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PRINT: ISSN 0972-3757 ONLINE: ISSN 2456-6330

Int J Hum Genet, 23(2-3): 140-146 (2023)
DOI: 10.31901/24566322.2023/23.2-3.857

Mutational Analysis in *SNCA* and Chromosomal Aberration in Parkinson's Disease (PD) Patients of Tamil Nadu Population

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KEYWORDS Chromosomal Aberration. Parkinson's Disease (PD). Rating Scales. *SNCA*. 22q11.2

ABSTRACT Parkinson's disease (PD) is an age-related disorderwhich deteriorates dopaminergic neurons that control balance and movement. The study aimed at identifying the chromosomal alterations and SNCA mutation in *n*=126 PD subjects with equivalent number of control subjects. The subjects were characterized as late-onset (n=92), early-onset (n=22) and juvenile (n=12). In this study primarily, severity and stages of PD were analysed using the Unified Parkinson's Disease Rating Scale (UPDRS) and Hoehn and Yahr (HY) scale. The UPDRS shows significant values in all the three age groups whereas HY scaling showed significancy in late-onset alone. Cytogenetic analysis with 22q11.2 deletion was observed in late-onset subject with higher significance and point mutation in *SNCA* with A53T and A30P was significant in late-onset and early-onset subjects. Therefore, the researchers conclude that genetic alterations have strong correlation with PD and it is necessary for therapeutic researches in PD.

INTRODUCTION

Parkinson's disease (PD) is the second-most common neurodegenerative condition affecting-dopaminergic (DA) neurons in the substantia nigra region (Iyer et al. 2021; Dhivya and Balachandar 2017). The distinguishing pathological characteristics of the substantia nigra are the presence of Lewy bodies, intraneural inclusions of protein α-synuclein (–Syn) which is renowned biomarker in PD (Mohana Devi et al. 2020; Jayaramayya et al. 2020). As per world-wide prevalence data, PD ranges from 41 per 100,000 to more than 1900 per 100,000 in the age group ≥80 (Dhivya et al. 2016). However, in India, a decade ago, the prevalence is about 14-27 per 100,000 population (Singhal et al. 2003). The standard motor symptoms include tremor, bradykinesia, postural instability and rigidity which are significant in diagnosing PD. Non-motor symptoms such as olfactory dysfunction and constipation have also been reported in Parkinson's disease (Dhivya et al. 2022; Vellingiri et al. 2022). Ageing is the primary factor in PD occurrence since the incidence increase when aged above 50 (Dhivya et al. 2020). Typically, when PD manifests after the age of 50 is known as late-onset and before the age of 50 termed as early-onset; the people affected between the age 15-25 is termed as juvenile. Apart from ageing factor, other risk factors such as hereditary, life-style and environmental factors plays a vital role in PD pathogenesis (Dhivya et al. 2021a; Dhivya

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et al. 2021b). Various researches have been conducted around the world to investigate PD pathogenesis where the therapies had temporarily alleviated PD but not completely. The research on genetic biomarkers is continuously flourishing in examining PD pathogenesis in which the higher end researches can be initiated to develop therapeutic approaches.

Studies on chromosomal abnormalities in PD have been minimally focused and findings have showed that 22q11.2 deletion closely associated with the PD pathophysiologicaly it raises the awareness and observance for the deletion in PD research. The deletion was not suspected until a case report was identified in PD who was in early-onset stage (Zaleski et al. 2009; Ogaki and Ross 2014). From molecular investigations, *SNCA* is the common and first gene reported to be involved in PD. The risk factors and SNCA has close interaction with higher risk. The gene codes for the protein −Syn and its association with PD has been widely studied in various populations. Among many polymorphisms in *SNCA*, point mutation was reported first in an autosomal dominant pattern with a modified −Syn structure (Polymeropoulos et al. 1997). Till now, there is no exact findings on functional aspect of −Syn but also it assumed to be involved in the release and transportation of dopamine as well as in fibrillization of tau protein (Ferese et al. 2015). In the current study, the researchers have inspected the severity of P Dusing rating scales and further molecular investigations were carried out for 22q11.2 deletion and SNCA alterations in PD subjects from the state of Tamil Nadu.

MATERIAL AND METHODS

Subject Recruitment

Totally 252 subjects were enlisted in this study which comprises of n=126 PD subjects and n=126 controls. The PD subjects were divided into male n=79 (62.7%) and female n=47 (37.3%). The PD subjects were then categorized into juvenile, early-onset and late-onset. The behavioural changes in each age group were examined based on the severity of the symptoms and the stage of PD.To compare the influence of the causative factors and genetic alterations in these populations, healthy participants were selected from a similar geographical area. This study has been proposed to the ethical committee of Bharathiar University, Coimbatore and it was approved. The consent was obtained from participating subjects. The 10 mL of blood was obtained from 126 PD patient's and healthy controls. The UPDRS scale was used to assess PD signs and behaviour, and the evolution and severity of PD were classified using five stages of HY scaling combined with a review of the patient's hospital records.

Inclusion Criteria

- The PD participants were chosen in accordance with the four decisive signs of the disease and their family history. Pedigree analysis was framed to decipher the consanguineous effect and previous family history with PD, signs and associated symptoms and the mode of transmission.
- The study also included PD participants who were exposed to a variety of environmental and lifestyle factors.
- PD subjects with multiple system atrophy (MSA), progressive supranuclear palsy (PSP), Myoclinical epilepsy, vascular parkinsonism, hypothyroidism, diabetes, essential tremor and Huntington chorea were also engaged within the study.
- The study included control participants with no neurological disorders, a clear family history of surgical intervention or head trauma, and proper lifestyle practices.

Exclusion Criteria

Participants were excluded from the study if they had high blood pressure, respiratory problems, pulmonary obstructive disorder, queasiness caused by persistent obstructive pulmonary disease, allergies, or had undergone carcinogenic therapy.

UPDRS and HY Scaling

Tools like the UPDRS and HY rating scales have been used to evaluate PD symptoms, stages and behaviour. The activities of daily living (ADL), the motor, and the part on mentation, behaviour, and mood were all covered in the in-

terviews with three different age groups. Resulted in different severity in deferent age groups and category. In HY analysis late-onset group displayed stage 4, whereas early-onset group falls into 3 stage and juvenile PD subjects found in the 2 stage. The PD patients in study were recruited based on the UPDRS and HY criteria.

Blood Collection

The blood sample were collected from the PD subjects as well as the control groups by venipuncture process, in heparin and EDTA tubes, from various hospitals and neurological centres in Chennai, Madurai, Mayiladuthurai and Viluppuram districts of Tamil Nadu, India.

Chromosomal Analysis

Karyotyping was carried out by using 0.5ml of heparinised peripheral blood. Metaphase spreads were performed using the standard cytogenetic techniques by (Moorhead et al. 1960). Lymphocytes were cultured in 5.0 ml of RPMI 1640 media vial which contain 1.0ml AB serum, and 0.2ml of PHA. The culture was incubated for 72 hours at 37 °C and inverted the culture tube twice a day to enable the mixing of the medium and the cells in the culture. At 71st hour, 150 µl of 0.1 percent colchicine was added in the culture and incubated to stop the cell cycle in the metaphase stage.

Harvesting and Slide Preparation

The Lymphocyte harvesting was started at the 72 hour of the culture by centrifuging at 1000 rpm for 20 minutes. The pre-warmed 6 ml of hypertonic solution (KCl) was added to the pellets and taken for centrifuge for 10 minutes at 1000rpm. Then the cells were fixed by the 1ml of freshly prepared fixative (3:1; Methanol: Glacial Acetic Acid). The fixed cells were washed until obtaining the clear suspension. The new slides were kept in the concentrated nitric acid for overnight and then washed with the running water. Then the slides were placed in the distilled water at 4°C. The cells suspension were placed on the slides in dropwise using the Pasture pipette and allow slides to dry at 37°C in the slide warmer.

GTG Banding

The cell suspension-fixed slides were treated for 5-20 seconds with 0.5 percent trypsin reagent. The slides were then treated with 10 percent Giemsa after rinsed in PBS (Sigma, USA). The excess stain were rinsed in the distilled water and slides were allowed to dried finally slides were observed under microscope.

Genotyping

From the study participants, isolation of blood DNA is done by using the standard protocol and the DNA quantification was resoluted at 260-280nm (Lahiri and Nurnberger Jr 1991). Following agarose gel electrophoresis to determine the quality of DNA. For PCR amplification of SNCA gene, primer sequences of exon 4 were used. Following 5 minutes of initial denaturation at 94°C, denaturation was fixed for 15 seconds at 94°C, 1 minute of amplification at 60°C, and 1 minute of extension at 72°C. The final extension time was 10 minutes at 72°C. The amplified products were given for Sanger sequencing and the products were resoluted under the genetic analyser.

Statistical Analysis

IBM-SPSS software was used for the statistical analysis. Statistical significance was calculated by chi-square test and t test. The strength of association was analysed using Odds ratios (OR) and confidence intervals methods. The Mean and standard deviation obtained values were used to assess the difference between the PD subjects and healthy controls. ANOVA was used to calculate the level of significance.

RESULTS

Table 1 denotes the general characteristics of PD subjects (n=126) with equal number of age-matched control subjects in all three age categories. The obtained values on mean ± SD in the age group of males were 66.29 ±14.84 and in females 63.92 ± 14.37 appropriately. In this study samples were obtained from 18 to 92 aged subjects. The age of onset of PD was ascertained prospectively as the age at which the first symptoms appeared.The PD patients were fur-

Table 1: General characteristics of PD patients and control subjects

General characteristics	*PD subjectsn (%)*	*Mean ± SD*	*Control n (%)*	*Mean ± SD*
No. of subjects		126		126
Gender				
Male	79 (62.7%)	66.29 ± 14.84	79 (62.7%)	66.29 ± 14.84
Female	47 (37.3%)	63.92 ± 14.37	47 (37.3%)	63.92 ± 14.37
Age Category				
Late-onset (≥50) n=92			*Group I (≥50) n=92*	
Male	53 (57.6%)	63.20 ± 13.88	53 (57.6%)	63.20 ± 13.88
Female	39 (42.3%)	64.09 ± 14.72	39 (42.3%)	64.09 ± 14.72
Early-onset (<50) n=22			*Group II (<50) n=22*	
Male	14 (63.3%)	46.85 ± 16.13	14 (63.3%)	46.85 ± 16.13
Female	8 (36.3%)	43.35 ± 15.60	8 (36.3%)	43.35 ± 15.60
Juvenile (18-30) n=12			*Group I (≥50) n=92*	
Male	12 (100%)	28.39 ± 14.65	12 (100%)	28.39 ± 14.65
Female	0	0	0	0

Table 1 depicts the total subjects recruited in PD which categories based on age as late-onset, early-onset and juvenile groups.

ther classified into three groups juvenile (18-30; *n*=12), early-onset (<50; *n*=22) and late-onset (≥50; *n*=92). Based on the gender each PD groups were classified, were the late-onset was with total male *n*=53 (57.6%; 63.20 ± 13.88) and female *n* =39 (42.4%; 64.09 ± 14.72). Early-onset with male *n* =14 (63.6%; 46.85 ± 16.13) and female *n* =8 (36.4%; 43.35 ± 15.60). Juvenile PD has been observed only in males with *n*=12 (100%; 28.39± 14.65).

Table 2 depicts the rating scales of UPDRS and HY in PD subjects. The UPDRS scaling and HY scaling with total stages 4 have been investigated in three age groups of PD. The total subjects noticed with each part has been mentioned in the table. As per the analysis, chi-square test showed the significance in "activities of daily living" and "motor symptoms" in all three aged groups of PD. In HY scaling, the significance of increased staging of severity of the symptoms was noticed in late-onset PD group than other two groups.

Table 3 shows the statistical evidence with mean ± SD of chromosomal abnormalities in PD subjects. In this study's PD subjects, the researchers' identified 22q11.2 deletion in late-onset subjects than in early-onset cases. The chromosomal type aberration (CSA) was observed in late-onset male with 1.8 ± 1.48; chromatid type aberration (CTA) was observed with 0.6 ± 0.54 and total chromosomal aberration (TCA) was 2.4 ± 2.02. The early-onset subjects were noticed with mean ± SD in males with CSA showing 1.0±1.11; CTA with 0.2 ± 0.10 and TCA 1.2 ± 1.21.

Table 2: UPDRS and HY scaling of PD patients

Scaling	*Late-onsetn (%)*	*χ^2 (p value)*	*Early-onsetn (%)*	*χ^2 (p value)*	*Juvenilen (%)*	*χ^2 (p value)*
UPDRS						
1. Mentation, behaviour and mood	33 (35.8%)	0.34 (0.14)	9 (40.9%)	0.21 (0.11)	3 (25%)	1.36 (0.11)
2. Activities of daily living	86 (93.4%)	0.12 (**0.03**)*	17 (77.2%)	0.23 (**0.04**)*	8 (66.6%)	1.33 (**0.02**)*
3. Motor symptoms	92 (100%)	0.11 (**0.03**)*	22 (100%)	0.25 (**0.03**)*	12 (100%)	1.27 (**0.03**)*
HY						
Stage 1					9 (75%)	0.82 (0.37)
Stage 2	49 (53.2%)	1.03 (**0.001**)*	15 (68.1%)	1.37 (0.24)	3 (25%)	0.16 (0.12)
Stage 3	31 (33.6%)	0.27 (**0.001**)*	7 (31.8%)	1.11 (0.10)		
Stage 4	12 (13.0%)	0.13 (**0.03**)*				

* p<0.05 which is significant to the severity of PD symptoms

Table 3: Depicts the statistical evidences of chromosomal abnormalities in PD subjects

PD cases	*Gender*	*Total (%)*	*CSAMean ± SD*	*CTAMean ± SD*	*TCAMean ± SD*
Late-onset (*n*=92)	Male	3 (3.26%)	1.8 ± 1.48	0.6 ± 0.54	2.4 ± 2.02
	Female	1 (1.08%)	-	-	-
Early-onset (*n*=22)	Male	2 (9.09%)	1.0±1.11	0.2 ± 0.10	1.2 ± 1.21
	Female	-			

Table 3 depicts the total PD patients with chromosomal alterations representing with Mean ± SD

Table 4 shows the genotype variant c.209G>A in late-onset (n=34) with 36.9 percent showing a significance of 0.03 and early-onset showing (n=12) with 54.5 percent showing 0.04 significancy. The genotype c.157G>A in n=19 (20.6%) subjects in late-onset showing 0.03 significance. Whereas the c.88G>C genotype was insignificant in late-onset subjects.

DISCUSSION

Though numerous research studies have been conducted on PD, still the etiological findings remain unknown. This study aims to investigate chromosomal alterations and appraise the genetic alterations in standard gene *SNCA* in PD patients by considering medical records and lifestyle factors. Severity of the symptoms and stages depends on the age and intensity of genetic mutation. In this study the researchers observed that advancement of symptoms and severity were varied in the late-onset and early-onset PD on-set groups. The first missense mutation was discovered in A53T of SNCA with familial PD in Italian relatives (Polymeropoulos et al. 1997). Later the same variants have been noted in Asia and Greek population (Tan et al. 2003; Bozi et al. 2014). In another study A53T mutation was observed in the 7 exon of SNCA gene in autosomal dominant PD European and American children (Vaughan et al. 1998). Comparable to this, researchers discovered a G-A transition with the A53T variant in exon 4.Bewilderingly, some Brazilian research observed no A53T mutation in *SNCA* gene of the PD Subjects (Teive et al. 2001; Camargos et al. 2009; Moura et al. 2012). Similarly, three more studies in India found no evidence of SNCA mutation (Nagar et al. 2001; Padmaja et al. 2012; Kadakol et al. 2014). With the exception of cognitive decline, a A53T mutation was showned in the German family with a clinical profile identical to the idiopathic PD. On the other hand, no additional non-motor symptoms seem to have been reported (Krüger et al. 1998). Similar motor deficits and alterations to dopaminergic system was seen in a mouse model of homozygous mutant A30P*A53T (Kilpeläinen et al. 2019). Previously, the A53T mutation was linked to clinical manifestations in patients with early-onset parkinsonism (Dhivya et al. 2016).

The present report examined the SNCA A53T*A30P mutations in patients exhibiting parkinsonian features. Although 126 PD cases are linked with clinical features of PD, in that twenty-three cases showed evidence of dementia, which links to the Hurtig et al. (2000) study, where –Syn inclusions are to blame for dementia and closely related to Parkinson's disease.Some of the research states that dementia is arise due to the duplication of SNCA gene (Singleton et al. 2003; Chartier-Harlin et al. 2004). These previous literatures were strongly confirmed that PD

Table 4: *SNCA* alterations in PD subjects

Genotype	*Late-onset*			*Early-onset n (%)*			*Juvenile*		
	n (%)	*OR*	*P value*	*n (%)*	*OR*	*P value*	*n (%)*	*OR*	*P value*
c.209G>A	34 (36.9%)	0.71	**0.03***	12 (54.5%)	1.13	**0.04***	4 (33.3%)	0.35	0.10
c.157G>A	19 (20.6%)	1.12	**0.03***	4 (18.1%)	0.77	0.11	-		
c.88G>C	14 (15.2%)	0.43	0.13	1 (4.5%)	-				

* p<0.05 which is significant to the severity of PD symptoms

is closely associated with dementia. There are few studies which are investigating the SNCA mutation dependent pathogenesis and the p.A30P SNCA mutation-based alterations in neuronal function is carried out in the patient-derived isogenic cell models (Barbuti et al. 2021). The mutated α-Syn (A30P) causes the DNA silencing and transcriptional deregulation was observed in one the animal model research. In the same study says that the mutated α-Syn (A30P) may also plays a vital role to maintain the integrity of dopaminergic neurons, it mays leads to the dementia with lewy body and PD (Cabrero et al. 2021). Additionally, a recent investigation found that A30P/A53T double mutated α-Syn mouse model shows that age based neuroinflammation and neurodegeneration in PD analysis (Rauschenberger et al. 2022).

Chromosomal alterations were analysed in this study, which resulted that deletion was observed in the chromosome 22q region. The previous literatures says that, while providing the antipsychotic drugs to Parkinsonian features infancy with 22qdeletion shows that development of schizophrenia was observed (Krahn et al. 1998). Similarly, the antipsychotic treatments may arise risk in developing PD which was proposed by Butcher and colleagues, while analysing the epidemiological studies related to the 22q11.2 deletion in early onset PD (Butcher et al. 2013). Some of the current studies state that early onset patients over the age of 45 show that 0.49 percent of deletion-based alterations in chromosomes (Mok et al. 2016). As a result of the researchers' findings, loss in 22q11.2 was discovered, which is one of the unique deletion syndrome in the PD research. Physicians should aware of the 22q deletion patients and should give prompt diagnosis and therapy.

CONCLUSION

In the current study, the chromosomal aberration 22q11.2 deletion is an interesting and unusual finding in PD research. Furthermore, the deletion should be investigated along with genetic alterations and its association with *SNCA* might have implications in neurodegeneration process. From this study's study subjects, the SNCA alterations has been significant with PD occurrence hence more PD genes should be examined in a multidisciplinary method in order to determine the genetic predisposition in PD research.

RECOMMENDATIONS

Genetic counselling is essential and it is been recommended since it provides information about disease onset and helpful to provide appropriate medications based on the symptoms. Genetic risk assessment was poorly developed for the early-onset PD cases. Psychological counselling is also important to assess the psychological impact which will helpful in providing the adequate prevention and treatment strategies for the PD patients. The SNCA alterations has shown significant with PD occurrence hence more PD genes should be examined in a multidisciplinary method in order to determine the genetic predisposition in PD pathogenesis.

REFERENCES

Barbuti PA, Ohnmacht J, Santos BF, Antony PM, Massart F et al. 2021. Gene-corrected p. A30P SNCA patient-derived isogenic neurons rescue neuronal branching and function. *Scientific Reports*, 11(1): 21946. doi: 10.1038/s41598-021-01505-x

Bozi M, Papadimitriou D, Antonellou R, Moraitou M, Maniati M et al. 2014. Genetic assessment of familial and early onset Parkinson's disease in a Greek population. *European Journal of Neurology*, 21(7): 963-968.

Butcher NJ, Kiehl TR, Hazrati LN, Chow EW, Rogaeva E et al. 2013. Association between early-onset Parkinson disease and 22q11.2 deletion syndrome: Identification of a novel genetic form of Parkinson disease and its clinical implications. *JAMA Neurology*, 70(11): 1359-1366.

Cabrero FR, Morrison EH 2021. *Lewy Bodies*. Treasure Island (FL): StatPearls Publishing.

Camargos ST, Dornas LO, Momeni P, Lees A, Hardy J et al. 2009. Familial Parkinsonism and early onset Parkinson's disease in a Brazilian movement disorders clinic: Phenotypic characterization and frequency of SNCA, PRKN, PINK1, and LRRK2 mutations. *Movement Disorders: Official Journal of the Movement Disorder Society*, 24(5): 662-666.

Chartier-Harlin MC, Kachergus J, Roumier C, Mouroux V, Douay X et al. 2004. α-synuclein locus duplication as a cause of familial Parkinson's disease. *The Lancet*, 364(9440): 1167-1169.

Dhivya V, Ramkumar S, Illakiyapavai D, Sangeetha M, Ganesan S et al. 2016. Screening of genetic mutations in early onset parkinsonism patients: A family-based study in Tamil Nadu population. *International Journal of Human Genetics*, 16(3-4): 158-165.

Dhivya V, Balachandar V 2017. Cell replacement therapy is the remedial solution for treating Parkinson's disease. *Stem Cell Investigation*, 4: 59.

Dhivya V, Iyer M, Narayanasamy A, Siva K, Vellingiri B 2020. Kynurenine pathway in Parkinson's disease-An update. *E Neurological Sci*, 21: 100270

Dhivya V, Iyer M, Krishnan P, Wilson R, Vellingiri B 2021a. A late-onset Parkinson's disease in tribes in India-A case report. *Brain Disorders*, 3: 100015.

Dhivya V, Iyer M, Wilson R 2021b. The association between multiple risk factors, clinical correlations and molecular insights in Parkinson's disease patients from Tamil Nadu population. *India Neurosci Lett*, 755: 135903.

Dhivya V, Iyer M, Narayanasamy A, Kamalakannan S, Valsala Gopalakrishnan A, Vellingiri B 2022. Genotypic-Phenotypic analysis, metabolic profiling and clinical correlations in Parkinson's Disease patients from Tamil Nadu population, India. *Journal of Molecular Neuroscience*, 72(8): 1724-1737.

Ferese R, Modugno N, Campopiano R, Santilli M, Zampatti S et al. 2015. Four copies of SNCA responsible for autosomal dominant Parkinson's disease in two Italian siblings. *Parkinson's Disease*, 2015: 546462.

Hurtig HI, Trojanowski JQ, Galvin J, Ewbank D, Schmidt ML et al. 2000. Alpha-synuclein cortical Lewy bodies correlate with dementia in Parkinson's disease. *Neurology*, 54(10): 1916-1921.

Iyer M, Subramaniam MD, Venkatesan D, Cho SG, Ryding M et al. 2021. Role of RhoA-ROCK signaling in Parkinson's disease. *European Journal of Pharmacology*, 894: 173815.

Jayaramayya K, Iyer M, Venkatesan D, Balasubramanian V, Narayanasamy A et al. 2020. Unraveling correlative roles of dopamine transporter (DAT) and Parkin in Parkinson's disease (PD)-A road to discovery? *Brain Research Bulletin*, 157: 169-179.

Kadakol GS, Kulkarni SS, WaliGM, Gai PB 2014. Molecular analysis of α-synuclein gene in Parkinson's disease in North Karnataka, India. *Neurology India*, 62(2): 149.

Kilpeläinen T, Julku UH, Svarcbahs R, Myöhänen TT 2019. Behavioural and dopaminergic changes in double mutated human A30P*A53T alpha-synuclein transgenic mouse model of Parkinson's disease. *Scientific Reports*, 9(1): 1-13.

Krahn LE, Maraganore DM, Michels VV 1998. Childhood-onset schizophrenia associated with parkinsonism in a patient with a microdeletion of chromosome 22. *Mayo Clinic Proceedings*, 73(10): 956-959. doi: 10.4065/73.10.956

Krüger R, Kuhn W, Müller T, Woitalla D, Graeber M et al. 1998. AlaSOPro mutation in the gene encoding α-synuclein in Parkinson's disease. *Nature Genetics*, 18(2): 106-108.

Lahiri DK, Nurnberger Jr JI 1991. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Research*, 19(19): 5444.

Mohana Devi S, Mahalaxmi I, Aswathy NP, Dhivya V, Balachandar V 2020. Does retina play a role in Parkinson's Disease? *Acta Neurologica Belgica*, 120(2): 257-265.

Mok KY, Sheerin U, Simón-Sánchez J, Salaka A, Chester L, Escott-Price V et al. 2016. Deletions at 22q11. 2 in idiopathic Parkinson's disease: A combined analysis of genome-wide association data. *The Lancet Neurology*, 15(6): 585-596.

Moorhead PS, Nowell PC, Mellman WJ, Battips DT, Hungerford DA1960. Chromosome preparations of leukocytes cultured from human peripheral blood. *Experimental Cell Research*, 20(3): 613-616.

Moura KC, Junior MC, de Rosso AL, Nicaretta DH, Pereira JS et al. 2012. Exon dosage variations in Brazilian patients with Parkinson's disease: Analysis of SNCA, PARKIN, PINK1 and DJ-1 genes. *Disease Markers*, 32(3): 173-178.

Nagar S, Juyal RC, Chaudhary S, Behari M, Gupta M et al. 2001. Mutations in the α synuclein gene in Parkinson's disease among Indians. *Acta neurologicascandinavica*, 103(2): 120-122.

Ogaki K, Ross OA 2014. Chromosome 22q11.2 deletion may contain a locus for recessive early-onset Parkinson's disease. *Parkinsonism & Related Disorders*, 20(9): 945-946.

Padmaja MV, Jayaraman M, Srinivasan AV, Srisailapathy CS, Ramesh A 2012. The SNCA (A53T, A30P, E46K) and LRRK2 (G2019S) mutations are rare cause of Parkinson's disease in South Indian patients. *Parkinsonism & Related Disorders*, 18(6): 801-802.

Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A et al. 1997. Mutation in the α-synuclein gene identified in families with Parkinson's disease. *Science*, 276(5321): 2045-2047.

Rauschenberger L, Behnke J, Grotemeyer A, Knorr S, Volkmann J, Ip CW2022. Age-dependent neurodegeneration and neuroinflammation in a genetic A30P/A53T double-mutated α-synuclein mouse model of Parkinson's disease. *Neurobiology of Disease*, 171: 105798.

Singhal B, Lalkaka J, Sankhla C 2003. Epidemiology and treatment of Parkinson's disease in India. *Parkinsonism Relat Disord*, 9: 105–109.

Singleton AB, Farrer M, Johnson J, Singleton A, Hague S et al. 2003. α-Synuclein locus triplication causes Parkinson's disease. *Science*, 302(5646): 841-841.

Tan EK, Tan C, Shen H, Chai A, Lum SY et al. 2003. Alpha synuclein promoter and risk of Parkinson's disease: microsatellite and allelic size variability. *Neuroscience Letters*, 336(1): 70-72.

Teive HA, Raskin S, Iwamoto FM, Germiniani F, Baran MH et al. 2001. The G209A mutation in the alpha-synuclein gene in Brazilian families with Parkinson's disease. *Arquivos de Neuro-Psiquiatria*, 59: 722-724.

Vaughan JR, Farrer MJ, Wszolek ZK, Gasser T, Durr A et al.1998. Sequencing of the α-synuclein gene in a large series of cases of familial Parkinson's disease fails to reveal any further mutations. *Human Molecular Genetics*, 7(4): 751-753.

Vellingiri B, Suriyanarayanan A, Abraham KS, Venkatesan D, Iyer M et al. 2022. Influence of heavy metals in Parkinson's disease: An overview. *Journal of Neurology*, 269(11): 5798-5811.

Zaleski C, Bassett AS, Tam K, Shugar AL, Chow EW, McPherson E 2009. *The Journal of Medical Genetics- Part A*, 149(3): 525.

Paper received for publication in January, 2023
Paper accepted for publication in May, 2023