Mutational analysis of the PINK1 gene in early-onset parkinsonism in Europe and North Africa

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Parkinson’s disease is a frequent disorder caused primarily by the loss of dopaminergic neurons of the substantia nigra. Mutations in the PTEN-induced kinase (PINK1) gene, in addition to those in parkin and DJ-1, have been found in families with recessive early-onset Parkinson’s disease. We screened for parkin and PINK1 mutations in a panel of 177 autosomal recessive Parkinson’s disease families with ages at onset ≤ 60 years, mostly from Europe. In 7 unrelated families, we identified 10 pathogenic PINK1 mutations (5 missense, 2 nonsense and 3 frameshift deletion mutations), 8 of which were novel. All the mutations were in the homozygous or compound heterozygous states. Interestingly, pseudo-dominant inheritance was observed in a family with two different mutations. The clinical characteristics of 12 PINK1 patients and 114 parkin patients were similar, even for signs such as dystonia at onset and increased reflexes, which were thought to be specific to parkin. In contrast, onset in patients with PINK1 mutations was earlier and increased reflexes were found more frequently than in patients without PINK1 or parkin mutations. These results suggest that PINK1 is the second most frequent causative gene in early-onset Parkinson’s disease with a slowly progressive phenotype, indistinguishable from early-onset patients with parkin mutations.

Keywords: early-onset parkinsonism; PINK1; parkin; mutation

Abbreviations: EOP = early-onset parkinsonism; PINK1 = PTEN-induced putative kinase; UPDRS = Unified Parkinson’s Disease Rating Scale


Introduction

Parkinson’s disease, the second most frequent neurodegenerative disorder, is caused primarily by loss of dopaminergic neurons of the substantia nigra. Studies of familial forms of the disease have led to the identification of a growing number of responsible loci/gene, which offer new insight into the pathological mechanisms underlying the degenerative process (Polymeropoulos et al., 1997; Kitada et al., 1998; Leroy et al., 1998; Singleton et al., 2003; Paisan-Ruiz et al., 2004; Valente et al., 2004a; Zimprich et al., 2004). Three of the ten loci/gene identified so far are responsible for autosomal recessive early-onset parkinsonism (EOP). Mutations in the parkin gene (PARK2) are a relatively frequent cause of parkinsonism with onset before the age of 50, and a prevalence of ~50% in families with autosomal recessive EOP and 15% of patients with isolated EOP has been reported (Lucking et al., 2000; Periquet et al., 2003). The phenotype of patients with parkin mutations is mild and is found mostly in cases with onset before the age of 45 (Lohmann et al., 2003). DJ-1 mutations (PARK7) are much less common than parkin mutations (frequency <1%), but the phenotype is similar (Abou-Sleiman et al., 2003; Bonifati et al., 2003; Hague et al., 2003; Ibanez et al., 2003). Recently,
mutations in the PTEN-induced putative kinase (PINK1) at the PARK6 locus have been described in familial and sporadic EOP. Several studies have described point mutations or deletions in the PINK1 gene, which are less frequent than parkin but more frequent than DJ-1 mutations (Hatano et al., 2004; Healy et al., 2004; Rogaeova et al., 2004; Rohe et al., 2004; Valente et al., 2004a; Valente et al., 2004b; Bonifati et al., 2005; Klein et al., 2005).

The PINK1 gene encodes a 581 amino acid protein, which is highly homologous to the serine–threonine kinases of the calcium/calmodulin family (Valente et al., 2004a). Its ubiquitously expressed transcript is predicted to encode a 34 amino acid mitochondrial targeting motif and a highly conserved protein kinase domain. The function of the PINK1 protein has not yet been determined, but its location in mitochondria suggests that it may phosphorylate mitochondrial proteins in response to cellular stress protecting against mitochondrial dysfunction (Valente et al., 2004a).

To further evaluate the pathogenic role of PINK1 in EOP in Europe, and also in North Africa, we performed linkage analyses in a series of 177 families with autosomal recessive EOP. After exclusion of parkin, among the 87 remaining families, 34 with putative linkage to PARK6 were examined for PINK1 mutations. In order to better define the PINK1 phenotype, we compared clinical data of patients with PINK1 mutations to those of patients with parkin mutations and with those of patients without PINK1 or parkin mutations.

Subjects and methods

Patients

We selected 177 index cases according to the following criteria: (i) at least two signs of the parkinsonian triad (resting tremor, bradykinesia, rigidity); (ii) a good response to levodopa therapy; (iii) age at onset ≤60 years in at least one affected family member and (iv) inheritance compatible with autosomal recessive transmission. Exclusion criteria were the presence of extensor plantar reflexes, ophthalmoplegia, early dementia or early autonomic failure. A majority of the index cases (n = 124) were from families with at least two affected members and 53 were isolated cases without family histories of Parkinson’s disease but were consanguineous. They originated from France (n = 91), Italy (n = 26), Portugal (n = 9), Great Britain (n = 8), The Netherlands (n = 5), Spain (n = 2), Germany (n = 2), Poland (n = 1), North Africa (n = 16) or other countries (n = 17). Neurological examinations were performed with a standardized protocol, including the Unified Parkinson’s Disease Rating Scale (UPDRS) motor score, the Hoehn and Yahr scale and the Mini Mental State Examination (MMSE). The patients were videotaped. Local ethics committees approved the study and written consent was obtained from all participants. Peripheral blood was collected from each patient and DNA was extracted from leucocytes according to standard procedures.

Genetic studies

We did not screen for DJ-1 mutations, since they were extremely rare in our population of patients with Parkinson’s disease (Ibanez et al., 2003). A group of 143 patients were screened for parkin mutations, which were found on both alleles in 66 index cases and one parkin mutation (on a single allele) in 24 by direct sequencing of the parkin gene and exon dosage methods as described elsewhere (Periquet et al., 2003). The remaining 53 patients without parkin mutations and an additional 10 patients who were excluded from linkage to the PARK2 locus by haplotype analysis as previously described (Periquet et al., 2001), as well as 24 who were considered to be suitable for PARK6 haplotype analysis, although they had not been tested for parkin mutations, were screened for PINK1 mutations.

For linkage analysis, the D1S478 microsatellite and two newly developed short tandem repeat markers (D1S3788 and D1S3787) were typed at the PARK6 locus. The D1S478 and D1S3788 markers were positioned ~300 and 0.3 kb, respectively, from the 5′ end and D1S3787 ~3.5 kb from the 5′ end of the PINK1 gene. Putative linkage was determined by the presence of either haplotype identity for the markers or homozygosity in consanguineous families (n = 34). All the genotypes were generated by polymerase chain reaction (PCR) using fluorescently labelled primers and analysed on an ABI 3730 automatic DNA analyser. Sequences of the primers and PCR conditions are available upon request. Haplotypes were constructed manually allowing for a minimum number of recombinations.

In index cases of families putatively linked to PARK6 locus, the eight exons of PINK1 and their exon/intron boundaries were amplified by PCR, using standard methods and previously described primers and PCR conditions (Valente et al., 2004a). Bidirectional dideoxy chain terminator sequence products were loaded on an ABI 3730 automated sequencer and analysed with DNA Sequencing Analysis (version 5.1) and Seqscape (version 2.1.1) softwares (Applied Biosystems). Nucleotide position and the predicted protein sequences were derived from the mRNA sequence (accession number NM8032409). Multiple sequence alignments of the human PINK1 protein and its closest homologues were produced with the ClustalW program using the European Bioinformatics Institute server (http://www.ebi.ac.uk/clustalw/).

Available members of families with PINK1 mutations were analysed to verify segregation of the mutation. All PINK1 exons, except for exons 2 and 4, were analysed by direct sequencing in 174 healthy controls of European origin (mean age at examination: 62.7 ± 9.5 years) to verify the absence of the newly identified PINK1 mutations.

Statistical analysis

Comparisons between patient groups were made with the chi-square test for qualitative variables and ANOVA for quantitative variables.

Results

The genotypes of 34 out of the 87 families tested were compatible with linkage to PARK6. Seven of the index patients (five familial and two isolated cases, four of whom were from France), who were unrelated, had PINK1 mutations (Table 1). The overall frequency of PINK1-positive families in the 177 families analysed was 4% (7 out of 177). The frequency of patients with parkin mutations in the population specifically tested for parkin was 59% (90 out of 153).
Ten different mutations were identified. Two have already been described (E240K, Rogaeva et al., 2004; R492X, Hatano et al., 2004), and eight were novel.

We found three homozygous missense mutations (E240K, G386A, and G409V) in exons 3 and 6 in three consanguineous families, two originating from France and one from Sri Lanka (Fig. 1 and Table 1). We also detected three small deletions of 1, 4 and 32 bp corresponding to mutations L519fsX522 (exon 8), C549fsX553 (exon 8) and K24fsX54 (exon 1), respectively (Table 1). The C549fsX553 and K24fsX54 mutations were present in the compound heterozygous state in a French family whereas the L519fsX522 frameshift deletion in exon 1 is located within the predicted 34 amino acid mitochondrial targeting motif upstream of the cleavage site for mitochondrial processing peptidase. Premature termination by this mutation could lead to a truncated protein that lacks 527 amino acids, including the whole highly conserved protein kinase domain. In addition, the G386A mutation is located within the putative chelation site at residues 384–386. The other mutations are also certainly pathogenic, because they result in variably truncated proteins. Interestingly, the K24fsX54 frameshift deletion in exon 1 is located within the predicted 34 amino acid mitochondrial targeting motif upstream of the cleavage site for mitochondrial processing peptidase. Premature termination by this mutation could lead to a truncated protein that lacks 527 amino acids, including the whole highly conserved protein kinase domain. On the other hand, the L519fsX522 and C549fsX553 frameshift mutations, which both affect exon 8 of PINK1, are predicted to lead to a protein lacking the 59 and 28 C-terminal amino acids, respectively. The Q456X and R492X nonsense mutations are also predicted to truncate the C-terminus of the PINK1 protein containing the end of the protein kinase domain and the putative phosphorylation site at threonine residue 545.

One PINK1 substitution, A383T, present in the heterozygous state in a 42-year-old consanguineous Moroccan patient with onset at age 36, was not found on control chromosomes but is probably a neutral variant since it substitutes a hydroxyl residue for an aliphatic residue that is not highly conserved between species. The fact that no second mutation was found in this patient also supports the hypothesis that this variant is not pathogenic.

We also identified eight common single nucleotide polymorphisms (SNPs) in cases and controls including intronic variants [IVS1-65C > G, IVS1-7G > A, IVS4-5A > G, 3'-untranslated region (3'-UTR) 38T > A], a synonymous SNP in exon 1 (L63L) and three non-synonymous SNPs
(Q115L in exon 1, A340T in exon 5, N521T in exon 8) (Valente et al., 2004a; Klein et al., 2005). In addition, we identified three as yet undescribed PINK1 polymorphisms. These novel changes of unknown pathogenic significance comprise two intronic SNPs: the IVS6 +43C > T variant present in the homozygous state in a single consanguineous patient and in the heterozygous state in 13 out of 174 (7.5%) individual controls and the other frequent variant 5'-UTR 38T > A found in patients and controls. Sequencing of the exon 6 of PINK1 revealed a silent substitution (c.1173 T > C;
D391D) present only in the heterozygous state in 2 out of 174 controls (1.1%).

Clinical characteristics

There were 12 patients in the 7 families with PINK1 mutations. The mean age at onset was 35 ± 8 years, and ranged from 21 to 52. All had the typical signs of Parkinson’s disease but with a younger age at onset and slower disease progression than in idiopathic Parkinson’s disease. We compared the phenotype of 12 patients with PINK1 mutations to those with mutations in the parkin gene including index cases and available family members (n = 114). Patients with PINK1 mutations had clinical findings similar to those with mutations in the parkin gene, although the latter had a tendency toward earlier onset. The median age at onset in patients with parkin mutations was 31, whereas that of patients with PINK1 mutations was 35 (Table 2 and Fig. 3).

Table 2

Comparison of clinical characteristics of patients with PINK1 or parkin mutations and patients without parkin or PINK1 mutations

<table>
<thead>
<tr>
<th></th>
<th>Patients with 2 PINK1 mutations</th>
<th>Patients with 2 parkin mutations</th>
<th>Non-PINK/non-parkin</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex ratio (male/female)</td>
<td>6:6</td>
<td>12</td>
<td>114</td>
<td>45:34</td>
</tr>
<tr>
<td>Age at onset (years)</td>
<td>34.8 ± 8.3 (21–52)</td>
<td>30.3 ± 10.4 (7–58)</td>
<td>46.8 ± 12.1 (12–60)</td>
<td>79 0.001</td>
</tr>
<tr>
<td>Age at examination (years)</td>
<td>55.8 ± 13.1 (36–73)</td>
<td>47.7 ± 13.2 (16–89)</td>
<td>60.4 ± 13.3 (22–81)</td>
<td>76 0.26</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>20.9 ± 12.7 (4–45)</td>
<td>16.8 ± 10.9 (1–58)</td>
<td>13.8 ± 8.6 (1–54)</td>
<td>76 0.015</td>
</tr>
<tr>
<td>Signs at onset %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micrographia</td>
<td>8</td>
<td>1/12</td>
<td>23</td>
<td>25/107 32</td>
</tr>
<tr>
<td>Bradykinesia</td>
<td>75</td>
<td>9/12</td>
<td>58</td>
<td>62/107 57</td>
</tr>
<tr>
<td>Rest tremor</td>
<td>50</td>
<td>6/12</td>
<td>63</td>
<td>67/107 54</td>
</tr>
<tr>
<td>Dystonia</td>
<td>17</td>
<td>2/12</td>
<td>28</td>
<td>26/94 5</td>
</tr>
<tr>
<td>Clinical signs at examination %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rigidity</td>
<td>100</td>
<td>10/12</td>
<td>94</td>
<td>105/112 95</td>
</tr>
<tr>
<td>Bradykinesia</td>
<td>100</td>
<td>10/12</td>
<td>95.5</td>
<td>107/112 100</td>
</tr>
<tr>
<td>Rest tremor</td>
<td>75</td>
<td>9/12</td>
<td>75</td>
<td>82/109 76</td>
</tr>
<tr>
<td>Increased reflexes LL</td>
<td>33</td>
<td>4/12</td>
<td>29</td>
<td>27/92 11</td>
</tr>
<tr>
<td>UDPRS III off</td>
<td>33 ± 3</td>
<td>3</td>
<td>38 ± 23</td>
<td>34     37 ± 17</td>
</tr>
<tr>
<td>UDPRS III on</td>
<td>22 ± 10</td>
<td>8</td>
<td>25 ± 20</td>
<td>82     29 ± 18</td>
</tr>
<tr>
<td>Hoehn and Yahr on</td>
<td>2.5 ± 1.2</td>
<td>7</td>
<td>2.3 ± 0.9</td>
<td>82     2.8 ± 1.2</td>
</tr>
<tr>
<td>MMSE† (/30)</td>
<td>28.4 ± 2.2 (23–30)</td>
<td>28.6 ± 2.2 (18–30)</td>
<td>26.6 ± 4.2 (10–30)</td>
<td>47 0.19</td>
</tr>
</tbody>
</table>

*Comparison between patients with PINK1 mutations and patients without parkin or PINK1 mutations; P non-significant between patients with parkin mutations and patients with PINK1 mutations (values not shown in the table); †no test possible since 100% occurrence in both groups; ‡MMSE, Mini Mental State Examination.

Fig. 2 Multiple alignments of PINK1 protein homologues in Clustal format. Missense mutations changing amino-acid highly conserved through evolution are highlighted. (Ensembl gene IDs: Homo sapiens ENST00000289840, Pan troglodytes ENSPTRT00000000572, Rattus norvegicus ENSRNOT000000020820, Mus musculus ENSMUST00000030536, Danio rerio ENSDART00000035872, Fugu rubripes SINFRUT00000129020, Anopheles gambiae ENSSANGT00000003537, Drosophila melanogaster CG4523-RA, Caenorhabditis elegans EEED8.9; and Macaca fascicularis gi15451340, dbjBAB64474.1)

C125G E240K L369P G386A G409V

HomoSapiens RAVSAQEIQQNTMSQDEVPAS KSDNHIYVEAD-P VIADCCLLAD YVDRCCNGCLM
PanTroglodytes RAVSAQEIQQNTMSQDEVPAS KSDNHIYVEAD-P VIADCCLLAD YVDRCCNGCLM
MacacaFascicularis RAVSAQEIQQNTMSQDEVPAS KSDNHIYVEAD-P VIADCCLLAD YVDRCCNGCLM
RattusNorvegicus RAASAQEIQQSKMSQDEVPAS KSDNHIYVEAD-S VIADCCLLAD YVDRCCNGCLM
MusMusculus RAASAQEIQQSKMSQDEVPAS KSDNHIYVEAD-S VIADCCLLAD YVDRCCNGCLM
DanioRerio TSSAQDIEIQA RSGMDEVPSC KSDNHIYELDF-N VITDCCCLAD YVDRCCNGCLM
FuguRubripes KSDAQDIEIQA RSGMDEVPSC KSDNHIYELDF-N VITDCCCLAD YVDRCCNGCLM
AnophelesGambiae ELEAYWEIRHKAMYKETVPK KSDNHIYELIR-P VITDCCCLAD EIDKGNVALM
DrosophilaMelanogaster ELEGYIREIREAYKETVPKR SNSNYIEIQ-D VIADCCCLAD DVDKGNAAALM
CaenorhabditisElegans RNVQLVLRQDOKGMMLEAPYP KSDNHIYELDFD VVADCCICLAD EVSLGNNAKTM
2.8 ± 1.2; \( P = 0.49 \) after a significantly longer disease duration (20.9 ± 12.7 versus 13.8 ± 8.6 years; \( P = 0.015 \)) compared with patients without \( PINK1 \) or \( parkin \) mutations, who had been included using the same criteria. As in cases positive for \( parkin \), dystonia at onset was more frequent in cases positive for \( PINK1 \) compared with those without \( PINK1 \) or \( parkin \) mutation (17% versus 5%, ns). Increased reflexes in the lower limbs (33% versus 11%, \( P = 0.04 \)) were more frequent in the group with \( PINK1 \) mutations than in the patients without \( PINK1 \) or \( parkin \) mutation. It is interesting to note that the phenotype may vary within the same family. In family SAL-727 with two truncating mutations, the ages at onset differed by 21 years.

There were no significant phenotypical differences between patients with truncating \( PINK1 \) mutations compared with those with missense mutations, but the number of patients was too small to detect slight differences (Table 1). Some families, however, might have specific features. Three affected women from family FPD-125 had worsening of their motor signs with changes in hormonal status, although two had a homozygous Q456X mutation and the other heterozygous Q456X and C125G. This was not noted in the three other women with \( PINK1 \) mutations. Psychiatric manifestations were noted in three patients and were severe in the two sisters with the homozygous Q456X mutation.

**Case reports**

**FPD-071-1**

This woman was born in 1929 on Reunion Island, where her family, of French origin, lived for two generations. Her parents were related. After vaccination for yellow fever, she developed, at age 21, rest tremor in the upper limbs that predominated in the right arm, which was diagnosed as a psychosomatic disease. Seventeen years later, treatment with trihexyphenidyl and ponatal was initiated without benefit. The following year, after worsening of gait, treatment was changed to a combination of trihexyphenidyl and orphenadrine hydrochloride. The patient reported some improvement of symptoms. At age 41, she was treated with levodopa and improved dramatically. Several months later she developed severe dopa-induced dyskinesias and treatment was stopped. At that time, the patient started to be depressed. She was hospitalized for 7 years in a psychiatric department and used her anti-parkinsonian treatment irregularly. The clinical signs of parkinsonism were very mild, and the diagnosis of Parkinson’s disease was not made until 20 years after disease onset. At age 56, clinical symptoms worsened and cognitive impairment was noticed. At age 66, she had tremor, akinesia and her cognitive functions were impaired but were not tested. She died at the age of 68 from septicaemia after a urinary infection. She was found to have a homozygous G386A mutation in \( PINK1 \).

**FPD-125**

**FPD-125-8**

This woman of Italian origin developed akinesia on the right side associated with dystonia on the right foot at age 24, a few weeks after hormonal treatment for hyperpilosity. She has always noted an increase of clinical signs in relation with hormonal changes (menstruation, p-pill, etc...). The patient reported having some unspecific symptoms at the age of 12, affecting coordination and her ability to run. At the ages of 16 and 26, she was treated for depression and anxiety. At age 34, clinical examination showed mild rest and action tremor associated with an akinetic-rigid picture. She complained about painful episodes of torticollis. She improved on 500 mg of levodopa and deprenyl per day. Her UPDRS score under treatment was 22 out of 108. Two years later the patient was hospitalized for major depression, panic attacks and social phobia that were treated with citalopram. The parkinsonian symptoms did not change, but episodes of levodopa-induced fluctuations, dyskinesia and painful dystonia increased at age 42. The UPDRS score improved from 30 out of 108 to 2 out of 108 when levodopa was increased to 800 mg/day. Because of the side effects of treatment, the daily dose of medication was decreased to 600 mg of levodopa and 7.5 mg bromocriptine that improved the painful dystonia and dyskinesia. She was homozygous for Q456X \( PINK1 \) mutation.

**FPD-125-6**

Her sister (FPD-125-8), who developed bilateral akinesia in the legs at age 36 during her first pregnancy, also described a relation between hormonal events and the aggravation of...
clinical signs. Two years later during her second pregnancy at the age of 39, she developed axial rigidity and complained about episodes of torticollis. Clinical examination showed a very mild rest tremor in the hands associated with an akinetic-rigid syndrome that affected the lower more than the upper limbs. Her UPDRS score under 100 mg of levodopa and 1 mg of dopergin per day was 14/108. The results of other clinical examinations were normal. At age 47, she was treated with 300 mg of levodopa daily. She had levodopa-related dyskinesias, fluctuations and foot dystonia, but her clinical signs had not progressed. Since the age of 30, she has received medication for recurrent episodes of depression. She had the same homozygous Q456X mutation as her sister.

**FPD-125-2**

Her mother developed mild hemi-parkinsonism at age 41. She reported marked pre-menstrual worsening of motor disability. At age 71, clinical examination showed no relevant progression of the disease. The patient was treated with 300 mg of levodopa and 7.5 mg of selegiline daily. Her UPDRS score was 22.5/108. She had some urinary urgency. Her Mini Mental Score was normal (27/30). She was compound heterozygote with the mutations Q456X/C125G.

**Family SAL-727**

Four of seven siblings of this family of French origin were affected. The oldest brother developed clinical signs at age of 52, the second at 40 and the youngest sister at 31. The third oldest brother was reported to be affected and died at age 55 from a heart attack; no detailed clinical information about him was available. All three surviving patients started the disease with bradykinesia of the lower limbs. After 21, 27 and 36 years of disease evolution, clinical examinations showed typical, but very mild, Parkinsonism treated with low daily doses of levodopa (500, 450 and 600 mg). The sustained improvement under treatment was estimated at 90%. The two brothers died at the ages of 83 and 78 years from cancer. They carried two mutations K246X54/C549fsX553 both of which are highly deleterious for the PINK1 protein in contrast to the very mild clinical picture.

**Discussion**

Several studies have reported pathogenic mutations in the *PINK1* gene associated with familial and sporadic EOP in different countries (Hatano et al., 2004; Healy et al., 2004; Rogaeva et al., 2004; Rohe et al., 2004; Valente et al., 2004a; Valente et al., 2004b; Bonifati et al., 2005; Klein et al., 2005). We extended these findings to France and some non-European countries (Algeria, Sri Lanka). In a series of 177 index patients with autosomal recessive Parkinson’s disease and an age at onset ≤60 years in at least one affected member, we performed PARK6 haplotype analyses in 87 families in which mutations in the *Parkin* gene were excluded or not tested. In the 34 putative PARK6-linked families, we found seven unrelated cases, four from France (five families and two isolated cases), with mutations in *PINK1* gene. Ten mutations were identified, eight of which were new (C125G, G386A, G409V, L369P, Q456X, L519fsX522, C549fsX553, and K246fsX54). One of the families, in which the PINK1 genotype was different in two generations, is the first reported case of pseudo-dominant inheritance of this disease.

The overall frequency of PINK1-positive families was 21% (7 out of 34) of putative PARK6-linked families and 4% (7 out of 177) of all the families in the series tested. The relative frequency may be under-estimated, however, because we did not perform any exon dosage assay, and may have missed large exon deletions or multiplications. Nevertheless, our results are in accordance with previous studies reporting relative frequencies of PINK1 mutations (0–15%), which varied according to the origin of the EOP populations screened (Hatano et al., 2004; Healy et al., 2004; Rogaeva et al., 2004; Valente et al., 2004b; Bonifati et al., 2005; Klein et al., 2005). This is much lower than the frequency of parkin (90 out of 153; 59%) in our series, *PINK1* is still, however, the second most common gene responsible for autosomal recessive EOP after *parkin*.

All the mutations found in the study, five missense, two nonsense and three frameshift, were homozygous or compound heterozygous. Their pathogenicity is established, because (i) they cosegregate with the disease; (ii) they are predicted to truncate the PINK1 protein or to change evolutionarily conserved amino acids and (iii) they were not found on a large series of chromosomes from healthy controls. Furthermore, 6 out of 10 of the mutations were in the predicted kinase domain of the PINK1 protein. The kinase activity of PINK1 has been confirmed recently and several mutations located within the highly conserved kinase domain have been shown to destabilize PINK1 and dramatically reduce its kinase activity (Beilina et al., 2005). Most interestingly, another mutation, the K246X54 frameshift deletion, fell within the predicted N-terminal mitochondrial targeting peptide. Recent studies *in vitro* (Beilina et al., 2005) have shown that PINK1 is processed at the N-terminus consistent with mitochondrial import. Mutations at this site may affect the localization of the protein.

In addition to the eight polymorphisms also present in the control population, we detected a unique new variant (A383T) present in a single patient. The A383 residue is not highly conserved and its presence in the heterozygous state in a single consanguineous patient indicates that it is probably a chance finding. It is of note that the rate of heterozygous mutations in this study (1 out of 177) is similar to that observed by Healy (1 out of 290) (Healy et al., 2004) but much lower than in other studies where frequencies of ~5% were reported (Valente et al., 2004b; Bonifati et al., 2005; Klein et al., 2005). The causative role of these single heterozygous mutations remains a matter of debate. Previous
comparisons of patients with 1 or 2 PINK1 mutations suggested that onset is earlier in the latter (Valente et al., 2004b; Bonifati et al., 2005). This is controversial (Klein et al., 2005). This observation would support the notion that heterozygous mutations in genes causing autosomal recessive forms with an early onset can also cause later onset Parkinson’s disease, as has been suggested for patients with single parkin mutations (Hedrich et al., 2002; Hoenicka et al., 2002; West et al., 2002; Oliveira et al., 2003). PET studies have shown evidence of dopaminergic dysfunction, increased DAT binding (Kessler et al., 2005), decreased 18F-dopa uptake (Khan et al., 2002) or decreased [123I]FP-CIT SPECT tracer uptake (Klein et al., 2005), in asymptomatic carriers of heterozygous PINK1 mutations that may predispose to late onset disease.

The presence of a single PINK1 mutation might also be fortuitous in the patients and unrelated to their disease, but this hypothesis cannot be evaluated without knowledge of the frequency of carriers of the different variants in the general population. In support of this hypothesis, however, the six heterozygous members of four families in which patients had two PINK1 mutations had no clinical symptoms of parkinsonism at age 39, 67, 69, 69, 73 and 81. Furthermore, none of the non-examined parents of PINK1 patients who were obligate heterozygous carriers were known to have Parkinson’s disease (Fig. 1). These observations indicate that if a single PINK1 mutation is a risk for later onset Parkinson’s disease, it has greatly reduced penetrance.

So far, 29 patients with homozygous or compound heterozygous PINK1 mutations have been reported (Hatano et al., 2004; Healy et al., 2004; Rogaeva et al., 2004, Rohe et al., 2004; Valente et al., 2004a, b; Albanese et al., 2005; Bonifati et al., 2005; Kessler et al., 2005; Klein et al., 2005; Li et al., 2005). With the addition of our 12 new patients, the phenotype consists of typical parkinsonian features with young age at onset (32.9 years ± 7.5, range 18–52 years, n = 41) and an excellent and sustained response to levodopa therapy. In case FPD-071-1, a good response to levodopa therapy (65%) was observed even after 45 years of disease duration. Disease progression is very slow and in several patients there is apparently no clinical worsening over periods of several decades. Cognitive impairment, if present, appears only after long disease durations. As was previously observed in patients with parkin mutations, the age at onset is variable even within the same family (Lohmann et al., 2003).

In the present study, we have compared for the first time patients with parkin and PINK1 mutations. They are phenotypically indistinguishable, although patients with PINK1 mutations tend to have a better response to levodopa, a less severe disease as evaluated by the UPDRS-score and longer mean disease duration. Interestingly, the presence of dystonia at onset and brisk reflexes which were initially considered as typical of parkin carriers appear to be at least as frequent in patients with PINK1 mutations. Although the overall phenotype is early onset Parkinson’s disease, behavioural and mood disorders, increased reflexes, dystonia at onset, early dyskinesias may be more frequent than in idiopathic Parkinson’s disease.

Although there were no clear correlations between specific phenotypic traits and a particular type of mutation, it is of note that three women from the same family reported worsening of their motor signs with changes in hormonal status. Two of them also had severe psychiatric manifestations including depression.

As no neuropathological information on PINK1-positive patients is available at present, it cannot be determined whether Lewy bodies are absent as in most parkin-positive patients and whether neuronal loss is limited to the substantia nigra.

In conclusion, this study of a large series of families and isolated cases with autosomal recessive EOP has established the frequency of PINK1 mutations at 4% in the mostly French population. Although less frequent than parkin, it is the second most frequent cause of autosomal recessive Parkinsonism. They are indistinguishable on the basis of clinical criteria, the typical clinical picture being early-onset Parkinson’s disease with slow progression and a good and sustained response to levodopa. Both disorders, however, show significant group differences compared with patients recruited according to the same criteria but who screened negative for both these genes. Since only two of the ten mutations identified in this study were already reported, the mutation spectrum appears to be large and most mutations have so far been private. Genetic testing for PINK1 is justified in EOP after exclusion of the most frequent parkin mutations.

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References


