Journal of Molecular Neuroscience
https://doi.org/10.1007/s12031-022-02085-9

Whole-Exome Sequencing Study of Consanguineous Parkinson's Disease Families and Related Phenotypes: Report of Twelve Novel Variants

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Received: 23 August 2022 / Accepted: 15 November 2022

Abstract

Parkinson's disease (PD) is a common progressive neurodegenerative disorder with motor and nonmotor symptoms. Recent studies demonstrate various susceptibility loci and candidate genes for familial forms of the disease. However, the genetic basis of the familial form of early-onset PD (EOPD) is not widely studied in the Iranian population. Therefore, the present study aimed to investigate the possible causative genetic variants responsible for developing EOPD among Iranian patients. Iranian patients with a clinical diagnosis of Parkinson's disease were evaluated, and 12 consanguineous families with at least two affected individuals with early-onset PD (EOPD) were chosen to enroll in the present study. An expert neurologist group examined these families. Whole-exome sequencing (WES) was performed on PD patients, and the possible causative genetic variants related to the development of PD were reported. Exome sequencing (WES) was performed on every PD patient and revealed that patients had novel genetic variants in *PRKN*, *PARK7*, and *PINK1* genes. All the genetic variants were in homozygous status and none of these variants were previously reported in the literature. Moreover, these genetic variants were "pathogenic" based on bioinformatic studies and according to the American College of Medical Genetics (ACMG). The present research revealed some novel variants for EOPD among the Iranian population. Further functional studies are warranted to confirm the pathogenicity of these novel variants and establish their clinical application for the early diagnosis of EOPD.

Keywords Parkinson's disease · Genetics · Whole-exome sequencing · Genotype · Causative variants

Introduction

Parkinson's disease (PD) is a common progressive neurodegenerative disorder with a considerable global burden. The estimated prevalence is reported to be 12.9 million by 2040 (Dorsey et al. 2018; Trist et al. 2019). The main motor symptoms of PD are stemmed from the loss of dopaminergic neurons in the substantia nigra caused by the accumulation of Lewy bodies in the midbrain neurons (Trist et al. 2019). PD patients also develop a variety of nonmotor impairments

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Published online: 15 December 2022

because of the involvement of other brain areas (Miller and Cronin-Golomb 2010). Interaction of both environmental and genetic risk factors is responsible for the development of PD (Dorszewska 2013). Pathogenic variants in fifteen genes (*SNCA*, *LRRK2*, *VPS35*, *GBA*, *RAB39B*, *PRKN*, *PINK1*, *DJ-1*, *ATP13A2*, *PLA2G6*, *DNAJC6*, *SYNJ1*, *FBXO7*, *VPS13C*, *PTRHD1*) have been firmly established as genetic causes in PD (Guadagnolo et al. 2021).

Moreover, genome-wide association studies (GWAS) and linkage studies introduce novel candidate genes as risk factors of PD (Grenn et al. 2020). Among the several causative variants reported for autosomal recessive PD, *PARK2 (PRKN)*, *PARK6 (PINK1)*, and *PARK7* (DJ-1) are the most common causes of early-onset PD. In addition to the rare mutations, common susceptibility variants like single-nucleotide polymorphisms (SNPs) are reported in some specific genes, including *LRRK2* and *SNCA* (Bonifati et al. 2002). However, the genetic variants identified thus far explain only a minority of PD heritability (Ohnmacht et al. 2020). Up to now, the genetic diagnosis of the majority of PD patients remains unresolved.

Moreover, heritability has been tackled through different approaches within the last years, and recent line of evidence suggested common variants with moderate/weak effect sizes on PD susceptibility. Even more, next-generation sequencing studies (mostly whole-exome sequencing (WES)) identified rare causative mutations in a different population (Pierce et al. 2020). The genetic architecture and mutational spectrum of PD varies based on the ethnic and genetic background of the population (Gandhi et al. 2005). Therefore, recent population-specific and large-scale genomics studies focused on inter-individual variations, especially PD endophenotypes (Gandhi et al. 2005; Yemni et al. 2019; Li et al. 2020). Integrating WES and functional evidence in PD revealed several novel candidate genes, especially in early-onset PD (EOPD), suggesting that a broad spectrum of genetic variants is linked to EOPD (Yemni et al. 2019; Li et al. 2020). Consanguineous marriage is an old tradition in Iranian culture, constituting a considerable percentage of marriages in Iran (Hosseini-Chavoshi et al. 2014). Therefore, the increase of genetic homozygosity in specific populations causes the appearance of rare mutations in recessive diseases (Bittles 2010). To date, there are still limited studies on the genetic architecture of EOPD among the Iranians, which are considered among understudied populations in genome studies. Wide-scale genomic studies in such populations were usually costly and decreasing the cost of genotyping studies is balancing the studies over every population revealing novel genetic variants (Mulder et al. 2020; Dehghani et al. 2021). Therefore, to address the gaps in knowledge about the genetics of EOPD in the Iranian population, we performed an extensive WES screening of the common and rare genetic variants causing familial EOPD among consanguineous Iranian families from mainland Iran. In addition, we also examined the clinical features of those patients to understand the connection between genotype and clinical phenotype.

Materials and Methods

Participants

The present study has been approved by the ethics committee of Mashhad University of Medical Sciences and took place in the neurology and neurogenetic clinics of Ghaem Hospital (Mashhad, Iran) and the academic center for education and culture genetic counseling clinic (Khorasan branch, Mashhad Iran). As part of a large multi-center study, we chose our main study population with EOPD from a database of 500 clinical records of Iranian patients with clinical diagnoses of PD. Among these patients, 52 patients had familial EOPD with consanguineous marriage. Thirty five patients agreed to undergo complete neurological examination and underwent WES. Figure 1 describes the study flowchart and the number of novel and previously reported variants related to PD.

PD diagnosis was confirmed by two expert neurologists (ASH and MSH) based on MDS clinical criteria for clinically established PD (Postuma et al. 2015). Different potential clinical findings of patients were evaluated based on a variety of clinical rating scales including Unified Parkinson's Disease Rating Scale 1, 2, and 3 (UPDRS1, 2, and 3) (Goetz et al. 2007), Hoehn and Yahr (1967), the Schwab and England Activities of Daily Living scale (Schwab 1969), EuroQol 5D 5L (EQ-5D5L) (Group TE 1990), Epworth sleepiness scale (Johns 1994), Alertness section of MSQ Mayo Clinic Sleep scale (Boeve et al. 2011), Freezing of gait scale (Boeve et al. 2011), Non-Motor Symptom scale (NMSS) (Boeve et al. 2011), Beck Anxiety Inventory (BAI) (Leentjens et al. 2011), Beck Depression Inventory (BDI) (Leentjens et al. 2011), Frontal Assessment Battery (FAB) (Leentjens et al. 2011), Montreal Cognitive Assessment (MoCA) (Nasreddine et al. 2005), and Starkstein Apathy Scale (Starkstein et al. 1992). Early-onset PD was considered the initiation of the primary symptoms before 50 years of age. Among the patients with EOPD, 12 families with at least two affected individuals were enrolled in the present study. Selected families had a history of consanguinity and mainly were from eastern parts of Iran, and they belonged to different ethnicities including Fars, Kurd, and Baluch. All participants provided their written informed consent to participate in this study. Content and procedures of written information consent were inspected thoroughly within the ethics approval procedure. The study protocol adhered to the Declaration of Helsinki. The patients' genomic DNA

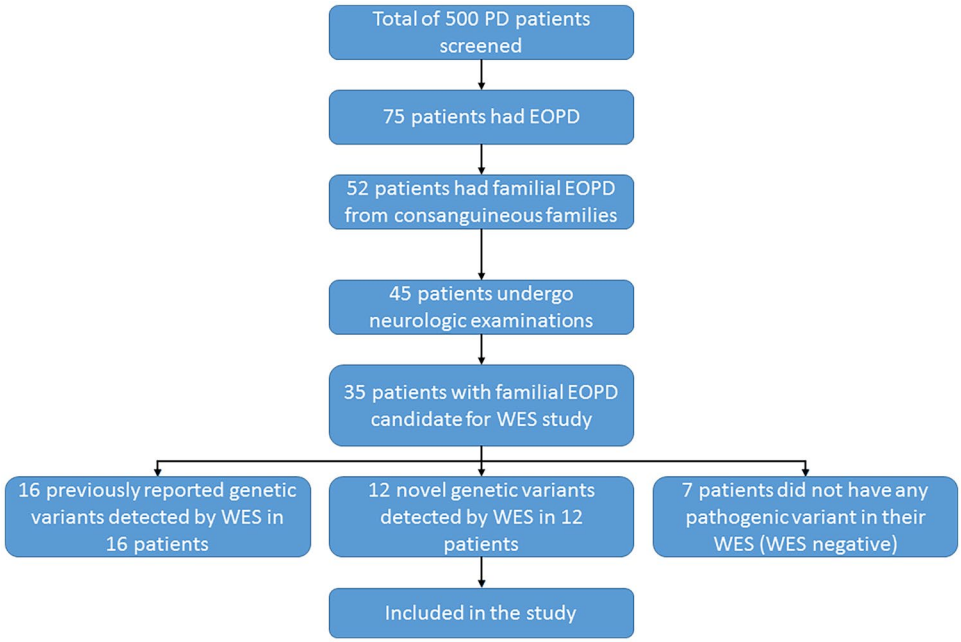


Fig. 1 The study flowchart indicating the final number of reported novel and previously known genetic variants causing PD

were isolated from whole blood, using standard procedures (Miller et al. 1988).

Whole-Exome Sequencing

The probands (one affected individual per family) underwent whole-exome sequencing WES at the sequencing core of Helmholtz Center Munich (Munich, Germany). Paired-end 100-bp libraries were prepared from lymphocyte-derived genomic DNA and captured using the SureSelect Human All Exon v6 kit (Agilent Technologies, CA, USA) according to the manufacturer's protocol. Samples were run on a HiSeq4000 (Illumina, CA, USA), generating on average ~ 10 Gb of uniquely mapped reads. Average coverage was ~ 100-fold, with ~ 97% of the target sequences covered at least 20-fold. Data processing was performed with the validated in-house pipeline, which implements Burrows-Wheeler-Aligner for sequence mapping (GRCh37/hg19) and SAMtools, GATK, ExomeDepth (CNV detection), and custom scripts for variant calling and annotation (Plagnol et al. 2012a). CNV detection was performed by ExomeDepth (ver 1.1.15) R software package. The software uses read depth for calling CNVs from BAM files and compared with our reference set. The reference set for the ExomeDepth considered 10 healthy individuals with Iranian decent (Plagnol et al. 2012b). Copy number variations formally known as deletion, duplication, and normal copies in every exon and the Bayes factor representing the likelihood ratio of CNVs to normal copies were calculated. CNVs with high Bayes factor were more likely to be true positive. Common CNVs among patients and reference were excluded. The CNVs were interpreted based on the Database of Genomic Variants and Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources (DECIPHER). After quality control and removing sequencing artifacts, we filtered data for non-synonymous variants and insertions-deletions (indels). The minor allele frequency (MAF) threshold was considered 0.1%, using control data from gnomAD (Karczewski et al. 2020), and in-house exome collections consisted of 20,000 samples with mean age of 49.6 years (male:female of 1:2). Prioritized variants were visually verified with the Integrative Genomics Viewer and the analysis did not screen repeat expansions. At first, every gene listed in the Parkinson's Disease and Complex Parkinsonism (version 1.108) panel was screened (the genes were listed in Supplementary Table 1) (Martin et al. 2019). In the next step, to check other possible variants, we performed the filter algorithm based on the frequency of the remaining variant, its impact, and pathogenicity. In order to evaluate the clinical significance of variants, publicly available databases including the Human Gene Mutation Database (HGMD) (Stenson et al. 2003), Varsome (Kopanos et al. 2019), and ClinVar (Landrum et al. 2018) were used. Pathogenicity prediction tools including Polymorphism Phenotyping v2 (PolyPhen-2) (Adzhubei et al. 2013), Sorting Intolerant from Tolerant (SIFT) (Sim et al. 2012), MutationTaster2 (Schwarz et al. 2014), Mutation Assessor (Reva et al. 2007), PROVEAN (Choi and Chan 2015), and Combined Annotation Dependent Depletion (CADD) (Rentzsch et al. 2019) were used to predict the impact of variants on structure and function in protein and messenger RNA (mRNA). For splice site variants, MaxEntScan (http://hollywood.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html) algorithm was used which implements the maximum entropy distribution

approach. The delta consensus values of 20% or more is considered significant based on empirical studies (Houdayer et al. 2008). Also, all the candidate variants were assessed for population frequency in the Iranome database, which includes information on the frequency of variants in the data of 800 exome cases from the eight main ethnic groups in Iran (http://www.iranome.ir/). Variants considered likely related to the patient's phenotype were confirmed by Sanger sequencing and the identified variants were sequenced in all available family members.

Sanger Sequencing and Segregation Analysis

Probands and at least three members per family were sequenced for variant validation and segregation studies. All the primers were designed by online Primer3 software, and PCR products were amplified under standard conditions. ExoSAP-IT™ (Applied Biosystem, Foster City, CA, USA) was used to purify the amplicons. Sanger sequencing was performed on the ABI 3500 genetic analyzer platform (Applied Biosystems) using the Big-Dye Terminator v3.1 Cycling Sequencing Kit (Applied Biosystems). Sequence data were analyzed and aligned with reference sequences using SeqScape™ Software v4.0 (Thermo Fisher Scientific, Waltham, MA, USA) and Chromas Lite v2.01 software.

Results

Among the patients with familial EOPD, most were male (9 versus 3 patients), and the mean ± standard deviation age of the study participants was 37.08 ± 6.06 years. Most of the participants were non-smokers (84%), non-alcohol users (92%), non-caffeine users (84%), and non-opium users (92%). Genetic variants and the results of clinical findings of patients with novel mutations are summarized in Table 1.

WES of 12 consanguineous EOPD families revealed 12 homozygous variants in three genes linked to autosomal recessive PD. All the probands were offspring of consanguineous marriages. Among the 12 pedigrees, six families (50%) had homozygous variants in *PRKN* gene (c.8del, c.171G > C, c.412 + 1G > T, c.356_357del, c.429C > A, and c.534 + 1G > T), two families (17%) had homozygous variants in *PARK7* gene (c.192 + 1G > C and c.181del), and homozygous variants in *PINK1* gene (c.669del, c.1223del, c.1124-2A > T, and c.488del) were found in four families (33%). Half of all variants were frameshift deletion causing a premature termination site in the protein sequence. One third of all patients had splice site substitution that disrupts the mechanism of exon–intron splicing with delta consensus values greater than 20% (Supplementary Table 2). Also, we demonstrated that two families (17%) had a missense variant and a family harboring a nonsense variant which makes a premature stop codon. All the 12 patients had novel homozygous variants in *PRKN*, *PARK7*, and *PINK1* genes. According to ACMG criteria, only one family was found with a likely pathogenic variant (c.171G > C) and the remaining families had pathogenic variants (Tables 2 and 3). There were no CNVs detected in Parkinson-related genes among the study population. The patient's family pedigrees apparently were autosomal recessive and these inheritance patterns were approved by the variants in the mentioned genes. Furthermore, segregation analysis by Sanger sequencing in at least three available members of each family demonstrated strict genotype–phenotype correlation for all variants. Family pedigrees and the status of their genotype in related members are shown in Fig. 2 and Supplementary Fig. 1.

The patients' genetic variants and their clinical phenotypes based on the questionnaires score and demographic data are summarized in Table 1.

Discussion

The present study revealed 12 novel genetic variants in *PINK1*, *PARK7*, and *PRKN* genes in 12 Iranian consanguineous families with EOPD. The present study revealed that most of the patients with EOPD had variants in *PRKN* gene. *PRKN* encodes a protein which is a component of multiprotein E3 ubiquitin ligase complex. Mutation in *PRKN* gene has been linked to the development of PD and autosomal recessive PD. Although different mutations inherited as homozygous or compound heterozygous forms cause PD phenotype, rare heterozygous mutations have also been related to an increased risk of developing EOPD (Lesage et al. 2008). Similar findings have been reported for heterozygous variants in other genes, including *PINK1*, which was the second most common gene with pathogenic variant (Marder et al. 2010). Patients carrying homozygous or compound heterozygous mutations in *PRKN* develop cardinal signs of PD at an earlier age. These patients may develop further clinical symptoms including dystonia or show coexisting comorbidities including depression and other psychiatric disorders (Hayashi et al. 2000). Even the patients in the same family may develop variable PD phenotype following the loss of *PRKN* function (Hayashi et al. 2000). Therefore, the patients may show different ages of onset, progression, presentations, and even responses to treatment with the same genetic mutation (Hayashi et al. 2000). Lücking et al.'s study on EOPD patients carrying *PRKN* gene mutation demonstrated that patients with missense mutations did not have different clinical manifestations from those with truncating mutations, suggesting that other genetic or environmental factors may contribute to the PD phenotype of patients (Lücking et al. 2000). Although the initial mutations of

Table 1 New genetic variations and their corresponding clinical findings

#	Gene	Variant	Gender	Age of onset	Schwab	EQ-5D5L	Epworth	Alertness	Freezing of gait	NMS	BAI	BDI	UPDRS1	UPDRS2	UPDRS3	Hoehn and Yahr stage	FAB	MoCA	Starkstein apathy
1	PRKN	c.8del p.Val3GlyfsTer41	M	34	50	50	1	6	4	4	0	3	15	8	64	0	3	2	0
2		c.171G>C p.Gln57His	F	31	80	40	7	10	4	11	12	5	8	7	22	2	11	20	8
3		c.412+1G>T ——	M	39	90	80	6	9	0	1	2	2	0	3	27	0	13	24	8
4		c.356_357del p.Leu119ArgfsTer7	M	30	80	80	2	10	1	1	6	6	4	2	16	2	16	21	7
5		c.429C>A p.Tyr143Ter	M	38	90	85	3	7	3	4	9	4	6	8	33	0	9	28	12
6		c.534+1G>T ——	M	33	80	80	8	5	9	9	16	14	8	15	55	2	10	26	26
7	PARK7	c.192+1G>C ——	F	42	80	50	8	10	8	7	28	21	15	17	53	4	11	22	5
8		c.181del p.Ala61GlnfsTer28	M	39	40	40	14	8	1	12	11	6	13	12	38	3	0	17	28
9	PINK1	c.669del p.Asn223LysfsTer12	M	44	70	45	4	6	2	4	13	8	7	4	34	4	12	16	11
10		c.1223del p.Gly408AlafsTer6	F	38	60	35	8	5	3	2	6	5	6	11	29	0	10	17	21
11		c.1124-2A>T ——	M	37	70	40	6	8	7	3	14	11	4	9	37	3	9	19	17
12		c.488del p.Gly163ValfsTer22	M	40	70	50	5	7	10	5	10	6	7	6	22	2	7	15	9

Table 2 List of genetic variants identified in 12 Iranian families

Family ID	Gene	Location	Transcript	cDNA	Protein	Zygosity	Novelty	ACMG	ACMG criteria
F01	PRKN (PARK2)	Chr6 162,864,505	NM_004562.3	c.8del	p.Val3GlyfsTer41	Hom	Yes	Pathogenic	PVS1, PM2, PP1
F02		Chr6 162,864,342		c.171G > C	p.Gln57His	Hom	Yes	Likely pathogenic	PM1, PM2, PP1, PP3
F03		Chr6 162,683,556		c.412 + 1G > T	——	Hom	Yes	Pathogenic	PVS1, PM2, PP1
F04		Chr6 162,683,612		c.356_357del	p.Leu119ArgfsTer7	Hom	Yes	Pathogenic	PVS1, PM2, PP1
F05		Chr6 162,622,268		c.429C > A	p.Tyr143Ter	Hom	Yes	Pathogenic	PVS1, PM2, PP1
F06		Chr6 162,622,162		c.534 + 1G > T	——	Hom	Yes	Pathogenic	PVS1, PM2, PP1
F07	PARK7(DJ1)	Chr1 8,025,486	NM_001123377.1	c.192 + 1G > C	——	Hom	Yes	Pathogenic	PVS1, PM2, PP1
F08		Chr1 8,025,474		c.181del	p.Ala61GlnfsTer28	Hom	Yes	Pathogenic	PVS1, PM2, PP1
F09	PINK1	Chr1 20,964,616	NM_032409.3	c.669del	p.Asn223LysfsTer12	Hom	Yes	Pathogenic	PVS1, PM2, PP1
F10		Chr1 20,975,094		c.1223del	p.Gly408AlafsTer6	Hom	Yes	Pathogenic	PVS1, PM2, PP1
F11		Chr1 20,974,996		c.1124-2A > T	——	Hom	Yes	Pathogenic	PVS1, PM2, PP1
F12		Chr1 20,964,435		c.488del	p.Gly163ValfsTer22	Hom	Yes	Pathogenic	PVS1, PM2, PP1

Hom, homozygous

PRKN gene were large homozygous deletions, missense and small deletion/insertion have also been reported in the literature (Mata et al. 2004). A study in the Japanese population demonstrated that among 184 PD patients, *PRKN* gene deletions are present in 40% of patients younger than 40 with a family history of PD. They also demonstrated that none of PD patients older than 40 did not carry *PRKN* gene deletions. Moreover, half of the patients with PRKN-related PD did not have any consanguinity and heredity (Ujike et al. 2001). Our study demonstrated that half of the Iranian families with EOPD had homozygous variants in *PRKN* gene (c.8del, c.171G > C, c.412 + 1G > T, c.356_357del, c.429C > A, and c.534 + 1G > T) which have not been previously reported in other populations. Although genetic variants in *PRKN* have not been widely studied in the Iranian population, studies on other populations including Hispanics reported that 255delA is the second most common mutation in PD patients. Ten percent of Hispanic PD patients who were younger than 40 or had a recessive pattern for PD had 255delA homozygous mutation in the *PRKN* gene (Muñoz et al. 2002). Other ethnic populations, including the Caribbean Hispanic population, did not report such a frameshift mutation, suggesting the 255delA mutation as an ancestral European mutation (Periquet et al. 2001).

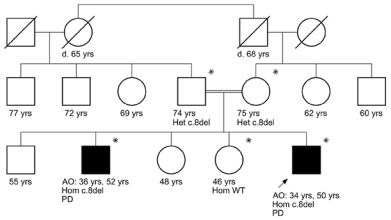
The *PINK1* was the second gene carrying novel variants in our Iranian population. *PINK1* is the second most common gene responsible for autosomal recessive EOPD after *PRKN* (Gandhi and Plun-Favreau 2017). The gene encodes a protein called *PTEN*-induced putative kinase 1 which is located in the mitochondria. The two specific regions of this protein, which are the kinase domain and mitochondrial-targeting motif domains, are crucial for the appropriate function of the protein in human cells (Zhou et al. 2008). Most of the genetic alterations in *PINK1* eliminating the kinase domain lead to loss of function of the protein and development of mitochondrial malfunction. Our study demonstrated four patients with novel homozygous mutations in *PINK1* (c.669del, c.1223del, c.1124-2A > T, and c.488del) which none of them were previously reported in the literature. All of the four variants were located in the kinase domain of *PTEN* protein which is linked to the development of PD phenotype. Either homozygous or compound heterozygous mutations in *PINK1* mutation cause a disease resembling sporadic PD with akinetic rigidity responding to levodopa (Gandhi and Plun-Favreau 2017). Similar to *PRKN*

Table 3 Allele frequencies and pathogenicity scores for all variants identified

Family ID	Gene transcript	Variant	gnomAD	Iranome	PhyloP score	PolyPhen-2	SIFT	Mutation-Taster	Mutation-Assessor	PROVEAN	CADD score
F01	PRKN (PARK2) NM_004562.3	c.8del p.Val3GlyfsTer41	NF	NF	7.205	NA	NA	D	NA	NA	35.0
F02		c.171G > C p.Gln57His	NF	NF	3.487	PD	Dam	D	H	D	32.0
F03		c.412 + 1G > T ——	NF	NF	4.524	NA	NA	D	NA	NA	27.0
F04		c.356_357del p.Leu119ArgfsTer7	NF	NF	5.378	NA	NA	D	NA	NA	42.0
F05		c.429C > A p.Tyr143Ter	NF	NF	3.459	NA	NA	D	NA	NA	31.0
F06		c.534 + 1G > T ——	NF	NF	4.216	NA	NA	D	NA	NA	29.5
F07	PARK7(DJ1) NM_001123377.1	c.192 + 1G > C ——	NF	NF	8.221	NA	NA	D	NA	NA	31.2
F08		c.181del p.Ala61GlnfsTer28	NF	NF	6.720	NA	NA	D	NA	NA	44.0
F09	PINK1 NM_032409.3	c.669del p.Asn223LysfsTer12	NF	NF	2.341	NA	NA	D	NA	NA	30.3
F10		c.1223del p.Gly408AlafsTer6	NF	NF	8.946	NA	NA	D	NA	NA	46.1
F11		c.1124-2A > T ——	NF	NF	8.349	NA	NA	D	NA	NA	32.0
F12		c.488del p.Gly163ValfsTer22	NF	NF	8.299	NA	NA	D	NA	NA	40.6

NF not found, *NA* not available, *PD* probably damaging, *D* deleterious, *H* high

Family: 01



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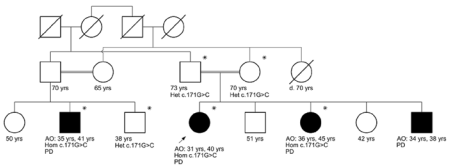

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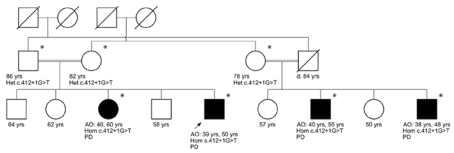

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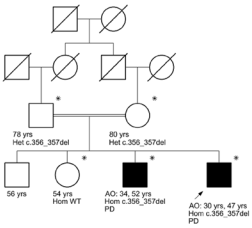

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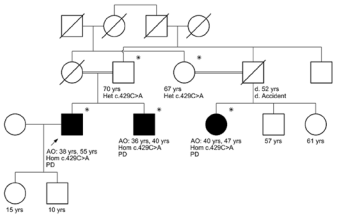

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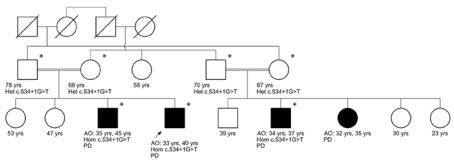

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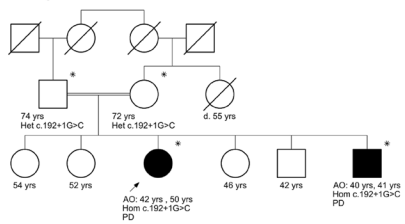

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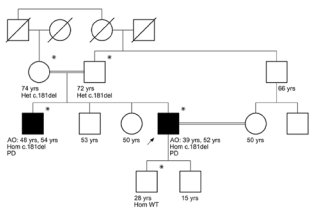

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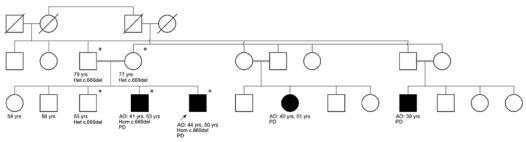
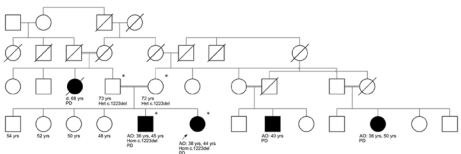
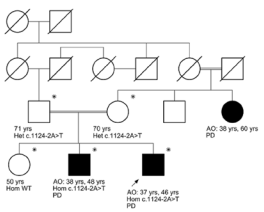


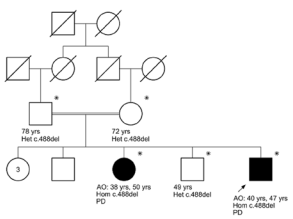
Family: 10



Family: 11



Family: 12



◂**Fig. 2** Pedigrees showing genotype–phenotype co-segregation for all detected variants. AO, age of onset; WT, wild type allele; Hom, homozygous; Het, heterozygous. *Identified individuals that were sequenced in segregation analysis by Sanger sequencing

mutations, patients with heterozygous mutations in *PINK1* are more likely to develop PD phenotype. However, in contrast to homozygous patients, the heterozygous tend to have milder clinical PD phenotype and later age of onset (Hedrich et al. 2006). Homozygous mutations in *PINK1* genes causing PD have been reported in multiple cohort studies on different populations. A study on 414 PD patients who were negative for *PRKN* mutations from 13 Asian countries demonstrated that 10 PD patients from 9 families had PINK1 mutations. The frequency of homozygous mutation in *PINK1* in families with autosomal recessive PD was reported to be 4.26% (Kumazawa et al. 2008). Other large studies from Europe demonstrated similar findings regarding the role of homozygous mutations of *PINK1* in the development of EOPD (Valente et al. 2004, 2001).

The third gene carrying novel genetic variants in EOPD was *PARK7*. Alongside *PRKN* and *PINK1*, *PARK7* is a gene that is responsible for most cases of autosomal recessive PD in a common biological pathway (Taipa et al. 2016). The gene encodes DJ-1 causing levodopa-responsive parkinsonism with juvenile or early onset (Delva et al. 2021). Although the mechanism of developing PD in patients carrying *PARK7* mutations is not widely studied, there are some different activities suggested for the normal DJ-1 protein. The main function of the protein is acting as a glyoxalase for restoring the function of proteins damaged by oxidative stress (Andreeva et al. 2019). Moreover, the protein plays an important role in maintaining mitochondrial function in responding to reactive oxygen species and also protects neurons from oxidative stress (Dolgacheva et al. 2019). PD patients with *PARK7* mutations may have disrupted oxidative stress response to electron transport chain dysfunction in the mitochondrion. Therefore, the negative effect of reactive oxygen species and dyshomeostasis of iron results in damaging of dopaminergic neurons (Chin et al. 2021). While only two patients (17%) in our study carried novel mutations in the *PARK7* gene (c.192 + 1G > C and c.181del), other studies reported much less prevalence. The prevalence of EOPD caused by *PARK7* has been reported to be less than 1% in the USA (Kasten et al. 2018). Both the missense and truncating mutations in *PARK7* cause complete lack or inactivation of the DJ-1 protein which is responsible for specific PD phenotype with early leg dystonia, slow disease progression, and favorable response to levodopa (Bonifati et al. 2003). Most of the patients affected with *PARK7* mutations age between 24 and 40 years but earlier presentations have also been reported (Taipa et al. 2016). Similar to sporadic PD, *PARK7*-related EOPD patients may develop dense Lewy bodies in intralaminar regions of the thalamus and predominant Lewy bodies in deep cortical layers (Taipa et al. 2016).

Limitations

Although analysis of exome data is not the accurate way of studying CNVs, further molecular diagnostic techniques including multiple ligation probe assay (MLPA) (although both using ExomeDepth and MLPA study copy numbers, using MLPA is more accurate) and genomic array should be considered to validate the absence of CNVs. One of the limitations of our study is the lack of using such confirmatory methods because of financial shortcoming. Moreover, for the splice site variants, and variants that have not finished complete co-segregation analysis within corresponding families, the computational studies were considered, and alongside of the other reported variants in our population, we believe that further functional studies and replication in larger samples for confirming our findings are warranted.

In conclusion, this paper represents 12 new mutations in patients with EOPD. The present study is the first research on patients with EOPD in the Iranian population, which has a high rate of consanguineous marriages. Further researches with bigger sample sizes are recommended to investigate the prevalence of these new mutations in different regions of Iran and to clarify their phenotype-genotype association.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12031-022-02085-9.

Author Contribution Mohammad Soudyab and Ariane Sadr-Nabavi designed the study; Reza Jafarzade Esfehani conducted data collection and the main literature search and wrote the first draft of the paper. Mohammad Soudyab and Vahid Nouri were responsible for statistical analyses. Mohammad Shariati, Ariane Sadr-Nabavi, Neda Shalaei, and Shabnam Vafadar developed the conceptual idea of the paper. Michael Zech and Julianne Winkelmann provided critical feedback on the manuscript, and suggested additional analyses and critical revisions. Ali Shoeibi and Ariane Sadr-Nabavi edited the manuscript for clarity and precision. All authors read and approved the final version of the manuscript.

Availability of Data and Materials DNA sequencing data have been deposited in the Academic Center for Education, Culture, and Research (ACECR) archive and will be made available upon reasonable request for academic use and within the limitations of the provided informed consent by the corresponding author upon acceptance. Every request will be reviewed by the board of the ACECR; the researcher will need to sign a data access agreement with the ACECR after approval.

Declarations

Ethics Approval and Consent to Participate The present study has been approved by the ethics committee of Mashhad University of Medical Sciences (IR.MUMS.MEDICAL.REC.1397.650).

Competing Interests The authors declare no competing interests.

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