ELSEVIER

Parkinsonism and Related Disorders 13 (2007) 359–361

Parkinsonism & Related Disorders

www.elsevier.com/locate/parkreldis

Case report

Novel features in a patient homozygous for the L347P mutation in the *PINK1* gene

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Received 17 July 2006; received in revised form 7 August 2006; accepted 8 August 2006

Abstract

The purpose of this study was to assess the genotype-phenotype of *PINK1* mutations. We genotyped eight known mutations in three clinic-based cohorts with Parkinsonism and found one homozygous p.L347P mutation in *PINK1*. Clinically, hypo-osmia and profound diurnal variation of symptoms were identified as novel features; fluorodopa positron emission tomography revealed striking decline in striatal fluorodopa uptake. We suggest that it may be possible to clinically separate this form of Parkinsonism from dopa-responsive dystonia and *Parkin*-related Parkinsonism. Furthermore, as this mutation has only been reported in Filipinos (two originated from Luzon island), our results support the hypothesis of a common founder.

Keywords: Parkinson's disease; *PINK1*; PARK6; Phenotype; F-DOPA PET

1. Introduction

The PARK6 locus was mapped first in a Sicilian family with an autosomal recessive form of Parkinsonism. Mutations in the *PTEN induced putative kinase 1* (*PINK1*) gene were subsequently identified as the cause of this form of Parkinsonism [1]. Causative mutations in *PINK1* have now been identified in Japanese, Israeli, Filipino, Italian, Irish, American and Taiwanese families, indicating that mutations in *PINK1* are distributed world-wide [2–4]. To date, the majority of patients with homozygous or compound heterozygous mutations in the *PINK1* gene have been described as having typical Parkinsonism, but atypical features such as hyperreflexia, psychiatric disturbances, marked orthostatic hypotension, dystonia at onset, and dementia have also been observed. This suggests that the clinical phenotype may be more complex than is seen in typical PD.

The present study was undertaken to determine the prevalence of known *PINK1* mutations in several patient groups in a clinic-based population. Although only one patient was found to carry a homozygous *PINK1* mutation, this case is of particular interest because a detailed description of the phenotype of this particular mutation (p.L347P) has not been previously reported. Furthermore, this is the first such case with a confirmed *PINK1* mutation to undergo fluoro-L-dopa positron emission tomography (F-Dopa PET). The clinical findings in this patient suggest it may be possible to differentiate mutations in the *PINK1* gene from other genetic forms of Parkinsonism based on clinical features. Finally, our patient originated from the Philippines, which is of particular interest as the two patients previously reported with this mutation are also of Filipino origin, raising the possibility of a founder effect.

2. Patients and methods

We screened 299 patients from the outpatient clinic at the Parkinson's Institute from three different cohorts; (i) 21 patients with the diagnosis of Parkinsonism before age 40 years (13 men, eight women, current age range 27–62 years), (ii) 38 subjects with a positive family history (iii) 228 patients

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1353-8020/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.parkreldis.2006.08.009

with typical, late-onset Parkinson's disease (PD) (157 men, 71 women, current age range 50–94 years). One hundred and sixty-two control samples were obtained from spouses/significant others of patients. After written informed consent approved by our institutional review board, blood was collected, and genomic DNA purified.

The known mutations in the *PINK1* gene as of the initiation of the study, namely p.Q239X, p.R246X, p.H271Q, p.G309D, p.L347P, p.E417G, p.R492X, and c.1573_1574insTTAG were genotyped using the Sequenom mass spectrometry technique. Positive results were confirmed by sequencing on an ABI prism 377 DNA Sequencer XL machine (PE Biosystems, USA). To exclude a deletion on the corresponding chromosome, a quantitative gene dosage assay was developed using Power SYBR Green on an ABI Prism 7000. Primers and conditions are available upon request. Sequencing for the DSC-3 C/T change in exon 4 of the *DYT3* multiple transcript system was performed using published primers and cycling conditions [5].

3. Results

One patient was homozygous for the p.L347P mutation in the *PINK1* gene (Fig. 1). No other mutations were detected in 460 patients and controls. We excluded a larger deletion of exon 5 on the corresponding allele by quantitative gene dosage. We performed sequence analysis for DSC-3 of the *DYT3* multiple transcript system because of the Filipino background of our patient, but did not detect the characteristic sequence change found in patients with a DYT3/Lubag phenotype.

4. Case report

A 36 year-old Filipino woman first noticed a resting tremor of her left leg and mild gait difficulties soon after giving birth to her second daughter at age 30. When first seen at our Institute (by JT) at age 34, the shaking had spread to her arms, and she reported a dramatic diurnal variation of her symptoms, such that on awakening she was virtually symptom free through mid-morning. By mid-day she would typically become fully symptomatic. There was no family history of Parkinsonism. Examination revealed a 7–8 per second resting tremor of all limbs and a postural tremor of the upper extremities. Her gait was moderately slowed with a "stuttering" quality. She exhibited prominent axial rigidity and mild dystonia of both feet and her right arm. Because she responded to a low dose of levodopa/carbidopa (50/12.5 mg/d), the possibility dopa-responsive dystonia (DRD) was raised. However, a fluorodopa PET scan showed a profound loss of uptake in the striatum, with putamen more affected than the caudate (Fig. 2).

By age 36, she was taking 2 mg of ropinerol three times per day but was having increasing difficulty managing day-to-day activities. She was also experiencing episodes of profuse sweating. Examination revealed no cognitive impairment, but there was evidence of mild depressed (Hamilton Depression Scale: 11). Her sense of smell was diminished (Score 7/12) on the Brief Smell Identification Test™ (B-SIT, Sensonics, Inc., Haddon Heights, NJ, USA). Deep tendon reflexes were symmetrically brisk. Her

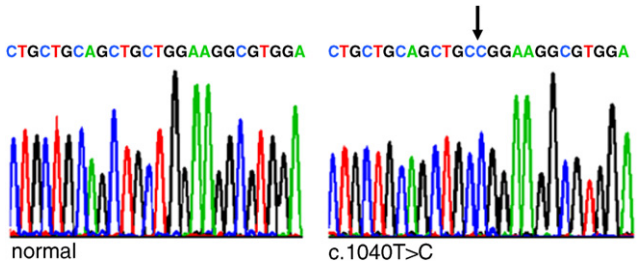

Fig. 1. Electropherogram of the p.L347P mutation in exon 5 of the *PINK1* gene. Left panel shows the normal sequence, right panel the mutation.

UPDRS score was 32 in the off state. She exhibited an intermittent low-amplitude resting tremor of both upper extremities (left slightly worse), and a mild to moderate postural tremor of both upper extremities. Tone was markedly increased in her neck and upper extremities and moderately increased in the legs symmetrically.

5. Discussion

While only one *PINK1* mutation (p.L374P) was identified in this study, this case proved to be interesting and potentially important for several reasons. First, although sleep benefit has been described in other patients with *PINK1* mutations, the diurnal variation in our patient was dramatic early in her illness, as she was virtually asymptomatic for the first few hours each morning, before becoming fully symptomatic by mid-day. In our experience, this degree of diurnal variation is exceptional in any form of Parkinsonism.

This patient was also of interest because she was diagnosed as having DRD early on in her illness. In retrospect, this is not surprising given her young onset, response to L-dopa, diurnal fluctuations, brisk reflexes, dystonic features, and moderate bilateral postural tremor, all of which are all consistent with the phenotype of DRD. These clinical features can also be seen in patients with mutations in the *Parkin* gene (PARK2), which may have a clinically similar phenotype with dystonia at onset, brisk tendon reflexes, and diurnal fluctuation. In fact, Ibanez and colleagues [6] have suggested that patients with Parkin and PINK 1 mutations may be clinically indistinguishable. Furthermore, Parkin mutations and DRD can have a very similar clinical presentation as well; this was highlighted in a series of 22 families with clinically diagnosed DRD, in which three families were found to have mutations in the Parkin gene [7].

Two clinical tests were helpful in narrowing the diagnosis in our patient. First, our patient's B-SIT smell test was markedly abnormal (0%tile compared to control group). This suggested that she did not have a *PARK2* mutation, in which smell is spared. If further studies show that defective olfaction is a consistent feature in patients with *PINK1* mutations, this could be useful to differentiate

these two conditions. The second diagnostic tool, the F-Dopa PET scan, showed marked decline in fluorodopa uptake in the putamen and nucleus caudate, virtually excluding a diagnosis of DRD, and suggesting severe nigrostriatal dysfunction. In a study from Khan and colleagues [8], homozygous and unaffected heterozygous patients linked to PARK6 also exhibited reduction (85%) of fluorodopa uptake on PET scanning in the posterior dorsal putamen, similar to idiopathic PD. The heterozygotes showed a significant 20–30% reduction in caudate and putamen fluorodopa uptake compared to controls but within normal limits [8]. Interestingly, both the PARK6 homozygotes and our patient with the *PINK1* mutation demonstrated reduced F-DOPA uptake in the caudate nucleus. In idiopathic PD F-DOPA uptake in the caudate nucleus is usually much less affected, unless the patient is suffering from atypical Parkinsonism (i.e. multiple system atrophy). Taken together, these observations suggest that it might be possible to dissect the clinical phenotypes between these three conditions by assessing olfaction and carrying out functional imaging thus allowing more accurate prediction of changes in certain genes based on clinical findings.

Perhaps the most important aspect of this report relates to the origin of the patient, who proved to be Filipino. This is only the third reported case of Parkinsonism with a homozygous p.L347P substitution in the *PINK1* gene. Both of the previously reported individuals with this mutation were Filipino as well [9,10]. These observations raise the possibility of a founder effect. At least two of these patients (ours and [9] (personal communication Tony Lang, December 2005)) were from the island Luzon of the Philippines. Interestingly, Rogaeva and colleagues [9] surveyed a population of 50 Filipino controls and found three heterozygotes for the p.L347P mutation, but none in 80 white controls. Further protein analysis for *PINK1* showed marked instability in vitro for p.L347P [11], excluding the possibility of a benign variant. This suggests a carrier frequency of 6% in this population [9], although this observation needs to be replicated and confirmed in a larger cohort. If confirmed, this carrier frequency in the Filipino population would be in the same range as seen for Gaucher disease in Ashkenazi Jews or sickle cell anemia in African-Americans and could therefore have important public health implications in this population.

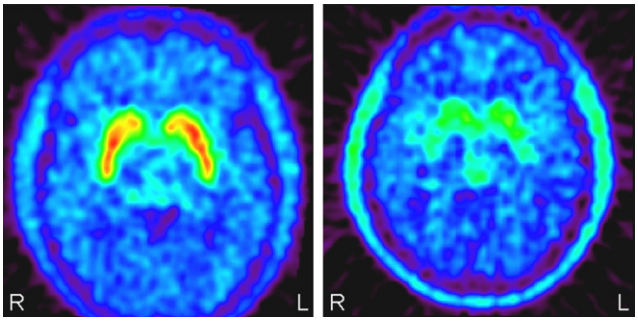

Fig. 2. F-DOPA PET of the patient (right) and normal control (left). Marked loss of uptake is shown in the striatum (putamen > caudate), which is symmetric in the putamen (−6.2 SD right and −6.1 SD left), but more prominent on the left caudate (−3.2 SD left and −1 SD right) in our patient. Arrow points to the mutation c.1040T > C (Ref. Seq. NM_032409.1).

Acknowledgments

We are indebted to the families and patients of this study for their participation. Without their interest, this study would not have been possible. We thank Venus Ilagan, Tracy L. Stewart, Grace S. Liang, and Neng C. Huang for their help ascertaining patients. This study was supported by the Draper Family fund.

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