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Original Article

Association of Brain-derived Neurotrophic Factor (rs6265) Gene Polymorphism with Susceptibility to Epilepsy

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Abstract

Objective: This study aimed to measure serum brain-derived neurotrophic factor (BDNF) and neuronal nitric oxide synthase (nNOS) levels in Egyptian children with epilepsy, to calculate the frequencies of *BDNF* gene polymorphisms to elucidate their usefulness as biomarkers for diagnosing epilepsy and assessing drug response.

Methods: Serum BDNF and nNOS levels were measured by enzyme-linked immunosorbent assay in 60 epileptic children as well as in 30 healthy children of the same age and sex. The BDNF rs6265 polymorphism was evaluated by genomic TaqMan genotyping.

Results: The genotyping distribution of the *BDNF* gene did not differ significantly between the controls and epileptic cases. The homozygous (G/G) responded to treatment far better. Mean serum BDNF was substantially less than the controls. However, children with epilepsy had considerably higher mean serum nNOS concentration.

Conclusion: BDNF genotypes have a big impact on responsiveness to therapy. In epileptic children, the mechanism of epileptogenesis is influenced by serum BDNF and nNOS.

Keywords: Brain-derived neurotrophic factor, neuronal nitric oxide synthase, gene polymorphism, epilepsy

INTRODUCTION

Epilepsy is a neurological condition that affects 0.5-1% of people globally, it results in aberrant neuronal discharge.[1,2] Fisher et al.[3] described the independent occurrence of two or more unprovoked seizures is known as epilepsy. They are not classified as febrile or neonatal seizures, either separately or in combination and are typified by involuntary motor, sensory, or autonomic seizures. Various pieces of evidence have suggested that epilepsy may be caused by genetic variations, even if the exact cause of the condition is still unknown. Over 70% of people with epilepsy are thought to have hereditary susceptibility.[4] The effectiveness of treatment can be impacted by genetic anomalies that change electrical impulses, channel function, neuronal excitability, and possibly even the pharmacokinetics of antiepileptic drugs (AEDs).[5]

The most abundant neurotropic factor in the brain is a tiny dimeric protein called brain-derived neurotrophic factor (BDNF). While it has a modest affinity for the p75 receptor, it has a high binding affinity for the tyrosine kinase receptor B. Several intracellular cascades, such as the signaling route for mitogen-activated protein kinase are activated because of these attachments promote both development and survival in a range of neurons. Research has demonstrated that it weakens inhibitory gamma-aminobutyric acid synapses while strengthening excitatory (glutamatergic) ones. BDNF enhances neurogenesis and contributes to activity-dependent synaptic plasticity such as learning and memory.[6]

The 5′ proregion of the human BDNF protein is altered by a single-nucleotide polymorphism (SNP) that replaces methionine (met) at codon 66 (Val66met) with valine (Val), which is irrelevant to position 196 of exon 2 (rs6265). This polymorphism affects activation dependent BDNF synthesis at the synapse, axonal transport, and intracellular packing of pro-BDNF.[7,8]

There is evidence linking neuronal nitric oxide synthase (nNOS) to epileptogenesis. Both kainic acid-induced seizure rat models and a status epilepticus mouse model generated by electrical stimulation exhibited increased nNOS levels.[9,10] According to Akyuz et al.,[11] nNOS

inhibition may have an anticonvulsant impact since it raises the epileptic threshold when nNOS activity is blocked.

This study aimed to assess the association of the *BDNF* (rs6265) gene polymorphism with epilepsy susceptibility in Egyptian patients, and to evaluate serum BDNF and nNOS levels as biomarkers of disease severity and treatment response in childhood epilepsy.

METHODS

Patients

A total of 90 Egyptian youngsters (58 boys and 32 girls) were enrolled in this comparative case-control study. The study included 60 children with epilepsy and 30 apparently healthy, demographically and ethnically matched controls. Between December 2021 and October 2022, patients with epilepsy were selected from our institution's pediatric neurology outpatient clinic.

Based on the children's medical history and an electroencephalogram (EEG), the diagnosis of epilepsy was reached. Following a third seizure within two months, all study participants were placed on antiepileptic medication, and they underwent clinical, biochemical, and EEG evaluations every three months.

The cases studied were chosen randomly from children who had experienced seizures over the previous 12 months. The parents provided a written informed consent prior to the children's enrolment in the study. Every patient underwent a thorough clinical examination and medical history taking.

Exclusion Criteria

The following were the exclusion criteria:

- Patients who are older than 15 years old.
- Patients who have epilepsy because of diseases including meningitis or encephalitis, head trauma, brain tumors, or inadequate oxygen exposure during birth.
- Patients with developmental abnormalities including autism and neurofibromatosis.
- Patients with an unpredictable seizure frequency.
- Patients with inadequate medical records.

MAIN POINTS

- The combined evaluation of genetic and biochemical markers may contribute to improved understanding of epilepsy pathophysiology and potential biomarker development.
- Children with epilepsy exhibited significantly lower serum brain-derived neurotrophic factor (BDNF) levels and significantly higher neuronal nitric oxide synthase (nNOS) levels compared with healthy controls.
- No significant difference was observed in the distribution of the BDNF rs6265 (Val66Met) polymorphism between patients and controls; however, patients with the homozygous G/G genotype showed a significantly better response to antiepileptic therapy than heterozygous G/A patients.
- Serum BDNF and nNOS levels were identified as significant predictors of treatment response and resistance, supporting their potential role as biomarkers in pediatric epilepsy.

Blood Samples

Early in the morning, during the interictal phase, blood samples were taken. Five milliliters of blood were collected from patients by venipuncture. Samples were divided into two parts. The first part was added to ethylenediamine-tetra acetic acid (EDTA) tube, and the other part was added to plane tube. The EDTA tubes were preserved at -80 ^{o}C until the extraction of genomic DNA. The plane tubes were centrifuged, and sera were obtained and frozen at -20 ^{o}C.

I. BDNF Genotyping

1) The Extraction of DNA

QIA amp DNA mini kit (QIAGEN, Lot No. 169017038) was used to extract the DNA. DNA was extracted according to manufacturer's instructions.

2) SNP Genotyping

SNP genotyping was done using TaqMan SNP genotyping assays.

Assay ID: C_11592758_10.

Catalog number: 4351379.

SNP ID: rs6265.

Product Description

TaqMan SNP genotyping assays provide optimized assays for genotyping SNPs. The products use the 5´nuclease assay for amplifying and detecting specific SNP alleles in purified genomic DNA samples. Each assay allows researchers to genotype individuals for a specific SNP.

SNP Genotyping Assay Contents

The 40✕SNP genotyping assay contains:

- Sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest.
- Two TaqMan® minor groove binder probes:
  - One probe labeled with VIC® dye detects the allele 1 sequence.
  - One probe labeled with FAM™ dye detects the allele 2 sequence.

Context sequence (VIC/FAM):

TCCTCATCCAACAGCTCTTCTATCA (G/A) GTGTTCGAAAGTGTC AGCCAATGAT

II. Measuring BDNF Serum Level

SinoGeneClon Biotech enzyme-linked immunosorbent assay (ELISA) kit for quantitative determination of human BDNF concentrations (Cat: SG-00371) was utilized to measure the serum level of BDNF using the ELISA. According to the manufacturer's instructions, the intra-assay coefficient of variation was <8%, and the inter-assay coefficient of variation was <10%.

III. Measuring nNOS Serum Level

To measure the blood level of nNOS, we used the ELK Biotechnology ELISA kit for quantitative detection of human nNOS concentrations (Cat: ELK1619). According to the manufacturer's instructions, the intra-assay coefficient of variation was <8%, and the inter-assay coefficient of variation was <10%.

nNOS assay specificity and limitations: the ELISA kit used was specific for nNOS with no reported cross-reactivity with endothelial NOS or inducible NOS isoforms. Limitations of the method include its indirect nature and inability to assess enzymatic activity.

Samples were analyzed as single measurements due to limited sample volume and in accordance with the manufacturer's recommendations. Assay reliability was ensured by standardized procedures and acceptable intra- and inter-assay coefficients of variation.

Statistical Analysis

The collected data was coded and checked before entering data into a computer. The statistical package for the social sciences version 23 software was used to statistically analyze the data, which was then shown in tables and graphs. Following a Kolmogorov-Smirnov test for normality, the quantitative data were displayed as mean and standard deviation for a normal distribution and median and IQ for a non-normal one. Frequencies and percentages of the qualitative data were displayed, and the p-value was obtained by comparing them using chi-square. A p-value of less than 0.05 denoted statistical significance in every analysis.

Clinical Definitions

Treatment response was categorized based on previously published criteria: seizure freedom was defined as excellent response, ≥75% reduction in seizure frequency as very good response, these thresholds are consistent with established definitions of clinically meaningful seizure reduction reported in prior studies (World Health Organization, practical neurology, neurology reviews).

1. Excellent Response for Treatment Response in Epilepsy

Definition: complete seizure freedom during the follow-up period (i.e., 0% seizure frequency compared to baseline).

2. Very Good Response

Definition: ≥75% reduction in seizure frequency compared to baseline.

In some cases, patients had to change medication due to incomplete control of seizures, or they had to add a 2nd medication.

Ethics Clearance and Participation Consent

The study complied with the Declaration of Helsinki's ethical guidelines; the study protocol was approved by Sohag University Faculty of Medicine Medical Research Ethics Committee (approval no: IRB00013006, date: 11/10/2021) and was registered on ClinicalTrials.gov (ID: NCT05096871). The parents provided written informed consent prior to the children's enrolment in the study.

RESULTS

This study involved the enrollment of 60 epileptic patients (61.7% males and 38.3% females) and 30 controls. The mean age of the patients was 7.8±3.6 years, with a range of 2 to 15 years. Seventy percent of children with epilepsy had generalized tonic-clonic seizures, while 18.3% had focal seizures. The remaining children had syncopal attacks (5%) and absence seizures (6.7%). The illness lasted an average of 3.6±2.5 years, with a range of 1 to 10.5 years. All patients in this study were on AED as shown in Table 1.

Serum BDNF and nNOS concentrations in control group and epileptic children. The study patients' group had a significantly lower mean BDNF concentration (6.7±0.87 ng/mL) compared to the controls (9.49±1.43 ng/mL) ($p<0.001$). Additionally, the epileptic children's mean serum nNOS concentration was significantly higher (5.8±2.9 ng/mL) compared to the controls (1.6±2.91.24 ng/mL) ($p<0.001$) as shown in Table 2.

There was no significant difference in the genotyping distribution of the BDNF gene between the epileptic cases and the controls ($p=0.643$), as shown in Table 3.

The genotyping frequencies in both the cases and controls are not significantly different from what would be expected if the population is in the Hardy-Weinberg equilibrium. The Hardy-Weinberg equation of cases (p-allele freq. =0.80/q-allele freq. =0.12) and controls (p-allele freq. =0.83/q-allele freq. =0.17).

Based on *BDNF* gene polymorphism when comparing patients with heterozygous G/A and homozygous G/G, there were no significant differences in age, height, weight, or duration of epilepsy ($p=0.435$, 0.265, 0.352, and 0.457 respectively) as shown in Table 4.

Table 1. Participant demographic information and attributes

Variables	Mean±SD	Range
Age (years)	7.8±3.6	2-15
Duration of the disease (years)	3.6±2.5	1 to 10.5
	n	**Percent**
Gender (cases)		
Male	37	61.7%
Female	23	38.3%
Types of seizures		
GTCC	42	70%
Focal	11	18.3%
Syncopal attack	3	5%
Absence seizures	4	6.7%
Response to treatment		
Excellent	9	15%
Very good	12	20%
On regular treatment	1	1.7%
Add second medication	26	43.3%
Change medication	12	20%

The data are displayed as a number (%), median (range), and mean±SD.
GTCC: Generalized tonic clonic seizures, SD: Standard deviation

Table 2. Comparison of serum BDNF and serum neuronal NOS concentrations (ng/mL) between the cases and controls

Parameter	Epileptic cases n=60	Control group n=30	p-value by Mann-Whitney U test
Serum BDNF (ng/mL) (mean±SD)	6.7±0.87	9.49±1.43	<0.001***
Median (IQR)	6.7 (6.0:7.4)	9.0 (8.6:10.6)	
Range	5:8.1	7.4:12.2	
Serum nNOS (ng/mL) (mean±SD)	5.8±2.9	1.6±1.24	<0.001***
Median (IQR)	5.7 (3.6:8.17)	1.0 (0.7:2.2)	
Range	0.7:12.3	0.5:5.7	

***: p<0.001 for extremely significant, and NS for non-significant p>0.05. For extremely significant, use NOS.
nNOS: Neuronal nitric oxide synthase, BDFN: Brain-derived neurotrophic factor, SD: Standard deviation, IQR: Interquartile range, NS: Non-significant

Table 3. Genotyping distribution of *BDNF* gene in epileptic cases and control group in the study

Genotyping		Groups		Total	p-value by chi-square
		Epileptic cases n=60	Control group n=30		
Heterozygous G/A	**No (% within groups)**	23 (38.3%)	10 (33.3%)	33 (36.7%)	*0.643 (NS)
Homozygous G/G	**No (% within groups)**	37 (61.7%)	20 (66.7%)	57 (63.3%)	
Total	**Count**	60	30	90	

*: Non-significant p>0.05 denotes non-significant.
NS: Not significant, BDFN: Brain-derived neurotrophic factor

Table 4. Comparison of age, weight, height, duration of epilepsy among cases (n=60) according to BDNF genotype

Parameter	*BDNF* gene polymorphism		p-value by independent t-test
	Homozygous G/G n=37	Heterozygous G/A n=23	
Age (years)	8.13±3.8	7.3±3.5	0.435 (NS)
Weight (Kg)	28.59±10.6	25.4±10.4	0.265 (NS)
Height (cm)	126.59±19.2	121.9±17.5	0.352 (NS)
Duration of epilepsy (years)	3.85±2.7	3.36±2.1	0.475 (NS)

Non-significant p>0.05 is indicated by NS.
BDFN: Brain-derived neurotrophic factor, NS: Not significant

Table 5 shows that there was no significant difference between the homozygous and heterozygous epileptic cases in terms of family history (p=0.958), seizure type (p=0.411), or EEG characteristics (p=0.416), on the other hand on assessing the patients' response to therapy, it shows that the homozygous group had significantly better response to medication (p=0.032).

The results also revealed that age, height, weight, and duration of sickness did not substantially correlate with serum BDNF or serum nNOS, while as shown in Table 6 there were positive moderate correlations with response to medication in the serum BDNF dependent and nNOS dependent groups. No correlations were found between other parameters.

Table 7 demonstrates that there was excellent overall negative correlation between BDNF and nNOS levels and slightly moderate negative correlation among cases. However, there were non-significant negative correlations among control groups.

According to the linear regression analysis, the results demonstrated that height, weight, age and disease duration were non-significant risk factors associated with serum BDNF concentrations. While serum BDNF and serum nNOS were significant predictors of treatment response (p=0.000 and 0.010, respectively) as shown in Table 8.

Table 9 demonstrates that resistance to antiepileptic therapy was significantly predicted by the serum BDNF levels (p=0.000) with cut-off point <6.87 ng/mL, area under curve (AUC) =0.946, sensitivity 81.6%, specificity 95.2% and accuracy rate 94.6%. Serum nNOS was also a significant predictor of resistance to antiepileptic treatment with p=0.000, (cut-off point >5.05, AUC=0.819, sensitivity 87.9%, specificity 81.0% and accuracy rate 81.9%).

Figure 1 shows the allelic discrimination plot for SNP rs6265 where the scatter plot displays the results of genotyping using a TaqMan SNP assay. Each point represents an individual sample plotted according to normalized fluorescence intensities for the G and A alleles. Two distinct clusters are observed: samples homozygous for the G allele (red, G/G) and heterozygous samples (green, G/A). No clear cluster corresponding to homozygous A/A (blue) was detected, and no samples fell into the undetermined category. This distribution suggests that in the analyzed population, the G allele is prevalent, with individuals observed as either homozygous G/G or heterozygous G/A, while the A/A genotype was absent in this dataset.

DISCUSSION

The complex neurological disorder known as epilepsy is characterized by frequent, unexpected seizures and temporary

Table 5. Cross tabulation of family history, type of seizure, EEG features and response to medication in 60 cases according to genotyping

Family history		**Genotyping**		**Total**	**X^2**	**p-value**
		Homozygous G/G	**Heterozygous G/A**			
No	**Count (%)**	32 (86.5%)	20 (87%)	52 (86.7%)	0.003	0.958
Yes	**Count (%)**	5 (13.5%)	3 (13%)	8 (13.3%)		
Total	**Count**	37	23	60		
Type of seizures		**Homozygous G/G**	**Heterozygous G/A**	**Total**	**X^2**	**p-value**
GTCC	**Count (%)**	26 (70.3%)	16 (69.6%)	42 (70%)	2.87	0.411 (NS)
Focal	**Count (%)**	8 (21.6%)	3 (13%)	11 (18.3%)		
Syncopal attacks	**Count (%)**	2 (5.4%)	1 (4.3%)	3 (5%)		
Absence seizures	**Count (%)**	1 (2.7%)	3 (13)	4 (6.7%)		
Total	**Count**	37	23	60		
EEG features		**Homozygous G/G**	**Heterozygous G/A**	**Total**	**X^2**	**p-value**
Generalized activity	**Count (%)**	27 (73%)	16 (69.6%)	43 (71.7%)	1.75	0.416 (NS)
Focal activity	**Count (%)**	6 (16.2%)	2 (8.7%)	8 (13.3%)		
Normal	**Count (%)**	4 (10.8%)	5 (21.7%)	9 (15%)		
Total	**Count**	37	23	60		
Response to medication		**Homozygous G/G**	**Heterozygous G/A**	**Total**	**X^2**	**p-value**
Excellent	**Count (%)**	8 (21.6%)	1 (4.3%)	9 (15%)	10.57	0.032*
Very good	**Count (%)**	10 (27%)	2 (8.7%)	12 (20%)		
On regular treatment	**Count (%)**	1 (2.7%)	0 (0%)	1 (1.7%)		
Add second medication	**Count (%)**	14 (37.8%)	12 (52.2%)	26 (43.3%)		
Change medication	**Count (%)**	4 (10.8%)	8 (34.8%)	12 (20%)		
Total	**Count**	37	23	60		

*$p<0.05$ (significant), NS: a non-significance level of $p>0.05$.
GTCC: Generalized tonic clonic seizures, EEG: Electroencephalography

Table 6. Eta correlation of family history, sex, EEG features and response to treatment with serum BDNF and serum neuronal nitric oxide synthase 60 cases

Correlation coefficient	**Serum-BDNF (dependent)**	**Serum neuronal nitric oxide synthase (dependent)**
Sex (n=90)	eta=0.014 p=0.91 (NS)	eta=0.48 (NS) p=0.48 (NS)
Type of seizures (n=60)	eta=0.303 p=0.315 (NS)	eta=0.2 (NS) p=0.90 (NS)
EEG features (n=60)	eta=0.278 p=0.143 (NS)	eta=0.106 (NS) p=0.5 (NS)
Family history (n=60)	eta=0.001 p=0.9 (NS)	eta=0.06 (NS) p=0.608 (NS)
Response to tilt table test (n=60)	eta=0.754 p=0.000***	eta=0.547 (NS) p=0.000***

NS: a non-significance level of $p>0.05$, ***: $p<0.001$ (very highly significant), weakly correlated (0.2-0.4); moderately correlated (0.4-0.6); and excellently correlated (0.6-1).
NS: Not significant, EEG: Electroencephalography, BDFN: Brain-derived neurotrophic factor

disruptions in brain activity. It is mostly brought on by pathologic neuronal discharges.[12]

BDNF has been identified over the past decade as one of the key neurotrophic factors that may regulate neuronal morphology and synapse formation, contributing to the central nervous system's neuroprotective function. Studies using transgenic mouse models have also shown that increased neuronal excitability and epilepsy risk may be influenced by brain overexpression of BDNF.[13] Since SNPs are among the most significant genetic alterations that could control BDNF expression and metabolism, recent studies have increasingly focused on these variants. Several studies have shown that the most prevalent polymorphism in the *BDNF* gene, rs6265 G>A, results in an amino acid change from Val to met, which may contribute to various central nervous system disorders including Alzheimer's disease, Parkinson's disease, depression, and bipolar disorder.[7,14-17]

Although the role of neuronal nitric oxide in epilepsy is well established, its anticonvulsant or proconvulsant effects remain controversial. While numerous studies have investigated the relationship between proinflammatory cytokines or nNOS and

Table 7. Spearman's rho correlation of serum BDNF and serum neuronal NOS

Correlation between BDNF and nNOS	Correlation coefficient	p-value
Overall (n=90)	-0.714**	0.000
Cases (n=60)	-0.444**	0.000
Controls (n=30)	-0.207 (NS)	0.2

**At the 0.01 level (2-tailed), the correlation is significant, NS is not.
NS: Not significant, NOS: Nitric oxide synthase, BDFN: Brain-derived neurotrophic factor

Table 8. Linear regression analysis of important study parameters as predictors of response to treatment

Parameters		Unstandardized coefficients		Standardized coefficients	t	Significant
		B	Std. error	Beta		
1	**(Constant)**	3.573	0.803		4.451	0.000
	Age	0.024	0.035	0.179	0.672	0.504 (NS)
	Weight	-0.011	0.009	-0.250	-1.254	0.215 (NS)
	Height	0.003	0.007	0.119	0.420	0.676 (NS)
	Disease duration	-0.001	0.023	-0.007	-0.056	0.956 (NS)
	Serum BDNF	-0.361	0.055	-0.653	-6.599	0.000***
	Serum NOS	0.044	0.016	0.265	2.665	0.010**

Dependent variable: response to medication. Strong significance by **p<0.01 and very high significance by ***p<0.001. Significantly not (NS: p>0.05).
NOS: Nitric oxide synthase, BDFN: Brain-derived neurotrophic factor, Std: Standard

Table 9. Sensitivity, specificity and accuracy rate of serum neuronal nitric oxide synthase and serum BDNF conc. as predictors of resistance to epileptic treatment in studied patients

Variable	Cut-off point	Area under the curve	p-value	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Accuracy rate
Serum BDNF (ng/mL)	<6.87	0.946	0.000***	81.6%	95.2%	96.9%	75%	94.6%
Serum nNOS (ng/mL)	>5.05	0.819	0.000***	87.9%	81.0%	88.2%	69.2%	81.9%

***p<0.001 (very highly significant).
nNOS: Neuronal nitric oxide synthase

epilepsy, the potential role of these factors in pediatric absence epilepsy remains unclear.[18] Accordingly, the present study was conducted to evaluate the association between epilepsy susceptibility and the *BDNF* rs6265 gene polymorphism.

In the current study, epileptic patients had a mean disease duration of 3.6±2.5 years, with a range of 0.5-10.5 years. Among the epileptic cases, 23 patients were female (38.3%) and 37 were male (61.7%). The control group comprised 21 males (70.0%) and 9 females (30.0%). Statistical analysis revealed no significant difference in sex distribution between the case and control groups (p=0.436). These findings are consistent with previous studies reporting a slightly higher risk of epilepsy in males. For example, Karabiber et al.[19] reported a male-to-female ratio of 1.42:1 in Turkish children aged 1-12 years, Wong[20] reported a ratio of 1.22:1, and Aaberg et al.[21] observed a marginally higher incidence of epilepsy in males. However, other studies, such as that by Topbaş et al.,[22] have reported an equal or slightly higher prevalence among females.

The results of the present study demonstrated that blood BDNF levels were significantly lower in epileptic patients compared with the control group (p<0.001). In contrast, serum nNOS levels were significantly higher in epileptic patients than in controls (p<0.001). These findings are consistent with the study by Poniatowski et al.,[23] which reported significantly lower serum BDNF levels in patients with generalized tonic-clonic seizures compared with controls. However, these results partially contradict the meta-analysis by Nowroozi et al.,[14] which found no significant differences in BDNF levels between patients with epilepsy and control subjects, except for lower BDNF levels observed in individuals with partial epilepsy.

Numerous studies investigating nNOS have reported inconsistent findings. Ibragic et al.[24] found no statistically significant difference in nNOS levels between patients with epilepsy and control subjects. In contrast, Kovács et al.,[25] Ribeiro et al.,[26] and Arhan et al.,[27] reported higher nNOS levels in newly diagnosed patients with epilepsy. These discrepancies may be attributed to the heterogeneity of epilepsy, as well as differences in study populations, sample sizes, and methodological approaches.

In the present study, the genotypic distribution of the *BDNF* gene was evaluated in both patients with epilepsy and controls. The heterozygous G/A genotype was observed in 38.3% of epileptic cases, while the homozygous G/G genotype was present in 61.7%. Among controls, 33.3% were heterozygous for G/A and 66.7% were homozygous for G/G. No statistically significant difference

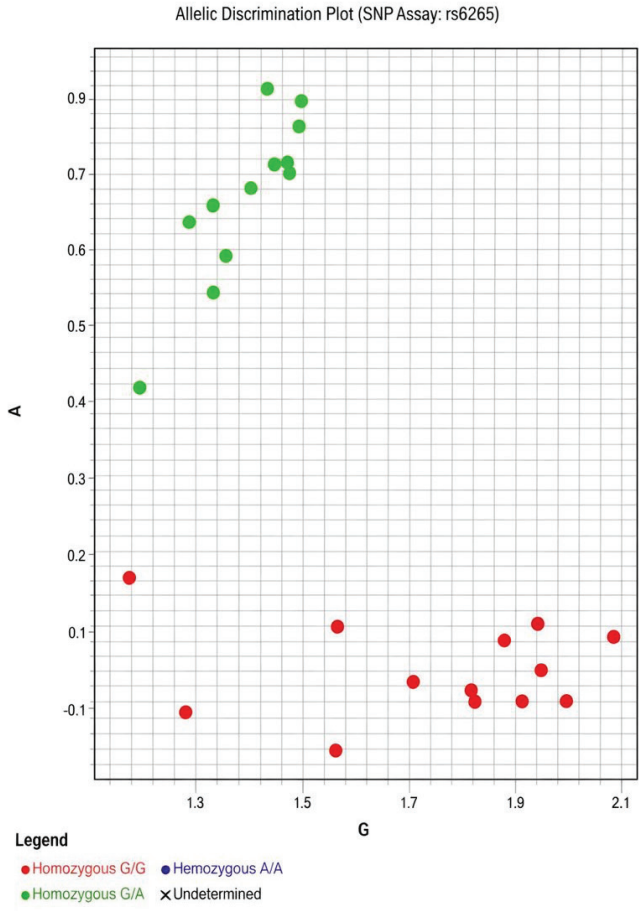

Figure 1. The allelic discrimination plot for SNP rs6265
SNP: Single-nucleotide polymorphism

was observed in the distribution of BDNF genotypes between epileptic patients and controls (p=0.643).

The BDNF Val66Met polymorphism affects activity-dependent secretion and intracellular trafficking of BDNF and has been implicated in epilepsy. Several studies have reported an association between the BDNF Val66Met polymorphism and epilepsy susceptibility.

Previous studies suggest that the impact of BDNF polymorphisms on epilepsy may vary across populations. Xu et al.[17] and Sha'ari et al.[28] reported that Asian populations may be more susceptible to epilepsy due to BDNF polymorphisms. Similar associations have been observed in patients with fragile X syndrome[29] and in Japanese cohorts.[30] Nevertheless, conflicting findings have also been reported, as Lohoff et al.[31] failed to replicate the Japanese data, and Bragatti et al.[32] found no significant clinical effect of the polymorphism in temporal lobe epilepsy.

In the present study, patients carrying the homozygous genotype showed a significantly better response to antiepileptic medication than heterozygous carriers (p=0.032), with no significant differences between groups in age, height, weight, or disease duration.

Consistent with these findings, Zeev et al.[33] reported that individuals homozygous for the wild-type BDNF allele (Val/Val) had milder disease severity than heterozygous carriers (Val/Met). In Rett syndrome patients with the p.R168X mutation, the presence of the BDNF polymorphism was associated with increased disease severity and a higher seizure risk. Together, these data suggest that the BDNF Val66Met polymorphism may influence both disease severity and treatment response in epilepsy.[7,8,34]

Study Limitations

Notwithstanding the noteworthy discoveries and contributions of the present investigation, it is important to recognize certain limitations:

1. Sample size: The study's findings may not be as broadly applicable as they could be due to its comparatively small sample size. More solid results and increased conclusion reliability would come from a larger sample size.

2. Selection bias: Because study participants were chosen from a certain demographic or medical environment, selection bias may have been introduced. This might limit the results' ability to be applied to a larger population and compromise the sample's representativeness.

3. Measurement variability: The measurement of serum BDNF and nNOS may have inherent variability due to assay methods or laboratory procedures. Variations in sample handling, storage, and analysis could introduce measurement errors that might affect the accuracy and reliability of the results. Moreover, a potential limitation of this study is the reliance on ELISA-based measurement of nNOS, which reflects circulating protein levels rather than direct enzymatic activity or tissue expression.

4. Generalizability: The findings of this study might be specific to the studied population or setting and may not be directly applicable to other populations or geographic regions. Further studies with diverse populations are needed to validate the results across different contexts.

5. Confounding factors: Case-control studies may be prone to confounding variables, where the observed association between the exposure (serum BDNF and nNOS levels) and outcome (response to treatment) may be influenced by other variables that were not accounted for in the study design or analysis.

6. Temporality: Case-control studies are retrospective in nature, meaning that the exposure and outcome are assessed simultaneously or after the occurrence of the outcome. This makes it challenging to establish a clear temporal relationship between exposure and outcome, limiting the ability to determine causality.

It is important to consider these limitations when interpreting the findings of a case-control study and to recognize the need for further research, such as prospective cohort studies, to confirm the observed associations and address these limitations.

CONCLUSION

This study demonstrates that pediatric epilepsy is associated with decreased serum BDNF levels and increased nNOS levels, supporting the involvement of neurotrophic and nitric oxide-related pathways in epilepsy pathophysiology. While no significant difference was observed in the overall distribution of the BDNF rs6265 (Val66Met) polymorphism between patients and controls,

the polymorphism was associated with clinical response to treatment. Homozygous carriers showed a significantly better response to antiepileptic therapy than heterozygous carriers, suggesting a potential influence of BDNF genetic variation on disease severity and therapeutic outcome. These findings underscore the relevance of BDNF as a potential biomarker and therapeutic modifier in pediatric epilepsy, warranting further large-scale studies to validate these results.

Ethics

Ethics Committee Approval: The study protocol was approved by Sohag University Faculty of Medicine Medical Research Ethics Committee (approval no: IRB00013006, date: 11/10/2021).

Informed Consent: The parents provided written informed consent prior to the children's enrolment in the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.A.S., Concept: A.M.F., S.K.A-M., N.S.A., A.A.S., R.S.Y., Design: A.M.F., S.K.A-M., N.S.A., A.A.S., R.S.Y., Data Collection or Processing: A.M.F., S.K.A-M., A.A.S., Analysis or Interpretation: A.M.F., S.K.A-M., N.S.A., A.A.S., R.S.Y., Literature Search: A.M.F., S.K.A-M. A.A.S., R.S.Y., Writing: N.S.A., R.S.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

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