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Association between the Val66Met (rs6265) polymorphism of the brain-derived neurotrophic factor (BDNF) gene, BDNF protein level in the blood and the risk of developing early-onset Parkinson's disease

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Brain-derived neurotrophic factor (BDNF) is involved in the maintenance of dopamine level and the survival of dopaminergic neurons, which may affect the functionality of brain structures responsible for motor and cognitive function. The aim of the study was to assess the association of individual and combined single nucleotide polymorphism (SNP) in the rs6265 BDNF (Val66Met), rs397595 DAT (SLC6A3), and rs4680 COMT (Val158Met) genes with early-onset of Parkinson's disease (PD) patients. Moreover, we assessed the association between the BDNF Val66Met polymorphism and the level of BDNF protein in the serum of patients with PD and controls. The study involved 163 patients with idiopathic PD divided into early onset (<55 years) and late-onset (>55 years) groups and 91 healthy age-matched people (Control). The SNP were determined using the TaqMan Real-Time PCR method. Serum BDNF levels were determined by ELISA assay. The risk of developing early PD in people with the BDNF genotype AG increases threefold in comparison with the carriers of the BDNF genotype GG. In PD patients and healthy people with the BDNF genotypes AG and AA, a lower serum BDNF level was found compared to those with the BDNF genotype GG in both groups. The results of our study indicate that the presence of the Val66Met BDNF gene polymorphism is associated with reduced blood BDNF levels and an elevated risk of developing early-onset PD. This effect appears to be more pronounced in men.

Key words: BDNF Val66Met gene polymorphism, BDNF concentration, early-onset PD

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease (Kelly et al., 2019). Based on estimates derived from healthcare statistical data, the annual incidence of PD ranges from 5/100,000 to over 35/100,000 new cases (Twelves et al., 2003; Simon et al., 2020). PD prevalence also increases with age, starting at less than

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1 percent in both men and women aged 45–54 years and increasing to 4 percent in men and 2 percent in women aged over 85 (Marras et al., 2018). This observation is in line with the results of a meta-analysis involving four North American populations, indicating a 5- to 10-fold increase in PD morbidity from the sixth to the ninth decades of life (Marras et al., 2018). Although the estimated incidence and prevalence of PD vary to a large extent between reported studies, there is a consistent trend showing higher rates in men than in women. Interestingly, there has also been a significant increase in the incidence of idiopathic PD under the age of 45 in recent years (Pinter et al., 2015; Marras et al., 2018; Simon et al., 2020).

PD is caused by the loss of dopaminergic neurons (Zhu et al., 2022), which leads to a reduction in the levels of the neurotransmitter dopamine (DA) (Fahn, 2006; Sung & Nicholas, 2013). This phenomenon inevitably leads to impairment of motor functions, which are associated with deficits in voluntary movement and manifest as bradykinesia, slowness of simple repetitive movements, reduced stride length, smaller range of arms movement, gait freezing, micrographia and facial movement deficits (Sung & Nicholas, 2013; Postuma et al., 2015; Simon et al., 2020).

Brain-derived neurotrophic factor (BDNF) is a neuropeptide involved in the maintenance of DA levels and the survival of dopaminergic neurons and thereby can affect motor and cognitive functions of the brain (Palasz et al., 2019). Although BDNF is expressed in the cortex, hippocampus, midbrain, amygdala, hypothalamus, pons, medulla oblongata, and spinal cord, the main sources of BDNF are the dopaminergic neurons in the pars compacta of the substantia nigra and striatum (Canals et al., 2001; Aid et al., 2007; Edelmann et al., 2014). BDNF plays an important role in neuron development (Scalzo et al., 2010), synaptic plasticity, and memory processes (Andero et al., 2014; Edelmann et al., 2014; Boschen & Klintsova, 2017) by activating the antiapoptotic pathway and upregulating tyrosine hydroxylase (TH) and DA synthesis (Palasz et al., 2020). On the other hand, elevated levels of DA can accelerate the process of dopamine-induced oxidative stress (Fukuchi et al., 2010). The neuroprotective effect of BDNF is the result of activation of the TrkB/MAPK/ERK1/2/IP3K/Akt pathway, which leads to attenuation of apoptosis as well as glutamate and nitric oxide (NO) neurotoxicity and oxidative stress-induced cell damage (Klintsova et al., 2004; Numakawa, 2010) It needs to be highlighted that all of the above-mentioned changes occur in PD and are a well-known feature of this disease (Rahmani et al., 2019). A decrease in BDNF levels in the blood and brain was observed in patients with depression or suffering from PD (Wang et al., 2017). As reviewed by Pałasz et al. (2020), a decrease in BDNF levels in PD may contribute to the overexpression of alpha-synuclein (ASN) and inhibition of DA synthesis.

The PD etiology is not well understood but is likely to involve both genetic and environmental factors. Previous genetic research indicates that carrying the Val66Met BDNF gene polymorphism may increase the risk of PD (Fischer et al., 2018; Michałowska et al., 2020). Both the heterozygous AG major allele (Val/Met) and the homozygous AA minor allele Met/Met of the BDNF single nucleotide polymorphism (SNP) result in a dose-dependent homozygous/heterozygous decrease in the activity-dependent release of BDNF by disrupting BDNF transport and packaging it into secretory vesicles, whereas constitutive levels of BDNF secretion remain unaffected (Chen et al., 2004; Baj et al., 2013). Work with cultured neurons has shown that the Val66Met polymorphism results in an approximate 18% decrease in the activity-dependent secretion of BDNF in transfected cells carrying one 66Met allele and a 29% decrease in those transfected with two 66Met alleles (Mercado et al., 2021). The rs6265 SNP (also called Val66Met) in the BDNF gene is a common genetic variant that can be of risk of PD and impact the therapeutic response in individuals with PD (Liu et al., 2019; Mercado et al., 2021). However, Mariani et al. (2015) in their meta-analysis found no association between the Val66Met polymorphism and the risk of developing PD.

It can therefore be assumed that these genetic risk factors might underlie the variability in the clinical response to the early-onset PD. To examine this hypothesis, we evaluated the association of individual and combined SNPs in the rs6265 BDNF (Val66Met), rs397595 DAT (Dopamine transporter) (SLC6A3), and rs4680 COMT (Catechol-O-Methyltransferase, Val-158Met) genes with PD in both early- and late-onset PD patients. Moreover, we assessed the association between the BDNF Val66Met gene polymorphism (rs6265) and BDNF serum levels in PD patients and controls.

METHODS

Participants

The study comprised 200 consecutive PD patients recruited from the outpatient clinic of the Department of Neurology at the Military Institute of Medicine in Warsaw (Poland). The diagnosis of PD was based on the United Kingdom Parkinson's Disease Society Brain Bank diagnostic criteria (Fahn, 2011; Martinez-Martin et al., 2013) and confirmed by a certified neurologist specializing in PD. The study included only patients with

idiopathic PD. One hundred sixty-three patients were included in the PD group, and 91 healthy individuals were included in the control group. The PD group was categorized into an early-onset PD group and a late-onset PD group based on specific age criteria. Early-onset PD included patients who experienced symptom onset before the age of 55, while late-onset PD encompassed those with symptom onset after the age of 55. The early-onset PD group comprised 70 patients (34 women and 36 men), and the late-onset PD group had 93 patients (53 women and 40 men). All patients exhibited at least two of the four cardinal signs of PD: bradykinesia, resting tremor, rigidity and postural impairment. The disease severity and motor symptoms were evaluated using the Unified Parkinson's Disease Rating Scale (UPDRS) part III (motor examination) (Martinez-Martin et al., 2013; Postuma et al., 2015). The progression of PD was evaluated using the Hoehn and Yahr scale (H-Y scale; Fahn, 2011).

The control group was recruited from the Caucasian healthy community and had no history of neurological disease or other chronic diseases. The groups were matched by age and sex.

This study protocol was conducted in accordance with the Declaration of Helsinki and approved by the relevant local Bioethics Committee of Military Institute of Medicine in Warsaw and Bioethics Committee of Jerzy Kukuczka of University of Health Education in Katowice (2/2017). All participants signed written informed consent prior to their inclusion in the study.

Blood sampling and serum BDNF level examination

Serum BDNF concentration was measured in a group of 40 PD patients randomly selected from the early-onset group, 40 from the late-onset cases and 40 participants selected randomly from control group. In total, 5 ml of peripheral blood was collected between 8:00 and 9:00 AM following an overnight fast and prior to clinical assessment. Blood samples were transferred into nonanticoagulated tubes and then set aside for clot formation at room temperature for 30 min. Subsequently, serum was obtained by centrifugation at 1500 rpm (1620 × g) for 15 min and then stored at –80°C until assay.

The serum concentration of total BDNF in patients and controls was measured using a sandwich BDNF enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions and according to a previously published protocol (Piotrowicz et al., 2020). The BDNF concentration is presented as the equivalent of human recombinant protein and measured in ng of protein per ml of serum. The limit of detection was set at 20 pg/ml. Each measurement was repeated twice, ensuring a mean inter-assay precision, presented as the coefficient of variation, of approximately 8%.

Genotyping

Genotyping was performed using a polymerase chain reaction (PCR) following a previously published protocol (Mogi et al., 1999; Michałowska et al., 2020). The SNPs for rs6265 BDNF (Val66Met), rs397595 DAT (SLC6A3) and rs4680 COMT (Val158Met) were determined by using a prevalidated allelic discrimination TaqMan Real-Time PCR Assay (Applied Biosystems, Forest City, CA, USA) following the manufacturer's instructions. Amplification was performed using a protocol with 40 cycles of 15 s at 95°C for denaturation and 1 min at 60°C for annealing/extension. An initial incubation of 10 min at 95°C was applied. Data analysis was performed according to the manufacturer's instructions using SDS software (Applied Biosystems, Forest City, CA, USA). The distribution of individuals within each genotype group are in esupp tables; these were consistent with an exact test of the Hardy-Weinberg equilibrium.

Statistical analysis

Statistical analysis of clinical outcomes for the two abovementioned patient groups was performed using the Mann–Whitney U test, with significance set at a p-value below 0.05. The analysis was performed using the Statistica software package (Statistica v.10, StatSoft, Inc., www.statsoft.com).

For analyses of allele and genotype frequencies between the control and two PD patient groups, the chi-square test was used. The Hardy-Weinberg equilibrium (HWE) analyses and polymorphism association with PD groups were performed in the Autonomous System Numbers (ASN) stats program IPv6. Pearson's chi-squared test or Fisher's exact test was used to observe genotype frequencies obtained from the data and the expected genotype frequencies obtained using HWE. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated using multinomial logistic regression (SPSS v.13, USA). The differences were considered significant if the p value was < 0.05.

Multivariate analyses (MVA) analysis Orthogonal Partial Least Squares Discriminant analysis OPLS_DA) was performed using the SIMCA software package (Version 17, Sartorius Stadim Data Analytics AB, Sweden).

The goodness of fit is reported as the cumulative score across all of the components $R^{2,}$ which is explained by the model, and Q^2, which allows prediction. The variable importance in the projection (VIP) value of each variable in the model was calculated to indicate its contribution to the classification. Those v variables with VIP values greater than 1.0 were considered significantly different.

RESULTS

Clinical characteristics of PD patients

The demographic and relevant clinical features of all participants, PD patients and the control group are presented in Table 1. One hundred sixty-three PD participants (87 females and 76 males) and 91 controls (47 females and 44 males) were enrolled in the study, with mean age of 65.4±10.5 and 56.0±9.0, respectively. Statistical analysis did not show any difference in demographic parameters between control and PD patients (P>0.05). The mean disease duration was 10.2±6.2 years (Table 1). Statistical analysis did not show any difference in age, age of onset or duration between groups of PD patients. The UPDRS III and Hoehn and Yahr score differences between male and female PD patients were not statistically significant (P>0.05) (Table 1).

All patients were diagnosed with idiopathic disease and were treated with anti-parkinsonian therapy.

The PD patients were divided into an early-onset PD group and a late-onset PD group. The early-onset PD group included patients under 55 years of PD onset with idiopathic PD, and the mean age of onset was 48.2±8.7 years (Table 1), while the late-onset PD group included patients over 55 years of PD onset, and the mean age of onset was 60.6±13.2 years (Table 1). The late-onset PD patients were significantly older than the PD young onset group (P<0.005). Early-onset PD was found in 70 (42.94% of all) PD patients, including 34 (48.57%) females and 36 (51.43%) males, with a mean age of 59.8 ± 9.15 years and duration of 13.1±12.4 (range 0.5-8) years (Table 1). Late-onset PD was found in 93 (57.05%) PD patients, including 53 (56.98%) females and 40 (43.02%) males, with a mean age of 70.4±10.4 and duration of 9.98±11.2 (range 0.5-8) years (Table 1). There were significant differences in age and duration between the PD groups (P<0.05); the early-onset group had a longer PD duration than the late-onset group. There were no differences in UPDRS III and Hoehn and Yahr scale scores or disease onset between males and females in the PD group (Table 1).

Table 1. Characteristics of Parkinson's disease (PD) patients and control participants.

Groups	Sex	Age (year) score mean ± SD	Age at PD onset (years) score mean ± SD	Duration of PD (years) score mean ± SD	Motor impairment level according to the Hoehn and Yahr scale score mean ± SD	UPDRS III scale score mean ± SD
PD	All n=163	65.4± 0.5	54.6±12.0	10.2±6.2	2.45±0.7	25.12±12.1
	Female n=87	66.3±12.8	55.5±13.0	9.6±6.7	2.48±0.8	24.12±12.1
	Male n=76	64.8±8.4	53.7±10.7	10.7±5.5	2.43±0.7	25.6±12.6
PD Early onset subgroup	All n=70	59.8±9.15	48.2±8.7	13.1±12.4	2.61±0.76	27.3±15.8
	Female n=34	58.9±8.8	47.4±6.7	11.5±7.4	2.67±0.84	25.62±14.7
	Male n=36	59.6±6.9	47.4±5.5	12.2±5.24	2.5±0.67	25.1±12.8
PD Late onset subgroup	All n=93	70.4±10.4	60.6±13.2	9.98±11.2	2.45±0.77	25.22±13.9
	Female n=53	71.1±12.7	61.7±14.5	10.3±14.3	2.35±0.79	24.71±15.0
	Male n=40	69.5±6.5	59.5±11.3	9.45±5.6	2.3±0.8	26.0±12.8
Control participants	All n=91	56±9.0	–	–	–	–
	Female n=47	56±6.5				
	Male n=44	61±7.3				

UPDRS III – Unified Parkinson's Disease Rating Scale part III (motor examination).

Genetic SNP polymorphisms for BDNF (rs6265, Val66Met) gene in all, early-onset and late-onset PD patients and control participants

The distribution of BDNF genotypes significantly deviated from the Hardy-Weinberg equilibrium test and was significantly different in all PD patients compared to the control group (P<0.05, Table 2). Multinomial logistic regression analysis indicated significant differences in the distribution of BDNF genotypes (P<0.05) between control and PD patients. A higher frequency of the BDNF AG genotype was observed in patients (P<0.05). Namely, among 163 patients, 90 (55.22%) had the GG genotype, 70 (42.94%) had the AG genotype and 3 (1.84%) had the AA genotype (Table 2). The corresponding numbers for 91 healthy controls were 63 (69.2%) with GG, 25 (27.5%) with AG and 3 (3.3%) with AA genotypes.

Multinomial logistic regression revealed an almost twofold increased risk for PD patients carrying the heterozygous AG genotype of the BDNF (Val66Met) gene polymorphism (OR=1.88, 95% CI=1.08-3.29, p=0.03), while no such association was observed with the mutant homozygous AA genotype, probably due to the low frequency of homozygous mutant genotypes and smaller sample size (Table 2).

The distribution of BDNF genotypes significantly deviated from the Hardy-Weinberg equilibrium test and was significantly different in patients with early-onset PD compared to the control group (P<0.006) (Table 2). A higher frequency of the BDNF AG genotype was observed in patents with PD (P<0.006). Among 70 patients with early-onset PD, 28 (40.0%) had the GG genotype, 42 patients (57.14%) had the AG genotype and 2 patients (2.86%) had the AA genotype. Multinomial logistic regression revealed an almost threefold increased risk for early-onset PD cases carrying the heterozygous AG genotype of the BDNF (Val66Met) polymorphism (OR=2.79, 95%, CI=1.44-5.39, p=0.003), while no such association was observed with mutant homozygosity, probably due to the low frequency of homozygous mutant genotypes and smaller sample size (Table 2).

Among 93 late-onset patients with PD, 62 patients (66.66%) had the GG genotype, 30 patients (32.26%) had the AG genotype, and 1 patient (1.08%) had the AA genotype. The BDNF GG, AG and AA genotype frequency distributions in late-onset patients with PD were similar to those in the control group. Multinomial logistic regression revealed an almost 1.5-fold increased risk for late-onset PD cases carrying the heterozygous AG genotype of polymorphism BDNF (Val66Met) (OR=1.38, 95%, CI=0.74-2.60, p=0.38) but was not statistically significant (Table 2).

The influence of sex on genetic SNP polymorphisms for BDNF (rs6265, Val66Met) in all, early-onset and late-onset PD patients and controls

Sex did not influence the BDNF genotype distribution in either the PD patient or control groups (P>0.05) (Table 3). A higher frequency of the BDNF AG genotype was observed in female and male patients with PD (P<0.03 and P<0.05, respectively). Among 91 female patients with PD, 58 patients (63.74%) had the

Table 2. Genotype frequencies of rs6265 BDNF (Val66Met) gene polymorphisms in PD patients and control participants.

Group	BDNF gene polymorphism Genotypes	PD all patients n (%)	Controls n (%)	χ^2 PD / controls	p value PD / controls	OR (95% CI)	p value PD *versus* controls
All	GG	90 (55.22%)	63 (69.2%)	3.47 / 0.071	0.010 / 0.78	0.50 (0.31-0.94)	0.04*
	AG	70 (42.94%)	25 (27.5%)			1.88 (1.08-3.29)	0.03*
	AA	3 (1.84%)	3 (3.3%)			0.92 (0.21-3.97)	–
Early onset subgroup	GG	28 (40.00%)		4.732 / 0.071	0.296 / 0.78	0.37 (0.19-0.71)	0.004***
	AG	40 (57.14%)				2.79 (1.44-5.39)	0.003***
	AA	4 (2.86%)				0.86 (0.14-5.30)	1.0
Late onset subgroup	GG	62 (66.66%)		0.32 / 0.071	0.989 / 0.78	0.74 (0.40-1.35)	0.40
	AG	30 (32.26%)				1.38 (0.74-2.60)	0.38
	AA	1 (1.08%)				0.97 (0.19-4.97)	–

*p<0.05 means significance, ***p<0.001 means very significance; BDNF, brain-derived neurotrophic factor; PD, Parkinson's disease; $\chi 2$, chi-square, OR, odds ratio; 95% CI, 95% confidence interval.

GG genotype, 33 patients (36.26%) had the AG genotype and 0-none (0%) had the AA genotype. Among seventy-two male patients with PD, 32 patients (52.63%) had the GG genotype, 37 patients (40.78%) had the AG genotype, and 3 patients (6.57%) had the AA genotype (Table 3).

The distribution of BDNF genotypes significantly deviated from the Hardy-Weinberg equilibrium test and was significantly different in females and males in the early-onset group of patients with PD compared to the control group (P<0.04, P<0.03), respectively (Table 3).

We observed similar BDNF GG, AG and AA genotype frequency distributions in females and males from the late-onset group of PD patients compared to the control group (P<0.65, P<0.89, respectively (Table 3). The distribution of BDNF genotypes was consistent with the Hardy-Weinberg equilibrium test in females and males of late-onset PD patients (Table 3).

Dopamine transporter (DAT) and Catechol-O-Methyltransferase (COMT) gene single nucleotide polymorphism in PD patients and control participants

Tables 4-5 summarizes the distributions of the genotypes involved in dopaminergic transmission for both PD patients and controls. The distribution of DAT and COMT genotypes in the PD groups and controls was confirmed to be the Hardy–Weinberg equilibrium test.

The frequencies of DAT genotypes did show significant deviation from Hardy-Weinberg equilibrium in analyzed SNPs in all PD patients (P>0.01), early-onset PD (P<0.26) and late-onset PD patients (P<0.008) compared to the control group (Table 4).

The frequencies of DAT genotypes showed significant deviation from Hardy-Weinberg equilibrium in all PD patients (P<0.01) and the late-onset PD group (P<0.008). The frequency of the genotypes

Table 3. Genotype frequencies of rs6265 BDNF (Val66Met) gene polymorphisms in PD patients and controls participants depending of sex.

Gene BDNF	Sex	Genotypes	PD patients n (%)	Controls n (%)	χ^2 PD / controls	p value PD / controls	OR (95% CI)	p value PD *vs.* controls
All PD	♀	GG	58 (63.74%)	32 (68.1%)	5.52 / 0.183	0.01 / 0.67	0.69 (0.33-1.47)	0.45
		AG	33 (36.26%)	13 (27.65%)			1.76 (0.81-3.8)	0.20
		AA	0 (0%)	2 (4.25%)			–	–
	♂	GG	32 (52.63%)	31 (70.45%)	0.95 / 0.16	0.054 / 0.89	0.46 (0.21-1.02)	0.08
		AG	37 (40.78%)	12 (27.27%)			1.83 (0.82-4.19)	0.19
		AA	3 (6.57%)	1 (2.28%)			3.02 (0.34-26.79)	–
Early onset subgroup 70	♀	GG	18 (52.95%)		3.218 / 0.207	0.07 / 0.65	0.53 (0.21-1.31)	0.24
		AG	18 (47.05%)				2.32 (0.91-5.88)	0.11
		AA	0 (0%)				–	–
	♂	GG	10 (38.90%)		2.25 / 0.016	0.13 / 0.89	0.26 (0.10-0.67)	0.009**
		AG	22 (55.55%)				3.33 (1.30-8.48)	0.01*
		AA	2 (5.55%)				2.52 (0.22-29.08)	1.0
Late onset subgroup 93	♀	GG	40 (64.15%)		2.52 / 0.207	0.11 / 0.65	0.84 (0.36-1.92)	0.84
		AG	15 (35.85%)				1.46 (0.62-3.42)	0.50
		AA	0 (0%)				–	–
	♂	GG	20 (65%)		1.27 / 0.016	0.26 / 0.89	0.78 (0.31-1.95)	0.76
		AG	22 (27.5%)				1.01 (0.39-2.65)	0.82
		AA	1 (7.5%)				3.48 (0.35-34.90)	–

*p<0.05 means significance , **p<0.01 means very significance; BDNF, brain-derived neurotrophic factor; PD, Parkinson's disease; χ2 , chi-square, OR, odds ratio; 95% CI, 95% confidence interval.

of DAT were similar in females and males of all PD groups and late-onset PD groups (Table 4). Multinomial logistic regression revealed almost 3.59; 4.54 and 2.94-fold increased risk for all PD, early-onset and late-onset PD group cases carrying the homozygous GG genotype of polymorphism BDNF (Val66Met) (OR=3.59 95% CI=1.44-8,95, p=0.006; OR=2.94 95%, CI=1.10-7.90, p=0.04), respectively. Female sex also did not influence the distribution of DAT genotypes (P>0.05) (Table 4).

We did not observe differences in the distribution of COMT (P=0.919) genotypes between PD patients and controls (P>0.05) (Table 5). The frequencies of COMT genotypes did not show significant deviation from

Table 4. Genotype frequencies of rs397595 DAT (*SLC6A3*) gene polymorphisms in PD patients and controls participants depending of sex.

Gene DAT		Genotypes	PD patients n (%)	Controls n (%)	χ^2 PD / controls	p value PD / controls	OR (95% CI)	p value PD *versus* controls
PD	All	AA	72 (44.17%)	58 (63.74%)	9.79 / 1.28	0.001 / 0.26	0.45 (0.26-0.76)	0.004
		AG	58 (35.58%)	27 (29.66%)			1.30 (0.75-2.27)	0.41
		GG	33 (20.25%)	6 (6.60%)			3.59 (1.44-8.95)	0.006**
	♀	AA	37 (22.70%)	25 (53.20%)	4.64 / 0.08	0.03 / 0.76	0.60 (0.30-1.22)	0.22
		AG	34 (37.36%)	18 (38.30%)			0.96 (0.46-1.98)	0.92
		GG	20 (21.98%)	4 (8.50%)			3.02 (0.97-9.45)	0.08
	♂	AA	35 (48.62%)	33 (75.00%)	5.04 / 1.55	0.02 / 0.21	0.32 (0.14-0.72)	0.009
		AG	24 (33.33%)	9 (20.45%)			1.94 (0.80-4.69)	0.20
		GG	13 (18.05%)	2 (4.55%)			4.62 (0.99-21.59)	0.06
Early onset subgroup	All	AA	25 (35.72%)		2.51 / 1.28	0.11 / 0.26	0.32 (0.16-0.60)	0.0007
		AG	28 (40.00%)				1.58 (0.82-3.05)	0.22
		GG	17 (24.28%)				4.54 (1.68-12.25)	0.03*
	♀	AA	12 (33.33%)		0.36 / 0.08	0.54 / 0.76	0.44 (0.18-1.08)	0.11
		AG	16 (44.44%)				1.29 (0.53-3.11)	0.73
		GG	8 (22.23%)				3.07 (0.84-11.17)	0.15
	♂	AA	13 (38.24%)		2.74 / 1.55	0.09 / 0.21	0.21 (0.078-0.54)	0.002
		AG	12 (35.29%)				2.12 (0.77-5.86)	0.22
		GG	9 (26.47%)				7.56 (1.51-37.83)	–
Late onset subgroup	All	AA	47 (50.54%)		6.99 / 1.28	0.008 / 0.26	0.58 (0.32-1.04)	0.09
		AG	30 (32.26%)				1.13 (0.60-2.11)	0.82
		GG	16 (17.20%)				2.94 (1.10-7.90)	0.04*
	♀	AA	25 (45.45%)		0.73 / 0.08	0.39 / 0.76	0.73 (0.34-1.60)	0.55
		AG	22 (40.00%)				1.07 (0.48-2.38)	1.00
		GG	8 (14.55%)				1.82 (0.51-6.51)	0.52
	♂	AA	20 (52.64%)		6.55 / 1.55	0.01 / 0.21	0.37 (0.14-0.94)	0.05
		AG	10 (26.31%)				1.39 (0.50-3.88)	0.71
		GG	8 (21.05%)				5.6 (1.11-28.26)	–

*p<0.05 means significance, DAT, dopamine transporter; PD, Parkinson's disease; χ2, chi-square, OR, odds ratio; 95% CI, 95% confidence interval.

Hardy-Weinberg equilibrium (P>0.05) in all PD, early-onset and late-onset PD groups. The frequencies of the genotypes of COMT were similar in females and males in both groups of PD (Table 5). Multinomial logistic regression indicated that individual SNP polymorphisms in COMT did not influence the prevalence of early-onset or late-onset PD in patients (P=0.508). Sex also did not influence the distribution of these genotypes (P>0.05) (Table 5).

Orthogonal partial least squares discriminant analysis (OPLS-DA) of genetic SNP polymorphisms for BDNF, DAT and COMT in all, early-onset and late-onset PD patients and controls

We assessed interactions between polymorphism genes (BDNF, DAT and COMT) involved in dopaminergic transmission and PD using OPLS-DA. OPLS-DA indicated that it is also possible to build a model that differentiates control and PD patients based on genetic data and movement disorders. The model consisted of one predictive component, two orthogonal components and one component unrelated to genes and groups. The cumulative variation R^2 is equal to 0.662 and Q^2cum (predictions) is 0.095. The validation test by CV-ANOVA had a calculated p value of 0.04. The most important genes variable influence on the projection (VIP>1) were the BDNF (AG) and BDNF (AA) genotypes, which were correlated with the PD group, while the BDNF (GG) genotype was correlated with the control group (P<0.05).

The OPLS-DA of three groups, control and PD with early onset and PD with late-onset, shows that it is possible to differentiate all groups upon gene analysis. For control and PD with early-onset groups, statistical analysis allowed us to build a model that consisted of one predictive and one orthogonal component. The cumulative R^2 was 0.55. The most different polymorphisms and VIP parameters were BDNF (GG),

Table 5. Genotype frequencies of rs4680 COMT (Val158Met) gene polymorphisms in PD patients and control participants.

Gene COMT		Genotypes	PD patients n (%)	Controls n (%)	χ^2 PD / controls	p value PD / controls	OR (95% CI)	p value PD *versus* controls
Early onset subgroup	All	AA	20 (28.57%)	27 (29.36%)	0.21 / 0.09	0.64 / 0.76	0.96 (0.48-1.91)	1.00
		AG	33 (47.14%)	47 (51.08%)			0.85 (0.46-1.59)	0.74
		GG	17 (24.28%)	18 (19.56%)			1.31 (0.62-2.79)	0.59
	♀	AA	9 (25.00%)	24 (50.0%)	0.0 / 3.0	1.00 / 0.08	0.33 (0.61-5.27)	0.04*
		AG	18 (50.00%)	16 (33.33%)			2.00 (0.82-4.86)	0.18
		GG	9 (25.00%)	8 (16.67%)			1.66 (0.57-4.86)	0.50
	♂	AA	11 (32.35%)	10 (22.75%)	0.07 / 0.82	0.79 / 0.36	1.62 (0.59-4.45)	0.49
		AG	16 (47.06%)	25 (56.80%)			0.64 (0.26-1.56)	0.45
		GG	7 (20.59%)	9 (20.45%)			1.00 (0.33-3.05)	0.79
Late onset subgroup	All	AA	27 (29.03%)		1.24 / 0.09	0.26 / 0.76	0.98 (0.52-1.85)	0.92
		AG	51 (54.84%)				1.16 (0.65-2.07)	0.72
		GG	15 (16.13%)				0.79 (0.37-1.68)	0.67
	♀	AA	16 (29.10%)		0.009 / 3.0	0.92 / 0.08	0.41 (0.18-0.92)	0.04*
		AG	27 (49.0%)				1.93 (0.87-4.29)	0.16
		GG	12 (21.80%)				2.55 (1.02-6.40)	0.06
	♂	AA	13 (34.21%)		2.26 / 0.82	0.13 / 0.36	1.76 (0.66-4.67)	0.36
		AG	22 (57.89%)				1.05 (0.43-2.51)	0.88
		GG	3 (7.90%)				0.33 (0.08-1.33)	0.19

*p<0.05 means significance, **p<0.01 means very significance; COMT, catechol-O-methyltransferase; PD, Parkinson's disease; χ2 , chi-square, OR, odds ratio; 95% CI, 95% confidence interval.

BDNF (AG), and DAT (AA). In the early-onset PD group, there were polymorphisms of BDNF (AG), DAT (AG) or DAT (GG), and COMT (AG). In controls, BDNF (GG), DAT (AA), and COMT (AA) polymorphisms mainly occurred.

For the control and PD with late-onset groups, OPLS-DA allowed us to build a model that consisted of one predictive and three orthogonal to data and one orthogonal to group components. The cumulative R^2 was 0.72. The most different polymorphisms and VIP parameters were BDNF (AG), BDNF (GG), and DAT (AA). In the late-onset PD group, there were polymorphisms of BDNF (AG), DAT (GG) or DAT (AG), and COMT (AG). In controls, BDNF (AA), BDNF (GG), DAT (AA), and COMT (AA) polymorphisms mainly occurred.

For the early-onset and late-onset PD groups, OPLS-DA allowed us to build the model that consisted of one predictive and one orthogonal to data and one orthogonal to group components. The cumulative R^2 was 0.56. The most different polymorphisms, VIP parameters, were BDNF (GG) and BDNF (AG). Although both groups of patients with early-onset and late-onset PD differed from controls in BDNF polymorphism, late-onset PD patients had a higher incidence of GG polymorphism than early-onset PD patients. Therefore, this polymorphism differentiates the two groups of patients the most.

Association of the BDNF genotype with serum BDNF concentration in the control and PD groups

Serum BDNF concentrations differed depending on the genotype in the control group and in the group of patients with PD (Table 6). The highest serum concentrations of BDNF were observed in the control group and in PD patients with the GG genotype and were 57.05±1.19 and 46.70±1.05 ng/ml, respectively (Table 6). Significantly decreased serum BDNF concentrations (P<0.05) were found in subjects with the AG and AA genotypes in both PD groups and were 31.68±1.44 and 31.04±4.37 ng/ml, respectively. Moreover, PD patients with GG and AG genotypes had significantly lower serum BDNF concentrations than the controls (P<0.05) (Table 6). In patients with early-onset and late-onset PD, similar changes were observed depending on the presence of genotype (GG and AG). In the AG genotype, BDNF levels were lower than those in the GG genotype in both early- and late-onset PD (P<0.05). In addition, statistically lower BDNF levels were found in early-onset PD patients matched by age in both genotypes (GG and AG) (P<0.05). The AA genotype was present in four patients with early-onset PD and one with late-onset PD (Table 6).

Table 6. Comparison of brain-derived neurotrophic factor (BDNF) serum concentration between genotypes of Val 66Met BDNF gene polymorphism PD patients and control participants.

Group	Genotypes _(n)	BDNF level [ng/ml serum]
Control	GG (n=20)	57.05 ± 1.19
	AG (n=16)	45.23 ± 1.33**
	AA (n=4)	40.46 ± 2.66**
PD	GG (n=87)	46.70 ± 1.05
	AG (n=46)	31.68 ± 1.44**#
	AA (n=5)	31.04 ±4.37**#
Early onset subgroup	GG (n=19)	48.90 ± 1.77&&
	AG (n=19)	34.56 ± 2.19**#&
	AA (n=2)	31.27 ± 6,75**#&
Late onset subgroup	GG (n=20)	45.80 ± 128&
	AG (n=19)	29.65 ± 1.88**#&
	AA (n=1)	29.22 ± 5.64**#&

**P<0.01 *vs.* genotype of GG, #P<0.05 *vs.* respective genotype of control, &P<0.01 *vs.* respective genotype of PD.

DISCUSSION

In recent years, a body of evidence has indicated that the BDNF gene might play a role in susceptibility to PD, possibly due to decreased BDNF mRNA expression and protein content in the substantia nigra and brain (Mogi et al., 1999). This conclusion is mainly based on studies conducted on elderly patients (those over 65 years of age) with an idiopathic PD, which is the most distinctive case of this disease (Palasz et al., 2020). However, in recent years, studies have shown that PD is increasingly common in younger populations. There is evidence that the clinical course of idiopathic PD in this group is markedly different from that of older patients (less than 65) in response to levodopa treatment (Quinn et al., 1987; Fischer et al., 2022). It concerns earlier onset of dyskinesias, more frequent fluctuations in clinical symptoms (scale H&Y and UPDRS), more severe involuntary movement and very rare occurrence of dementia (Quinn et al., 1987; Postuma et al., 2015; Kolicheski et al., 2022). To identify potential factors associated with the abovementioned variations in PD, we examined whether polymorphisms in the BDNF, DAT and COMT genes may at least in part contribute to different clinical courses of idiopathic PD in young and old patients.

BDNF is an important activator of tyrosine kinase receptor (Trk), while COMT is involved in the hydro-

lysis of dopamine, and DAT removes dopamine from the synaptic cleft, depositing it into surrounding cells, thus terminating the dopamine signal (Chen et al., 2006). It was shown that the BDNF rs6265 SNP variant results in reduced activity-dependent release of BDNF through reduced vesicular packaging (Chen et al., 2004). Moreover, despite not having a direct effect on protein structure, the BDNF genotypes AG and AA are associated with brain atrophy (Chen et al., 2006; Cagni et al., 2017) and cognitive dysfunction (Wang et al., 2019). Some studies have revealed that BDNF stimulates neuronal growth, is involved in nigrostriatal dopamine neuron survival and upregulates TH and dopamine synthesis in PD (Palasz et al., 2020). Previous clinical studies have shown that decreased serum BDNF level in PD patients is significantly correlated with the I-123-FP-CIT (N–ω -fluoropropil-2ß-carbometoxi-3β-[4-yodofenil]nortropane) uptake ratios and neurodegeneration of the dopaminergic system (Hernández-Vara et al., 2020). ^{123}I-FP-CIT is a cocaine analogue that binds itself to the pre-synaptic dopamine transporters, allowing the study of nigrostriatal dopaminergic system integrity which is commonly affected in parkinsonian syndromes and preserved in other mimicking disorders, e.g.: essential tremor and in drug-induced parkinsonism (Hernández-Vara et al., 2020).

A common observation of these studies is that the abovementioned changes can lead to impaired motor and cognitive functions in patients with PD (Hernández-Vara et al., 2020). Our previous study confirmed the association of rs6265 BDNF (Val66Met) with the risk of PD and suggested a synergic effect of rs6265 BDNF (Val66Met), rs397595 DAT (*SLC6A3*), and rs4680 COMT (Val158Met) polymorphisms on the occurrence of motor fluctuations and dyskinesias (motor levodopa-induced complications, MLIC) in some patients treated with L-DOPA (Michałowska et al., 2020).

Dopaminergic (rs1076560 DRD2 G>T and rs4680 catechol-methyltranspherase (COMT) Val158Met) or brain derived neurotrophic factor (rs6265 BDNF Val66Met) genetic polymorphisms are associated with gait function and medication responsiveness in Parkinson's disease. Catechol-O-methyltransferase (COMT) and monoamine oxidases (MAO) are involved in the metabolism of amine neurotransmitters and play a central role in therapeutic response to levodopa. The allele A (Met) of a common SNP in the COMT gene, the Val158Met (rs4680), is linked to increased thermolability and reduced activity of the enzyme (Lotta et al., 1995). The factors listed above may affect human movement control to varying degrees.

Association of the Val66Met BDNF gene polymorphism with early-onset PD

In the present study, approximately 42% of the PD patients recruited into our studies developed idiopathic PD before the age of 55. We found that the BDNF gene Val66Met (rs6265) and DAT gene (rs397595) polymorphisms, but not the COMT gene polymorphism, were associated with early-onset PD. OPLS-DA indicated that the most important gene (VIP>1) was BDNF gene Val66Met (rs6265). The BDNF genotypes of AG and AA were correlated with the PD group, while the BDNF genotype GG was correlated with the control group ($P<0.05$). Our current data support the hypothesis that the AG/AA BDNF genotype, which may result in an insufficient supply of BDNF, could contribute to the neurodegenerative processes in PD particularly in patients under the age of 55. The main new achievement of the present study is to provide new and relevant data that in younger patients, alongside environmental risk factors (single or collective), variability in BDNF polymorphisms also plays an important role in contributing to the development of PD symptoms (Tables 2, 3). In line with this inference are previous studies indicating that a decrease in BDNF levels is associated with the BDNF Val66Met polymorphism, which is further associated with reduced motor cortex plasticity (Fischer et al., 2018; Huangi et al., 2021) and leads to impaired synaptic plasticity in the nigrostriatal system (Wang et al., 2019). These changes may play a role in the development of MLIC in PD patients (Michałowska et al., 2020).

Previous epidemiological studies have shown an increasing risk of developing idiopathic PD before the age of 55, which may be due to an interaction with both environmental factors and genetic alterations (Simon et al., 2020). Because some studies have observed an association between a positive family history and early-onset PD, this feature suggests an important role of genetic alterations in the clinical progression of this disease. Targeted research on this topic has led to the identification of numerous genes associated with early-onset PD, often referred to as Parkinson's disease genetics (Simon et al., 2020; Riboldi et al., 2022). Initial data revealed that mutations in the PARKIN, PINK, DJ-1 and LRRK2 genes are linked to autosomal recessive early-onset PD (Simon et al., 2020; Riboldi et al., 2022). The results of our research are in agreement with these observations by showing the possible involvement of some genetic predisposition in the pathogenesis of PD in younger patients (Table 1). Our study suggest that susceptibility to develop PD is greater in patients with the BDNF Val66Met polymor-

phism (Table 2). Multinomial logistic regression revealed an almost threefold increased risk for early-onset PD cases carrying the heterozygous AG genotype of the BDNF (Val66Met) polymorphism (OR=2.79, 95%, CI=1.44-5.39, p=0.003). Furthermore, the presence of DAT AA increased the risk of developing PD at a young age (Table 4). Because our patients with early-onset PD did not have a family history of this disease, it seems that gene–environment interactions are necessary to initiate the cascade of events leading to the early-onset idiopathic PD. An increase in the risk of developing early-onset PD due to environmental factors may be contributed by having a specific polymorphism (Simon et al., 2020), or conversely, reduced risk of PD may occur in the presence of specific genetic variants (Kolicheski et al., 2022).

The association between the BDNF Val66Met polymorphism and susceptibility to PD was previously investigated with inconsistent results. Several meta-analyses found no association between SNPs and the risk of developing PD (Jiménez-Jiménez et al., 2014; Wang et al., 2019). One exception discussed in a meta-analysis was the association between the BDNF SNP and PD, which was found in European but not in Asian populations (Urbina-Varela et al., 2020). Our data suggest that the SNP BDNF Val66Met polymorphism may contribute to the early onset of neurodegenerative processes in PD, leading to disturbances in motor and nonmotor functions. Previous studies have indicated an association between the Val66Met SNP and reduced plasticity of the motor cortex (Huangi et al., 2021) as well as impaired synaptic plasticity in the nigrostriatal system , which could play a role in the development of the MLIC in PD patients (Michałowska et al., 2020). In an unmedicated cohort of early-stage PD subjects, specific genotypes for the BDNF rs10501087, rs1491850, and rs11030094 SNPs are associated with a prolonged time to the initiation of symptomatic therapy for PD motor symptoms, suggesting a slower rate of disease progression. A prospective clinical trial examining various BDNF SNP is warranted to determine what effects, if any, exist on PD progression and for their future use in clinical trial design (Fischer et al., 2022). In fact, our study reported an increased risk of developing PD in individuals under 55 years old.

Association of the Val66Met BDNF gene polymorphism with late-onset PD

We observed a significant deviation from Hardy-Weinberg equilibrium in the frequencies of DAT genotypes in the late-onset PD group, while the frequencies of BDNF genotypes remained consistent with Hardy-Weinberg equilibrium. The frequency of the genotypes of DAT were similar in females and males of all PD groups and late-onset PD groups (Table 4). We did evidence associations between individual DAT (rs397595) polymorphisms and all PD and late-onset PD, but OPLS-DA indicated that the most important VIP parameters were BDNF (AG) and DAT (AG) in late-onset PD. The link between DAT polymorphisms and susceptibility to PD might consist of changes in DAT expression and synaptic uptake of potential neurotoxins responsible for nigral cell death (Tipton & Singer, 1993). In one study (Nishimura et al., 2002) involving Japanese PD patients, no evidence of an association between the 1215A/G polymorphism in the DAT gene and PD was found. In contrast, two other studies (Nishimura et al., 2002) confirmed such an association. Additionally, the 10-copy genotype of the 40-pb VNTR polymorphism in the same gene was suggested as a protective factor for the development of PD in male Taiwanese individuals (Lin et al., 2003).

After the initial discovery of SNCA (Synuclein Alpha) gene mutations as a cause of PD more than 20 years ago, focused research has led to the identification of multiple genes linked to PD onset and progression. Gene-environment interactions may be essential in triggering the sequence of events that lead to the onset of PD and, in certain cases, modifying the risk of developing PD. Examples include stress exposure and specific polymorphisms that increase PD risk (Fischer et al., 2022; Kolicheski et al., 2022), or the relationship between smoking, reduced PD risk, and how this can be influenced by specific genetic variants.

A major question that remains unanswered by current genetic-environment studies is whether early-onset an late-onset PD are distinct disease entities or different staging on the same spectrum of disease. As discussed herein, there are clinical and neuropathologic differences in the phenotypic manifestation of disease signs, but it is not clear whether these are simply a reflection of overall health (biological age) in younger patients. Age is recognized as the major risk factor for late-onset PD, but this is absent in patients with early-onset PD and may reflect a stronger genetic/environmental component in the latter.

CONCLUSIONS

The results of our study indicate that the presence of the Val66Met BDNF gene polymorphism is associ-

ated with reduced blood BDNF levels and an elevated risk of developing early-onset PD (prior to age 55). This effect appears to be more pronounced in men.

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