Research Report

DRD3 Predicts Cognitive Impairment and Anxiety in Parkinson's Disease: Susceptibility and Protective Effects

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Abstract.
Background: A possible genetic contribution of dopamine D3 receptor (DRD3) to cognitive impairment in Parkinson's disease (PD) has yet to be investigated.
Objective: To explore the effects of rs6280 (Ser9Gly) genotype on PD patients' cognitive performance and to clarify possible interactions with psychopathology.
Methods: Two hundred and fifty-three consecutive PD patients underwent neurological and neuropsychological evaluations, which included: Unified Parkinson's Disease Rating Scale (UPDRS), Hoehn & Yahr scale (H&Y), Dementia Rating Scale-2 (DRS-2), and Hospital Anxiety and Depression Scale (HADS). rs6280 polymorphism was genotyped for all PD patients and for 270 ethnically matched healthy volunteers (HC). Non-parametric group comparisons and logistic regressions were used for data analyses.
Results: rs6280 genotype did not differ between PD and HC groups. PD patients with rs6280 CC genotype had more impaired cognitive performance (i.e., <1st percentile of demographically adjusted norms) on DRS-2 subscales Initiation/Perseveration and Construction than those with TT genotype. These associations remained statistically significant when other covariates (e.g., demographic features, disease duration, severity of motor symptoms in OFF and ON states, anti-parkinsonian medication, and psychopathology symptoms) were taken into consideration. PD patients with rs6280 TC had less anxiety (i.e., HADS Anxiety ≥ 11) than those with TT ($p = 0.012$). This association was also independent of other covariates.
Conclusions: Study findings suggest that rs6280 CC genotype predisposes to executive dysfunction and visuoconstructional deficits, whereas the heterozygous genotype protects from anxiety in PD. These effects do not appear to be dependent of one another. rs6280 is not a genotypic susceptibility factor for PD.

Keywords: Parkinson's disease, dopamine D3 receptor, cognition, anxiety

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INTRODUCTION

Cognitive dysfunction is a common and debilitating nonmotor symptom of Parkinson's disease (PD). The cognitive profile of patients with PD varies considerably, however cognitive deficits in the early stages of the disease (e.g., planning, initiation, monitoring of goal-directed behaviors, and working memory) are frequently associated with frontal–striatal dysfunction [1]. The conversion to dementia has been linked to posterior cortical dysfunction [2]. The neuropathological substrate of cognitive decline and dementia in PD has been a topic of great interest and debate in recent years.

DRD3, a G-protein couple receptor, is expressed mainly in neurons and glial cells throughout the central nervous system with marked expression in limbic areas. DRD3 modulates dopamine release and activation of signaling pathways through its autoreceptor function. The literature has provided evidence that DRD3 is involved in cognitive and social functions [3] and is implicated in mood disorders [4]. Studies in animal models suggest that when the D3 R function increases (e.g., through agonism, increased receptor expression), it impairs cognition and when D3 R function is reduced (e.g., through antagonism, lack of the receptor) it improves cognitive performance [3]. However, a neuropathological study in PD patients found that a reduction in DRD3 is related to pre-mortem presence of dementia and poor response to anti-parkinsonian medication [5].

The impact of DRD3 gene variation on measures of dopamine receptor binding is not yet completely understood. Though, it is known that DRD3 function can be modulated by the functional rs6280 polymorphism ($25T > C$), that leads to the missense substitution, of a serine for a glycine aminoacid in the N-terminal of DRD3 receptor (Ser9Gly). DRD3 predominantly acts as a presynaptic autoreceptor that inhibits dopamine release. The higher affinity of the glycine aminoacid (rs6280C allele) leads to an increased intracellular signaling and a decrease in extrasynaptic dopamine concentration under conditions of tonic dopamine release. The glycine autoreceptor may also predispose to reward-dependent elevated phasic dopamine signaling [6]. Clinically, the rs6280C allele in PD has been associated with visual hallucinations [7], impulse control disorders [8], and enhanced loss aversion in the absence of active impulse control disorder [9]. The rs6280C allele has also been associated with executive dysfunction in psychotic patients [10, 11] and

substance abusers [12]. The poor cognitive functioning, specifically executive dysfunction, in first time psychotic episode carriers of the rs6280C allele is accompanied by lower grey matter volume in the hippocampus [11]. The rs6280C allele has also been linked to depression and anhedonia symptoms, including in PD patients [4]. This association was accompanied by increased resting-state activation (i.e., amplitude of low-frequency fluctuations) in the right medial frontal cortex [4].

These set of findings suggests that DRD3 receptor plays an important role in the intricate modulation of dopamine signaling pathways involved in the fine-tuning of cognitive functions and emotional processing. The main goals of the present study were to explore the effects of rs6280 genotype on cognitive test performance of PD patients and to clarify possible interactions with psychopathology symptoms. As observed in non-PD psychotic patients, we expected that PD patients with rs6280 CC and/or CT genotype would have poorer performance in executive functions than those with r6280 TT genotype.

METHODS

Subjects

Our cohort of PD patients has been previously described as part of a comprehensive observational study [13]. Briefly, from a series of 322 consecutive non-surgically treated PD subjects, only one patient refused to participate in the study. From this cohort ($n = 321$), 6 died prior to assessment (2 patients from respiratory infection, 1 from heart disease, and 3 from unknown cause), 2 were excluded because they developed other debilitating conditions, and 13 moved geographically to a region not dependent from our center or could not be reached between inclusion and assessment. For the present study, 47 patients were *a posteriori* excluded, due to illiteracy (i.e., had less than 3 years of education) ($n = 27$), the rs6280 genotyping could not be conducted ($n = 16$), patient's inability to complete the Dementia Rating Scale-2 (DRS-2) ($n = 4$), and/or change in the diagnosis ($n = 3$). A total of 253 PD participants were included in the study.

A series of 270 ethnically matched volunteers, recruited from the community (i.e., healthy blood donors), integrated the healthy control (HC) group. HC subjects only participated in the genotyping component of the study.

All participants (or legal representatives) were informed about the nature of the study and gave their consent for participation, in accordance with the Declaration of Helsinki. The study was approved by local ethics committee.

Procedures

Assessment protocol

Clinical records were reviewed to determine age at disease onset and to identify current medication. Age at the first motor symptom was considered age at PD onset. Current anti-parkinsonian medication was converted to levodopa equivalent dose (LED) [14]. Two movement disorder specialists (AM, NVC) evaluated patients' motor symptoms after at least 12 h without anti-parkinsonian medication (OFF state) and under the effect of their habitual morning dose of medication (ON state), using the Unified Parkinson's Disease Rating Scale (UPDRS) [15] and Hoehn & Yahr scale (H&Y) [16]. Each PD patient was classified as having tremor-dominant or non-tremor-dominant phenotype [17].

A trained neuropsychologist, blinded to the motor assessment scores, applied the Portuguese versions of DRS-2 [18] and Hospital Anxiety and Depression Scale (HADS) [19]. The DRS-2 is a measure of general cognitive status. It consists of 24 brief subtests whose scores are combined into five subscales of attention, initiation/perseveration, construction, conceptualization, and memory. DRS-2 total and subscales scores were adjusted for age and education, according to the national norms, and the first percentile of norms was used as cut-off for cognitive impairment. For those medically treated (98% of the patients), the neuropsychological tests were applied under the effect of their regular anti-parkinsonian medication (in ON state).

History of appendectomy was explored as a covariate, because a prior study with the same dataset revealed that history of appendectomy was a risk factor for cognitive impairment (i.e., DRS-2 Conceptualization and Memory subscales) in late onset PD [20]. History of appendectomy had been obtained *a posteriori* via phone contact, with the patient or a family member.

DNA extraction and genotyping

Peripheral blood samples were collected in 5% ethylenediaminetetraacetic acid (EDTA) tubes (VACUETTE®, Portugal). Genomic DNA was obtained from proteinase-K-treated peripheral blood leukocytes using a salting-out procedure as previously described [21]. The rs6280 polymorphism was genotyped using a pre-designed TaqMan allelic discrimination assay (C_949770_20; Thermo Fisher Scientific, USA) and NzySpeedy qPCR mastermix (Nzytech, Portugal) in a Rotor Gene 6000 RT-PCR machine (Qiagen, Germany).

Statistical analyses

Descriptive statistics were used for group characterization. Chi-square test and Mann-Whitney test were applied to compare PD group with HC group. Multiple logistic regression analyses were used to explore group differences regarding rs6280 genotype, while taking into consideration sex as a covariate.

Chi-square test and Kruskal-Wallis test were applied to compare the demographic and clinical characteristics of PD patients according to rs6280 genotype. Simple and multiple logistic regressions were used to explore predictors of impairment in cognitive measures, other than rs6280. The following independent variables were included in the multiple logistic regression model: rs6280 genotype, sex, age, education, history of appendectomy, age at disease onset ($\geq$55), disease duration ($\leq$5 years, 6–10 years, and $\geq$11 years), levodopa equivalent dose, dopamine agonist, anti-depressant, UPDRS-II at OFF state, UPDRS-III at OFF and ON states, H&Y at OFF and ON states, non-tremor dominant, HADS Anxiety $\geq$ 11, and HADS Depression $\geq$ 11. Due to collinearity issues, UPDRS-III at ON state, and H&Y at OFF and ON states were not included in the initial regression model. In separate analyses, UPDRS-III OFF state was replaced by UPDRS-III ON, H&Y OFF, and H&Y ON in the regression model. Backward selection method was applied, with a threshold for variable removal of $p > 0.100$.

RESULTS

rs6280 in PD and HC

The PD group ($n = 253$) had a higher frequency of males (53.8% vs. 37.4%, $p < 0.001$) than the HC group ($n = 270$). However, the frequency of rs6280 TT, TC, and CC of the PD group (respectively, 46.2%, 38.3%, and 15.4%) was not statistically different ($p = 0.279$) from the HC group (respectively, 41.2%, 45.2%, and 13.6%). The allelic frequency (i.e., he relative frequency of an allele in a given group) of C in the PD cohort was 34.6% and in the HC group

Table 1
Characterization of the PD sample ($n = 253$). DRD3 genotype stratification

		Total Sample ($n = 253$)	rs6280			
			TT ($n = 117$)	TC ($n = 97$)	CC ($n = 39$)	p
Sex	Male	136 (53.8%)	68 (58.1%)	48 (49.5%)	20 (51.3%)	0.427
Age		68 (62, 76)	69 (61, 77)	68 (63, 76)	67 (60, 73)	0.498
Education		4 (4, 8)	4 (4, 9)	4 (4, 6)	4 (4, 8)	**0.047**
History of appendectomy		28 (11.2%)	14 (12.0%)	8 (8.5%)	6 (15.8%)	0.460
Age at disease onset		60 (53, 69)	60 (51, 70)	60 (54, 69)	61 (51, 67)	0.668
Disease duration		6 (4, 11)	7 (4, 11)	6 (4, 12)	6 (5, 10)	0.976
Levodopa equivalent dose		740 (450, 1100)	740 (410, 1213)	720 (430, 1075)	800 (480, 1250)	0.724
Dopamine agonist		109 (43.1%)	44 (37.6%)	46 (47.4%)	19 (48.7%)	0.262
Anti-depressant		84 (33.2%)	38 (32.5%)	32 (33.0%)	14 (35.9%)	0.924
UPDRS-I	Total	2 (1, 4)	2 (1, 3)	2 (1, 4)	2 (1, 5)	0.974
	Item 3 ≥ 2	50 (19.8%)	20 (17.1%)	23 (23.7%)	7 (17.9%)	0.458
UPDRS-II	OFF	14 (8, 20)	13 (8, 20)	14 (9, 18)	16 (10, 22)	0.272
	ON	8 (4, 12)	7 (4, 12)	8 (4, 12)	8 (5, 12)	0.603
UPDRS-III	OFF	23 (24, 39)	31 (22, 39)	30 (24, 39)	32 (26, 38)	0.729
	ON	20 (15, 27)	20 (15, 26)	19 (14, 27)	22 (15, 28)	0.826
H&Y OFF	OFF	2.5 (2, 3)	2.5 (2, 3)	2.5 (2, 3)	2.5 (2, 3)	0.763
	ON	2 (2, 2.5)	2 (2, 2.5)	2 (2, 2.5)	2.3 (2, 2.5)	0.537
Non-tremor dominant		189 (76.9%)	87 (74.4%)	72 (74.2%)	30 (76.9%)	0.941
DRS-2	Total	40 (15.8%)	19 (16.2%)	13 (13.4%)	8 (20.5%)	0.581
	Attention	34 (13.4%)	15 (12.8%)	14 (14.4%)	5 (12.8%)	0.935
	Initiation/Perseveration	26 (10.3%)	9 (7.7%)	8 (8.2%)	9 (23.1%)	**0.016**
	Construction	27 (10.7%)	9 (7.7%)	8 (8.2%)	10 (25.6%)	**0.004**
	Conceptualization	18 (7.1%)	10 (8.5%)	3 (3.1%)	5 (12.8%)	0.097
	Memory	31 (12.3%)	15 (12.8%)	9 (9.3%)	7 (17.9%)	0.366
HADS	Anxiety ≥ 11	45 (18.3%)	27 (23.5%	9 (9.8%)	9 (23.1%)	**0.028**
	Depression ≥ 11	51 (20.7%)	21 (18.3%)	18 (19.6%)	12 (30.8%)	0.235

Missing data: history of appendectomy could not be determined in 4 patients; 5 patients were not medicated with anti-parkinsonian medication, therefore neither UPDRS-II, UPDRS-III nor H&Y were applied in ON; and HADS was not applied to 7 patients, due to patients' inability to complete the questionnaire. DRS-2 scores are presented as frequency of impairment (i.e., performance < 1st percentile). Chi-square test and Kruskal-Wallis test was used for group comparisons.

was 36.2%. The genotypes TT or TC were recorded in 84.6% of PD patients and in 86.4% of HC subjects ($p = 0.555$), whereas the genotypes TC or CC were recorded in 53.8% of PD patients and 58.8% of HC subjects ($p = 0.242$). These negative findings regarding rs6280 were confirmed by multiple logistic regression analyses, with sex as covariate.

PD group characteristics

PD group ($n = 253$) was composed by 136 men (54%) and 117 women (46%). The group's median (25th, 75th percentiles) age was 68 years (62, 76) and of education was 4 years (4, 8). A positive history of appendectomy was recorded in 11.2% of PD patients. The median age at disease onset was 60 years (53, 69) and disease duration at the time of the assessment was 6 years (4, 11). The median levodopa equivalent dose was 740 mg (450, 1100) and 43% of PD patients were taking dopamine agonists (94.5% were taking ropinirole and their median dose was 12 mg). Eighty-four patients (33.2%) were taking anti-depressant at the time of the assessment and 50

(19.8%) had a UPDRS-I item 3 score ≥ 2. The median UPDRS-II, UPDRS-III, and H&Y scores OFF medication were respectively 14 (8, 20), 23 (24, 39), and 2.5 (2, 3). The median UPDRS-II, UPDRS-III and H&Y scores ON medication were respectively 8 (4, 12), 20 (15, 27), and 2 (2, 2.5). A non-tremor dominant phenotype was observed in 76.9% of PD patients. The frequencies of impaired performance on DRS-2 and of psychopathology symptoms on HADS are presented in Table 1.

rs6280 and PD group demographic and clinical characteristics

As presented in Table 1 and 2, sex, age, history of appendectomy, age at disease onset, disease duration, levodopa equivalent dose, dopamine agonist, anti-depressant medication, UPDRS-I, UPDRS-I item 3 score ≥ 2, UPDRS-II OFF and ON, UPDRS-III OFF and ON, and H&Y OFF and ON did not vary significantly with rs6280 genotype ($p > 0.05$). The median education for the three genotypes was 4 years.

rs6280 and PD cognitive impairment

As presented in Table 1 and 2, PD patients with rs6280 CC genotype, in comparison to those with TT or TC genotypes, had more impaired cognitive performance (i.e., <1st percentile of demographically adjusted norms) on DRS-2 subscales Initiation/Perseveration (23.1% vs. 7.7% and 8.2% respectively, Chi-square test $p = 0.016$) and Construction (25.6% vs. 7.7% and 8.2% respectively, Chi-square test $p = 0.004$). No other significant association was found between rs6280 genotype and cognitive test performance, namely DRS-2 Total and subscales Attention, Conceptualization, and Memory.

rs6280 CC genotype remained associated with impaired performance on DRS-2 subscales Initiation/Perseveration (adjusted odds = 5.244; 95%CI: 1.400, 19.637; $p = 0.014$) and Construction (adjusted odds = 6.891; 95%CI: 2.087, 22.759; $p = 0.002$) in comparison to TT genotype, when sex, age, education, history of appendectomy, age at disease onset (≥ 55 years), disease duration (≤ 5 years, 6–10 years, and ≥ 11 years), levodopa equivalent dose, dopamine agonist intake, anti-depressant intake, UPDRS-II score OFF medication, UPDRS-III score OFF medication, non-tremor dominant, HADS Anxiety ≥ 11, and HADS Depression > 11 were considered as covariates. The same pattern of results was observed for rs6280 genotype, when UPDRS-III OFF medication was replaced by UPDRS-III ON medication, H&Y OFF or H&Y ON in the regression model.

Other predictors of cognitive impairment in PD

As shown in Table 2, simple logistic regressions revealed that higher scores on UPDRS-I, UDRS-II OFF and ON, UPDRS-III OFF and ON and H&Y OFF and ON were associated with impaired cognitive performance on DRS-2 Total and all subscales. Higher levodopa equivalent dose also increased the odds of impaired performance on DRS-2 Total and subscales Initiation/Perseveration and Memory. Older age, history of appendectomy, older age at disease onset, longer disease duration, UPDRS-I item $3 \geq 2$ and non-tremor dominant phenotype, and more severe symptoms of anxiety and depression were also related to more cognitive impairment on DRS-2 Total and/or subscales. Higher education and dopamine agonists were associated with lower odds of impairment on DRS-2 Total and/or subscales. No significant association was found between cognitive measures and anti-depressant intake.

rs6280 and psychopathology in PD

As shown in Table 1, the frequency of anxiety varied according to rs6280 genotype. A simple logistic regression revealed that patients with rs6280TC genotype had less anxiety (i.e., HADS Anxiety subscale ≥ 11) than those with the rs6280TT (9.8% vs. 23.5%; odds = 0.353, $p = 0.012$). This result remained statistically significant (adjusted odds = 0.242; 95%CI:0.096, 0.613; $p = 0.003$) even when other covariates as sex, age, education, history of appendectomy, age at disease onset (≥ 55 years), disease duration (≤ 5 years, 6–10 years, and ≥ 11 years), levodopa equivalent dose, dopamine agonist intake, anti-depressant, UPDRS-II score OFF medication, UPDRS-III score OFF medication, non-tremor dominant, and DRS-2 Total were taken into consideration. The same pattern of results was observed for rs6280 genotype, when UPDRS-III OFF medication was replaced by UPDRS-III score ON medication, H&Y OFF or H&Y ON in the regression model.

As shown in Tables 1 and 3, the frequency of depression, as measured by HADS Depression subscale ≥ 11, was not statistically different for rs6280 TT (18.3%), TC (19.6%), and CC (30.8%) genotypes ($p = 0.235$). Consistently, the frequency of UPDRS-I item $3 \geq 2$ did not vary significantly between genotypes: rs6280 TT (17.1%), TC (23.7%), and CC (17.9%) ($p = 0.458$).

Other predictors of cognitive impairment and psychopathology in PD

As shown in Table 3, simple logistic regressions revealed that worse non-motor and motor experiences of daily living as measured by UPDRS-I and UPDRS-II OFF and ON medication, more severe symptoms on UPDRS-III OFF and ON medication, more advanced disease stage as measured by H&Y OFF and ON medication, and more cognitive difficulties on the DRS-2 were associated with more symptoms of anxiety and depression (i.e., HADS Anxiety ≥ 11, and HADS Depression ≥ 11). Female sex, younger age at assessment and disease onset, and longer disease duration were associated with more anxiety (i.e., HADS Anxiety ≥ 11). Depression (i.e., HADS Depression ≥ 11 and UPDRS-1 item $3 \geq 2$) was related to fewer years of education and was more common in PD patients with non-tremor dominant motor phenotype. UPDRS-1 item $3 \geq 2$, but not HADS Depression ≥ 11, was associated with female

Table 2
Predictors of cognitive impairment on DRS-2 Total and subscales. Simple logistic regressions analyses

		Total			Attention			Initiation/ Perseveration			Construction			Conceptualization			Memory		
		OR	95%CI	p	OR	95%CI	p	OR	95%CI	p	OR	95%CI	p	OR	95%CI	p	OR	95%CI	p
rs6280	TT																		
	TC	0.80	0.37, 1.12	0.563	1.15	0.52, 2.51	0.524	1.08	0.40, 2.91	0.881	1.08	0.40, 2.91	0.881	0.34	0.09, 1.28	0.111	0.70	0.29, 1.67	0.415
	CC	1.33	0.53, 3.34	0.542	1.00	0.34, 2.96	>0.999	3.60	1.31, 9.87	**0.013**	4.14	1.54, 11.13	**0.005**	1.57	0.50, 4.92	0.436	1.49	0.56, 3.97	0.428
Sex	Male	0.84	0.43, 1.64	0.604	0.73	0.36, 1.51	0.401	1.43	0.62, 3.28	0.403	0.56	0.25, 1.25	0.155	0.40	0.15, 1.11	0.079	0.78	0.37, 1.66	0.523
Age		1.10	1.05, 1.15	**<0.001**	1.02	0.98, 1.06	0.285	1.06	1.01, 1.10	**0.012**	1.08	1.03, 1.13	**0.001**	1.08	1.03, 1.14	**0.004**	1.07	1.02, 1.11	**0.002**
Education		0.86	0.75, 0.98	**0.024**	0.88	0.77, 1.01	0.070	0.91	0.79, 1.05	0.187	0.74	0.57, 0.95	**0.019**	0.89	0.75, 1.07	0.213	0.95	0.84, 1.06	0.335
History of appendectomy		1.55	0.59, 4.12	0.376	1.06	0.34, 3.27	0.918	2.31	0.79, 6.78	0.127	1.03	0.29, 3.69	0.960	3.79	1.23, 11.71	**0.021**	2.87	1.10, 7.48	**0.031**
Age at disease onset ≥ 55 years		2.90	1.16, 7.21	**0.023**	0.79	0.37, 1.69	0.545	1.99	0.72, 5.49	0.183	2.10	0.76, 5.77	0.150	2.34	0.66, 8.34	0.189	0.93	0.41, 2.08	0.854
Disease duration	≤5 years																		
	6–10 years	1.71	0.71, 4.12	0.233	0.91	0.36, 2.28	0.844	1.43	0.53, 3.88	0.484	1.25	0.45, 3.50	0.666	3.52	0.90, 13.72	0.070	2.36	0.76, 7.34	0.138
	≥11 years	3.07	1.31, 7.20	**0.010**	1.77	0.76, 4.16	0.189	1.79	0.66, 4.90	0.256	2.27	0.86, 5.97	0.098	3.79	0.94, 15.20	0.060	6.47	2.26, 18.54	**<0.001**
Levodopa equivalent dose		1.00	1.00, 1.00	**0.043**	1.00	1.00, 1.00	0.130	1.00	1.00, 1.00	**0.020**	1.00	1.00, 1.00	0.270	1.00	1.00, 1.00	0.657	1.00	1.00, 1.00	**0.001**
Dopamine agonist		0.15	0.06, 0.40	**<0.001**	0.91	0.44, 1.90	0.809	0.28	0.10, 0.77	**0.014**	0.20	0.07, 0.60	**0.004**	0.15	0.03, 0.67	**0.013**	0.50	0.22, 1.13	0.096
Anti-depressant		1.82	0.92, 3.62	0.087	1.11	0.52, 2.38	0.781	1.85	0.81, 4.19	0.143	2.03	0.91, 4.54	0.086	1.67	0.64, 4.41	0.298	1.79	0.84, 3.83	0.135
UPDRS-I	Total	1.68	1.44, 1.95	**<0.001**	1.38	1.22, 1.56	**<0.001**	1.91	1.58, 2.31	**<0.001**	1.38	1.21, 1.56	**<0.001**	1.50	1.29, 1.75	**<0.001**	1.53	1.33, 1.76	**<0.001**
	Item 3 ≥ 2	3.05	1.46, 6.37	**0.003**	1.87	0.83, 4.21	0.133	6.19	2.65, 14.47	**<0.001**	2.74	1.17, 6.41	**0.021**	3.68	1.37, 9.87	**0.010**	3.06	1.37, 6.82	**0.006**
UPDRS-II	OFF	1.12	1.08, 1.17	**<0.001**	1.09	1.05, 1.14	**<0.001**	1.16	1.10, 1.22	**<0.001**	1.09	1.05, 1.14	**<0.001**	1.13	1.07, 1.20	**<0.001**	1.14	1.09, 1.20	**<0.001**
	ON	1.20	1.13, 1.27	**<0.001**	1.15	1.09, 1.21	**<0.001**	1.22	1.14, 1.31	**<0.001**	1.14	1.08, 1.20	**<0.001**	1.20	1.12, 1.28	**<0.001**	1.18	1.11, 1.25	**<0.001**
UPDRS-III	OFF	1.08	1.05, 1.12	**<0.001**	1.07	1.04, 1.11	**<0.001**	1.10	1.06, 1.14	**<0.001**	1.07	1.03, 1.10	**<0.001**	1.09	1.05, 1.12	**<0.001**	1.08	1.05, 1.12	**<0.001**
	ON	1.13	1.08, 1.18	**<0.001**	1.10	1.05, 1.14	**<0.001**	1.14	1.08, 1.20	**<0.001**	1.07	1.03, 1.12	**0.001**	1.11	1.06, 1.17	**<0.001**	1.09	1.05, 1.14	**<0.001**
H&Y	OFF	3.12	1.99, 4.90	**<0.001**	2.31	1.47, 3.61	**<0.001**	2.90	1.76, 4.76	**<0.001**	3.28	1.99, 5.42	**<0.001**	3.12	1.78, 5.48	**<0.001**	3.89	2.35, 6.44	**<0.001**
	ON	8.86	3.82, 20.54	**<0.001**	3.40	1.79, 6.46	**<0.001**	4.56	2.21, 9.44	**<0.001**	5.20	2.44, 11.11	**<0.001**	4.57	2.16, 9.71	**<0.001**	5.78	2.66, 12.54	**<0.001**
Non-tremor dominant		3.53	1.20, 10.34	**0.022**	1.68	0.66, 4.27	0.274	9.60	1.27, 72.38	**0.028**	1.55	0.56, 4.29	0.394	–	–	–	11.89	1.59, 89.04	**0.016**
HADS	Anxiety ≥ 11	2.56	1.17, 5.60	**0.019**	3.48	1.55, 7.85	**0.003**	2.13	0.82, 5.53	0.120	2.20	0.89, 5.43	0.088	9.29	3.17, 27.19	**<0.001**	2.59	1.12, 5.60	**0.027**
	Depression ≥ 11	2.83	1.33, 6.01	**0.007**	2.02	0.88, 4.62	0.095	3.42	1.40, 8.33	**0.007**	2.73	1.16, 6.45	**0.022**	4.35	1.55, 12.24	**0.005**	3.03	1.35, 6.79	**0.008**

DRS-2 subscales were recoded: >1st percentile – 0 vs. ≤1st percentile –1. Appendectomy was coded: without past history–0 and with past history–1. Age at disease onset was recoded into: <55 years–0, ≥55 years–1. Disease duration was recoded into ≤5 years–0, 6–10 years–1, and ≥11 years–2. Non-tremor dominant was coded: no–0 and yes–1. HADS Anxiety and Depression were recoded into <11 – 0 and ≥11 – 1. Missing data: history of appendectomy could not be determined in 4 patients; 5 patients were not medicated with anti-parkinsonian medication, therefore neither UPDRS-II, UPDRS-III nor H&Y were applied in ON; and HADS was not applied to 7 patients, due to patients' inability to complete the questionnaire. All patients with impaired DRS-2 Conceptualization subscale had non-tremor dominant phenotype.

Table 3
Predictors of anxiety and depression. Simple logistic regressions analyses

		Anxiety			Depression			UPDRS-1 item 3 ≥ 2		
		OR	95%CI	p	OR	95%CI	p	OR	95%CI	p
rs6280	TT									
	TC	0.35	0.16, 0.80	**0.012**	1.09	0.54, 2.19	0.812	1.41	0.75, 2.64	0.281
	CC	0.96	0.41, 2.31	0.978	1.99	0.87, 4.56	0.104	1.33	0.57, 3.08	0.505
Sex	Male	0.49	0.25, 0.94	**0.033**	0.62	0.33, 1.16	0.133	0.28	0.15, 0.53	**<0.001**
Age		0.97	0.94, 1.00	**0.038**	1.03	1.00, 1.06	0.102	1.02	0.99, 1.05	0.137
Education		0.90	0.81, 1.00	0.059	0.88	0.79, 0.98	**0.024**	0.86	0.77, **0.95**	**0.003**
History of appendectomy		0.79	0.26, 2.40	0.671	1.73	0.71, 4.23	0.226	2.29	1.03, 5.11	**0.042**
Age at disease onset ≥ 55 years		0.38	0.20, 0.75	**0.005**	1.23	0.62, 2.44	0.550	0.57	0.31, 1.04	0.067
Disease duration	≤5 years									
	6–10 years	2.09	0.92, 4.77	0.079	1.81	0.86, 3.81	0.120	2.20	1.05, 4.60	**0.037**
	≥11 years	2.83	1.23, 6.54	**0.015**	1.83	0.83, 4.02	0.133	2.96	1.42, 6.19	**0.004**
Levodopa equivalent dose		1.00	1.00, 1.00	0.142	1.00	1.00, 1.00	0.169	1.00	1.00, 1.00	**0.024**
Dopamine agonist		1.31	0.68, 2.50	0.420	0.58	0.31, 1.11	0.102	0.60	0.33, 1.10	0.099
Anti-depressant		1.64	0.84, 3.18	0.144	1.75	0.93, 3.29	0.083	3.72	1.96, 7.06	**<0.001**
UPDRS-I	Total	1.33	1.17, 1.53	**<0.001**	1.51	1.30, 1.74	**<0.001**	2.01	1.67, 2.42	**<0.001**
	Item 3 ≥ 2	2.39	1.14, 4.78	**0.021**	6.14	3.04, 12.40	**<0.001**	–		
UPDRS-II	OFF	1.10	1.06, 1.15	**<0.001**	1.08	1.04, 1.13	**<0.001**	1.11	1.06, 1.15	**<0.001**
	ON	1.15	1.09, 1.22	**<0.001**	1.13	1.07, 1.20	**<0.001**	1.13	1.08, 1.19	**<0.001**
UPDRS-III	OFF	1.04	1.01, 1.08	**0.007**	1.06	1.03, 1.09	**<0.001**	1.05	1.03, 1.08	**<0.001**
	ON	1.06	1.02, 1.10	**0.005**	1.09	1.04, 1.13	**<0.001**	1.07	1.038, 1.11	**<0.001**
H&Y	OFF	1.73	1.12, 2.65	**0.008**	2.13	1.45, 3.39	**<0.001**	2.04	1.42, 2.93	**<0.001**
	ON	2.66	1.33, 4.92	**0.001**	3.18	1.82, 7.08	**<0.001**	2.42	1.46, 4.01	**<0.001**
Non-tremor dominant		1.43	0.65, 3.17	0.376	3.02	1.22, 7.48	**0.017**	5.82	2.03, 16.72	**0.001**
DRS-2	Total	2.48	1.17, 5.60	**0.018**	2.80	1.33, 6.01	**0.006**	3.26	1.74, 6.10	**<0.001**

HADS Anxiety and Depression were recoded into <11 – 0 and ≥11 – 1. Appendectomy was coded: without past history–0 and with past history–1. Age at disease onset was recoded into: <55 years–0, ≥55 years–1. Disease duration was recoded into ≤5 years–0, 6–10 years–1, and ≥11 years–2. Non-tremor dominant was coded: no–0 and yes–1. DRS-2 Total was recoded: >1st percentile – 0 vs. ≤1st percentile –1. Missing data: HADS was not applied to 7 patients, due to patients' inability to complete the questionnaire; history of appendectomy could not be determined in 4 patients; and 5 patients were not medicated with anti-parkinsonian medication, therefore neither UPDRS-II, UPDRS-III nor H&Y were applied in ON state.

sex, longer disease duration, and higher levodopa equivalent dose. No significant association was found with dopamine agonist.

DISCUSSION

Study results revealed that PD patients with rs6280 CC genotype are more likely to have significant deficits on DRS-2 Initiation/Perseveration and Construction subscales [22]. The association between cognitive deficits and CC genotype remained statistically significant when demographic features, appendectomy history, disease duration, severity of non-motor and motor symptoms and disease stage (OFF and ON states), anti-parkinsonian medication, psychoactive medication, motor phenotype, and psychopathology symptoms were taken into consideration. These findings are consistent with the literature results from non-Parkinsonian psychotic patients [10, 11] and substance abusers [12]. Noteworthy, no association was found between rs6280 genotype and DRS-2 Total score in our cohort, suggesting that DRD3 polymorphism does not have a global impact on cognition in PD.

DRS-2 Initiation/Perseveration subscale explores the ability to initiate, maintain and terminate goal-directed behaviors through verbal (e.g., semantic fluency) and motor (e.g., copying alternating manual movements and graphomotor sequences) tasks, whereas the Construction subscale consists of copying simple designs. Notably, deficits in semantic fluency and copy of designs are known predictors of dementia in PD [23].

There are reports that global cognitive decline in PD, as measured by DRS-2 Total score, is accompanied by an Alzheimer's disease pattern of brain atrophy (i.e., hippocampus and parietal–temporal cortex) [24]. There is some evidence that frontal lobe regions and the nucleus accumbens may be particularly involved in the performance of Initiation/Perseveration and Construction subscales in PD [25]. Dopamine D3 receptors are predominantly expressed in the nucleus accumbens [26], which is believed to regulate cognitive and socio-emotional processes by integrating information from limbic structures and the prefrontal cortex [27].

The increased impairment on certain cognitive tests by PD individuals with rs6280 CC genotype may reflect the higher affinity for dopamine, the increased dopamine-mediated cyclic adenosine monophosphate (cAMP) response, and the prolonged

mitogen-associated protein kinase (MAPK) signal characteristic of the Gly variant [28]. The presence of the Gly aminoacid attenuates the function of the D3 receptor [29]. An inverted-U curve between dopaminergic signaling and cognition has been hypothesized [30, 31], where too little or too much dopaminergic signaling impairs cognitive performance. In the present study, almost all patients were ON medication at cognitive assessment. Dopaminergic drugs may produce variable effects on cognitive performance, depending on the baseline levels of dopamine in certain brain regions, which may partly reflect genetic predisposition, and on the specific cognitive processes involved [30, 31]. Increasing levels of dopamine may benefit performance on some cognitive tasks and have a detrimental effect on others [30].

In the present study, PD patients taking dopamine agonist had better cognitive performance than those without dopamine agonist. An important confounder is that, in clinical practice, dopamine agonists are usually administered in monotherapy or adjunct therapy to younger patients in the early or stable PD [32], which is consistently related to more intact cognition in PD. Nonetheless, the significant association between dopamine agonist and cognition remained statistically significant, even when other covariates were taken into account. The preferred dopamine agonist in our cohort was ropinirole, a non-ergoline dopamine agonist with high D2/D3 affinity. The limited evidence available suggests that ropinirole may have relatively small adverse effects on cognition [33–35]. Ropinirole has been shown to selectively reduce proactive inhibition (i.e., slowing of motor activity in anticipation of stopping), but not reactive inhibition (i.e., response to sudden sensory cues and serves to abruptly stop motor activity) in healthy adults [36]. A reduction in proactive inhibition could potentially favor semantic fluency in PD patients [37]. There is evidence that pramipexole, another agonist with D3 affinity used in our cohort, may have negative effects on multiple cognitive functions, including semantic fluency [33, 38].

In our cohort, a screening measure detected anxiety in 18% and depression in 21% of PD patients. These frequencies are roughly similar to those reported by a previous study that also used screening tools and applied conservative cut-offs [39]. Though, these figures are lower than the estimated prevalence of clinically significant anxiety and depression symptoms reported by meta-analyses [40, 41]. Consistent with the literature, anxiety in PD patients was associ-

ated with female sex, younger age at disease onset and at assessment, and more severe motor symptoms [39, 42]. Depression was also associated with higher UPDRS-II, UPDRS-III, and H&Y scores [43]. Study findings provided further support to the notion that lower education and poorer cognitive performance are associated with anxiety and depression in PD patients [43]. No significant effect of levodopa equivalent dose or dopamine agonist was observed on psychopathology symptoms. Though, PD patients taking dopamine agonists tended to have lower odds of depression (i.e., HADS Depression ≥ 11 and UPDRS-1 item 3 ≥ 2). Dopamine agonists are currently recommended to treat depression in PD [44].

PD patients with the rs6280 TC genotype had lower risk of anxiety than patients with the rs6280TT genotype. Another study explored the relationship between rs6280 and anxiety symptoms in PD and reported no significant association between the C allele and symptoms of anxiety [45]. Anxiety and depression are frequently comorbid in PD [46]. Theoretically, anxiety is associated with a state of physiological hyperarousal, whereas symptoms of anhedonia and the absence of positive affect are specific to depression [47, 48]. The pathophysiology of PD-related anxiety is complex and remains to be elucidated [46, 49]. Though, it is reasonable to speculate that rs6280 TC genotype may directly or indirectly protect from anxiety in PD patients treated with anti-parkinsonian medication.

The rs6280 was not statistically related to depression symptoms (i.e., HADS Depression ≥ 11 and UPDRS-1 item 3 ≥ 2) nor with anti-depressant medication. Though, there is evidence in the literature that rs6280 polymorphism may be implicated in the pathogenesis of depression. A study found that PD patients with the rs6280C allele had more severe depression symptoms (i.e., with predominant anhedonia) than those without the rs6280C allele. Moreover, these depression symptoms were positively correlated with activation of the right medial frontal gyrus [4].

DRD3 genotypic composition was not statistically different in our cohort of PD patients and a population-based HC group, supporting the null hypothesis regarding the rs6280 polymorphism and the risk of PD [50, 51]. Though, there are reports in the literature that rs6280 CC genotype maybe more frequent in PD patients [8].

The consecutive recruitment of non-surgically treated PD patients, the neurological evaluation in both OFF and ON states by movement disorders specialists, and the cognitive assessment in ON state conducted by a neuropsychologist blinded to the neurological and the genetic results are strengths of the study. DRS-2 was completed by most patients of the cohort (including bedridden and/or with very low cognitive functioning), which reduces the risk of sample bias. As a single-center study, the generalizability of the research findings is limited. The replication of the study in other populations is necessary. Nevertheless, the rs6280 allelic frequency found in our population (allele C = 34.6%) is aligned with those reported for the European population (https://www.ncbi.nlm.nih.gov/snp/rs6280#frequency_tab) [52] The present research focused on the effects of the rs6280 genotype on PD patients' cognitive test performance, while taking into consideration other known predictors (e.g., demographic characteristics, appendectomy history, motor symptoms, dopaminergic treatment, psychoactive medication, and psychopathology symptoms). However, the list of potential covariates was not exhaustive. For instance, other polymorphisms (e.g., COMT158, APOE, E326K) that are known to affect the cognitive trajectory of patients with idiopathic PD [53, 54] were not explored.

The cognitive evaluation was limited to the DRS-2, a global scale commonly used both in clinical practice and research. DRS-2 scores (both Total and subscales) were adjusted to the demographic characteristics of the individuals based on norms and a conservative cut-off was applied. This approach limits the risk of overestimating cognitive impairment, but also increases the insensitivity to milder cognitive deficits. DRS-2 is currently recommended by Movement Disorders Society to evaluate global cognitive performance in PD patients, because it is considered reliable, valid, and sensitive to change in PD and evaluates cognitive domains commonly affected in PD, such as executive abilities, attention, visuospatial skills, and memory [55]. As a global assessment scale, ceiling effects may occur. However, it is considered a clinically valid measure to differentiate levels of cognitive impairment in demented patients [56]. DRS-2 subscales are generally considered valid measures of the respective constructs [22, 57–61]. Though, the reported indicators of construct validity are relatively modest, thus probably reflecting the non-specificity to a single cognitive domain for each subscale and the screening nature of the tests. The interpretation of the study findings is also limited by the cross-sectional nature of the study. A longitudinal design may clarify the cognitive trajectories of

PD patients according to their rs6280 genotype.

In conclusion, PD patients with rs6280 CC genotype are more likely to have deficits on measures of executive and visuoconstructional functions than those with TT genotype, suggesting a genetic predisposition to the development of impairment in cognitive domains related to the frontal lobes. The current study also revealed that rs6280 TC genotype may play a protective role on anxiety, but not on depression. These novel findings ought to be confirmed in other PD cohorts.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

DATA AVAILABILITY

The data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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