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## Association of brain-derived neurotrophic factor (Val66Met) polymorphism with the risk of Parkinson's disease and influence on clinical outcome

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Parkinson's disease (PD) is a common neurodegenerative disease. Motor symptoms of rigidity, tremor, and bradykinesia and non-motor symptoms like the cognitive deficit, autonomic dysfunction, dementia, anxiety and depression all contribute to morbidity. Emerging shreds of evidence suggest the role of BDNF (Val66Met) polymorphism in PD risk and associated cognitive deficit. Hence, the current study is aimed to investigate the role of BDNF Val66Met in the risk of PD development and associated cognitive abnormalities. A total of 269 PD cases and 271 healthy, age, ethnicity and gender- matched controls were recruited in the study. Genomic DNA was isolated, amplified and SNP was identified using the RFLP method and validated by Sanger's sequencing. There was a significant association of BDNF Val66Met with PD risk in both Dominant and recessive models (GG vs GA+AA: OR: 1.47, CI: 1.04-2.09,  $P = 0.03$ , GG+GA vs AA: OR: 2.32, CI: 1.07-5.00,  $P = 0.02$ ). The main nonmotor symptom *i.e.* cognitive impairment was significantly associated with the variant genotype of BDNF Val66Met Polymorphism (GG vs GA+AA: OR: 1.47, CI: 1.04-2.09,  $P = 0.03$ , GG+GA vs AA: OR: 2.32, CI: 1.07-5.00,  $P = 0.02$ ). We found a significant association of variant genotype with disease severity, the activity of daily living as assessed by S & E score as it was found to better with wild genotype and a significant decrease in quality of life with homozygous mutant genotype. We did not find significant differences in disease duration, absolute levodopa response among the genotypes. Our results implicate BDNF Val66Met polymorphism is associated with the risk of PD, cognitive impairment, poor quality of life and greater disease severity in PD.

**Keywords:** BDNF polymorphism, Bradykinesia, Cognitive impairment, Dementia, MoCA, Parkinson's disease

Parkinson's disease (PD) is one of the common neurodegenerative diseases, presenting with tremors, rigidity, bradykinesia, abnormal posture and gait as the cardinal features. The non-motor symptoms of PD include olfactory dysfunction, cognitive impairment, sleep disorder, pain, depression, anxiety, autonomic nervous dysfunction *etc.*, which can occur during PD or earlier than motor symptoms, totally reducing the quality-of-life<sup>1</sup>. Among the non-motor symptoms, cognitive impairment represents an important non-motor characteristic of the disease. In a study, the development of cognition

was shown to be 8.5% in a year, which reached 47.4% in 6 years duration of the disease<sup>2</sup>.

The pathophysiology of PD-related cognitive impairment is still unknown. It is believed that environmental and genetic factors contribute to the development of this symptom<sup>3</sup> and it is reported to be related to many mechanisms, such as reactive microgliosis, altered protein handling, oxidative stress, and mitochondrial dysfunction<sup>4</sup>. Among the environmental factors, aging seems to be an essential contributor to impaired cognition<sup>5</sup>. From the literature and neuropathological findings, cognitive impairment of PD was shown to be correlated with the number of cortical Lewy bodies, neurofibrillary tangles, senile plaques (Alzheimer's disease- related pathological changes) and cerebrovascular lesions. At present, the specific susceptibility genes for cognitive impairment of PD are still unknown.

The Brain- Derived Neurotrophic Factor is a protein encoded by the BDNF gene, found on chromosome 11<sup>6,7</sup>. BDNF has several known single

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**Abbreviations:** aLR, absolute Levodopa response; BDNF, Brain derived neurotrophic factor; EOPD, Early onset Parkinson's disease; H & Y, Modified Hoehn and Yahr scale; LOPD, Late onset Parkinson's disease; MoCA, Montreal cognitive assessment; PD, Parkinson's disease; PDQ 39, Parkinson's disease questionnaire; S & E, Schwab and England score; UPDRS III, Unified Parkinson's disease rating scale.

nucleotide polymorphisms (SNP) of which Val66Met is most frequently studied polymorphism in neuropathological conditions. A common SNP - Val66Met (rs6265)<sup>8</sup> is a point mutation in the coding sequence, where guanine to adenine substitution at position 196, results in a valine to methionine *i.e.*, an amino acid switch at 66<sup>th</sup> codon and is unique to humans<sup>8,9</sup>. This substitution interferes with normal translation of BDNF mRNA and normal intracellular trafficking<sup>8</sup>. This mutation results in a reduction of hippocampal tissue which is reported in a high number of individuals suffering from learning and memory disorders<sup>9</sup>, anxiety disorders<sup>10</sup>, and neurodegenerative diseases such as Alzheimer's and Parkinson's<sup>11</sup>. The 'A' allele is associated with abnormal packaging of the precursor of BDNF and decreased mature BDNF production in cells<sup>12,13</sup>.

In recent studies, investigation of genes involved in neuroplasticity in association with cognitive functions showed interesting results<sup>14</sup>. Results from the studies have shown that brain-derived neurotrophic factor (BDNF), can significantly enhance the dopaminergic neurons' tolerance against the acidic environment, and is significantly down-regulated in the ventral substantia nigra of PD patients<sup>15</sup>. Emerging evidences have indicated the possible role of BDNF protein on cognition in normal and neuropathological conditions. It is evident from the literature that BDNF promotes survival, differentiation, and maintenance of neuronal cells and also acts as a mediator in long-term potentiation (LTP), which is involved with memory formation in the hippocampus<sup>16</sup>. The role of genetic polymorphisms of the BDNF gene in cognitive impairment associated with PD is still unclear. BDNF G196A (Val66Met, rs6265) polymorphism is one of the most frequently studied BDNF gene polymorphisms. A study in hippocampal mice cells shows the functional polymorphism of BDNF (Val66Met) impair BDNF secretion<sup>17,9</sup>. Val66Met polymorphism has been reported to be involved in impaired episodic memory function<sup>16,8</sup>. The Causative and curative roles of BDNF (Val66Met) and its mechanism in cognitive impairment in PD have been described<sup>18</sup>. In a study, the functional Val66Met BDNF polymorphism was not associated with PD susceptibility<sup>19</sup>, but it is associated with cognitive impairment<sup>20</sup> and planning ability in PD<sup>21</sup>. In a study, it was shown that proBDNF initiate the heteromer formation of sertolin and neurotrophic receptors such as TrkA or TrkB ,

thereby triggers the neuronal cell death<sup>22</sup>. However, in other studies, BDNF Val66Met polymorphism was found to have no association with cognitive functioning in PD<sup>23,24</sup>. Because of conflicting and inconclusive findings<sup>20,25-29</sup>, the present study is aimed to investigate the role of BDNF Val66Met with PD susceptibility and its association with cognitive impairment in south Indian PD patients.

## Materials and methods

### Study subjects

The current study was conducted in 306 PD patients visiting outpatient unit of Department of Neurology, Nizam's Institute of Medical Sciences, Hyderabad, Telangana. A total of 271 healthy age-and gender matched controls were recruited in the study and of 306 PD patients genotyping was done in 269 subjects, who had accepted for genetic analysis. All the patients were recruited, based on UK Parkinson's disease society brain bank clinical criteria (except the presence of family history which is not considered exclusion criteria). Patients who had secondary Parkinson's disease (drug- induced), atypical Parkinsonian syndromes and other neurological disorders were excluded from the study. Baseline characteristics such as gender, age, height, weight, body mass index, disease duration, age at onset of all of the patients were obtained using a self-designed questionnaire and medical records. Clinical parameters like disease severity by Hoehn and Yahr (H & Y) Scale, Motor performance by Unified Parkinson's disease rating scale (UPDRS)- part III scores in "OFF" (12 h without dopaminergic medication) and "ON" (best response state after levodopa challenge) states, cognition by MoCA (Montreal Cognitive Assessment), activity of daily living by S & E and Quality of life by PDQ39 Scoring were assessed in each patient. This study was approved by the Institutional Ethics Committee (IEC) of Nizam's Institute of Medical Sciences (NIMS), (EC/NIMS/18/2017), Hyderabad, India. The informed consent was obtained from all the subjects.

### Sample collection

Five milliliter blood samples were collected from both cases and controls. Blood was subjected to centrifugation at 3000 rpm for 10 min for the separation of plasma and serum at 4°C temperature and stored at -20°C for further analysis. Genomic DNA was extracted from whole blood using the standard phenol-chloroform extraction protocol.

### Genotyping of BDNF Val66Met Polymorphism

Genotyping of BDNF Val66Met polymorphism was determined by using standard PCR method<sup>30</sup> and the primers used were i) BDNF forward: 5'- AGA AGAGGAGGCTCCAAAGG -3' and ii) BDNF reverse: 5'- AAACATCCGAGGACAAGG TG-3' . Ten microliters volume was used for each PCR reaction, containing 2 picomole/ $\mu$ L of each primer, 5  $\mu$ L of Takara Emerald Amp GT polymerase chain reaction (PCR) master mix, together with 3–4  $\mu$ L of 50 ng/ $\mu$ L genomic DNA using Eppendorf master cycler. PCR conditions for the amplification were as follows: initial denaturation at 94°C for 5 min, followed by 40 cycles of 30 sec at 94°C, 15 sec at 54.8°C and 45 sec at 72°C for an extension, and a final extension of 10 min at 72°C. The amplified PCR fragments were run on 3% agarose gel and were visualized using an ultraviolet transilluminator for genotyping. The PCR amplification results in 249-bp length amplicon which are subjected to restriction digestion with NlaIII enzyme (New England Biolabs, MA, USA) at 37°C for 120 min. Fragments of 203 bp and 46 bp correspond to the wild G/G genotype, whereas, 126 bp, 77 bp, 46 bp fragments correspond for mutant A/A genotype and 203 bp, 126 bp, 77 bp, 46 bp fragments correspond for the G/A genotype. These were separated by 3% agarose gel electrophoresis and were visualized using an ultraviolet transilluminator for genotyping. Genotype was cross-checked in 10% of the samples by Sanger's sequencing and found 100% concordance.

### Clinical assessment

These were the assessments performed Hoehn and Yahr (H&Y): PD severity was determined by modified H & Y staging. Stage (0-5 severity). MoCA: Montreal Cognitive Assessment (Range: 0-30,  $\geq$ 26 normal,  $<$ 26 impaired cognition), S & E: Schwab and England activities of daily living scale (100-0% disability), PDQ 39: Parkinson's disease questionnaire (higher the score worse will be the quality of life).

UPDRS III: Unified Parkinson's disease rating scale part III clinician-scored monitored motor evaluation in 'on' and 'off' states, aLR: absolute Levodopa response measured as change in UPDRS III (OFF-ON). aLR%: Mean Levodopa response measured as a percentage change in UPDRS III (OFF-ON)/OFF\*100), Tremor dominant, a kinetic rigidity and mixed type PD patients were divided.<sup>31</sup>

### Statistical analysis

Genotype frequencies were tested for the deviation from Hardy–Weinberg equilibrium using chi– square ( $\chi^2$ ) test<sup>32</sup>. Simple gene counting was performed to find out the allele and genotype frequency distributions between the cases and controls. The test of associations was performed by Fisher's exact two-tailed test using the online tool Vassar Stats Calculator ([www.faculty.vassar.edu/lowry/VassarStats.html](http://www.faculty.vassar.edu/lowry/VassarStats.html)). GraphPad Prism Software 5.0 (San Diego, USA) was used for generating bar graphs. Statistical significance for all the tests was considered if a two-tailed *P*- value is  $<$  0.05.

## Results

### Demographic characteristics of PD patients

The demographic characteristics of cases and controls are shown in Table 1. There were 210 male patients and 96 female patients. Among the PD cases, there were 18 familial PD cases and 288 had Sporadic PD. Data were further segregated into early– onset PD (EOPD) ( $\leq$ 50 years) and late-onset PD (LOPD) ( $>$ 50 years) and results show that 15 and 3 familial PD cases, 132 and 156 sporadic PD cases were in EOPD and LOPD groups, respectively. The mean age of cases was  $56.92 \pm 10.97$  years and controls were  $56.12 \pm 10.59$  years, respectively. The mean age of EOPD patients was  $48.44 \pm 8.29$  years whereas,  $64.76 \pm 6.35$  years in LOPD patients which is statistically significant ( $P = < 0.0001$ ). The disease duration of PD was  $7.08 \pm 4.38$  years. The disease duration was significantly high in patients with EOPD,  $8.52 \pm 4.93$  years as compared to LOPD group,  $5.75 \pm 3.29$  years ( $P = < 0.0001$ ).

The distribution of symptoms of PD *i.e.*, right side onset was reported in 47.4% cases, whereas left side onset was observed in 42.5% cases and 10.1% reported bilateral onset of PD. Further the data was segregated among EOPD and LOPD groups and founds 46.9% and 47.8% cases reported right side onset, 44.9%, 40.3% patients with left side onset and 8.2%, 11.9% cases of bilateral onset, respectively. Symmetric onset of PD was observed in 10.5% of patients whereas 89.5% of patients reported asymmetric onset of PD among which 8.2% and 11.9% cases of symmetric onset, 91.8% and 88.1% cases of asymmetric onset were reported each in EOPD and LOPD groups. Cardinal features of PD such as tremors were dominant in 16% cases among which 17% cases in EOPD group and 15.1% cases in

LOPD group, whereas akinetic rigidity was dominant in 72.9% cases out of which 74% in EOPD group, 71.7% in LOPD group, and mixed symptoms were seen in 11.1% cases out of which 9% in EOPD group, 13.2% in LOPD group, respectively. Major diagnostic criteria for PD UPDRS III motor examination revealed, the mean OFF score as  $52.96 \pm 11.65$ , which was distributed significantly high  $55 \pm 12.07$  with the EOPD group ( $P = 0.0031$ ) and  $51.07 \pm 10.95$  with LOPD group, whereas mean ON score  $15.39 \pm 6.76$ , where  $15.27 \pm 6.34$  in EOPD group and  $15.49 \pm 7.14$  in LOPD group was observed. The mean change in Levodopa response (aLR) was  $37.56 \pm 9.10$ , which was found significantly ( $P = <0.0001$ ) less  $39.72 \pm 9.20$  with LOPD compared to  $35.57 \pm 8.57$  EOPD group. The mean of aLR was calculated by aLR% and

it was found to be significantly reduced with LOPD group ( $P = 0.034$ ).

The disease severity score of PD *i.e.*, H & Y stage was  $2.68 \pm 0.76$  whereas  $2.69 \pm 0.76$  was observed with EOPD group and  $2.68 \pm 0.76$  with LOPD group, respectively. The measure of cognitive impairment *i.e.*, MOCA score was  $27.59 \pm 2.77$  where the EOPD group showed  $27.45 \pm 2.29$  in EOPD group and  $27.64 \pm 2.84$  with the LOPD group. The S & E score was  $67.54 \pm 16.07$ , with  $67.19 \pm 16.62$  EOPD group and  $67.86 \pm 15.60$  with LOPD group was observed. Quality of life questioner *i.e.*, PDQ 39 was  $36.85 \pm 29.82$  where  $67.24 \pm 28.86$  and  $60.72 \pm 30.43$  was observed with EOPD and LOPD groups, respectively and was found significantly ( $P = 0.05$ ) decreased quality of life with LOPD group (Tables 1 & 2). There was no significant difference between male and female patients with all the above parameters.

Table 1 — Demographic data in PD cases and controls

Parameters	PD Cases	Control	P value
Age (Years)	$56.92 \pm 10.97$	$56.12 \pm 10.59$	0.42
Male	210 (68.6%)	180 (66.6%)	
Female	96 (31.4%)	90 (29.4%)	
Age at onset (Years)	$49.83 \pm 11.73$	-	-
Disease duration (Years)	$7.08 \pm 4.38$	-	-
EOPD	147 (48.0%)	-	-
LOPD	159 (52.0%)	-	-
Familial PD	18 (5.9%)	-	-
Sporadic PD	288 (94.1%)	-	-
Consanguinity	80 (2.6%)	-	-
Right side onset	145 (47.4%)	-	-
Left side onset	130 (42.5%)	-	-
Bi lateral onset	31 (10.1%)	-	-
Symmetric onset	32 (10.5%)	-	-
Asymmetric onset	274 (89.5%)	-	-
Tremor Dominant PD	49 (16.0%)	-	-
Akinetic Rigidity dominant PD	223 (72.9%)	-	-
Mixed symptom PD	34 (11.1%)	-	-
UPDRS 3 OFF	$52.96 \pm 11.65$	-	-
UPDRS 3 ON	$15.39 \pm 6.76$	-	-
aLR	$37.56 \pm 9.10$	-	-
aLR%	$71.32 \pm 9.87$	-	-
H & Y	$2.68 \pm 0.76$	-	-
S & E	$67.54 \pm 16.07$	-	-
MoCA	$27.59 \pm 2.77$	-	-

aLR, absolute Levodopa response; aLR%, absolute Levodopa response percentage; EOPD, Early-onset Parkinson's disease; PDQ 39, Parkinson's disease questionnaire; LOPD, Late-onset Parkinson's disease; MoCA, Montreal cognitive assessment; PD, Parkinson's disease; PDQ 39, Parkinson's disease questionnaire; S & E, Schwab and England score; UPDRS III, Unified Parkinson's disease rating scale; \*P Value  $\leq 0.05$  considered as significant.

#### Association of BDNF Val66Met polymorphisms with PD

The distribution of genotype frequency of BDNF Val66Met polymorphism was in accordance with Hardy-Weinberg Equilibrium in both cases and controls ( $P > 0.05$ ). Allele and genotype frequencies of BDNF Val66Met polymorphism are given in (Table 3). The genotype frequencies of BDNF Val66Met polymorphism shows higher frequency of homozygous wild G/G genotype with 57.6% genotypic distribution in cases and 66.8% in controls, followed by heterozygous G/A genotype with 34.2% genotype distribution in cases and 29.5% in controls, and homozygous mutant A/A genotype with 8.2% in cases and 3.7% in healthy controls group, respectively. The genotype distribution between cases and controls showed a significant difference in both dominant and recessive models (GG vs GA+AA: OR: 1.47, CI: 1.04-2.09,  $P = 0.03$ ; GG+GA vs AA: OR: 2.32, CI: 1.07-7-5.00,  $P = 0.02$ ). The allelic distribution of wild 'G' allele was 74.72% in cases and 81.55% in controls, whereas 'A' allele frequency was 25.28% in cases and 18.45% in healthy control group, respectively which was found significant between cases and controls (OR: 1.49, CI: 1.11-2.00,  $P = 0.008$ ) (Table 3).

#### Association of Age, age at onset and disease duration with variant genotypes

The age and age at onset were compared between the wild and variant genotypes, where we could not find a significant difference between wild, heterozygous and mutant variants (Age: G/G:  $56.68 \pm 0.87$  years, G/A:  $58.62 \pm 1.18$  years, A/A:  $55.15 \pm$

Table 2 — Demographic data distribution in EOPD and LOPD groups

Parameters	EOPD (N=147)	LOPD (N=159)	P Value
Age (Years)	48.44 ± 8.29	64.76 ± 6.35	<0.0001*
Age at onset (Years)	39.90 ± 7.23	59.00 ± 6.40	<0.0001*
Disease Duration (Years)	8.52 ± 4.93	5.75 ± 3.29	<0.0001*
Male	96 (65.3%)	114 (71.7%)	-
Female	51 (34.7%)	45 (28.3%)	
Familial PD	15 (10.2%)	3 (1.9%)	
Sporadic PD	132 (89.8%)	156 (98.1%)	
Consanguinity	31 (21.0%)	12 (7.5%)	
RT side onset	69 (46.9%)	76 (47.8%)	
Lt side onset	66 (44.9%)	64 (40.3%)	
Bi lateral onset	12 (8.2%)	19 (11.9%)	
Symmetric onset	12 (8.2%)	19 (11.9%)	
Asymmetric onset	135 (91.8%)	140 (88.1%)	
Tremor Dominant	25 (17.0%)	24 (15.1%)	
Akinetic Rigidity dominant	109 (74.0%)	114 (71.7%)	
Mixed symptom	13 (9.0%)	21 (13.2%)	
UPDRS 3 OFF	55 12.07	51.07 ± 10.95	0.0031*
UPDRS 3 ON	15.27 ± 6.34	15.49 ± 7.14	0.7765
aLR	39.72 ± 9.20	35.57 ± 8.57	<0.0001*
aLR%	72.56 ± 9.23	70.18 ± 10.32	0.0348*
H & Y	2.69 ± 0.76	2.68 ± 0.76	0.9085
S & E	67.19 ± 16.62	67.86 ± 15.60	0.6903
MOCA	27.45 ± 2.29	27.64 ± 2.84	0.522
PDQ 39	67.24 ± 28.86	60.72 ± 30.43	0.05*

aLR, absolute Levodopa response; aLR%, absolute Levodopa response percentage; EOPD, Early-onset Parkinson's disease; PDQ 39, Parkinson's disease questionnaire; LOPD, Late-onset Parkinson's disease; MoCA, Montreal cognitive assessment; PD, Parkinson's disease; PDQ 39, Parkinson's disease questionnaire; S & E, Schwab and England score; UPDRS III, Unified Parkinson's disease rating scale; \*P Value ≤ 0.05 considered as significant.

1.975 years), Age at onset: G/G: 49.26 ± 0.94 years, G/A: 51.54 ± 1.300 years, A/A: 48.55 ± 1.95 years). The mean disease duration was compared and there was no significant difference between wild, heterozygous and mutant variants: G/G: 7.41 ± 0.37 years, G/A: 7.07 ± 0.45 years, A/A: 6.63 ± 0.55 years, respectively.

#### Association of UPDRS III Off and On scores with variant genotypes

The mean OFF and ON scores were compared between the variant genotypes, and found to have no significant difference between the wild, heterozygous and mutant variants, UPDRS III OFF score: G/G: 52.98 ± 0.94, G/A: 53.23 ± 1.18, A/A: 57.85 ± 1.99

Table 3 — Frequency distribution of BDNF Val66Met polymorphism

Genotype	Cases Frequency (N=269)	Controls Frequency (N=271)
GG	155 (57.6%)	181 (66.8%)
GA	92 (34.2%)	80 (29.5%)
AA	22 (8.2%)	10 (3.7%)
G	401 (74.72%)	442 (81.55%)
A	135 (25.28%)	100 (18.45)
Model	OR (CI at 95% confidence)	P value
Dominant (GG vs GA+AA)	1.47 (1.04-2.09)	0.03*
Recessive (GG+GA vs AA)	2.32 (1.07-5.00)	0.02*
Allelic distribution G vs A	1.49 (1.11-2.00)	0.008*

OR, odds ratio; CI, Class interval; GG, wild type; GA, Heterozygous; AA, mutant genotype; G, wild type allele; A, mutant allele; frequency expressed in %, \*P Value ≤ 0.05 considered as significant.

whereas UPDRS III ON score: G/G: 15.05 ± 0.50, G/A: 15.96 ± 0.88, A/A: 19.79 ± 1.14.

#### Association of aLR and aLR % with variant genotypes

aLR and aLR% are the Levodopa response patterns observed in PD patients after medication. There was no significant difference between the different genotype variants with aLR: G/G: 37.94 ± 0.77, G/A: 37.27 ± 0.92, A/A: 38.06 ± 1.37 and with aLR%: G/G: 71.75 ± 0.77, G/A: 70.71 ± 1.25, A/A: 69.85 ± 1.45 in wild, heterozygous and mutant variants, respectively.

#### Association of BDNF Val66Met with H & Y stage

The disease severity score *i.e.*, modified H & Y stage when compared between the genotypes of BDNF Val66Met polymorphism, and found a significantly high disease severity with the variant genotype (A/A: 2.90 ± 0.14, P = 0.01) compared to wild type (G/G: 2.57 ± 0.05). When the wild and heterozygous genotypes were compared, the heterozygous genotype carrying the variant allele (G/A: 2.90 ± 0.08, P = 0.008) showed significantly greater disease severity compared to wild genotype (G/G: 2.57 ± 0.05) (Table 4).

#### Association of BDNF Val66Met with S & E score

The Schwab and England ADL (Activities of Daily Living) scale is a method of assessing the capabilities of people suffering from impaired mobility where the scale assesses the difficulties patients have completing daily activities. The S & E score was

found better with wild ( $69.47 \pm 1.21$ ) genotype compared to mutant genotypes ( $65.76 \pm 2.85$ ), but could not reach significance. When wild genotype ( $69.47 \pm 1.21$ ) was compared to heterozygous ( $64.20 \pm 1.89$ ), we found a significant ( $P = 0.01$ ) decrease in S & E score with heterozygous genotype carrying variant allele (Table 4).

**Association of BDNF Val66Met with Tremor dominant, Akinetic rigidity, and mixed symptom PD group**

Higher number of patients were showing patients showing akinetic rigidity dominance (71.7%) compared to tremor dominant (16.3%) and mixed symptom (11.9%). Genotype wise these symptoms were analysed, where 20.6% were tremor dominant, 67.7% akinetic rigidity dominant and 11.6% cases were with mixed symptom in GG genotype, 14.13% tremor dominant, 77.17 % akinetic rigidity and 8.7% were with mixed symptoms in GA genotype. 77.27% were akinetic rigidity, 22.72% were with mixed symptoms in AA genotype but we could not find a single case with tremor predominant with AA genotype (Table 5).

**Association of BDNF Val66Met with MoCA**

Montreal Cognitive Assessment (MoCA) score is used as a screening assessment for detecting cognitive impairment. When MoCA was compared between wild (G/G:  $28.13 \pm 0.15$ ) and mutant (A/A:  $25.48 \pm 0.40$ ,  $P = <0.0001$ ) genotypes, cognition was significantly more impaired with variant genotype. A significant difference in MoCA score was also observed with heterozygous genotype (G/A:  $27.64 \pm 0.25$ ) compared to homozygous variant genotype

Table 4 — Association of BDNF Val66Met polymorphism with H & Y and S & E score distribution

Genotype	H & Y Mean $\pm$ SD (N)	P Value
GG vs GA	$2.57 \pm 0.05$ (155) vs $2.90 \pm 0.08$ (92)	0.0008*
GG vs AA	$2.57 \pm 0.05$ (155) vs $2.90 \pm 0.14$ (22)	0.0158*
GA vs AA	$2.90 \pm 0.08$ (92) vs $2.90 \pm 0.14$ (22)	0.9917
	S & E Mean $\pm$ SD (N)	
GG vs GA	$69.47 \pm 1.21$ (155) vs $64.20 \pm 1.89$ (92)	0.0158*
GG vs AA	$69.47 \pm 1.21$ (155) vs $65.76 \pm 2.85$ (22)	0.2093
GA vs AA	$64.20 \pm 1.89$ (92) vs $65.76 \pm 2.85$ (22)	0.6547

GG, wild type; GA, Heterozygous; AA, mutant genotype; H & Y, modified Hoehn and Yahr scale; S & E, Schab England score; \*P Value  $\leq 0.05$  considered as significant.

(A/A:  $25.48 \pm 0.40$ ,  $P = <0.0001$ ) (Table 6). Cognitive impairment is more prevalent among akinetic rigid subgroup of PD patients. When the patients of PD were segregated into Tremor dominant (TD), Akinetic Rigidity (AR) and Mixed symptom (MD) groups, the impact of variant genotype on cognitive impairment persisted in the subgroups. Cognition was significantly worse in patients with mutant genotypes in AR group (G/G:  $28.04 \pm 0.60$ , A/A:  $25.68 \pm 0.44$ ,  $P < 0.0001$ ) and MD group (G/G:  $27.61 \pm 0.60$ , A/A:  $24.40 \pm 0.92$ ,  $P = 0.01$ ) and none of the mutant group had tremor predominant PD (Table 7).

**Association of BDNF Val66Met with PDQ39**

The 39-item Parkinson's disease Questionnaire (PDQ-39) is widely used to assess the quality of life of PD patients. When PDQ-39 was compared between wild ( $59.68 \pm 2.19$ ) and variant ( $74.36 \pm 4.68$ ) genotypes, we noted a significant decrease in the quality of life of PD patients with homozygous mutant genotype (Table 6).

Within the tremor dominant group genotype, wise PDQ39 was compared where no significant difference was found between wild and heterozygous variants. When genotype wise PDQ 39 was compared within the akinetic rigidity dominant group, significantly reduced quality ( $75.11 \pm 5.44$ ) of life was found with heterozygous ( $P = 0.03$ ) compared to ( $62.33 \pm 2.78$ ) wild genotype, similarly significantly reduced quality

Table 5 — Genotype wise Frequency distribution of Tremor dominant, Akinetic rigidity and mixed symptom PD group

Genotype	Tremor Dominant PD	Akinetic Rigidity dominant PD	Mixed symptom PD
GG	32 (20.6%)	105 (67.7%)	18 (11.6%)
GA	13(14.13%)	71 (77.17%)	8 (8.70 %)
AA	-	17 (77.27%)	5 (22.72%)

GG, wild type; GA, Heterozygous; AA, mutant genotype; PD= Parkinson's disease \*P Value  $\leq 0.05$  considered as significant.

Table 6 — Association BDNF Val66Met polymorphism with MOCA and PDQ 39

Genotype	MoCA Mean $\pm$ SD (N)	P Value
GG vs GA	$28.13 \pm 0.15$ (155) vs $27.64 \pm 0.25$ (92)	0.087
GG vs AA	$28.13 \pm 0.15$ (155) vs $25.48 \pm 0.40$ (22)	$< 0.0001^*$
GA vs AA	$27.64 \pm 0.25$ (92) vs $25.48 \pm 0.40$ (22)	$< 0.0001^*$
	PDQ 39 Mean $\pm$ SD (N)	
GG vs GA	$59.68 \pm 2.19$ (155) vs $65.52 \pm 3.21$ (92)	0.5058
GG vs AA	$59.68 \pm 2.19$ (155) vs $74.36 \pm 4.68$ (22)	$0.0054^*$
GA vs AA	$65.52 \pm 3.21$ (92) vs $74.36 \pm 4.68$ (22)	0.1343

GG, wild type; GA, Heterozygous; AA, mutant genotype; MoCA, Montreal Cognitive assessment; PDQ 39, Parkinson's disease questionnaire; \*P Value  $\leq 0.05$  considered as significant.

Table 7 — Association of BDNF Val66Met polymorphism with MOCA in tremor predominant, akinetic rigidity, and mixed symptom cases

Genotype	Tremor dominant MoCA Mean ± SD (N)	P Value	Akinetic rigidity MoCA Mean ± SD (N)	P Value	Mixed symptom MoCA Mean ± SD (N)	P Value
GG vs GA	28.72 ± 0.23 (32) vs 27.77 ± 0.46 (13)	0.050*	28.04 ± 0.18 (105) vs 27.42 ± 0.32 (71)	0.0758	27.61 ± 0.60 (18) vs 29.13 ± 0.39 (8)	0.1259
GG vs AA	28.72 ± 0.23 (32) vs Nil	-	28.04 ± 0.18 (105) vs 25.68 ± 0.44 (17)	< 0.0001*	27.61 ± 0.60 (18) vs 24.40 ± 0.92 (5)	0.0183*
GA vs AA	27.77 ± 0.46 (13) vs Nil	-	27.42 ± 0.32 (71) vs 25.68 ± 0.44 (17)	0.0027*	29.13 ± 0.39 (8) vs 24.40 ± 0.92 (5)	0.0002*

GG, wild type; GA, Heterozygous; AA, mutant genotype; MoCA= Montreal cognitive assessment, \*P Value ≤0.05 considered as significant.

Table 8 — Association of BDNF Val66Met polymorphism with PDQ39 in tremor predominant, Akinetic rigidity and mixed symptom cases

Genotype	Tremor dominant PDQ 39 Mean ± SD (N)	P Value	Akinetic rigidity PDQ 39 Mean ± SD (N)	P Value	Mixed symptom PDQ 39 Mean ± SD (N)	P Value
GG vs GA	49.63 ± 3.61 (32) vs 54.77 ± 6.71 (13)	0.4718	62.33 ± 2.78 (105) vs 75.11 ± 5.44 (71)	0.0373*	62.06 ± 6.54 (18) vs 54.00 ± 7.95 (8)	0.4788
GG vs AA	49.63 ± 3.61 (32) vs Nil	-	62.33 ± 2.78 (105) vs 75.46 ± 5.00 (17)	0.0297*	62.06 ± 6.54 (18) vs 79.60 ± 7.28 (5)	0.196
GA vs AA	54.77 ± 6.71 (13) vs Nil	-	75.11 ± 5.44 (71) vs 75.46 ± 5.00 (17)	0.9616	54.00 ± 7.95 (8) vs 79.60 ± 7.28 (5)	0.0500*

GG, wild type; GA, Heterozygous; AA, mutant genotype; PDQ 39= Parkinson's disease questionnaire, \*P Value ≤0.05 considered as significant.

of life ( $75.46 \pm 5.00$ ) found with mutant genotype ( $P = 0.02$ ) compared to ( $75.46 \pm 5.00$ ) wild genotype. Whereas the significantly poor quality of life ( $79.60 \pm 7.28$ ) with mutant variant ( $P = 0.05$ ) compared to heterozygous variant ( $54.00 \pm 7.95$ ) within mixed symptom group (Table 8).

## Discussion

Parkinson's disease although, more common in people >65 years, can affect the younger people too.<sup>33,34</sup> PD is multifactorial in origin, resulting in progressive degeneration of the dopaminergic neurons of the nigrostriatal pathway, neuro-inflammation, Lewy bodies' deposits and the damage of the neural circuits that predominantly control movement, along with other functions.<sup>35-37</sup> The origin of the motor and non-motor complications of PD can be due to several genetic alterations that have been described, including  $\alpha$ -synuclein, SNCA, PINK 1, DJ-1, LRRK2, ATP13A2, PLA2G6, FBX07, VPS35, and BDNF genes.<sup>38,39</sup> Independent of the etiologic origin, patients with PD can develop various non-motor symptoms which can cause major morbidity and cognitive dysfunction is a major contributor. BDNF gene mutations are reported to be associated with such non-motor complications in PD.

BDNF gene encodes the protein Brain-Derived Neurotrophic Factor, involved in neuroplasticity<sup>14</sup>

which promotes survival, differentiation, and maintenance of neuronal cells. To the best of our knowledge, there are no studies available to date exploring the role of BDNF Val66Met polymorphism in South Indian PD subjects. Hence, the present study was carried out to explore the association of BDNF Val66Met variants with PD risk and cognitive impairment. We found a significant increase of "A allele" frequency in PD patients (25.28%) compared to the controls (18.45%) and established a significant association of BDNF Val66Met "A/A genotype" as well as "A allele" in PD. Worldwide, the distribution of alleles have been reported, with the highest prevalence of Val/Val allele (from 59 to 72%), followed by Val/Met (from 25 to 38%) and a lower prevalence of the Met/Met allele (from 2 to 4%)<sup>40-42</sup>. The cause of PD is associated with numerous molecular factors linked to the survival and vulnerability of the dopaminergic neurons (DN) of the substantianigra (SN)<sup>35,43,44</sup>. In a study by Howells *et al.*<sup>45</sup>, patients with PD had low levels of BDNF mRNA, suggesting that low BDNF mRNA levels may contribute to the death of dopaminergic neurons and to the development of the disease. The regulation of BDNF expression has become a key strategy for the rescue of damaged dopaminergic neurons in PD.

In our study, we also noted the association

of BDNF Val66Met polymorphism with cognitive impairment in PD patients. BDNF homozygous Met/Met genotype showed a significant higher frequency of cognitive impairment than Val/Val homozygotes. This finding is supported by Foltynie *et al.*<sup>21</sup> in his study in 2005, where 291 patients were investigated for the impact of BDNF Val66Met on cognitive impairments of the patients and the results of this investigation were the association of the BDNF polymorphism with planning ability in PD. A higher risk of cognitive impairment in PD patients was reported to be associated with the BDNF Val66Met allele<sup>20</sup>.

From the epidemiological studies, it is evident that higher age, severe motor symptoms, older age at the onset, longer disease duration, and male gender were likely to be risk factors of PD cognitive dysfunction<sup>46,47</sup>. A recent study<sup>48</sup> suggested that different genetic factors could play a role in cognitive impairment. In the experimental knockout mice model, it has been observed that the inhibition of BDNF signalling can affect spatial learning and memory<sup>49</sup>. Recently, it has been detected that subjects with BDNF Met-allele show decreased cognitive flexibility when compared to homozygous carriers of the BDNF Val-allele<sup>50</sup>. In a meta-analysis, it was observed that BDNF Val66Met was significantly associated with cognitive impairment in PD patients specifically in Caucasians<sup>51</sup>. In our study, there was no difference in gender prevalence, the age at onset, disease duration and the motor disability (UPDRS III scores) among the various alleles. The impact of the Met/Met genotype on cognitive impairment persisted after matching for the PD phenotypic subsets as well as the age of onset.

In our study Met/Met genotype was not found in tremor predominant group and a kinetic rigidity was found predominant with the same genotype. Impaired cognition was found predominant with a kinetic rigidity group which was highly expressed with Met/Met genotype.

The emerging evidence indicates that the BDNF polymorphism may modifies the clinical course of PD or the prevalence of PD. Our results (Cases Met/Met genotype frequency: 8.2 %, whereas Controls Met/Met genotype frequency 3.7%) were supported by a Japanese association study on 20 candidate gene SNPs and PD, revealed that the frequency of Met/Met genotype is higher in PD patients than healthy controls<sup>52</sup>. The homozygous mutant genotype

(Met/Met:  $25.48 \pm 0.40$ ) and heterozygous genotypes (Val/Met:  $27.64 \pm 0.25$ ) were significantly associated with cognitive dysfunction, which was in accordance with two studies where the homozygous mutant genotype and heterozygous genotypes were shown to associated with higher prevalence of cognitive impairment in PD patients<sup>53,54</sup>. The disease severity was found significantly higher with Met/Met genotype and Val/Met genotype compared with Val/Val genotype<sup>53,54</sup>. In the line of with these findings, we also found that Met/Met genotype is associated with disease risk and severity.

The clinical outcome measured as change in the quality of life utilising PDQ39 questioner reveals that Met/Met genotype was significantly associated with poor quality of life compared to homozygous Val genotype. The activity of daily living measured by The Schwab and England ADL (Activities of Daily Living) scale was significantly lower with Met/Met genotype. Regular follow-up of these patients in particularly patients carrying variant genotype may help in better management.

Although our study is limited by only performing a screening test of cognitive impairment, we found irrefutable proof of the association of Met/Met genotype with poor cognition as well as the disease severity and quality of life. Therefore, given the importance of cognitive impairment in PD and the apparent influence of genetic factors in this condition, further prospective studies are required to explicate the role of genetic factors on cognitive impairment in PD.

## Conclusion

We found significant association of BDNF Val66Met polymorphism with PD risk in both dominant and recessive models. Consistent with the literature, we also observed the cognitive impairment in patients carrying BDNF Val66Met polymorphism. In PD patients, an increase in disease severity and decrease in quality of life and activity of daily living were also observed with this polymorphism.

## Conflict of Interest

All authors declare no conflict of interest.

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