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Using whole exome sequencing in determining the genetic cause of Parkinson disease in an Iranian family

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Abstract

Objective: Parkinson's disease (PD) is the second most common neurodegenerative disorder. Identification of PD and Parkinson-plus genes will inform us for further elucidation of the disease mechanisms and therapeutic approaches. Accordingly, in this study, an Iranian pedigree with familial PD was selected to investigate the underlying genetic causes by whole exome sequencing (WES). *Methods:* WES was performed on two affected family members to identify shared pathogenic putative variants. WES finding was confirmed by Sanger sequencing in two sisters. *Results:* Prioritizing genes related to parkinsonism identified one homozygous pathogenic mutation: g.20655C>T, c.1366C>T (p.Q456X) on the 7th exon of *PINK1* gene. This homozygous C to T transition which introduces a premature termination codon (PTC) was predicted to be disease causing.
Conclusion: In the present study, we report the successful application of WES to identify the molecular pathogenesis of autosomal recessive PD, which is a genetically heterogeneous disorder. The presence of this mutation in patients with family history draws attentions to the importance of genetic counseling.

Keyword: Whole exome sequencing; Parkinson; *PINK1* gene

INTRODUCTION

After Alzheimer's disease, Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting about 4.5 million people over age 50 in 2005.[1-3] It is attributed to a progressive loss of dopaminergic neurons of the substantia nigra and is defined by tremor, rigidity, and slowness of movements.[4,5] The Origin of PD and its pathogenic mechanisms remained undetermined but mitochondrial dysfunction has been firmly incriminated in PD pathogenesis.[6] Over the last 18 years, the knowledge and classification of neurodegenerative disorders, including PD[2,5] has been changed.

It is evident that genetic factors play an important role in this disease. Families with inherited PD constitute 10–15% of all patients. They have been useful sources in analyzing the genetic etiology of this disease.[5,7] Familial PD was determined with well-defined exonic mutations in the genes *PARK2*, *SNCA*, *PARK7*, *PINK1*, *ATP13A2*, and *LRRK2*.[8] Accordingly, the most prevalent autosomal dominant PD is linked to mutations in the leucine-rich repeat kinase 2 genes (LRRK2), which was elucidated in over 10% of all familial forms.[1] Furthermore, the cause of familial autosomal recessive PD is ascribed to four genes, parkin (*PARK2*), *PINK1* (*PARK6*), *DJ- 1 (PARK7*), and *ATP13A2* (*PARK9*).[9] A key model in defining mitochondrial pathology in PD is the function loss in PTEN-induced putative kinase 1 (PINK1).[10] *PINK1* gene has 8 exons that encode the 581 amino acid protein phosphatase and tensin homolog (PTEN)-induced kinase 1 (*PINK1*).[11] *PINK1* is a serine/threonine kinase localized to mitochondria and apply a neuroprotective function.[10] Biochemical as well as genetic studies of *PINK1* and *PARKIN*, which their variants cause recessively inherited PD, demonstrate that these genes are produced and cooperate in the same pathway to guarantee mitochondrial integrity and function; supporting

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previous evidence of mitochondrial role in PD. PINK1 protein accumulates on the outer membrane of dysfunctional mitochondria, and with its kinase activity recruits parkin from the cytosol. Then, parkin gene ubiquitinates mitochondrial outer membrane proteins to mediate selective removal of labeled mitochondrion by autophagic machinery (mitophagy).[11]

The estimation of PD risk in over 500 nuclear families revealed that in up to 60% of idiopathic PD patients the phenotype could be elucidated by genetic factors.[12] Additionally, family based segregation of a pathological mutation can be followed longitudinally for translational clinical and therapeutic research; it can be of major importance for the discovery of disease modifiers or biomarkers. Identification of PD and Parkinson-plus genes will inform us for further elucidation of the disease mechanisms and therapeutic strategies.[13] Due to the high rate of consanguineous marriage in countries like Iran, the frequency of autosomal recessive forms of PD are probably higher than in western countries in which most researches has been done. Additionally, studying PD genetics in underrepresented populations can further clarify the underlying genetic etiology. Lim's study revealed differences in genetic factors and clinical presentation between PD in the Western Pacific Region and in Europe and North America.[14] Another study conducted in Middle East, North Africa, and South Asia (MENASA) in the field of PD showed that unique genotypes, environment factors, historical, geographical, and familial factors could affect the disease phenotype in these regions.[15] Ultimately, researchers will enlist the genetic classification to predict and prevent penetration of such genes into the next generation. In this study, an Iranian pedigree with familial PD was selected to investigate the underlying genetic causes through whole exome sequencing (WES).

METHODS

This study was carried out in accordance with the International Ethical Guidelines and Declaration of Helsinki and approved by ethics committee of Isfahan medical university. Informed consent was obtained from patients before the test.

Four of seven siblings (VI:7, VI:11, VI:12, VI:13), the product of consanguineous marriage (first cousin) from Isfahan province, Iran, diagnosed with PD were the subjects of this study. The proband (VI:13) also had an affected first cousin with a similar clinical presentation (Figure 1). After obtaining informed consent, members of family underwent a standardized

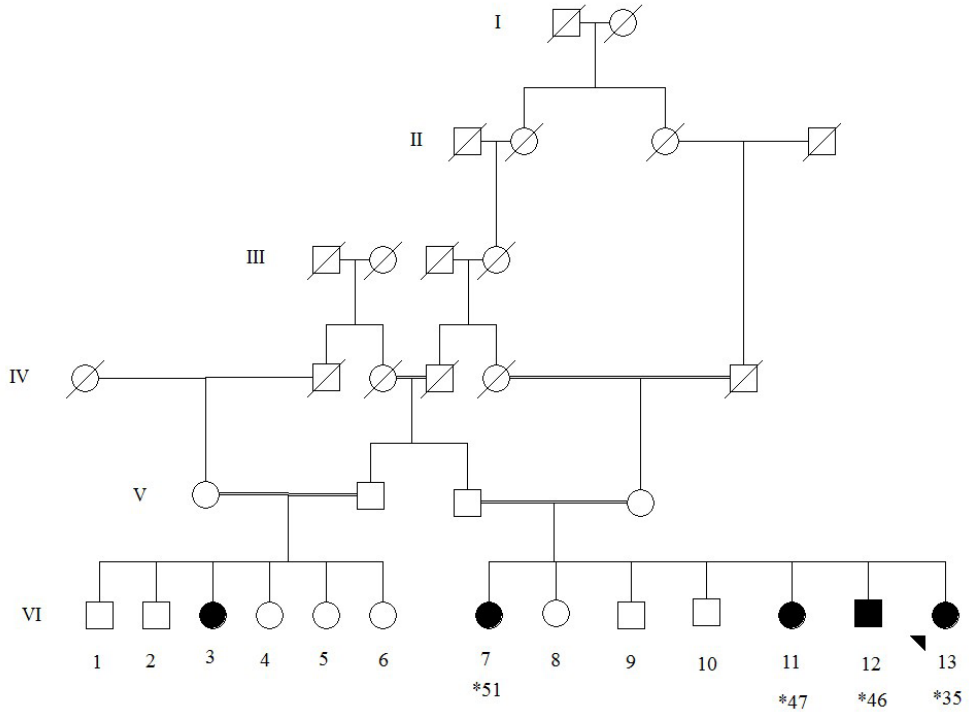

Figure 1. Autosomal recessive pattern of inheritance for the PD patients

neurological examination by a single and experienced neurologist. Diagnosis of PD was established according to clinical examinations, presence of resting tremor, bradykinesia, and/or muscle rigidity. In addition to these typical symptoms, clinical symptoms of two patients was presented as atypical features including one of them had history of ataxia and the other one showed dystonic tremor. When the patients were seem, they claimed that it did not take long time until their onset of symptoms. At baseline the Hoehn–Yahr scale (HY) in four stages of 1–1.5, 2–2.5, 3, and 4-5[16,17] and also motor part of the Unified Parkinson's Disease Rating Scale III (UPDRS III)[18] were used to rate off-phase parkinson symptoms. Secondary parkinsonism and other parkinson-plus syndromes were excluded.

Serum levels of 25OHD (we defined vitamin D insufficiency as a 25OHD concentration of less than 30.0 ng/mL and vitamin D deficiency as a 25OHD concentration of less than 20.0 ng/mL) and parathyroid hormone (PTH) (normal range: 10– 65 IU/L) were analyzed by enzyme immunoassay (Biomerica, CA, and IDS, UK). Other laboratory data of peripheral blood including calcium (normal range: 8.2–10.6mg/dL), phosphorus (normal range: 2.5–4.5mg/dL), and alkaline phosphatase (ALP) (normal range: 64–306mg/dL) were performed by spectrophotometric methods (Hitachi 902 autoanalyzer).

Genetic analysis

High quality genomic DNA was extracted from whole peripheral blood samples of two affected sisters (VI11 and VI13) by using Exgene Blood SV mini kit (Geneall Biotechnology Co Ltd, South Korea) and its quality and quantity determined using agarose gel and Nanodrop 2000 instrument (Thermo Fisher Scientific Inc., USA), respectively. WES was performed on two affected family members to identify shared pathogenic putative variants. Also, other affected members were not interested to take part in this study. DNA capturing and paired-end sequencing with 100X coverage was performed in macrogen company (South Korea). Coding regions were captured with Agilent SureSelect V5 Target Enrichment Kit (Agilent Technologies, Inc., Santa Clara, CA, USA) and sequenced with Illumina HiSeq 4000 platform (Illumina, San Diego, CA, USA). In the data analysis process, briefly, the variant analysis was done via mapping the FASTQ to the reference genome (UCSC hg19) using the Burrows-Wheeler Alignment software (http://bio-bwa.sourceforge.net/) with default parameters. Variants were called with Genome Analysis Tool Kit software (https://gatk.broadinstitute.org/) and annotated using Annovar software. Variants with a minor allele frequency (MAF) > 0.01 were filtered in databases such as exome aggregation consortium (ExAC) (http://exac.broadinstitute.org), 1000 genomes project phase 3 database (https://www.internationalgenome.org/), dbSNP version 147, HGMD (http://www.hgmd.cf.ac.uk/ac/index.php), exome sequencing project (ESP) (https://evs.gs.washington. edu/), and Iranome (http://www.iranome.ir/) to downstream analysis according to their chromosomal location, mode of inheritance, functional consequences, inheritance pattern, and clinical presentation. Computational predictive tools such as MutationTaster (http://www.mutationtaster.org/), PROVEAN (http://provean.jcvi.org/index.php), SIFT Indel (http://sift-dna.org/), DDIG Indel (http://sparks-lab.org/ddig/), and PANTHER (http://www.pantherdb.org/) were used to predict the pathogenicity of the detected variant.

Sanger sequencing

WES finding was confirmed by Sanger sequencing in two sisters (VI11 and VI13). The entire coding region of the exon 7 of *PINK1* gene was amplified by Forward primer: 5´ GAGAATGCAAGTCCTGTCAC 3´ and Reverse primer 5´ACTGCTAGGATTCTGACTATGC 3´.

RESULTS

Clinical and Paraclinical results

The patients show the presence of resting tremor, bradykinesia, and/or muscle rigidity. At baseline the Hoehn–Yahr scale (HY) in four stages of 1–1.5, 2–2.5, 3, and 4-5[16,17] and also motor part of the Unified Parkinson's Disease Rating Scale III (UPDRS III)[18] were used to rate off-phase parkinsonian signs. Secondary parkinsonism and other parkinson-plus syndromes were excluded. Serum levels of 25OHD and parathyroid hormone (PTH) were analyzed by enzyme immunoassay (Biomerica, CA, and IDS, UK). Other laboratory data of peripheral blood including calcium, phosphorus and alkaline phosphatase (ALP) were analyzed by spectrophotometric methods (Hitachi 902 autoanalyzer) (Table 1).

Whole Exome Sequencing results

WES of patients VI11 and VI13 identified a total number of 66,762,786 and 68,205,202 reads with

Table 1: Clinical characteristic and laboratory findings of patient's peripheral blood analysis

No	Tested	Age (Year)	Weight (Kg)	Height (cm)	UPDRSIII score	Hoehn & yahr score	Calcium (mg/dL)	Phosphor (mg/dL)	Alkaline Phosphatase(mg/dL)	PTH (IU/L)	25OH Vitamin D (ng/mL)
1	VI 7	51	73	150	14	1.5	9.6	3.2	193	52	24.1
2	VI 11	47	85	154	25	2	10	3.9	180	31	86.3
3	VI 12	46	84	179	20	1	9.4	3.7	136	49	78.3
4	VI 13	35	52	165	28	3	9.5	3.6	167	40	102.6

Parkinson's Disease Rating Scale III (UPDRSIII), PTH, Parathyroid hormone

the average 100 bp reads length respectively. Mean Depth of Target Regions was 74.4 and 78.0, while 74.3% and 75.7% of reads mapped to 50Mb target regions and 86.6% and 88.2% of target reads have sequencing depth more than 30X respectively.

A total number of variants in final VCF files of patients VI11 and VI13 were 88,507 and 89,349 respectively. An in house performed to find the genetic cause of Parkinson disease stepwise filtering process was done. According to the assumption that common variants with frequency >0.01 are not likely to be the causative pathogenic mutations, these variants filtered out at the first step. The common variants in two affected sisters, total number of 7373, were selected at the second step. According to variant effect's, synonymous, upstream / downstream and intronic variants except the 20-30bp flanking each exon, were excluded. Prioritizing genes related to Parkinsonism identified one homozygous pathogenic mutation: g.20655C>T, c.1366C>T (p.Gln456*) (rs45539432) on the 7th exon of *PINK1* gene. This homozygous C to T transition which introduces a premature termination codon (PTC) subsequently, was predicted to be disease causing/deleterious by Mutation Taster, conservative predictive tools such as phyloP and phastCons and CADD- Phred score was T: 37. Sanger sequencing results confirmed the inherited mutation in both affected family members (Figure 2).

This mutation has been reported previously as the causative mutation in autosomal recessive form of PD. This finding is consistent with the diagnosis of autosomal recessive form of PD. In addition, we found other variants related to PD in both sisters (Table 2). None of other found variants were met the criteria for further investigation due to genomic location, inheritance pattern, and pathogenicity score.

DISCUSSION

WES is a new emerging approach which facilitates the identification of causative genes in Mendelian disorders even when enough information about the mode of inheritance or confined clinical diagnosis is not available. In this study, WES followed by a stepwise in-house filtering process determined a pathogenic c.1366C>T (p.Q456X)(rs45539432) mutation on *PINK1* gene in an Iranian family with PD. The detected variant has been previously reported as the pathogenic variants in autosomal recessive early-onset PD.[19-21]

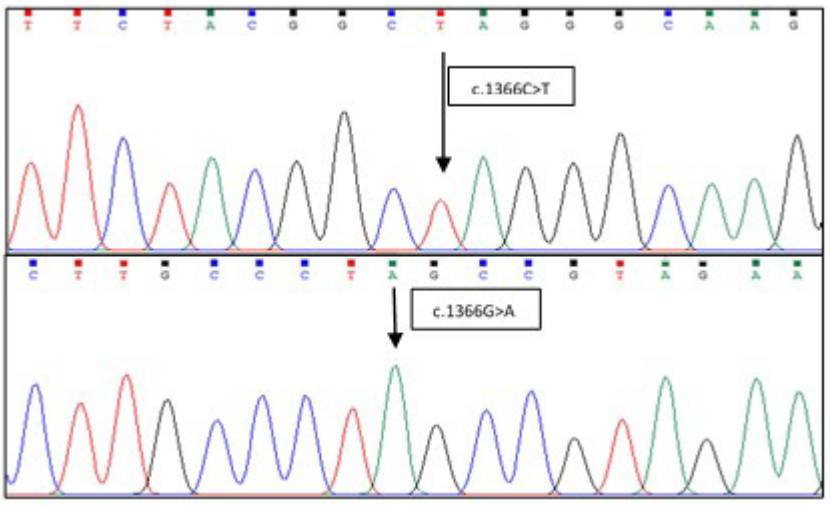

Figure 2. Inherited mutation in both affected family members by Sanger sequencing

The *PINK1* gene is related to early-onset of PD compatible with the early age of onset – 37 and 47- in two affected family members. This type is often indistinguishable from idiopathic form of PD in clinical manifestations including rigidity, bradykinesia, and rest tremor.[22]

This mutation was first described in one Italian family. Bonifati et al, Showed the absence of mutant mRNA in peripheral leukocytes and lymphoblastoid cell lines of heterozygous carriers, consistent with the lack of mutant allele expression or mRNA instability.[23] The pathogenic variant Q456X has been described in a large german family with 4 affected patients with varying onset age (between 39-61 years).[20] Furthermore, in Ishihara-Paul's *et al*. study, Q456X variant of *PINK1* was found in homozygote state in 4 affected individuals with PD.[21] The third report of this variant was revolving around a family with two affected members both of whom were in homozygous state for p.Q456X substitution.[19]

In this study, Q456X was rarely found in healthy controls and was not pathogenic in heterozygote carriers. Public databases were used for comparison. For example, the frequency of T as minor allele in ExAC, 1000 genomes, and GO-ESP was T=0.00002/3, T=0.0002/1 and T=0.00008/1 respectively. Moreover, the penetrance and pathogenicity in the described variant were complete, therefore they were not checked in other healthy family members.

Nonsense mutations Q456X cause premature stop codon in exon 7, and a reduction in PINK1 mRNA levels, which is likely caused by nonsense-mediated mRNA decay or predicted to truncate the C-terminus of the PINK1 protein especially kinase domain and the residue threonine 545 which is presumed to be the phosphorylation site. However, this leads to loss of quality control over defective mitochondrial and fidelity which requires the collaboration between PINK1 and PARK proteins. As a result mitochondrial bioenergetics respiration could be affected and subsequently it would result in reduced mtDNA levels, defective ATP production, impaired mitochondrial calcium handling, and increased free radical generation; taken together the susceptibility to apoptosis in neuronal cells would be increased.[24,25] Previous studies illustrated the different clinical phenotypes and the onset age in mutation carrier patients.[20] Most PD cases are considered as multifactorial entities; the interaction between multiple genetic and environment factors. However, 5–10% of patients are now known to follow monogenic forms of the disease and inherited in autosomal dominant or recessive pattern. Rarely. *Pink1* mutations associated with autosomal recessive forms of PD and it is the second most common cause of AR early onset PD and consequently two mutations, one on each allele, are required to manifest the phenotype. As carriers are clinically normal in autosomal recessive disease and regarding the high rate of consanguineous marriage in Iran, genetic counselors should be fully aware of the risk inherited in homozygosity in children. Additionally, the two patients in our study showed features, history of ataxia and dystonic tremor, which are non-typical features for autosomal recessive early-onset of PD. Severe and mild dystonia is previously reported as a symptom related to the L347P mutation in the PINK1 gene[26,27], but it is also reported in PARK2 and

Table 2: Other polymorphic variants with MAF<0.01 identified in genes related to Parkinson disease in the studied family

Gene	RefSeq	Genomic Location	Exon/ total exons	mRNA changes	AminoAcid changes	ID	1000G MAF	Zygosity
ATP13A2	NM_022089.3	chr1:17323070	12/28	c.1196-79C>T	.	rs183590584	A:0.0034/17	HET
DNAJC6	NM_001256864.1	chr1:65864329	13/18	c.2039-167T>A	.	.	.	HET
GIGYF2	NM_001103147.1	chr2:233636135	9/30	c.533-4987A>G	.	.	.	HET
GIGYF2	NM_001103147.1	chr2:233680403	21/31	c.2227G>A	p.Ala743Thr	.	.	HET
SNCA	NM_000345.3	chr4:90756550	2/5	c.121+148G>C	.	rs7681440	C: 40%	HET
PARK2	NM_004562.2	chr6:161970150	8/11	c.934-121_934-116delCACACA	.	rs111859679	-: 33%	HET
PARK2	NM_004562.2	chr6:161970150	8/11	c.934-121_934-118delCACA	.	rs68042180	- : 0.50	HET
TBP	NM_003194.4	chr6:170871037	3/8	c.216_218delACA	p.Gln73del	rs71815788	CAA: 25%	HOM
TBP	NM_003194.4	chr6:170871052	3/8	c.228G>A	p.Gln76Gln	rs112083427	A: 22%	HET
LRRK2	NM_198578.3	chr12:40703087	30/50	c.4317+52_4317+53insGT	.	rs10650388	**-0.49**	HOM
LRRK2	NM_198578.3	chr12:40753044	46/50	c.6844-18_6844-17insT	.	rs111612315	-: 41%	HOM
ATXN2	NM_002973.3	chr12:111993667	2/24	c.768+20C>T	.	.	.	HET
SYNJ1	NM_003895.3	chr21:34050939	12/31	c.1627+15delT	.	rs201012663	-: 14%	HET

The following abbreviations are used: MAF, Mutation Annotation Format

PARK14, and in atypical parkinsonism).[28] Ataxia, as a cerebellar sign, is reported in multiple system atrophy (MSA), which is a neurodegenerative disorder and one type of atypical parkinsonism.[28]

In conclusion, the current report is the first description of this variant in Iran. It both underlines the relevance of variant *PLINK1,* 1366 C>T in familial PD and the importance of genetic counseling as consanguinity in Iran is common.

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DISCLOSURES

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Conflict of interest: None

REFERENCES

1. Spatola M, Wider C. Genetics of Parkinson's disease: the yield. *Parkinsonism Relat Disord* 2014;20:S35-S38.
2. Sundal C, Fujioka S, Uitti RJ, Wszolek ZK. Autosomal dominant Parkinson's disease. *Parkinsonism Relat Disord* 2012;18:S7-S10.
3. Wider C, Skipper L, Solida A, *et al*. Autosomal dominant dopa-responsive parkinsonism in a multigenerational Swiss family. *Parkinsonism Relat Disord* 2008;14(6):465-70.
4. Davie CA. A review of Parkinson's disease. *Br Med Bull* 2008;86(1):109-27.
5. Liu Z, Guo J, Li K, *et al*. Mutation analysis of CHCHD2 gene in Chinese familial Parkinson's disease. *Neurobiol Aging.* 2015;3118. 36(11):3117. e7-3117.e8.
6. Fallaize D, Chin L-S, Li L. Differential submitochondrial localization of PINK1 as a molecular switch for mediating distinct mitochondrial signaling pathways. *Cell Signal* 2015;27(12):2543-54.
7. Verstraeten A, Theuns J, Van Broeckhoven C. Progress in unraveling the genetic etiology of Parkinson disease in a genomic era. *Trends Genet* 2015;31(3):140-9.
8. Gasser T. Genetics of Parkinson's disease. *Curr Opin Neurol* 2005;18(4):363-9.
9. Tomiyama H, Li Y, Yoshino H, *et al*. Mutation analysis for DJ-1 in sporadic and familial parkinsonism: screening strategy in parkinsonism. *Neurosci Lett* 2009;455(3):159-61.
10. Kostic M, Ludtmann MH, Bading H, *et al*. PKA phosphorylation of NCLX reverses mitochondrial calcium overload and depolarization, promoting survival of PINK1-deficient dopaminergic neurons. *Cell Rep* 2015;13(2):376-86.
11. Pickrell AM, Youle RJ. The roles of PINK1, parkin, and mitochondrial fidelity in Parkinson's disease. *Neuron* 2015;85(2):257-73.
12. Hamza TH, Payami H. The heritability of risk and age at onset of Parkinson's disease after accounting for known genetic risk factors. *J Hum Genet* 2010;55(4):241-3.
13. Hong KW, Oh B-S. Overview of personalized medicine in the disease genomic era. *BMB Rep* 2010;43(10):643-8.
14. Lim SY, Tan AH, Ahmad-Annuar A, *et al*. Parkinson's disease in the Western Pacific Region. *Lancet Neurol* 2019;18(9):865-79.
15. Khalil H, Chahine L, Siddiqui J, Aldaajani Z, Bajwa JA. Parkinson's disease in the MENASA countries. *Lancet Neurol* 2020;19(4):293-94.
16. Chitsaz A, Maracy M, Basiri K, *et al*. 25-hydroxyvitamin D and severity of Parkinson's disease. *Int J Endocrinol* 2013;2013:4.
17. Hoehn MM, Yahr MD. Parkinsonism: Onset, progression and mortality. *Neurology* 1967;17(5):427-42.
18. Goetz C. Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS): a new scale for the evaluation of Parkinson's disease. *Revue Neurologique* 2010;166(1):1-4. (in French)
19. Siuda J, Jasinska-Myga B, Boczarska-Jedynak M, *et al*. Early-onset Parkinson's disease due to PINK1 p. Q456X mutation–clinical and functional study. *Parkinsonism Relat Disord* 2014;20(11):1274-8.
20. Hedrich K, Hagenah J, Djarmati A, *et al*. Clinical spectrum of homozygous and heterozygous PINK1 mutations in a large German family with Parkinson disease: role of a single hit? *Arch Neurol* 2006;63(6):833-8.
21. Ishihara-Paul L, Hulihan M, Kachergus J, *et al*. PINK1 mutations and parkinsonism. *Neurology* 2008;71(12):896-902.
22. Kasten M, Kertelge L, Brüggemann N, *et al*. Nonmotor symptoms in genetic Parkinson disease. *Arch Neurol* 2010;67(6):670-6.
23. Bonifati V, Rohe C, Breedveld G, *et al*. Early-onset parkinsonism associated with PINK1 mutations Frequency, genotypes, and phenotypes. *Neurology* 2005;65(1):87-95.
24. Abramov AY, Gegg M, Grunewald A, Wood NW, Klein C, Schapira AHV. Bioenergetic consequences of PINK1 mutations in Parkinson disease. *PLoS One* 2011;6(10):e25622.
25. Gegg ME, Cooper JM, Schapira AH, Taanman J-W. Silencing of PINK1 expression affects mitochondrial DNA and oxidative phosphorylation in dopaminergic cells. *Plos One* 2009;4(3):e4756.
26. Tan AH, Lohmann K, Tay YW, *et al.* PINK1 p. Leu347Pro mutations in Malays: Prevalence and illustrative cases. *Parkinsonism Relat Disord* 2020;79:34-9.
27. Doostzadeh J, Tetrud J, Allen-Auerbach M, Langston J, Schüle B. Novel features in a patient homozygous for the L347P mutation in the PINK1 gene. *Parkinsonism Relat Disord* 2007;13(6):359-61.
28. Balestrino R, Schapira AH. Parkinson disease. *Eur J Neurol* 2020;27(1):27-42.