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Analysis of *PRKN* Variants and Clinical Features in Polish Patients with Parkinson's Disease

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Abstract: The etiology of Parkinson's disease (PD) is still unclear, but mutations in *PRKN* have provided some biological insights. The role of *PRKN* mutations and other genetic variation in determining the clinical features of PD remains unresolved. The aim of the study was to analyze *PRKN* mutations in PD and controls in the Polish population and to try to correlate between the presence of genetic variants and clinical features. We screened for *PRKN* mutations in 90 PD patients and 113 controls and evaluated clinical features in these patients. We showed that in the Polish population 4% of PD patients had *PRKN* mutations (single or with additional polymorphism) while single heterozygous polymorphisms (S167N, E310D, D394N) of *PRKN* were present in 21% of sporadic PD. Moreover, 5% PD patients had more than one *PRKN* change (polymorphisms and mutations). Detected *PRKN* variants moderately correlated with PD course and response to L-dopa. It also showed that other *PARK* genes (*SNCA*, *HTRA2*, *SPR*) mutations probably may additionally influence PD risk and clinical features. *PRKN* variants are relatively common in our Polish series of patients with PD. Analysis of the *PRKN* gene may be useful in determining clinical phenotype, and helping with diagnostic and prognostic procedures in the future.

Keywords: PARK, *PRKN*, Genetic variants, Clinical features, Parkinson's disease.

INTRODUCTION

The molecular mechanisms underlying Parkinson's disease (PD) pathogenesis, especially sporadic PD (SPD), are poorly understood, but it is believed that both genetic and environmental factors are included in the PD pathogenesis. Mutations and polymorphisms in some PARK genes, including *PRKN* (PARK2) encoding the Parkin protein, have been described as being associated with PD [1-3].

Parkin is a cytoplasmic protein that plays an important role in the proper functioning of mitochondria and the ubiquitin-proteasome system, where it functions as an E3 ligase ubiquitin, directing proteins to degradation in the proteasome and consequently preventing cell apoptosis [4]. It has been shown that Parkin is an enzyme with a Ubl domain N′ terminus, followed by two Really Interesting New Gene (RING) finger domains separated by an In Between Ring (IBR) domain (Fig. **1**). It is believed that the motif of two RING fingers separated by an IBR domain is related to the catalytic function of the Parkin [3]. It is suggested that loss or decrease of Parkin function caused by genetic changes in the *PRKN* gene may affect the risk of PD.

Mutations of the *PRKN* gene were firstly identified in Japanese families with autosomal recessive juvenile Parkinsonism, and since then more than 180 allelic variants of this gene have been found including point mutations, deletions and multiplications of exons [1, 3]. It has been shown that *PRKN* point mutations and polymorphisms occur at different frequencies in Caucasian, African and Asian populations [1-8]. To date, there has only been one report investigating *PRKN* variants in the Polish population, and then only in early onset PD (EOPD; 20-40 years old) [9]. However, variants of *PRKN* in late onset PD (LOPD) in the Polish population have not been known so far. Moreover, although many reports have indicated the important role of *PRKN* exon 2 and 4 deletions in PD [5-7], we did not detect any deletion of these exons in the Polish population [4], similar to the case for the American and German populations [2, 8].

The results of similar studies in other populations suggest that the presence of *PRKN* mutations may be connected with a milder course of PD and a good response to L-dopa therapy [10-12]. On the other hand, some authors showed a higher incidence of dyskinesia in the presence of the *PRKN* mutation in PD [10-12]. Therefore, the importance of various *PRKN* variants remains unclear, not only for disease manifestation but also clinical features in PD and the level of the Parkin protein. Further, so far the search for a correlation between the presence of *PRKN* genetic variants and clinical features in PD has not been conducted in the Polish population. In our previous study, we investigated only the presence of genetic variants of *PRKN* mainly in LOPD in the Polish population [4]. Here we analyze *PRKN* variants with polymorphism and mutation distinction clearly based on allele frequency [according to the Human Gene Mutation Database (HGMD)], and for the first time we try to correlate

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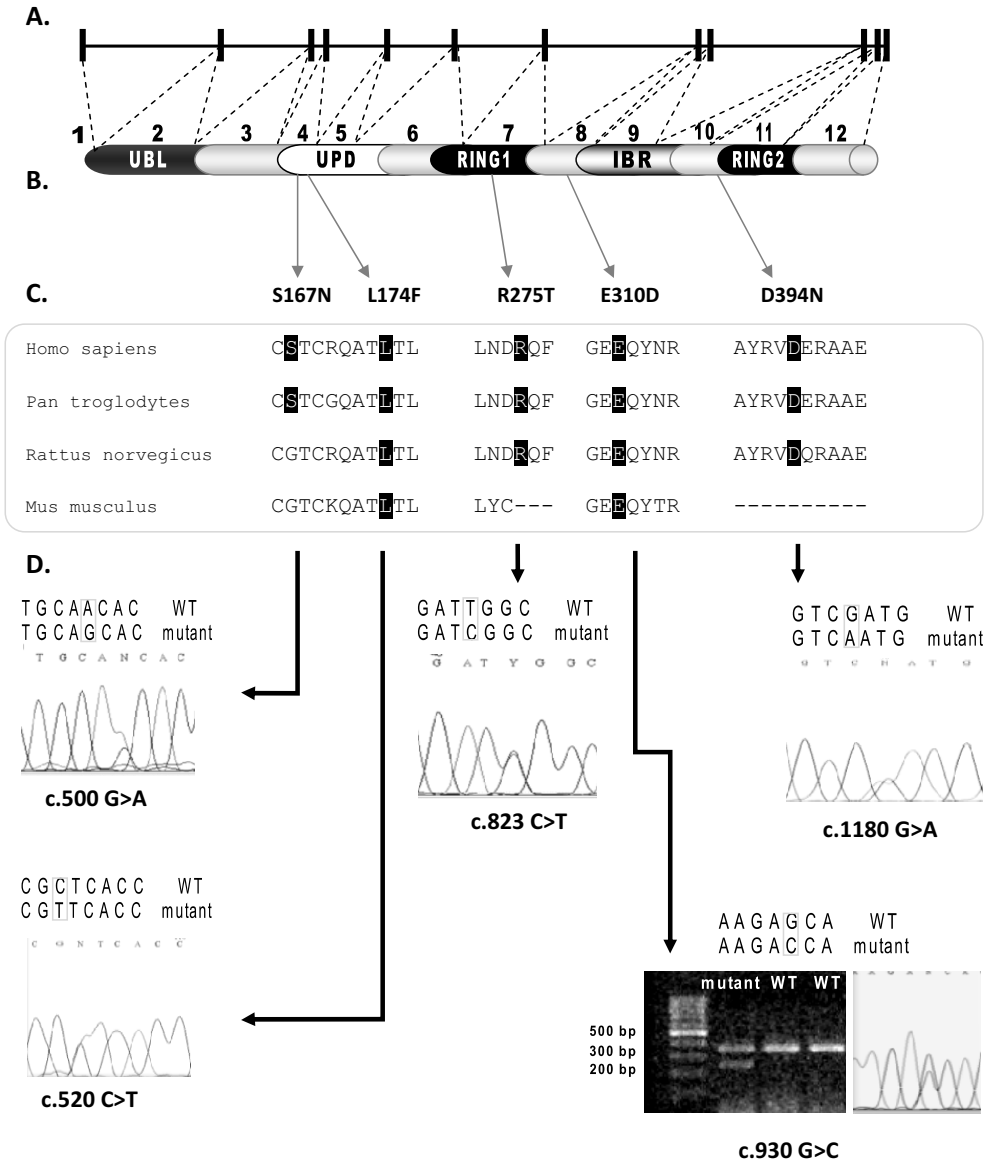

Fig. (1). Organization of Parkin and detected variants of *PRKN* gene.
A. organization of the *PRKN* gene; **B.** domains of the Parkin protein; **C.** locations of five detected missense substitutions are indicated by arrows. Variants L147F and E310D occurred at evolutionarily conserved amino acids while S167N, R275T and D394N at limited conserved amino acids; **D.** results of sequencing and restriction analysis of *PRKN* gene. Wild-type and mutant sequences were detected.

the presence of previously detected *PRKN* variants (including multiple substitutions and novel mutation) with clinical features in Polish PD patients.

The aim of this study was to analyze allelic variants of the *PRKN* gene in both PD patients and controls in the Polish population as well as to analyze clinical features in PD patients with detected mutations and polymorphisms of the *PRKN* gene. Because there are suggestions in the literature that there may be some possible interactions between Parkin and other proteins related with PD (Fig. **2**), we analyzed also the coexistence of mutation and polymorphisms in *PRKN* and other PARK genes (*SNCA*- synucleine alpha, *HTRA2*- high temperature requirement A2, *LRRK2*- leucine-rich repeat kinase 2 and *SPR*- sepiapterin reductase), which we previously studied in PD Patients and described in our book chapter [4].

PATIENTS AND METHODS

Patient Selection and Samples

A total of 203 subjects were included in this study: 90 SPD patients (42 female and 47 male), mean age 61.9±10.1 years (selected from among 180 consecutive patients with Parkinsonism presenting to the Neurology Clinic of the Poznan University of Medical Sciences, Poland), including 8 EOPD and 82 LOPD cases. The control group was composed of 113 healthy individuals matched for age and gender (mean age 55.5±9.5 years) who did not exhibit neurological diseases or symptoms of dementia. All subjects had a negative family history of PD, had no A30P mutation in the *SNCA* gene, were evaluated by experienced neurologists and diagnosed based on modified UK PD brain bank criteria [13]. The stage of the disease was evaluated according to the

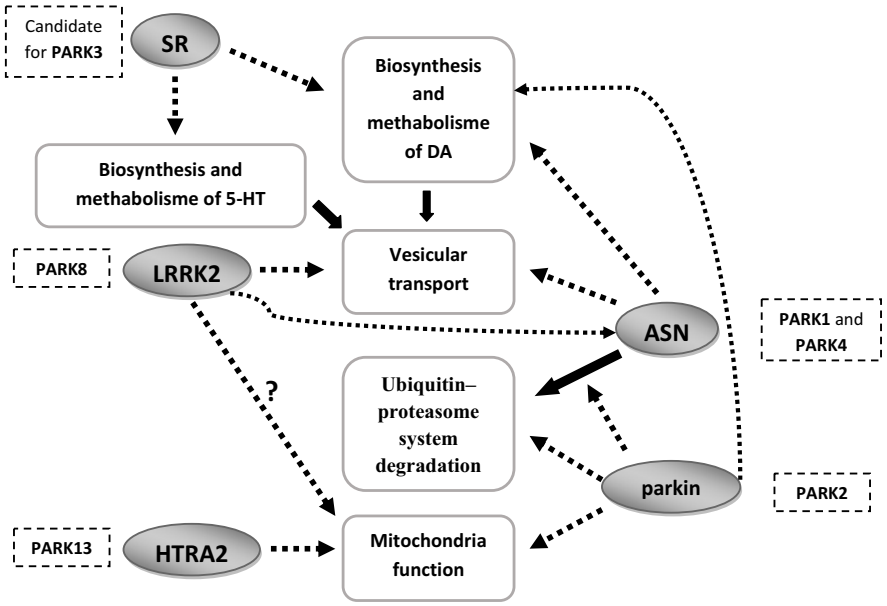

Fig. (2). Interactions between Parkin and selected proteins and genes involved in PD pathogenesis.
SR – sepiapterin reductase; LRRK2 – leucine-rich repeat kinase 2; HTRA2 – high temperature requirement A2; ASN – alpha-synuclein; DA – dopamine; 5-HT – serotonin; PARK1-4,8,13 – loci related to PD.

Hoehn-Yahr scale, while cognitive function was evaluated using the Montreal Cognitive assessment (MoCA) scale. Patients with PD were treated with L-dopa in a dose up to 500 mg per day for the first 5 years of treatment, and 800-1500 mg per day in subsequent years of treatment. All participants provided written informed consent. The study was approved by a local ethics committee.

Analysis of the *PRKN* Genotype and Phenotype

Genomic DNA was extracted from venous blood using standard protocols and stored at -80 °C. Genotyping of *PRKN* was performed using PCR/RFLP, high resolution melting analysis (HRM) and sequencing method as previously described [4].

HRM analysis was used to screen for mutations in exons 4, 7 and 11 of *PRKN* using LightCycler 480 Real-Time PCR system (Roche, USA) and High Resolution Master Mix (Roche, USA). Primers for HRM analysis were generated using the online software Primer3 (http://www-genome.wi.mit.edu/cgibin/primer/primer3_www.cgi) based on the published genomic sequence of the *PRKN* gene and presented in (Table **1**). Melting curves and difference plots were analyzed by 3 investigators blinded to phenotype. For the samples with shifted melting curves, PCR products were cleaned and sequenced in the forward and reverse directions using the 3130xl Genetic Analyzer (Applied Biosystems HITACHI, USA) and reads were aligned to the human reference genome with BioEdit Software (Tom Hall Ibis Biosciences, Canada). Coding DNA mutation numbering is relative to NM_004562.2.

Screening for P37L and P37= (c.110C>T, c.111G>A) in exon 2 and E310D in exon 8 of *PRKN* was performed by PCR/RFLP analysis using specific primers (5'-TTTCCC AAATATTGCTCTA-3', 5'-GCAGTGTGGAGTAAAGTTCAAGG -3' and 5'-CTAAAGAGGTGCGGTTGGAG-3'; 5'- GGAGCCC AAACTGTCTCATT-3' respectively) and MvaI and HaeIII, respectively, (Fermentas, Canada) as the restriction enzymes. All detected variants were confirmed by sequencing of the PCR product.

Parkin protein levels were evaluated using ELISA commercial kits (Uscn Life Science Inc., China) according to the manufacturer's protocol, as was previously described in detail [4]. The plate(s) were read at 450 nm on an EPOCH Multi-Volume Spectrophotometer (BioTek, USA) and the results were analyzed using Gen5 2.1 Software (BioTek, USA).

Statistical Analysis

The chi-square test and Fisher's exact test, univariate odds ratio (OR) and logistic regression analysis in Statistica for Windows software were used to evaluate the results of the study.

RESULTS

Analysis of *PRKN* gene in PD patients and healthy controls detected five missense substitutions: two mutations, one novel (L174F) and one known (R275T); two polymorphisms (S167N, D394N) and one substitution (E310D) with unclear nature (in the studied population this was probably a polymorphism) [4]. All detected substitutions were in a heterozygous configuration. The frequencies of the detected allelic variants are presented in (Table **2**). For the single D394N polymorphism, a significant frequency difference between PD patients and controls was demonstrated, while the R274T mutation was present only in one PD patient and the novel L174F mutation only in two PD patients and was associated with earlier age of PD onset (about 40 years old in both patients, Table **3**). Additionally, the analysis of the amino acid sequence of Parkin (encoded by the *PRKN* gene) revealed that substitutions L174F and E310D were located in

Table 1. Sequences of the primers for exons 4, 7 and 11 *PRKN* HRM analysis.

PRKN exon	Primer Sequence	Reference Sequence	Design Method	Size of Product
4	GCATTATTAGCCACTTCTTCTGC	NG_008289.1 NM_004562.2	PRIMER 3+	181 bp
	TGCTGACACTGCATTTCCTT			
7	TCCTGGTTTTCCAGTGCAAC	NG_008289.1 NM_004562.2	PRIMER 3+	123 bp
	AAGGCAGGGAGTAGCCAAGT			
11	CCGACGTACAGGGAACATAAA	NG_008289.1 NM_004562.2	PRIMER 3+	167 bp
	GGACAGGGCTTGGTGGTT			

Table 2. Frequency of *PRKN* substitutions in PD patients and controls [4].

Variant				
SINGLE HETEROZYGOUS POLYMORPHISMS	**PD patients**	**Controls**	**OR (95% CI)**	**p**
S167N	3%	1%	-	>0.05(F)
E310D	9%	6%	-	>0.05(C)
D394N	**8% ***	**2% ***	**4,68** (0.948-23.114)	**<0.05(C)**
SINGLE HETEROZGOUS MUTATION				
L147F	1%	0%	-	-
MORE THAN ONE *PRKN* CHANGE	**Mutation (exon)**		**Polymorphism (exon)**	
patient 1	**R275T (7)**		E310D (8)	
patient 2			S167N (4)	
patient 3			E310D (8), D394N (11)	
patient 4	**L174F (4)**		E310D (8), D394N (11)	
patient 5			S167N (4), E310D (8), D394N (11)	
COEXISTING OF MUTATION IN OTHER PARK GENES	***PRKN* polymorphism**		**Other genes mutation**	
patient 6	S167N		S213T *SPR* [4]	
patient 7	E310D		A141S *HTRA2* [4]	
patient 8	E310D		G399S *HTRA2* [4]	

OR – odds ratio; CI – confidence interval; F – Fisher's exact test; C – chi-square test; * p<0.05– statistically significant differences for specific p compared with controls.

Table 3. Clinical features in PD patients with *PRKN* allelic variants.

PD patient	Age	*PRKN* substitution/s	Duration of the disease	Stage of the disease on the H-Y scale	Response to L-dopa therapy	Cognitive disorders	Symptoms of depression	Dyskinesias	Fluctuations	Autonomic disorders	Parkin level
1	71	S167N	>10	2	g	-	-	-	-	+	Udr
2	59	S167N	>10	3	fg	-	-	+	+	-	Udr
3	68	S167N	>10	3	g	+	-	-	-	-	Udr
4	54	S167N	<5	2	p	+	+	-	-	-	Udr
5	46	L147F	>10	2	g	-	-	+	-	+	Udr

(Table 3) contd….

PD patient	Age	*PRKN* substitution/s	Duration of the disease	Stage of the disease on the H-Y scale	Response to L-dopa therapy	Cognitive disorders	Symptoms of depression	Dyskinesias	Fluctuations	Autonomic disorders	Parkin level
6	46	S167N, L147F	5-10	2	fg	-	+	-	-	-	Udr
7	62	S167N, E310D, D394N	>10	2	fg	-	+	-	-	-	0.073
8	63	R275T, E310D	<5	1	g	-	-	-	-	-	Udr
9	41	E310D	<5	2	g	-	-	-	-	-	0.069
10	57	E310D	>10	1	p	+	+	-	-	-	Udr
11	66	E310D	5-10	3	fg	-	-	-	-	+	Udr
12	76	E310D	5-10	3	g	-	+	+	+	-	Udr
13	59	E310D	<5	1	g	-	-	-	-	-	0.037
14	69	E310D	<5	2	g	-	-	-	-	-	0.083
15	51	E310D	5-10	2	g	-	+	-	+	-	Udr
16	73	E310D	<5	2	p	+	+	-	-	+	Udr
17	67	E310D	<5	1	g	-	-	-	-	-	Udr
18	51	E310D	>10	1	p	-	-	-	-	-	Udr
19	49	E310D, D394N	<5	2	fg	-	-	-	-	+	0.061
20	73	E310D, D394N	<5	2	g	-	-	-	-	-	0.018
21	55	D394N	<5	2	vg	-	-	-	-	-	Udr
22	59	D394N	>10	4	g	+	+	+	+	+	Udr
23	65	D394N	<5	2	g	-	+	-	-	-	Udr
24	66	D394N	>10	3	g	-	-	-	-	-	Udr
25	61	D394N	>10	2	g	+	-	-	-	-	Udr
26	62	D394N	<5	1	g	-	-	-	-	-	Udr
27	68	D394N	<5	1	g	-	-	-	-	-	Udr

vg – very good, g – good, fg – fairly good, p – poor, H-Y scale – Hoehn-Yahr scale, "+" – present, "-" - apsent.

a conserved region, whereas substitutions S167N, R275T and D394N were located in a limited conserved region of this protein (Fig. **1**).

Moreover, the presence of detectable levels of Parkin was demonstrated only in PD patients with either the E310D substitution or the E310D and D394N (0.018-0.069 ng/ml) substitutions who had had PD less than five years, while in the patient with S167N, E310D and D394N changes, Parkin was present (0.073 ng/ml) even in patients who had PD for over 10 years (Table **3**).

Correlations between the presence of a *PRKN* polymorphism and response to L-dopa therapy, as well as progress of the disease in PD patients are presented in (Table **4**). Generally, the presence of a single S167N polymorphism was associated with late onset of PD (mean age 63 years old). Also, a moderate positive correlation was shown (Spearman, R+0.554; $p<0.05$) between the presence of the S167N substitution and response to L-dopa therapy in PD patients with duration of the disease more than ten years.

Table 4. Correlation between the presence of detected *PRKN* polymorphisms and response to L-dopa pharmacotherapy and stage of the disease in Hoehn-Yahr scale in patients with Parkinson's disease.

PD Patients				
Polymorphism	**Duration of the disease**			**P**
	< 5 years	**5-10 years**	**> 10 years**	
	Responce to L-dopa therapy			
S167N	-	-	**R+0.554**	**p<0.05**
E310D	-	-	*	0.05
D394N	**R+0.385**	-	-	**p<0.05**
	Stage of the disease in Hoehn-Yahr scale			
E310D	-	-	**R-0.458**	**p<0.05**

* results near statistical significance; R - Spearman correlation coefficient for p<0.05.

Additionally, an L174F mutation was detected only in PD patients with early onset of the disease (before 40 years old, independent of the co-occurrence of additional mutations in *PRKN*). However, the R275T mutation was detected only in one PD patient, who also had a substitution in exon 8 (E310D). Late onset of the disease (after 60 years old) was observed in this patient.

Furthermore, regardless of the presence of additional genetic changes, in PD patients who had PD for more than ten years there was a negative moderate correlation between the presence of E310D and the stage of the disease on the Hoehn-Yahr scale (Spearman, R-0.458; p<0.05). Simultaneously, in these patients a trend was observed for a better response to L-dopa therapy at the border level of significance (Table **4**).

We also showed that the D394N polymorphism was associated with late onset of the disease (mean age for patients with a single D394N: 62 years old).

Additionally, it was shown that in PD patients the presence of polymorphism D394N of *PRKN* positively (but also in a moderate degree) correlated with the response to L-dopa therapy in the early period of the disease (less than 5 years) [Spearman, R+0.385; p<0.05; (Table **4**)].

Interestingly, in 5% of the PD patients there was more than one change in the *PRKN* gene, while in control subjects we detected only single *PRKN* substitutions (Table **2**). In three PD patients with single heterozygous polymorphism of *PRKN* there were previously detected mutations in other PARK genes (*HTRA2* in two cases and *SPR* in one case, described previously in our book chapter) as it is shown in (Table **1**) [4].

DISCUSSION

Previously we identified 5 missense heterozygous *PRKN* substitutions (S167N, L174F, R275T, E310D, D394N), of which L174F was a novel mutation, and R275T and E310D variants were detected for the first time in the Polish population [4]. However, a correlation analysis of the detected *PRKN* genetic variants with clinical features in PD in the Polish population has not been conducted so far. Here we conducted genetic analysis with distinction between polymorphisms and mutations, and carried out a detailed correlation between the occurrence of particular genetic changes and clinical features of the disease.

Mutations of *PRKN* (R275T and L174F), similarly to another study in a Caucasian population, were detected in the Polish population similarly to other study in Caucasian population in almost 4% of PD patients and were present only in PD patients, which may indicate a high penetrance of these mutations [8]. Although the localization of the L174F mutation in the unique Parkin domain (UPD, Fig. **1**) suggests that this mutation may not significantly impair the activity of Parkin, it may in fact be one of the factors increasing the risk of PD via a different mechanism; a hypothesis that should be confirmed in further studies. However, high penetrance of the R275T mutation has been previously suggested and has been confirmed by our findings [8].

Surprisingly, the E310D substitution was detected at a high frequency in the Polish population, which may be geographically conditioned. According to previous reports by Bardien *et al.*, it appears that this variant may have incomplete penetrance or may lead to preclinical changes in the central nervous system and may increase the risk of PD, probably in combination with other genetic or environmental factors [16]. It is also suggested that the E310D substitution may have a polymorphic nature.

Generally, we showed that single heterozygous polymorphisms (S167N, E310D, D394N) of the *PRKN* gene were present in 21% of spontaneous PD (SPD) compared to 8% in controls. We demonstrated a significantly higher frequency of the D394N polymorphism in LOPD cases as compared with the controls, indicating that this polymorphism may increase the risk of PD over four-fold. However, the results of these studies were not confirmed by the study of EOPD in the Polish population, most likely due to the age of the controls, which was below 40 years, in whom PD manifestation in the future may be likely in the presence of *PRKN* changes [9].

It is of note that we detected more than one *PRKN* variant in 5% of the PD patients, thus suggesting that the presence of more than one heterozygous *PRKN* change may be necessary for the manifestation of PD, and thus confirming a previously proposed hypothesis [10]. On the other hand, it cannot be ruled out that one *PRKN* variant may be sufficient to increase the PD risk and to induce preclinical changes in the *substantia nigra* [17]. It also seems that in patients with three changes in *PRKN* (S167N, E310D, D394N), despite the long duration of the disease, Parkin probably maintained a protective effect that may have resulted in a milder and slower course of disease progression.

As has been suggested, the phenotype of PD caused by *PRKN* mutations is typically characterized by a good response to L-dopa, slowly evolving course, and early age of PD onset [18, 19]. Conversely, some authors suggest that *PRKN* mutation carriers are clinically indistinguishable from other EOPD patients, except for a lower L-dopa equivalent dose (LED) and later development of L-dopa-related motor complications [11, 20], but very little is known to date about clinical features in LOPD patients with *PRKN* variants. Although the young age of onset is undoubtedly the best clinical indicator of Parkin-related PD, some reports have also indicated late onset of PD in patients with *PRKN* mutations [14, 15, 19, 21]. Presently, it is assumed that Parkin-related PD displays wide intrafamilial variability of age at onset [10, 15, 21, 22]. In our patient with changes in exons 7 and 8, LOPD was been reported, which is contrary to the previous reports that indicated EOPD in patients with the R275T mutation [10, 14]. However, the novel mutation L174F was identified only in patients with EOPD, similarly to some other exon 4 mutations [10, 14, 15]. Moreover, in patients with L174F, a slow progression of PD was noted, probably due to the action of compensatory mechanisms [14]. However, in the patient with the single L174F mutation, a good response to L-dopa therapy was additionally reported, which was better than in the co-occurrence of an additional polymorphism in exon 4 of *PRKN* (S167N). On the other hand, in patients with a single L174F mutation the occurrence of dyskinesia and autonomic disorders was also reported, in contrast to patients with two substitutions in exon 4. Whereas in the patient with coexisting substitutions in exons 7 (R275T) and 8 (E310D), good response to L-dopa therapy was reported, which is consistent with previous reports by Khan *et al.* [14]. Interestingly, in patients with the R275T mutation, Khan *et al.* observed psychiatric disorders, including depression, while in our study we observed no symptoms of depression or other cognitive disorders, dyskinesia, fluctuations or autonomic disorders. Generally, we showed that in PD patients with more than one *PRKN* change (mutations and polymorphisms), additional symptoms (like cognitive impairment, depression, dyskinesia, fluctuations and autonomic disorders) were less frequently than in the presence of a single *PRKN* polymorphism. Therefore, it seems that the presence of more than one genetic change in the *PRKN* gene may modify clinical features in PD [23]. Interestingly, according to recent reports, a wide variability in onset age and phenotype might be observed even within the same family (variation of up to 20 years in the age of onset has been observed) [11, 24], thus indicating that there are strong modulating factors which are either environmental or genetic. We also confirmed that other genetic factors probably may additionally increase the risk of PD manifestation, namely in our 3 PD patients with *PRKN* polymorphisms and previously detected mutation in the *HTRA2* or *SPR* gene (Fig. **2**) [4]. To date, patients carrying mutations and polymorphisms in two genes related with PD (including *LRRK2/PRKN* and *PINK1/PRKN* digenic mutation carrier) were described as very rare [25, 26]. In the present study, in patients with the E310D *PRKN* substitution with an additional *HTRA2* mutation (previously detected and described in our book chapter) [4], slower progression of the disease was observed (1st stage on the Hoehn-Yahr scale irrespective of PD duration), and no additional symptoms. Therefore, it seems that the mutation of *HTRA2* may rather be associated with a milder course of PD while in the PD patient with the S167N variant, the additional *SPR* mutation was probably associated with depressive symptoms. It seems that the presence of depressive symptoms in this patient may be connected rather with a mutation in *SPR*, which encodes sepiapterin reductase, an enzyme related to the biosynthesis of dopamine and serotonin, the latter of which is associated with the occurrence of depressive disorders when its level is reduced [27-31].

On the other hand, so far it has been suggested that in PD cases associated with the *PRKN* mutation the clinical phenotype is usually similar; however, Khan *et al.* showed that although some clinical themes may be common and overlapping, Parkin-related PD is also clinically heterogeneous and probably may be mutation-dependent, which is consistent with the results of our study [14, 32, 33]. We showed that in PD patients with various mutations and polymorphism of *PRKN* gene, differences in clinical features are present. It seems that the S167N polymorphism probably may be related with a better response to L-dopa therapy in late period of PD, while polymorphism D394N at the beginning of the disease; whereas the E310D polymorphism may presumably be related with slower progression of the disease. Nevertheless, the demonstrated correlations are moderate and definitely require confirmation in studies on larger groups.

CONCLUSION

In conclusion, 4% of Polish PD patients demonstrated the presence of mutations in *PRKN* genes, while 5% of PD patients had more than one change in the *PRKN* gene (mutation and polymorphisms). Furthermore, S167N, E310D and D394N were the most frequently (21%) detected *PRKN* polymorphisms in the analyzed population. However, the observed high frequency of the E310D variant may be geographically conditioned and requires study in larger groups. It seems that *PRKN* variants are relatively common in our Polish population of patients with PD. It also seems that variants of the *PRKN* gene probably correlate moderately with the course of the disease, response to therapy and the presence of neuropsychiatric symptoms. Moreover, we suggest that the coexistence of more than one mutation and/or polymorphism in *PRKN* or other genes may additionally influence the risk of PD and clinical features. Finally, it also seems that clinical features in *PRKN*-related PD may be associated with the type of genetic change(s) present (mutation/polymorphism). However, this hypothesis should be confirmed in further studies. Nonetheless, analysis of the *PRKN* gene presumably may be useful in determining clini-

cal phenotype, and helping with diagnostic and prognostic modalities in the future.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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