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Original Article

Genetics Section

Mutation Analysis of NOS1 Exon 18 Polymorphisms with the Risk of Parkinson's Disease: A Cross-sectional Study

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ABSTRACT

Introduction: Nitric Oxide Synthase 1 (NOS1) has been implicated in Parkinson's Disease (PD) pathogenesis through its role in neuronal signaling and oxidative stress. However, evidence of specific genetic contributions in Indian populations remains limited.

Aim: The present study aimed to examine whether genetic variations in exon 18 of the NOS1 gene are associated with PD in a South Indian cohort.

Materials and Methods: The present observational cross-sectional study was conducted between March 2022 and May 2023 at a Tertiary Care Hospital, Karnataka, India. One hundred clinically diagnosed PD patients underwent detailed neurological assessment and 1 mL venous blood collection. Deoxyribonucleic Acid (DNA) was extracted and exon 18 of NOS1 (chromosome no 12) was amplified by PCR and sequenced using Sanger sequencing. Variants were analysed and verified using bioinformatics tools and reference sequences.

Results: The cohort comprised 65 males and 35 females, with 64 cases classified as sporadic, 34 idiopathic, and 2 familial. Demographic data showed that 67% were urban residents. Sequencing of exon 18 detected a synonymous single nucleotide variant (g.119313C>T, rs1047735) in three patients: two heterozygous and one homozygous. This variant (c.2706C>T) results in p.His902=, without amino acid change.

Conclusion: Only synonymous variants were found. Although these variants do not change the amino acid sequence, they may influence NOS1 regulation at the RNA level. Larger case-control studies with functional analyses and broader genomic approaches such as whole-exome sequencing are necessary to clarify whether NOS1 exon 18 variants contribute to PD susceptibility.

Keywords: Inherited mutations, Synonymous variants, Oxidative stress

INTRODUCTION

Parkinson's Disease (PD) is a chronic neurodegenerative disorder and the second most common neurodegenerative condition worldwide. It is primarily characterised by a combination of motor symptoms-resting tremor, rigidity, bradykinesia, postural instability and non-motor manifestations such as sleep disturbances, autonomic dysfunction, depression, and cognitive impairment. The prevalence of PD increases with age, affecting about 1% of individuals over 60 years [1-3]. The disease pathology is marked by the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta, leading to dopamine deficiency in the striatum. This deficiency disrupts the basal ganglia circuits, which normally integrate voluntary motor control and other cognitive functions. Another hallmark of PD pathology is the presence of Lewy bodies containing aggregated α-synuclein [4-6].

PD is multifactorial. Inherited mutations in specific genes (such as SNCA, LRRK2, PARK2, and GBA) account for a small proportion of familial cases. However, most cases are sporadic and are thought to result from complex interactions of multiple low-effect genetic factors with environmental triggers including pesticide exposure, heavy metals, head trauma, and oxidative stress [7].

Recent genetic studies have identified several candidate genes potentially associated with increased PD susceptibility, including NOS1, ABCB1, PON1, PON2, CYP1A1, and CYP1A2 [8]. Among these, NOS1 (neuronal nitric oxide synthase) is of particular interest. Nitric oxide (NO), produced by neuronal nitric oxide synthase (nNOS), plays a dual role: under physiological conditions, it is an important neuromodulator, whereas excessive NO production leads to oxidative and nitrosative stress, damaging neurons. Postmortem studies have reported increased NOS1 expression and activity in PD brains, suggesting a potential role in neurodegeneration [9,10].

The NOS1 gene, located on chromosome 12q24.22, has 33 exons. Genetic variants in this gene could influence NO production and neuronal vulnerability. Previous studies on exons 18 and 29 have been inconsistent, with some reporting associations and others showing no link [1,11-15]. In the author's earlier work (Kadakol et al., 2023), analysis of exon 29 in a North Karnataka population showed no significant association with PD [11]. Data from southern India on exon 18 variants is scarce, hence the present study aimed to examine whether genetic variations in exon 18 of the NOS1 gene are associated with PD in a South Indian cohort.

MATERIALS AND METHODS

The present observational cross-sectional study was conducted in the Department of Neurology at a Tertiary Care Center affiliated with BLDE (Deemed to be University), Vijayapura, Karnataka, India, for a study period from March 2022 to May 2023. Ethical approval was obtained from the Institutional Ethics Committee (Ref: BLDE(DU)/IEC/507/2022-23), and all participants provided informed written consent.

Inclusion and exclusion criteria: A total of 100 consecutive patients were recruited based on clinical diagnosis of PD using the UK Parkinson's Disease Society Brain Bank criteria. Inclusion criteria included patients older than 40 years with a confirmed diagnosis of idiopathic PD and willingness to participate. Patients with secondary parkinsonism, atypical parkinsonian syndromes, psychiatric illness, or unwillingness to give blood samples were excluded.

Study Procedure

Each participant underwent a detailed neurological examination, and data were recorded in a structured proforma capturing:

- Demographic details (age, sex, residence, ethnicity)
- Clinical characteristics (age of onset, disease duration, primary motor and non-motor features)
- Family history of neurodegenerative disorders
- History of exposure to pesticides, heavy metals, and other risk factors
- Lifestyle information such as smoking and alcohol intake

Sample collection and DNA extraction: From each participant, 1 mL of peripheral blood was collected into K2 Ethylenediaminetetraacetic acid (EDTA)-coated vacutainers. Samples were processed within 24 hours. DNA was isolated using the Nucleospin DNA extraction kit (Germany) according to manufacturer's instructions. DNA quality and concentration were checked spectrophotometrically.

PCR amplification of exon 18: The targeted region was exon 18 of NOS1 (NG_011991.2). Primers used are detailed in [Table/Fig-1]. PCR was performed in a 20 µL reaction mixture containing genomic DNA, ready-to-use master mix (Takara, Japan), primers, and nuclease-free water. Cycling parameters included an initial denaturation at 95°C for 5 min, 35 cycles of 95°C for 30 s, annealing at 60°C for 45 s, extension at 72°C for 1 min, followed by a final extension of 72°C for 5 min. PCR products were visualized by electrophoresis on 2% agarose gels.

Primer	Sequence	Product size (bp)	Tm (°C)
Forward	CCCACAGTCCCTTAGATGA	268	60
Reverse	GGGTGTGGGGAGCATTATT	268	60

[Table/Fig-1]: Primer sequences for amplification of exon 18 of NOS1 gene.

Sanger sequencing and variant analysis: Bidirectional sequencing of PCR products was performed on an ABI 3500xl genetic analyzer using both forward and reverse primers. Chromatograms were inspected manually for quality, and sequences were aligned with the reference using Variant Reporter software. Identified variants were annotated using dbSNP and ClinVar. The potential effect of synonymous mutations on RNA splicing and stability was assessed using Human Splicing Finder and ESEfinder.

STATISTICAL ANALYSIS

Data was analysed using descriptive statistics and variables were presented in the form of numbers and percentages.

RESULTS

Demographic characteristics: Out of 100 patients, 65 were male (65%) and 35 female (35%). Sixty-seven percent resided in urban areas. Ethnically, the largest group was Lingayat 47 (47%), followed by Brahmins 15 (15%), Muslims 10 (10%), and others 25 (26%). In terms of age distribution, the majority were 55 (55%) were between 60-79 years followed by those aged 40-59 years were 37 (37%). A small proportion of patients belonged to the age groups 20-39 years were 3 (3%) and 80-92 years were 5 (5%) [Table/Fig-2,3].

Features	n (%)
Male	65 (65)
Female	35 (35)
Sporadic	64 (64)
Idiopathic	34 (34)
Familial	2 (2)
Urban	67 (67)
Rural	33 (33)
Lingayat	47 (47)
Brahmin	15 (15)
Muslim	10 (10)
Others	25 (25)

[Table/Fig-2]: Baseline characteristics of Parkinson's disease patients (n=100).

Age group (years)	n (%)
20-39	3 (3)
40-59	37 (37)
60-79	55 (55)
80-92	5 (5)

[Table/Fig-3]: Age distribution of patients (n=100).

Clinical features: As shown in [Table/Fig-4], tremor was the predominant symptom 90 (90%), followed by slowness in activities 32 (32%) and dyskinesia 8 (8%). Less frequently observed symptoms included walking difficulty 5 (5%), bradykinesia 3 (3%), rigidity 3 (3%), general weakness 2 (2%), frequent falls 1 (1%), and difficulty in speech 1 (1%). These findings confirm the variability in clinical presentation of PD among patients.

Features	n (%)
Tremors	90 (90)
Slowness of activities	32 (32)
Dyskinesia	8 (8)
Walking difficulty	5 (5)
Bradykinesia	3 (3)
Rigidity	3 (3)
Weakness	3 (3)
Frequent falls	1 (1)
Difficulty in speech	1 (1)

[Table/Fig-4]: Clinical features of Parkinson's disease patients (n=100).

Genetic findings: Exon 18 of NOS1 was successfully amplified and sequenced in 76 samples. Sequencing revealed a single synonymous variant g.119313C>T (rs1047735) in three samples: Two heterozygous and one homozygous [Table/Fig-5]. This variant corresponds to c.2706 C>T and results in p.His902=, a synonymous substitution [Table/Fig-6]. No missense, nonsense, or frameshift mutations were detected.

Sample ID	gDNA position	cDNA position	Amino acid position	Variant type	Zygosity
32 P	g.119313 C>T	c.2706 C>T	p.His902=	Synonymous	Homozygous
34 P	g.119313 C>T	c.2706 C>T	p.His902=	Synonymous	Heterozygous
38 P	g.119313 C>T	c.2706 C>T	p.His902=	Synonymous	Heterozygous

[Table/Fig-5]: Synonymous variant detected in NOS1 exon 18.

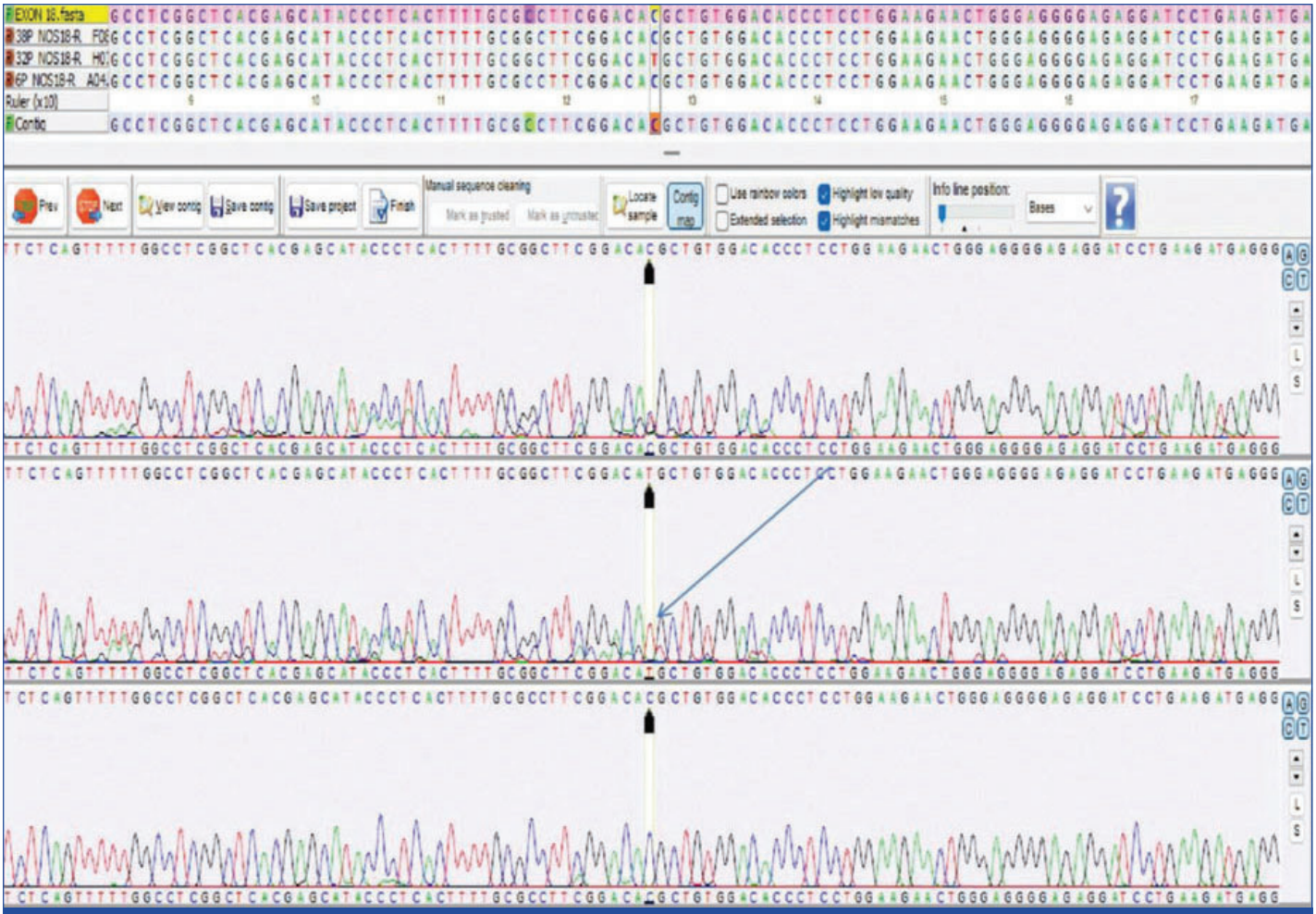
DISCUSSION

The present study provides insights into the contribution of NOS1 exon 18 variants in South Indian PD patients. The sequencing identified a synonymous variant in three cases and no pathogenic coding variants.

While synonymous mutations do not alter the amino acid sequence, they are not always neutral. These mutations may affect splicing patterns, mRNA stability, codon usage bias, and translation speed, which can subsequently alter protein structure or expression. Several bioinformatic studies have highlighted the functional consequences of synonymous variants, emphasizing their relevance in neurodegenerative diseases.

Comparison with Previous Studies

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterised by the selective loss of dopaminergic



[Table/Fig-6]: Electropherogram showing heterozygous C>T substitution at position g.119313 in exon 18 of NOS1.

neurons in the substantia nigra, leading to motor and non-motor dysfunctions. Although the aetiology of PD is multifactorial, genetic studies have contributed significantly to understanding the disease pathogenesis [16,17]. The prevalence of PD in the Indian population is reported to be lower than in Western countries, with estimates in Karnataka showing an incidence of 7 per 100,000 individuals in the general population and 134 per 100,000 among individuals aged above 50 years [18-20]. Genome-Wide Association Studies (GWAS) have identified several genetic variants associated with PD susceptibility, including polymorphisms in NOS1, ABCB1, and other genes [21]. Increased nitrite levels and elevated NOS1 activity have been proposed as potential mechanisms contributing to neurodegeneration, supporting a possible functional role for synonymous variants [13].

The present study findings are consistent with earlier studies by Levecque et al., [1], Drozdzik M et al., [12], Gupta SP et al., [14], Huerta C et al., [22], Huang H et al., [15] and Hague S et al., [23], which found no direct causal role for exon 18 variants in PD. Similarly, in our previous publication on exon 29 of NOS1 [11], no significant association was observed.

The present study is one of the few to evaluate NOS1 exon 18 in a South Indian cohort. Given the ethnic and genetic diversity of India, such region-specific studies add valuable data to global research.

Limitation(s)

- **Lack of a control group:** Without a control group, no meaningful association or statistical significance can be inferred.
- **Sample size:** The small cohort limits the generalizability and detection of rare variants.
- **Single exon studied:** The analysis was restricted to exon 18. Pathogenic variants in other exons or regulatory regions cannot be ruled out.
- **Functional studies not performed:** While bioinformatic tools suggest possible regulatory effects of synonymous mutations, functional validation (e.g., expression analysis, in vitro assays) was beyond the scope.

CONCLUSION(S)

In this South Indian PD cohort, exon 18 of the NOS1 gene showed only synonymous variants. These findings highlight the need for large-scale, multi-exon sequencing and functional studies to better understand the genetic basis of PD. Future studies should include a case-control design with larger multiethnic cohorts, NGS technologies (whole-exome or whole-genome sequencing), and functional assays to clarify whether synonymous variants in NOS1 influence PD risk.Although the current study does not establish a causal relationship, it contributes regional genetic data and underscores the importance of exploring synonymous mutations.

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