Juvenile-Onset Parkinsonism as a Result of the First Mutation in the Adenosine Triphosphate Orientation Domain of PINK1

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Background: Mutations in the PTEN-induced putative kinase 1 (PINK1) gene at 1p36 have been involved in autosomal recessive early-onset parkinsonism.

Objective: To describe the clinical and genetic features of the largest kindred reported to date with early-onset parkinsonism associated with the PINK1 gene.

Design: Clinical and genetic study.

Setting: Collaborative study.

Patients: Eight patients from Sudan with particularly early onset (ages 9-17 years) and phenotypes varying from dopa-responsive dystonia–like to typical early-onset parkinsonism.

Main Outcome Measures: The PINK1 genotype and Parkinson disease status of all available family members.

Results: The disease was caused by a novel mutation, p.A217D, located in the highly conserved adenosine triphosphate orientation site of the PINK1 kinase domain.

Conclusion: This study extends the phenotypic and molecular spectrum of the PINK1 gene and the geographic origin of patients with PINK1 gene mutations.

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Parkinson disease, the second most prevalent neurodegenerative disorder, results from the selective loss of dopaminergic neurons in the nigrostriatal pathway of the brain. The identification of single genes linked to the disease in familial forms of Parkinson disease has yielded crucial insight into the physiopathologic mechanisms underlying neurodegeneration. Mutations in 3 genes have been identified as being responsible for autosomal recessive early-onset parkinsonism (AREOP). The most frequent of these are mutations in the parkin gene (PARK2), with a prevalence of about 50% in families with AREOP. Clinically, parkin mutations usually manifest before the age of 45 years, and the phenotype is mild. Mutations in the second gene (DJ1) causing AREOP (PARK7) are found in approximately 1% of these patients and result in a similar phenotype. The third and most recently described mutation in AREOP is in the PTEN-induced putative kinase 1 (PINK1) gene (PARK6), with an intermediate frequency and clinical characteristics similar to that of parkin.

Mutations in the PINK1 gene have been reported in several countries, most recently in Algeria and Sri Lanka. We report herein a large inbred Sudanese kindred in which 8 individuals, 5 of whom were living, had AREOP associated with a novel PINK1 mutation. Apart from a family from Algeria, we are not aware of a study that describes this form of AREOP in North Africa or the Middle East, where autosomal recessive diseases are prevalent because of the high rate of consanguinity.

Methods

Patients

The kindred originated from North Sudan and the sibships are currently living in Khartoum. Neurological examinations were performed by one of us (M.A.M.S.) at the homes of the 3 branches of the family with a total of 5 living affected individuals. The patients were videotaped. The family history was negative for essential tremor and Alzheimer disease. There was also no medical history of use of neuroleptic drugs before onset of the disease, cerebral ischemia, encephalitis, or drug intoxication in the affected individuals. Also, none of the affected individuals had ophthalmoplegia, dementia, features of autonomic failure, or pyramidal syndrome on examination. Clinical data from the 3 deceased patients was obtained by interviewing their relatives. Peripheral blood samples were collected from each patient, their living parents, and most of their unaffected siblings. Informed consent was obtained from all par-
Participants. Extraction of DNA from leukocytes was performed in Khartoum by one of us (M.M.M.) according to standard procedures. Extracted DNA samples were shipped to Paris, France, for genetic studies.

**GENETIC STUDIES**

Parkin and DJ1 loci were excluded using microsatellite markers as previously described.6,7 The 8 exons of PINK1 and their exon-intron boundaries were amplified by means of polymerase chain reaction analysis, using standard methods and previously described primers and polymerase chain reaction conditions.8 Bidirectional dyeoxy chain terminator sequence products were loaded on an automated sequencer (ABI3730; Applied Biosystems Inc, Foster City, Calif) and analyzed with commercially available software (DNA Sequencing Analysis, version 5.1 and SeqScape, version 2.1.1 [Applied Biosystems Inc]). Nucleotide position and the predicted protein sequences were derived from the messenger RNA sequence (GenBank accession No. NM_032409).

Multiple sequence alignments of the human PINK1 protein and its closest homologues were produced with the ClustalW program (http://www.ebi.ac.uk/clustalw/).

To verify the absence of the newly identified PINK1 mutation in the general population, we analyzed PINK1 by means of direct sequencing in 197 healthy controls, 131 of European origin and 66 of Northern African origin.

The pedigree includes 5 generations with multiple inbreeding loops (Figure 1). The disease was known to have affected 8 family members (6 women and 2 men) in the last 2 generations, and ended fatally in 3 (all women; individuals IV:13, IV:22, and IV:26).

Family member IV:13 started to show symptoms of the disease at the age of 17 years. She was reported to have died suddenly at 25 years of age. Onset was at 10 years of age in her cousin, family member IV:22, who died at 20 years of age after a short febrile illness. Likewise, family member IV:26 died at 20 years of age after a febrile illness, with onset of her disease at 15 years of age.

A summary of the demographic and clinical features of the disease in the 5 living affected individuals is shown in the Table. Their age at onset ranged from 9 to 14 years (mean ± SD age, 12.6 ± 2.1 years). The mean age at onset in the 3 deceased individuals was 17 years (25, 20, and 20 years of age). Figure 1. Pedigree of the kindred with mutations in the PTEN-induced putative kinase 1 gene. ID indicates identification; WT, wild-type. *Family members available for study. The siblings are listed in chronological birth order (oldest to youngest).
set, including that of the deceased patients, was 13.1 years (range, 9-17 years; SD, 2.6 years). The other notable features were (1) the presence of dystonia at onset with a remarkable response to levodopa in 2 patients, which led to the diagnosis of dopa-responsive dystonia; (2) the existence of diurnal fluctuations in all 5 patients; (3) the slow progression and the sustained response to treatment in all patients, even after durations of as long as 20 years; (4) the presence of striatal toe on plantar reflex in 2 patients; and (5) the absence of other atypical neurological signs (eg, dementia or hallucinations, dysautonomia, pyramidal signs, or ophthalmoplegia) in all patients.

Sequencing of the PINK1 gene revealed an homozygous transversion c.650C>G in exon 2 in all the 5 living patients, causing an alanine-to-aspartic acid substitution at position 217 (p.A217D) (Figure 1). All available unaffected relatives were heterozygous carriers or had 2 normal alleles. None of the heterozygous carriers had signs of parkinsonism. This mutation, which was not found in 394 sequenced control chromosomes, is located in the highly conserved LAIK amino acid sequence at position 216-219, corresponding to the adenosine triphosphate (ATP) orientation domain (Figure 2).

**CASE REPORTS**

**Family Member IV:16**

The proband (family member IV:16) was first examined at the age of 26 years. His disease started at the age of 13 years with symmetrical bradykinesia and dystonia of the lower limbs. His symptoms worsened as the day progressed and improved after a night’s sleep or a nap. Despite his motor difficulties, he managed to perform his job as a porter. On examination, he was found to have mild parkinsonian features (bradykinesia, resting tremor, and rigidity). He responded dramatically to combination therapy consisting of 250 mg of levodopa and 25 mg of carbidopa (Sinemet-275) taken as one fourth of a tablet 4 times daily. He was initially diagnosed as having Segawa disease (levodopa-responsive dystonia). Soon thereafter, he developed dyskinesia, which he could tolerate after adjustment in the timing and dosage of his treatment. At 31 years of age, he had to leave his job because of progressive motor disability. At 44 years of age, he was still mobile and could manage with 250 mg of levodopa and 25 mg of carbidopa (Sinemet-275) taken as half of a tablet 5 times daily. When examined half an hour after levodopa therapy, he was mobile, and no rigidity was detected. However, he had mild slowing with reduction in amplitude on finger taps and a monotonic but understandable speech. Tendon jerks and plantar reflex were normal. An upward flinging of the big toe was interpreted as a striatal toe or a pseudo-Babinski sign. Serum copper and ceruloplasmin levels and cranial computed tomography and magnetic resonance imaging findings were normal.

**Family Member IV:20**

Family member IV:20 was first examined at 18 years of age. Onset was at 13 years of age, with bradykinesia of the left side associated with dystonia of the left foot. His symptoms also worsened progressively during the day and improved after sleep. When examined, he was found to have features of mild Parkinson disease. He responded dramatically to levodopa and required only 100 mg of levodopa and 10 mg

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**Table. Clinical Features of 5 Living Patients With Early-Onset Parkinsonism Associated With PINK1 Mutations**

<table>
<thead>
<tr>
<th>Features</th>
<th>IV:1</th>
<th>IV:4</th>
<th>IV:16</th>
<th>IV:20</th>
<th>V:2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td>F</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td><strong>Onset of disease</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age, y</strong></td>
<td>14</td>
<td>14</td>
<td>13</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td><strong>Symptom/sign</strong></td>
<td>Difficulty in initiating movements</td>
<td>Bradykinesia, tremor</td>
<td>Bradykinesia, dystonia</td>
<td>Bradykinesia, dystonia</td>
<td>Bradykinesia, shuffling gait</td>
</tr>
<tr>
<td><strong>Asymmetry</strong></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Age at last examination, y</strong></td>
<td>44</td>
<td>36</td>
<td>46</td>
<td>38</td>
<td>16</td>
</tr>
<tr>
<td><strong>Disease duration, y</strong></td>
<td>30</td>
<td>22</td>
<td>33</td>
<td>25</td>
<td>7</td>
</tr>
<tr>
<td><strong>Signs at examination</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bradykinesia</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Rigidity</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Rest tremor</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td><strong>Diurnal fluctuation</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Brisk tendon jerks in the lower limbs</strong></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>†</td>
<td>+</td>
</tr>
<tr>
<td><strong>Levodopa treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Current dosage, mg/d</strong></td>
<td>187.5</td>
<td>250</td>
<td>625</td>
<td>100</td>
<td>187.5</td>
</tr>
<tr>
<td><strong>Improvement ≥30%</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Duration of treatment, y</strong></td>
<td>2</td>
<td>2</td>
<td>20</td>
<td>20†</td>
<td>2</td>
</tr>
<tr>
<td><strong>Ascertained adverse effects</strong></td>
<td>Minimal dyskinesia that disappeared later</td>
<td>Very remarkable dyskinesia</td>
<td>Remarkable dyskinesia</td>
<td>Remarkable dyskinesia</td>
<td>Minimal dyskinesia</td>
</tr>
</tbody>
</table>

**Abbreviations:** F, female; M, male; PINK1, PTEN-induced putative kinase 1 gene; +, present; −, absent.

*Indicates not regularly treated.
†Indicates contracture deformities.
‡Indicates not regularly treated.

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of carbidopa (Sinemet-110) taken as one fourth of a tablet 3 to 4 times daily. At 36 years of age, he had stopped taking levodopa because of severe levodopa-induced dyskinesias. Instead, he received trihexyphenidyl hydrochloride (2 mg taken twice daily). On examination, he was confined to bed with rigidity of the upper and lower limbs associated with contracture deformities at the elbow and knee joints. In addition, he had a masklike face and choreic movements of the upper and lower limbs. Speech was monotonic and slurred. Cognition seemed to be intact. Plantar reflexes revealed striatal toe. Findings of various investigations, including serum levels of copper and ceruloplasmin and cranial computed tomography, were normal.

Family Member V:2

Family member V:2 was 14 years of age when first examined at her home. The onset of the disease was at 9 years of age, with bradykinesia of the left side (which later became generalized) associated with a shuffling gait, which caused her to leave school. Overall, she felt better in the morning than at midday. On examination (at about 1:30 PM), she could walk slowly with short steps but while holding on to the wall. Speech was characterized by a slight loss of expression and volume. She had cogwheel rigidity in the upper and lower limbs. Deep tendon jerks were brisk. Plantar reflexes were flexor. She was markedly improved with the dosage of levodopa.

Family Member IV:1

Family member IV:1 was first examined at the age of 42 years. Onset of the disease was at 14 years of age, with difficulty in initiating movements and heaviness of her legs, mainly on the left side. The symptoms worsened as the day progressed. On examination, she had mild anemia and decreased volume of speech. She had mild slowing and reduced amplitude of finger taps and mild rest tremor in the upper and lower limbs. Gait examination revealed mild shuffling. Deep and plantar reflexes were normal. Cognitive functions were intact. She was markedly improved with one fourth of a tablet of 250 mg of levodopa and 25 mg of carbidopa (Sinemet-275). When undergoing evaluation at 44 years of age, she was asymptomatic and had minimal levodopa-induced dyskinesia that disappeared after adjusting the dosage of the treatment. She was treated with 250 mg of levodopa and 25 mg of carbidopa (Levocar) taken as one fourth of a tablet 3 times daily, and 5 mg of trihexyphenidyl hydrochloride at night.

Family Member IV:4

Family member IV:4 was first examined at 34 years of age. Her symptoms started at 14 years of age with bradykinesia and heaviness of the right leg associated with tremor of the right hand. Her condition worsened with age, but her mobility was better in the morning than the evening. Maintenance therapy consisted of trihexyphenidyl hydrochloride, 2 mg 3 times daily, and phenothiazine, 25 mg twice daily, with no improvement in her condition. When undergoing evaluation at 34 years of age, she was able to walk but with difficulty and had a masklike face and mono-
tonic, slurred but understandable speech. She had a flexed posture of her elbows with associated dystonic posture of both hands, and moderate rest and action tremor of the hands. She had a marked rigidity of the upper and lower limbs. She had difficulty maintaining her posture while sitting, with retropulsion and a tendency to fall unless supported by the examiner. Deep and plantar reflexes were normal. She showed remarkable improvement in her symptoms after therapy consisting of 250 mg of levodopa and 25 mg of carbidopa (Sinemet-275) taken as one fourth of a tablet 3 times daily. When examined at 36 years of age, she maintained full mobility with therapy consisting of 250 mg of levodopa and 25 mg of carbidopa (Levocar) taken as approximately one fifth of a tablet 5 times daily. Generalized chorea was noted 2 hours after taking levodopa, and lasted for about 15 minutes.

**COMMENT**

The present study describes, to our knowledge, the largest kindred to date with early-onset parkinsonism associated with PINK1 mutations, in which 8 individuals in 2 generations were affected with the disease. It is also the first PINK1 kindred of Arab descent outside Algeria.

The clinical features of the present patients are similar to those previously described for PINK1 or parkin mutations. However, the ages at onset in the present group (9-17 years) are much younger, because all of the patients were juvenile cases and are outside the 95% confidence interval calculated from previously reported ages at onset (18.2-47.6 years). In addition, the presence of a pseudo-Babinski sign (striatal toe) has not been reported previously in patients with PINK1 mutations. The presence of early dyskinesias constituted a significant comorbidity in the family and prompted 1 of the patients (family member IV.20) to stop using levodopa for a considerable period of time. During a period of 18 years, he became wheelchair bound and developed contracture deformities. Three patients died between the ages of 20 and 25 years, but this might reflect the high prevalence of intercurrent infections in a developing country.

Finally, we have identified a novel PINK1 missense mutation (p.A217D). It is most likely pathogenic because it segregates with the disease, changes a highly conserved amino acid in the middle of a stretch of conserved amino acids, is a nonconservative substitution, and was not found in a large series of healthy controls. Moreover, it is located within the kinase domain inside the predicted ATP orientation site, very close to the highly conserved Lys-219 residue that has been shown, when mutated, to inhibit the kinase activity of the protein and abolish the ability of PINK1 to reduce basal caspase-3 activity. It might be postulated that the important functional changes caused by this mutation are responsible for the consistent juvenile onset of the disease in the 3 sibships of this family, which is significantly earlier than what has been observed in other cases with PINK1 mutations.

In conclusion, this study extends the phenotypic and molecular spectrum of PINK1-associated AREOP and the geographic origin of patients with the PINK1 gene mutation.

**REFERENCES**