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RESEARCH

Gene Panel Sequencing Analysis Revealed a Strong Contribution of Rare Coding Variants to the Risk of Parkinson's Disease in Sporadic Moroccan Patients

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Abstract

Parkinson's disease (PD) is a neurodegenerative movement disorder which can be either familial or sporadic. While it is well known that monogenic mutations are not a very common cause of PD, GWAS studies have shown that an additional fraction of the PD heritability could be explained by rare or common variants. To identify the rare variants that could influence the risk of PD in the Moroccan population, a cohort of 94 sporadic PD patients negative for the *LRRK2* G2019S mutation was subjected to NGS gene panel sequencing, and gene dosage using the MLPA method. Mean age of onset at enrollment was 51.7 ± 11.51 years, and 60% of patients were men. We identified 70 rare variants under 0.5% of frequency in 16 of the 20 genes analyzed, of which 7 were novel. Biallelic disease-causing variants in genes with recessive inheritance were found in 5 PD cases (5.31%), whereas 13 patients (13.8%) carried likely pathogenic variants in genes with dominant inheritance. Moreover, 8 patients (8.5%) carried a single variant in *MAPT* or *POLG*, whereas co-occurrence of rare variants involving more than one gene was observed in 28 patients (30%). PD patients with variants in recessive genes had a younger mean age at onset than patients with dominant ones (33.40 (12.77) vs. 53.15 (6.63), $p < 0.001$), while their clinical features were similar. However, patients with rare variants in the risk factor genes or in more than one gene tended to have less resting tremor ($p < 0.04$), but more dystonia ($p < 0.006$) and dementia ($p < 0.002$) than those without any rare variants in known PD-associated genes. Our results showed a significant enrichment of rare variants particularly in *LRRK2*, *VPS13C*, *POLG*, and *MAPT* and underline their impact on the risk of sporadic form of the disease.

Keywords Parkinson's disease · Sporadic form · Gene-panel analysis · Rare variants · Morocco

Introduction

Parkinson's disease (PD) is considered as a growing health problem; its prevalence is expected to double from 6.2 million cases in 2015 to 12.9 million cases by 2040 (Dorsey and Bloem 2018). PD is a neurodegenerative disease characterized clinically by motor symptoms as bradykinesia, rest tremors, rigidity, loss of balance, and non-motor symptoms such as psychiatric symptoms, sensory symptoms, and gastrointestinal and autonomic symptoms, in addition to sleep disorders among others (Balestrino and Schapira 2020). The greatest PD risk factor is advancing age, but environment and genetics factors can also affect disease risk and progression.

PD genes were identified historically with linkage and genetic studies of familial forms. Studies on these families have made possible the discovery of several PD causing

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genes, including *SNCA*, *LRRK2*, and *VPS35* with an autosomal dominant mode of inheritance, and *PRKN*, *DJ-1*, *PINK1*, *ATP13A2*, *FBXO7*, *PLA2G6*, *DNAJC6*, *SYNJ1*, and *VPS13C* with an autosomal recessive mode of inheritance, and provided interesting insight on the pathophysiological mechanisms of the disease (Blauwendraat et al. 2020). Several further studies have suspected other genes to cause PD including *HTRA2*, *EIF4G1*, *DNAJC13*, *TMEM230*, *GIGYF2*, and *LRP10* and were the subject of controversy because most of them lack replication and functional validation studies (Blauwendraat et al. 2020).

However, monogenic mutations are not a very common cause of PD and only account for around 5–10% worldwide. Indeed, over the past decade, the development of sequencing technologies has allowed to discover a large number of variants in many loci associated with the disease through genome-wide association studies (GWAS) conducted on idiopathic PD (Kia et al. 2021; Chang et al. 2017; Blauwendraat et al. 2020). Among these, *GBA* and *MAPT* have been reported as major genetic risk factors for PD (Pan et al. 2021; Huang et al. 2022; Billingsley et al. 2018; Bandres-Ciga et al. 2016).

The largest and most significant GWAS, recently performed by Nalls et al. (2019) on samples from European ancestry, identified 90 independent risk loci associated with Parkinson's disease. Their results have estimated to about 22% the Parkinson's disease heritability, which is driven by common genetic variants. In addition, several studies have shown that some of these loci, notably the Mendelian genes discovered in the familial forms, contain rare causal variants that could explain an additional fraction of the missing heritability (Satake et al. 2009; Nalls et al. 2014; Chang et al. 2017; Zheng et al. 2020; Loesch et al. 2021).

The Moroccan population is considered one of the oldest populations of *Homo sapiens* by the discovery of fossils in the Jebel Irhoud region dated to 300,000 years ago (Hublin et al. 2017). Whole-genome sequencing of only three present-day Moroccans identified over 200,000 SNV absent from 1000G, gnomAD databases, and the African Genome Variation Project, suggesting that the Moroccan population would have more genetic variability and therefore would be an ancient population (Crooks et al. 2020). Since most genetic variations are population-specific, studying the genetic architecture of Parkinson's disease in such an old population can help identify novel causative and rare variants. In previous work on PD patients of Moroccan origin, we showed that the *LRRK2* G2019S mutation was the most common and represented globally about 40%, with a high rate in familial forms (Bouhouche et al. 2017a) and which originated in a Berber founder who lived at least 5000 years ago (Ben El Haj et al. 2017). Nevertheless, the disease in the familial patients negative for G2019S was mainly due to causative mutations in recessive genes, particularly in *PRKN* and *PINK1*, and rarely in dominant ones (Bouhouche et al. 2017b; Smaili et al. 2021). Several variants reported by these studies were novel and potentially pathogenic.

The aim of this study was to assess in a Moroccan cohort of 94 PD sporadic cases, without the *LRRK2* G2019S mutation, the contribution of the pathogenic and rare variants in known PD genes to the etiology of idiopathic PD. To reach this objective, a combination of multiplex ligation-dependent probe amplification (MLPA) and NGS gene panel sequencing was applied.

Patients and Methods

Subjects and Clinical Assessment

Among the 210 PD patients recruited from the Movement Disorder Unit of the Department of Neurology (Specialties Hospital, Rabat, Morocco), as part of a project to study the genetic bases of PD, only 94 were sporadic and negative for the *LRRK2* G2019S mutation. These patients were subjected to a structured clinical interview by neurologists and fulfilled the criteria of the United Kingdom Parkinson's Disease Society Brain Bank. Collected data included demographic data (age and sex), age at onset, disease duration, initial symptoms, symptoms at examination, clinical form, motor complications, and non-motor features. Symptoms at examination were evaluated based on UPDRS III in ON state. A score ≥ 1 was mandatory for each item to consider patient having resting tremor (item 20), akinesia (items 23, 24, 25, and 26 or 27), rigidity (item 22), gait impairment (item 29), and postural instability (item 30). Motor complications were appreciated according to UPDRS IV, including dyskinesia and motor fluctuation. The clinical form was typed as tremor dominant, akinetic-rigid, or mixed form according to criteria used by Rajput et al. (2009). Tremor dominant form referred to patients in whom the tremor was the dominant finding over the other symptoms. Patients with important bradykinesia and rigidity with no visible tremor were ranked as akinetic-rigid form, and those who had the same degree of bradykinesia, rigidity, and tremor were classified as mixed form. Patients were classified into early onset PD when the age of onset was before their fifty years old and late onset PD when the disease began after 50. Non-motor symptoms were evaluated using MDS non-motor rating scale. We assessed cognitive impairment, psychiatric features, autonomic dysfunctions (constipation, urinary dysfunctions, and orthostatic hypotension), sleep disorders, and pain. A subscale of 1 or more for each item classified patient as having the corresponding non-motor symptom.

The Biomedical Research Ethics Committee of the Medical School of Rabat (CERB) approved this study, and all

patients and controls provided a written informed consent in accordance with the Declaration of Helsinki.

Genetic Analysis

Genomic DNA was extracted from peripheral blood leukocytes using the Wizard® Genomic DNA Purification Kit from Promega. The quality and quantities of DNA were assessed by the NanoDrop One and the Qubit dsDNA HS Assay Kit on Qubit fluorometer (ThermoFisher Scientific).

The multiplex ligation-dependent probe amplification (MLPA) was used to detect exon-rearrangements in PD genes for all negative G2019S patients, using the P-051 kit according to the manufacturer protocol. MLPA data were analyzed using the Coffalyser software (MRC-Holland, Amsterdam, The Netherlands).

Gene panel next-generation sequencing (NGS) containing 20 genes associated with PD and overlapping phenotypes (Supplementary Material) was performed in all patients using the Ion Proton System (Thermo Fisher Scientific). Library and template preparation were performed on the Ion Chef System, using an Ion PI Chips v2 for chip loading, and sequencing runs were performed on Ion proton machine. Data of runs were imported in BAM format files and uploaded into the Ion Reporter Software for variant analysis and annotations (Thermo Fisher Scientific). Thirty-three healthy control individuals of Moroccan origin were also screened by the 20-gene panel as a reference for the study population and have also served to eliminate the mutational errors inherent to the sequencing technology used.

The strategy used for variant prioritization and classification is shown in Fig. 1. Exon deletions identified by MLPA were considered as pathogenic. Among the variants obtained with the Ion Reporter Software from NGS sequencing, we only selected functional variants including non-synonymous substitutions (missense and non-sense variants), splice site variants, and small insertions and deletions that were covered by > 50 reads. INDELS causing frameshift, splice, and non-sense variants were all considered as pathogenic, according to the American College of Medical Genetics and

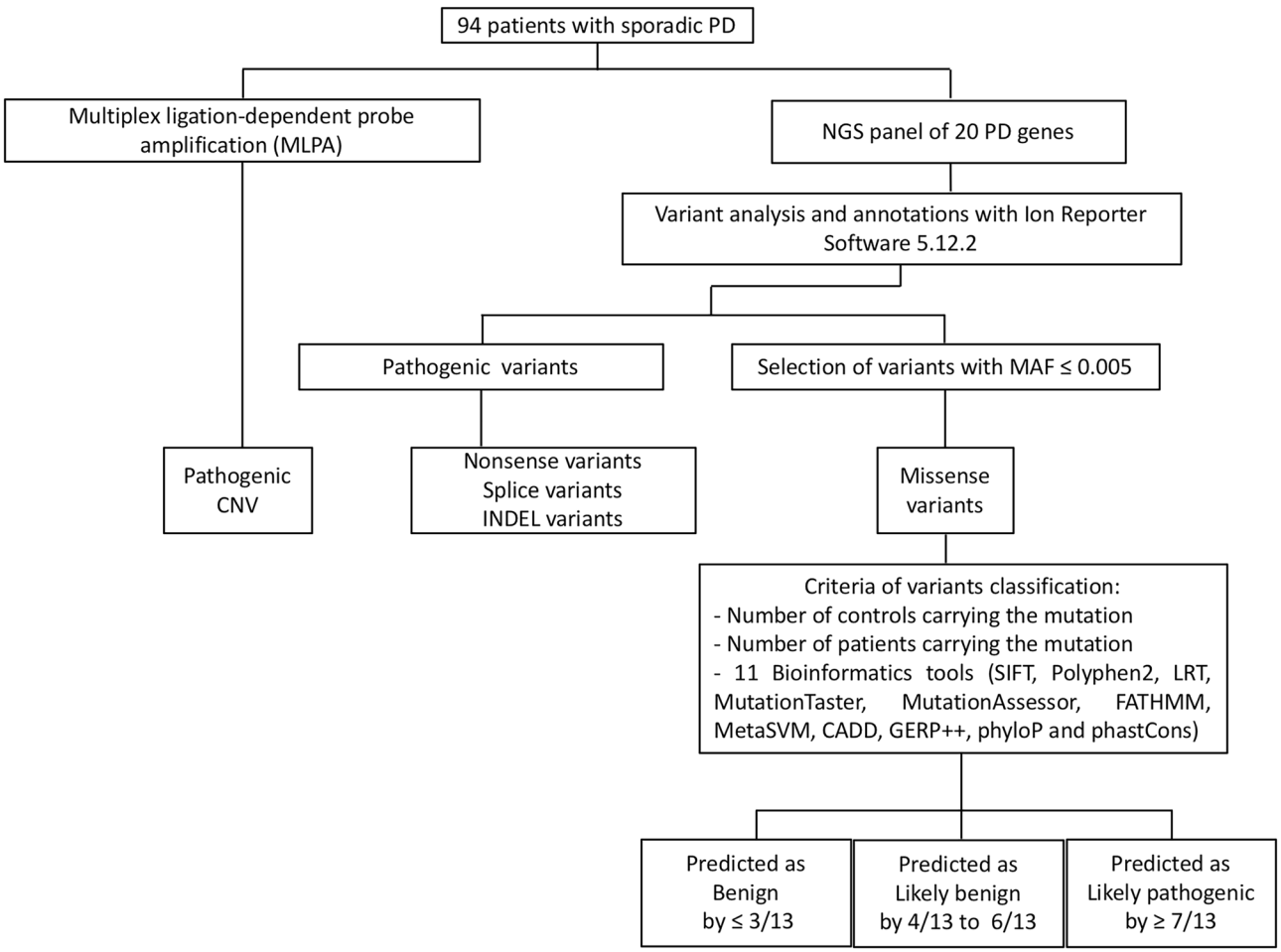

Fig. 1 Summary of genetic analysis strategy and criteria for variant prioritization and classification

Table 1 Summary of demographic data

Series	*N*	Age, mean ± SD (range) in years	AAO, mean ± SD (range) in years	Male/female ratio
Total PD	94	58.85 ± 11.03	51.7 ± 11.51	57: 37
LOPD (AAO > 50)	55	66.67 ± 6.53	59.60 ± 5.79	32: 23
EOPD (AAO ≤ 50)	39	50.72 ± 9.75	40.56 ± 7.66	25: 14
Control individuals	33	40.24 ± 11.80		17: 16

Abbreviations: *AAO* age at onset, *EOPD* early-onset Parkinson's disease, *LOPD* late-onset Parkinson's disease, *N* number

Genomics (ACMG) guidance for pathogenicity classification (Richards et al. 2015). Regarding missense variants, we only retained rare variants with MAF less than 0.5% in public database (1000G, GnomAD and ExAC) in Caucasians. They have been classified as likely pathogenic, likely benign or benign based on 13 criteria, including the number of patients as well as controls carrying the variant, and 11 bioinformatics tools (Fig. 1). Filtered candidate variants were validated by Sanger sequencing. Exons containing each selected variant were amplified by PCR. Amplicons were sequenced using the BigDye terminator cycle sequencing kit v3.1 and run on a SeqStudio genetic analyzer (Thermo Fisher Scientific). The obtained electrophoregrams were analyzed by SeqScape Software v4.1.

Results

Summary of Demographic Data

A total of 94 patients with sporadic PD who fulfilled the inclusion and exclusion criteria of PD were enrolled into this study. Among them, 15 (16%) were from consanguineous marriage. In our cohort, the mean age of onset at enrollment was 51.7 ± 11.51 years and 60% of patients were men. Thirty-nine patients (41.49%, 39/94) had early-onset PD (EOPD) with a mean age of onset of 40.56 ± 7.66 years, and fifty-five (58.51%, 55/94) were late-onset PD (LOPD) with a mean age of onset of 59.60 ± 5.79 years. The mean age at recruitment of the 33 controls was 40.24 ± 11.80 years, of them 52% were males (Table 1).

Summary of Variants Identified

All PD patients were screened with MLPA and NGS gene panel sequencing consisting of genes strongly associated with PD, genes with high genetic risk or genes with low confidence for PD (Supplementary File). A total of 70 rare variants were identified in 64/94 (68.08%) PD isolated cases (Fig. 2a). Of these variants, 5 (7%) were loss of function, all considered as pathogenic including non-sense and exon deletion mutations, and 65 were missense (93%) (Fig. 2b). For missense variants, only 4 out of 65 (6%) were reported pathogenic in the ClinVar database. The remaining 61 missense variants were predicted likely pathogenic beyond 6 out of the 13 criteria for variant prioritization and classification used. By applying our workflow, 40 of the 65 (61.5%) identified missense rare variants were pathogenic or likely pathogenic and 25 (38.5%) were benign or likely benign (Fig. 2c).

These 70 rare variants were found in 16 of the 20 genes analyzed, of which about half were found in genes with autosomal recessive (AR) inheritance (Fig. 3). Loss-of-function mutations were only found in the *PRKN*, *PINK1*, and *DJ-1* genes, while missense variants were more frequent

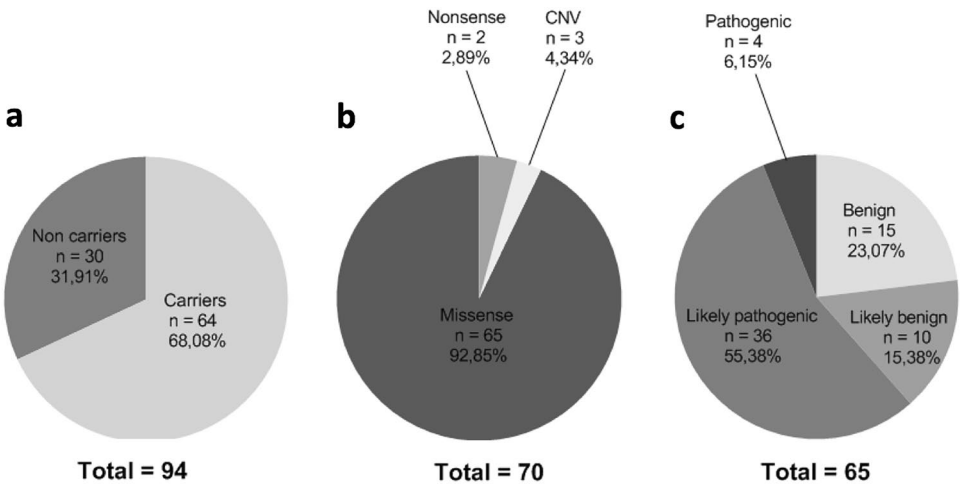

Fig. 2 Pie plot showing the distribution and frequencies of rare variants identified in PD isolated cases and their classification. **A** Frequency of carriers vs non-carriers. **B** Type and frequency of the identified rare variants. **C** Frequency of the pathogenicity classification status of missense rare variants

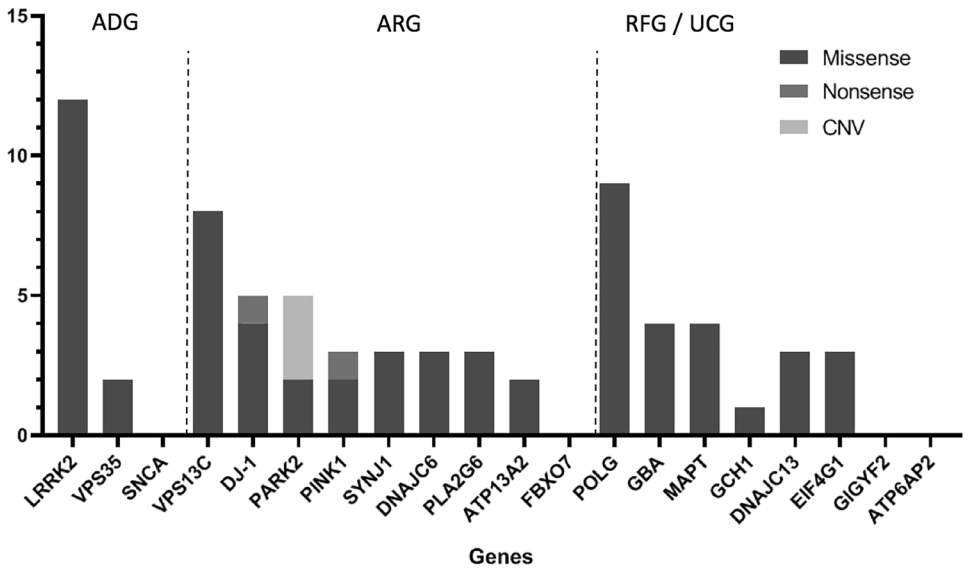

Fig. 3 Frequency and distribution of identified rare variants in our PD isolated case cohort using a 20-gene sequencing panel. ADG: autosomal dominant genes; ARG: autosomal recessive genes; RFG/UCG: Risk factor and unconfirmed PD genes

in *VPS13C*. In genes with autosomal dominant (AD) inheritance, most of the rare variants (12/14) were found in *LRRK2*. Twenty-four rare variants were observed in the risk factor and unconfirmed genes (RFG/UCG), with *POLG* being the most common. No pathogenic or rare variants were found in *SNCA*, *FBXO7*, *GIGYF2*, and *ATP6AP2*.

Findings in Dominant PD-Associated Genes

The *LRRK2* was the gene most frequently mutated with 12 rare variants, of them 9 were likely pathogenic according to the used classification criteria and are located between exons 17 and 34, except for the p.Leu119Pro variant located in the N-terminal region and with the highest CADD score. Of these, two variants were newly identified, the p.Lys739Arg in exon 18 and the p.Glu1492Lys in exon 31 (Fig. 4a). Two variants were frequently found, the likely pathogenic p.Pro1262Ala variant identified in 6 patients, and the p.Tyr2189Cys variant identified in 5 patients and classified variant of unknown significance (VUS) in ClinVar database. Only two rare variants were found in the *VPS35* gene, the p.Lys382Arg and p.Ala737Val located in the SNXS domain, both absent in controls and classified likely pathogenic (Fig. 4b).

Thirteen patients in our cohort (13.8%) carried likely pathogenic variants in AD genes. Five of the eleven patients mutated for *LRRK2* were EOPD and the other six were LOPD, whereas the two patients mutated for *VPS35* were both LOPD (Table 2). It should be noted that one patient (3913) was homozygous for both the novel p.Glu1492Lys variant and the p.Pro1262Ala in *LRRK2*, and two other patients (3711 and 3880) have two different variants in *LRRK2* at heterozygous state (Supplementary file).

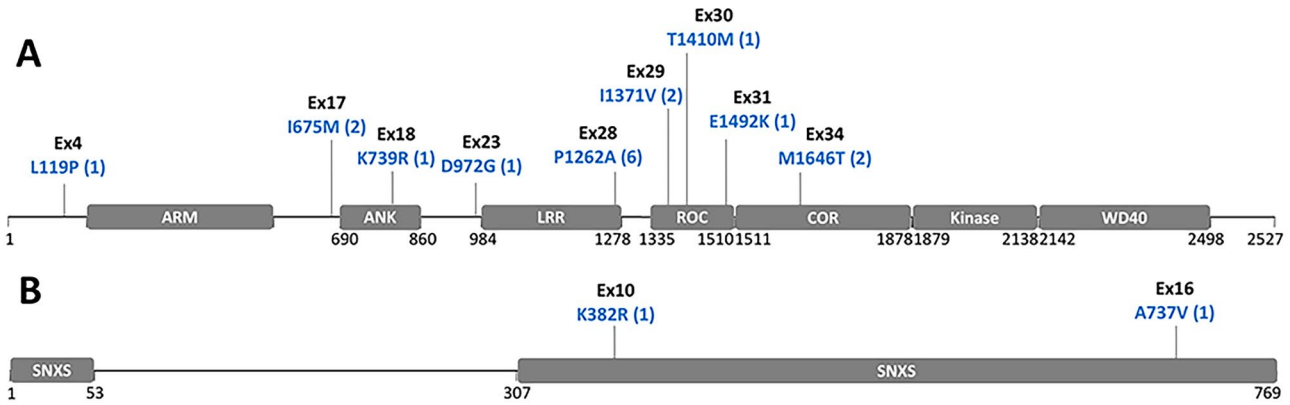

Fig. 4 Schematic representation of the **A** LRRK2 and **B** VPS35 proteins with the likely pathogenic variants (in blue) putatively contributing to PD. Numbers in brackets indicate the number of variant carriers

Table 2 Frequencies of dominant gene variant carriers for rare likely pathogenic variants in early-onset and late-onset PD patients

	EOPD ($n=39$)	LOPD ($n=55$)	All ($n=94$)
LRRK2	5 (12.82%)	6 (10.90%)	11 (11.70%)
VPS35	0 (0%)	2 (3.63%)	2 (2.12%)
SNCA	0 (0%)	0 (0%)	0 (0%)

Findings in Recessive PD-Associated Genes

In the 9 known PD-causing genes tested with AR inheritance, MLPA analysis detected 3 exon deletions in *PARK2* gene, while 29 rare variants were identified by NGS gene panel analysis. Among these, twenty of them were classified as pathogenic or likely pathogenic. Most of them were found in the *VPS13C*, *PRKN*, *PINK1*, and *DJ-1* genes (Fig. 5). Biallelic variants were present in only 5 of the 94 PD cases (5.31%), including two patients homozygous for the pathogenic p.Gln456Ter and p.His271Gln variants in *PINK1*, one patient carrying a compound heterozygous deletion of exons 3 and 5 in *PRKN*, one patient compound heterozygous for two novel pathogenic variants, Tyr141Ter and Thr110Pro in *DJ-1*, and finally one patient homozygous for the likely pathogenic p.Pro309Ala variant in *VPS13C* (Table 3). It should be noted that the *DJ-1* Thr110Pro variant has been classified as pathogenic since it was identified in a patient with another pathogenic stop variant and that we previously found it in the homozygous state in a familial form in which segregation has been verified (Smaili et al. 2021). The four patients with biallelic variants in *PARKN*, *PINK1*, and *DJ-1* had a very young AAO (under 30 years old) in comparison with the patient with homozygous variant in *VPS13C* (AAO = 56 years).

We identified 10 patients with single heterozygous rare variants in 5 AR PD-genes, with no other rare variants in the twenty-panel genes analyzed. Four variants were likely pathogenic and occurred in the *DJ-1* and *VPS13C* genes in 5 patients, and four others were benign or likely benign occurred in *PLA2G6*, *PINK1,* and *DNAJC6* in 5 patients

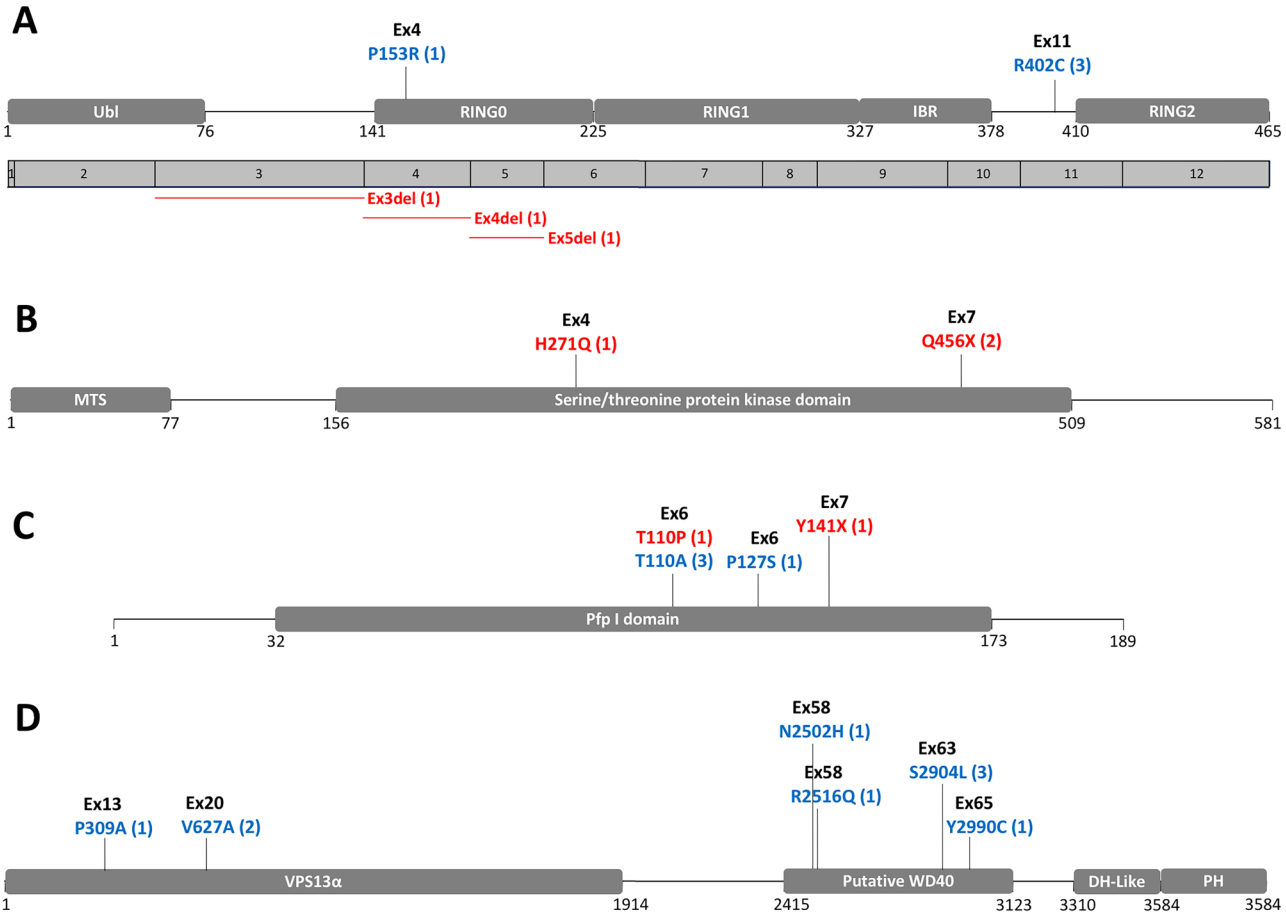

Fig. 5 Schematic representation of the **A** PRKN, **B** PINK1, **C** DJ-1, and **D** VPS13C proteins with the pathogenic (in red) and likely pathogenic (in blue) variants putatively contributing to PD. Numbers in brackets indicate the number of variant carriers

Table 3 Frequencies of biallelic recessive gene variant carriers for pathogenic and likely pathogenic variants in early-onset and late-onset PD patients

	EOPD ($n=39$)	LOPD ($n=55$)	All ($n=94$)
PRKN			
Homozygous	0 (0%)	0 (0%)	0 (0%)
Compound heterozygous	1 (2.56%)	0 (0%)	1 (1.06%)
PINK1			
Homozygous	2 (5.12%)	0 (0%)	2 (2.12%)
Compound heterozygous	0 (0%)	0 (0%)	0 (0%)
DJ-1			
Homozygous	0 (0%)	0 (0%)	0 (0%)
Compound heterozygous	1 (2.56%)	0 (0%)	1 (1.06%)
VPS13C			
Homozygous	0 (0%)	1 (1.81%)	1 (1.06%)
Compound heterozygous	0 (0%)	0 (0%)	0 (0%)

(Supplementary file). The AAO of these patients was significantly later than cases with biallelic variants (50.70 (SD = 9.53) vs. 33.4 (SD = 12.77), $p = 0.0009$).

Patients with Rare RFG/Multigenic Variants

Thirty-six patients have a single rare variant in the risk factor genes or in several genes among the 20 genes tested, including the RFG/UCG and Mendelian genes (MG). In these patients, twenty-four rare variants were discovered in the RFG/UCG. Among them, 9 were found in *POLG*, 4 in *MAPT* and *GBA* each, and only one in *GCH1*. Six rare variants were found in the unconfirmed PD genes with 3 in *EIF4G1* and 3 in *DNAJC13* (Fig. 6a), and most of them are benign.

Five likely pathogenic variants were found in more than half of the patients (20/36, 56%), including 2 variants in *MAPT* (p.Arg222Ser identified in 7 patients, 1 of whom was homozygous, and p.Ser427Phe found in 5 patients), and 3 variants in *POLG* (p.His613Tyr in 4 patients, p.Thr251Ile and p.Tyr831Cys in 2 patients each). Two already reported pathogenic variants (p.Leu483Pro and p.Arg502Cys) and one novel (p.Thr247Ser) were identified in *GBA* in 3 patients. All these variants were not found in any of the 66 chromosomes of control individuals (Supplementary file).

Eight of the 36 patients carried a single variant in *MAPT* or *POLG*, whereas co-occurrence of rare variants involving more than one gene was observed in 28 patients. Seven of them carried 2 to 4 rare variants in MG, mainly in genes with AR inheritance, and the remaining 21 patients carried 2 to 4 rare variants in the RFG/UCG and MG. In this latter group of patients, pathogenic and likely pathogenic variants were mainly found in *POLG* and *MAPT*, and additionally one or more variants in the MG and UCG (Fig. 6b, Supplementary file).

The patient with the youngest AAO and the most severe phenotype (3855) presents 3 rare variants in *PRKN* (p.Arg402Cys), *POLG* (p.Met1195Ile), and *LRRK2* (p.Leu119Pro). He also carried additionally other common variants in these genes, including *PRKN* p.Ser167Asn and p.Val380Leu variants, and the *POLG* p.Gln1236His variant, as well as the *MAPT* H2 haplotype. All these 7 alleles were found at the heterozygous state in the patient. Segregation analysis in living and healthy parents aged 55 and 60 years showed that the two variants *POLG* p.Met1195Ile and *PRKN* p.Ser167Asn were inherited from the father, and the other 5 alleles from the mother.

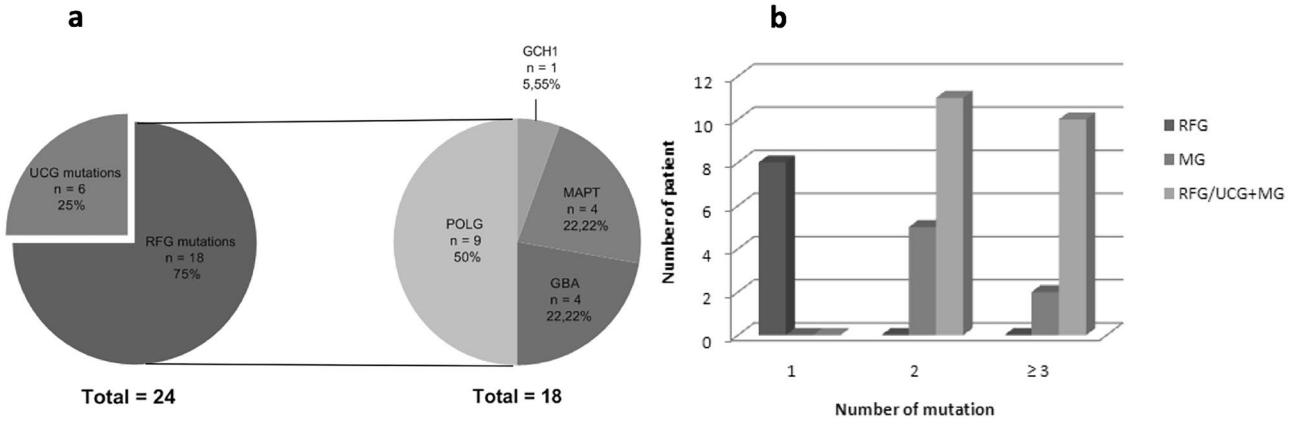

Fig. 6 Pie plot and histogram showing the distribution and frequencies of rare variants identified in risk factor (RFG), unconfirmed (UCG), and Mendelian (MG) genes. **a** Pie plot showing the frequency of rare variants in RFG and UCG. **b** Histogram showing the number of patients carrying one or additionally more variants in RFG/UCG and Mendelian genes (MG)

Clinical Findings

The clinical data of the 13 dominant gene variant carriers were compared to the 5 recessive gene variant carriers and are shown in Table 4. The mean age at onset for patients carrying biallelic variants in recessive genes was 33.4 years (SD = 12.77), compared to an age at onset of 53.15 years (SD = 6.63) for patients carrying variants in dominant genes, and the difference was highly significant ($p < 0.001$). However, we did not find any differences between the two groups for either motor or non-motor symptoms. Note that the clinical form of all 5 patients with AR inheritance was mixed presenting with tremor and akinesia, compared to about half for patients with AD inheritance, but the difference was not significant ($p = 0.063$) because of the small number of patients per group.

Table 5 presents the comparison of clinical data of the 36 patients carrying RFG/multigenic rare variants versus the 30 patients without any rare variants in the 20 genes analyzed. There was no difference in age at onset ($p = 0.822$) and disease duration ($p = 0.070$) between the two groups. Carriers of RFG/multigenic variants reported slightly less resting tremor at diagnosis than patients without any variants in the genes tested ($p = 0.041$), while the distribution of the clinical form remains not significantly different between the two groups ($p = 0.895$). However, they experienced more dystonia ($p = 0.006$) and more cognitive decline ($p = 0.002$). The rest of the clinical criteria were not significantly different between the two groups.

Discussion

In this study, we combined MLPA and next-generation sequencing panel of 20 genes to study the genetic bases of 94 sporadic cases with Parkinson's disease of Moroccan origin.

Table 4 Comparison of clinical data between patients with variants in autosomal dominant (AD) and autosomal recessive (AR) PD genes

Variable	AD gene mutation positive ($n = 13$)	AR gene biallelic mutation positive ($n = 5$)	p value
Sex male, N (%)	8 (61.5)	3 (60.0)	0.952
Mean age at onset, years (SD)	53.15 (6.63)	33.40 (12.77)	**< 0.001**
Mean disease duration, years (SD)	7.92 (4.35)	7.20 (5.80)	0.281
Initial symptom			
Tremor	8 (61.5)	3 (60.0)	
Akinesia	5 (38.5)	1 (20.0)	0.226
Tremor and akinesia	0 (0.00)	1 (20.0)	
Symptoms at exam (%)			
Resting tremor	11 (84.6)	3 (60.0)	0.261
Akinesia	10 (76.9)	4 (80.0)	0.888
Rigidity	8 (61.5)	3 (60.0)	0.952
Gait impairment	3 (23.1)	1 (20.0)	0.888
Postural instability	4 (30.8)	1 (20.0)	0.641
Clinical form			
Tremor dominant	6 (46.1)	0 (00.0)	
Akinetic	1 (07.6)	0 (00.0)	0.063
Mixed	6 (46.1)	5 (100)	
Motor complications (%)			
Dyskinesia	6 (46.1)	4 (80.0)	0.196
Motor fluctuation	9 (69.2)	3 (60.0)	0.710
Dystonia	7 (53.8)	1 (20.0)	0.196
Non-motor features (%)			
Cognitive decline	2 (15.4)	0 (00.0)	0.352
Sleep disorder	6 (46.1)	0 (00.0)	0.063
Psychiatric features	4 (30.8)	3 (60.0)	0.255
Pain	2 (15.4)	1 (20.0)	0.814
Urinary dysfunction	7 (53.8)	1 (20.0)	0.196
Constipation	4 (30.8)	0 (00.0)	0.160
Orthostatic HypoTA	4 (30.8)	1 (20.0)	0.648

Table 5 Comparison of clinical data between patients with variants in risk factor and multigenic PD genes and patients without mutation in the 20 genes of the panel tested

Variable	Risk factor/multigenic mutation positive ($n=36$)	All gene panel mutation negative ($n=30$)	p value
Sex male, N (%)	21 (58.3)	19 (63.3)	0.802
Mean age at onset years (SD)	53.11 (11.8)	52.17 (11.1)	0.822
Mean disease duration, years (SD)	8.89 (6.61)	7.30 (4.74)	0.070
Initial symptom			
Tremor	18 (50.0)	20 (66.7)	
Akinesia	9 (25.0)	3 (10.0)	0.242
Tremor and akinesia	9 (25.0)	7 (33.3)	
Symptoms at exam (%)			
Resting tremor	18 (50.0)	23 (76.7)	**0.041**
Akinesia	22 (61.1)	23 (76.7)	0.197
Rigidity	20 (55.6)	15 (50.0)	0.805
Gait impairment	16 (44.4)	13 (43.3)	1.000
Postural instability	16 (44.4)	19 (63.3)	0.145
Clinical form			
Tremor dominant	6 (16.7)	6 (20.0)	
Akinetic	10 (27.8)	9 (30.0)	0.895
Mixed	20 (55.6)	15 (50.0)	
Motor complications (%)			
Dyskinesia	7 (19.4)	9 (30.0)	0.392
Motor fluctuation	16 (44.4)	13 (43.3)	1.000
Dystonia	8 (22.2)	0 (00.0)	**0.006**
Non-motor features (%)			
Cognitive decline	15 (41.7)	2 (6.70)	**0.002**
Sleep disorder	15 (41.7)	17 (56.7)	0.323
Psychiatric features	13 (36.1)	11 (36.7)	1.000
Pain	15 (41.7)	7 (23.3)	0.189
Urinary dysfunction	13 (36.1)	11 (36.7)	1.000
Constipation	14 (38.9)	10 (33.3)	0.798
Orthostatic HypoTA	9 (25.0)	8 (26.7)	1.000

Our objective is to analyze the contribution of rare variants in known PD genes on the disease risk in this North African population. Overall, we found 70 rare variants below the frequency of 0.5%; among them, 7 variants were novel including two in *DJ-1* (p.Tyr141X and p.Thr110Pro), in *PLA2G6* (p.Asn150His and p.His477Tyr), in *LRRK2* (p.Lys739Arg and p.Glu1492Lys), and one in *VPS13C* (p.Asn2502His). Four of 94 patients with sporadic PD (4.25%) are carriers of known pathogenic variants in different Mendelian genes, and all of them concern genes with AR inheritance, notably in *PINK1*, *PRKN*, and *DJ-1*. This rate becomes 10.25% (4/39) if only isolated cases with EOPD are considered. Therefore, knowing that the pathogenic *LRRK2* G2019S variant alone representing 28% of sporadic PD patients in Morocco (Bouhouche et al. 2017a) was previously excluded in our cohort, the frequency of known pathogenic variants in Moroccan population is substantially higher than that reported in other ethnic groups (Koziorowski et al. 2010; Spataro et al. 2015; Benitez et al. 2016; Bandres-Ciga et al. 2016; Kim and Alcalay 2017; Tan et al. 2019). When we applied our criteria for variant prioritization and classification, 40 of the 65 (61.5%) identified rare missense variants were classified pathogenic/likely pathogenic. Thirteen patients out of 94 (13.8%) carried likely pathogenic variants in known PD genes with AD inheritance, including 8 in *LRRK2* and 2 in *VPS35*. Another patient carried the rare likely pathogenic variant p.Pro309Ala in *VPS13C* at homozygous state, bringing to 5 the number of PD patients which may be explained by the detected variants in known Mendelian genes with AR inheritance. Therefore, a monogenic cause of sporadic PD in our Moroccan cohort may account for 18 patients among the 94 tested (19%), involving four recessive (*PINK1*, *DJ-1*, *PRKN*, and *VPS13C*) and two dominant (*LRRK2* and *VPS35*) genes, without counting *LRRK2* G2019S, the most common variant. In a previous study conducted on a series of PD patients

with a familial and consanguineous form, we have shown a Mendelian inheritance rate of more than 45%, in whom pathogenic variants in *PARK2* and *PINK1* were the most frequent (Bouhouche et al. 2017b). In another study conducted on the prevalence of the *LRRK2* G2019S mutation in Morocco, 12% of carriers were from consanguineous marriage, of which 2% were familial forms and 10% sporadic forms (Bouhouche et al. 2017a). Therefore, the high rate of Mendelian heritability of PD in the Moroccan population is due on the one hand to the impact of consanguinity and the high prevalence of the *LRRK2* G2019S mutation on the other hand.

In addition, seven patients in our cohort carried two or three rare variants in different Mendelian genes. These results confirm earlier studies that the genetic bases of familial and sporadic forms of PD are not quite different. While Mendelian forms of the disease are due to single high-penetrance mutations in particular genes, sporadic forms may additionally be due to many large effect variants in different genes. These genes are usually involved in the mitochondria and ubiquitin pathways, as previously reported (Obeso et al. 2010; Spataro et al. 2015; Bayne and Trempe 2019).

Besides patients with only rare variants in Mendelian genes, twenty-one other patients (22%) carried all at least a rare likely pathogenic variant in *POLG* or *MAPT*, and additional rare variants in Mendelian genes, particularly in *LRRK2* and *VPS13C*. Therefore, the co-occurrence in patients of rare variants in these four genes appear to be most common in the Moroccan population affecting PD risk. These genes, in addition to *GBA*, have been reported to be among the most decisive genetic risk factors for sporadic PD (Bandres-Ciga et al. 2020; Borsche et al. 2021; Kanaya et al. 2021). However, the frequency of pathogenic/likely pathogenic variants in *GBA* was very low in our population, confirming previous studies (Nishioka et al. 2010).

Our study focused only on the contribution of rare variants in the risk of PD. However, it is well known that sporadic forms of PD are the result of the involvement of both rare large effect and common moderate effect variants (Spataro et al. 2015; Bandres-Ciga et al. 2020; Hustad and Aasly 2020). Indeed, we have shown in this study that the patient with the most severe clinical phenotype and the youngest AAO carried both rare and common variants in Mendelian and risk factor genes inherited separately from his two parents, knowing that we have only analyzed the known Mendelian and risk factor PD genes. In the same way, GWAS studies carried out over the last few years have shown that the genetic basis of PD could be explained by a continuum from a single pathogenic allele to the sum of multiple low-risk alleles in several genes (Petrucci et al. 2014; Bandres-Ciga et al. 2020; Zheng et al. 2020).

Furthermore, we found 10 patients (11%) who carried a single rare heterozygous variant in Mendelian recessive genes. It has been reported that heterozygous variants in recessive inherited genes could be a risk factor for late-onset PD (Klein and Lohmann-Hendrick et al. 2007; Jia et al. 2022). Moreover, these patients could carry other variants in other PD loci, not analyzed by our NGS gene panel. Finally, thirty patients in our cohort (32%) have no rare variants under 0.5% in the 20 genes tested, suggesting that there may be other high-risk genes that have not yet been identified and thus further genetic heterogeneity for PD in the Moroccan population.

For the same mean disease duration, the clinical features of the two groups of patients with AR and AD inheritance variants were similar. The only statically significant difference was related to the mean age at onset, where the AR group was younger. It is well known that biallelic recessive variants, particularly in *PRKN*, *PINK1*, *DJ-1*, and *VPS13C*, are responsible for EOPD (Guadagnolo et al. 2021). However, our patient homozygous for the p.Pro309A variant in *VPS13C* started the disease later at 56 years old. This rare variant, which has never been reported in the homozygous state, has been classified as likely pathogenic and could be considered a weak-effect allele responsible for LOPD. This AR group of patients all had a mixed clinical form without atypical signs evolving as an idiopathic PD, and none of them presented with cognitive decline at the time of examination. These results are in agreement with the data of the literature, particularly in the large cohort studies which showed that the *PRKN*, the *PINK1* and the *DJ-1* are considered to be the most common causative recessive genes, responsible for pure PD, and that should be assessed in patients with EOPD, particularly in populations with high inbreeding (Bonifati 2012; van der Merwe et al. 2015; Lesage et al. 2020; Guadagnolo et al. 2021). Regarding patients with AD inheritance, *LRRK2* was as expected the most frequently mutated. PD patients with *LRRK2* rare variants were about half EOPD and half LOPD, while the only two patients with *VPS35* variants were LOPD. These patients presented overall with idiopathic PD with less dementia and good response to different therapeutic options and a benign course, as previously reported in other populations (Yahalom et al. 2020; Guadagnolo et al. 2021). We found no patients with rare variants in *SNCA* in our PD isolated case series, as well as in other previous studies on familial forms (Smaili et al. 2021).

We also compared the clinical findings between the group of patients with rare RFG/multigenic variants and the group without rare variants in the genes tested. For a comparable disease duration and mean age at onset, there was less resting tremor, more dystonia, and cognitive decline in the RFG/multigenic group. This group of patients appears to be clinically heterogeneous, and the significant presence of cognitive decline could be explained by the presence of variants in *MAPT* in a significant number of patients. Indeed, *MAPT* has been reported as a genetic risk factor for cognitive impairment (Harrington et al. 2021; Gonzalez-Latapi et al. 2021).

In conclusion, our genetic analysis revealed that up to 5% of patients in our Moroccan cohort with sporadic PD carry known pathogenic mutations in Mendelian genes with recessive inheritance. Moreover, we demonstrated that rare variants showed significant enrichment in about 50% of our cohort, confirming their influence on the risk of PD. Among the sequenced genes, the Mendelian genes *LRRK2* and *VPS13C* and the risk factor genes *POLG* and *MAPT* could be responsible for the enrichment in rare variants, having an important cumulative impact on the risk of PD.

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Author contributions HT, MR NM HND and WR acquired the clinical data. IS carried out NGS and Sanger experiments, and prepared the figures. AhB performed bioinformatics analysis and interpreted the results. RR performed statistical analysis. AhB and HT wrote the paper. AhB, WR and AlB revised the manuscript. AhB conceptualized and designed the study. All authors reviewed the manuscript.

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Data Availability The datasets generated or used in the current study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Declarations

Ethics Approval All research was approved by the ethics committee of biomedical research of Medical School and Pharmacy of Rabat (CERB).

Consent to Participate Written informed consent was obtained from all study participants.

Conflict of Interest The authors declare no competing interests.

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