Eukaryotic translation initiation factor *EIF4G1* p.Ser637Cys mutation in a family with Parkinson's disease with antecedent essential tremor

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Abstract. Essential tremor (ET) and Parkinson's disease (PD) are common chronic movement disorders that can cause a substantial degree of disability. However, the etiology underlying these two conditions remains poorly understood. In the present study, Whole-exome sequencing of peripheral blood samples from the proband and Sanger sequencing of the other 18 family members, and pedigree analysis of four generations of 29 individuals with both ET and PD in a nonconsanguineous Chinese family were performed. Specifically, family members who had available medical information, including historical documentation and physical examination records, were included. A novel c.1909A>T (p.Ser637Cys) missense mutation was identified in the eukaryotic translation initiation factor 4γ1 (*EIF4G1*) gene as the candidate likely responsible for both conditions. In total, 9 family members exhibited tremor of the bilateral upper limbs and/or head starting from ages of ≥40 years, 3 of whom began showing evidence of PD in their 70s. Eukaryotic initiation factor 4 (eIF4)G1, a component of the translation initiation complex eIF4F, serves as a scaffold protein that interacts with many initiation factors and then binds to the 40S ribosomal subunit. The *EIF4G1* (p.Ser637Cys) might inhibit the recruitment of the mRNA to the ribosome. In conclusion, the results from the present study suggested that *EIF4G1* may be responsible for the hereditary PD with 'antecedent ET' reported in the family assessed.

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Introduction

Parkinson's disease (PD) has a prevalence of 0.5-1% after 65 years of age and 1-3% after 80 years of age worldwide (1). The main clinical symptoms of PD include heterogeneous motor (including tremor at rest, bradykinesia, rigidity and postural instability) and non-motor signs (including cognitive decline, depression, anxiety, dysautonomia, sleep disturbances and anosmia) with heterogeneous pathological characteristics (including mild-motor predominant PD, diffuse malignancies PD and intermediate PD) (2,3). Essential tremor (ET) is defined as an action tremor lasting for ≥3 years, which primarily involves both upper limbs and can be with or without tremors in other locations. ET mainly occurs in the absence of other neurological disorders, such as dystonia, ataxia or parkinsonism (4). Systematic review and meta-analysis revealed that the prevalence of ET increased with advancing age, and the global prevalence of ET was 2.87% in people aged ≥80 years (5,6). Notably, according to the Consensus Statement on the Classification of Tremors, individuals with long-term ET may eventually develop other neurological disorders, such as dystonia or PD (4).

Although PD is considered to be a sporadic disorder, 10-30% patients with PD report having a first-degree family members with PD (7). Previous linkage and sequence analyses performed in patients with familial PD have identified *SNCA*, *LRRK2*, *GIGYF2*, *VPS35*, eukaryotic translation initiation factor 4γ1 (*EIF4G1*), *DNAJC13*, *CHCHD2* and *TMEM230* as autosomal dominant pathogenic genes, and *PARK2*, *PINK1*, *DJ-1*, *ATP13A2*, *PLA2G6*, *FBXO7*, *DNAJC6*, *SYNJ1* and

VPS13C as autosomal recessive pathogenic genes (8-10). Additionally, other genes in which mutations have been shown to be associated with PD include *UBTF*, *GRN*, *FAM171A2*, *PODXL* and *PTRHD1*, and mutations in *RAB39B* have been reported to cause X-linked PD (11,12). A systematic analysis performed in patients with sporadic PD identified autosomal dominant deleterious mutations in the *SNCA*, *LRRK2*, *GIGYF2*, *VPS35*, *EIF4G1*, *DNAJC13* and *CHCHD2* genes (13). In particular, although mutations in the *EIF4G1* gene have been identified in both patients with familial and sporadic PD, the role of *EIF4G1* in PD etiology remains elusive.

The *EIF4G1* gene is located on chromosome 3q27.1 and covers an ~20.8-kb genomic region with 31 coding exons (14). It encodes a 1,599-amino acid eIF4G1 protein, which is abundantly and ubiquitously expressed as a subunit of the translation initiation complex eIF4F. eIF4G1 serves as a scaffold protein that interacts with poly(A)-binding protein, eIF3, eIF4E, the RNA helicase eIF4A and the 40S ribosomal subunit (15). It serves an important role in signal transduction (16), cell growth and mortality (17) and the translation of mRNAs associated with these aforementioned processes (18). Overexpression of the eIF4G1 protein has been associated with several malignant disorders in humans (such as breast cancer, lung cancer, multiple myeloma, pancreatic ductal adenocarcinoma and chronic lymphocytic leukemia), whereas decreased eIF4G1 protein expression results in the reduction of overall protein and reduced ATP production (19). Furthermore, the degradation of eIF4G1, triggered by the activation of calpain, has been reported to result in decreased protein synthesis and increased neuronal cell death after ischemic injury *in vitro* (20).

Clinically, ET is defined by the presence of isolated action tremors, whereas PD is characterized by the presence of bradykinesia with either resting tremor or rigidity (21). Patients with longstanding ET who later develop a PD phenotype are thereby referred to as having PD with 'antecedent ET' (22). Previous studies have been conducted on patients with familial PD harboring *EIF4G1* mutations; these patients are clinically characterized by a relatively long, mild course and retain a high level of cognition (23,24).

In the present study, a linkage and sequence analysis was performed on a Chinese family with members exhibiting ET or PD with antecedent ET. A novel mutation in EIF4G1 was discovered in familial cases of ET and PD, thereby broadening the range of pathogenic mutations associated with these conditions.

Materials and methods

Clinical characteristics. The ethical review board of the Affiliated Hospital of Jining Medical University (Jining, China) approved the study protocol (approval no. 2023-09-C031), and written informed consent was obtained from all participating subjects. Between April and June 2023, data on the family history of all the members comprising this family was gathered. A total of 29 members were in this family (two members are deceased), including 15 males and 14 females. All living members ranged in age from 4-75, with a median ages of 37±21.25. In this investigation, a cohort of 19 individuals who underwent exome sequencing were ultimately enrolled, while individuals who were deceased or did not undergo genetic sequencing were excluded from the analysis. Neurological examinations were performed by three neurologists specializing in movement disorders without knowledge of the participants' genotype. The assessment of motor function in the nervous system encompassed evaluations of muscle strength, tone, involuntary movements, coordination (including finger-nose tests, rapid alternating movements, heel-knee-tibia tests, Romberg's sign and gait), as well as examinations of nerve reflexes (such as abdominal, biceps, triceps, radioperiosteal, knee, Achilles tendon, Babinski, Gordon, Oppenheim, Hoffmann, Kernig, Brudzinski and nuchal rigidity reflexes) and sensory function (including superficial, deep, and cortical sensations). The laboratory tests conducted encompassed blood routine analysis, electrolyte levels, liver and kidney function assessments, blood glucose monitoring, and ceruloplasmin evaluation.

Patients were diagnosed with ET according to the published Classification of Tremors by the Task Force on Tremor of the International Parkinson and Movement Disorder Society (4). Patients were diagnosed with PD based on the United Kingdom PD Society Brain Bank clinical diagnostic criteria (25), where severity was assessed using the Hoehn and Yahr scale (26) and the Movement Disorder Society-sponsored revision of the Unified PD Rating Scale Part III (UPDRS III) (27). Cognitive impairment was assessed using the Mini-Mental State Examination (MMSE) (28).

Whole-exome sequencing (WES). To identify the gene responsible for ET and PD in the family, 5 ml peripheral blood was collected from the most severely affected patient, Case II-2 and sent to Beijing Kangso Medical Inspection Co., Ltd. for WES. A FlexiGene DNA kit (cat. no. 51206; Qiagen China Co., Ltd.) was used to extract genomic DNA from the peripheral blood samples. Quality testing of the extracted DNA, library construction, hybrid capture and sequencing were performed as previously described (29). Agarose gel electrophoresis was used to assess the extent of DNA degradation, the presence of RNA, and protein contamination (data not shown). The DNA concentration was precisely quantified utilizing a Qubit 2.0 fluorometer (cat. no. 32866; Thermo Fisher Scientific, Inc.). The quantified genomic DNA was randomly sheared into 180-280 bp segments followed by adaptor ligation and cohesive end trimming. Next, the DNA library was amplified using the *Trans*NGS® Index Primers (384) Kit (cat. no. 3KI241; TransGen Biotech Co., Ltd.) according to the manufacturer's protocol: Initial denaturation was at 98˚C for 3 min; followed by 5 cycles of 30 sec at 98˚C, 35 sec at 60˚C and 30 sec at 72˚C, with a final extension at 72˚C for 3 min. The subsequent adaptor-specific primers were employed for the amplification of the DNA library: Forward, 5'-AATGATACGGCGACCACCGAGATCTACACTAGCTGCCACACTCTTTCCCTACACGACCTCTTCCGATC-s-T-3' and reverse 5'-CAAGCAGAAGACGGCATACGAGATTCCGCGAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3' (-s-represents a phosphorothioate bond). The PCR products were subsequently purified using *MagicPure* Size Selection DNA Beads (cat. no. EC401; TransGen Biotech Co., Ltd.) in accordance with the manufacturer's instructions. Subsequent DNA fragments were subjected to liquid-phase hybridization with up to 500,000 biotin-labeled Agilent SureSelect Human

All Exon V6 probes (cat. no. 5190-8863; Agilent Technologies, Inc.), followed by capture using streptomycin magnetic beads and amplification utilizing the SureSelect Target Enrichment System (Agilent Technologies, Inc.) under specific thermocycling conditions: Initial denaturation at 98˚C for 2 min; subsequent 15 cycles of 30 sec at 98˚C, 30 sec at 62˚C and 1 min at 72˚C, concluding with a final extension at 72˚C for 10 min. Adaptor-specific primers were employed for the amplification process: Forward, 5'-AATGATACGGCGACC ACCGA-3' and reverse primer, 5'-CAAGCAGAAGACGGC ATACGA-3'. The products were purified using *MagicPure* Size Selection DNA Beads as aforementioned. The effective concentration (3 nM) of the library was accurately quantified using the Qubit 2.0 fluorometer and an Agilent Technologies 2200 TapeStation qPCR (7500 Fast Dx Real-Time PCR Instrument; Thermo Fisher Scientific, Inc.). Subsequently, single-read sequencing was performed on a NextSeq500 (Illumina, Inc.).

Bioinformatics analysis. Data analysis was performed as described in previous studies (29,30). Alignment between sequencing reads and the human reference genome (version hg19) was performed using the Burrows-Wheeler Alignment tool (version 0.7.15; https://github.com/lh3/bwa). Single-nucleotide variants and small insertion or deletion variants were detected using the GATK (v3.6; https://www.broadinstitute.org). CODEX (v1.14.1, https://www.bioconductor.org/packages/release/bioc/html/CODEX.html), XHMM (v1.0, https://zzz.bwh.harvard.edu/xhmm/index.shtml) and Kangso Sequencing Copy Number Variation Detection Software v1.0 (Beijing Kangso Medical Inspection Co., Ltd.) were used to detect the possible copy number variations (31,32). The RefSeq (reference genome version, GRCH37/Hg19; https://www.ncbi.nlm.nih.gov/refseq), Ensembl (April 2021 update; https://www.ensembl.org/index.html) and UCSC Genome Browser (reference genome version, GRCH37/Hg19; https://genome.ucsc.edu) were employed for the annotation of genes in the present study. Frequencies of annotated variants in populations were investigated using the 1000G (2015 update; http://www.1000genomes.org), dbSNP (v150; https://www.ncbi.nlm.nih.gov/SNP), and ExAC (v0.3; ExAC is now in gnomAD; www.gnomad-sg.org) databases. Impacts of any mutations on eIF4E function were investigated using the SIFT (version 2; https://sift.bii.a-star.edu.sg), PolyPhen2 (version 2; http://genetics.bwh.harvard.edu/pph2) and MutationTaster (NCBI 37/Ensembl 69; http://www.mutationtaster.org) tools (33,34). The Online Mendelian Inheritance in Man (https://www.omim.org/), Human Gene Mutation (http://www.hgmd.org) and ClinVar databases (https://submit.ncbi.nlm.nih.gov/clinvar/) were used to perform disease-related annotations. SWISS-MODEL (http://swissmodel.expasy.org/interactive) was used to predict the secondary and tertiary structures of the mutated and wild-type eIF4F proteins. The American College of Medical Genetics and Genomics (ACMG) Variation Interpretation Guidelines were used to classify the variants (pathogenic, likely pathogenic, benign, likely benign, and variants of uncertain significance) and conduct clinical analyses (35).

Sanger sequencing verification. In total, 2 ml peripheral blood was collected from 19 participants and partners of Case II-1 and Case II-2, and sent to Beijing Kangso Medical Inspection Co., Ltd. for Sanger sequencing verification. The remaining family members (10/29) declined to be tested. According to the WES results of a severely affected patient, namely Case II-2, the c.1909A>T mutation in the *EIF4G1* gene was selected for further validation. Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast) was used for primer design (36) according to the gene sequence from GenBank (accession no. NM_198241). The primer sequences used were: Forward, 5'-CCGTGAGTTCCTGCTTGGTT-3' and reverse, 5'-CTTGCGTGGTTCTTTTCGGG-3' (Tianyi Huiyuan Biotechnology Co., Ltd.). The c.1909A>T mutation was amplified by PCR using a EasyTaq PCR SuperMix (cat. no. AS111; Beijing TransGen Biotech Co., Ltd.), under the following thermocycling conditions: Initial denaturation at 95˚C for 10 min; followed by 35 cycles of 30 sec at 95˚C, 30 sec at 60˚C and 45 sec at 72˚C; with a final extension at 72˚C for 5 min. The amplicons were subsequently subjected to Sanger sequencing utilizing an ABI 3730xl DNA analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.). The resultant sequences were then compared with the findings of WES, with false-positive variants identified through next-generation sequencing being omitted.

Whole-exome sequence accession numbers in ClinVar. The novel c.1909A>T (p.Ser637Cys) missense mutation in *EIF4G1* was deposited in ClinVar (https://submit.ncbi.nlm.nih.gov/clinvar; accession no. SCV004039569).

Imaging examinations. MRI examinations were conducted using a 3.0T MRI scanner (Ingenia CX; Philips Healthcare) with T1-weighted scan, T2-weighted scan and 3D fluid-attenuated inversion recovery performed in all 19 sequenced family members, with 8 symptomatic cases undergoing additional high-resolution three-dimensional (3D) susceptibility-weighted imaging (SWI) sequence. The presence or absence of the swallow tail sign (STS) was assessed in the cross-sectional images on the 3D SWI sequence in nigrosome-1, which is located within the dorsolateral substantia nigra (SN). Previous studies have shown that the absence of STS is both a highly specific and sensitive indicator for the presence of PD (37,38).

Results

Clinical features. The family lived in eastern China, and there were no consanguineous marriages in the family. There were a total of 29 individuals in this family, comprising 15 males and 14 females. The age range of living family members in the study varied from 4 to 75 years, with a median age of 37±21.25. Case II-1 and Case II-2 were admitted to the Affiliated Hospital of Jining Medical University for the first time in April 2023. In total, 19 individuals from this family spanning three generations were clinically assessed in May 2023 at the Affiliated Hospital of Jining Medical University and were genetically sequenced (Fig. 1). The first generation, consisting of two members, has passed away. A total of eight family members (III-6,9,11, IV-1,8-11) chose not to participate in the study. Based on the aforementioned diagnostic criteria, three patients were diagnosed with PD [I-2 (deceased), II-1,2] and six were diagnosed with ET (III-1-3,5,8, IV-2) in June 2023. All three

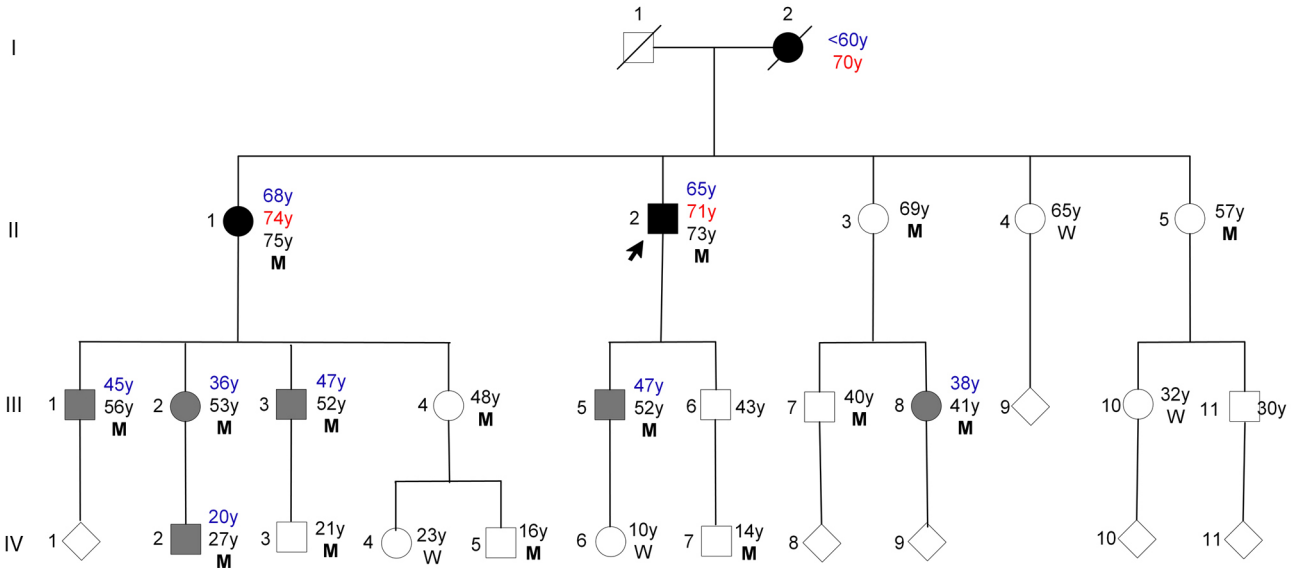

Figure 1. Pedigree of a family with ET and PD. Individuals with PD are represented with filled black shapes, whereas those with ET are represented with filled gray shapes. Numbers in blue indicate the age of onset of ET, whereas those in red indicate the age of PD onset. Numbers in black indicate the age of participants as of Jan 2024. A diagonal line in each shape signifies a deceased family member, with squares representing males, circles representing females and diamonds indicating undisclosed gender. The individual who underwent exome sequencing (II-2) is indicated with an arrow. ET, essential tremor; PD, Parkinson's disease; M, c.1909A>T (p.Ser637Cys) missense mutation carrier; W, wild-type.

patients with PD reported having a multiple-year history of tremors before the clinical appearance of PD signs.

Case I-2 died at the age of 75 years. According to the patient's grandchildren, they had trembling of the hands in their 60s. These symptoms then worsened as they advanced to >70 years of age, manifesting as involuntary shaking of limbs at rest, movement retardation and a tendency to fall. The patient was unable to take care of themself and was almost completely bedridden before death. Neither the individual's parents nor their three siblings had a similar disease history. Based on the patient's disease history, a diagnosis of clinically possible PD may be considered in accordance with the criteria established by the Movement Disorder Society (39).

Case II-1 is a 75-year-old woman who had been otherwise healthy until aged 68 years, when they first experienced action tremor in their hands, which has persisted thereafter (for 7 years). The past medical history of this patient included hypertension. The patient presented with postural tremor in the hands and hyposmia. However, one year prior to the hospital visit, at 74 years of age, the individual experienced the onset of resting tremor in the upper left limb, followed by the gradual development of resting tremor in the lower left limb and upper right limb. These symptoms were accompanied by mild bradykinesia, decreased arm swing during walking, a decline in short-term memory and loss of smell. PD was initially diagnosed in the patient at the age of 75 on June 5, 2023 at the age of 75 with a UPDRS III score of 35 and Hoehn-Yahr stage II when they were completely independent. The patient was initially treated with oral levodopa (50 mg) and benserazide hydrochloride (12.5 mg) three times daily in June 2023. Due to the suboptimal control of resting tremor, the dosage of oral medication was escalated to levodopa (100 mg) and benserazide hydrochloride (25 mg) after a two-week period. The patient underwent biweekly post-treatment monitoring. One month later, the patient responded well to medication with an UPDRS III score of 18 on July 3, 2023 at the age of 75.

Case II-2 appears as the most severely affected living patient within the present study. This 73-year-old male individual initially presented with tremor in his hands eight years ago, at the age of 65, prior to hospitalization. The medical history included a diagnosis of type II diabetes. Initially presented with intention tremor, the patient's symptoms exacerbated during periods of nervousness and were less noticeable at rest. Subsequently, two years prior to his hospitalization (at the age of 71), the patient developed resting tremor in his upper right limb, which subsequently extended to the lower right limb and upper left limb. These symptoms were accompanied by bradykinesia, a stiff facial expression, reduced arm swing during walking, shuffling gait and occasionally coughing when drinking water. PD was first diagnosed on June 12, 2023 at the age of 73 with a UPDRS III score of 45 and Hoehn-Yahr stage II when the patient was completely independent. The treatment regimen was identical to that received by his older sister, namely levodopa and benserazide hydrochloride. However, during the two subsequent follow-up visits, which occurred every two weeks, the patient exhibited poor response to the prescribed drug therapy with a UPDRS III score of 45 on July 9, 2023 at the age of 73. Subsequently, the patient was recommended undergoing a levodopa challenge test (LCT) or be treated with arotinolol to control the tremors. The patient refused LCT due to concerns about drug withdrawal reactions and adverse effects of LCT, but accepted the addition of arotinolol. Therefore, arotinolol (10 mg) was given once daily, which alleviated the tremors substantially according to a telephone follow-up after 2 weeks. At the 2-month follow-up after starting the treatment, the patient was satisfied with their symptom improvement with a UPDRS III score of 21 on August 6, 2023 at the age of 73.

Case III-1-3, 5, 8 and IV-2 all had action (postural and kinetic) tremors in their hands, with or without head tremors, which worsened with nervousness and disappeared with

Table I. Clinicopathological features of affected individuals within the family.

Individual ID	I-2	II-1	II-2	III-1	III-2	III-3	III-5	III-8	IV-2
Sex	F	F	M	M	F	M	M	F	M
Age at ET onset, years	<60	68	65	45	36	47	47	38	20
Age at PD onset, years	70	74	71	-	-	-	-	-	-
Age at examination, years	/	75	73	56	53	52	52	41	27
Disease duration, years	>15	7	8	11	17	5	5	3	7
Resting tremor	+	+	+	-	-	-	-	-	-
Action tremor	+	+	+	+	+	+	+	+	+
MMMSE scoring	/	22	23	26	28	24	30	30	30
UPDRS III scoring	/	35	45	-	-	-	-	-	-
Hoehn-Yahr stage	/	II	II	-	-	-	-		-
Concomitant diseases	/	Hypertension	Type II diabetes mellitus	-	-	-	-	-	-

'/' denoted absence of testing, while '-' signified a negative outcome. ET, essential tremor; MMSE, Mini-Mental State Examination; PD, Parkinson's disease; UPDRS III, Unified PD Rating Scale Part III; F, female; M, male.

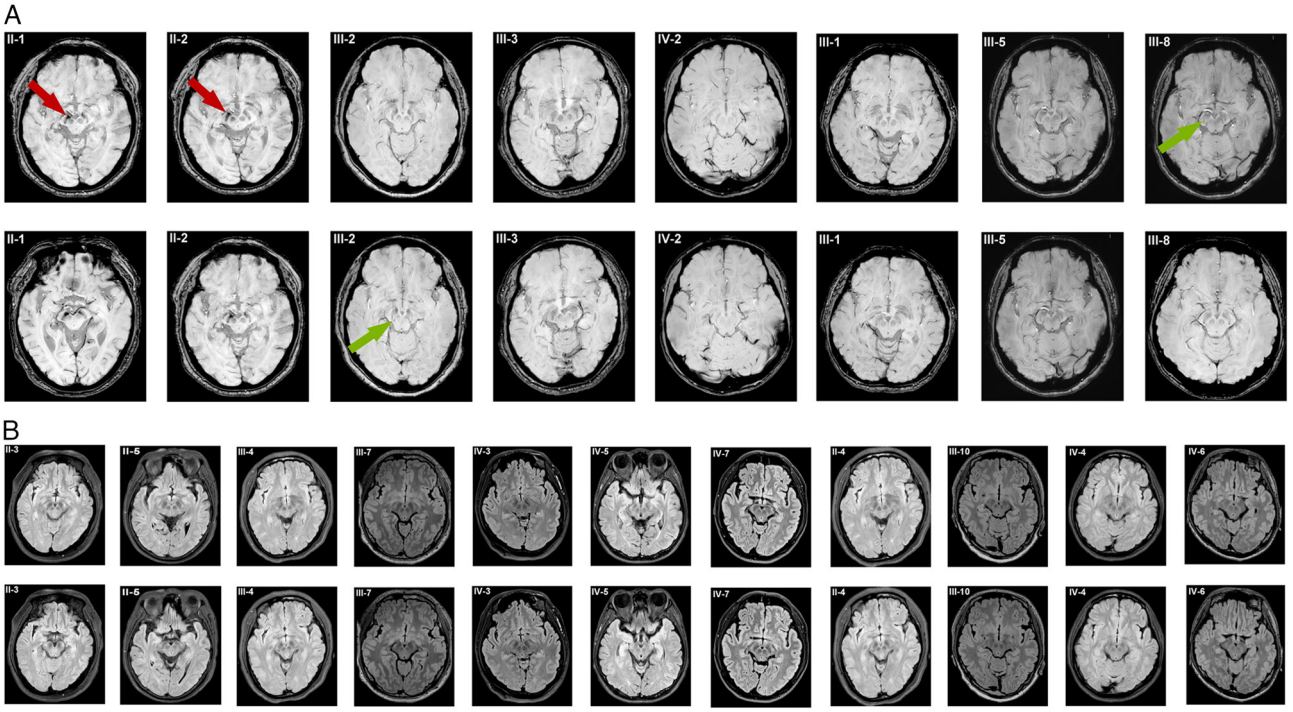

Figure 2. The cranial 3T MRI of 19 included family members. (A) Cranial 3T MRI with high-resolution 3D susceptibility-weighted imaging findings for Cases II-1, -2, III-1-3, -5, -8, and IV-2 in the same family. Cases II-1 and Case II-2 showed reduced signals in the SN region and a typical loss of the swallow tail sign. Case III-2 showed slightly reduced signal in the SN with an identified swallow tail morphology. Case III-3 showed slightly reduced signal in the SN with a faint swallow tail morphology. Cases III-1, III-5, -8 and IV-2 showed normal hyperintense signals in the SN region and a clear swallow tail morphology. (B) Cranial 3T MRI with high-resolution 3D fluid-attenuated inversion recovery findings for other family members (II-3-5; III-4, -6, -7 and -9-11; IV-1 and -3-7) did not show any obvious pathognomonic alterations. SN, substantia nigra. The red arrow points to the SN region and the green arrow points to the STS region.

drinking alcohol, but did not show clinical manifestations of PD. The time to action tremor onset in these patients was earlier (at 40-50 years of age) compared with their parents and grandparents. The other individuals (II-3, 4, 5; III-4, 7, 10; and IV-3, 4, 5, 6, 7) did not show any clinical manifestations of ET and/or PD. The remaining 8 members (III-6, 9, 11, IV-1 and 8-11) declined participation in the study and consequently did not undergo WES, laboratory tests and MRI examination, precluding an analysis of their respective conditions.

Neurological assessments of all participants indicated that Case II-1 exhibited mild bradykinesia, diminished arm swing during ambulation and cognitive decline in short-term

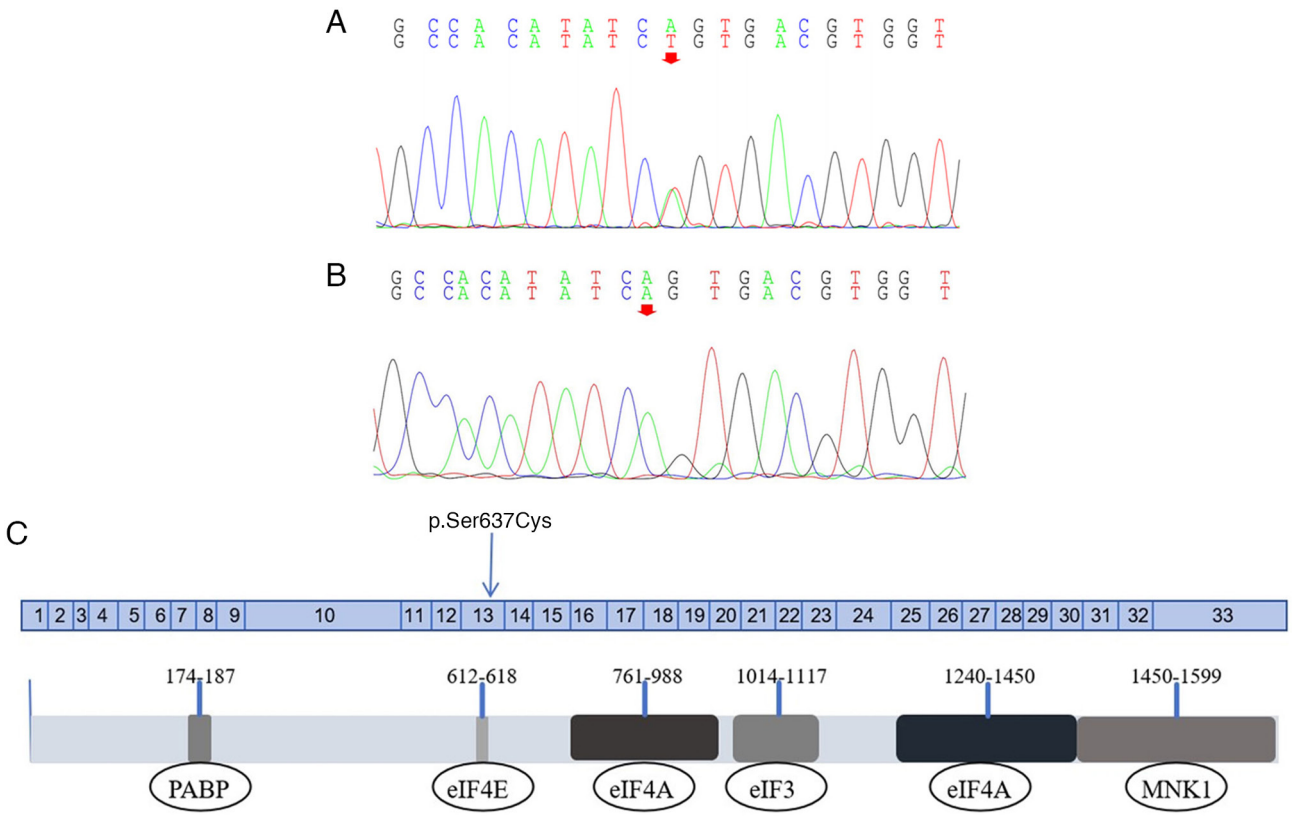

Figure 3. Sanger sequencing results. (A) c.1909A>T missense mutation in the *EIF4G1* gene from Case II-2. (B) A wild-type *EIF4G1* gene from partners of Case II-2. EIF4G1, eukaryotic translation initiation factor 4γ1. (C) Diagram of the novel mutation c.1909A>T (p.Ser637Cys) in the *EIF4G1* gene.

memory. Case II-2 displayed bradykinesia, facial rigidity, decreased arm swing during walking and a shuffling gait, while Cases III-1-3, 5, 8 and IV-2 demonstrated action tremors in the hands, with or without accompanying head tremors and tremors could exacerbated by anxiety. All patients had normal tendon reflexes, negative pathological signs and normal limb sensations. Examination of the cranial nerves revealed that Case II-1 exhibited anosmia and the other patients had no abnormalities. General physical examinations and routine laboratory tests did not reveal any obvious pathognomonic alterations. The MMSE results showed that none of the patients had cognitive impairment. The information of 8 symptomatic patients (2 patients with PD and 6 patients with ET) and 1 deceased patient with PD are listed in Table I, whereas those of the other asymptomatic patients are not listed.

Imaging features. The results of cranial 3T MRI of 19 included family members are displayed in Fig. 2. Both Case II-1and II-2 showed multiple ischemic degeneration foci, old infarct foci with softening foci in the bilateral cerebral hemispheres and basal ganglia. There was also evidence of bilateral hemispheric atrophy and lateral periventricular white matter degeneration. Specifically, Case II-1 displayed a significant decrease in signal intensity in both SN regions, along with absence of the bilateral STS. In contrast, Case II-2 showed a notable reduction in signal intensity in the right substantia nigra and a slight decrease in signal intensity in the left substantia nigra, accompanied by bilateral disappearance of the STS.

Case III-2 showed small degenerative foci of the right frontal lobe, slightly reduced signal in the bilateral SN and a visually identified STS. Case III-3 showed ischemic degeneration foci in the center of the right semiovale and slightly reduced signal in the bilateral SN, with a faintly present STS. Case IV-3 showed no abnormalities and a positive bilateral STS. Cranial 3T MRI (Fig. 2) did not reveal any obvious pathognomonic alterations in the other family members.

Gene discovery. WES was performed in Case II-2. A missense mutation was identified in the *EIF4G1* gene, specifically c.1909A>T in exon 13 on chr3:184040722 (Fig. 3). Using Sanger sequencing, a total of 15 family members, namely II-1-3 and -5; III-1-5,-7 and -8; and IV-2,-3,-5 and-7, were found to carry the same heterozygous missense mutation in *EIF4G1*. No other family members, nor the partners of Case II-1 and Case II-2, had this c.1909A>T variant. This mutation resulted in a serine-to-cysteine substitution at position 637 (Ser637Cys), near the eIF4E binding region (Fig. 3C). To the best of our knowledge, this variant has not been previously reported in the Online Mendelian Inheritance in Man, Human Gene Mutation or ClinVar databases. However, no changes in the secondary or tertiary structure were predicted at the protein level according to SWISS-MODEL.

Subsequent SIFT and PolyPhen2 analyses indicated that the p.Ser637Cys mutation is potentially 'Deleterious' and 'Possibly damaging', respectively. MutationTaster software identified the Ser637Cys mutation as a 'disease causing' mutation. According to the ACMG criteria (35), this variant was considered as having uncertain significance. In particular, it was categorized as pathogenic moderate (PM1; located in a mutational hot spot and/or critical and well-established

functional domain; e.g., active site of an enzyme, without benign variation), as it was located in a mutational hot spot and/or critical and well-established functional domain, such as the active site of an enzyme, without benign variation. Furthermore, it was categorized as benign supporting 4, since there were multiple lines of computational evidence suggesting no impact on the gene or gene product in terms of conservation, evolutionary and splicing impact.

Discussion

In the present study, the clinical details of a family of patients with ET or PD with antecedent ET are described. There are 29 members in this family, where the 2 members in the first generation have died. A total of 19 participants underwent a systematic physical examination, laboratory tests, imaging tests and genetic sequencing. At first, one of the deceased patients (Case I-2) was diagnosed with PD according to the description provided by their grandchildren. In addition, 2 members of the family were diagnosed with PD, both of whom had a ≥6-year tremor history before the PD signs appeared. By contrast, 6 family members were diagnosed with ET, with the other 11 family members currently asymptomatic. Consistent with the previously reported clinical characteristics of patients with *EIF4G1* variants, the patients in the present family showed late-onset PD with mild progression, cognitive preservation and a good response to L-levodopa treatment (23,40). However, unlike such previously reported cases, the patients with PD in the present family all had ET prior to PD onset.

Case II-1 and II-2 both showed reduced signals in the SN region and a typical loss of the STS in cranial 3T MRI with high-resolution 3D SWI. Nigrosome-1 is the largest cluster of dopaminergic neurons and is located in the dorsolateral SN (41). It is considered to be a hyperintense structure on high-resolution 3D SWI sequences on 3T MRI and is surrounded by hypointense portions in the SN and medial lemniscus (41). Such imaging features result in the appearance of an STS (42). Degeneration of nigrosome-1 in patients with PD results in the loss of normal hyperintense signals within the dorsolateral SN, which appears as an absence of the STS (43). However, the third-generation patients with ET in the present study showed slightly reduced signals in the SN with a faint or identifiable STS morphology. In the fourth-generation patients with ET, normal hyperintense signals in the SN region and the STS morphology were both clearly observed. Therefore, in the family in the present study, there is a gradual decrease in signal within the SN region and disappearance of the STS with increasing age and disease progression.

Further genetic testing revealed that all family members with confirmed PD or ET had the c.1909A>T (p.Ser637Cys) variant in the *EIF4G1* gene. The inheritance mode was consistent with it being autosomal dominant. Previously, mutations/variants in the *EIF4G1* gene were deemed to be associated with autosomal dominant PD (ADPD) (22,23). The missense mutation p.Arg1205His in *EIF4G1* was found to segregate with disease in a large French family with ADPD. This variant was found to perturb eIF4G1-eIF3E binding, which is a crucial step in recruiting the 40S ribosomal subunit (23,44). In another study, the p.Ala502Val variant has been shown to disrupt eIF4G1-eIF4E binding, which may affect the binding of mRNA to ribosomes (22,45). Subsequently, p.E462delInsGK was identified in two affected siblings as a possible novel disease-causing agent of ADPD (46). The discovery of variants p.Gly686Cys in sporadic PD patients and p.Arg1197Trp in healthy individuals challenges the assumed pathogenicity characteristic of these variants (47). In another study with 975 patients with PD and 1,014 healthy controls, novel non-synonymous variants p.Thr318IIe, p.Val541Gly and p.Gly698Ala were predicted to be 'probably or possibly damaging' by Polyphen2, whereas p.Gly698Ala was predicted to be 'disease causing' by MutationTaster (47). In the family of the present study, although the p.Ser637Cys mutation was considered as having uncertain significance according to the ACMG criteria, it was predicted to be 'possibly damaging' by Polyphen2 and 'disease causing' by MutationTaster.

In addition, the p.Arg1205His mutation was also identified in three healthy controls (47), which raises questions regarding the potential of the *EIF4G1* variant to cause PD. Subsequent studies in patients with sporadic PD from different ethnic backgrounds, including Asian (48,49), African (50) and European (51) showed mutations in the *EIF4G1* gene are not a significant or frequent risk factor for Parkinson's disease. However, the incomplete penetrance of p.Arg1205His in the *EIF4G1* gene was similar to the p.Gly2019Ser mutation in the *LRRK2* gene for late-onset PD, which should be considered (52). Therefore, members of the family in the present study who were harboring the p.Ser637Cys variant but did not show any clinical manifestations of PD may have incomplete penetrance of the *EIF4G1* variant. In addition, the onset of PD is age-dependent (53) and most family members in the present study had not reached the average age of onset (age at onset 61.7; standard deviation ±8.57) for late onset PD in 26 Italian families (24). Therefore, members in this family will require regular annual follow-ups for the next 20 years.

A variant of the *PARK1* gene (p.Ala53Thr) was previously identified in members of a family afflicted with PD, which showed Mendelian segregation. This suggests that PD may have a strong genetic component (54). To date, ~30 genes have been reported to be associated with PD (55). Genome-wide association studies and candidate gene association studies have collectively identified ≥90 common variants of independent loci that modify disease risk (56). In addition, accumulating evidence has suggested that exposure to various toxins and changes in dietary habits may affect the occurrence and progression of PD (57). Studying this complex interplay between genetic and environmental factors will improve the understanding of the pathophysiology of PD. The present study reported the clinical features and sequencing analysis results of a family with ET or PD with 'antecedent ET'. The emergence of a new mutation in *EIF4G1* in a family with tremors suggested that the role of *EIF4G1* variants in familial PD should be considered.

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Availability of data and materials

The c.1909A>T (p.Ser637Cys) missense mutation in *EIF4G1* generated in the present study may be found in ClinVar under accession no. SCV004039569 or at the following URL: (https://submit.ncbi.nlm.nih.gov/clinvar/). The other data generated in the present study may be requested from the corresponding author.

Authors' contributions

RHL, YK and QXK designed the study. XYX, LY, YYJ, XCW and JG collected the data. RHL, YK and XYX contributed to the data analysis and interpretation. RHL drafted the manuscript. YK and QXK contributed to the revision of the manuscript. XYX and LY confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by The Ethics Committee of The Affiliated Hospital of Jining Medical University (approval no. 2023-09-C031; Jining, China). All participants provided written informed consent to participate.

Patient consent for publication

All participants provided written informed consent for the publication of any associated data as well as any accompanying images.

Competing interests

The authors declare that they have no competing interests.

References

1. Nussbaum RL and Ellis CE: Alzheimer's disease and Parkinson's disease. N Engl J Med 348: 1356-1364, 2003.
2. Hayes MT: Parkinson's disease and Parkinsonism. Am J Med 132: 802-807, 2019.
3. Fereshtehnejad SM, Zeighami Y, Dagher A and Postuma RB: Clinical criteria for subtyping Parkinson's disease: Biomarkers and longitudinal progression. Brain 140: 1959-1976, 2017.
4. Bhatia KP, Bain P, Bajaj N, Elble RJ, Hallett M, Louis ED, Raethjen J, Stamelou M, Testa CM and Deuschl G; Tremor Task Force of the International Parkinson and Movement Disorder Society: Consensus Statement on the classification of tremors. From the task force on tremor of the International Parkinson and Movement Disorder Society. Mov Disord 33: 75-87, 2018.
5. Song P, Zhang Y, Zha M, Yang Q, Ye X, Yi Q and Rudan I: The global prevalence of essential tremor, with emphasis on age and sex: A meta-analysis. J Glob Health 11: 04028, 2021.
6. Louis ED and Ferreira JJ: How common is the most common adult movement disorder? Update on the worldwide prevalence of essential tremor. Mov Disord 25: 534-541, 2010.
7. de Lau LM and Breteler MM: Epidemiology of Parkinson's disease. Lancet Neurol 5: 525-535, 2006.
8. Clarimón J, and Kulisevsky J: Parkinson's disease: From genetics to clinical practice. Curr Genomics 14: 560-567, 2013.
9. Funayama M, Ohe K, Amo T, Furuya N, Yamaguchi J, Saiki S, Li Y, Ogaki K, Ando M, Yoshino H, *et al*: CHCHD2 mutations in autosomal dominant late-onset Parkinson's disease: A genome-wide linkage and sequencing study. Lancet Neurol 14: 274-282, 2015.
10. Lesage S, Drouet V, Majounie E, Deramecourt V, Jacoupy M, Nicolas A, Cormier-Dequaire F, Hassoun SM, Pujol C, Ciura S, *et al*: Loss of VPS13C function in autosomal-recessive Parkinsonism causes mitochondrial dysfunction and increases PINK1/Parkin-Dependent mitophagy. Am J Hum Genet 98: 500-513, 2016.
11. Nalls MA, Blauwendraat C, Vallerga CL, Heilbron K, Bandres-Ciga S, Chang D, Tan M, Kia DA, Noyce AJ, Xue A, *et al*: Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: A meta-analysis of genome-wide association studies. Lancet Neurol 18: 1091-1102, 2019.
12. Puschmann A: New genes causing hereditary Parkinson's disease or Parkinsonism. Curr Neurol Neurosci Rep 17: 66, 2017.
13. Yang N, Zhao Y, Liu Z, Zhang R, He Y, Zhou Y, Xu Q, Sun Q, Yan X, Guo J and Tang B: Systematically analyzing rare variants of autosomal-dominant genes for sporadic Parkinson's disease in a Chinese cohort. Neurobiol Aging 76: 215.e1-215.e7, 2019.
14. Yan R and Rhoads RE: Human protein synthesis initiation factor eIF-4 gamma is encoded by a single gene (EIF4G) that maps to chromosome 3q27-qter. Genomics 26: 394-398, 1995.
15. Jackson RJ, Hellen CU and Pestova TV: The mechanism of eukaryotic translation initiation and principles of its regulation. Nat Rev Mol Cell Biol 11: 113-127, 2010.
16. Raught B, Gingras AC, Gygi SP, Imataka H, Morino S, Gradi A, Aebersold R and Sonenberg N: Serum-stimulated, Rapamycin-sensitive phosphorylation sites in the eukaryotic translation initiation factor 4GI. EMBO J 19: 434-444, 2000.
17. Ramírez-Valle F, Braunstein S, Zavadil J, Formenti SC and Schneider RJ: eIF4GI links nutrient sensing by mTOR to cell proliferation and inhibition of autophagy. J Cell Biol 181: 293-307, 2008.
18. Haimov O, Sehrawat U, Tamarkin-Ben Harush A, Bahat A, Uzonyi A, Will A, Hiraishi H, Asano K and Dikstein R: Dynamic interaction of eukaryotic initiation factor 4G1 (eIF4G1) with eIF4E and eIF1 Underlies Scanning-Dependent and -independent translation. Mol Cell Biol 38: e00139-18, 2018.
19. Howard A and Rogers AN: Role of translation initiation factor 4G in lifespan regulation and age-related health. Ageing Res Rev 13: 115-124, 2014.
20. Vosler PS, Gao Y, Brennan CS, Yanagiya A, Gan Y, Cao G, Zhang F, Morley SJ, Sonenberg N, Bennett MV and Chen J: Ischemia-induced calpain activation causes eukaryotic (translation) initiation factor 4G1 (eIF4GI) degradation, protein synthesis inhibition, and neuronal death. Proc Natl Acad Sci USA 110: 18102-18107, 2013.
21. Algarni M and Fasano A: The overlap between Essential tremor and Parkinson disease. Parkinsonism Relat Disord 46 (Suppl 1): S101-S104, 2018.
22. Tarakad A and Jankovic J: Essential Tremor and Parkinson's disease: Exploring the relationship. Tremor Other Hyperkinet Mov (NY) 8: 589, 2019.
23. Chartier-Harlin MC, Dachsel JC, Vilariño-Güell C, Lincoln SJ, Leprêtre F, Hulihan MM, Kachergus J, Milnerwood AJ, Tapia L, Song MS, *et al*: Translation initiator EIF4G1 mutations in familial Parkinson disease. Am J Hum Genet 89: 398-406, 2011.
24. Gialluisi A, Reccia MG, Modugno N, Nutile T, Lombardi A, Di Giovannantonio LG, Pietracupa S, Ruggiero D, Scala S, Gambardella S, *et al*: Identification of sixteen novel candidate genes for late onset Parkinson's disease. Mol Neurodegener 16: 35, 2021.
25. Gibb WR and Lees AJ: The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 51: 745-752, 1988.
26. Hoehn MM and Yahr MD: Parkinsonism: Onset, progression and mortality. Neurology 17: 427-442, 1967.
27. Goetz CG, Tilley BC, Shaftman SR, Stebbins GT, Fahn S, Martinez-Martin P, Poewe W, Sampaio C, Stern MB, Dodel R, *et al*: Movement disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): Scale presentation and clinimetric testing results. Mov Disord 23: 2129-2170, 2008.

28. Norris D, Clark MS and Shipley S: The mental status examination. Am Fam Physician 94: 635-641, 2016.
29. Wang XC, Liu RH, Wang T, Wang Y, Jiang Y, Chen DD, Wang XY, Hou TS and Kong QX: A novel missense mutation in SPAST causes hereditary spastic paraplegia in male members of a family: A case report. Mol Med Rep 27: 79, 2023.
30. Liu YD, Ma MY, Hu XB, Yan H, Zhang YK, Yang HX, Feng JH, Wang L, Zhang H, Zhang B, *et al*: Brain proteomic profiling in intractable epilepsy caused by TSC1 truncating mutations: A small sample study. Front Neurol 11: 475, 2020.
31. Fromer M, Moran JL, Chambert K, Banks E, Bergen SE, Ruderfer DM, Handsaker RE, McCarroll SA, O'Donovan MC, Owen MJ, *et al*: Discovery and statistical genotyping of copy-number variation from whole-exome sequencing depth. Am J Hum Genet 91: 597-607, 2012.
32. Jiang Y, Oldridge DA, Diskin SJ and Zhang NR: CODEX: A normalization and copy number variation detection method for whole exome sequencing. Nucleic Acids 43: e39, 2015.
33. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS and Sunyaev SR: A method and server for predicting damaging missense mutations. Nat Methods 7: 248-249, 2010.
34. Schwarz JM, Cooper DN, Schuelke M and Seelow D: MutationTaster2: Mutation prediction for the deep-sequencing age. Nat Methods 11: 361-362, 2014.
35. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, *et al*: Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17: 405-424, 2015.
36. Tsai FM, Lin YJ, Cheng YC, Lee KH, Huang CC, Chen YT and Yao A: PrimerZ: Streamlined primer design for promoters, exons and human SNPs. Nucleic Acids Res 35: W63-W65, 2007.
37. Liu X, Wang N, Chen C, Wu PY, Piao S, Geng D and Li Y: Swallow tail sign on susceptibility map-weighted imaging (SMWI) for disease diagnosing and severity evaluating in Parkinsonism. Acta Radiol 62: 234-242, 2021.
38. Kim DS, Tung GA, Akbar U and Friedman JH: The evaluation of the swallow tail sign in patients with Parkinsonism and gait disorders. J Neurol Sci 428: 117581, 2021.
39. Postuma RB, Berg D, Stern M, Poewe W, Olanow CW, Oertel W, Obeso J, Marek K, Litvan I, Lang AE, *et al*: MDS clinical diagnostic criteria for Parkinson's disease. Mov Disord 30: 1591-1601, 2015.
40. Deng H, Wu Y and Jankovic J: The EIF4G1 gene and Parkinson's disease. Acta Neurol Scand 132: 73-78, 2015.
41. Damier P, Hirsch EC, Agid Y and Graybiel AM: The substantia nigra of the human brain. I. Nigrosomes and the nigral matrix, a compartmental organization based on calbindin D (28K) immunohistochemistry. Brain 122: 1421-1436, 1999.
42. Cosottini M, Frosini D, Pesaresi I, Donatelli G, Cecchi P, Costagli M, Biagi L, Ceravolo R, Bonuccelli U and Tosetti M: Comparison of 3T and 7T susceptibility-weighted angiography of the substantia nigra in diagnosing Parkinson disease. AJNR Am J Neuroradiol 36: 461-466, 2015.
43. Schwarz ST, Afzal M, Morgan PS, Bajaj N, Gowland PA and Auer DP: The 'swallow tail' appearance of the healthy nigrosome-a new accurate test of Parkinson's disease: A case-control and retrospective cross-sectional MRI study at 3T. PLoS One 9: e93814, 2014.
44. Villa N, Do A, Hershey JW and Fraser CS: Human eukaryotic initiation factor 4G (eIF4G) protein binds to eIF3c, -d, and -e to promote mRNA recruitment to the ribosome. J Biol Chem 288: 32932-32940, 2013.
45. Sonenberg N and Hinnebusch AG: Regulation of translation initiation in eukaryotes: Mechanisms and biological targets. Cell 136: 731-745, 2009.
46. Lesage S, Condroyer C, Klebe S, Lohmann E, Durif F, Damier P, Tison F, Anheim M, Honoré A, Viallet F, *et al*: EIF4G1 in familial Parkinson's disease: Pathogenic mutations or rare benign variants? Neurobiol Aging 33: 2233.e1-2233.e5, 2012.
47. Schulte EC, Mollenhauer B, Zimprich A, Bereznai B, Lichtner P, Haubenberger D, Pirker W, Brücke T, Molnar MJ, Peters A, *et al*: Variants in eukaryotic translation initiation factor 4G1 in sporadic Parkinson's disease. Neurogenetics 13: 281-285, 2012.
48. Li K, Tang BS, Guo JF, Lou MX, Lv ZY, Liu ZH, Tian Y, Song CY, Xia K and Yan XX: Analysis of EIF4G1 in ethnic Chinese. BMC Neurol 13: 38, 2013.
49. Chen Y, Chen K, Song W, Chen X, Cao B, Huang R, Zhao B, Guo X, Burgunder J, Li J and Shang HF: VPS35 Asp620Asn and EIF4G1 Arg1205His mutations are rare in Parkinson disease from southwest China. Neurobiol Aging 34: 1709.e7-e8, 2013.
50. Blanckenberg J, Ntsapi C, Carr JA and Bardien S: EIF4G1 R1205H and VPS35 D620N mutations are rare in Parkinson's disease from South Africa. Neurobiol Aging 35: 445.e1-e3, 2014.
51. Gagliardi M, Annesi G, Tarantino P, Nicoletti G and Quattrone A: Frequency of the ASP620ASN mutation in VPS35 and Arg1205His mutation in EIF4G1 in familial Parkinson's disease from South Italy. Neurobiol Aging 35: 2422.e1-e2, 2014.
52. Kumari U and Tan EK: LRRK2 in Parkinson's disease: Genetic and clinical studies from patients. FEBS J 276: 6455-6463, 2009.
53. Marchetti B, Tirolo C, L'Episcopo F, Caniglia S, Testa N, Smith JA, Pluchino S and Serapide MF: Parkinson's disease, aging and adult neurogenesis: Wnt/β-catenin signalling as the key to unlock the mystery of endogenous brain repair. Aging Cell 19: e13101, 2020.
54. Lunati A, Lesage S and Brice A: The genetic landscape of Parkinson's disease. Rev Neurol (Paris) 174: 628-643, 2018.
55. Bandres-Ciga S, Diez-Fairen M, Kim JJ and Singleton AB: Genetics of Parkinson's disease: An introspection of its journey towards precision medicine. Neurobiol Dis 137: 104782, 2020.
56. Polymeropoulos MH, Higgins JJ, Golbe LI, Johnson WG, Ide SE, Di Iorio G, Sanges G, Stenroos ES, Pho LT, Schaffer AA, *et al*: Mapping of a gene for Parkinson's disease to chromosome 4q21-q23. Science 274: 1197-1199, 1996.
57. Pirooznia SK, Rosenthal LS, Dawson VL and Dawson TM: Parkinson disease: Translating insights from molecular mechanisms to neuroprotection. Pharmacol Rev 73: 33-97, 2021.