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Genetic analysis of DJ-1 in a cohort Parkinson's disease patients of different ethnicity

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Abstract

Mutations in the DJ-1 gene have been described in autosomal recessive Parkinson's disease patients (ARPD) of European ancestry and young onset (YOPD) Ashkenazi Jewish and Afro-Caribbean patients. There is little information on the prevalence of DJ-1 mutations amongst Asian PD populations. In this study, we examined for DJ-1 mutations in consecutive YOPD and ARPD in a multi-ethnic cohort (Chinese, Malays, and Indians) of PD patients in a tertiary referral center. Sequence analysis of all the exons and the exon and intron boundaries of the DJ-1 gene were carried out. We did not find any DJ-1 mutations in these patients. A number of intronic variants with genotype frequency ranging from 15 to 90% were detected. Unlike Parkin, pathogenic DJ-1 mutations appear to be restricted to certain populations and are unlikely to be of clinical importance in our Asian cohort.

Keywords: DJ-1; Mutation; Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disease characterized clinically by tremor, bradykinesia and rigidity. Genetic variants of a number of candidate genes may be associated with an increased risk of PD [18]. Importantly, mutations of the alpha synuclein, parkin, ubiquitin-C-hydrolase ligase 1 and Nurr1 genes have been described in familial PD [3,10–13,16]. DJ-1 is an eight-exon gene spanning 24 kb, the open reading frame is encoded within exons 2–7 and exon 1 is alternatively spliced (1a/b). Mutations in the DJ-1 gene have recently been reported in two separate consanguineous families of Italian and Dutch ancestry [3]. The first, a homozygous 12-kb deletion within exons 1–5 leads to a loss of the gene product and causes parkinsonism in a large Dutch family. The second, a homozygous missense mutation in exon 7 leads to a substitution of a highly conserved leucine for a proline at position 166 (L166P) that segregates with parkinsonism in a small consanguineous Italian family.

Subsequently DJ-1 mutations have been demonstrated in young onset PD (YOPD) patients [1,5]. Two other studies found no DJ-1 mutations in a Caucasian ARPD population [6,9]. To our knowledge, there has not been any published information on the prevalence of DJ-1 mutations amongst a cohort of Asian PD patients. Such data is important to determine if DJ-1 mutations are confined to certain isolated ethnic populations and if a founder effect exists.

In this study, we conduct a mutational analysis of DJ-1 to determine if this gene is of any clinical importance in our YOPD and ARPD patients. We also provide a concise summary of the findings of DJ-1 mutational analysis in the literature.

We recruited consecutive YOPD (age of onset <50 years of age) and probable ARPD (history of one or two affected family members) from the movement disorder clinic in a tertiary hospital. All patients were examined by a movement disorder specialist and satisfied the UK PD Society Brain Bank Clinical Diagnostic Criteria of PD [8]. Exclusion criteria were extensor plantar reflexes, ophthalmoplegia, early dementia, or early autonomic failure. Written informed

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consent was taken from all study patients. The institutional ethics committee approved this study.

DNA was extracted from peripheral lymphocytes according to standard protocols. Specific primer sets were designed for each of the exons. PCR were carried out under the following conditions: denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s and elongation at 72 °C for 60 s each, and a final elongation step of 10 min at 72 °C. PCR was carried out in a final volume of 25 μl including 1.5 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphate, 0.4 μM forward and reverse primers, 1 U Taq polymerase, and 60 ng of DNA. According to the manufacturers' instructions, bidirectional dideoxy chain terminator sequencing was done (BigDye, Applied Biosystems, Warrington, UK). Electrophoreses of the products were carried out using the ABI 3100 automated DNA sequencer (Applied Biosystems). We also screened for exons 1–5 deletions.

Primers

Exon 1
F TGA GGC CAA GGC GGC GTG AGT
R TTC TGA GCC AGC GGG ACA CCG T

Exon 2
F GTT GTC TAT GAA AAC CGT TTC CCT AGG AAG
R TGG ATC AAC AGG TAT TTA TTC TTA TGT CAT CTC TG

Exon 3
F GAT TGT GTC ACT GCC CTC TAG CC
R GTC CTC ACC CTC TTA ATC TGT CAG G

Exon 4
F AAA CAC CAT TCC GTC ATG TGG ATA CAC CTT
R TTG TTT CTA TTT TTT TTT CAC AGC CTC CTC CC

Exon 5
F TTA TGA GAA ATG CCT TGC TTG GGT TTA AGA ATA TA
R AAT GAA AGG CTA CCC CAC CCA CCA A

Exon 6
F ACA TGG GCT TTT CTA TAT CT
R GCC TGG GCG ATG GAG CGA GA

Exon 7
F AAG AGC TTG GAG TGC CTA GTA AAT GTT TTT GAA T
R ATA TGG ACA GTT AAG AGC TGC AAA TGA AGG T

A total of 65 index patients were included. The majority of them were ethnic Chinese, followed by Malays and Indians. Forty of them were YOPD (age of onset <50 years), with mean age of onset of 42.33 ± 5.95 years, with the majority of them men. In addition, there were 25 probable index ARPD patients, with an age range from 36 to 85 years, with predominant men (Table 1).

Table 1
Demographics of study patients

Demographics	Number
Sample size	65
Race	
Chinese	57 (87.7%)
Malay	7 (10.8%)
Indian	1 (1.5%)
Young onset PD	40
Mean age of onset (years) (range)	42.33 ± 5.95 (29–51)
Gender	
Men	29 (44.6%)
Women	11 (16.9%)
Autosomal recessive PD	25
Mean age of onset (years) (range)	57.9 ± 13.1 (36–85)
Gender	
Men	16 (64%)
Women	9 (36%)

Sequence analysis did not demonstrate any pathogenic mutations in the DJ-1 gene. Mutations as described previously (3) were also not found in our samples. However, a number of likely polymorphic variants were found. Some of these included IVS 4 + 90 T → G, IVS 4 + 105 G → A, IVS 4 +106 G → A, IVS 5 + 101 G → A, and IVS 6 + 1798 T → C. The frequency of the genotypes at these loci ranged from 15 to 90% (Table 2). There was no significant difference in the distribution of the genotypes between YOPD and ARPD.

This study did not demonstrate any mutations in the DJ-1 gene in a cohort of YOPD and ARPD patients, suggesting that DJ-1 mutations are likely to be confined to certain genetically isolated populations such as in the Netherlands where it was originally described [3]. We identified several intronic variants (which likely represent polymorphisms and probably not pathogenic) but none in the coding sequence. Two recent case control studies on DJ-1 exon 1 and intron 1 polymorphic variants did not demonstrate any significant association of these variants with risk of PD [4,15].

Though rare, DJ-1 mutation is the second most common (after Parkin) cause of YOPD and ARPD, and its discovery has provided some useful insight into the pathophysiology of

Table 2
Frequency of the intronic variants

	Wild		Hetero		Homo	
IVS4 + 90	TT	7.10%	GT	59.50%	GG	33.30%
IVS4 + 105	GG	7.10%	GA	59.50%	AA	33.30%
IVS4 + 106	GG	7.10%	GA	59.50%	AA	33.30%
IVS5+ 101	GG	10.60%	GA	57.40%	AA	31.90%
IVS6 + 1798	TT	84.60%	TC	12.80%	CC	2.60%

Hetero: heterozygote; homo: homozygote.

PD. DJ-1 protein becomes acidic under cell stress condition and it is sumoylated (a ubiquitin like protein modification). The potential antioxidant and chaperone activity of DJ-1 could be affected by the high instability of the mutant protein [14]. DJ-1 is not an essential component of Lewy bodies and Lewy neurites, is expressed predominantly by astrocytes in human brain tissue [2]. DJ-1 has also been demonstrated to co-localize within a subset of pathological tau inclusions [17]. Hence DJ-1 may be involved in pathologic processes in various neurodegenerative diseases and this may involve the ubiquitin-proteosome pathway.

Our findings in an Asian cohort support observations in four studies in either YOPD or ARPD of predominant Caucasian PD patients [1,5,6,9]. Two such studies found none of their 39 ARPD and 125 ARPD patients carried the DJ-1 mutations [6,9]. However in one report, the authors found one patient with a compound heterozygous DJ-1 mutation, amongst 107 YOPD [5]. The fourth study [1] determined the prevalence DJ-1 mutation to be about 1% (2/185) in YOPD. One patient was an Ashkenazi Jew who had a homozygous missense mutation in exon 2 resulting in a substitution of a conserved methionine for an isoleucine (M26I). The second patient with Afro-Caribbean roots had a heterozygous mutation in exon 7 (D149A) together with a homozygous C/T substitution in exon 4 leading to a abolishment of a predicted exonic splice enhancer site (G78G). In addition, three heterozygous variants were found amongst 190 sporadic later onset PD patients. One of the variant (R98R) may represent a rare polymorphism [7]. Taken together, it appears that compared to the parkin gene, routine priority screening for DJ-1 mutations in all PD patients may not be necessary or cost effective.

We recognize some limitations of our screening technique. Sequencing the coding regions may sometimes miss heterozygous exon deletions, duplications, and genomic rearrangements. However, our findings can be compared to data from published studies as similar screening techniques were employed. Further gene dosage studies will be particularly useful.

In conclusion, we did not find any DJ-1 mutations amongst YOPD and ARPD patients, suggesting that DJ-1 is unlikely to be of clinical importance in our Asian cohort. A number of intronic variants were detected but unlikely to be pathogenic.

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