

## SHORT REPORT

# Mutational screening of the *parkin* gene among South Indians with early onset Parkinson's disease

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*Parkin* mutations are commonly encountered in multiethnic populations with familial early onset Parkinson's disease (PD) and less frequently in sporadic PD. A total of 102 patients (recruited from a hospital) with early onset PD from an ethnically homogeneous Indian population (age of onset  $\leq 50$  years) including both familial ( $n=20$ ) and sporadic ( $n=82$ ) cases were screened for *parkin* mutations. There were 105 normal controls. A homozygous missense mutation, Thr240Met, was found in exon 6 of one case and homozygous deletions of exons 8 and 9 were found in another index case. These constituted 2% of all early onset PD, 10% of all familial early onset PD, and 25% of all autosomal recessive early onset PD patients. No mutations were found in the patients with sporadic early onset PD, but seven exonic changes (three novel) were identified among 23 sporadic cases. These may represent heterozygous pathogenic changes. Use of more advanced techniques, including gene dosage estimation, and studies in Indian populations of different ethnic backgrounds may detect more mutations in future studies. This is the first report of *parkin* mutations from India and the first report from a non-white, non-oriental population of early onset PD.

Parkinson's disease (PD) is a neurodegenerative disorder affecting 2% of individuals older than 65 years. Genetic as well as environmental factors are implicated in its aetiology. The discovery of new genes such as  *$\alpha$ -synuclein*, *parkin*, *UCH-L1*, *DJ-1*, *NR4A2*, *PINK1*, *Dardarin* and their mutations and many genetic loci have highlighted the contribution of genetics in PD.<sup>1–6</sup> Many patients with early onset PD in diverse ethnic groups have mutations in the *parkin* gene.<sup>7–10</sup>

A variety of deletions, insertions, and point mutations in the *parkin* gene have been linked to early onset PD.<sup>11</sup> In a study of multiethnic families with autosomal recessive early onset PD, 49% were found to have *parkin* mutations.<sup>8</sup> In sporadic PD with age of onset less than 45 years, 18% constituted *parkin* mutations.<sup>9</sup> Most reports of *parkin* mutations are from Japan, Europe, and North America except for a few of North African families which were studied in a multiethnic population from Europe.<sup>8</sup> There are no large series of *parkin* mutations from non-white, non-oriental populations or any previous Indian studies. Therefore the aim of our study was to search for *parkin* mutations in ethnically identical South Indian patients with early onset PD and to characterise the phenotype associated with these mutations.

## METHODS

### Subjects

A total of 102 patients with clinically definite PD and age of onset of symptoms 50 years or less (early onset PD) participated in the study.<sup>12</sup> A movement disorders specialist

(AK) examined all the patients and secondary causes of parkinsonism were excluded. All clinical data were prospectively collected. We interviewed the proband and one or two close relatives to collect the details of the family tree and to fill in a family history questionnaire to identify PD in the family. The affected family members of the proband were examined when available or, alternatively, their medical and treatment charts were verified to identify affected people in the family. The control group consisted of 105 ethnically identical normal healthy volunteers, unrelated to the patients. They were examined for neurological diseases before inclusion in the study. The ethics committee of the hospital approved the study and all participants gave written informed consent.

### Molecular analysis

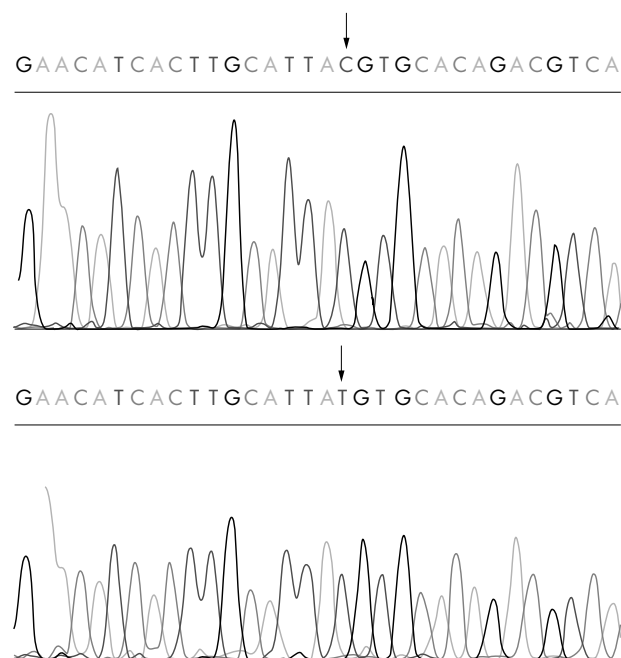
We extracted genomic DNA from 10 ml peripheral blood samples by a standard phenol-chloroform method. Primers flanking the 12 exons of the *parkin* gene were designed and the 12 coding exons and their flanking intronic sequences were amplified in 102 PD probands. The amplified products were screened by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) using the Genephor system and ready-to-use gel 12.5/24 according to the manufacturer's instructions (Amersham Biosciences; Uppsala, Sweden). We analysed the gels by standard silver staining, and samples exhibiting mobility shifts were sequenced on an ABI3100 Genetic Analyzer (Applied Biosystems, Foster City, USA using big dye terminator cycle sequencing reagents. Sequences were compared using Clustal X version 1.8. <http://www.molbiol.ox.ac.uk/documentation/clustalx/clustalx.html>).

## RESULTS

Among the 102 patients with PD (74 men, 28 women; mean (SD) age of disease onset of 40 (8) years; mean (SD) disease duration of 7.7 (5) years), there were 20 index cases of familial early onset PD (two or more affected family members) and 82 sporadic cases (no affected family member in at least three generations). Eighty six patients were on levodopa/carbidopa and the rest were on dopamine agonist treatment. All were responsive to dopaminergic treatment. Eight of the familial cases had an autosomal recessive mode of inheritance, whereas the index case and one of the parents was affected in the remaining 12 families. There was no consanguinity in any sporadic or familial case.

### Familial PD

We identified two different *parkin* mutations in two index cases with a possible autosomal recessive pattern of inheritance in both. One had homozygous deletions of exons 8 and 9. The same mutation was later identified in a single affected sibling. The second case had homozygous 820 C→T transition in exon 6 of *parkin*, which leads to a Thr240Met amino acid change in the crucial ring finger domain of the



**Figure 1** Chromatogram showing wild-type and Thr240Met mutation in the *parkin* gene.

*parkin* protein (fig 1). The mutation was not found in the controls. One familial PD case was a compound heterozygote for Arg334Cys and Glu444Gln (1101 C→T transition), but we could not determine the pathogenicity of this mutant combination.

The proband with exons 8 and 9 was 33 years old when symptoms appeared as action dystonia of the right foot followed by stiffness of the limbs of the same side and slowness of gait. The opposite side began to get stiff three years later. She was prescribed levodopa/carbidopa (150 mg daily) from elsewhere and had significant relief. Peak dose dyskinesias and wearing-off motor fluctuations appeared within six months of treatment. At 40 years of age, her Unified Parkinson's Disease Rating Scale (UPDRS) motor score (subset III) was 30. Folstein's Mini-mental State Examination (MMSE) score was 30. Her only affected sibling

tested positive for the same mutation later and developed dystonia of the right foot with diurnal fluctuation at 45 years followed by reduced arm swing and rest tremor of the ipsilateral hand. Her UPDRS motor score was 13 and her MMSE score was 30. She was started on 6 mg ropinirole daily, with 50% relief of symptoms.

The proband with the Thr240Met missense mutation was a man who had stiffness of asymmetrical onset in his left leg at 40 years and postural instability with falls within six months of onset of disease. He had occasional rest tremor of the left foot and slowness of all movements, and the disease spread to the opposite side within a year. His UPDRS motor score was 34 and his MMSE score was 28. He was also depressed. He was treated elsewhere with 300 mg levodopa/carbidopa, with 60% relief of symptoms. He had mild wearing-off motor fluctuations within a year of treatment but had no dyskinesias. Neither of these patients had other neurological signs.

### Sporadic early onset PD

There were no mutations in the sporadic cases, and we detected four known and three novel exonic changes in these cases (table 1). One case had two changes, 226G→A (Arg42His) and 1101C→T (Arg33Cys). These seven exonic changes occurred in 23 different patients and might represent heterozygous pathogenic changes.

We found two novel intronic variants in two separate patients (see table 1). However, on the basis of an analysis performed using splice site predictor software that evaluates the effect of such polymorphisms on the pre-RNA splicing ([http://www.fruitfly.org/seq\\_tools/splice.html](http://www.fruitfly.org/seq_tools/splice.html)), these did not appear to be functionally significant.

### DISCUSSION

The two *parkin* mutations found in our series are rare but have been reported. The Thr240Met heterozygous mutation was previously reported in an Italian patient with isolated early onset parkinsonism and in another case in a multiracial study from North America.<sup>13, 14</sup> Descriptions of the phenotypes of these cases are not available. Deletion of exons 8 and 9, the second type of mutation found in our study, has been reported in Algerian families in association with an earlier age of onset of disease (mean age 13 years).<sup>8, 14</sup> Our patient with deletions of exons 8 and 9 had disease onset in the fourth decade and her affected sibling in the fifth decade. The

**Table 1** Mutations and changes identified in the *parkin* gene in 102 patients with early onset Parkinson's disease

	Nucleotide change	Amino acid change	Type of mutation	Allele frequency (%)	Status
<b>Mutations identified</b>					
Exon 6	820C→T	Thr240Met	Missense	0.98	Known <sup>7</sup>
Exons 8 and 9	Homozygous deletion		Frame shift	0.98	Known <sup>8</sup>
<b>Polymorphisms identified</b>					
<i>Exonic</i>					
Exon 2	226G→A	Arg42His	Missense	0.98	Novel
Exon 4	601G→A	Ser167Asn	Missense	7.4	Known <sup>7</sup>
Exon 9	1101C→T	Arg334Cys	Missense	1.47	Known <sup>9</sup>
	1118G→A	Ala339Ala	Neutral	0.49	Known <sup>9</sup>
	1177G→A	Gly359Asp	Missense	0.49	Novel
Exon 10	1239G→C	Val380Leu	Missense	5.39	Known <sup>7</sup>
Exon 12	1430G→C	Glu444Gln	Missense	1.26	Novel
<i>Intronic</i>					
Intron 2	IVS2+25T→C			10.3	Known <sup>7</sup>
	IVS2+35G→A			0.49	Novel
Intron 9	IVS9+20C→A			0.49	Novel
Intron 3	IVS3-20T→C			63.7	Known <sup>7</sup>
Intron 7	IVS7-35G→A			0.98	Known <sup>7</sup>

phenotypes of our cases were not different from the classic descriptions of *parkin* mutations.

We did not find any *parkin* mutations in all 82 of our sporadic cases. In studies reporting higher frequencies of *parkin* mutations among patients with sporadic PD under 45–50 years, the majority of mutations were in the younger age groups.<sup>13</sup> In our series only 13% of the patients with sporadic PD were younger than 30 years at disease onset. This difference might partially account for the absence of *parkin* mutations among our patients. We found seven exonic changes in our patients with sporadic PD which might represent heterozygous pathogenic states. The relative low frequency of *parkin* mutations in our study is an underestimation because we did not perform gene dosage studies in our cohort. Future studies using more advanced techniques which include gene dosage studies and conducted in the ethnically diverse populations of India might reveal a higher frequency of mutations. Most of the reported data so far are from multiethnic families and the relative frequency of *parkin* linked cases is as follows: French 16%, Italian 13%, North African 21%, and Brazilian 8%.<sup>13</sup> Recently, based on a study in Serbian patients, it has been reported that the frequency of *parkin* mutation depends on the ethnic origin of patients.<sup>15</sup> A small study from the USA recently found a low frequency of *parkin* mutations among Caucasian patients with early onset PD.<sup>16</sup>

## CONCLUSION

We identified two rare homozygous mutations of the *parkin* gene in the first non-white series of PD cases, and we report the first *parkin* mutations in Indian patients with possible autosomal recessive early onset PD. The phenotypes of our cases were not at variance with the typical presentations of *parkin* linked PD. We found seven exonic changes including three novel changes in separate patients among our patients with sporadic PD. These may represent pathogenic heterozygous states. The homozygous deletional mutations in exons 8 and 9 in the *parkin* gene identified in one of our patients can result in the formation of a truncated protein. The Thr240Met missense found in another patient can compromise the function of the parkin protein. Our finding of truncation/missense mutations provides additional evidence that the phenotype of this form of parkinsonism is due to impairment of function of the parkin protein.

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