Parkin mutations and phenotypic features in Czech patients with early-onset Parkinson's disease

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Abstract

OBJECTIVES: Mutations in several genes such as parkin can be detected in up to 20% of patients with early-onset Parkinson's disease (EOPD). The aim of our study was to determine the frequency of *parkin* alterations and phenotypic characteristics in Czech EOPD patients.
METHODS: A total of 45 EOPD individuals (age at onset <45 years) were phenotyped and screened for *parkin* mutations.
RESULTS: In total, 19 patients (42.2%) were carriers of previously described heterozygous genetic alterations. *Parkin* mutations (Ex2del, R402C) were identified in two (4.4%) cases, non-pathogenic variant A82E plus polymorphism D394N occurred in one (2.2%) patient and *parkin* polymorphisms (3× S167N, 1× R334C, 7× V380L, 4× D394N) were found in 15 (34.9%) individuals. Furthermore, the G2019S mutation in the *LRRK2* gene was found in one (2.2%) subject.
CONCLUSION: The clinical characteristics of our patients correspond to previous descriptions of EOPD phenotype. This is the first report on EOPD-associated genetic alterations among Czech patients. Our results support the hypothesis that single heterozygous *parkin* variants may act as risk factors for EOPD.

Abbrevation :

PD	- Parkinson's disease
EOPD	- early-onset Parkinson's disease
MLPA	- multiplex ligation-dependent probe amplification
PCR	- polymerase chain reaction
SIFT	- sorting intolerant from tolerant
H-N	- Hoehn and Yahr stage

ORIGINAL ARTICLE

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder, affecting more than 1% of the population over the age of 60 years (de Lau & Breteler 2006). Clinically, it is characterized by motor symptoms such as resting tremor, rigidity, bradykinesia, and postural instability, as well as non-motor impairment as is autonomic dysfunction or cognitive defict (Jankovic 2008). The mean age at onset of PD is usually between 60–70 years (Twelves *et al.* 2003), however about 5–10% of cases start before the age of 45 years (Schrag & Schott 2006). The clinical phenotype of early-onset PD (EOPD) differs from classic-onset PD in several features such as slower disease progression, more frequent occurrence of dystonia, excellent treatment response, and early development of levodopa-induced dyskinesias (Schrag & Schott 2006). Multiple risk factors are supposed in the etiology of PD, including harmful environmental influences and genetic alterations. So far, four recessively inherited genes have been reported to be associated with EOPD – *parkin* (PARK2), *PINK1* (PARK6), *DJ-1* (PARK7) and *ATP13A2* (PARK9) (Lesage & Brice 2009). *Parkin* mutations are presented in up to 50% of patients with familial EOPD as well as 18% of sporadic EOPD cases (Lucking *et al.* 2000; Periquet *et al.* 2003). To date, more than 200 different mutations of the *parkin* gene have been identified (Hedrich *et al.* 2004). The aim of our study is to determine the frequency of genetic alterations in the *parkin* gene and to evaluate phenotypic characteristics in Czech EOPD patients.

MATERIAL AND METHODS

Patients and clinical assessments

A total of 45 unrelated Czech patients (36 males, 9 females) with EOPD (age of onset ≤45 years) were recruited from the Movement Disorders Center, Prague, Czech Republic. The diagnosis of PD was based on the UK Brain Bank diagnostic criteria with the exception of positive family history that has not been regarded as an exclusion criterion (Hughes *et al.* 1992). Written informed consent was obtained from all individuals. Phenotypic data were assessed by personal interview and neurological examination. Clinical evaluations included the severity of PD (Hoehn and Yahr stage; H-N), the presence of motor and non-motor (Non-Motor Symptoms Scale; NMSS) symptoms, as well as the occurrence of levodopa-induced dyskinesias and motor fluctuations (Unified Parkinson's Disease Rating Scale; UPDRS IV). The study was reviewed by the ethics commitee of the General Teaching Hospital, Prague, Czech Republic.

Molecular studies

For genetic analysis, venous blood sample was collected from each patient. Genomic DNA was isolated from peripheral blood leukocytes using standard procedures.

Sequence analysis

All 12 exons of the *parkin* gene were amplified from the patient's genomic DNA by the polymerase chain reaction (PCR). For amplification, previously described primers were used (Kitada *et al.* 1998), except for the exon 2 primers (primer sequences available on request). All fragments (both strands) were analyzed on ABI PRISM 3100/3130 Genetic Analyzers (Applied Biosystems). *Parkin* gene variants (polymorphisms and mutations) were numbered relative to Genbank mRNA sequence (accesion number NM_004562). The effects of observed amino acid substitutions on protein function were predicted using SIFT (Sorting Intolerant From Tolerant; http://sift.jcvi.org).

Gene-dosage analysis

To indentify exon rearrangements in the *parkin* gene, we analyzed gene dosage using multiplex ligation-dependent probe amplification (MLPA), SALSA MLPA kit P051 and P052B (MRC-Holland). This probe mix contains probes for all exons of *parkin*, 7 exons (out of 8) of *PINK1* and 5 exons (out of 7) of *DJ-1* gene. Additionally, probes for three other PD-associated genes (*SNCA*, *UCH-L1*, and *LRRK2*) are included. The MLPA assay was performed according to the manufactuer's protocol. The fragments were analyzed on a CEQ 8000 capillary sequencer (Beckman Coulter, Inc., USA) with the Fragment Analysis software. For each sample, the relative peak area was calculated and compared with controls using the Coffalyser v9.4 software (MRC-Holland).

RESULTS

Phenotypic characteristics of patients

The mean age of the onset was 35.6 ± 4.5 (range 20–45) years, average disease duration amounted to 17.2 ± 7.2 (range 6–36) years. The mean H-N stage was 2.4 ± 0.8 (range 1–5). A positive family history was present in 8 (17.8%) cases. Dystonia occurred in 29 patients (64.4%), depression was found in 26 (57.8%) individuals. An excellent response to dopaminergic therapy was reported by 38 (84.4%) of patients. Dyskinesias were registered in 30 (66.7%) cases, motor fluctuations in 39 (86.7%); these symptoms occured after a mean interval of 9.1 ± 4.2 (range 1–18) and 7.6 ± 4.1 (range 1–23) years, respectively. The overview of the results and the comparison between groups of patients with and without alterations in the *parkin* gene are shown in Table 1.

Genotypic characteristics of patients

In total, 19 patients (42.2%) were carriers of previously described heterozygous genetic alterations. *Parkin* mutations (Ex2del, R402C) were identified in two (4.4%) cases, *parkin* non-pathogenic variant (A82E) plus polymorphism (D394N) occurred in one (2.2%) patient, and *parkin* polymorphisms (3× S167N, 1× R334C, 7× V380L, 4× D394N) were found in 15 (34.9%) individuals. In addition, the G2019S mutation

Tab. 1. Phenotypic characteristics of patient groups according to the presence of *parkin* alterations.

Charakteristics	All patients	Patients without *parkin* alterations	Patients with *parkin* alterations
No. of patients *(% of whole group)*	45	**27** (60.0%)	**18** (40.0%)
Sex *(male / female; % men)*	**36/9** (80.0%)	**20 / 7** (74.1%)	**16/2** (88.9%)
Age at onset *(mean, SD) yrs*	**35.6** (± 4.5)	**34.6** (± 4.8)	**37.1** (± 3.6)
Age at examination *(mean, SD) yrs*	**51.3** (± 7.7)	**52.2** (± 8.7)	**50.1** (± 5.8)
Disease duration *(mean, SD) yrs*	**15.5** (± 7.2)	**17.3** (± 8.2)	**12.9** (± 4.3)
H-Y stage *(mean, SD)*	**2.4** (± 0.8)	**2.3** (± 0.7)	**2.5** (± 0.9)
Positive family history	**8** (17.8%)	**4** (14.8%)	**4** (22.2%)
Symptoms at onset			
Rigidity & bradykinesia	**30** (66.7%)	**15** (65.6%)	**15** (83.3%)
Tremor	**18** (40.0%)	**12** (44.4%)	**6** (33.3%)
Pain	**8** (17.8%)	**5** (18.5%)	**3** (16.7%)
Micrographia	**6** (13.3%)	**5** (18.5%)	**1** (5.6%)
Dystonia	**3** (6.7%)	**1** (3.7%)	**2** (11.1%)
Motor symptoms at examination			
Rigidity	**43** (95.6%)	**25** (92.6%)	**18** (100.0%)
Bradykinesia	**42** (93.3%)	**24** (88.9%)	**18** (100.0%)
Micrographia	**42** (93.3%)	**24** (88.9%)	**18** (100.0%)
Gait disturbances	**41** (91.1%)	**23** (85.2%)	**18** (100.0%)
Tremor	**36** (80.0%)	**21** (77.8%)	**15** (83.3%)
Dysarthria	**34** (75.6%)	**21** (77.8%)	**13** (72.2%)
Dystonia	**29** (64.4%)	**15** (55.6%)	**14** (77.8%)
Falls	**16** (35.6%)	**19** (33.3%)	**7** (38.9%)
Non-motor symptoms at examination			
Depression	**26** (57.8%)	**12** (44.4%)	**14** (77.8%)
Daytime sleepines	**26** (57.8%)	**14** (51.9%)	**12** (66.7%)
Nighttime insomnia	**19** (42.2%)	**11** (40.7%)	**8** (44.4%)
Urinary impairment	**17** (37.8%)	**8** (29.6%)	**9** (50.0%)
Altered sexual function	**17** (37.8%)	**8** (29.6%)	**9** (50.0%)
Cognitive deficit	**16** (35.6%)	**8** (29.6%)	**8** (44.4%)
Sleep benefit	**16** (35.6%)	**10** (37.0%)	**6** (33.3%)
Gastrointestinal impairment	**14** (31.1%)	**7** (25.9%)	**7** (38.9%)
Orthostatic faintness	**13** (28.9%)	**3** (11.1%)	**10** (55.6%)
Pain	**10** (22.2%)	**7** (25.9%)	**3** (16.7%)
Response to treatment and motor complications			
Excellent response to dopaminergic therapy	**38** (84.4%)	**23** (85.2%)	**15** (83.3%)
Dyskinesias (peak of dose)	**30** (66.7%)	**18** (66.7%)	**12** (66.7%)
- Interval of development *(mean, SD) yrs*	**9.1** (± 4.2)	**9.7** (± 4.8)	**8.3** (± 3.0)
Motor fluctuations (wearing off)	**39** (86.7%)	**23** (85.2%)	**16** (88.9%)
- Interval of development *(mean, SD) yrs*	**7.6** (± 4.1)	**8.8** (± 4.5)	**5.9** (± 2.5)

in the *LRRK2* gene was found in one (2.2%) subject. No patients had exon rearrangements of *PINK1* or *DJ-1*. All amino acid substitutions were predicted by SIFT to be tolerated with the exception of R402C in the *parkin* gene and G2019S in the *LRRK2* gene. The genetic findings are summarized in Table 2.

DISCUSSION

The phenotypic characteristics of Czech EOPD patients do not substantially differ from previous descriptions of EOPD phenotype (Khan *et al.* 2003; Schrag & Schott 2006). Our results confirmed a high presence of dystonia (64.4%), depression (57.8%), and excellent response to dopaminergic therapy (84.4%) among patients with EOPD. Relative to other studies (Lucking *et al.* 2000; Khan *et al.* 2003; Macedo *et al.* 2009), we found a later development of levodopa-induced dyskinesias (mean = 9.1 years). Distinct clinical differences between patients with *parkin* alterations and non-variant carriers have not been observed (Table 1). These findings support the suggestion that patients with *parkin* mutations are clinically indistinguishable from non-mutation carriers with EOPD (Klein *et al.* 2005). On the other hand, individuals with *parkin* alterations had more frequent incidence of non-motor symptoms, such as depression (77.8%), orthostatic faintness (55.6%), urinary impairment (50.0%) and sexual dysfunction (50.0%).

The genotypic characterization was based on molecular analysis of the *parkin* gene whose mutations are the most frequent genetic cause of EOPD (Lucking *et al.* 2000; Periquet *et al.* 2003). Importantly, about 50% of them represent exon rearrangements which are not detectable with conventional methods if occurring in heterozygous state (Hedrich *et al.* 2001). In consideration of this fact, the molecular analysis was performed using both sequence analysis and gene-dosage analysis by MLPA, which provides sensitive detection of exon rearrangements (Scarciolla *et al.* 2007).

Two previously described *parkin* mutations (Ex2del, R402C) (Lucking *et al.* 2000; Bertoli-Avella *et al.* 2005) were identified in our sample. Although the R402C mutation is considered to be pathogenic, it has been also shown in healthy subject (in heterozygous state) (Schlitter *et al.* 2006). The substitution A82E (Hedrich *et al.* 2001) was predicted by SIFT to be non-pathogenic. This is supported by *in vitro* study where A82E variant did not affect the cellular distribution of the protein (Cookson *et al.* 2003). We have also found four already reported polymorphisms (S167N, R334C, V380L, D394N) (Abbas *et al.* 1999; Satoh & Kuroda 1999; Wang *et al.* 1999; Lucking *et al.* 2000), all of them were predicted by SIFT to be non-pathogenic, however substitution R334C was recently shown to cause structural rearrangement of the protein (Beasley *et al.* 2007). Even though the association of these polymorphisms with PD is debatable, several studies suggest that homozygous S167 and V380L polymorfisms might be risk factors for PD (Lucking *et al.* 2003; Biswas *et al.* 2007). However, in our sample all *parkin* variants occurred only in the single heterozygous state, with the exception of compound heterozygosity for substitutions A82E and D394N in one patient. The intronic and regulatory sequences of the *parkin* gene were not analyzed, therefore, a second undetected mutation cannot be fully excluded.

With regard to recessive inheritance of *parkin* associated EOPD, the presence of homozygous or compoud heterozygous *parkin* mutations is required to cause the disease. Although the clinical significance of single heterozygous *parkin* mutations, promoter, and coding polymorphisms remains unclear, partial evidence sug-

Tab. 2. Genetic alterations found in patients.

Genetic alteration	n (%)	State	Pathogenicity of amino acid substitution (*)	Type of alteration
Total genetic alterations	**19** (42.2%)	-	-	-
Parkin alterations	**18** (40.0%)	-	-	-
Ex2del	1 (2.2%)	heterozygous	-	mutation
A82E / D394N	1 (2.2%)	compound heterozygous	both tolerated	non-pathogenic variant + polymorphism
S167N	3 (6.7%)	heterozygous	tolerated	polymorphism
R334C	1 (2.2%)	heterozygous	tolerated	polymorphism
V380L	7 (15.6%)	heterozygous	tolerated	polymorphism
D394N	4 (8.9%)	heterozygous	tolerated	polymorphism
R402C	1 (2.2%)	heterozygous	not tolerated	mutation
LRRK2 alteration	**1** (2.2%)	-	-	-
G2019S	1 (2.2%)	heterozygous	not tolerated	mutation

(*) Pathogenicity of amino acid substitution was predicted using SIFT

gests that these genetic alterations also contribute to PD risk (West *et al.* 2002; Lucking *et al.* 2003; Klein *et al.* 2007). Unaffected carriers of heterozygous *parkin* mutations show presynaptic dopaminergic dysfunction in the striatum (Hilker *et al.* 2001; Khan *et al.* 2002) and hyperechogenicity of the substantia nigra (Walter *et al.* 2004; Hagenah *et al.* 2007). These preclinical changes suggest that heterozygous *parkin* alterations might play a substantial role in the pathogenesis of PD. High incidence of heterozygous *parkin* variants in our sample (37.8%) encourages this hypothesis. In contradiction with the suggestion above, several studies have demonstrated a similar frequency of heterozygous *parkin* mutations in cases and healthy controls, and do not support the notion that these alterations are risk factor for PD (Lincoln *et al.* 2003; Chien *et al.* 2006; Kay *et al.* 2007). Future research should answer the question whether heterozygous *parkin* mutations may cause or increase the susceptibility to PD.

In addition, the heterozygous point mutation G2019S in the *LRRK2* gene (dominantly inherited) was identified in one patient. This substitution is the most frequent pathogenic mutation in PD (Kachergus *et al.* 2005), predominantly associated with late-onset phenotype, but also described in EOPD (Hedrich *et al.* 2006). No patients had exon rearrangements in the EOPD associated genes *PINK1* and *DJ-1*, analysis of the *ATP13A2* gene were not performed.

This is the first report on PD-associated variants (mutations and polymorphisms) among Czech patients with EOPD. The drawn data provide a valuable source of information for further genetic studies, such as the determination of the frequency of *PINK1*, *DJ-1*, and *ATP13A2* alterations in the EOPD sample or evaluation of heterozygous *parkin* variants among age-matched healthy controls.

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