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Incidence of mutations in the *PARK2*, *PINK1*, *PARK7* genes in Polish early-onset Parkinson disease patients

Częstość występowania mutacji w genach PARK2, PINK1, PARK7 *w grupie polskich pacjentów z chorobą Parkinsona o wczesnym początku*

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Abstract

Background and purpose: Parkinson disease (PD) is a complex disease, comprising genetic and environmental factors. Despite the vast majority of sporadic cases, three genes, i.e. *PARK2*, *PINK1* and *PARK7* (*DJ-1*), have been identified as responsible for the autosomal recessive form of early-onset Parkinson disease (EO-PD). Identified changes of these genes are homozygous or compound heterozygous mutations. The frequency of *PARK2*, *PINK1* and *PARK7* mutations is still under debate, as is the significance and pathogenicity of the single heterozygous mutations/variants, which are also detected among PD patients. The aim of the study was to analyze the incidence of autosomal recessive genes *PARK2*, *PINK1*, *PARK7* mutations in Polish EO-PD patients.

Material and methods: The analysis of the *PARK2*, *PINK1* and *PARK7* genes was performed in a group of 150 Polish EO-PD patients (age of onset < 45 years). Mutation analysis was based on sequencing and gene dosage abnormality identification.

Results: Mutations were identified only in the *PARK2* and *PINK1* genes with the frequency of 4.7% and 2.7% of subjects, respectively. In *PARK2*, point mutations and exons'

Streszczenie

Wstęp i cel pracy: Pomimo że wśród osób z chorobą Parkinsona (ChP) dominują przypadki sporadyczne, trzy geny: *PARK2*, *PINK1* i *PARK7*, zostały scharakteryzowane jako odpowiedzialne za występowanie autosomalnie recesywnej postaci ChP o wczesnym początku. Mutacje w tych genach mogą mieć charakter zmian homozygotycznych lub heterozygotycznych złożonych, dotyczących obu alleli. U części pacjentów identyfikuje się tylko mutacje jednoalleliczne, a ich udział w patogenezie ChP pozostaje wciąż sprawą kontrowersyjną. Celem badania jest analiza częstości występowania i rodzaju mutacji w genach *PARK2*, *PINK1* i *PARK7* w polskiej populacji chorych na ChP o wczesnym początku.

Materiał i metody: Analizę występowania mutacji w genach *PARK2*, *PINK1* i *PARK7* przeprowadzono w grupie 150 polskich pacjentów z ChP o wczesnym początku (wiek w chwili wystąpienia objawów choroby < 45 lat). Badanie obejmowało analizę sekwencji kodujących wszystkich genów oraz identyfikację rearanżacji (delecje/duplikacje) w ich obrębie. W analizach mutacji wykorzystano bezpośrednie sekwencjonowanie genów oraz metodę MLPA (*multiplex ligation-dependent probe amplification*).

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rearrangements, and in *PINK1* only missense mutations were detected. In both genes mutations were found as compound heterozygous/homozygous and single heterozygous. EO-PD patients' genotype-phenotype correlation revealed similarities of clinical features in mutation carriers and non-carriers.
Conclusions: The frequency of the *PARK2*, *PINK1*, *PARK7* mutations among Polish EO-PD patients seems to be low. The role of single heterozygous mutations remains a matter of debate and needs further investigations.

Key words: early onset Parkinson disease, *PARK2*, *PINK1*, *PARK7*.

Wyniki: Mutacje zidentyfikowano tylko w genach *PARK2* i *PINK1* z częstością odpowiednio 4,7% i 2,7%. W genie *PARK2* znaleziono mutacje punktowe oraz rearanżacje, natomiast w *PINK1* tylko mutacje punktowe typu zmiany sensu. Zidentyfikowane mutacje występują zarówno jako heterozygotyczne złożone/homozygotyczne, jak i jednoalleliczne. Korelacja fenotypowo-genotypowa wśród pacjentów z ChP o wczesnym początku jest podobna zarówno dla nosicieli, jak i dla osób bez mutacji.
Wnioski: Częstość występowania mutacji w genach *PARK2*, *PINK1* i *PARK7* wśród polskich pacjentów z wczesną postacią ChP wydaje się mała. Rola pojedynczych heterozygotycznych mutacji w patogenezie ChP o wczesnym początku pozostaje sprawą dyskusyjną i w celu jej wyjaśnienia potrzebne są dalsze badania.

Słowa kluczowe: choroba Parkinsona o wczesnym początku, *PARK2*, *PINK1*, *PARK7*.

Introduction

Parkinson disease (PD) is one of the most common neurodegenerative disorders, comprising genetic and environmental factors. Typical onset of PD is about the age of 60 but 5% of patients develop the early onset form of PD (EO-PD). The choice of the early onset definition is arbitrary and usually refers to an age < 45 years, but in the literature it may be defined as < 40 or even 50 years [1-3].

The majority of PD cases are sporadic, but monogenic recessive or dominant forms caused by mutations in several genes have been described [3]. Mutations in *PARK2* (OMIM 602544), *PINK1* (OMIM 608309), and *PARK7* (OMIM 606324) are responsible for the autosomal recessive form of PD (AR-PD) [4-7].

PARK2-PD is characterized by a broad range of clinical phenotypes, some atypical signs, early onset, slower progression, good response to levodopa, but often with more severe dopa-induced complications, and no dementia [8,9]. According to published data, mutations in *PARK2* are a common cause of EO-PD worldwide (10-20% of patients) [10]. The frequency of *PARK2* mutations in the European population has been established at 50% in familial and 19% in sporadic cases [11]. *PINK1-PD* is an early onset parkinsonism with slow progression, and excellent reaction to levodopa [12]. The reported frequency of *PINK1* mutations among AR-PD cases is approximately 4.5% [13]. The knowledge on the *DJ1*-PD (*PARK7*) features is limited so far, as very few such patients have been reported. In addition to parkinsonism, clinical characteristics include psychiatric symptoms, short stature, and brachydactyly [14]. The incidence of *PARK7* mutations in some ethnic groups has been estimated as 1-2% of EO-PD [15-18].

In all EO-PD genes (*PARK2*, *PINK1* and *PARK7*) mutations were identified as homozygous, compound heterozygous and single heterozygous. The role of single heterozygous variants in "recessive genes" remains still controversial, as they are found in PD patients and healthy controls [19,20].

Material and methods

Subjects

One hundred and fifty EO-PD patients (42% females, 58% males) of Polish origin from different centers covering central and northern and southern Poland with onset of PD before the age of 45 entered the study. Neurologists experienced in diagnostics of movement disorders performed neurological examination. The clinical diagnosis of PD patients was established according to UK Parkinson's disease Brain Bank criteria [21]. The patients' age ranged from 25 to 68 years (mean: 45.8; standard deviation [SD]: 8.4), their age at disease onset varied between 20 and 44 years (mean: 36.8; SD: 5.4) and disease duration ranged from 0.5 to 30 years (mean: 8.9; SD: 6.3). Most patients presented a sporadic form of PD (80%), while the familial cases constituted another 20%. Patients were classified as familial cases if at least one of their first- or second-degree rela-

Table 1. Clinical features of patients with early-onset Parkinson disease: non-carriers and *PARK2, PINK1* gene mutation carriers

	Non-carriers	*Parkin* mutation carriers	*Pink* mutation carriers
Number	141	7	4
Positive family history [%]	20	57	25
Women [%]	41	57	75
Age at onset [years]; mean ± SD	34.9 ± 4.9	32.1 ± 5.4	28 ± 0
Disease duration [years]; mean ± SD	7.9 ± 5.9	9.7 ± 7.7	18.0 ± 14.1
Hoehn and Yahr stage	2.0 ± 0.8	2.3 ± 0.7	2.7 ± 0.3
Daily dose of levodopa [mg]; mean ± SD	784 ± 494	557 ± 528	666 ± 400
MMSE score	28.7 ± 1.8	29.5 ± 1.2	29.5 ± 0.7
Dyskinesia [%]	50	43	100
Fluctuations [%]	58	57	50
Symptoms at onset [%]:			
Rest tremor	46	43	0
Bradykinesia	25	0	50
Rigidity	17	14	50
Other (pain, dystonia)	13	43	0

SD – standard deviation; MMSE – Mini-Mental State Examination

tives had a diagnosis of PD but clear AR pattern of disease inheritance was observed in 3 cases (2%) only. Clinical data of the patients are presented in Table 1.

Control group

The control group consisted of 230 unrelated subjects (56% females, 44% males), healthy volunteers with no evidence of neurological/neurodegenerative disorder history in the family, aged from 18 to 73 years (mean: 31; SD: 11.2). All participants signed an informed consent form. The ethics committees of the Warsaw Medical University and Institute of Mother and Child approved the study.

Methods

Molecular analysis was performed on genomic DNA extracted from patients' venous blood. All coding exons of the *PARK2*, *PARK7* and *PINK1* genes were amplified by polymerase chain reaction (PCR) and products directly sequenced. In the case of novel mutations in the *PINK1* gene, the direct sequencing of appropriate exons in the control group was performed. To detect rearrangements of the genes, multiplex ligation-dependent probe amplification (MLPA) was performed using the commercially available kits SALSA MLPA P051/P052 (MRC-Holland). Sequence analysis was performed using Surveyor v.3.2 software in comparison to reference sequences: NM_004562, NM_007262, NM_032409 [NCBIRefSeq,http://www.ncbi.nlm.nih. gov/nuccore]. MLPA peak analysis, normalization and calculation of dosage ratio were performed with the Gene Markerv.1.51 software (both SoftGenetics LLC). Mutations were referred to data in Human Gene Mutation Database (HGMD Professional; http://www.hgmd. cf.ac.uk) and Parkinson Disease Mutation Database (PDmutDB; http://www.molgen.ua.ac.be/PDmutDB). Statistical analysis was performed using SPSS version 14.0 software. The impact of the newly identified *PINK1* gene mutations on the protein structure and function was analysed with the PolyPhen-2 v.2.1 software using the HumVar model (http://genetics.bwh.harvard.edu/pph2).

Results

Mutations were found only in the *PARK2* and *PINK1* genes. In *PARK2*, changes were detected in 7 patients (4.7%); the frequency of *PINK1* mutations was even lower (2.7%).

In the *PARK2* gene, the mutations were found as homozygous in one case, compound heterozygous in four

Table 2. Mutation in the *PARK2* and *PINK1* gene identified among patients with early-onset Parkinson disease (EOPD)

ID	Sex	Age at onset [years]	Parkinson disease in family	*PARK2*	*PARK7*	*PINK1*
31703 GA	F	24	None	Ex3_4del/p.Gln34ArgfsX5	–/–	–/–
30716 KL	M	26	Two brothers (EO-PD)	Ex3del/Ex4_7del	–/–	–/–
29293 KE	F	33	None	Ex2_5dup/p.Lys211Asn	–/–	–/–
24702 KB	F	37	None	p.Gln34ArgfsX5/p.Gln34ArgfsX5	–/–	–/–
20300 BM	F	31	None	Ex4_7del/ p.Gln34ArgfsX5	–/–	–/–
19134 KW	M	36	Father (LOPD)	Ex2dup/–	–/–	–/–
26260 ZR	M	38	None	p.Arg275Trp/–	–/–	–/–
19642 LH	F	28	Two brothers (EOPD)	–/–	–/–	p.Ile3658Asn/p.Ile368Asn
17788 RM	F	31	None	–/–	–/–	p.Lys186Asn/–
16655 AI	M	28	None	–/–	–/–	p.Ser535Leu/–
23866 OJ	F	39	None	–/–	–/–	p.Gly411Ser/–

LOPD – late-onset Parkinson disease

cases and in 2 cases as single heterozygous (Table 2). Thus, the frequency of the *PARK2* mutations in our EO-PD concerns about 4.0% of alleles. In one patient we also found homozygous mutation p.Asp394Asn/p.Asp394Asn. This abnormality, if it occurs as single heterozygous, is considered as polymorphism [rs1801334, dbSNP, http://www.ncbi.nlm.nih.gov/SNP], but as homozygous it shows an association with familial PD [22,23].

In the *PINK1* gene, potentially pathogenic variants were detected only in four patients: one case of homozygous mutation p.Ile368Asn/ p.Ile368Asn and heterozygous p.Ser535Leu/– (both novel, not detected in control Polish group) and two known, but controversial, single heterozygous changes p.Lys186Asn and p.Gly411Ser. All identified mutations were localized in functional domains of *PINK1* protein. The *in silico* analysis performed using the PolyPhen-2/HumVar tool confirmed potential pathogenicity of both novel mutations p.Ile368Asn and p.Ser535Leu. These two substitutions were predicted as "probably damaging", with the corresponding probability scores reaching 0.99 and 1.0.

EO-PD patients' genotype-phenotype correlation revealed similarities of clinical features in mutation carriers and non-carriers (Table 1). Asymmetrical tremor was a dominant symptom at the onset of the disease in both groups. The patient with single *PARK2* mutation and 25-year duration of disease had severe fluctuations and dyskinesias. Both subjects with double mutation had no dyskinesias. Response to levodopa treatment was excellent for all of them.

Discussion

Mutations in all analyzed genes were found in 7.4% of examined EO-PD patients. This frequency is similar to the mutation frequency of *PARK2*, *PINK1*, *PARK7* genes (6.9%) in a large study ($n = 953$ PD patients, age at onset < 51 years) [3]. Analysis of 187 Dutch patients with PD (age at onset ≤ 50) revealed 8.5% of subjects with mutation of these genes [24], but analysis of a group of 127 Chinese patients with EO-PD showed mutations in 18.1% of cases [25]. This variable incidence may reflect the ethnic differences.

Published data on *PARK2* mutations in sporadic young onset cases (age at onset < 45 years) also give a variable frequency. Analysis of 146 Italian unrelated EO-PD patients (disease onset < 40) found 8.2% of cases with homozygous/compound heterozygous point mutations and/or exon rearrangements and 2.7% of subjects with a single mutation in the *PARK2* gene [26]. Again, in a Chinese group of 127 EO-PD patients any mutation was found in 12.6% of cases [25]. Hakansson *et al*. [27] analyzed a group of 60 Swedish patients with EO-PD and described only one case with a single

mutation (1.5%), but among 187 Dutch patients (age at onset ≤ 50) this frequency was higher (5.9%) [24]. Other EO-PD patient surveys presented *PARK2* mutations at a frequency of 2.7% in Queensland, Australia, 6.6% in South African and 5.5% in Korean cohorts [28-30]. Frequency of *PARK2* mutation in a cohort of mixed populations ($n = 953$) was 6.3% [3]. Those data, as well as ours (4.7%), may suggest that the *PARK2* gene mutations are less frequent than has been expected based on previous studies [11].

PINK1 gene mutations are probably very rare, with local higher distribution. Alcalay *et al*. [3] found no *PINK1* mutation in 953 EO-PD patients. One percent of subjects with *PINK1* mutation was found in a Dutch and 3.1% in a Chinese EO-PD population [24,25]. Our results with 2.7% of patients with mutations seem to be about average and may suggest that the *PINK1* gene mutations are less frequent than in earlier reports.

The *PARK7* mutations are rare, representing perhaps less than 1-2% in EO-PD cases [15-18].

A large study of a group of 953 PD patients with age at onset < 51 years showed mutations of *PARK7* genes in 0.2% of subjects [3]. In the Chinese cohort of sporadic EO-PD patients mutations were more frequent and were found in 2.4% of subjects [25]. In our cohort of 150 EO-PD patients, similarly to some other studies, no mutations in the *PARK7* gene were found [1,30].

As reported elsewhere, we found the phenotype of the *PARK2* mutation carriers indistinguishable from non-carriers. The clinical hallmark of the *PARK2* mutation carriers was a good response to levodopa and a lack of dementia. Lohmann *et al.* [32] analyzed groups of 44 EO-PD patients with or without mutation and found no difference in both groups except for significantly lower daily doses of dopaminergic treatment in *PARK2* mutation carriers. Typical clinical presentation of *PINK1*-PD is an early-onset parkinsonism with slow progression, and excellent reaction to levodopa, not differing from typical PD features.

In our group we identified five patients with only a single heterozygous *PARK2* or *PINK1* mutations. Many authors have also found such mutations in their patients in these genes as well as in *PARK7* [1,2,10,24]. There are several potential ways to elucidate this issue. A second mutation may be localized in regions of the gene that were not screened (promoter, introns) or in another gene that acts in the same pathway in the pathogenesis of PD. Some single heterozygous variants could act as loss-of-function mutations by lowering the biological activity of the encoded protein (haploinsufficiency). But the most important thing is that these heterozygous mutations may result in increased risk for developing PD.

The frequency of heterozygous *PARK2* mutations in healthy subjects is about 3.7% [33]. These subjects could develop parkinsonian syndromes later [33]. *PARK2*, *PINK1* and *PARK7* genes underline the importance of mitochondrial dysfunction and oxidative stress in PD [34]. Positron emission tomography studies with the use of 18F-dopa showed a 20-30% reduction of its uptake when compared with controls, which may support the concept of increased risk of PD development in the next years for these carriers [35].

Conclusions

1. The frequency of the *PARK2*, *PINK1*, *PARK7* mutations among Polish EO-PD patients seems to be low.
2. The role of single heterozygous mutations remains a matter of debate and needs further investigations.

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Disclosure

Authors report no conflict of interest.

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