PINK1 mutations and parkinsonism

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ABSTRACT

Background: *PINK1* loss-of-function causes recessive, early-onset parkinsonism. In Tunisia there is a high rate of consanguineous marriage but *PINK1* carrier frequency and disease prevalence have yet to be assessed.

Objectives: The frequency of *PINK1* mutations in familial parkinsonism, community-based patients with idiopathic Parkinson disease (PD) (non-familial PD), and control subjects was determined. Demographic and clinical characteristics of individuals with *PINK1* homozygous or heterozygous variants, or without *PINK1* mutations, were compared.

Methods: A total of 92 kindreds (with 208 affected and 340 unaffected subjects), 240 nonfamilial PD, and 368 control participants were recruited from the Institut National de Neurologie, Tunis. Clinical examinations included Hoehn &Yahr, UPDRS, and Epworth scales. *PINK1* sequencing and dosage analysis was performed in familial index patients, the variants identified screened in all subjects. Parkin and LRRK2 genes were also examined.

Results: Four *PINK1* homozygous mutations, three novel (Q129X, Q129fsX157, G440E, and one previously reported; Q456X), segregate with parkinsonism in 46 individuals in 14 of 92 families (15%). Six of 240 patients with nonfamilial PD were found with either homozygous Q456X or Q129X (2.5%) substitutions. In patients with familial disease, *PINK1* homozygotes were younger at disease onset (36 ± 12 years) than noncarriers (57 ± 15 years) and more often had an akinetic-rigid presentation at examination and slow progression.

Conclusions: Segregation of *PINK1* mutations with parkinsonism within families, and frequency estimates within population controls, suggested only four *PINK1* mutations were pathogenic. Several *PINK1* sequence variants are potentially benign and there was no evidence that *PINK1* heterozygosity increases susceptibility to idiopathic Parkinson disease. ***Neurology*® 2008;71:896-902**

GLOSSARY

AAO = age at onset; **CRF** = case report forms; **ET** = essential tremor; **PD** = Parkinson disease; **UPDRS** = Unified Parkinson's Disease Rating Scale.

Parkinson disease (PD) is a neurodegenerative syndrome with a complex, multifactorial etiology, although causal mutations in several genes have been implicated in families with a Mendelian pattern of disease inheritance.[1] The PARK6 locus was linked to chromosome 1p35-36 in a Sicilian kindred[2] and mutations in the PTEN induced putative kinase 1 (*PINK1*) were subsequently identified.[3] *PINK1* encodes a mitochondrial serine-threonine protein kinase[4] and is one of three genes including parkin (*PRKN*) and *DJ-1* implicated in autosomal recessive, early-onset forms of parkinsonism.

The frequency of neurodegenerative disorders including idiopathic PD is higher in Tunisia than in most other countries.[5] In this region unique geographic and sociocultural factors,

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including large pedigrees, low rates of migration, and high rates of consanguinity, facilitate genotype-phenotype correlations in genetic disease.

Current findings originated as a genome-wide linkage study of familial parkinsonism in Tunisia that identified a significant linkage peak on chromosome 1p35-36. The *PINK1* gene was subsequently sequenced and the segregation and frequency of any variants identified were examined within families, nonfamilial PD, and control subjects. The clinical characteristics of individuals with and without *PINK1* mutations are described.

METHODS

Study population. The Institut National de Neurologie, Tunis, provides a specialized neurologic service to the entire country of Tunisia.[6] A total of 92 families were recruited, comprised of 76 multiplex kindreds which include clinical data and DNA samples from 208 patients with parkinsonism, 340 unaffected subjects, and 27 with an uncertain diagnosis. The remaining 16 familial index cases (singleton families) were derived from pedigrees consisting of affected siblings, parent-offspring pairs, avuncles, first cousins, or half-first cousins once removed. In these 16 kindreds DNA was only available from one affected subject and unaffected family members. Subjects in the study also included 240 nonfamilial patients with PD and 368 control participants. The site obtained local ethics committee approval before beginning subject recruitment. Subjects were informed of all aspects pertaining to their participation in the study, and gave either written or proxy consent, prior to their inclusion.

Physical examinations were performed by neurologists specialized in movement disorders. Patients with PD and control subjects without a family history of parkinsonism were collected from all regions of Tunisia, through the Institut National de Neurologie, Tunis. For our family-based study the proband was examined at the study site, and additional family members were recruited via the proband. Inclusion criteria were age at assessment older than 18 years, with at least one other affected first- to third-degree blood relative (excluding a monozygotic twin).

Individuals were diagnosed as "affected" if they satisfied the United Kingdom Parkinson's Disease Society Brain Bank criteria,[7] "unaffected" if all signs of parkinsonism were absent, "controls" if all signs of parkinsonism were absent and there was no family history of parkinsonism, and "uncertain" if only one parkinsonian sign or abnormal feature was present. Most of the latter subjects were diagnosed with essential tremor (ET). Standardized case report forms (CRF) were used for clinical and demographic data collection and included data on Hoehn and Yahr staging,[8] the Unified Parkinson's Disease Rating Scale (UPDRS)[9], and the Epworth scale.[10] Approximately half of the patients (105/208) completed a simplified CRF including section III of the UPDRS but without Hoehn and Yahr staging. Duration of disease was calculated by subtracting the age at onset (AAO) in medical records from the age at examination. For a minority of patients AAO was not documented (n = 42/208, primarily as past medical records were not available for 33/208 [16%] of affected family members), and was estimated either from their clinical history or by self-report. Confirmatory diagnoses of 35 affected members from 18 pedigrees with familial parkinsonism and 6 patients with nonfamilial PD were performed by independent movement disorders specialists and were completely concordant.

Mutation detection. DNA was extracted by standard procedures from a peripheral venous blood sample.[11] The original genome scan was carried out using 1116 microsatellite markers spaced an average of 4 centiMorgans across the genome.[12] MERLIN was used for nonparametric multipoint linkage analysis.[13]

All eight *PINK1* exons were subsequently sequenced in one familial index case from each of the families recruited. Quantitative analysis of *PINK1* exon dosage was also performed by real-time PCR, controlled using a DNA sample hemizygous for chromosome 1p35-36,[14] a gift from Dr. Enza-Maria Valente (CSS-Mendel Institute, Rome, Italy). ABI probes were designed for the 14 nonsynonymous coding changes that were found in <10% of sequenced individuals. These probes were then run through all remaining family members, 240 nonfamilial patients and 372 control subjects. Parkin (*PRKN*) sequencing and dosage analysis was also performed for all exons for any families with *PINK1* homozygous mutations using published methods.[15,16] In addition, *LRRK2* point mutations including 6055G>A (G2019S) were assayed as previously described.[17]

RESULTS

The linkage analysis included 69 multiplex families with 174 affected individuals genotyped at <5 cM resolution. The maximum lod score on chromosome 1 was 5.7 from multipoint nonparametric linkage analysis. The lod-1 interval spanned 7 MB from D1S199 to D1S2749 which corresponds to the physical position of the *PINK1* locus. To determine if the linkage signal was due to mutations within *PINK1*, the gene was successfully sequenced in 89 familial index cases. Fourteen nonsynonymous coding changes were identified and assessed within other family members from 92 families (76 multiplex families and an additional 16 singleton families), 240 nonfamilial patients with PD and 368 control subjects (table 1). Eight substitutions have been identified previously, including Q115L, P196L, A340T, A383T, G411S, E476K, Q456X, and N521T. Novel variants identified in this study included Q129X, Q129fsX157, T145M, R152W, G227R, and G440E. Of these changes, Q129X, Q129fsX157, G440E, and Q456X appear to be pathogenic as they were found in a homozygous state in 46 out of 50 (92%) of the affected individuals within 14 families (figure). Excluding these kindreds from linkage analysis abolished the chromosome 1p35-36 signal.

Evidence for pathogenicity of the other 10 *PINK1* mutations was equivocal. One coding substitution N521T was common (heterozygous frequency of 24.8%, homozygous frequency 4.1% in controls) and did not segregate with parkinsonism in the families. This was excluded from further analyses of the demographic and clinical characteristics of *PINK1* carriers. Other substitutions were uncommon (<5% frequency) in controls and generally found in a heterozygous state with the exception of homozygous

Table 1 Frequency of *PINK1* mutations in Parkinson disease family probands, nonfamilial patients, and control subjects*

	Q115L	Q129fsX157†	Q129X†	T145M	R152W	P196L	G227R	A340T	A383T	G411S	G440E†	Q456X†	E476K	N521T
dbSNP assignment	ss69356465	ss69356467	ss69356466	ss69356468	ss69356469	rs35802484	ss69356470	rs3738136	ss69356471	ss69356473	ss69356474	ss69356475	ss69356476	rs1043424
Probands	92	92	92	92	92	92	92	92	92	92	92	92	92	92
Heterozygous	1 (1.1)	0 (0.0)	0 (0.0)	1 (1.1)	1 (1.1)	1 (1.1)	1 (1.1)	6 (6.7)	1 (1.1)	1 (1.1)	0 (0.0)	1 (1.1)	1 (1.1)	29 (32.2)
Homozygous	0 (0.0)	1 (1.1)	2 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.1)	7 (7.8)	0 (0.0)	2 (2.2)
Nonfamilial patients	239	232	236	228	231	234	232	235	236	239	240	234	239	231
Heterozygous	2 (0.8)	0 (0.0)	0 (0.0)	1 (0.4)	1 (0.4)	0 (0.0)	0 (0.0)	12 (5.1)	3 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	5 (2.1)	73 (31.6)
Homozygous	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	5 (2.1)	0 (0.0)	14 (6.1)
Control subjects	367	365	363	353	363	360	361	365	367	368	366	365	362	343
Heterozygous	13 (3.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)	18 (4.9)	8 (2.2)	0 (0.0)	0 (0.0)	2 (0.5)	3 (0.8)	85 (24.8)
Homozygous	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	14 (4.1)

Values represent number (%).
*Excludes unsuccessful mutation screening results for each mutation; total nonfamilial patients (n = 240), control subjects (n = 372), and probands (n = 92).
†Columns highlight putative pathogenic mutations.

Figure Four pedigrees are shown representative of families with homozygous *PINK1* Q129X, Q129fsX157, G440E, or Q456X mutations

Squares represent males, circles are females, diamonds are where gender has been disguised. Deceased individuals are shown with a diagonal line, affected subjects have a quarter-filled quadrant, and consanguineous marriages have a double horizontal line between parents. +/+ and +/− represent homozygous and heterozygous *PINK1* mutation carriers.

inheritance of Q115L, A383T, and E476K in three control subjects aged 64, 49, and 49 years at examination. Nonfamilial patients were also identified with homozygous substitutions Q129X (n = 1, AAO 38 years) and Q456X (n = 5, average AAO 38 ± 9) which are putatively pathogenic.

Demographic characteristics are reported for the affected and unaffected members of families with parkinsonism, nonfamilial patients with PD, and control subjects, stratified by *PINK1* status (table 2). All participants were of Arab-Berber ethnicity with the exception of one family which originated from Turkey and two from Southern Europe. All *PINK1* mutations were in individuals of Arab-Berber descent. Within families, unaffected individuals with homozygous *PINK1* mutations were marginally younger at examination (34 ± 5 years) than the onset age of affected homozygous *PINK1* individuals (36 ± 12 years); therefore some may yet develop parkinsonism.

Subsequent analyses focus on subjects with pathogenic mutations, Q129X, Q129fsX157, G440E, and Q456X. Clinical characteristics of patients with parkinsonism with *PINK1* homozygous and heterozygous mutations were compared to those without *PINK1* variants (table 3). The mean age at onset of parkinsonism in familial *PINK1* homozygous patients (36 ± 12) was younger than in familial PD

Table 2 Demographic characteristics of subjects focused on four putatively pathogenic *PINK1* mutations

	N	N with 2nd CRF	Mean age at exam ± SD, y	Median age at exam (range), y	Male, n (%)	Female, n (%)
Control subjects						
Homozygous	0	0	NA	NA	NA	NA
Heterozygous	2	2	50 ± 2	50 (48-51)	2 (100)	0 (0)
No *PINK1* mutations	366	366	56 ± 11	55 (29-90)	185 (51)	181 (49)
Nonfamilial patients						
Homozygous	6	6	52 ± 11	56 (35-61)	3 (50)	3 (50)
Heterozygous	0	0	NA	NA	NA	NA
No *PINK1* mutations	234	234	64 ± 11	66 (25-85)	113 (48)	121 (52)
14 Families with 4 *PINK1* mutations*						
Affected						
Homozygous	46	21	53 ± 14	51 (26-77)	24 (52)	22 (48)
Heterozygous	1	0	76	76	0 (0)	1 (100)
Unaffected						
Homozygous	4	2	34 ± 5	33 (28-41)	1 (25)	3 (75)
Heterozygous	54	27	55 ± 17	61 (19-86)	19 (35)	35 (65)
78 Families without 4 *PINK1* mutations*						
Affected	159	83	66 ± 14	69 (28-96)	81 (51)	78 (49)
Unaffected	260	142	54 ± 17	53 (21-93)	115 (44)	145 (56)

*Four homozygous mutations that segregate with disease within families and are likely to be pathogenic: Q456X, Q129X, Q129fsX157, and G440E.
CRF = case report form.

with heterozygous *PINK1* mutations (69 ± 8 years, Wilcoxon rank sum test $p < 0.005$) or affected family members without any of the four potentially pathogenic *PINK1* mutations (57 ± 15 years, Wilcoxon rank sum test $p < 0.0001$). The mean age at onset of PD in six individuals with nonfamilial PD and *PINK1* homozygous mutations (38 ± 9 years) was younger than in nonfamilial PD without any of the four mutations (58 ± 12 years, Wilcoxon rank sum test $p < 0.001$).

In familial patients with homozygous *PINK1* mutations, in contrast to patients without any *PINK1* mutation, the duration of parkinsonism was longer (Wilcoxon rank sum test $p < 0.0001$) and Hoehn and Yahr scores were not significantly different (table 3). The notable exception were higher mean Hoehn & Yahr scores in nonfamilial patients with homozygous *PINK1* mutations and with a very long duration of disease, although the sample size was too small for significance (n = 6, $p = 0.1$). The majority of familial patients without *PINK1* mutations recalled tremor as a first feature (83%) whereas gait or balance problems were infrequent (14%). In contrast, familial *PINK1* homozygotes noted their first symptom as either tremor (54%) or deterioration in gait or balance (37%). At examination, the type of parkinsonism in most affected individuals were classified as mixed, with both rigidity and tremor present, but *PINK1* homozygotes more often had akinetic-rigid parkinsonism (31%), compared to heterozygotes (0%) or individuals without *PINK1* mutations (13%). Clinical differences on first presentation and subtype at examination between *PINK1* homozygotes and *PINK1* heterozygote or patients without *PINK1* mutations were statistically significant ($p < 0.0002$). Data on cognition and dementia are limited to neurologist observations as more than 60% of the study population was unable to read and therefore their MMSE scores were not valid.[18]

Some families carried more than one *PINK1* sequence variation. One familial affected individual (onset age 74) was a compound heterozygote for both Q129X and A340T substitutions; another familial patient (onset age 60) was a compound heterozygote for Q456X and A383T, whereas two unaffected individuals (ages 44 and 69) from two families were compound heterozygous for both Q456X and A340T. In two of the eight families with the Q456X mutation, the proband did not screen positive for the mutation but it was identified in other family members.

Within the 14 families with *PINK1* mutations, *PRKN* sequencing and dosage analysis was performed and *LRRK2* point mutations including 6055G>A (G2019S) were assayed. There are seven

Table 3 Characteristics of patients with Parkinson disease (PD) with *PINK1* homozygous and heterozygous mutations compared to those without *PINK1* mutations

	PD patients from 92 families*			Nonfamilial patients†	
	PINK1 homozygotes	*PINK1* heterozygotes	No *PINK1* mutations*	*PINK1* homozygotes	No *PINK1* mutations
N Individuals	46	3	159	6	234
Mean age at onset ± SD, y	36 ± 12	69 ± 8	57 ± 15	38 ± 9	64 ± 11
Median age at onset (range)	35 (13-59)	72 (60-74)	60 (9-87)	37 (29-54)	66 (25-85)
Mean duration PD ± SD, y	17 ± 11	10 ± 7	8 ± 7	14 ± 11	6 ± 6
First symptom, n (%)					
Tremor	24 (54)	3 (100)	132 (83)	3 (50)	169 (72)
Deterioration in gait/balance	17 (37)	0 (0)	23 (14)	3 (50)	25 (11)
Muscle cramping or dystonia	3 (7)	0 (0)	1 (1)	0 (0)	2 (1)
Other	1 (2)	0 (0)	3 (2)	0 (0)	38 (16)
Type of PD					
Akinetic-rigid	14 (31)	0 (0)	20 (13)	2 (33)	35 (15)
Mixed	25 (54)	2 (67)	124 (78)	3 (50)	170 (73)
Tremor-dominant	7 (15)	1 (33)	15 (9)	1 (17)	29 (12)
Hoehn & Yahr,‡ mean ± SD (n)					
"On" medication	1.6 ± 0.9 (19)	NA	1.9 ± 1.0 (38)	2.8 ± 0.3 (3)	2.3 ± 0.8 (66)
"Off" medication	2.0 ± 0.0 (1)	2.0 ± 0.0 (1)	2.0 ± 1.0 (27)	4.0 ± 0.0 (1)	2.7 ± 0.8 (120)
UPDRS III,‡ mean ± SD (n)					
"On" medication	38.3 ± 24.6 (19)	NA	45.4 ± 22.3 (38)	40.0 ± 7.0 (3)	47.5 ± 18.6 (66)
"Off" medication	62.0 ± 0.0 (1)	62 ± 0 (1)	58.0 ± 21.6 (27)	75.0 ± 0.0 (1)	61.1 ± 20.9 (120)
Ever on a dopa agonist, n (%)	40 (87)	2 (67)	122 (77)	NA	195 (84)

*Fourteen families had affected individuals with one of four putatively pathogenic mutations, Q456X, Q129X, Q129fsX157, and G440E, with homozygotes affected by disease. Seventy-eight families had no homozygous mutations, but did have two affected individuals with heterozygous mutations.
†Of 240 patients with nonfamilial PD, 6 were homozygous for one of four potentially pathogenic *PINK1* mutations, and none were heterozygous.
‡Only assessed on the second version of the patient report form.
UPDRS = Unified Parkinson's Disease Rating Scale.

heterozygous *PINK1* carriers who also inherited either M192L (n = 2), P153R (n = 4), or D394N (n = 1) heterozygous *PRKN* substitutions. However, all were unaffected with a mean age at examination of 54 ± 19, range 26–73 years. Of three homozygous *PINK1* carriers with heterozygous P153R *PRKN* substitutions, two were affected at 30 and 36 years while the other remains unaffected at examination at 35 years. In one family, one affected (age 56 years at onset) and one unaffected individual (age 62 years at examination) also carried a heterozygous Lrrk2 G2019S substitution.

DISCUSSION This report describes the largest group of *PINK1* carriers identified to date and all are from a single population background. A total of 14 *PINK1* variants were found in Tunisian patients with familial parkinsonism. Of these four *PINK1* mutations, Q129X, Q129fsX157, G440E, and Q456X, were found to segregate with disease and were homozygous in affected individuals within 14 families. Three of these are novel substitutions. All four substitutions were rarely found in 368 control subjects and never in a homozygous state. Segregation of parkinsonism within these 14 families explains the linkage signal on chromosome 1p35-36 and provides genetic evidence that Q129X, Q129fsX157, G440E, or Q456X inherited in a recessive homozygous fashion are pathogenic. Two of these four mutations,

Q129X and Q456X, were also present in patients with seemingly nonfamilial PD (Q129X 0.4%, Q456X 2.1%). Of the other 10 variants N521T is a common polymorphism but the rest are uncommon; within nonfamilial patients with PD only A340T was found in a homozygous state, and homozygous Q115l, A383T, and E476K substitutions were identified in control subjects.

Our subsequent analysis focused on the four pathogenic homozygous *PINK1* substitutions identified in 14 families, as other mutations are rare, do not segregate with disease within pedigrees, and may be benign. Overall, *PINK1* homozygotes had a younger mean onset age than heterozygotes and those without any of the four potentially pathogenic *PINK1* mutations. However, despite longer disease duration, familial *PINK1* homozygotes did not show greater symptom severity based on Hoehn & Yahr or UPDRS III scales. *PINK1* homozygotes were less likely to have tremor as a first symptom or at examination than other patients with PD, and deterioration in gait and balance were noted more frequently; *PINK1* homozygous patients were also more likely to develop akinetic-rigid parkinsonism, compared with other patients with PD. In idiopathic PD, deterioration in gait and balance are often associated with a faster rate of cognitive decline and may be considered a risk factor for incident dementia.[19] Cognitive decline and dementia were difficult to gauge but were not pronounced within the 14 families with *PINK1* substitutions. *PINK1* heterozygous mutations may be associated with the presence of psychosis[20]; however, while psychiatric variables were assessed through a self-report of medical history, there were a large number of missing responses. Further study of cognitive and psychiatric aspects of *PINK1* parkinsonism may be worthwhile.

In the literature, it remains controversial whether *PINK1* heterozygous mutations are a risk factor for idiopathic PD.[21,22] Cross-sectional imaging studies provide support that *PINK1* haploinsufficiency may contribute to nigrostriatal dysfunction but it is not clear whether the impairment is developmental or the consequence of a slowly progressive neurodegenerative process.[23] Similarly, many studies have suggested *PRKN* heterozygous mutations are a risk factor for idiopathic PD, but community-based studies remain equivocal.[16,24] In our study, of the many heterozygous *PINK1* carriers with one of four pathogenic mutations (including parents of homozygous affected individuals who must be at least obligate heterozygotes) few had parkinsonism (table 2). This suggests that heterozygote mutations may not be a risk factor for PD, at least within the age range that this study was able to sample.

In good agreement with our observations, functional data from RNAi knockdown of *PINK1* expression in mice to less than 10% of the endogenous gene and protein does not lead to dopaminergic neuronal death or dysfunction, whereas targeted, complete germ-line deletion of *PINK1* can lead to impaired dopamine release and reduced synaptic plasticity in the striatum.[25,26] In *Drosophila* removal of the *PINK1* homologue results in a more dramatic albeit nondopaminergic phenotype, including male sterility, apoptotic muscle degeneration, and mitochondrial fragmentation that may be rescued by parkin overexpression suggesting PINK1 and parkin function, at least in part, in the same pathway.[27] In our study there was no evidence for a joint effect of *PINK1* and *PRKN* on disease susceptibility or age at onset although the number of subjects with mutations in both genes was limited. Nevertheless, it will be important to elucidate the potential interactions between these proteins and the convergent pathways in which they function.

The frequency of *PINK1* mutations described may be atypical and limited to the Arab-Berber sample. However, as clinical studies of the *PRKN* gene illustrate, genetic mutations generally have the same effects in different ethnicities.[28] Testing for *PINK1* mutations in patients in Tunisia may have practical utility in diagnosis and patient care. Within affected kindreds, *PINK1* carrier status for pathogenic mutations might contribute to genetic counseling. However, it is important to note that not all *PINK1* coding variants are pathogenic, only when inherited in a homozygous state, and that the majority of mutations identified appear benign. Similarly, within families or within the general community, there was no evidence that *PINK1* heterozygous carriers are at increased risk of idiopathic PD.

Affected and symptomatic individuals within the *PINK1* pedigrees described may now enable biomarker identification and validation. Assuming findings are generalizable such biomarkers would facilitate the design of future neuroprotection (delayed disability) trials in idiopathic PD, and the development of novel therapeutic strategies aimed at more than symptomatic relief.

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