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Article

Phenotypic Differences among Recently Diagnosed Drug-Naïve Patients with Parkinson's Disease with or without *SNCA* Polymorphisms

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Abstract: Some studies show that patients with mutations in the SNCA gene, which codifies for the alpha-synuclein protein, show a particular phenotype. The effects of SNCA Single Nucleotide Polymorphism (SNPs) in recently diagnosed, drug-naïve patients with PD have been less explored. Therefore, we set out to explore the differences in the clinical characteristics of recently diagnosed drug-naïve sporadic PD patients with or without SNCA rs3910105 or rs356181 SNPs. Patients with a clinical diagnosis of PD in the Parkinson's Progression Markers Initiative (PPMI) database entered the study. We excluded those with missing data, dementia, psychiatric conditions, a diagnosis change over the first five years from the initial PD diagnosis, or with a familial history of PD. Subjects were evaluated with the MDS-Unified PD Rating Scale (MDS-UPDRS), DAT imaging, the Geriatric Depression Scale (GDS), the State-Trait Anxiety Inventory (STAI), the Montreal Cognitive Assessment (MoCA), the SCOPA-AUT for autonomic function, the Epworth Sleepiness Scale (ESS), the RBD Questionnaire, and the University of Pennsylvania Smell Identification Test (UPSIT). We included 308 PD patients fulfilling all inclusion and exclusion criteria. A logistic regression analysis and Machine-Learning models did not disclose any difference between patients either with or without the SNCA rs3910105 SNP or with or without the SNCA rs3910105 polymorphism. Our results suggest that the SNCA polymorphisms rs3910105 and rs356181 have no impact on the phenotype of idiopathic, sporadic, recently diagnosed, drug naïve PD patients.

Keywords: single nucleotide polymorphism; Parkinson's disease; alpha-synuclein; phenotype; pathophysiology; PD-related variants

1. Introduction

Parkinson's disease (PD) is the second most frequent neurodegenerative disorder worldwide [1]. Patients are affected by motor and non-motor symptoms, the latter being the most disturbing [2]. The major motor symptoms of the disease are bradykinesia, resting tremor, rigidity, and postural abnormalities [3]. Non-motor symptoms include mood disorders, troubled sleep, dysautonomia,

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cognitive disfunction and pain, among others [3]. Pathogenically, PD likely encompasses many genetic–molecular entities, resulting in lesions in different structures within the central or peripheral nervous system. The deposition of alpha-synuclein in the cellular soma, leading to the formation of Lewy bodies, appears to be one of the main events leading to neurodegeneration and, eventually, dementia [2]. Other factors contributing to the neurodegenerative process include mitochondrial dysfunction, synaptic alterations, the disruption of calcium homeostasis, and neuroinflammation [2].

PD can be sporadic or, when autosomal mutations are present, familial [4, 5]. However, recent evidence indicates that genetic mutations also contribute to sporadic PD in non-negligible ways [6]. A recent meta-analysis of genome-wide association studies (GWAS) included the analysis of 7.8M single nucleotide polymorphisms (SNPs) in 37.7K cases, 18.6K UK Biobank proxy-cases (having a first-degree relative with PD), and 1.4M controls [6]. The authors could identify 90 variants that explained 16–36% of the heritable risk of PD depending on prevalence.

Interestingly, patients with mutations in the *SNCA* gene, which codifies for the alpha-synuclein protein, show a particular phenotype. For example, in a study involving 230 PD patients, the *SNCA* (Synuclein alpha gene) rs356182 GG genotype was associated with a more tremor-predominant phenotype and predicted a slower rate of motor progression [7]. The *SNCA* p.Ala53Thr variant among monogenic forms of PD manifests with a rapidly evolving rate of PD progression and early emergence of levodopa complications, while the p.His50Gln variant often manifests as a tremor-dominant subtype with cognitive impairment [8]. Patients with this variant are also frequently affected by a high rate of psychotic symptoms and depression, early onset of cognitive decline, and autonomic dysfunction [8]. REM (rapid eye movement) sleep behavior disorder can also be found more frequently among patients with the *SNCA*-A53T_rs104893877 variant [9]. The effects of *SNCA* SNPs in recently diagnosed, drug-naïve patients with PD have been less explored. Therefore, we set out to explore the differences in the clinical characteristics of recently diagnosed drug-naïve sporadic PD patients with or without *SNCA* rs3910105 or rs356181 SNPs.

2. Materials and Methods

2.1. Participants

The Parkinson's Progression Markers Initiative (PPMI) is an ongoing multicenter observational study focused on identifying disease biomarkers in PD patients attending clinical centers all over the world [10]. Identifying markers of disease progression through this initiative serves to accelerate therapeutic trials aimed at reducing PD disabilities. The review board of each clinical center participating in the initiative approves the study protocol and all participating patients are required to sign a written informed consent form. Information is de-identified and shared with all investigators. We extracted information only from each participant's baseline visit.

For our study, we selected patients with a clinical diagnosis of idiopathic PD based on the UKPDBBS or the MDS criteria within two years before the date of inclusion. Patients with missing data, demented patients, those being treated with any antiparkinsonian drug, those whose PD diagnosis was changed during the first five years after the initial diagnosis, those with psychiatric conditions, or those with a familial history of PD were excluded.

2.2. Patient evaluation

Subjects were evaluated with the MDS-Unified PD Rating Scale (MDS-UPDRS) and additional clinical tests for cognition, depression, anxiety, autonomic function, sleep, and olfaction [10]. All patients underwent DAT imaging. Depression was assessed using the Geriatric Depression Scale (GDS), and anxiety with the State-Trait Anxiety Inventory (STAI). Cognitive testing included the Montreal Cognitive Assessment (MoCA). Autonomic function was examined using the SCOPA-AUT. Diurnal somnolence and REM sleep behavior disorder (RDB) were assessed using the Epworth Sleepiness Scale (ESS) and RBD Questionnaire, respectively. Finally, hyposmia/anosmia was evaluated using the University of Pennsylvania Smell Identification Test (UPSIT).

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2.3. Genomic data processing

As part of the screening/baseline visit, whole-genome sequencing was performed on whole blood-extracted DNA samples using a Macrogen Inc. sequencer [10]. One microgram of each DNA sample was fragmented using the Covaris System and prepared following the Illumina TruSeq DNA Sample preparation guide to obtain a final library of 300-400 bp average insert size. Libraries were multiplexed and sequenced on the Illumina HiSeq X platform. Paired-end read sequences were aligned to the GRCh37-hs37d5 genome using the Burrows-Wheeler aligner-maximal exact matches algorithm (BWA-MEM v0.7.13). The Bamsormadup2 tool (v2.0.87) was used to filter duplicates and sort aligned bam files. After filtering duplicated read sequences, the reads were realigned and recalibrated using the GATK pipeline (v3.5). A haplotype caller in the GATK pipeline was used to call variants, including single nucleotide variants (SNVs) and small indels, and to generate genome VCFs. Using the hg38 aligned cohort VCF files from the whole-genome sequencing data, genotype information was extracted with BCF tools and PLINK. We focused on the *SNCA* rs3910105 and rs356181 SNPs.

2.4. Statistical analysis

Numerical variables were expressed as means ± standard deviation and the categorical ones in percentages. Differences between PD patients with and without *SNCA* polymorphisms were analyzed with Analysis of Variance (ANOVA) or the Chi-square test. P-values were corrected for multiple comparisons using the Benjamini-Hochberg method. All analyses were performed using R Statistical Software (v4.1.2; R Core Team 2021).

2.5. Machine-Learning models

We designed machine learning models to predict SNP variants and the number of alleles based on the variables evaluated in the dataset. The data was divided into training and test sets using an 80-20 split ratio. Decision trees, boosted decision trees, random forest, and Support-Vector Machine (SVM) models were trained on the training set, and a grid search was conducted to optimize the hyperparameters of each model. Each model performance was evaluated on the test set, using accuracy (the proportion of cases classified correctly), recall (the true positive rate), and the F1-Score (the harmonic mean of accuracy and recall). Analyses were carried out in Python, using the scikit-learn library and its corresponding methods.

3. Results

3.1. Characteristics of patients with or without SNCA SNPs

Three hundred and eight patients met all inclusion and exclusion criteria. Of them, 146 (47.4%) and 51 (16.5%) had 1 or 2 GG minor alleles in the rs3910105 SNP, respectively. In terms of the rs356181 SNP, 135 (43.8%) and 106 (34.4%) had one or two AG minor alleles, respectively. Three hundred and two patients (98.0%) had one or two minor alleles in one or both SNPs. One hundred and thirty-six patients (44.1%) had one or two minor alleles in both SNPs.

There were no differences between patients either with or without the *SNCA* rs3910105 SNP (Table 1) or with or without *SNCA* rs3910105 polymorphism (Table 2).

Table 1. Characteristics of PD patients with the *SNCA* rs3910105 SNP.

Genotype	AA (N=111)	GA (N=146)	GG (N=51)	P-value
Sex				
Female	32 (28.8%)	55 (37.7%)	23 (45.1%)	0.213
Male	79 (71.2%)	91 (62.3%)	28 (54.9%)	
Years of education	15.7 (2.98)	15.5 (3.03)	15.2 (2.98)	0.784

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Age	62.3 (9.48)	61.4 (9.53)	63.1 (9.27)	0.719
Age at PD onset	61.8 (9.40)	60.9 (9.48)	62.7 (9.13)	0.664
Results of the DAT scan				
Caudate contralateral	1.84 (0.552)	1.78 (0.606)	1.86 (0.503)	0.821
Caudate ipsilateral	2.16 (0.533)	2.09 (0.659)	2.17 (0.566)	0.772
Putamen contralateral	0.691 (0.275)	0.661 (0.224)	0.715 (0.356)	0.637
Putamen ipsilateral	0.967 (0.380)	0.922 (0.399)	0.949 (0.370)	0.837
Elixhauser comorbidity score	0.901 (2.48)	1.40 (3.08)	0.902 (2.39)	0.471
Non-motor symptoms				
ESS score	5.40 (3.14)	5.68 (3.32)	6.80 (3.81)	0.104
Diurnal somnolence	12 (10.8%)	21 (14.4%)	13 (25.5%)	
GDS score	2.03 (2.32)	2.36 (2.53)	3.00 (2.73)	0.151
Depression	14 (12.6%)	19 (13.0%)	13 (25.5%)	
RBD Questionnaire score	3.98 (2.48)	4.15 (2.70)	4.62 (3.11)	0.584
Probable RBD	39 (35.1%)	55 (37.7%)	21 (41.2%)	
SCOPA gastrointestinal score	2.17 (2.10)	2.06 (1.97)	2.25 (1.96)	0.941
SCOPA urinary score	4.50 (3.24)	4.06 (2.85)	3.88 (2.45)	0.544
SCOPA cardiovascular score	0.432 (0.921)	0.418 (0.651)	0.608 (0.827)	0.505
SCOPA thermoregulation score	1.13 (1.32)	1.14 (1.30)	1.41 (1.83)	0.641
SCOPA visual score	0.378 (0.557)	0.418 (0.722)	0.392 (0.532)	0.969
SCOPA sexual dysfunction score	1.30 (1.63)	1.05 (1.39)	1.33 (1.85)	0.563
STAIT anxiety score	22.0 (8.82)	23.3 (9.54)	24.9 (9.95)	0.301
Anxiety	4 (3.6%)	16 (9.6%)	3. (5.9%)	0.303
UPSIT	23.2 (7.78)	22.6 (8.78)	21.8 (7.64)	0.788
MoCA score	27.2 (2.47)	27.3 (2.31)	27.3 (2.20)	0.995
Cognitive deterioration	32 (28.8%)	50 (34.2%)	16 (31.4%)	0.790
MDS-UPDRS I	1.21 (1.85)	1.09 (1.36)	1.55 (1.46)	0.358
Motor assessment				
Hoehn & Yahr	1.55 (0.500)	1.58 (0.496)	1.67 (0.476)	0.572
MDS-UPDRS II+III	26.1 (10.8)	26.8 (10.6)	30.2 (13.2)	0.190
Oro-buccal score	2.65 (2.44)	2.56 (2.44)	3.10 (2.57)	0.608
Eating score	1.45 (0.842)	1.29 (0.807)	1.61 (1.06)	0.139
Mobility score	1.74 (1.76)	1.88 (1.94)	2.69 (2.44)	0.521
Axial score	4.43 (2.43)	4.47 (2.23)	5.25 (2.68)	0.186
Resting tremor	2.76 (2.43)	2.68 (2.36)	2.78 (2.32)	0.991
Postural and kinetic t	1.44 (1.55)	1.49 (1.47)	1.69 (1.62)	0.812
Rigidity	3.81 (2.61)	3.77 (2.60)	3.86 (2.85)	0.998
Bradykinesia right	2.85 (2.13)	2.81 (2.49)	2.94 (2.24)	0.989
Bradykinesia left	2.44 (2.44)	2.92 (2.48)	2.88 (2.64)	0.469
Bradykinesia infra	2.55 (2.01)	2.94 (2.15)	3.35 (2.56)	0.177
PD subtype				
Tremor dominant	98 (88.3%)	116 (79.5%)	42 (82.4%)	0.431
PIGD	6 (5.4%)	17 (11.6%)	3 (5.9%)	
Indeterminate	6 (5.4%)	13 (8.9%)	6 (11.8%)	

ESS: Epworth Sleepiness Scale; GDS= Geriatric Depression Scale; REM= Rapid-Eye movement; STAI= State-Trait Anxiety Inventory; UPSIT= University of Pennsylvania Smell Identification Test; MoCA= Montreal Cognitive Assessment; UPDRS= Unified PD Rating Scale; PD= Parkinson's Disease; PIGD= Postural-Instability Gait Disorder.

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Table 2. Characteristics of PD patients with the *SNCA rs356181* SNP.

Genotype	GG (N=67)	AG (N=135)	AA (N=106)	P-value
Sex				
Female	28 (41.8%)	47 (34.8%)	35 (33.0%)	0.691
Male	39 (58.2%)	88 (65.2%)	71 (67.0%)	
Years of education	15.3 (3.12)	15.6 (3.15)	15.6 (2.74)	0.936
Age	63.3 (8.14)	62.0 (9.52)	61.4 (10.1)	0.646
Age at PD onset	62.8 (8.02)	61.4 (9.47)	60.8 (10.1)	0.610
Results of the DAT scan	6.48 (6.30)	6.59 (6.51)	6.25 (5.74)	0.981
Caudate contralateral	1.87 (0.532)	1.77 (0.593)	1.84 (0.563)	0.645
Caudate ipsilateral	2.23 (0.499)	2.06 (0.663)	2.15 (0.566)	0.281
Putamen contralateral	0.723 (0.316)	0.650 (0.232)	0.693 (0.275)	0.316
Putamen ipsilateral	0.962 (0.365)	0.936 (0.399)	0.940 (0.386)	0.975
Elixhauser comorbidity score	0.910 (2.74)	1.56 (3.27)	0.755 (1.94)	0.136
Non-motor symptoms				
ESS score	6.35 (3.48)	5.41 (3.32)	5.84 (3.33)	0.321
Diurnal somnolence	12 (17.9%)	20 (14.8%)	14 (13.2%)	0.851
GDS score	2.72 (2.75)	2.16 (2.36)	2.36 (2.52)	0.523
Depression	11 (16.4%)	21 (15.6%)	14 (13.2%)	0.939
RBD Questionnaire score	4.54 (2.95)	3.95 (2.59)	4.21 (2.66)	0.542
Probable RBD	28 (41.8%)	50 (37.0%)	37 (34.9%)	0.778
SCOPA gastrointestinal score	2.18 (1.84)	2.17 (2.06)	2.07 (2.06)	0.980
SCOPA urinary score	3.99 (2.19)	4.03 (3.06)	4.52 (3.17)	0.561
SCOPA cardiovascular score	0.493 (0.726)	0.474 (0.818)	0.406 (0.790)	0.886
SCOPA thermoregulation score	1.25 (1.47)	1.23 (1.49)	1.07 (1.25)	0.790
SCOPA visual score	0.388 (0.650)	0.452 (0.688)	0.340 (0.550)	0.598
SCOPA sexual dysfunction score	1.33 (1.97)	1.15 (1.48)	1.15 (1.36)	0.876
STAIT anxiety score	24.4 (9.34)	23.1 (9.27)	22.2 (9.55)	0.524
Anxiety	4 (6.0%)	10 (1.4%)	7 (6.6%)	0.984
UPSIT	22.0 (7.40)	22.4 (8.62)	23.5 (8.26)	0.669
MoCA score	27.4 (2.23)	27.4 (2.24)	27.1 (2.56)	0.769
Cognitive deterioration	22 (32.8%)	43 (31.9%)	33 (31.1%)	0.997
MDS-UPDRS I	1.15 (1.34)	1.22 (1.49)	1.23 (1.81)	0.990
Motor assessment				
Hoehn & Yahr	1.64 (0.483)	1.56 (0.498)	1.57 (0.498)	0.732
MDS-UPDRS II+III	29.6 (13.0)	26.4 (10.6)	27.1 (11.2)	0.250
Oro-buccal score	2.76 (2.43)	2.60 (2.57)	2.73 (2.34)	0.967
Eating score	1.49 (0.975)	1.33 (0.741)	1.45 (0.951)	0.564
Mobility score	2.60 (2.41)	1.75 (1.81)	1.83 (1.86)	0.862
Axial score	5.10 (2.70)	4.39 (2.33)	4.51 (2.23)	0.241
Resting tremor	2.87 (2.36)	2.59 (2.24)	2.81 (2.55)	0.852
Postural and kinetic tremor	1.69 (1.57)	1.44 (1.38)	1.47 (1.66)	0.745
Rigidity	3.79 (2.57)	3.90 (2.82)	3.69 (2.44)	0.945
Bradykinesia right	3.13 (2.50)	2.75 (2.33)	2.78 (2.19)	0.716
Bradykinesia left	3.00 (2.62)	2.77 (2.44)	2.53 (2.50)	0.691
Bradykinesia infra	3.13 (2.40)	2.93 (2.17)	2.62 (2.06)	0.479

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PD subtype				
Tremor dominant	54 (80.6%)	112 (83.0%)	90 (84.9%)	0.962
PIGD	6 (9.0%)	11 (8.1%)	9 (8.5%)	
Indeterminate	7 (10.4%)	12 (8.9%)	6 (5.7%)	

ESS: Epworth Sleepiness Scale; GDS= Geriatric Depression Scale; REM= Rapid-Eye movement; STAI= State-Trait Anxiety Inventory; UPSIT= University of Pennsylvania Smell Identification Test; MoCA= Montreal Cognitive Assessment; UPDRS= Unified PD Rating Scale; PD= Parkinson's Disease; PIGD= Postural-Instability Gait Disorder.

3.2. Machine-Learning models

All machine learning failed to converge in a model with sufficient accuracy, recall, and F1-sscore (Table 3).

Table 3. Performance of machine-learning models in predicting possible RBD in the "validation" subsample.

Machine Learning model	Metric	*SNCA*_rs356181	*SNCA*_rs3910105
decision tree	Accuracy	0.34	0.34
	Recall	0.34	0.34
	F1-score	0.33	0.20
Boosted decision tree	Accuracy	0.35	0.35
	Recall	0.35	0.35
	F1-score	0.31	0.24
Random forest	Accuracy	0.36	0.34
	Recall	0.36	0.34
	F1-score	0.24	0.18
SVM	Accuracy	0.38	0.33
	Recall	0.38	0.33
	F1-score	0.22	0.16

4. Discussion

PD patients with autosomal mutations in the *SNCA* and those with sporadic PD bearing *SNCA* polymorphisms differ phenotypically from non-carrier PD patients. Indeed, in a study involving 230 PD patients, the *SNCA* rs356182 GG genotype was associated with a more tremor-predominant phenotype and predicted a slower rate of motor progression [7]. Regarding monogenic forms of PD, the *SNCA* p.Ala53Thr variant manifests with a rapidly evolving rate of PD and early emergence of levodopa complications, while the p.His50Gln variant often manifests as a tremor-dominant subtype, with cognitive impairment [8]. These latter patients are also frequently affected by a high rate of psychotic symptoms and depression, early onset of cognitive decline, and autonomic dysfunction [8]. REM sleep behavior disorder is also found more frequently among patients with the *SNCA*-A53T_rs104893877 variant [9]. However, a recent meta-analysis showed considerable variability of design among studies aimed at establishing the phenotypic consequences of *SNCA* mutations, and a general absence of any significant association [11]. Similarly, no major differences were found between idiopathic, sporadic, recently diagnosed, drug naïve PD patients with or without *SNCA* rs3910105 or rs356181 SNPs.

Our results are in agreement with those of Szwedo and colleagues, who analyzed the impact of SNCA polymorphisms on the clinical presentation of 433 newly diagnosed PD patients [9]. The effects of five SNCA polymorphisms (rs2870004, rs356182, rs5019538, rs356219, and rs763443) were analyzed by these authors. While the rs356219 was associated with faster cognitive decline, no significant associations were found between each of the five SNCA SNPs and the development of motor or

functional impairment. Considered together, these results suggest that common SNCA SNPs do not contribute to motor impairment.

Based on our findings, two hypotheses can be entertained. Either SNCA polymor-phisms do not significantly affect a patient's phenotype — supported by the results of the meta-analysis showing no overall associations between the polymorphisms and pheno-types[11] — or the effects of SNCA polymorphism are possibly stronger during later stages of the disease. The first scenario would imply that the association between *SNCA* polymorphisms and phenotypes is the result of biased studies. Alpha-synuclein deposition may result from previous pathological events like altered calcium homeostasis, synaptic changes, mitochondrial dysfunction, neuroinflammation, apoptosis, and failure in protein degradation [2]. Then, alterations in alpha-synuclein structure may gain importance in later disease stages, whereas factors affecting any of these mechanisms may be more important early in the disease, which would be in agreement with our second hypothesis.

5. Conclusions

We found that SNCA polymorphisms rs3910105 and rs356181 had no impact on the phenotype of idiopathic, sporadic, recently diagnosed, drug naïve PD patients. These findings agree with those of previous studies showing no major effects of other *SNCA* polymorphisms on the characteristic of early PD patients.

6. Future directions

Further studies are required to determine differences in the effects of SNCA SNPs between early and late stages of PD disease. Monitoring recently diagnosed patients would allow us to compare the characteristics of patients with or without polymorphisms at different stages of the disease, avoiding a major source of bias. The constraint of this and similar studies in terms of sample size could be surmounted by combining the results from different cohorts using meta-analytic techniques.

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