Research Article

BDNF Gene Polymorphism in Patients With Temporal Lobe Epilepsy

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Abstract

Objective: Brain-derived neurotrophic factor (BDNF), a neurotrophin which performs its activity through neurotrophic tyrosine kinase receptors, is one of the candidate genes for epilepsy. Our aim is to investigate brain-derived neurotrophic factor (BDNF) gene polymorphisms as an candidate gene in patients with temporal lobe epilepsy.

Methods: One hundred and seven patients included in the study were divided into two groups as mesial temporal sclerosis (MTS) (n:62) and non-MTS (n:45), and compared with 104 healthy controls. C-T substitution at position 240 in the BDNF gene which corresponds to the 270C/T polymorphism was analysed using a PCR-restriction fragment length polymorphism (RFLP) method.

Results: From a clinical point of view, the history of febrile convulsions and status epilepticus in medically intractable patients with MTS was statistically very significant in comparison to the other group (p<0.000), as expected. There was no statistical significance in the distribution of genotypes between the TLE group (MTS group and non-MTS group) and the control group individuals.

Conclusion: Hence, in patients with temporal lobe epilepsy, BDNF gene polymorphism was not detected. The indication of lack of correlation between BDNF gene polymorphism and TLE contributes to literature in that it makes it possible for future researchers to compare and contrast their results with studies that had not yielded statistically significant results.

Keywords: Brain-derived neurotrophic factor (BDNF), temporal lobe epilepsy, mesial temporal sclerosis, polymorphism, gene

Temporal Lob Epilepsili Hastalarda BDNF Gen Polimorfizmi

Özet

Amaç: Nörotrofi tirozin kinaz reseptörler yoluyla aktivitesini gösteren bir nörotrofin olan brain-derived neurotrophic factor (BDNF) (beyin kaynaklı sinir büyüme faktörü) epilepsi için aday genlerden birisidir. Amaç temporal lob epilepsili hastalarda aday gen olarak BDNF gen polymorfizmini araştırmaktır.

Metod: Mezyal temporal sklerozu olan (MTS) (n:62) ve olmayan (n:45) olarak iki gruba ayrılan yüzeyi hasta bu çalışmaya alınmış ve 104 sağlıklı kontrol ile karşılaştırılmıştır. 270 C/T polymorfizmine denk gelen BDNF geninde 240. pozisyonda C – T değişiminin analiz edilmesi için PCR-restriction fragment length polymorphism (RFLP) metodu kullanılmıştır.

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INTRODUCTION
The molecular and cellular bases of epileptogenesis have been poorly understood. Many experimental studies on temporal lobe epilepsy have been made to find out the underlying mechanism of epilepsy. In the last decade, genetics has been the pioneer subject for all medical fields including epilepsy research.

In recent years, neurotrophins are discovered to have an important role in epileptogenesis. Brain-derived neurotrophic factor (BDNF), a neurotrophin which performs its activity through neurotrophic tyrosine kinase receptors, is one of the candidate genes for epilepsy\(^{(1,14)}\).

BDNF has an important role on the growth and survival of developing neurons, regulating neuronal morphology and cytogenesis under basal conditions.

Although its function in the adult brain is not sufficiently known, pathological upregulation is known to cause epilepsy development in experimental studies\(^{(2,3,7)}\). Various stimuli such as seizure activity, ischemia, head trauma and stress also increase the expression of BDNF in mRNA and proteins\(^{(3)}\).

BDNF has been noted to increase in dentate gyrus and CA1-CA3 regions of hippocampus during seizures in animals\(^{(4,5)}\). BDNF-elicited hyperexcitability is also confirmed by whole cell patch clamp recordings taken from dentate gyrus of human TLE patients\(^{(18)}\).

However, this is yet to be tested in normal hippocampal tissues. All these experimental studies generally direct us to the clinical evidence. A recent study reported that BDNF gene polymorphisms showed positive association in patients with partial epilepsy in the Japanese population\(^{(6)}\). Afterwards, another study showed no correlation in patients with TLE from originally European ancestry. In this study, our aim is to investigate the importance of BDNF gene polymorphisms as candidate genes in temporal lobe epilepsy.

METHODS
This study involves 107 patients with Temporal lobe Epilepsy (TLE) who were diagnosed and followed by the Epilepsy outpatient clinic of the Neurology Department, Istanbul Faculty of Medicine according to the ILAE criteria. The patients were divided into two groups: those with mesial temporal sclerosis (MTS) (n:62) and non-MTS (n:45). Healthy individuals who neither had a disease nor took medicine constituted the control group. To retrieve the necessary information about each patient, either the patients or their family members were interrogated in detail.

The study was approved by the ethics committee of Istanbul Faculty of Medicine,
and all the patients and those in the control group gave their written informed consent.

DNA is extracted from 10 ml of whole blood using salting-out method which is done at Istanbul University, Institute of Experimental Medicine (DETAE), Genetics Department, from where the control group blood samples were also taken. C-T substitution at position 240 in the BDNF gene which corresponds to the 270C/T polymorphism was analysed using a PCR-restriction fragment length polymorphism (RFLP) method (15).

PCR primers used were forward primer BDNF-F (5 CAG AGG AGC CAG CCC GCT GCG -3) and reverse primer BDNF-R (5-CTC CTG CAC CAA GCC CCA TTC-3) . PCR conditions were 5 minutes at 94 oC, 1 minute at 60 oC, 1 minute at 72 oC for 35 cycles. Hinfl restriction enzyme digested samples were analysed by electrophoresis in 5% agarose gels.

The clinical features of the patients as well as their genotypes and allele frequencies were analysed statistically using SPSS 10.0, and potential correlation was calculated with chi-square. The results were compared with Hardy- Weinberg rule to observe compliance.

RESULTS

The clinical features of all patients are shown comparatively in the table 1. From a clinical point of view, the history of febrile convulsions and status epilepticus in medically intractable patients with MTS was statistically very significant in comparison to the other group (p<0.000), as expected.

The mean onset age of epilepsy in the MTS group was significantly younger than the non-MTS group. In the MTS group consanguinity was also more meaningful.

It was statistically significant that patients with non-MTS and, following epilepsy surgery, patients with MTS showed good response to monotherapy.

The genotype distribution obtained from 107 patients with TLE and 104 healthy individuals were 93,5% CC, 0%TT and 6,5% CT (Table 2). There was no statistical correlation between healthy and TLE patients.

The genotype frequencies obtained from 62 patients with MTS were 93,5% CC, 0% TT and 6,5% CT (Table 3). No significant difference from the control group was found.

The genotype frequencies obtained from non-MTS patients were 93,3% CC, 0% TT and 6,7% CT (Table 4). No significant difference from the control group was found in this group, either.

There was no statistical significance in the distribution of genotypes between the TLE group (MTS group and non-MTS group) and the control group individuals.

Moreover, no association was noted between the BDNF genotype and frequency of alleles and distribution of gender, history of birth, mental-motor developmental retardation, history of central nervous system infections, febrile convulsions, head trauma, loss of consciousness during head trauma, the history of systemic and psychiatric diseases, consanguinity, the history of epilepsy and febrile convulsions in family.

There was no statistical significance between the BDNF and either seizure features, type of auras, or treatment and prognosis of epilepsy. Moreover, there was no correlation between BDNF and patients who were/ are on remission, and patients with a history of secondary generalization, history of status epilepticus, monotherapy and polytherapy usage, patients who underwent epilepsy surgery, and patients who have recurrences after epilepsy surgery.
### Table 1 The clinical features of the study groups

<table>
<thead>
<tr>
<th></th>
<th>MTS</th>
<th>Non-MTS</th>
<th>Healthy control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/Male</td>
<td>30/32 (62)</td>
<td>24/21 (45)</td>
<td>104</td>
</tr>
<tr>
<td>Mean age</td>
<td>31.8±10.8 (16-76)</td>
<td>35.4±13.4 (16-66)</td>
<td></td>
</tr>
<tr>
<td>Head trauma</td>
<td>41.9% (26)</td>
<td>44.4% (20)</td>
<td></td>
</tr>
<tr>
<td>History of febrile seizure</td>
<td>61% (41) *</td>
<td>15.6% (7)</td>
<td></td>
</tr>
<tr>
<td>Family history of epilepsy</td>
<td>22.6% (14)</td>
<td>24.4% (11)</td>
<td></td>
</tr>
<tr>
<td>Consanguinity</td>
<td>29% (18)**</td>
<td>8.9% (4)</td>
<td></td>
</tr>
<tr>
<td>Mean seizure frequency/month</td>
<td>3-4/month</td>
<td>1-2/month</td>
<td></td>
</tr>
<tr>
<td>Onset age of epilepsy</td>
<td>12.4±11*** (0-63)</td>
<td>18.3±9.2 (1-44)</td>
<td></td>
</tr>
<tr>
<td>Aura</td>
<td>85.5% (53)</td>
<td>93.3% (42)</td>
<td></td>
</tr>
<tr>
<td>History of status epilepticus</td>
<td>24.2% (15)****</td>
<td>6.7% (3)</td>
<td></td>
</tr>
<tr>
<td>Patients on monotherapy</td>
<td>33.9% (21)</td>
<td>66.7% (30)****</td>
<td></td>
</tr>
<tr>
<td>Patients with surgery</td>
<td>%36.4 (28)******</td>
<td>%0</td>
<td></td>
</tr>
<tr>
<td>Medically intractable patients</td>
<td>%66.1(41)******</td>
<td>%20 (9)</td>
<td></td>
</tr>
</tbody>
</table>

(*p<0.000, **p<0.011, ***p<0.004, ****p<0.017, *****p<0.001, *******p<0.000, ********p<0.000)

### Table 2 Distribution of BDNF genotype and frequency of alleles in all TLE patients

<table>
<thead>
<tr>
<th>BDNF genotype</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>Frequency of C allele</th>
<th>Frequency of T allele</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLE (107)</td>
<td>93.5% (100)</td>
<td>6.5% (7)</td>
<td>0% (0)</td>
<td>97% (207)</td>
<td>3% (7)</td>
<td>P=0.875</td>
</tr>
<tr>
<td>Control (104)</td>
<td>94.2% (98)</td>
<td>5.8% (6)</td>
<td>0% (0)</td>
<td>97% (202)</td>
<td>3% (6)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3 Distribution of BDNF genotype and frequency of alleles in patients with MTS

<table>
<thead>
<tr>
<th>BDNF genotype</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>Frequency of C allele</th>
<th>Frequency of T allele</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTS (62)</td>
<td>93.5% (58)</td>
<td>6.5% (4)</td>
<td>0% (0)</td>
<td>97% (120)</td>
<td>3% (4)</td>
<td>P=0.85814</td>
</tr>
<tr>
<td>Control (104)</td>
<td>94.2% (98)</td>
<td>5.8% (6)</td>
<td>0% (0)</td>
<td>97% (202)</td>
<td>3% (6)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4 Distribution of BDNF genotype and frequency of alleles in non-MTS patients

<table>
<thead>
<tr>
<th>BDNF genotype</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>Frequency of C allele</th>
<th>Frequency of T allele</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-MTS (45)</td>
<td>93.3% (42)</td>
<td>6.7% (3)</td>
<td>0% (0)</td>
<td>97% (87)</td>
<td>3% (3)</td>
<td>P=0.83279</td>
</tr>
<tr>
<td>Control (104)</td>
<td>94.2% (98)</td>
<td>5.8% (6)</td>
<td>0% (0)</td>
<td>97% (202)</td>
<td>3% (6)</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

Although the generation of new neurons in human brain has been thought to be controversial, it is known that the abnormal projections observed in animal models known as mossy fiber sprouting are also seen in human epilepsy\(^{(10)}\). An overview of literature reveals confusing results about the role of BDNF both in epileptogenesis and in the protection from epilepsy. Experimental studies always do not show correlation with the clinical ones.

Elevated BDNF levels are reported in the kainic acid\(^{(13)}\), hilar lesion\(^{(9)}\), unilateral continuous hippocampal stimulation\(^{(17)}\), as well as in tissues resected from human epileptic brain\(^{(16)}\). On the other hand several reports show that intrahippocampal infusion of BDNF can diminish the development of epilepsy\(^{(11,12)}\). Although there is the possibility that BDNF infusion down-regulates TrkB signaling pathway, antiepileptic effect of BDNF is thought to be attributable to neuropeptide Y. The level of neuropeptide Y increases in the neocortex of TLE patients, in positive correlation with the level of BDNF\(^{(16)}\).

In one study, done in Japan, a positive association between the C240T polymorphism in the BDNF gene and partial epilepsy was found\(^{(6)}\). On the contrary, another study from the USA, in which patients with TLE and the controls were of European ancestry, showed no association\(^{(8)}\). The third study on this topic is our study which also showed no correlation between the distribution of BDNF genotype and frequency of alleles in patients with or without MTS compared with the controls. We observed no definitive effect of BDNF polymorphism on the clinical features of TLE in our population.

Even though the number of our patients might be considered enough to make a genetic study, a larger number of patients should be tested again to further verify the association between BDNF polymorphism and TLE. Moreover, as it has been noted earlier, other polymorphisms in the BDNF gene can reveal results to support its being a candidate gene for TLE\(^{(8)}\).

The other point that needs to be considered is the population factor. Our country is located between Europe and Asia and, is inhabited by people of mixed origins. With our ongoing study, we expect to find more statistically significant results. Hence, additional studies are needed to reveal more conclusive results about the involvement of BDNF in human epilepsy including TLE.

While it is true that this study does not yield statistically significant results about the effects of BDNF polymorphism on the clinical features of TLE, it is important to include studies which convey statistically insignificant results in literature. It will enable future researchers to compare and contrast their results with the extant studies and achieve more convincing results.

This article has been primarily co-authored by Gülcan Sargin and Candan Gürses

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