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Poszukiwanie nowych zmienności genetycznych u pacjentów z rodzinnymi parkinsonizmami w populacji polskiej.

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu

w dyscyplinie nauki medyczne

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Wykaz publikacji będących podstawą cyklu tematycznego pracy doktorskiej

Publikacja	**Rodzaj pracy**	**IF**	**MNiSW**
Milanowski Łukasz, Hoffman-Zacharska Dorota, Geremek Maciej, Friedman Andrzej, Figura Monika, Koziorowski Dariusz Mariusz. The matter of significance - Has the p.(Glu121Lys) variant of TOR1A gene a pathogenic role in dystonia or Parkinson disease? J Clinical Neuroscience. 2020 Feb;72:501-503. doi: 10.1016/j.jocn.2019.12.018.	Praca oryginalna	1,760	70
Milanowski Łukasz, Sitek Emilia, Dulski Jaroslaw, Cerquera-Cleves Catalina, Gomez Juan, Brockhuis Bogna, Schinwelski Michal, Kluj-Kozlowska Klaudia, Ross Owen A, Slawek Jaroslaw, Wszolek Zbigniew K. Cognitive and behavioral profile of Perry syndrome in two families. Parkinsonism and Related Disorders. 2020 Aug;77:114-120. doi: 10.1016/j.parkreldis.2020.05.019.	Praca oryginalna	3,926	100
Milanowski Łukasz, Lindemann Jennifer, Hoffman-Zacharska Dorota, Soto-Beasley Alexandra, Barcikowska Maria, Boczarska-Jedynak Magdalena, Deutschlander Angela, Klodowska Gabriela, Dulski Jaroslaw, Fedoryshyn Lyuda, Friedman Andrzej, Jamrozik Zygmunt Tadeusz, Janik Piotr Stanisław, Karpinsky Katherine, Koziorowski Dariusz Mariusz, Krygowska-Wajs Anna, Jasinska-Myga Barbara, Opala Grzegorz, Potulska-Chromik Anna Maria, Pulyk Aleksander, Rektorova Irena, Sanotsky Yanosh, Siuda Joanna, Slawek Jaroslaw, Smilowska Katarzyna, Szczechowski Lech, Rudzinska-Bar Monika, Walton Ronald, Ross Owen, Wszolek Zbigniew. Frequency of Mutations in PRKN, PINK1, and DJ1 in Patients With Early-Onset Parkinson Disease From Neighboring Countries in Central Europe. Parkinsonism and Related Disorders 2021 (in press) 10.1016/	Praca oryginalna	3,926	100

j.parkreldis.2021.03.026			
Milanowski Łukasz, Ross Owen, Friedman Andrzej, Hoffman-Zacharska Dorota, Gorka-Skoczylas Paulina, Jurek Marta, Koziorowski Dariusz Mariusz, Wszolek Zbigniew K. Genetics of Parkinson's disease in the Polish population. Neurologia i Neurochirurgia Polska 2021; 55:1-12. doi. 10.5603/PJNNS.a2021.0013	Praca poglądowa	1,025	40
	Sumaryczna punktacja	**10,637**	**310**

Spis treści

I. Lista i objaśnienie skrótów

AD-autosomalnie dominujący

AR-autosomalnie recesywny

ATP13A2-ATPaza transportująca kationy 13A2

CHCHD2- białko zawierające domenę zwinięta-cewka-helisa-zwinięta cewka-helisa 2

DATScan- Specjalistyczna tomografia komputerowa pojedynczego fotonu z użyciem radioznacznika znakującego transportera dopaminy

DCTN1-dynaktyna 1

DJ1-białkowa deglikaza DJ1

DNAJC- homolog DnaJ podrodzina C

EOPD-choroba Parkinsona o wczesnym początku

EIF4G1- czynnik inicjacji translacji eukariotycznej 4 gamma 1

FBXO7-białko F-box typu 7

GIGYF2- Białko GYF oddziałujące z GRB10 typu 2

GWAS-badanie asocjacyjne całego genomu

HTRA2- peptydaza serynowa-2 HtrA

LRRK2- kinaza druga związana z powtórzeniami bogatymi w leucynę

MLPA- zależna od ligacji multipleksowa amplifikacja sond

NHLBI- National Heart, Lung, and Blood Institute

OMIM- Online Mendelian Inheritance in Man

PD-choroba Parkinsona

PINK1-indukowana PTEN kinaza 1

PLA2G6-fosfolipaza A2 grupy 6

PRKN-parkina

SNCA- α-synukleina

TOR1A-torsyna 1A

UCH-L1- hydrolaza L1 ubikwityny

WES-sekwencjonowanie całoeksomowe

VPS- pęcherzykowe białko sortujące

II. Streszczenie w języku polskim

Wstęp

PD jest jednym z najczęstszych neurodegeneracyjnych zaburzeń ruchowych na świecie, dotykającym ludzi ze wszystkich grup etnicznych. Większość przypadków PD jest sporadyczna; jednak około 15% to postacie rodzinne. Genetyczną przyczynę PD udaje się zidentyfikować zwykle u pacjentów z wczesnym początkiem objawów lub u osób z dodatnim wywiadem rodzinnym. Celem cyklu publikacji jest analiza zmienności genetycznych związanych z parkinsonizmami w populacji polskiej.

Metodologia

Przegląd literatury został dokonany z użyciem bazy PubMed. Duplikacje w genie SNCA wykryto przy pomocy MLPA MRC-Holland. W pracy analizującej rodzinę z mutacją w genie *TOR1A* wykonano sekwencjonowanie całoeksomowe przy pomocy platformy Illumina Novaseq 6000. Celem potwierdzenia obecności wariantu wykonano u probandki sekwencjonowanie sangerowskie genu *TOR1A*. W pracy dotyczącej zespołu Perry'ego wykonano sekwencjonowanie sangerowskie exonu 2 genu DCTN1. W trzeciej pracy sekwencjonowanie sangerowskie wykonano w genach PRKN, PINK1, DJ1 oraz dokonano analizy rearanżacji eksomowych przy pomocy kitów MLPA MRC-Holland.

Wyniki

Praca przeglądowa posumowuje wiedzę dotyczącą dotychczas wykonanych badań genetycznych w chorobie Parkinsona w populacji Polskiej. W pierwszej pracy oryginalnej wykazano obecność mutacji p.(Glu121Lys) w genie TOR1A u probandki. Nie wykryto natomiast obecności mutacji w 23 opisywanych genach OMIM powiązanych z PD. Dokonano analizy dostepnych baz danych-wariant ten ujawniono u 3 pacjentów z bazy NHLBI (0,02%) oraz u 0.03% pacjentów w bazie gnomAD. Dodatkowo w bazie danych 600 WES Zakładu Genetyki Medycznej Instytutu Matki i Dziecka ujawniono ten wariant u 2 zdrowych mężczyzn (0,33%).

W drugiej publikacji opisano charakterystykę kliniczną oraz neuropsychologiczną dwóch rodzin z zespołem Perry'ego-polskiej i kolumbijskiej W polskiej rodzinie na podstawie

długoletnich, 11-letnich obserwacji udało się ustalić wariant behawioralny otępienia czołowo-skroniowego jako dominujący fenotyp neuropsychologiczny.

W trzeciej pracy analizowano 541 pacjentów z EOPD (Republika Czeska n=11, Niemcy n=38, Polska n=476, Ukraina n=16). Wśród wszystkich pacjentów 17.2% (n=93) miało dodatni wywiad rodzinny. Wśród pacjentów populacji polskiej pozytywny wywiad rodzinny był stwierdzony u 15.8% pacjentów (n=75). U 14 polskich pacjentów wykazano obecność mutacji homozygotycznej lub złożonej heteorzygotycznej w genie PRKN oraz 3 homozygotycznych w genie PINK1. Warianty PRKN p.Glu79Ter oraz p.Cys466Phe nie były wcześniej raportowane w innych populacjach w dostępnych bazach danych.

Wnioski

Podsumowując w populacji polskiej mogą występować charakterystyczne dla rodzinnych parkinsonizmów zmienności genetyczne. Identyfikacja specyficznych wariantów w znanych genach jest pierwszym krokiem do przyszłego odkrycia nowych genów w PD.

III. Streszczenie w języku angielskim

Title: **Novel genetic variants associated with familial forms of parkinsonism in Polish population.**

Introduction

PD is one of the most common neurodegenerative movement disorders in the world, affecting people of all ethnicities. Most cases of PD are sporadic; however, about 15% are familial forms. The genetic cause of PD is usually identified in patients with an early onset of symptoms or in those with a positive family history. The aim of the series of publications is to analyze the genetic variation associated with parkinsonism in the Polish population.

Methodology

In the review paper, the electronic database, PubMed, was searched. The *SNCA* duplication was revealed with MLPA-MRC-Holland kit usage. In the study analyzing a family with a *TOR1A* mutation, whole-exome sequencing was performed using the Illumina Novaseq 6000 platform. To confirm the presence of the variant, Sanger sequencing of the *TOR1A* was conducted in the proband. In the Perry syndrome paper, Sanger sequencing of *DCTN1*, exon 2 was performed. In the third study, Sanger sequencing of *PRKN*, *PINK1*, *DJ1* and the exome rearrangements analysis with MLPA MRC-Holland kit usage were carried .

Results

The review paper summarizes the current knowledge in the genetic studies conducted in Polish PD patients.

The first original article showed the presence of the p. (Glu121Lys) mutation in the TOR1A gene in proband. There were no other mutations in 23 previously described PD-related OMIM genes. The available databases were analyzed - this variant was revealed in 3 patients from the NHLBI database (0.02%) and in 0.03% of patients from the gnomAD database. Additionally, the database of 600 WES results from the Department of Medical Genetics of the Institute of Mother and Child revealed this variant in 2 healthy men (0.33%).

The second publication described the clinical and neuropsychological characteristics of two Perry syndrome families- Polish and Colombian. In the Polish family, eleven years of

observation revealed that the dominant neuropsychological phenotype was the behavioral variant of frontotemporal dementia.

In the third study, 541 EOPD patients were included (Czech Republic n = 11, Germany n = 38, Poland n = 476, Ukraine n = 16). Among all patients, 17.2% (n = 93) had a positive family history. In the Polish population, a positive family history was found in 15.8% of patients (n = 75). Fourteen Polish patients had a homozygous or complex heterozygous mutation in *PRKN* and 3 patients had homozygous mutations in the *PINK1*. The PRKN p.Glu79Ter and p.Cys466Phe variants have not been previously reported in other populations in the available databases.

Conclusions

To sum up, in the Polish population there may occur genetic variants characteristic for familial parkinsonism. The identification of specific variants in known genes is the first step to new parkinsonism's loci discovery in the future.

IV. Wprowadzenie

PD jest jednym z najczęstszych neurodegeneracyjnych zaburzeń ruchowych na świecie, dotykającym ludzi ze wszystkich grup etnicznych (1). Główne objawy ruchowe obejmują drżenie, sztywność, bradykinezję lub akinezę oraz niestabilność postawy (1). Patofizjologia tej choroby opiera się na degradacji neuronów dopaminergicznych istoty czarnej. Charakterystyczną cechą neuropatologiczną jest obecność ciał Lewy'ego złożonych z zagregowanych włókienek α-synukleiny (1). Jednak istnieje wiele różnych molekularnych szlaków prowadzących finalnie do PD. Diagnoza jest zwykle oparta na objawach klinicznych, ale do przydatnych narzędzi diagnostycznych należą metody radiologiczne, takie jak DATScan i pozytonowa tomografia emisyjna (2). Za występowaniem PD może odpowiadać wiele czynników. Częstość występowania PD różni się w różnych grupach etnicznych. Jedną z najczęstszych obserwacji jest to, że PD występuje znacznie częściej w populacjach zachodnich (3). Jednak w Azji i Afryce istnieją określone grupy etniczne, w których choroba Parkinsona jest powszechna (4). Czynniki wpływające na rozpowszechnienie choroby również różnią się w zależności od populacji. Jedna z różnic w częstości występowania może być związana z najważniejszym czynnikiem ryzyka PD- wiekiem (5). Grupy etniczne Europy Zachodniej są zwykle starsze niż podgrupy z krajów o niskich dochodach, więc częstość występowania PD w tych krajach jest wyższa (5). Ponadto opcje diagnostyczne i terapeutyczne są bardziej dostępne w krajach o wysokim dochodzie. Dodatkowo każda grupa etniczna ma charakterystyczne dla siebie podłoże genetyczne. Większość przypadków PD jest sporadyczna; jednak około 15% to postacie rodzinne (6). Genetyczną przyczynę PD udaje się zidentyfikować zwykle u pacjentów z EOPD, definiowaną jako wiek wystąpienia objawów poniżej 50 rż. lub u osób z dodatnim wywiadem rodzinnym (7). Dotychczas zidentyfikowano wiele loci genetycznych związanych z PD.

W bazie danych OMIM 23 geny zostały powiązane z monogenicznymi postaciami PD (8) (Tabela). W ostatnim badaniu GWAS zidentyfikowano znacznie więcej genów ryzyka PD- ponad 90 loci (9).

Polska jest państwem jednorodnym etnicznie; obecna populacja wynosi 38 milionów, a 97,1% deklaruje narodowość polską. Jednak w przeszłości na obecnych ziemiach polskich żyło wiele różnych grup mniejszościowych; granice zmieniały się wielokrotnie, powodując masowe migracje ludzi. Czynniki te doprowadziły do powstania wyjątkowego tła

genetycznego w naszym kraju. Polska ma znacznie starszą populację, a częstość występowania PD rośnie; w 2016 r. zgłoszono około 75 000 przypadków (5).

W populacji polskiej dotychczasowe badania genetyczne w chorobie Parkinsona analizowały zarówno geny powiązane z monogenowymi formami oraz geny będace czynnikami ryzyka PD. Podsumowanie dotychczasowych analiz genetycznych przeprowadzonych w populacji polskiej zawarta jest w pracy przeglądowej dołaczonej do cyklu obecnej pracy doktorskiej (10).

Cykl przedstawia wyniki trzech prac oryginalnych oraz jedną pracę poglądową:

- Genetics of Parkinson's disease in the Polish population (10).

-The matter of significance - Has the p.(Glu121Lys) variant of TOR1A gene a pathogenic role in dystonia or Parkinson disease (11)?

- Cognitive and behavioral profile of Perry syndrome in two families (12).

- Frequency of Mutations in PRKN, PINK1, and DJ1 in Patients With Early-Onset Parkinson Disease From Neighboring Countries in Central Europe (13).

Praca „ Genetics of Parkinson's disease in the Polish population" jest przeglądem literatury dotyczącym dotychczasowych wykonanych badań genetycznych w chorobie Parkinsona w populacji Polskiej.W pierwszym artykule oryginalnym przedyskutowano o potencjalnym wpływie mutacji w genie TOR1A p.Gly121Lys, dotychczas opisywanej u osób z uogólnioną dystonią na wystepowanie choroby Parkinsona. Druga praca oryginalna porusza problem zagadnień neuropsychologicznych u dwóch rodzin z zespołem Perry'ego-polskiej i kolumbijskiej. Zespół Perry jest chorobą dziedziczoną w sposób autosomalnie dominujący i na jego obraz kliniczny składają się 4 objawy tj. parkinsonizm, hipowentylacja ośrodkowa, utrata wagi oraz zaburzenia psychiatryczne (najczęściej depresja). Trzecia praca oryginalna analizuje występowanie 3 najczęstszych genów związanych z występowaniem choroby Parkinsona o wczesnym początku u pacjentów EOPD z 4 sąsiadujących krajów w Europie Centralnej: Czechach, Niemczech, Ukrainie oraz Polsce. Do analiz zostali włączeni zarówno pacjenci z jak i bez dodatniego wywiadu rodzinnego.

V. Założenia i cel pracy

W populacji polskiej mogą występować charakterystyczne dla kohorty zmienności genetyczne związane z parkinsonizmami.

VI. Omówienie cyklu

Praca przeglądowa jest podsumowaniem badań genetycznych dotychczas przeprowadzonych wśród pacjentów z PD w populacji polskiej. Dokonano analizy różnych grup zmienności genetycznych (zarówno warianty odpowiedzialne za monogenową PD, czynniki ryzyka PD jak i warianty związane z metabolizmem dopaminy) orz rózne kohorty pacjentów (przypadki sporadyczne, o wczesnym początku oraz postaci rodzinne). Dodatkowo zaprezentowano pierwszy przypadek polskiego pacjenta z duplikacją w genie *SNCA*

Celem pierwszej pracy oryginalnej była dyskusja nad patogennością mutacji w genie TOR1A i jej potencjalnym powiązaniu z rodzinną postacią PD. U dwojga członków rodziny (probandki oraz jej ojca) zdiagnozowano chorobę Parkinsona o późnym początku. Wykonano sekwencjonowanie całoeksomowe u probandki wykazało obecność mutacji p.(Glu121Lys) w genie *TOR1A.* Nie wykryto natomiast obecności mutacji w 23 opisywanych genach OMIM powiązanych z PD. Mutacje w genie TOR1A odpowiedzialne są za występowanie dystonii uogólnionej. Wariant p.Glu121Lys był dotychczas opisywany tylko w jednym badaniu, gdzie ujawniono go u jednego pacjenta spośród 162 serbskich pacjentów z pierwotna dystonią (14). W naszym opracowaniu poddaliśmy w wątpliwość jakąkolwiek patogenność tej mutacji. Na poparcie swojej tezy przytoczyliśmy fakt obecności tej mutacji w dostępnych bazach danych- wariant ten ujawniono u 3 pacjentów z bazy NHLBI (0,02%) oraz u 0.03% pacjentów w bazie gnomAD. Dodatkowo w bazie danych 600 WES Zakładu Genetyki Medycznej Instytutu Matki i Dziecka ujawniono ten wariant u 2 zdrowych mężczyzn (0,33%). W analizach *in silico* patogenność tego wariantu również jest kontrowersyjna-HGMD Proffesional uznaje ten wariant za powiązany z chorobą, co nie znajduje odzwierciedlenia w bazach (PolyPhen2, HumDiv/HumVar algorytms, MutationTaster oraz CADD), gdzie wariant opisywany jest jako niepatogenny. Baza ClinVar klasyfikuje tą mutacje jako VUS. Konkludując, wariant TOR1A p.Glu121Lys wydaje sie nie mieć wływu na rodzinną postać PD.

W drugiej publikacji opisano charakterystykę kliniczną oraz neuropsychologiczną dwóch rodzin z zespołem Perry'ego-polskiej i kolumbijskiej. Zespół Perry'ego jest chorobą o

dziedziczeniu autosomalnie dominującym oraz charakteryzuje się występowaniem 4 objawów klinicznych: parkinsonizmu niewrażliwego na leczenie lewodopą, utraty masy ciała, centralnej niewydolności oddechowej oraz zaburzeń psychicznych (głównie depresji). W dotychczasowej literaturze opisano tylko 23 rodziny z tą formą parkinsonizmu. Po raz pierwszy został dokonany dokładny przegląd objawów psychiatrycznych u pacjentów z zespołem Perry'ego. W polskiej rodzinie na podstawie długoletnich, 11-letnich obserwacji udało się ustalić wariant behawioralny otępienia czołowo-skroniowego jako dominujący fenotyp neuropsychologiczny.

W trzeciej pracy poddaliśmy analizie 541 pacjentów z EOPD (Republika Czeska n=11, Niemcy n=38, Polska n=476, Ukraina n=16). Wśród wszystkich pacjentów 17.2% (n=93) miało dodatni wywiad rodzinny. U wszystkich pacjentów wykonano sekwencjonowanie Sangerowskie genów *PRKN, PINK1* oraz *DJ1*, w których mutacje są najczęściej identyfikowaną przyczyną choroby Parkinson o dziedziczeniu autosomalnie recesywnym. Wśród pacjentów populacji polskiej pozytywny wywiad rodzinny był stwierdzony u 15.8% pacjentów (n=75). U 14 polskich pacjentów wykazano obecność mutacji homozygotycznej lub złożonej heteorzygotycznej w genie *PRKN* oraz 3 homozygotycznych w genie *PINK1*. Warianty PRKN p.Glu79Ter oraz p.Cys466Phe nie były wcześniej raportowane w innych populacjach w dostepnych bazach danych.

VII. Podsumowania i wnioski

Podsumowując w populacji polskiej mogą występować charakterystyczne dla rodzinnych parkinsonizmów zmienności genetyczne. Identyfikacja specyficznych wariantów w znanych genach jest pierwszym krokiem do przyszłego odkrycia nowych genów w PD.

VIII. Tabela

Tabela. Geny odpowiedzialne za monogenowe postacie choroby Parkinsona (8).

Locus	Gen	Lokalizacja (chromosom)	Dziedziczenie
PARK1/4	*SNCA*	4q22.1	AD
PARK2	*PRKN*	6q26	AR
PARK3	---	2p13	AD
PARK5	*UCH-L1*	4p13	AD
PARK6	*PINK1*	1p36.12	AR

PARK7	*DJ1*	1p36.23	AR
PARK8	*LRRK2*	12p12	AD
PARK9	*ATP13A2*	1p36.13	AR
PARK10	---	1p32	---
PARK11	*GIGYF2*	2q37.1	AD
PARK12	---	Xq21-q25	X-linked
PARK13	*HTRA2*	2p13.1	AD
PARK14	*PLA2G6*	22q13.1	AR
PARK15	*FBXO7*	22q12.3	AR
PARK16	---	1q32	---
PARK17	*VPS35*	16q11.2	AD
PARK18	*EIF4G1*	3q27.1	AD
PARK19	*DNAJC6*	1p31.3	AR
PARK20	*SYNJ1*	21q22.11	AR
PARK21	*DNAJC13/TMEM 230*	3q22.1	AD
PARK22	*CHCHD2*	7p11.2	AD
PARK23	*VPS13C*	15q22.2	AR

IX. Kopie opublikowanych prac

REVIEW ARTICLE

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Genetics of Parkinson's disease in the Polish population

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ABSTRACT

Introduction. Genetic forms of Parkinson's disease (PD) often cluster in different ethnic groups and may present with recognisable unique clinical manifestations. Our aim was to summarise the current state of knowledge regarding the genetic causes of PD and describe the first Polish patient with *SNCA* duplication.

Methodology. We searched the electronic database, PubMed, for studies between January 1995 and June 2020 that evaluated genetics in Polish patients with PD, using the search terms 'Parkinson's disease, 'Polish', 'genetics', 'mutations', and 'variants'.

Results. In total, 73 publications were included in the review; 11 genes responsible for monogenic forms and 19 risk factor genes have been analysed in the Polish population. Pathogenic variants were reported in four monogenic genes (*LRRK2*, *PRKN*, *PINK1*, and *SNCA*). Eight genes were associated with PD risk in the Polish population (*GBA*, *TFAM*, *NFE2L2*, *MMP12*, *HLA-DRA*, *COMT*, *MAOB*, and *DBH*). Multiplex ligation-dependent probe amplification and Sanger sequencing in *PRKN*, *PINK1*, *DJ1*, *LRRK2*, and *SNCA* revealed *SNCA* duplication in a 43-year-old Polish patient with PD examined by movement disorder specialists.

Conclusion. Only a limited number of positive results have been reported in genes previously associated with PD in the Polish population. In the era of personalised medicine, it is important to report on genetic findings in specific populations.

Key words: genetics, Parkinson's disease, Polish population, *SNCA* duplication

Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative movement disorders worldwide, affecting people of all ethnic groups [1]. The cardinal motor features include tremor, rigidity, bradykinesia or akinesia, and postural instability [2–4]. The pathophysiology of this disease is based on degeneration of dopaminergic neurons in the substantia nigra [1]. The characteristic neuropathological feature is the presence of Lewy bodies composed of aggregated α-synuclein fibrils. However, many different molecular pathways of dysfunction have been proposed leading to PD [1]. Diagnosis is usually based on clinical features, but radiological methods such as dopamine transporter scan and positron emission tomography are useful diagnostic tools [5, 6].

Multiple factors may be associated with the prevalence of PD. The frequency of PD and PD subtypes differ in different ethnic groups. One of the most common observations is that PD occurs much more frequently in Western populations. However, there are specific ethnic groups in Asia and Africa where PD is common [7]. The factors impacting upon disease prevalence also differ across populations [8, 9]. One difference in prevalence may be associated with the most important risk factor for PD: age [10]. Western European ethnic groups are usually older than subgroups from low-income countries, so the prevalence of PD is higher. Also, diagnostic and therapeutic options are more available in high-income countries [11, 12]. Furthermore, genetic background is characteristic for different ethnic groups [10]. Most PD cases are sporadic; however, about 15% are familial [1]. The genetic cause of PD

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is usually determined in patients with early-onset PD (EOPD) or in those with a positive family history. Many genetic loci associated with PD have been identified.

In the Online Mendelian Inheritance in Man (OMIM) database, 23 genes have been associated with monogenic forms of PD. The last genome-wide association study (GWAS) identified more risk genes than the 23 already in the OMIM database; > 90 risk loci [13].

Poland is ethnically homogenous; the current population is 38 million and 97.1% declare Polish nationality. However, in the past, many different minority groups have lived in current Polish territories; the borders have changed many times, resulting in massive migrations of people. These factors have led to the presence of a unique genetic background in this country. Poland has a substantial older population and the occurrence of PD is increasing; approximately 75,000 cases were reported in 2016 [14].

Many genetic PD loci associated with different pathways have been studied in the Polish population. Patients have been recruited in five main PD centres in Poland (Supplementary Fig. 1).

The aim of this review was to summarise the genetic studies that have been conducted in Polish patients with PD. The electronic database, PubMed, was searched for articles published between January 1995 and June 2020 relating to studies that evaluated genetics in Polish patients with PD. Review articles and meta-analyses were also investigated, and their reference lists were examined for possible inclusion. Our search was limited to human studies. We used the following search terms: 'Parkinson's disease', 'Polish', 'genetics', 'mutations', and 'variants'. We also describe a new Polish patient with *SNCA* duplication. The blood specimen from this patient was collected with institutional review board approval, and informed consent was signed.

Monogenic forms of PD

In monogenic forms of PD, the disease is inherited dominantly or recessively by mutation of a single gene. The monogenic forms of PD are responsible for about 30% of familial forms and 3-5% of sporadic cases [15]. Several genes from this group have been reported in Polish populations (Tab. 1) [16–30].

Autosomal recessive PD genes

Many studies of monogenic PD forms in Polish populations have analysed the three most common autosomal recessive genes reported in EOPD: *PRKN, PINK1*, and *DJ1* [16, 20, 23, 24, 31]. Though typical age at onset for PD is above 60 years, EOPD is defined in different ways. While the European Parkinson's Disease Association defines 'early' as age at onset younger than 40, the American Parkinson's Disease Association defines it as age at onset younger than 50. EOPD is reported in about 5% of patients [32]. Summaries of monogenic PD forms are provided in Table 1 and Figure 1.

PRKN (OMIM 602544, PARK2)

The *PRKN* gene is associated with the autosomal recessive form of EOPD [33]. *PRKN* encodes the protein responsible for quality control of mitophagy. PRKN is an E3 ubiquitin ligase that participates in ubiquitin-proteasome interaction. Mutations in *PRKN* result in degradation of damaged mitochondria, leading to oxidative stress that can damage the substantia nigra dopaminergic cells [15]. According to published data, the mutations in *PRKN* are present in a large proportion of EOPD worldwide (up to 18% of patients) [15]. *PRKN* PD type is characterised by a broad range of clinical phenotypes, some atypical signs, but generally has early onset, slower progression, better response to levodopa, and often more severe drug-induced adverse effects [34]. Sometimes in the clinical phenotype in carriers, parkinsonism is not a dominant symptom [31].

Several studies have analysed *PRKN* in Polish populations. The first case-control study of 79 patients with EOPD (onset < 40 years) and 204 controls revealed two patients with homozygous or compound heterozygous mutation and one with heterozygous mutation (3.8%) [24]. A study of 150 patients with EOPD (onset < 45 years) reported *PRKN* mutations in 4.7% [23]. Gaweda-Walerych et al. [20] identified only one heterozygous *PRKN* deletion; however, from 344 patients with PD (171 EOPD), Ambroziak et al. [16] identified five compound heterozygous and three heterozygous mutations.

PINK1 (OMIM 608309, PARK6)

PINK1 (phosphatase and tensin homolog-induced putative kinase 1) is another common cause of early-onset parkinsonism worldwide. It was first described in a large Italian family and is the second most commonly identified mutation in patients with autosomal recessive EOPD [35]. *PINK1* protein strongly cooperates with *PRKN* in mitochondrial quality control to identify, label, and remove damaged organelles. *PINK1* is responsible for ubiquitin phosphorylation at Ser65. The endogenous Ser65 phosphopeptide is only detected with *PINK1* and together cause a decrease in mitochondrial membrane potential [27].

In the first Polish *PINK1* genetic study, only four patients with EOPD (2.67%) were carriers of *PINK1* mutations (one homozygote) [23]. Another study analysed molecular characteristics of *PINK1* p.Gln456Ter mutation present in two family members. This mutation can lead to a decrease in mRNA and loss of protein function [29, 36]. One molecular study revealed that previously described *PINK1* p.Ile368Asn cannot be stabilised on the outer mitochondrial membrane upon mitochondrial stress, and due to conformational changes in the active site, does not exert kinase activity towards ubiquitin [17]. In 748 Polish patients with PD, 0.94% were carriers of *PINK1* p.Gly411Ser mutation, which increased PD risk via dominant-negative mechanism [27].

Table 1. Autosomal recessive and autosomal dominant inherited genes analysed in Polish populations

Gene	Chromosome localisation	Results	Study group
Autosomal recessive			
PRKN	6q26	2 homozygotes/compound heterozygotes and 1 heterozygote	79 EOPD, age < 40 y [24]
		5 compound heterozygotes, 2 heterozygotes	150 EOPD, age < 45 y [23]
		5 compound heterozygotes, 3 heterozygotes	344 PD (171 EOPD, age < 45 y; 173 LOPD) [16]
		No pathogenic mutations	104 EOPD, age ≤ 50 y [20]
PINK1	1p36.12	1 homozygote, 3 heterozygotes	150 EOPD, age < 45 y [23]
		PINK1 p.Gln456Ter in both patients	2 family members affected [29]
		PINK1 p.Ile368Asn in both patients	2 family members affected [17]
		0.94% p.Gly411Ser *PINK1* mutation carriers	748 PD [27]
DJ1	1p36.23	No pathogenic mutations	150 EOPD, age < 45 y [23]
Autosomal dominant			
LRRK2	12q12	1 G2019S heterozygote	100 sporadic PD [22]
		No pathogenic variants	174 sporadic PD [18]
SNCA	4q22.1	No p.Ala30Pro, p.Glu46Lys, p.Ala53Thr, or multiplication p.Ala18Thr in 1 patient, p.Ala29Ser in 1 patient	629 PD [21]
		SNCA duplication in patient with EOPD[a]	1 sporadic PD[a]
VPS35	16q11.2	No pathogenic mutations	346 PD [30]
DNAJC13	3q22.1	No pathogenic mutations	702 PD (9.23% positive family history) [25]
CHCHD2	7p11.2	No pathogenic mutation	394 PD [26]
EIF4G1	3q27.1	p.Ala502Val in 1 patient (variant of uncertain pathogenicity)	397 PD [19]
HTRA2	2p13.1	No pathogenic mutations	101 PD [28]

EOPD — early-onset PD; LOPD — late-onset PD; PD — Parkinson's disease; [a]New patient

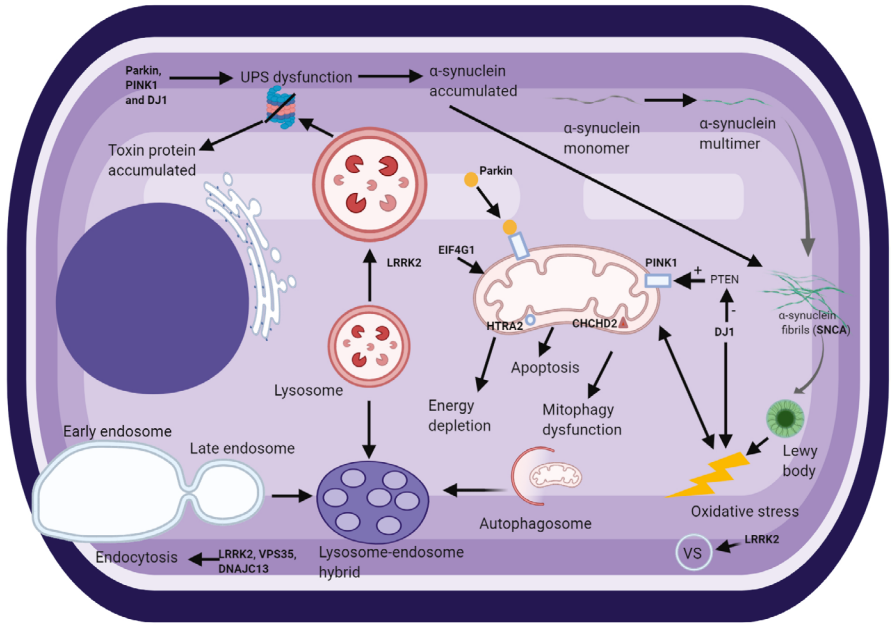


Figure 1. Main pathways associated with Parkinson's disease pathophysiology explored in Polish patients. Bold indicates protein encoding by genes responsible for monogenic forms of PD
ER — endoplasmic reticulum; SV — synaptic vesicle; UPS — ubiquitin-proteasome system

DJ1 (OMIM 602533, PARK7)

The third most commonly reported EOPD gene is *DJ1*; however, it is much rarer than *PRKN* and *PINK1*. It has been reported in only a few populations [37]. As with *PRKN* and *PINK1, DJ1* participates in mitochondrial quality control. *DJ1* increases the expression of two mitochondrial proteins, UCP4 and UCP5, which decrease mitochondrial membrane potential, reduce reactive oxygen species production, improve mitochondrial functions, and protect the neuronal cells [38]. No *DJ1* variants have been reported in Polish populations [23].

Autosomal dominant PD genes

Autosomal dominant inherited genes generally cause medium-onset to late-onset parkinsonism or PD, with few or no additional symptoms. The characteristic feature is incomplete penetrance of these genes [1].

LRRK2 (OMIM 600907, PARK8)

LRRK2, a large (7,584 bp) gene that encodes leucine-rich repeat kinase 2, is the most common genetic cause of PD. The main purpose of this protein remains unknown, but it may involve such cellular functions as neurite outgrowth, cytoskeletal maintenance, vesicle trafficking, autophagic protein degradation, and the immune system. The well-established association with autosomal dominant PD had six variants. The first families identified with mutation in *LRRK2* were in Japan and the US [39, 40]. The most commonly reported *LRRK2* mutation is the p.Gly2019Ser variant, detected in 30% and 13% of Arab-Berber and Ashkenazi Jewish familial cases of PD, respectively [41, 42]. It has also been reported in up to 6% of familial and 2% of sporadic European PD cases [43]; however, in the Polish population it is rather rare. A study screening for *LRRK2* variants in a European population only found them in one Polish family [22], while another study performed in 174 Polish patients did not reveal any pathogenic variants in this gene [18].

VPS35 (OMIM 601501, PARK17)

VPS35 (vacuolar protein sorting 35 homolog) is a rare cause of autosomal dominant PD. The first reported variant, p.Asp620Asn, was described in Swiss and Austrian families with late-onset PD [30, 44]. The encoding protein is responsible for transmembrane receptor recycling and protein transport between the endoplasmic reticulum and the trans Golgi network. The functional protein cooperates with two other proteins, VPS26 and VPS29, to create a highly conservative, active complex. All three genes were analysed in 356 Polish patients with PD, but no variants in *VPS26* and *VPS29* were found [45]. The original paper describing a *VPS35* variant in a PD family also included analysis of 346 patients with PD and did not reveal any other pathogenic variants [30].

SNCA (OMIM 163890, PARK1)

SNCA mutation was first described in mixed Greco-Italian and Greek families [46]. Initially, point mutations were reported, then multiplications [47]. The clinical phenotype is consistent with late-onset PD with a positive family history and is associated with a good response to levodopa treatment. Occasionally, patients have multiple system atrophy phenotype. Fifty-nine families with *SNCA* duplications have been described worldwide [48]. In some patients with duplications, there is no family history and the phenotype is variable. Patients with triplications usually have earlier age at onset and more severe clinical symptoms [49]. From 629 Polish probands, two sporadic cases with variants, p.Ala18Thr and p.Ala29Ser, were reported, but P.Ala30Pro, p.Glu46Lys, and p.Ala53Thr and multiplication variants were not discovered [21]. The clinical phenotype was characterised by a good response to levodopa, at least at the beginning of the disease. Post mortem of the patient with p.Ala29Ser mutation revealed Lewy bodies and neuritis [21].

We recently identified the first Polish patient with *SNCA* duplication. A 43-year-old right-handed man was referred to the neurology clinic. He had been suffering from right hand tremor for two years. Neurological examination revealed hypomimia, slow speech with dysarthria, bradykinesia, rigidity, and rest tremor on the right side. He reported anosmia and mild drooling, but denied any sleep disturbances. Family history was negative for PD. The patient was diagnosed with PD and initial levodopa treatment (200 mg daily) was implemented, with good response. Because of the younger age at onset (< 50), multiplex ligation-dependent probe amplification in *PRKN*, *PINK1*, *DJ1*, *LRRK2*, and *SNCA* and Sanger sequencing in *PRKN* were performed, revealing a heterozygous *SNCA* duplication (Fig. 2).

Candidate familial PD genes

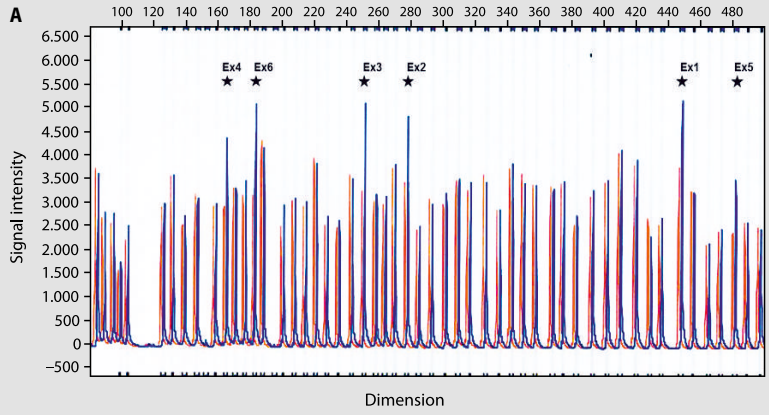
Additional genes have been identified as possible causes of PD. Analyses of autosomal-dominant PD families initially identified *DNAJC13*, *CHCHD2*, *EIF4G1*, *LRP10*, *NUS1*, and *HTRA2* as causative genes; however, data from the case-control study did not support this observation [50]. These genes were also analysed in Polish populations (Tab. 1).

DNAJC13 (OMIM 616361, PARK21)

The first variant in this gene was observed in a Dutch-German-Russian Mennonite family [51]. *DNAJC13* (DnaJ [Hsp40] homolog, subfamily C, member 13 protein) is associated with recycling and functioning of the lysosomal system. In a population of 702 Polish patients with PD with 9.23% positive family history, no pathogenic variants were observed [25].

CHCHD2 (OMIM 616710, PARK22)

Heterozygous mutations in *CHCHD2* (coiled-coil-helix-coiled-coil-helix domain containing 2) were identified first in Japanese families with autosomal dominant patterns of inheritance of PD. The protein is responsible for cytochrome c oxidase activity by acting as a transcription factor to regulate cytochrome c oxidase expression, thereby facilitating mitochondrial electron transport chain flux under low oxygen



B

	Probe Name	Bin Size	Height Ratio
1	X	100.5	1.102
2	Y	105.0	1.128
3	**Ctr1_05q31**	127.2	1.023
4	TNFRSF9	133.0	1.011
5	PINK1_ex05	140.2	1.065
6	PINK1_ex01	148.0	0.971
7	SNCA A 30P	153.5	–1
8	**Ctrl 15q21**	159.3	1.010
9	SNCA_ex04	166.0	1.482
10	PINK1_ex02	171.7	0.998
11	ATP13A2_ex09	177.8	1.094
12	SNCA_ex06	183.8	1.609
13	Ctrl_9p13	189.0	0.965
14	LRRK2_G2019S	196.2	–1
15	PARK2_ex 4	201.4	1.182
16	PINK1_ex03	208.4	1.019
17	ATP13A2_ex 02	215.2	1.033
18	**Ctr1_8q12**	221.7	0.975
19	PINK1_ex07	228.5	1.071
20	PARX2_ex1	235.8	1.054
21	PARK7_ex06	244.0	0.978
22	SNCA ex 03	251.5	1.566
23	Ctr1_18q21	258.9	1.044
24	LPA Downstream	264.6	1.052
25	**PARK7_ex2**	270.5	1.016
26	SNCA_ex2	278.2	1.402
27	PARK2_ex02	285.6	1.028
28	**Ctr1_3p22**	293.4	0.969
29	PARK2_ex8	302.1	1.082
30	PINK1_ex06	309.8	1.007
31	PARK7_ex4	317.0	1.049
32	PARK2_ex09	326.9	0.951
33	**Ctrl_2q36**	335.4	0.997
34	PARK2 ex6	343.1	1.028
35	**PARK7_ex1**	350.5	0.948
36	PARK2_ex10	357.8	0.994
37	PARK7_ex 5	368.5	0.981
38	PARK2_ex11	375.0	1.013
39	**Ctr1_11p14**	383.1	1.076
40	PARK2_ex12	393.1	1.048
41	PINK1_ex4	402.2	1.014
42	PARK7_ex03	410.5	1.015
43	**PARK2_ex5**	420.3	1.027
44	PARK7_ex01	428.9	0.856
45	**Ctrl_6p12**	435.8	1.053
46	SNCA_ex1	448.1	1.377
47	PARK7_ex7	455.6	0.991
48	PINK1_ex08	465.0	1.012
49	PARK2_ex3	472.6	1.011
50	**SNCA_ex5**	481.4	1.463
51	PARK2_ex 7	488.9	1.004
52	**Ctrl_2p13**	497.1	0.983

Figure 2. Detection of the *SNCA* gene duplication in EOPD patient with multiplex ligation-dependent probe amplification (MLPA) method. Reaction was performed with SALSA MLPA Probe mixes P051 (MRC Holland). Dosage analysis was performed with GeneMarker Software v.2.7.0 (SoftGenetics, LLC). **A**. Trace comparison — overdosage of all SNCA exons of patient's sample in relation to control. This panel shows the differences in peak height between patient's sample (blue) and control (red) for all SNCA exons. **B**. Report table — reporting peak ratio for all probes, duplication of SNCA exons (high ratio > 1.3) are indicated in positions 9,12, 22,24, 26,46 and 50. **C**. Ratio plot — visualization of the peak ratios. Normal relative probe signals are between the green lines (0.7–1.3), and are depicted in green. Aberrant relative probe signals are depicted in red

conditions and inhibiting mitochondria-mediated apoptosis. In a study of 394 Polish patients with PD, there were no definite pathogenic variants in this gene [26].

EIF4G1 (OMIM 614251, PARK18)

EIF4G1 encodes the protein, eIF4F, a component of the translation initiation complex. In a cohort of 397 Polish patients with PD, p.Ala502Val variant with unknown pathogenicity was identified in a single case [19]. However, further analysis of this locus did not support its pathogenicity [52].

HTRA2 (OMIM 610297, PARK13)

The Htra2 protein, a serine protease located in mitochondria, is responsible for apoptosis, especially during stress conditions. This protein is also an element of Lewy bodies. *HTRA2* was first reported in German familial and sporadic PD cases [53], but in 101 Polish patients with PD, no pathogenic variants were reported [28].

Risk factor genes

In addition to the genes responsible for familial forms of PD listed in the OMIM database, other genetic loci have been identified that increase the risk of PD occurrence. Some genes can be included as both monogenic and risk factor genes. Most mutations of *SNCA* are responsible for monogenic forms of PD, but some polymorphisms (e.g. rs356219) are risk factors for PD [54]. The last GWAS revealed about 90 genomic regions that can be associated with PD prevalence [13]. However, risk factor genes were analysed in a population of less than 1,000 Polish patients with PD, and so the study was underpowered [55]. While GWAS PD studies are conducted mainly in European populations, Polish patients with PD are not often included in the analysis [13].

GBA

GBA encoding glucocerebrosidase is one of the first risk factors described in PD. The encoding protein is a lysosomal hydrolase located in the lysosomal membrane and is involved

in the degradation of a sphingolipid glucocerebroside. Mutations in both alleles are responsible for Gaucher's disease, which is characterised by glucocerebroside accumulation and secondary macrophage accumulation [56]. Heterozygous carriers of *GBA* variants had increased risk of PD, and the highest prevalence of *GBA* mutations occurred in Ashkenazi Jewish patients. *GBA* variants were found in 19% of patients with PD and 3% of the general population [56]. In the first study conducted in a Polish population, 4.07% of *GBA* carriers were reported in a group of 270 non-demented patients with PD [57]. The second study revealed 16 carriers (11.6%) among 138 Polish patients with PD [58]. It is known that dementia occurs more often in *GBA* mutation carriers (60.0% *vs.* 19.6%) [58].

APOE

Apolipoprotein E plays a key role in lipid metabolism. *APOE* is considered one of the most important genetic risk factors for Alzheimer's disease (AD). Three common polymorphisms (ε2, ε3, and ε4) and six genotypes (ε2/ε2, ε3/ε3, ε4/ε4, ε2/ε3, ε2/ε4, ε3/ε4) have been identified in *APOE*, and ε3 is the most common allele. The potential impact of these variants was studied in the context of the occurrence of dementia in PD, rather than disease prevalence [59]. In a Polish population with PD, Pierzchlinska et al. [60] revealed no statistically significant correlation between *APOE* genotypes and dementia. Another study of 407 Polish patients with PD found no statistically significant differences in the distribution of *APOE* genotypes [60].

Other genetic analysis in Poland

We found other studies of Polish populations that do not fit into the gene groups described above. They describe mutations in mitochondrial DNA and genes associated with the immune system or with dopamine metabolism. All pathways analysed in Polish populations are set out in Table 2 [57, 58, 60–78].

Mitochondrial dysfunction has been implicated in PD pathogenesis [79]. The mutations causing mitochondrial dysfunction in nuclear DNA also risk variants in mitochondrial DNA [70]. Some changes in mitochondrial DNA may modify risk of PD. Mitochondrial transcription factor A (*TFAM*) has been shown to decrease reactive oxygen species [80]. The intronic variant rs2306604 increased risk of PD in an analysis of 326 patients with PD [67]. Mitochondrial DNA can be divided into haplogroups, restricted to particular populations and geographical areas.

Multiple European haplogroups, including J, K, U, and some super-haplogroups (e.g. UK and JT), have been associated with a reduced risk of PD [70]. This observation was also made in a Polish population [81]. Haplogroup J was associated with a lower PD risk in men. Subcluster K1a was more prevalent in healthy controls, while K1c was more frequent in patients with PD (P = .025 and P = .011, respectively). Furthermore, the sublineages (U4 + U5a1 + K + J1c + J2) previously proposed to partially uncouple oxidative phosphorylation decrease PD risk (P = .027) [81]. No impact of *TOMM40* on disease occurrence was observed in 407 PD patients [71].

Oxidative stress is one of the best-known potential pathomechanisms of PD. *NFE2L2* encoding nuclear factor-erythroid 2–related factor 2 is responsible for regulation of the expression of many antioxidant pathway genes in the so-called *phase II response*. In a Polish case-control study, *NFE2L2* haplotypes decreased the risk of PD for heterozygous and homozygous carriers [78]. Matrix metalloproteinases are huge families of endopeptidases important in inflammation. One of these families is macrophage metalloelastase (*MMP12*), first identified as an elastolytic metalloproteinase secreted by inflammatory macrophages [82]. In 241 patients with PD, rs652438 G allele genotypes of *MMP12* decreased the risk of the disease [65].

One of the pathways previously associated with PD and strictly connected with oxidative stress is the immune system. In an analysis of the human leukocyte antigen region polymorphism *HLA-DRA* rs3129882 in 343 Polish patients with PD, the recessive model of GG genotype was observed to be protective [73]. In another case-control study (341 patients with PD and 315 controls), polymorphisms in *IL-10* (-1082G > A and -592C > A) were not risk factors for sporadic PD [63]. Although semaphorins are the proteins responsible for regulation of the immune system and tumour progression, rs7702187 SNP in SEMA5A was not a marker of PD risk in 235 Polish patients with PD [64]. The triggering receptor expressed on myeloid cells 2 (*TREM2*) is a member of the innate immune receptor of the TREM family. It is found on activated macrophages, immature dendritic cells, osteoclasts and microglia. While the *TREM2* p.Arg47His (rs75932628) variant has been associated with increased risk of PD in a Polish study, this variant was rare in patients with PD and no variants were reported in controls [74].

A few studies have been conducted on the variants encoding enzymes associated with dopamine metabolism pathway [61, 62, 75, 83]. Lack of dopamine in synapses is a main clinical indication of PD. Because levodopa is a basic treatment for PD, polymorphisms in these enzymes may impact upon response to this treatment. A couple of studies in Polish patients have analysed genes encoding enzymes associated with dopamine metabolism [61, 62]. Catechol-O-methyltransferase (COMT) and monoamine oxidase B (MAOB) are involved in dopamine degradation in synapses. A study of 210 Polish patients with PD found a significantly lower frequency of the *COMT* LL genotype responsible for high enzyme activity [61]. The combined haplotype of the *MAOB* G (G/G) and *COMT* HL genotypes showed a four-fold increase (P < .05) in the risk of PD in women [61]. Bialecka et al. [62] analysed the impact of these polymorphisms on response to treatment. Their five-year observational study of 95 Polish patients with PD analysed

Table 2. Genetic risk factors associated with PD analysed in Polish populations

Gene	Mechanism	Results
APOE	Responsible for lipid metabolism; pathological aggregation of proteins	No impact on PD and PDD occurrence [60]
GBA	Lysosomal hydrolase responsible for degradation of a sphingolipid glucocerebroside	2 studies: –4.07% in 270 non-demented patients with PD [57] –11.6% in 138 patients with PD [58]
Mitochondrial dysfunction		
TFAM	Mitochondrial DNA transcription factor	Intronic variant rs2306604 increased risk of PD in analysis in 326 patients with PD (OR, 1.789; 95% CI, 1.162-2.755; *P* = .008) [67]
TOMM40	Translocase of the outer mitochondrial membrane 40 homolog	No impact on PD occurrence [71]
Haplo-group J	Mitochondrial DNA	Associated with lower PD risk in men (OR, 0.19; 95% CI, 0.069-0.530; *P* = .0014) [70]
Oxidative stress and immune system		
NFE2L2	Regulation of expression of many antioxidant pathway genes	*NFE2L2* haplotypes decrease risk of PD-heterozygous (OR, 0.4; 95% CI, 0.3-0.6; *P* < .001), homozygous (OR, 0.2; 95% CI, 0.1-0.4; *P* < .001) [78]
MMP12	Matrix metalloproteinase secreted by inflammatory macrophages, responsible for inflammatory reaction	rs652438G allele genotypes decrease risk of disease (OR, 0.47; 95% CI, 0.26-0.85; *P* = .013) [65]
HLA-DRA	Human leukocyte antigen	rs3129882 GG genotype protective for PD occurrence (OR, 0.67; *P* = .04) [73]
IL-10	Modulatory effects against proinflammatory cytokines, especially INF-γ and TNF-α	No impact on PD occurrence [63]
SEMA5A	Regulation of immune system and tumour progression	No impact on PD occurrence [64]
TREM2	Found on activated macrophages, immature dendritic cells, osteoclasts, and microglia	No impact on PD occurrence [74]
Dopamine and other neurotransmitter metabolism		
COMT	Catecholo-O-metylotransferase, responsible for dopamine metabolism	Lower frequency of *COMT* LL in PD [61]
MAO-B	Monoamine oxidase B responsible for dopamine metabolism	*MAOB* G (G/G) and *COMT* HL genotype → fourfold increased risk of PD in women (*P* < .05) No impact on response to treatment [62]
DBH	Noradrenaline synthesis from dopamine in plasma	rs1611115 was observed more often (OR, 2.01; *P* = .01) [75]
MDR1	Responsible for regulating environmental xenobiotics concentration	No impact on PD occurrence [77]
Pathways associated with other neurodegenerative disorders		
STH	Impact on AD pathogenesis	No impact on PD occurrence [72]
GRN	Impact on FTD occurrence	No impact on PD occurrence [68]
MAPT	Microtubule-associated protein	No impact on PD occurrence [69]
CALB1	L-type voltage-operated calcium channels	No impact on PD occurrence [76]
DAPK1	Ca2 +/ calmodulin-dependent serine/threonine kinase that plays a proapoptotic role in programmed cell death cascade	No impact on PD occurrence [66]

AD — Alzheimer's disease; FTD — frontotemporal dementia; INF — interferon; OR — odds ratio; PD — Parkinson's disease; PDD — Parkinson's disease dementia; TNF — tumour necrosis factor

the presence of *COMT* L and *MAOB* G polymorphisms in two study groups: those receiving less than 500 mg/day of levodopa, and those receiving 500 mg/day or more during the observational period. No statistical differences were observed between these groups [62]. Another study examined differences in polymorphism distribution in dopamine B-hydroxylase (*DBH*), responsible for noradrenaline synthesis from dopamine in plasma [75]. In a study of 224 Polish patients, *DBH* -1021C > T; rs1611115 was observed more often in the study group than in controls [75]. Michalowska et al. analysed the occurrence of polymorphisms in genes associated with dopaminergic metabolism and their impact on risk of PD and motor levodopa-induced adverse effects. They found that rs6265 *BDNF* (p.Val66Met) was associated with risk of PD. Additionally, they observed a synergic effect of rs6265 *BDNF* (p.Val66Met), rs397595 *DAT* (SLC6A3), and rs4680 *COMT* (p.Val158Met) polymorphisms on the occurrence of motor levodopa-induced adverse effects [83]. In a study of 158 patients with PD and 139 controls, Tan et al. [77] analysed seven SNPs from *MDR1* responsible for regulating environmental xenobiotics, but found no significant differences between the two groups.

The correlation of eight SNPs localised in the chromosomal region 2q24.3, previously associated with PD risk, was analysed; however, a study of 713 Polish patients revealed no association with PD risk [84]. The saitonin p.Gln7Arg polymorphism previously associated with AD was analysed in 100 patients with PD, but no association with disease occurrence was observed [72]. An SNP in the progranulin gene (*GRN*; 3'UTR+78C > T; rs5848) associated with frontotemporal dementia was not found to be a risk factor for PD in 364 Polish patients [68]. Microtubule-associated protein τ was previously reported to be associated with AD and frontotemporal dementia; however, a study of 832 Polish patients with PD found no impact on disease presence with *MAPT* p.Ala152Thr variant [69]. Death-associated protein kinase 1, previously reported in AD, was also not observed in patients with PD patients [66]. Calbindin belongs to L-type voltage-operated calcium channels. It has been reported that rs1805874 SNP may increase the risk of PD in Japanese patients [85]; however, this observation was not confirmed in Polish or other European populations (Tab. 2) [76]. Locus 5q23 (D5S1462 and D5S2501) was identified in two large Polish families with levodopa responsive parkinsonism [86, 87].

Clinical implications

Our report summarises the prevalence of PD genetic factors in the Polish population, and presents the first case of *SNCA* duplication in this population. Many genes responsible for both familial forms of PD and increased risk of disease have been established in the Polish population. Data indicates that PD genes reported in other countries are rarely observed in this population.

The diagnosis of PD is still based on clinical examination. Detailed genetic characteristics of specific populations may lead to the discovery of new PD biomarkers [86]. With the increasing availability of personalised medicine, the number of clinical trials calling for specific mutation carriers will increase. Currently, there is an ongoing phase I clinical trial for *LRRK2* p.Gly2019Ser mutation carriers. Antisense oligonucleotide BIIB094 binds to *LRRK2* mRNA and causes its degradation (NCT03976349). Another trial analysed DNL201 particle inhibition of the LRRK2 protein (NCT03710707) [87]. The most explored gene in the context of clinical trials is *GBA*. There are six ongoing clinical trials (three phase 1 and three phase 2) with different mechanisms, including glucocerebrosidase activators, glucosylceramide synthases inhibitors, and adeno-associated virus gene therapy [87, 88]. In 2019, the Michael J. Fox Foundation announced funding for development for *PRKN* and *PINK1* [89]. In the 2019, the Michael J. Fox Foundation announced funding for development for *PRKN* and *PINK1* targeted therapy.

Future perspectives

Many PD genes have been extensively screened in the Polish population. The frequency of variants in known genes is low. However, some methodological approaches (GWAS or clinical exomes analysis) have not been conducted yet. Furthermore, there are new sequencing methods, such as long-read sequencing, which can directly sequence single molecules of DNA in real time, often without the need for amplification. This direct sequencing approach enables the production of reads that are considerably longer than those resulting from classical short-read sequencing, allowing the sequencing of parts of the genome that are yet to be discovered. Long-read sequencing will facilitate better genetic characterisation of all patients with PD.

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Short communication

The matter of significance – Has the p.(Glu121Lys) variant of *TOR1A* gene a pathogenic role in dystonia or Parkinson disease?

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ABSTRACT

Next Generation Sequencing (NGS), has now become a very powerful tool for decoding variants of genes involved in pathogenesis of number of human disorders. One of the challenges of this method is to decipher the real pathogenic variants from a number of identified, not related to the disorder in analyzed case. Another issue is recognition of new phenotypes previously unrecognized but related to new variants combinations' in known genes. The other aspect is the HGMD or ClinVar mutation databases usage in data interpretation. The aim of this paper is to discuss pathogenicity of p.(Glu121Lys) missense mutation in the *TOR1A* gene previously described as dystonia causing variant. The patient diagnosed with typical Parkinson disease and positive family history was included into analysis. Also the internal whole exome sequencing (WES) database containing 600 subjects who has performed WES due to different causes was searched. All subjects had WES performed on SureSelect Human All Exon v.6 enrichment, Illumina NovaSeq 6000 platform, (annotations according to internal Institute Mother and Child's pipeline). The *TOR1A* p.(Glu121Lys) heterozygous mutation was revealed in 1 patient diagnosed with PD and 2 healthy subjects who has no dystonia symptoms. To conclude the *TOR1A* p.Glu121Lys variant should not be recognized as clearly pathogenic now.

1. Introduction

NGS is increasingly being applied to clinical testing. The whole exome sequencing (WES) approach has shown promise in the identification of genetic cause especially in neurological disorders like primary dystonia (DYT) or Parkinson disease (PD) dependent from multiple genes. This is very powerful tool in identification of DNA variants. However, it produces a large number of rare variants of unknown importance, which could be related, but also unrelated to the phenotype under consideration. Recently WES seems to be also an attractive diagnostic tool in reverse phenotyping, particularly for genetic diseases with unusual phenotypes.

Dystonia is a movement disorder characterized by sustained muscle contractions frequently producing twisting, repetitive or patterned movements or abnormal postures. There are multiple forms of dystonia, among them the group of monogenic primary dystonias with more than 20 DYT-designated genes identified till now [1,2]. Although some of the genetic dystonias have distinct phenotypes, there is a considerable phenotypic overlap between them making clinical-based classification quite difficult. Additionally, some forms reveals parkinsonian symptoms, [3,4], so the parallel analysis of the genes related to both Parkinson Disease and dystonia is now the diagnostic method of choice.

The first gene associated with early - onset dystonia, currently known as DYT1-dystonia [MIM128100] was *TOR1A* gene [MIM 605204] encoding the protein TorsinA. DYT1 is a dominant disorder with a variable phenotypic severity and incomplete penetrance of 20–30%, caused by heterozygous mutations in the *TOR1A* gene. The most frequent is a tree base-pair deletion c.907_909GAG, causing in frame glutamate deletion p.(Glu303del), known as p.ΔE [5,6]. Additional point mutations have also been reported in *TOR1A* gene [7]. Among them p.(Glu121Lys) missense substitution described by Vulinovic et al. as putative pathogenic mutation [8]. Since 2017 a few cases of homozygous mutations in *TOR1A* have been recognized as a new an atypical presentation of *TOR1A* disease – severe arthrogryposis with developmental delay, strabismus and tremor [9,10]. This clearly indicates that *TOR1A* gene hides new

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surprises, but make us think about existing data and their involvement in phenotype.

The aim of this paper is to analyze and discus the pathogenic role of the mentioned p.(Glu121Lys) missense mutation in the *TOR1A* gene. This mutation is annotated as pathogenic variant, but could be also identified among individuals without signs of primary DYT1 dystonia.

2. Materials and methods

To identify the molecular background of familial case of PD in the case of 65-year-old female of Polish origin with typical PD and positive familial history (autosomal dominant inheritance) the WES analysis was performed (SureSelect Human All Exon v.6 enrichment, Illumina NovaSeq 6000 platform, annotations according to ZGM IMiD pipeline). Clinical evaluation was performed by experienced neurologist.

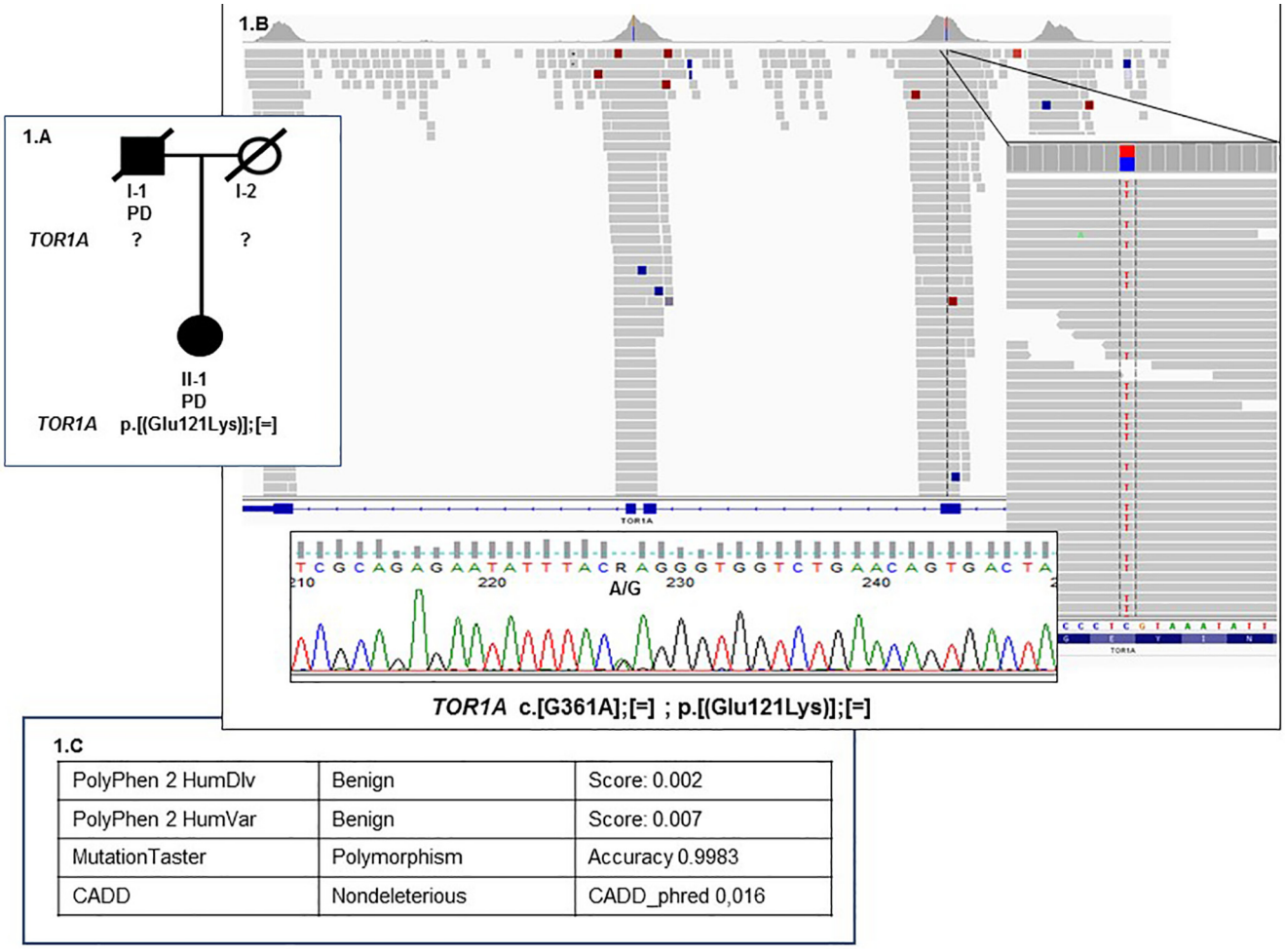
3. Results

Proband's symptoms appeared at the age of 55, primarily as a right-hand resting tremor. During an examination, she presented dysarthria, hypomimia, extra-pyramidal rigidity more severe on the right side, accompanied by resting, positional and kinetic tremor. She has no symptoms of dystonia. MRI revealed age-related vasogenic white matter changes in both hemispheres. EEG examination revealed generalized diffuse, sporadic changes in the posterior parts, especially in posterior temporal lobes. Psychological examination exposed mild cognitive impairment and deficits of executive functions. The patient has had a very good response to levodopa treatment and achieved 46 pointsin UPDRS III scale in state OFF versus 5 points in state ON. She has had drug-induced dyskinesias and end-dose fluctuations.

Performed analysis excluded mutations in known PD and PD associated genes – point mutations (WES) and rearrangements (MLPA), however revealed the presence of a heterozygous substitution, c.361G > A in the *TOR1A* gene, resulting in a missense mutation, p.(Glu121Lys) (Fig. 1). We identified this variant also in our WES database (two cases of healthy, unrelated adults among 600), with frequency – 0,33%.

4. Discussion

This variant is known and was reported in segmental dystonia including cervical dystonia and spasmodic dysphonia in 2014 by



1.C

PolyPhen 2 HumDiv	Benign	Score: 0.002
PolyPhen 2 HumVar	Benign	Score: 0.007
MutationTaster	Polymorphism	Accuracy 0.9983
CADD	Nondeleterious	CADD_phred 0,016

Fig. 1. Genetic finding of the *TOR1A* gene mutation p.Glu121Lys in PD proband and predictions, of the possible impact of an amino acid substitution on the TorsinA protein structure and function (analysis with different bioinformatic tools). 1.A Pedigree of the proband's family (filed symbols represent affected with PD), 1.B NGS data analyzed with IGV [http://software.broadinstitute.org/software/igv/] and Sanger sequencing data analyzed with Finch TV software [https://digitalworldbiology.com/FinchTV], both showing single nucleotide heterozygosity resulting in missense substitution p.Glu121Val in the TorsinA protein. 1.C Table with pathogenicity prediction data obtained for different prediction algorithms – PolyPhen2, MutationTaster and CADD.

Vulinovic's group in one patient from the cohort of 162 (0,6%) with familial primary dystonia's [8]. When discovered the variant was novel, but later it was reported in other 3 individuals from NHLBI Exome Sequencing Project (0,02%). It is present in Genome Aggregation Database (gnomAD), with frequency of 0,03%. (0.02% for European Non-Finish population). It is annotated in HGMD Professional (https://portal.biobase-international.com) and in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar) databases, however annotations are not concordant. In HGMD is annotated as pathogenic (DM – disease mutation; HGMD CM1310069) but in ClinVar as variant of uncertain significance. In silico pathogenicity predictions indicate rather variant's nonpathogenic character (PolyPhen2; HumDiv/HumVar algorytms – polymorphism, MutationTaster – polymorphism, CADD – nondeleterious), however according to ACMG Classification is finally rating as uncertain significance (VUS) [VarSome, htpps://varsome.com]. Vulinovic et al. performed functional assay for the novel TOR1A mutations in comparison to WT protein and the most common p. ΔE. Different methods had been used to analyze the TOR1A protein forms stability, subcellular localization, degradation pathways and inclusion forming. In the contrary to the other novel mutations p.Ala14_Pro15del and p. Arg288Gln the p.Glu121Lys behaved in the most tests like the WT protein, however showing increased stability and inclusion formation upon inhibition of the autophagy-lysosomal degradation pathway. Authors, according to the obtained results, concluded that analysed variants "*may be considered as a possible disease-causing mutation*" but also suggested, that further experiments should be done to confirm this status [8]. The pathogenicity of the p.Arg288Gln mutations was confirmed by other studies [11,12], but the other mutations were reported in dystonia only by Vulinovic et al. [8].

Although the original data on p.Glu121Lys pathogenicity were not strong enough to classify this missense mutation as pathogenic/disease causing one, the variant was annotated as pathogenic in HGMD.

This study reveals that, this classification may be exaggerated. Only analysis of large well clinically characterized cohorts can be a reliable method for proper clinical annotation of rare variants. It also shows that variants' annotations should be constantly updated. With the increasing number of sequenced patients the clinical annotation of the p.Glu121Lys variant may be changed due to enrichment in the cohorts of movement disorder patients compared to control individuals. To conclude the *TOR1A* p. Glu121Lys variant should not be recognized as clearly pathogenic now.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics

The study was approved by the local Medical University of Warsaw ethics committee (KB/230/2017).

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Cognitive and behavioral profile of Perry syndrome in two families

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ABSTRACT

Objective: Perry syndrome (PS) is a rare neurodegenerative disorder with autosomal dominant inheritance caused by point mutations in *DCTN1* and characterized by parkinsonism, hypoventilation, weight loss, and psychiatric symptoms. Even though behavioral manifestation is a main feature of PS, detailed neuropsychological assessment was not performed in this cohort. In this study, the neuropsychological profile of individuals from one Polish and one Colombian family are presented.

Methods: Detailed clinical and neuropsychological data were obtained from Polish and Colombian families. Clinical and neuropsychological examinations on the proband from the Polish family were performed 6 times over 11 years. Each of 3 individuals from the Colombian family received a clinical and neuropsychological assessment.

Results: The neurologic examination showed severe parkinsonism, levodopa-induced motor fluctuations, and dyskinesias in all cases. Respiratory insufficiency was observed in 2 patients and weight loss in 1 individual. Neuropsychological assessment revealed predominant deterioration of working memory and learning capacity in the Polish patient. He also demonstrated compulsive behaviors, such as excessive shopping and eating, but only in the "on" phase. In the Colombian family, attentional deficits were present in 2 out of 3 cases. Out of 4 reported cases apathy and depressed mood were present in 2 individuals. Two cases demonstrated impulsivity and one had episodes of hypomania.

Conclusions: Both of these families revealed relatively similar neurologic and neuropsychological profiles. The Polish patient's behavioral and neuropsychological profile was mostly compatible with a behavioral variant of frontotemporal dementia. Of note, not only depression and apathy, but also impulsivity can occur in PS.

1. Introduction

Perry syndrome (PS) is a rare familial form of autosomal dominant inherited parkinsonism with central hypoventilation, weight loss, and psychiatric features [1,2]. Further characteristic clinical symptoms of PS include a mostly limited response to levodopa, fatigue, sleep disturbances, and autonomic dysfunction, which are also observed in other parkinsonisms [3,4]. The most characteristic neuropsychiatric features include depression and apathy. Progression of the disease is rapid, with an average duration of about 5.5 (range, 2–14) years [1]. Mutations in the *DCTN1* gene were identified in 2009 as a cause of this disease [5]. *DCTN1* codes the dynactin p150Glued subunit, which is a protein co-operating with dynein in axonal transport [6,7]. The dynein-dynactin complex is responsible for retrograding along the microtubules. Damage to this protein results in inappropriate autophagosome-lysosome fusion and blockage of autophagic flux, causing neurodegeneration [8].

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The mutation in *DCTN1* has been previously reported as a cause of other diseases, including amyotrophic lateral sclerosis, frontotemporal dementia, progressive supranuclear palsy–like syndrome, and distal spinal and bulbar muscular atrophy, known as distal hereditary motor neuropathy 7B [6,9–15]. To date, 9 disease-causing mutations have been described in 23 families worldwide and the majority cluster between amino acids 67–74 within the N-terminal cytoskeleton-associated protein–glycine-rich (CAP-Gly) domain [6]. Therapeutic interventions are limited to symptomatic treatments. Parkinsonian symptoms are treated with carbidopa and levodopa administered orally or by intestinal infusion, but usually with poor response. A diaphragmatic pacemaker could be implemented in patients with respiratory insufficiency [16–18]. Selective serotonin reuptake inhibitors and other antidepressants are administered for anxiety and depression. The aim of this study is to present clinical and neuropsychological aspects of PS.

2. Methods

2.1. Clinical and genealogic studies

Clinical studies included medical chart reviews, interviews with patients and their relatives, and neurologic examinations performed by experienced movement disorder specialists. ^{123}I-ioflupane single-photon emission computed tomography was used to visualize striatal dopamine transporter concentration in the Polish patient.

2.2. Ethical standards

All ethical aspects of this study were approved by the institutional review boards of Mayo Clinic, Medical University of Gdansk, and Pontificia Universidad Javeriana.

2.3. Neuropsychological evaluation

The comprehensive neuropsychological and neurologic assessments were performed in one patient from the Polish family and in 3 patients from the Colombian family. Neuropsychological assessment addressed: language, visuospatial function, praxis, attention and working memory, episodic memory, and executive function. The detailed neuropsychological methodology is included in Supplementary Table 1.

3. Results

3.1. Polish family

In this study, we present an update on clinical and neuropsychological assessment of the PS proband of a Polish family (see: Fig. 1) [6]. A 53-year-old man (proband) first developed symptoms of psychomotor slowing at the age of 42 years (patient III-10). Subsequently, other symptoms appeared (Table 1). Motor symptoms were initially responsive to oral carbidopa-levodopa/dopamine agonist therapy. Dopamine transporter imaging identified bilateral reduction in radiotracer uptake in the striatum (see: Supplementary Fig. 1). Head MRI revealed diffuse symmetrical cortical and subcortical atrophy without any specific features. The diagnosis of PS was established in the proband after acute respiratory failure at the age of 50, when genetic testing revealed a *DCTN1* p.Gly71Glu mutation [6]. Since then, the proband has used assisted ventilation during the night; however, respiratory insufficiency has worsened, and the off-ventilator time has recently been reduced to no more than 1–2 h per day. No hormonal abnormalities were reported in this patient.

The proband has a strong family history of other relatives affected by similar symptoms. The proband's father (II-3) died at 52 years of age due to complications after a car accident and did not develop any neurologic symptoms (Fig. 1A). This may explain the incomplete penetrance of the disease in this family. Proband's aunt (II-2) was diagnosed with schizophrenia.

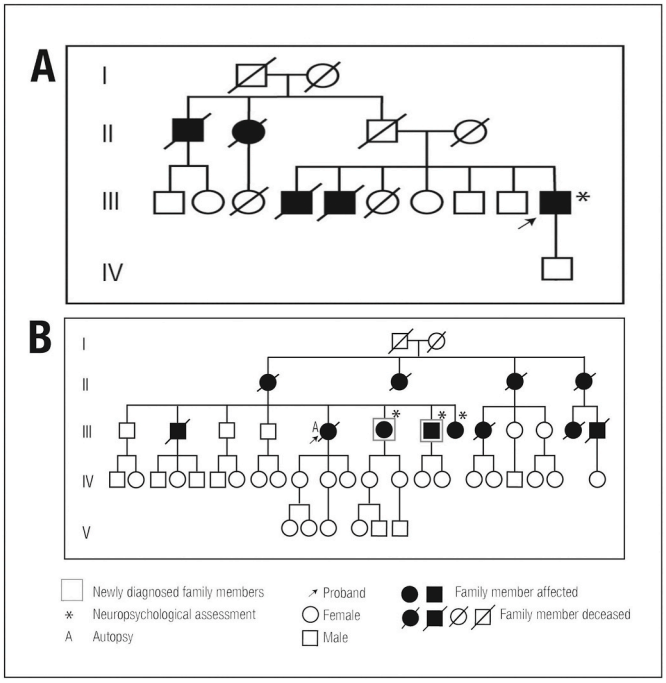

Fig. 1. Polish and Colombian Perry Syndrome Families. A, Updated pedigree of Polish family. B, Updated pedigree of Colombian family.

Behavioural symptoms such as apathy and social withdrawal were present at the disease onset at the age of 42 years. Six years after the onset of behavioral problems, apathy, disinhibition, and aggression were predominant (Frontal Behavioral Inventory total score, 21; negative behaviour score, 10; disinhibition, 11) He demonstrated compulsive shopping and cleaning, but only after taking dopaminergic medication. He ate compulsively and licked his plate afterward. Also, he could not discontinue motor activity easily when needed. However, empathy and warmth were preserved.

His cognitive functions (Supplementary Table 1 and Table 2) were initially quite stable. Executive and memory problems were prominent during the disease course. Ten years after onset, compulsive tendencies, inhibition, and set-shifting problems markedly contributed to the patient's disability. Of note, set-shifting problems and disinhibition were not so pronounced in psychometric testing early in the disease course. During the last assessment, due to the debilitating fatigue and deterioration of respiratory and motor function, only a written MMSE (combination of handwriting and typing) was performed, which indicated selective deterioration in working memory [19] and overelaboration in drawing (Supplementary Fig. 2) corresponding to executive problems (Table 1). Overall, his behavioral and neuropsychological profile shares features with the behavioral variant of frontotemporal dementia (bvFTD). The only features more in line with parkinsonian syndromes than with bvFTD were psychomotor slowing and on/off fluctuations that were also present in the cognitive domain (Table 2, Supplementary Table 2).

3.2. Colombian family

The Colombian family has been described previously [15,18], and since the last publication, 2 additional family members have been diagnosed with PS (Fig. 1B). A *DCTN1*p.Gly71Arg mutation has been revealed in all family members described below.

3.2.1. Patient III-6

The first newly diagnosed Colombian patient (III-6) is a woman who presented to the clinic in 2016, at age of 60 years, with a 12-months history of tremor that started in the left lower extremity and spread to her right hand, with subsequent slowness of movements. She reported

Table 1
Polish Patient's history (11 Years).

Age (Years Since Disease Onset)	Motor Symptoms and Neurologic Examination	Respiratory Problems	Behavior	Cognition
42 (0)	Slowing	None	Apathy; depression	Impaired verbal fluency
45 (3)	Severe bradykinesia; dysarthria; hypomimia; gait impairment; posture impairment; relatively good response to levodopa	None	Insomnia	Deterioration of planning and verbal memory
48 (6)	Progression of parkinsonism; rest tremor in left arm; Babinski sign in the right foot; wearing off; RLS; oromandibular dyskinesia; decreased response to levodopa	Tachypnea at night	Aggression; compulsive behaviors; disinhibition; hyperorality	Deterioration of working memory
50 (8)	"Round the houses" sign in vertical saccades; dysphagia; orthostatic hypotension; tonic-clonic seizures	Acute respiratory failure; nocturnal assisted ventilator support delivered through tracheostomy	Behavioral symptoms less pronounced	Relatively stable
52 (10)	No change	No change	No change	No change
53 (11)	Worsening of postural stability (unable to walk unassisted); preserved response to levodopa treatment and dyskinesia with a mixed pattern of dystonia and chorea; RBD, RLS, and dysautonomia symptoms	Off-ventilator time reduced to maximum 1–2 h	Difficult to assess due to overall disease severity	Further deterioration of working memory

Abbreviations: RBD, rapid eye movement sleep behaviour disorder; RLS, restless leg syndrome.

severe insomnia, depression, anxiety, and a 10-kg weight loss over the previous 6 months. Her daughter noticed apathy as well. No relevant hormonal abnormalities were observed in this patient. Three months prior, she had been started on 12.5/125 mg of carbidopa/levodopa twice daily with no improvement. Her family history included 2 brothers and 2 sisters with parkinsonism and respiratory failure, with similar symptoms observed in her mother and 3 maternal aunts (Fig. 1B). Neurologic examination revealed hypomimia and hypophonia, predominant right-sided rest tremor, global bradykinesia, and rigidity. A head MRI evidenced mild global cerebral atrophy without any specific pattern two years after disease onset. The carbidopa/levodopa dose was progressively increased to 25/250 mg 3 times daily with better control of her parkinsonian symptoms; insomnia and sleep fragmentation, albeit without major improvement, despite use of several medications. Over a 2-year period, she began to develop clear-cut motor fluctuations with mild-to-moderate levodopa-induced dyskinesias, requiring doses every 4 h. By that time, depression, anxiety, and apathy had gradually worsened, and the patient also complained of cognitive problems.

Neuropsychological tests were administered to the patient at the age of 63 years (4 years after the disease onset), showing cognitive deficits of attentional, amnestic, praxic, gnostic, and executive predominance, as well as a decrease in language processing. The patient had reduced and dysarthric speech output. In reading paralexias were present. Decreased processing speed was accompanied by problems in selective and sustained attention was well as in shifting the attentional focus. Testing also revealed deficits in verbal manipulation of information, verbal learning and delayed verbal recall. Disturbances in visuoperceptual and visuoconstructional skills as well as in visual memory were present. Regarding executive functions, the patient demonstrated aspontaneity, concrete thinking, impaired set-shifting and deficits in metacognitive skills. Severe depression was observed (Table 2 and Supplementary Table 3).

3.2.2. Patient III-7

The second newly diagnosed patient (III-7) is a man who was seen in 2015, at age of 58 years, with a 6-month history of rest tremor in the right hand, associated with slowness. He did not report either weight loss or respiratory or sleep disturbances and had no cognitive or psychiatric complaints. The thyroid function test was normal. Neurologic examination revealed mild hypomimia, associated with mild hand rest tremor and moderate bradykinesia, with no other notable alterations. The head MRI performed in the first year of the disease was normal. A total daily dose of 25/250 mg of oral carbidopa/levodopa, divided into 2 doses, was initiated, with a good initial response. However, over the next 2 years, the patient required a progressive increase in daily doses due to worsening motor symptoms and the onset of mild motor fluctuations, especially wearing off. Additionally, he started to complain of conciliation insomnia and occasional mild dyspnea, but his pulmonary function tests did not show substantial disturbances. Three years after disease onset, he was receiving a total of 75/750 mg of carbidopa/levodopa over 3 doses daily with relatively good control of tremor and bradykinesia, although he started to complain of some mild memory deficits. The patient's wife reported failure to inhibit motor action (e.g. ignoring traffic lights or obstacles while walking).

A neuropsychological testing was administered to the patient III-7 at the age of 61 years, revealing the following cognitive deficits: mild-to-moderate difficulties in attention, shown in occasional hypoprosexia, and failure in the ability to appropriately switch and divide his attentional resources. He presented with mild-to-moderate anomia without improvement with semantic cues. With regards to his executive functions, the patient displayed mild-to-moderate perseverative tendencies, associated with behavioral inhibition deficiencies, and moderate alterations in working memory. The patient showed deficient verbal learning, as the attention and concentration deficits interfered in the

Table 2
Summary of neuropsychological profile in four patients.

Function	Patient			
	Colombian III-6	Colombian III-7	Colombian III-8	Polish III-10
Age at evaluation (Years since disease onset)	63 (4)	61 (4)	53 (6)	from 45 (3) to 53 (7)
Language function				
Lexical and semantic competence				
Naming	mildly impaired	mildly impaired	mildly impaired	preserved
Verbal comprehension	preserved	impaired (multi-step commands)	NA	preserved
Verbal repetition	mostly preserved	preserved	NA	NA
Visuospatial skills and praxis				
Object perception	mildly impaired	preserved	preserved	preserved
Space perception	NA	preserved	preserved	preserved
Construction	impaired	preserved	preserved	mildly impaired due to executive and motor task demands
Ideomotor praxis	NA	preserved	NA	preserved
Attention, short-term and working memory, processing speed				
Attention & working memory -untimed	impaired	preserved	preserved	impaired early
Attention & working memory - timed; processing speed	impaired	mildly impaired	preserved	impaired later in the disease course
Memory				
Recent	preserved	preserved		preserved
Verbal learning	impaired	NA	preserved	impaired later in the disease course
Logical memory	impaired	impaired	NA	NA
Visual memory	impaired	NA	preserved	preserved
Academic skills				
Writing	mildly impaired	preserved	NA	graphomotor difficulties late in the disease course
Reading	mildly impaired	preserved	NA	NA
Calculation	mildly impaired secondarily to working memory deficits	preserved	NA	NA
Abstraction and executive function				
Abstract thinking and reasoning	selectively impaired visuospatial reasoning	preserved	preserved	preserved
Verbal fluency	preserved	preserved	preserved	impaired early
Cognitive flexibility	impaired	NA	preserved	mildly impaired early
Planning	NA	NA	NA	impaired later in the disease course
Emotional functioning and behavior	apathy severely depressed mood	impulsivity	anxiety hypomania	apathy depressed mood impulsivity

NA, not assessed.

encoding process and the dysexecutive symptoms affected his ability to retrieve or recall.

The mild cognitive impairment of the frontal subcortical type was observed in patient. However, he maintained his autonomy and independence, proper social and family relationships, and work. Regarding evidence of neurocognitive impairments in his daily life, the patient was not aware of any major alteration beyond occasional anomia, mild difficulties inhibiting inappropriate comments, perseverative behavior, and impaired learning of new information. The detailed results of all conducted tests are presented in Supplementary Table 3.

3.2.3. Patient III-8

The previously reported patient (III-8) is a 57- year-old woman whose symptoms started 10 years ago with symmetrical upper limb tremor and poor balance issues with good carbidopa/levodopa response [15]. She reported sleeping only about 2 h a day. In 2015, she developed dyskinesia and motor fluctuations. One year later, she had a deep brain stimulation device implanted to both subthalamic nuclei. Because of this procedure, her most actual brain imaging was CT and did not reveal any significant abnormalities. At the age of 54 years, she had a respiratory insufficiency episode and had been treated with diaphragmatic pacing with good response. However, due to recurrent infections, the diaphragmatic pacer was removed in 2019. Her other medical history include hypothyroidism treated with 50mcg of levothyroxine.

Neuropsychological evaluation was performed at the beginning of her disease at the age of 53 years, before the patient's deep brain stimulation procedure. Her performance on a brief assessment of general cognitive status was within normal limits. She performed in the average range on measures of semantic, phonemic, and action fluency. She had no problems with visuospatial perception, working memory, verbal learning, or visual memory. Her performance on tasks of attention and executive functioning was largely within normal limits. She reported history of hypomanic episodes and current severe restlessness with elevated mood and anxiety and reduced sleeping times. However, apathy was not observed (Table 2 and Supplementary Table 3).

4. Discussion

To our knowledge, this is the first description of neuropsychological profiling of patients with PS. We present 2 previously described families who underwent neuropsychological testing.

Cognitive impairment in PS has been evaluated [13,15,20–22], with 2 studies reporting scores in MMSE screening test of 23/30 and 18/30 [13,21]. While Ohshima et al. [23] reported no difficulties with daily activities, 1 patient developed mild cognitive impairment assessed by the Revised Hasegawa's Dementia Scale. In another analysis, the level of intelligence measured by the Wechsler Adult Intelligence Scale was slightly below the normal range [24]. In a study by Aji et al. [25], the Addenbrooke's Cognitive Examination-Revised revealed selective problems with memory and fluency. Frontal-subcortical pattern of deficits, evidenced by deficient verbal fluency and impairment on Trail Making Test, was also reported in another family [21].

All PS cases with some degree of cognitive impairment have shared typical frontostriatal profile, either isolated or accompanied by other deficits, mainly in memory. In all patients language was either preserved or mildly impaired. Predominant deficits concerned executive function and slowed and inefficient information processing, the latter corresponding to working memory and attentional impairments. Executive impairment manifested in some cases in set-shifting problems and disinhibition. Visuospatial function was either unaffected or affected in terms of construction, secondarily to attentional and/or executive factors.

In the 2 newly diagnosed patients from the Colombian family, the most dominant neuropsychological features were a frontal-subcortical type of mild cognitive impairment and depression. The previously described Colombian patient (III-8) did not present with any severe neuropsychological disturbances on examination; however, she was examined shortly after the symptom onset.

The Polish family proband was examined 6 times over 11 years. This patient's behavioral and neuropsychological profile shares features with behavioral variant of frontotemporal dementia, as he presented with 5 of the 6 typical features of that variant: behavioral disinhibition, apathy, stereotyped behaviors, hyperorality, and executive deficits, with sparing of visuospatial functions [26]. Some features in the Polish patient also corresponded more closely to PD due to on/off fluctuations as well as features of impulse control disorders (ICD). ICD symptoms were present only in the on phase. In the remaining cases some of bvFTD features were also present: behavioral disinhibition/impulsivity (Colombian III-7 and III-8), apathy (Colombian-III 6). Cognitive profile of all individuals presented, cannot be directly compared due to the fact that long follow-up was available only in one patient. Some deficits tend to develop over time and may not be present if the individual is tested only once, especially when early in the disease course (Table 2).

Frontal lobe involvement in PS could be previously implied due to: frontal lobe releasing signs on neurologic examination [13], behavioral symptoms [20,27], frontal dysexecutive syndrome [28], and frontal hypoperfusion [20] or hypometabolism [13] on neuroimaging. This FTD-like presentation of PS was recently classified as atypical [6]. Features of bvFTD, observed in PS, are not surprising. There is overlap between PS and frontotemporal dementia in terms of not only phenotype (behavioral symptoms), but also neuropathology (TDP-43 pathology) and genetics (*DCTN1* mutation having been reported also in motoneuron disease/amyotrophic lateral sclerosis [MND/ALS]). PS is not the only atypical parkinsonian syndrome in which the patient may be likely to fulfill Rasckovsky et al.'s criteria of bvFTD [29]. It is reported that 32% of patients with the diagnosis of progressive supranuclear palsy fulfill Rascovsky's criteria at the initial assessment. Patients with FTD associated with MAPT p.Pro301Leu mutations may share bvFTD characteristics and symptoms characteristic for progressive supranuclear palsy and corticobasal syndrome. For example, Polish proband reported in 2014 exhibited vertical gaze impairment and unilateral neglect [30]. This exemplifies that overlap in the clinical characteristics is rather a rule than an exception in the spectrum frontotemporal lobar degeneration.

Psychiatric comorbidity is a main symptom of PS. Depression or apathy are initial symptoms in more than 50% of patients [1]; however, the diagnosis of depression is usually made without detailed psychological testing. Depression is also a frequent symptom in Parkinson disease, but it is usually more severe in PS and occurs in most patients, with reports of suicide and suicidal thoughts [2,24,31]. In a study of 2 patients with PS, Beck's Depression Inventory scores were 9 and 23 points (63 points maximum), compatible with minimal and moderate depression, respectively [32]. Standard pharmacologic treatment with selective serotonin reuptake inhibitors is usually ineffective, suggesting that depression in PS may require stronger intervention [2]. Apathy, defined as a general lack of interest, initiative, and social withdrawal, is another common neuropsychiatric feature of PS.

Severe depression, previously described [1] as the most common psychiatric manifestation of PS, was observed in patient III-6 from the Colombian family and at disease onset in the Polish patient. Episodes of elevated mood observed in Patient III-8 may resemble fluctuations present in the Polish patient as they were also linked to “on” phase.

Obsessive compulsive behaviors have been observed in PS [21,33]. Impulse control disorders, such as compulsive shopping, have been described in patients with PS treated with dopamine agonists [20], and dysexecutive syndrome has been reported in those treated exclusively with levodopa [2,34]. Furthermore, deficient inhibitory control and episodes of disinhibited behavior have been described with no mention of dopaminergic treatment [25]. As the relationship between pharmacotherapy and ICD is unclear in PS and the disorder is caused by the TDP-43 pathology associated with FTD, we argue that FTD-like

behaviors in PS should be regarded as a sign of overlap between PS and FTD and not simply the adverse effect of dopaminergic treatment.

5. Conclusion

Depression and apathy are typical and usually severe symptoms of PS. Some patients may also develop disinhibition and overall behavioral profile sharing major features with behavioral variant of frontotemporal dementia. Our results indicate that neuropsychological function should be monitored in PS as mental struggles may also worsen due to severe, hard to treat medical symptoms of parkinsonism and respiratory insufficiency. The patients may present with selective frontostriatal cognitive profile or with more widespread deficits affecting memory.

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Author contributions

L.M. contributed to collection, analysis, and interpretation of data; drafting of the article; and generation of figures. E.J.S. contributed to conception and design; collection, analysis, and interpretation of data and critical revision of the article. J.D. contributed to collection of data and critical revision of the article. C.C.C. contributed to collection, analysis, and interpretation of data and critical revision of the article. J.D.G. collection, analysis, and interpretation of data and critical revision of the article. B.B. collection of data and generation of figures. M.S. contributed to collection of data and critical revision of the article. K.K–K. collection, analysis, and interpretation of data and critical revision of the article. O.A.R. contributed to experiments, collection of data, and critical revision of the article. J.S. contributed to conception and design, collection of data, and critical revision of the article. Z.K.W. contributed to conception and design; collection, analysis, and interpretation of data; critical revision of the article, and providing funding. All authors approved the final article.

Declaration of competing interest

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Appendix A. Supplementary data

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Short communication

Frequency of mutations in *PRKN*, *PINK1*, and *DJ1* in Patients With Early-Onset Parkinson Disease from neighboring countries in Central Europe

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ABSTRACT

Introduction: Approximately 10% of patients with Parkinson disease (PD) present with early-onset disease (EOPD), defined as diagnosis before 50 years of age. Genetic factors are known to contribute to EOPD, with most commonly observed mutations in *PRKN*, *PINK1*, and *DJ1* genes. The aim of our study was to analyze the frequency of *PRKN*, *PINK1*, and *DJ1* mutations in an EOPD series from 4 neighboring European countries: Czech Republic, Germany, Poland, and Ukraine.

Methods: Diagnosis of PD was made based on UK Brain Bank diagnostic criteria in departments experienced in movement disorders (1 from Czech Republic, 1 from Germany, 9 from Poland, and 3 from Ukraine). EOPD was defined as onset at or before 50 years of age. Of the 541 patients recruited to the study, 11 were Czech, 38 German, 476 Polish, and 16 Ukrainian. All cohorts were fully screened with Sanger sequencing for *PRKN*, *PINK1*, and *DJ1* and multiplex ligation-dependent probe amplification for exon dosage.

Results: PRKN homozygous or double heterozygous mutations were identified in 17 patients: 1 Czech (9.1%), 1 German (2.6%), 14 Polish (2.9%), and 1 Ukrainian (6.3%). *PINK1* homozygous mutations were only identified in

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3 Polish patients (0.6%). There were no homozygous or compound heterozygous *DJ1* mutations in analyzed subpopulations. One novel variant in *PRKN* was identified in the Ukrainian series.
Conclusion: In the analyzed cohorts, mutations in the genes *PRKN*, *PINK1*, and *DJ1* are not frequently observed.

1. Introduction

Understanding the genetic basis of monogenic forms of Parkinson disease (PD), representing about 10% of cases, has provided great insight into disease pathophysiology. PD is estimated to affect 1% of the population over the age of 55 years, but 3% over the age of 75 years. Typical onset of PD is at 60–65 years of age; however, between 10% and 15% of patients develop an early-onset form of the disease (EOPD) before 50 years of age [1,2].

The majority of late-onset PD and EOPD cases are sporadic. However, recessive mutations in autosomal inherited genes, such as *PRKN*, *PINK1*, and *DJ1*, are responsible for a high percentage of familial EOPD [1]. With the growing importance of personalized medicine, it is important to establish the frequency of *PRKN*, *PINK1*, and *DJ1* mutations in EOPD populations. For example, the Michael J. Fox Foundation introduced Parkinson's Research Strategy to Advance Therapeutic Development of *PINK1* and *PRKN* in 2019 [3]; the number of clinical trials will be growing and will likely include specific mutation carriers.

Understanding the genetic landscape of patients with EOPD, especially those in neighboring countries, may help to uncover novel or geographically associated variants that could contribute to further molecular characterization and classification of the disease. Czech Republic, Germany, Poland, and Ukraine are bordering countries in central Europe. Due to historical events, the human migration between these 4 countries may have elevated the frequency of specific mutations, and the presence of gene variants may be similar in these countries.

There were previous studies analyzing *PRKN, PINK1* and *DJ1* in Czech, German and Polish population (Supplemental Table 1). However, the available data in these countries have mostly been obtained from a small number of case studies, and to our knowledge, the distribution of EOPD gene mutations in bordering countries has never been compared. The aim of our study was to analyze the frequency of *PRKN*, *PINK1*, and *DJ1* mutations in patients with EOPD from 4 neighboring countries: Czech Republic, Germany, Poland, and Ukraine.

2. Materials and methods

2.1. Study population

Diagnosis of PD was made based on UK Brain Bank diagnostic criteria in departments with experience in movement disorders (1 from Czech Republic, 1 from Germany, 9 from Poland, and 3 from Ukraine). EOPD was defined as age of onset of 50 years or younger. Patients' samples were collected from Czech Republic, Germany, Poland, and Ukraine between January 1, 2001, and December 31, 2019 (Supplemental Figure). Detailed characteristics of 9 patients with *PRKN* or *PINK1* homozygous/double heterozygous mutations presented here have already been published (Supplemental Table 1). Study approval was obtained from the ethics review boards at each institute. Written informed consent was obtained from all subjects. All patients included into analysis were unrelated.

2.2. Molecular analysis

All patients with EOPD were fully sequenced for *PRKN* (Exons 1–12), *PINK1* (Exons 1–8), and *DJ1* (Exons 1–6), and exon dosage analysis was performed with multiplex ligation-dependent probe amplification (MLPA). The identified variants were labelled according to appropriate reference sequences: *PRKN* (NM_004562), *PINK1* (NM_032409), and *DJ1* (NM_007262). The impact of the newly identified mutations on protein structure and function was analyzed with PolyPhen-2 v.2.1 software using the HumVar model, Mutation Taster, and Combined Annotation Dependent Depletion score. Annotation of identified mutation was checked in Human Gene Mutation Database Professional and ClinVar. Synonymous, intronic-type variants and common variants with mean allele frequency (MAF) greater than 5% in the Genome Aggregation Database (gnomAD) were excluded from further analysis.

3. Results

The whole study group included 541 patients diagnosed with EOPD (Supplemental Figure). The mean (SD) age of disease onset was 40.4 (7.2), the mean (SD) age of inclusion in the study was 50.7 (9.8), and 216 (39.9%) patients were woman (Table 1). We observed 23 variants in all analyzed genes, with 1 variant reported in the Ukrainian cohort not previously described in analyzed databases (*PRKN*: c.443 G > C, p. Val148Leu). The Combined Annotation Dependent Depletion score for this mutation was 23. PolyPhen-2 predicted this variant as possibly damaging; however, analysis in Mutation Taster revealed it as benign p. Arg275Trp (rs34424986) and p.Asp394Asn (rs1801334) in *PRKN* (7 [1.3%] each) and p.Ala340Thr (rs3738136) in *PINK1* (19 [3.5%]) were the most common variants in the whole study group. p.Arg98Gln was the only reported variant in *DJ1*, observed in 2 German patients (0.4% of total study group) (Table 2). Gene rearrangements were analyzed for the whole study group. Exon deletions and duplications were only observed in *PRKN*, with Ex3del and Ex2del being the most commonly reported (3 [0.6% of total study group]). Rearrangements were reported in the Czech, German and Polish subpopulation (Supplemental Table 2).

PRKN homozygous or double heterozygous mutations were identified in 1 of 11 Czech (Ex2_4del/Ex3_4del; 9.1%), 1 of 38 German

Table 1
Demographic characteristics of studied populations with early-onset Parkinson's disease and respective relative frequencies of PRKN, PINK1, and DJ1 mutations.

Characteristic	Czech Republic (n=11)	Germany (n=38)	Poland (n=476)	Ukraine (n=16)	Total (N=541)
Age at study, mean (SD) (range)	55.3 (8.6) (42–68)	54.2 (10.9) (25–77)	49.9 (9.6) (25–78)	53.9 (7.8) (43–69)	50.7 (9.8) (25–78)
Sex, No. (%)					
Male	9 (81.8)	24 (63.2)	284 (59.7)	8 (50.0)	325 (60.1)
Female	2 (18.2)	14 (36.8)	192 (40.3)	8 (50.0)	216 (39.9)
Age at onset, mean (SD) (range)	44.1 (5.3) (32–49)	39.3 (7.5) (10–47)	40.4 (7.2) (12–50)	43.6 (6.0) (26–49)	40.4 (7.2) (10–50)
Positive family history (at least one other family member with Parkinson's disease), No. (%)	2 (18.2)	10 (26.3)	75 (15.8)	6 (37.5)	93 (17.2)
Total number of heterozygous/ homozygous carriers, No. (%)					
PRKN	1 (9.1)	3 (7.9)	31 (6.5)	3 (18.8)	38 (7.0)
PINK1	2 (18.2)	4 (10.5)	15 (3.2)	3 (18.8)	24 (4.4)
DJ1	0	2 (5.3)	0	0	2 (0.4)

(Ex2del/Ex2del; 2.6%), 14 of 476 Polish (p.Lys211Asn/p.Arg275Trp [n=2], Ex3del/Ex4_7del [n=1], Ex4_7del/p.Gln34ArgfsTer5 [n=1], Ex2_5dup/p.Lys211Asn [n=1], p.Gln34ArgfsTer5/p.Gln34ArgfsTer5 [n=1], Ex3del/p.Cys446Phe [n=1], Ex4del/p.Lys211Asn [n=1], Ex2del/Ex4del [n=1], Ex2del/Glu79Ter [n=1], p.Arg275Trp/p.Pro437Leu [n=1], Ex3del/Ex5_9del [n=1], Ex3_4del/p.Gln34ArgfsTer5 [n=1], Ex2dup/p.Gln34ArgfsTer5 [n=1]; 2.9%), and 1 of 16 Ukrainian (p.Gln34ArgfsTer5/p.Arg275Trp; 6.3%) patients. The compound heterozygosity was confirmed in 9 Polish *PRKN* patients based on the parent's cosegregation analysis. *PINK1* homozygous mutations were only identified in 3 Polish patients (p.Ile368Asn/p.Ile368Asn [n=1], p.Ala168Pro/p.Ala168Pro [n=1], p.Gln456Ter/p.Gln456Ter [n=1]; 0.6%). Of 20 patients with homozygous or double heterozygous mutations in *PRKN* and *PINK1*, 5 (25.0%) had exon rearrangements in both alleles, 7 (35.0%) had point mutation and exon rearrangement, and 8 (40.0%) had point mutations in both alleles (Supplemental Table 3).

4. Discussion

The obtained data indicate that mutations in the genes *PRKN*, *PINK1*, and *DJ1* are rare among analyzed Czech, German, Polish, and Ukrainian patients with EOPD.

The summary of previously published papers in analyzed populations is included in Supplemental Table 1. *PRKN* prevalence in the study groups was similar to previous studies. This analyzed Polish cohort has a larger population compared to previous studies (Supplemental Table 1). The few differences between our results and those published may be caused by use of a different definition of EOPD (age of onset ≤50 years rather than <40 or <45) (Supplemental Table 1). Additionally, Oczkowska et al. (Supplemental Table 1) published only 2 *PRKN* heterozygotes from the cohort of 8 patients with EOPD, and sequencing of all *PRKN* exons and MLPA were not performed. *PINK1* homozygous mutations were only seen in Polish study group, with similar prevalence to previous reports (Supplemental Table 1). In the Czech population, we revealed a similar *PRKN* gene occurrence to that previously reported in patients with age of onset younger than 40 and younger than 45 years (7.1% and 4.4%, respectively) (Supplemental Table 1). In the German population the prevalence of *PRKN* mutations was 11% (Supplemental Table 1). Lack of *PINK1* homozygous cases among German patients is contrary to previous reports, in which 0.056% were carriers of *PINK1* homozygous mutation (Supplemental Table 1). However, this difference may be due to the small sample size of the German cohort in our study. Additionally, previous reports of *DJ1* mutations in German patients have only been heterozygous (Supplemental Table 1). The high percentage of EOPD in the analyzed populations may be the result of referral bias at specialty centers, which attract a higher number of patients with a positive family history of EOPD, suggestive of genetic cause (Supplemental Figure).

To our knowledge, this is the first study to provide data from Ukrainian patients with EOPD. One double heterozygous (6.3%) and 2 heterozygous (12.5%) mutations were present in *PRKN* and 2 heterozygous (12.5%) in *PINK1*. No exon rearrangements were observed for any gene in this study group. One new variant, *PRKN* pVal148Leu (heterozygous), was described in the Ukrainian cohort; but the real pathogenicity of this variant is unknown. Lack of *PRKN* exonic deletion or duplication in the Ukrainian population was quite unexpected since reported 43.2% of all *PRKN* mutations can be structural variants [4]. This lack may be caused by the small size of the Ukrainian study group, and the real mutation distribution may not be reflected.

The role of heterozygous mutations remains contentious. Some studies have reported an increased frequency of carriers of heterozygous *PRKN* and *PINK1* variants in patients with PD compared to healthy controls [5,6]. In pathologic and positron emission tomography studies, decreases in dopaminergic neurons in heterozygous carriers have been observed [7,8]. In contrast, a large case-control sequencing analysis did not detect an enrichment of such variants in patients with PD [9].

To our knowledge, our study is the first to compare the frequency of EOPD genes between neighboring countries and to report the frequency of *PRKN*, *PINK1*, and *DJ1* in a Ukrainian population. This is also the largest analysis of patients with EOPD from Poland. Furthermore, patients came from different regions of Poland, so our study should reflect the real distribution of *PRKN*, *PINK1*, and *DJ1* throughout the country. A

Table 2
Coding sequence variants with MAF GnomAD <5%.

Variant	Genotype	AA	Exon	MAF Czech Republic (n=11)	MAF German (n=38)	MAF Polish (n=476)	MAF Ukraine (n=16)	MAF (GnomAD)
PRKN								
rs55777503	c.101_102del AC	p. Gln34ArgfsTer5	2	0	0	0.005252 (n=5)	0.0313 (n=1)	0.0004
rs-	c.235G > T	p.Glu79Ter	3	0	0	0.00105 (n=1)	0	0
rs55774500	c.245C > A	p.Ala82Glu	3	0	0	0.003151 (n=3)	0	0.00005
rs747624684	c.394_396dupCCA	p.Pro133dup	3	0	0	0.00105 (n=1)	0	0.00007
rs-	c.443 G > C[a]	p.Val148Leu[a]	4	0	0	0	0.0313 (n=1)	0
rs1801474	c.500G > A	p.Ser167Asn	4	0	0	0.00105 (n=1)	0	0.01012
rs137853060	c.633A > T	p.Lys211Asn	6	0	0	0.004202 (n=4)	0	0.0000004
rs34424986	c.823C > T	p.Arg275Trp	7	0	0	0.006303 (n=6)	0.0313 (n=1)	0.003241
rs1801334	c.1180G > A	p.Asp394Asn	11	0	0.0132 (n=1)	0.007353 (n=7)	0.0313 (n=1)	0.03326
rs55830907	c.1204C > T	p.Arg402Cys	11	0	0	0.00105 (n=1)	0	0.00221
rs149953814	c.1310C > T	p.Pro437Leu	12	0	0	0.00105 (n=1)	0	0.00003
	c.1337G > T	p.Cys446Phe	12	0	0	0.00105 (n=1)	0	0
rs182893847	c.1372A > C	p.Met458Leu	12	0	0	0.00105 (n=1)	0	0.0003
PINK1								
rs768091663	c.502G > C	p.Ala168Pro	2	0	0	0.002101 (n=2)	0	0.00004476
rs143204084	c.558G > C	p.Lys186Asn	2	0	0	0.004202 (n=4)	0	0.0006
rs772510148	c.838G > A	p.Ala280Thr	4	0	0	0.00105 (n=1)	0	0.000007892
rs202128685	c.935G > A	p.Arg312Gln	4	0	0	0.00105 (n=1)	0	0.00003159
rs3738136	c.1018G > A	p.Ala340Thr	5	0.0909 (n=1)	0.0526 (n=4)	0.014706 (n=14)	0.0625 (n=2)	0.04527
rs774647122	c.1103T > A	p.Ile368Asn	5	0	0	0.002101 (n=2)	0	0.00000009
rs45478900	c.1231G > A	p.Gly411Ser	6	0	0	0.002101 (n=2)	0.0625 (n=2)	0.002
rs45539432	c.1366C > T	p.Gln456Ter	7	0	0	0.002101 (n=2)	0	0.0008
rs146126901	c.1604C > T	p.Ser535Leu	8	0	0	0.003151 (n=3)	0	0.0000009
DJ1								
rs71653619	c.293 G > A	p.Arg98Gln	4	0	0.0263 (n=2)	0	0	0.0107

Abbreviation: AA, amino acid; gnomAD, Genome Aggregation Database; MAF, minor allele frequency.
[a] New variant.

previous report of EOPD in Poland had limited methodologies to analyze all exons rearrangements (Supplemental Table 1). Exon rearrangements were reported in more than 50% of our patients, so MLPA should always be the element of EOPD diagnosis.

There are some limitations to our study. The most noteworthy limitation is the difference in the sample size obtained for each cohort, with the largest number of patients in the Polish cohort. Czech, German, and Ukrainian study populations were small; however, the results from the Czech and German cohorts were similar to previously reported data. The other significant limitations of our study are lack of family co-segregation analysis or droplet digital PCR in double heterozygote patients.

In conclusion, this study revealed that mutations in *PRKN, PINK1*, and *DJ1* genes are not frequent in analyzed cohorts. All reported *PRKN* and *PINK1* variants described in Czech Republic, Germany, and Ukraine were observed in the Polish study population, except the newly reported variant in the Ukraine population (*PRKN* p.Val148Leu). DJ1 homozygous/compound heterozygous mutations are not reported in most countries, similarly to our cohorts. Further studies of larger cohorts of Ukrainian EOPD patients, which were not previously genetically characterized, are necessary to support the expected variants frequency found in our study. Low frequency of *PRKN*, *PINK1*, and *DJ1* in the studied groups may be due to the presence of other genes associated with EOPD. Future studies are warranted to better understand EOPD variants and facilitate discovery of potential biomarkers or molecular mechanisms for PD and help in qualification for current or future PD therapeutic trials [10,11].

Declaration of competing interest

L.M.M. reports receiving grant support from The Polish National Agency for Academic Exchange, during the conduct of the study. A.B.D. is supported by Allergan, Inc. (educational grant), by a gift from Carl Edward Bolch, Jr, and Susan Bass Bolch, and by the Sol Goldman Charitable Trust. O.A.R. is supported by the National Institutes of Health (NIH; R01 NS78086; U54 NS100693), the US Department of Defense (W81XWH-17-1-0249), the Little Family Foundation, the Mayo Clinic Center for Individualized Medicine, and the Michael J. Fox Foundation. Z.K.W. is partially supported by the Mayo Clinic Center for Regenerative Medicine, Mayo Clinic in Florida Focused Research Team Program, the gifts from The Sol Goldman Charitable Trust, and the Donald G. and Jodi P. Heeringa Family, the Haworth Family Professorship in Neurodegenerative Diseases fund, and The Albertson Parkinson's Research Foundation. He serves as PI or Co-PI Biogen, Inc. (228PD201), Biohaven Pharmaceuticals, Inc. (BHV4157-206 and BHV3241-301), and Neuraly, Inc. (NLY01-PD-1) grants. He serves as Co-PI of the Mayo Clinic American Parkinson Disease Association Center for Advanced Research.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2021.03.026.

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X. Opinia Komisji Bioetycznej

Komisja wyraża pozytywną opinię w sprawie przeprowadzenia wnioskowanych badań- na warunkach określonych we wniosku oraz dodatkowo zastrzegając:
1/ obowiązek przedstawienia Komisji:

- wszystkich zmian w protokole mających wpływ na przebieg oraz ocenę badania,
- wszystkich przypadków zdarzeń niepożądanych,
- zawiadomienia o przyczynach przedwczesnego zakończenia badania,
- sprawozdania w toku przeprowadzonych badań-za sześć miesięcy,
- raportu końcowego.

Komisja Bioetyczna
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KB/230/2017

Komisja Bioetyczna przy Warszawskim Uniwersytecie Medycznym
w dniu 12 grudnia 2017 r. po zapoznaniu się z wnioskiem:

dr hab. n. med. Dariusza Koziorowskiego
Klinika Neurologii Wydziału Nauki o Zdrowiu
ul. Kondratowicza 8, 03-242 Warszawa

dotyczącym: wyrażenia opinii w sprawie badania pt :„Poszukiwanie nowych zmienności genetycznych związanych z występowaniem rodzinnym choroby Parkinsona w populacji polskiej."

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Uwagi Komisji – *verte*

Komisja działa na podstawie art.29 ustawy z dnia 5.12.1996r. o zawodzie lekarza /Dz.U.nr 28/97 poz.152 wraz z późn.zm./, zarządzenia MZiOS z dn.11.05.1999r. w sprawie szczegółowych zasad powoływania i finansowania oraz trybu działania komisji bioetycznych /Dz.U.nr 47 poz.480/, Ustawy prawo farmaceutyczne z dnia 6 września 2001r. (Dz.U.Nr 126, poz. 1381 z późn. zm.) oraz Zarządzenie nr 56/2007 z dnia 15 października 2007r. w sprawie działania Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym /Regulamin Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym/.
Komisja działa zgodnie z zasadami GCP .
W załączeniu: skład komisji oraz lista obecności

Przewodniczący Komisji Bioetycznej

Prof. dr hab. n. med. Zbigniew Wierzbicki

*niepotrzebne skreślić

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7. Prof. dr hab. med. Krzysztof J. FILIPIAK .....
8. Dr n. med. Zygmunt JAMROZIK .....
9. Dr hab. n. med. Mariusz JASIK .....
10. Dr hab. n. med. Andrea HORVATH-STOLARCZYK .....
11. Dr Agnieszka PIECHAL .....
12. Mec. Ryszard PŁACZKOWSKI .....
13. Prof. dr hab. Joanna GÓRNICKA-KALINOWSKA .....
14. Alicja JAWORSKA .....
15. Ksiądz Władysław GRĘDOWSKI .....
16. inż. Iwona SIUDALSKA .....

SKŁAD OSOBOWY KOMISJI BIOETYCZNEJ
przy WARSZAWSKIM UNIWERSYTECIE MEDYCZNYM, ul. Żwirki i Wigury 61
w dniu 12 grudnia 2017

1. Prof. dr hab. n. med. Zbigniew WIERZBICKI
   Klinika Chirurgii Ogólnej i Transplantacyjnej
   02-006 Warszawa, ul. Nowogrodzka 59
   specjalizacja: chirurgia ogólna.

2.Prof. dr hab. n. med. Barbara GAJKOWSKA
Polska Akademia Nauk
Specjalizacja: biolog

3. Prof. dr hab. n. med. Jadwiga KOMENDER
   Klinika Psychiatrii Wieku Rozwojowego
   00-576 Warszawa, ul. Marszałkowska 24
   specjalizacja: psychiatria, pediatria

4. Prof. dr hab. n. med. Bożenna WOCIAL
   Katedra i Klinika Nadciśnienia Tętniczego
   i Angiologii
   02-097 Warszawa, ul. Banacha 1
   specjalizacja: biochemia kliniczna

5. Prof. nadzw. dr hab. n. med. Andrzej KAŃSKI
   II Zakład Anestezjologii i Intensywnej Terapii
   02-097 Warszawa, ul. Banacha 1a
   specjalizacja: anestezjologia

6. Prof. dr hab. n. med. Jadwiga DWILEWICZ-TROJACZEK
   Katedra i Klinika Hematologii, Onkologii i Chorób Wewnętrznych
   ul. Banacha 1a, 02-097 Warszawa
   specjalizacja: choroby wewnętrzne

7. Dr n. med. Zygmunt JAMROZIK
   Katedra i Klinika Neurologii
   02-097 Warszawa, ul. Banacha 1
   specjalizacja: neurologia

8. Prof. dr hab. n. med. Krzysztof J. FILIPIAK -nieobecny
   I Katedra i Klinika Kardiologii
   Ul. Banacha 1a, Warszawa
   specjalizacja: choroby wewnętrzne, kardiologia, hipertensjologia, farmakologia kliniczna

9. Dr hab. n. med. Mariusz JASIK
II Katedra i Klinika Położnictwa i Ginekologii
ul. Karowa 2, 00-315 Warszawa
specjalizacja: choroby wewnętrzne, farmakologia kliniczna, diabetologia

10. Dr hab. n. med. Andrea HORVATH-STOLARCZYK -nieobecna
Klinika Pediatrii
ul. Żwirki I Wigury 63a,
02-091 Warszawa
specjalizacja: pediatria

11. Mec. Ryszard PŁACZKOWSKI
radca prawny

12. Prof. dr hab. filoz. Joanna GÓRNICKA-KALINOWSKA
Zakład Etyki Instytutu Filozofii,
Wydział Filozofii i Socjologii
Uniwersytetu Warszawskiego

13. Alicja JAWORSKA
Oddział Intensywnej Terapii Kardiologii
Klinika Nadciśnienia Tętniczego
Przełożona pielęgniarek

14. Dr Agnieszka PIECHAL
Katedra i Zakład Farmakologii WUM
specjalizacja: farmakologia, neurologia

15. Ksiądz Władysław GRĘDOWSKI

16. inż. Iwona SIUDALSKA -sekretarz
02-091 Warszawa, ul. Żwirki i Wigury 61/pok.219/
tel. 5720303, fax 5720165

XII. Oświadczenia współautorów publikacji

Warm. 16.04.21

(miejscowość, data)

ŁUKASZ MILANOWSKI

(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „**Genetics of Parkinson's disease in the Polish population**" opublikowanej w Neurologii i Neurochirurgii Polskiej

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w tworzeniu projektu, wykonanie przeglądu literatury, badanie kliniczne pacjenta, przygotowanie pierwszej wersji manuskryptu, ocena artykułu przed oddaniem do recenzji.

Mój udział procentowy w przygotowaniu publikacji określam jako 85%.

Warszawa, 16.04.2021
(miejscowość, data)

Dariusz Koziorowski
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „**Genetics of Parkinson's disease in the Polish population**" opublikowanej w Neurologii i Neurochirurgii Polskiej

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w tworzeniu projektu, ocena artykułu przed oddaniem do recenzji.

Mój udział procentowy w przygotowaniu publikacji określam jako 2%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Łukasza Milanowskiego, którego udział procentowy wynosi 85%.

Dariusz Koziorowski

Wa 16.04.21

(miejscowość, data)

ANDRZEJ FRIEDMAN

(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „**Genetics of Parkinson's disease in the Polish population**" opublikowanej w Neurologii i Neurochirurgii Polskiej

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w tworzeniu projektu, ocena artykułu przed oddaniem do recenzji.

Mój udział procentowy w przygotowaniu publikacji określam jako 2%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Łukasza Milanowskiego, którego udział procentowy wynosi 85%.

Warszawa, 15.04.2021

(miejscowość, data)

Paulina Górka-Skrzypek

(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „**Genetics of Parkinson's disease in the Polish population**" opublikowanej w Neurologii i Neurochirurgii Polskiej

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w tworzeniu projektu, ocena artykułu przed oddaniem do recenzji.

Mój udział procentowy w przygotowaniu publikacji określam jako 2%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Łukasza Milanowskiego, którego udział procentowy wynosi 85%.

Paulina Górka-Skrzypek

Warszawa 12.04.2021
(miejscowość, data)

Marta Marek
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „**Genetics of Parkinson's disease in the Polish population**" opublikowanej w Neurologii i Neurochirurgii Polskiej

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w tworzeniu projektu, ocena artykułu przed oddaniem do recenzji.

Mój udział procentowy w przygotowaniu publikacji określam jako 2%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Łukasza Milanowskiego, którego udział procentowy wynosi 85%.

Warszawa 07.04.2021.

(miejscowość, data)

Dorota Hoffman-Zacharska

(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „**Genetics of Parkinson's disease in the Polish population**" opublikowanej w Neurologii i Neurochirurgii Polskiej

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w tworzeniu projektu, przygotowanie ryciny, ocena artykułu przed oddaniem do recenzji.

Mój udział procentowy w przygotowaniu publikacji określam jako 2%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Łukasza Milanowskiego, którego udział procentowy wynosi 85%.

INSTYTUT MATKI I DZIECKA
Zakład Genetyki Medycznej
Kierownik Pracy
dr hab. n. med.
Dorota Hoffman-Zacharska prof. IMiD

Jacksonville, FL; 3 kwietnia 2021 roku

Zbigniew K. Wszolek, M.D.

OŚWIADCZENIE

Jako współautor pracy pt. „**Genetics of Parkinson's disease in the Polish population**" opublikowanej w Neurologii i Neurochirurgii Polskiej

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w tworzeniu projektu, nadzór merytoryczny podczas przygotowywania artykułu, ocena artykułu przed oddaniem do recenzji.

Mój udział procentowy w przygotowaniu publikacji określam jako 5%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Łukasza Milanowskiego, którego udział procentowy wynosi 85%.

Zbigniew K. Wszolek.

Zbigniew K. Wszolek, M.D.
Consultant, Haworth Family Professor in Neurodegenerative Diseases
Professor of Neurology
Phone: 904-953-7229; FAX: 904-953-0757; Pager: 904-953-8173
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4500 San Pablo Road
Jacksonville, FL 32224

U.S.A.

Warszawa, 16.04.21

(miejscowość, data)

ŁUKASZ MILANOWSKI

(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „**The matter of significance - Has the p.(Glu121Lys) variant of TOR1A gene a pathogenic role in dystonia or Parkinson disease?**" opublikowanej w Journal of Clinical Neuroscience.

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w tworzeniu projektu badania, badanie kliniczne pacjenta, przygotowanie pierwszej wersji manuskryptu, ostateczna ocena artykułu przed oddaniem do recenzji.

Mój udział procentowy w przygotowaniu publikacji określam jako 51%.

Łukasz Milanowski

Warszawa 07.04.2021

(miejscowość, data)

Dorota Hoffman-Zacharska

(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „**The matter of significance - Has the p.(Glu121Lys) variant of TOR1A gene a pathogenic role in dystonia or Parkinson disease?**" opublikowanej w Journal of Clinical Neuroscience.

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w tworzeniu projektu badania, wykonanie eksperymentów, przygotowanie ryciny, ocena artykułu przed oddaniem do recenzji.

Mój udział procentowy w przygotowaniu publikacji określam jako 25%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Łukasza Milanowskiego, którego udział procentowy wynosi 51%.

INSTYTUT [illegible]
Zakład [illegible] Medycznej
Kierownik Pracowni Neurogenetyki

dr hab. n. med.
Dorota Hoffman-Zacharska prof. IMiD

Warszawa, 16.04.2021
(miejscowość, data)

Dariusz Koziorowski
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „**The matter of significance - Has the p.(Glu121Lys) variant of TOR1A gene a pathogenic role in dystonia or Parkinson disease?**" opublikowanej w Journal of Clinical Neuroscience.

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w tworzeniu projektu badania, ocena artykułu przed oddaniem do recenzji.

Mój udział procentowy w przygotowaniu publikacji określam jako 10....%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Łukasza Milanowskiego, którego udział procentowy wynosi 51%.

Dariusz Koziorowski

Wa 16.04.21

(miejscowość, data)

ANDRZEJ FRIEDMAN

(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „**The matter of significance - Has the p.(Glu121Lys) variant of TOR1A gene a pathogenic role in dystonia or Parkinson disease?**" opublikowanej w Journal of Clinical Neuroscience.

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w tworzeniu projektu badania, ocena artykułu przed oddaniem do recenzji.

Mój udział procentowy w przygotowaniu publikacji określam jako 10...%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Łukasza Milanowskiego, którego udział procentowy wynosi 51%.

Warszawa
16.04.2021
(miejscowość, data)

MONIKA FIGURA
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „**The matter of significance - Has the p.(Glu121Lys) variant of TOR1A gene a pathogenic role in dystonia or Parkinson disease?**" opublikowanej w Journal of Clinical Neuroscience.

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w tworzeniu projektu badania, ocena artykułu przed oddaniem do recenzji.

Mój udział procentowy w przygotowaniu publikacji określam jako ..2...%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Łukasza Milanowskiego, którego udział procentowy wynosi 51%.

Monika Figura

Warszawa, 16.04.21

(miejscowość, data)

ŁUKASZ MILANOWSKI

(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „**Cognitive and behavioral profile of Perry syndrome in two families**" opublikowanej w Parkinsonism and Related Disorders

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w tworzeniu projektu badania, zebranie materiału, przygotowanie pierwszej wersji manuskryptu, ocena artykułu przed oddaniem do recenzji.

Mój udział procentowy w przygotowaniu publikacji określam jako 60%.

Łukasz Milanowski

07.04.2021

(miejscowość, data)

Prof. dr hab. med. Jarosław Sławek
specjalista neurolog

JAROSŁAW SŁAWEK

(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „**Cognitive and behavioral profile of Perry syndrome in two families**" opublikowanej w Parkinsonism and Related Disorders

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w tworzeniu projektu badania, nadzór merytoryczny podczas prowadzenia badań, ocena artykułu przed oddaniem do recenzji.

Mój udział procentowy w przygotowaniu publikacji określam jako 1.....%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Łukasza Milanowskiego, którego udział procentowy wynosi 60%.

Prof. dr hab. med. Jarosław Sławek
specjalista neurolog

Jacksonville, FL; 3 kwietnia 2021 roku

Zbigniew K. Wszolek, M.D.

OŚWIADCZENIE

Jako współautor pracy pt. „**Cognitive and behavioral profile of Perry syndrome in two families**" opublikowanej w Parkinsonism and Related Disorders

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Mój udział procentowy w przygotowaniu publikacji określam jako 11%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Łukasza Milanowskiego, którego udział procentowy wynosi 60%.

Zbigniew K. Wszolek

Zbigniew K. Wszolek, M.D.
Consultant, Haworth Family Professor in Neurodegenerative Diseases
Professor of Neurology
Phone: 904-953-7229; FAX: 904-953-0757; Pager: 904-953-8173
E-mail: Wszolek.Zbigniew@mayo.edu

Mayo Clinic Florida
4500 San Pablo Road
Jacksonville, FL 32224

U.S.A.

Gdańsk 03/04/2021

(miejscowość, data)

Jarosław Dulib

(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „**Cognitive and behavioral profile of Perry syndrome in two families**" opublikowanej w Parkinsonism and Related Disorders

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w tworzeniu projektu badania, nadzór merytoryczny podczas prowadzenia badań, ocena artykułu przed oddaniem do recenzji.

Mój udział procentowy w przygotowaniu publikacji określam jako 20 %.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Łukasza Milanowskiego, którego udział procentowy wynosi 60%.

Z poważaniem

Jarosław Dulib

Gdańsk, 13.04.2021

(miejscowość, data)

Michał Schinwelski

(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „**Cognitive and behavioral profile of Perry syndrome in two families**" opublikowanej w Parkinsonism and Related Disorders

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w tworzeniu projektu badania, ocena artykułu przed oddaniem do recenzji.

Mój udział procentowy w przygotowaniu publikacji określam jako 1%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Łukasza Milanowskiego, którego udział procentowy wynosi 60%.

Warszawa, 16.04.21

(miejscowość, data)

ŁUKASZ MILANOWSKI

(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „**Frequency of Mutations in PRKN, PINK1, and DJ1 in Patients With Early-Onset Parkinson Disease From Neighboring Countries in Central Europe**" opublikowanej w Parkinsonism and Related Disorders

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w tworzeniu projektu badania, badanie kliniczne pacjentów, wykonanie eksperymentów, napisanie pierwszej wersji manuskryptu, ostateczna ocena artykułu przed oddaniem do recenzji.

Mój udział procentowy w przygotowaniu publikacji określam jako 51%.

Jacksonville, FL, USA; 3 kwietnia 2021 roku

Zbigniew K. Wszolek, M.D.

OŚWIADCZENIE

Jako współautor pracy pt. „**Frequency of Mutations in PRKN, PINK1, and DJ1 in Patients With Early-Onset Parkinson Disease From Neighboring Countries in Central Europe**" opublikowanej w Parkinsonism and Related Disorders

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w tworzeniu projektu badania, nadzór merytoryczny podczas prowadzenia badań, ocena artykułu przed oddaniem do recenzji.

Mój udział procentowy w przygotowaniu publikacji określam jako 20%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Łukasza Milanowskiego, którego udział procentowy wynosi 51%.

Zbigniew K. Wszolek.

Zbigniew K. Wszolek, M.D.
Consultant, Haworth Family Professor in Neurodegenerative Diseases,
Professor of Neurology
Phone: 904-953-7229, FAX: 904-953-0757, Pager: 904-953-8173
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Mayo Clinic Florida
4500 San Pablo Road
Jacksonville, FL 32224, U.S.A.

Warszawa 07.04.2021

(miejscowość, data)

Dorota Hoffman-Zacharska

(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „**Frequency of Mutations in PRKN, PINK1, and DJ1 in Patients With Early-Onset Parkinson Disease From Neighboring Countries in Central Europe**" opublikowanej w Parkinsonism and Related Disorders

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w tworzeniu projektu badania, wykonanie eksperymentów, ocena artykułu przed oddaniem do recenzji.

Mój udział procentowy w przygotowaniu publikacji określam jako 2%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Łukasza Milanowskiego, którego udział procentowy wynosi 51%.

INSTYTUT [illegible] I BIOLOGII
Zakład [illegible]
Kierownik [illegible]

dr hab. n. med.
Dorota Hoffman-Zacharska prof. IMiD

Gdańsk, 03/04/2021

(miejscowość, data)

Dariusz Dudka

(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „**Frequency of Mutations in PRKN, PINK1, and DJ1 in Patients With Early-Onset Parkinson Disease From Neighboring Countries in Central Europe**" opublikowanej w Parkinsonism and Related Disorders

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w tworzeniu projektu badania, badanie kliniczne pacjentów, ocena artykułu przed oddaniem do recenzji.

Mój udział procentowy w przygotowaniu publikacji określam jako 1...%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Łukasza Milanowskiego, którego udział procentowy wynosi 51%.

Z poważaniem

Dariusz Dudka

Prof. dr hab. med. Jarosław Sławek
specjalista neurolog
3478478

(miejscowość, data)

07.04.2021

JAROSŁAW SŁAWEK

(imię i nazwisko)

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Prof. dr hab. med. Jarosław Sławek

Warszawa 16.04.2021

(miejscowość, data)

Dariusz Koziorowski

(imię i nazwisko)

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Dariusz Koziorowski

Wro 16.04.21

(miejscowość, data)

ANDRZEJ FRIEDMAN

(imię i nazwisko)

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