Relative high frequency of the c.255delA parkin gene mutation in Spanish patients with autosomal recessive parkinsonism

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Patients with parkinsonism associated with parkin gene mutations usually present an autosomal recessive pattern of inheritance and have early onset disease, with a mean age at onset of around 30 years. The disease is also characterised by symmetrical involvement, slow progression, foot dystonia at onset, hyperreflexia, good response to levodopa therapy, and early levodopa induced dyskinesias. However, unusual features such as late onset disease, which can begin at an age similar to that of typical Parkinson’s disease (PD), a rapidly progressive course, or the presence of gait ataxia have been described in patients with parkin gene mutations.

Many parkin gene mutations (exonic deletions and multiplications, and point mutations) have been described in patients from different populations. We have searched for parkin gene mutations in our PD patients in order to identify new mutations and to define further the clinical spectrum associated with these mutations.

MATERIALS AND METHODS

Subjects
Patients fulfilling the clinical diagnostic criteria for PD proposed by the UK Parkinson’s Disease Society Brain Bank, except for the exclusion criteria of familial disorder, were recruited from the PD Movement Disorder Unit of the Hospital Clinic of Barcelona. Control subjects were recruited among healthy donors without familial history of PD or tremor. Parkinsonian patients with either age at onset ≤40 years or an autosomal recessive pattern of inheritance were selected for genetic study. The study was approved by the ethics committee of our hospital.

Genetic study
DNA was isolated from patients’ leukocytes by standard procedures. The different exons of the parkin gene were amplified by polymerase chain reaction (PCR) using primers and conditions as described. A single strand conformational polymorphism (SSCP) analysis of the PCR products was carried out through electrophoresis in two different conditions: 7.5% polyacrylamide gel at room temperature and 12% polyacrylamide gel at 4°C. The bands were visualised after silver staining. PCR products with an abnormal motility pattern were sequenced. The normal cDNA sequence of parkin gene (DNA Data Bank of Japan: AB009973) was obtained from the GenBank database (http://www.ncbi.nlm.nih.gov/Genbank).

Statistical assessment
Means were compared with the non-parametric Mann-Whitney U test. Frequencies were compared with the χ² test.

RESULTS
We identified 37 index patients (17 women, 20 men) fulfilling the criteria proposed for the screening of mutations in the parkin gene. Twenty four were familial PD cases, with other siblings affected, and 13 were sporadic early onset PD cases. Parkin gene mutations were found in seven index patients (19%; table). Six patients had homozygous mutations that consisted of two frameshift mutations (c.255delA found in four patients and c.972delG found in one patient) and one exon rearrangement (exon 5–6del). One patient had a heterozygous mutation (Val15Met). We sequenced the coding region of parkin gene in this patient and his brother, who also carried the mutation and was affected by the disease, but we did not find any other mutations. The Val15Met mutation was not found in 120 chromosomes belonging to 30 PD patients and 30 healthy control subjects, supporting the fact that this could be a new missense mutation. In addition to these mutations, we identified two polymorphisms in our patients. Val/Leu380 was found in two unrelated families with late onset parkinsonism and Asp/Asn394 in a patient with sporadic juvenile onset parkinsonism.

Abbreviations: PD, Parkinson’s disease; PCR, polymerase chain reaction; SSCP, single strand conformational polymorphism; UPDRS, unified Parkinson’s disease rating scale
Two of seven patients with parkin gene mutations were sporadic cases. Six patients were Spanish and one came from Morocco (PK-11). The mean age at onset (SD) was 32.8 (8.6) years in patients with mutations and 44.4 (15) years in patients without mutations (p = 0.02). Dystonia was only found in two patients with parkin gene mutations. These patients developed the disease at the age of 20 and 30 years, respectively. All patients had a good response to levodopa and/or other dopamine agonists. The mean (SD) motor score of the unified Parkinson's disease rating scale (UPDRS) in the "on" stage was 23.4 (8.6). One of three patients (PK-205) treated with levodopa showed choreiform "on" dyskinesias.

The c.255delA mutation was found in four unrelated patients. All patients but one were living in Barcelona but they were originally from different regions in Spain. Parents' consanguinity was confirmed only in one case (patient PK-21). The age at onset of the disease ranged from 30 to 41 years. All patients developed symmetrical parkinsonian signs (tremor, rigidity, and bradykinesia); however, the clinical features at onset varied among the patients. Only one patient (PK-184) presented with feet dystonia at onset. This patient is now 65 years old and she is treated only with the dopamine agonist bromocriptine. Another patient (PK-21) was initially diagnosed to have essential tremor because for several years he only exhibited postural tremor on his hands that responded partially to propanolol. He is now treated with very low doses of levodopa and selegiline. The third patient (PK-131) began her disease with tremor in the upper limbs, which was present at rest but increased in frequency notably when she held the arms outstretched. The last patient (PK-201) presented with slow gait and short steps and had an excellent response to bromocriptine, trihexyphenidyl, and selegiline, eight years after disease onset.

The genetic study of siblings of patients PK-21 and PK-131 lead us to identify heterozygous and homozygous carriers of the mutation. One homozygous carrier (L97–516) was a 47 year old woman who was the sister of patient PK-21. She did not present parkinsonian signs at the time of examination. However, another homozygous carrier (L00–379), a 42 year old woman, who was the sister of patient PK-131 and had not previously been diagnosed with PD, showed bradykinesia and slight symmetrical rigidity on the extremities, associated with slight postural tremor on her hands. None of the heterozygous carriers of the mutation presented parkinsonian signs.

Both homozygous and heterozygous mutated alleles showed a clear and reproducible pattern of bands by SSCP that were different from those obtained for normal alleles (fig 1). Thus, we considered that SSCP was an easy method to investigate the presence of the c.255delA mutation in normal subjects in order to estimate the prevalence of this mutation in our population. Subsequently, we screened for the c.255delA mutation in 100 healthy control subjects and found a single subject with an abnormal SSCP pattern suggesting the presence of the mutation in heterozygosity. The mutation was confirmed after sequencing the PCR product. Application of the Hardy-Weinberg principle allowed us to calculate an approximate genetic risk of 1/40 000. We therefore estimate that about 1000 individuals could be homozygous carriers for the mutation in the Spanish population.

**DISCUSSION**

We found parkin gene mutations in two sporadic early onset PD cases and in five familial PD cases with an autosomal...
We are aware that some mutations could have been missed in our study because of the approach we have used to detect mutations. Although the sensitivity of the SSCP analysis performed using two different conditions is high, it is not 100%. Moreover, heterozygous exon deletions or exon multiplications require a semiquantitative PCR for their detection. Unfortunately, this technique is not yet available in our laboratory. However, our work confirms the presence of parkin gene mutations in Spanish PD patients and contributes to the characterisation of the phenotype associated with these mutations. Moreover, it has allowed us to outline a relative high prevalence of the c.255delA mutation in our population. The clinical spectrum of parkin gene mutations remains open to discussion and further genetic and pathological studies are still needed to confirm our findings.

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Competing interests: none declared

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Received 19 February 2002
In revised form 31 July 2002
Accepted 31 July 2002

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