No	Yes

€ – 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.

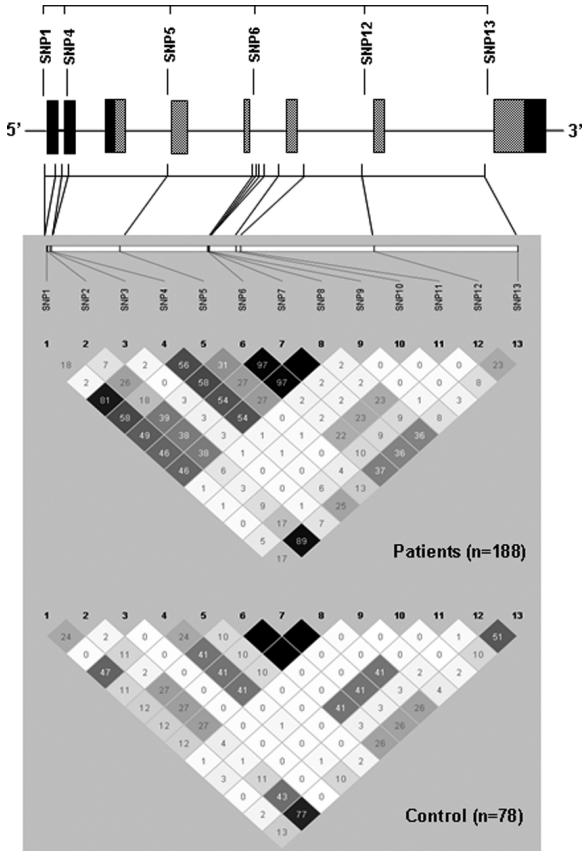

Fig. 3. Linkage Disequilibrium plot of 13 SNPs in *DJ-1* gene. SNP1 to 13 represent g. −21 G > A, g. 18 T > C, g. 168_185 ins/dup, g.213 G > T, IVS2-109 C > T, IVS4 + 30 T > G, IVS4 + 45 G > A, IVS4 + 46 G > A, IVS4-81 C ins, IVS4-98 G > A, IVS5 + 31 G > A, IVS5-216 G > A and IVS6-593 T > C, respectively. SNP6, SNP7, and SNP8 are in LD (r2 > 0.8) in both patient and control groups. SNP1, SNP4, SNP5, SNP6, SNP12, and SNP13 could be used for future association studies.

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 cosegregated 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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