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# Brain-derived neurotrophic factor (BDNF) val66met polymorphism influences clinical and neurocognitive outcomes in patients with mild to moderate traumatic brain injury

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**Abstract**

**Aim:** Traumatic Brain injury (TBI) is a major cause of injury and death annually in India. Most patients who sustain a mild or moderate head injury recover within weeks to months. Genetic variation in the Brain Derived Neurotrophic Factor (BDNF) gene among such individuals might be the cause of differential response and recovery from TBI. In our study, BDNF gene was examined in patients with TBI in order to see if a correlation could be found between BDNF Gene polymorphism and the extent of recovery from mild to moderate TBI. This study is a first of its kind in India.

**Materials and methods:** A Total of 115 patients who had suffered from mild to moderate head injury and who were admitted to the hospital emergency ward were enrolled in the study. On consent of the patients, 5 ml venous blood samples were collected from them and preserved in EDTA at -20°C. Polymerase Chain Reaction followed by Restriction Fragment Length Polymorphism and Agarose Gel Electrophoresis were used to genotype the BDNF gene and determine the allele types. They were then assigned a category based on the Glasgow Coma Scale through interviews and examination. Six months follow up data was available for 115 patients.

**Results:** A protective effect of the G allele in the gene with better performance in attention, executive function, memory, and overall cognition among patients with mild and moderate TBI was observed.

**Conclusions:** Our current study has demonstrated the role of the BDNF rs6265 Val66Met polymorphism in influencing clinical and neurocognitive outcomes in patients with mild and moderate TBI, where Met allele carriers had considerably low standard scores in clinical & cognitive domains.

**Keywords:** traumatic brain Injury, brain derived neurotrophic factor, polymerase chain reaction, restriction fragment length polymorphism

## Introduction

Traumatic Brain injury is a major cause of injury and death with nearly 1.5 to 2 million individuals injured annually in India 1. Most patients who sustain a mild or moderate head injury recover within weeks to months. A subgroup of patients, however, continues to experience disabling symptoms beyond this period, interfering with return to work or resumption of social activities 2. TBI is a leading cause of death and disability, with about 10 million people affected each year 3. Road traffic accidents are the leading cause (60%) of TBIs followed by falls (20%-25%) and violence (10%) 4. Alcohol involvement is known to be present among 15%-20% of TBIs at the time of injury 5.

An individual with traumatic brain injury (TBI) is someone who has a trauma-induced disruption of brain function 6. This is generally manifested by at least one of the following:

1. Any period of loss of consciousness
2. Any loss of memory for events immediately before or after the accident
3. Any alteration in mental state at the time of the accident (e.g. feeling dazed, disoriented, or confused),
4. Focal neurological deficit(s) that may or may not be transient.

TBI includes head-object collision (direct injury) and/or the brain undergoing an acceleration/deceleration movement (i.e. whiplash) without direct external trauma to the head 7. The severity of TBI can be determined on the basis of the Glasgow Coma Scale (GCS) and the duration of posttraumatic amnesia (PTA). The GCS, measures the level of consciousness and includes three categories: eyes opening (score 1-4), best verbal response (score 1-5), and best motor response (score 1-6) 8. The total scores of the GCS ranges from 3 to 15. Low scores indicate a low level of consciousness and vice-versa. PTA is usually seen in TBI cases where patients become disoriented and are unable to remember events post the injury 9. PTA is resolved only when continuous memory returns 10.The Finnish Adult TBI Guideline recommends the use of both the GCS and length of PTA. The following categories are suggested by the Guideline: mild (GCS 13-15 or PTA < 24 hours), moderate (GCS 9-12 or PTA 1 to 7 days), and severe (GCS $\leq$8 or PTA > 7 days), or very severe (PTA > 4 weeks) 11.

Following TBI, the brain attempts to activate repair mechanisms and stimulate neuroregeneration. This is facilitated by a family of neurotrophic factors that include nerve growth factor, glia-derived neurotrophic factor, neutrophin-3, and brain-derived neurotrophic factor (BDNF) 7. The human BDNF gene has been mapped to the short arm

of chromosome 11. (11p13). A single nucleotide polymorphism (SNP) at the 66th Codon of the BDNF gene produces a valine (Val) to methionine (Met) missense change (G196A: Val66Met) 7. This SNP alters the intracellular trafficking and packaging of pro-BDNF which in turn affects the regulated secretion and neuroplastic effect of mature BDNF 7. This polymorphism has also been linked to cognitive functioning and clinical pathology 12. In healthy individuals, Met allele has been linked to impaired episodic memory, working memory and hippocampal function 13. In clinical populations, the met allele has been associated with a wide range of neurodegenerative diseases such as Alzheimer's disease 8, 14.

The BDNF protein acts on certain neurons of the nervous system aiding their survival as well as stimulating the growth and differentiation of more neurons and synapses. BDNF is active in the hippocampus, cortex, and basal forebrain—areas vital to learning, memory, and higher thinking. It is also expressed in the retina, motor neurons, the kidneys, saliva, and the prostate 15. BDNF reduces the impact of Secondary Brain Injury (ischemic, inflammatory, cytotoxic and apoptotic processes) via alterations in BDNF induced gene expression in traumatized tissue [16]. In addition, BDNF increases long-term potentiation of neurons, decreases long-term depression and increases certain forms of short-term plasticity 16.

In the following study, BDNF gene polymorphism Val66Met was examined in patients with TBI in order to see if a correlation could be found between the polymorphism and the extent of recovery from mild to moderate TBI.

**Material and Methods**

This Study was conducted at the Neurogenetic Unit of Bangur Institute of Neurosciences, Institute of Psychiatry and the Department of Neurosurgery at Institute of Post Graduate Medical Education and Research and S.S.K.M. Hospital (Seth Sukhlal Karnani Memorial Hospital), Kolkata for a period of one year from February, 2018 to February, 2019. All patients (n=115) of mild & moderate (GCS 9 & above) head injury admitted in S. S. K. M. Hospital, Kolkata during this time period was included in this study. Patients who were less than 18 years of age, patients who had associated chest, abdomen, limb or penetrating brain injury and those with pre-existing psychomotor impairment were excluded from this study. All admitted patients were investigated clinically, radiologically and treated conservatively as per standard protocol.

Inclusion criteria of the TBI patients include mild to moderate head injury GCS≥9 (classified according to Glasgow Coma Scale) and age more than 18 years. Exclusion criteria are GCS<9 (Severe head Injury), associated chest, abdomen & limb injury, penetrating brain injury and patients with preexisting psychomotor impairment.

Patients of traumatic head injury were admitted in the emergency ward of S.S.K.M Hospital and post evaluation, standard treatment protocol was undertaken as per clinical and neuroimaging data. If necessary, they underwent emergency surgery for head injury. With their consent, 5 ml venous blood sample was collected from patients. All patients were followed up after discharge, once at 3 months and once at 6 months. They were allocated to one of the categories of Glasgow Outcome Scale after interview and examination.

The patients included in the study were evaluated for the following features: History of event-First observed, progress, route of referral and existing medical history; initial examination including Glasgow Coma Scale (GCS), the duration of post-traumatic amnesia (PTA), if any; Neuroimaging data and operative notes, surgical procedure was done and date of discharge or death and GCS at the time of discharge. In addition to genetic analysis, psychological tests were also performed. Neurological assessment using PGI Battery of Brain Dysfunction (PGIBBD) Neuropsychological battery was done at 3 & 6 months.The components of the PGI-Battery of Brain Dysfunction are as follows:

- PGI Memory Scale (PGIMS)
- Revised Bhatia Short Battery of Performance Tests of Intelligence (BSR-R)
- Verbal Adult Intelligence Scale (VAIS)
- Nahar–Benson test
- Bender visual motor Gestalt test (Bender–Gestalt test).

After getting a signed consent from the patient or from immediate family members, venous blood sample (4-5 ml) were collected from patient and preserved in EDTA at -20ºC for DNA isolation. Moreover, 2ml of venous blood sample was collected in vials not having any anticoagulants. Serum was isolated from these samples and stored at -20 ºC for ELISA. Genomic DNA was isolated from EDTA blood using the standard phenol-chloroform protocol.

100ng of DNA was used as a template for Polymerase Chain Reaction (PCR). The primer sequences are given below in Table 1. The PCR amplification was carried out in a final reaction volume of 25 µl containing ~100 ng of genomic DNA (dissolved in Tris EDTA Buffer), 1x PCR buffer, 2mM MgCl2, 0.2mM dNTPs, 10 picomoles forward primer, 10 picomoles reverse primer, 1.25 units Taq polymerase. After an initial denaturation at 95°C for 5 min., the reaction was continued for 30 cycles for 94°C for 30 sec., 60°C for 30 sec. and 72°C for 30 sec. Final elongation was done at 72°C for 5 min [17, 18]. Then Restriction Fragment Length Polymorphism (RFLP) was carried out using Restriction Enzyme Eco-721. The RFLP tubes were incubated for 18h at 37°C. The different digestion products were visualized as bands under UV light on 4% Agarose Gel using a gel documentation system (BIO-RAD).

**Table 1:** Nucleotide sequence of primers [17, 18]

| No. | Primer | Sequence (5→3) |
|---|---|---|
| 1 | Forward Primer | 5´- GAG GCT TGA CAT CAT TGG CT-3´ |
| 2 | Reverse Primer | 5´- CGT GTA CAA GTC TGC GTC CT -3´ |

The BDNF genotypes of the patients were categorized into three groups according to the allelic form present, i.e, AA [Methionine], GG [Valine] and GA [Valine and Methionine.]

ELISA was performed using a commercial human BDNF ELISA kit and blood serum levels of BDNF for the subjects. The samples were analyzed for any correlation with the genotypes.

Descriptive statistical analysis was performed to calculate the means with corresponding standard deviations (s.d.). Test of proportion was used to find the Standard Normal Deviate (Z) to compare the difference proportions. Chi-square ($\chi^2$) test was performed to find the associations between the variables. Corrected Chi-square ($\chi^2$) test was

performed to find the associations between the variables when one of the cell frequencies was less than zero. t-test was used to compare the difference between two means. Also One Way Analysis of variance (ANOVA) followed by post hoc Tukey's Test was performed with the help of Critical Difference (CD) at 5% and 1% level of significance to compare the mean values. Then $p<0.05$ was taken to be statistically significant.

**Observation and Results**

A total of 115 patients were included in our study for BDNF genotyping with a minimum 6 months follow up. Met allele and Val allele were found in 30.48% and 34.78% of the patients, respectively. In 61.74% of the patients both the alleles, Methionine with Valine was found.

**Table 2:** Distribution of BDNF Gene polymorphism

| Polymorphism | Amino Acid | Distribution | Total (%) |
|---|---|---|---|
| AA | Methionine | 4/115 | 3.48% |
| GA | Valine, Methionine | 71/115 | 61.74% |
| GG | Valine | 40/115 | 34.78% |

ANOVA-test showed that there was no significant difference in mean ages, loss of consciousness, GCS on admission and discharge of the patients of three groups ($p>0.05$). Also, Chi-square ($\chi^2$) test showed that there was no significant difference in gender of the patients of the three groups ($p>0.05$). Thus, the patients of the three groups were matched for all demographic parameters.

Dysfunction Rating Score (DRS) was allotted to the patients and is indicative of neurocognitive outcome. The mean dysfunction score (mean ± standard deviation) of the patients was 19.01±2.35 with range 17 – 27 and the median was 18. Most of the patients (83.3%) had dysfunction score ≤20. However, 16.7% of the patients had dysfunction score>20. One way ANOVA showed that there was significant difference in mean Dysfunction Rating Score and hospital stay of the patients of the three groups ($p<0.05$). As per Tukeys Test followed by ANOVA, the mean Dysfunction Rating Score of the patients with Methionine was the highest of all and the mean hospital stay of the patients with Valine was the highest of all.

**Table 3:** Comparison of Glasgow Outcome Score (GOS) of the patients according to the polymorphism and time interval

| Time Interval | Methionine (n=4) | | Methionine+Valine (n=71) | | Valine (n=40) | |
|---|---|---|---|---|---|---|
| | Mild disability (GOS-2) | Low disability (GOS-1) | Mild disability (GOS-2) | Low disability (GOS-1) | Mild disability (GOS-2) | Low disability (GOS-1) |
| At Discharge | 2 (50%) | 2 (50%) | 7 (9.8%) | 64 (90.1%) | 10 (25.0%) | 30 (75.0%) |
| At 3 month Follow up | 2 (50%) | 2 (50%) | 7 (9.8%) | 64 (90.1%) | 4 (10.0%) | 36(90.0%) |
| At 6 month Follow up | 1 (25%) | 3 (75%) | 4 (5.6%) | 67(94.3%) | 0 (0.0%) | 40 (100.0%) |

There was no significant difference in mean Glasgow Outcome Score at 3 months and at 6 months for Methionine containing form of BDNF (AA) ($t_{78}=1.25$; $p>0.05$). The mean Glasgow Outcome Score at 6 months was significantly higher than that of at 3 months for individuals with the Valine containing form of BDNF (GG) ($t_{78}=3.15$; $p<0.001$). The mean Glasgow Outcome Score at 6 months was significantly higher than that of at 3 months for individuals with Valine and Methionine (GA) ($t_{78}=3.15$; $p<0.001$).

Mild and moderate TBI patients with BDNF rs6265 Val homozygous allele showed significant differences in their clinical & neurocognitive performance and were more likely to perform better than the Met carriers in overall cognition. The Met allele carriers of BDNF rs6265 had considerably low standard scores in most clinical & neurocognitive domains observed longitudinally. No correlation has been found with BDNF genotypes and serum BDNF levels.

**Discussion**

S.S.K.M. Hospital is one of the main government tertiary care hospitals in West Bengal. Thus, patients from all over West Bengal are admitted here for various cases, traumatic brain injury being one of them. In our study, we genotyped the DNA of 115 patients for the BDNF gene Val66Met SNP (Single Nucleotide Polymorphism). We have analysed distribution of TBI in various age groups, sex, geographic distribution & mode of injury; its clinical outcome and neurocognitive outcome in relation to severity of brain injury. We have also analysed distribution of BDNF alleles in our study population and it's correlation with the clinical outcome and neurocognitive outcome of mild & moderate TBI over time. This is only study in India till now which checks the BDNF Polymorphism type and its effect on recovery from TBI.

We observed a possible protective effect of the G allele in rs6265, with better performance in the domains of attention, executive function, memory, and overall cognition among patients with mild and moderate TBI. The "finer" performance by those patients with the wildtype G allele in both neurocognitive and neurobehavioral measures, have been consistently reported by other studies involving other CNS pathologies as well [19-21].

While some studies have reported increased neurocognitive vulnerability in G allele homozygotes [22-24], we believe that the superior neurocognitive performance observed in our study might be due to the possibility that the G homozygous allele is associated with increased activity dependent secretion of BDNF, increased synaptic plasticity, and better hippocampus dependent memory and cognitive performance [21]. These mechanistic processes have been well explicated in the works of Egan [25] and Kauppi [26]. The divergent influence of haplotype specific variants cannot be overlooked as well. On the other hand, patients with the A allele in our study revealed mostly non-significant trends of impaired neurocognitive performance with some interactions seen across the time points in the overall cognition. Additionally, the memory domain saw statistically significant interaction over time and was also negatively associated with allele status, with evidence of regressing memory function at 6 months among the patients with the A allele. Longitudinal change in memory performance with evidence of deteriorating performance among the A minor allele group (Met carriers) was observed.

Strengths of the study include a relatively well characterized homogeneous group of patients in terms of injury type or severity, a short time frame from the time of injury to

clinical & neurocognitive testing, detection of early neuropsychological deficits in the acute stage, and a consistent reassessment interval at 6 months post trauma.

However, there are also certain limitations in our study that are worth mentioning. First, the method of dichotomizing the patients' allele carrier status category (wildtype G allele vs. A minor allele, both the homozygous and heterozygotes) may have unequally diminished the dual allele effect of the A minor allele homozygous vs. heterozygotes (Met/Met vs. Val/Met) on the neurocognitive performance. Additionally, the sample size representing each arms of the rs6265 polymorphism was rather small and should be increased in future longitudinal studies.

Our study has demonstrated the role of the BDNF rs6265 Val66Met polymorphism in influencing clinical and neurocognitive outcomes in patients with mild and moderate TBI. Findings were detrimentally profound among Met allele carriers. It was reported that Val66Met had less mature BDNF in the cortex. This could lead to neuro-inflammation and apoptosis after trauma. These experiments were done in mice [27]. Thus this type of study is the need of the hour for genetically targeted therapy in TBI. The identification of polymorphism is also important as Val66Met carriers have previously been shown to be more susceptible to nootropic drugs and less susceptible to antipsychotics [28]. Investigation of this polymorphism, in future studies may have significant influence over the ways in which TBI patients can be managed with personalized therapies and their outcomes predicted.

## Conclusion

In our study, a protective effect of the G allele instead of A allele in the BDNF gene at position 196 was observed, with better performance in attention, executive function, memory, and overall cognition among patients with mild and moderate TBI. Overall, our current study has demonstrated the role of the BDNF (rs6265) Val66Met polymorphism in influencing clinical and neurocognitive outcomes in patients with mild and moderate TBI, where Met carriers had considerably detrimental effects having low scores in clinical & cognitive domains.

## Ethical considerations

After the subjects' guardian had been given a complete description of the study, written informed consent was obtained. The protocol was approved by the Ethics Committee of I.P.G.M.E&R. and S.S.K.M. Hospital, Kolkata.

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## Conflict of Interest

Authors declare that there is no conflict of interest about the work.

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