Evidence of associations between brain-derived neurotrophic factor (BDNF) serum levels and gene polymorphisms with tinnitus

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Abstract

Background: Brain-derived neurotrophic factor (BDNF) gene polymorphisms are associated with abnormalities in regulation of BDNF secretion. Studies also linked BDNF polymorphisms with changes in brainstem auditory-evoked response test results. Furthermore, BDNF levels are reduced in tinnitus, psychiatric disorders, depression, dysthymic disorder that may be associated with stress, conversion disorder, and suicide attempts due to crises of life. For this purpose, we investigated whether there is any role of BDNF changes in the pathophysiology of tinnitus. Materials and Methods: In this study, we examined the possible effects of BDNF variants in individuals diagnosed with tinnitus for more than 3 months. Fifty-two tinnitus subjects between the ages of 18 and 55, and 42 years healthy control subjects in the same age group, who were free of any otorhinolaryngology and systemic disease, were selected for examination. The intensity of tinnitus and depression was measured using the tinnitus handicap inventory, and the differential diagnosis of psychiatric diagnoses made using the Structured Clinical Interview for Fourth Edition of Mental Disorders. BDNF gene polymorphism was analyzed in the genomic deoxyribonucleic acid (DNA) samples extracted from the venous blood, and the serum levels of BDNF were measured. One-way analysis of variance and Chi-squared tests were applied. Results: Serum BDNF level was found lower in the tinnitus patients than controls, and it appeared that there is no correlation between BDNF gene polymorphism and tinnitus. Conclusions: This study suggests neurotrophic factors such as BDNF may have a role in tinnitus etiology. Future studies with larger sample size may be required to further confirm our results.

Keywords: BDNF gene polymorphism, neurotrophic factor, serum BDNF level, tinnitus

INTRODUCTION

Tinnitus is the term that describes perceiving a sound in the ear or somewhere else inside the head without an external sound source.[1-3] It is quite frequent with a percentage of frequency between 6 and 30%, and this rate increases in correlation with the age.[1] The etiopathogenesis of tinnitus has yet to be understood thoroughly, and etiological reasons are of a great variety.[4,5] Tinnitus is regarded as an otological symptom. However, imaging and animal studies suggested that tinnitus development may be connected to the central auditory system and neural actions. Several theories are present that link tinnitus to neural actions such as spontaneous neural activity,[6,7] synchronous neural activity,[8,9] “bursting” neural activity,[10-12] and corresponding neuroplastic change.[6,13,14] These newly emerging pathophysiological understandings give way to developing treatment methods on a neuronal basis.[5]

Brain-derived neurotrophic factor (BDNF), which is one of the crucial factors in terms of neural plasticity, is one of the neurotrophic factors that have a key role in growing of neurons metamorphoses and survival thereof as well as the developing auditory canal, including the epithelium of the
inner ear.\textsuperscript{[3,15-17]} The \textit{BDNF} gene is approximately 70 kb long on the short arm (p) of the chromosome 11 (11p14.1).\textsuperscript{[18-20]} This gene codes pro \textit{BDNF}, a progenitor peptide that proteolytically converts into the mature protein.\textsuperscript{[21]} Single-nucleotide polymorphisms (SNPs) of the gene coding the \textit{BDNF} protein are frequently encountered. There exists studies that demonstrate SNPs of the \textit{BDNF} gene \textit{[rs6265 (Val66Met), rs2030324, and rs1491850]} are related to changes at \textit{BDNF} serum levels and scores of brainstem auditory evoked response (BAER) test.\textsuperscript{[17,22,23]}

In the \textit{rs6265} polymorphism, a transition of guanine to adenine occurs in the 196th nucleotide, resulting to a mutation of the 66th amino acid valine into methionine (Val66Met).\textsuperscript{[21,24]} It has been found that the Val66Met polymorphism has negative effects on prefrontal and hippocampal anatomy and functions. It has been expressed that people with Met allele carriers of the \textit{BDNF} gene have a smaller hippocampal volume and might have a tendency toward major depressive disorder (MDD).\textsuperscript{[25,26]} There exist numerous studies which demonstrate that the \textit{BDNF} Val66Met polymorphism affects \textit{BDNF} secretion and that, meanwhile, it might be related to various central neural system diseases such as Parkinson’s disease, Alzheimer’s disease, and epilepsy, as well as numerous psychiatric disorders such as schizophrenia, bipolar disorder, and obsessive compulsive disorder.\textsuperscript{[27-33]} Also, the relationship between the Val66Met polymorphism and variations of loudness dependence of auditory evoked potentials (LDAEP) has been demonstrated.\textsuperscript{[23]}

In addition to the studies demonstrating the relationship between the \textit{rs2030324} polymorphism in the noncoding intronic region of the \textit{BDNF} gene as well as \textit{rs1491850} polymorphism in the promoter region of the \textit{BDNF} gene, and mutations of LDAEP, various studies have been carried out to demonstrate the relationship between the \textit{rs1491850} polymorphism and the response to the treatment of mood disorders and MDD patients.\textsuperscript{[23,34,35]} It has been indicated that the \textit{rs2030324} polymorphism might have a relationship with tendency toward these diseases in schizophrenic patients, patients with asthma, and nicotine addicts.\textsuperscript{[36,37]}

All the data obtained from these studies make one hypothesize that there might be a correlation between tinnitus and BDNF polymorphisms and \textit{BDNF} serum levels. In this study, it was aimed to detect any possible role of \textit{BDNF} mutations in tinnitus pathophysiology by examining the relationship between \textit{BDNF} polymorphisms, mutations, and \textit{BDNF} serum levels.

### Materials and Methods

Our study group was composed of 65 patients (aged between 18 and 55 years) with tinnitus (30 females and 35 males) who applied to the University Medical School Hospital Ear-Nose-Throat (ENT) Outpatient Clinic and who had suffered tinnitus for at least 3 months. Participating patients in the research signed informed consent forms. A total of 13 of these patients with MDD according to the Diagnostic and Statistical Manual, Fourth Edition of Mental Disorders (DSM-IV) criteria were excluded from the group as they did not meet the criteria of the study and audiologic tests could not be completed. The control group in the study was composed of 13 female and 29 male subjects with no ENT or systemic diseases.

### Ethics

Permission for the study was granted by the Local Council of Ethics in April 2013.

### Sociodemographic Information and Interview Forms

In this study, the audiologic tests applied on the study subjects were performed according to Orenay-Boyacioglu \textit{et al.}\textsuperscript{[38]}

### Information Form

For patients, forms including information regarding the clinical diagnosis, existence of systemic diseases, and findings of otoscopic examination carried out by the ENT physician were filled at the ENT clinic in the University Medical Faculty Hospital.

### Personal Information Form

Personal information forms that includes patient’s name, last name, age, sex, level of education, profession, contact information, marital status, number of children, and information regarding their tinnitus story were collected, as well as questions of visual analog scale (VAS) that measures subjective perception of patients on a scale from 0 to 10. These questions were indicative of how the study subjects perceived their tinnitus intensity, incidence, duration, and the level of discomfort they experienced. VAS was used to evaluate the effect of tinnitus on quality of life of the study subjects with the following: VAS-1 for severity of tinnitus, VAS-2 for frequency and duration of tinnitus, VAS-3 for discomfort level, VAS-4 for attention-deficit, and VAS-5 for sleep disorders.

### Tinnitus Handicap Inventory

Tinnitus severity was evaluated by tinnitus handicap inventory (THI) that had three answer options, “yes”,

| Table 1: Evaluation of tinnitus handicap inventory (THI)\textsuperscript{[38]} |
|------------------|-----------------|-----------------|-----------------|
| Grade 1          | 0–16            | Weak            | Only heard in quiet environments |
| Grade 2          | 18–36           | Moderate        | Can easily be masked by ambient noise and for gettable with activity |
| Grade 3          | 38–56           | Middle          | Although noticed in background noise, does not interfere with daily activities |
| Grade 4          | 58–76           | Severe          | Almost always heard, can interfere with sleep and daily activities |
| Grade 5          | 78–100          | Disaster        | Always heard, interferes with sleep and daily activities |

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“no”, and “sometimes” scored as “4”, “0”, and “2”, respectively. The minimum score was 0, with a maximum of 100 [Table 1].

**Impedancemetric Assessment**

Otoscopic examinations were performed on the study subjects followed by tympanometric evaluations. Impedance measurements were carried out using a Model AZ 26 diagnostic immittance meter (Interacoustics, Middelfart, Denmark) and medical Telephonics TDH39 (Farmingdale, NY, USA) headphones. Middle-ear flexibility and pressure values for both ears were measured at 226 Hz probe tone. Ipsilateral and contralateral reflex threshold values were also determined.

**Audiologic Evaluation**

Audiologic tests were carried out by the University Medical School ENT Audiology Unit with an Interacoustics AC40 audiometer (Interacoustics). Pure tone average of 125 to 8000 Hz airway hearing thresholds was measured by using Telephonics TDH39P loudspeaker, and 500 to 4000 Hz bone conduction hearing thresholds were measured with the use of Radioear B71 (Middelfart, Denmark) vibrator, and finally averages of 500 to 1000 to 2000 to 4000 Hz thresholds were calculated.

**Determining Tinnitus Map**

For this purpose, the tinnitus frequency and intensity, the residual inhibition of tinnitus of patients, and the ability to mask tinnitus were determined with Interaudios AC40 audiometer. To measure the tinnitus frequency, patients were asked to match their tinnitus frequency with a test signal (125, 250, 500, 750, 1000, 2000, 3000, 4000, 6000, and 8000 Hz). The intensity of tinnitus was measured at the matched tinnitus frequency of each patient by a signal starting under hearing threshold with 5 dB increments until patients match the test signal intensity to their tinnitus intensity. To understand if the tinnitus can be masked ipsilaterally, a narrow band noise was used at the matched tinnitus frequency of each patient. The level of masking sound suppressing the tinnitus, minimal masking sound (MMS), was measured by increasing the pitch intensity by 5 dB increments. For residual inhibition, a pure tone 10 dB higher than the MMS at tinnitus frequency was applied ipsilaterally for 60 s.

**Depression Evaluation**

Depression levels of the patients were evaluated by Structured Clinical Interview (SCID-I) for DSM-IV axis I disorders in a psychiatry clinic. A fully structured interview that lasted 1.5 h on average was conducted, where the interviewer sincerely asked preformulated questions to the patient in a predefined order. In case relevant information emerges during the interview, the interviewer was free to ask for more information and to modify the rating appropriately.

**Brain-Derived Neurotrophic Factor Serum Measurement**

BDNF serum protein levels were measured by enzyme-linked immunosorbent assay (ELISA). For the analysis of BDNF serum levels, 8 to 10 cm\(^3\) of venous blood was taken into a jelly tube and centrifuged at 3600 rpm for 10 min at 4°C and then stored in two separate tubes at −80°C until tested. BDNF serum levels of the patient group and the control group were measured using a commercial ELISA kit (CYT306, Chemikine BDNF; Millipore, Billerica, Massachusetts, USA) according to the manufacturer’s instructions.

**Genotyping**

Two cubic centimeters of peripheral blood from the tinnitus and control subjects were drawn into EDTA tubes. DNA was isolated from the blood samples using DNeasy Mini Kit (Qiagen, Hilden, Germany). Determination of purity and amount of isolated patient DNA’s were carried out by spectrophotometer (Maestro Nano, Irvine, California, USA). Subjects with a DNA quality between 1.8 and 2.0 per A260/A280 nm were included in the study. Primer pairs of 5’-ACT GTC GAG AGC GTG AAT GG-3’ (forward, rs6265), 5’-AGA AGA GGA GGC TCC AAA GG-3’ (reverse, rs6265), 5’-CACCTC AAA CAT CAC ACA GCC-3’ (forward, 1491850), 5’-ACC AAA GGA GGT TTT CAG GAC ATT-3’ (reverse, 1491850), and 5’-GGA CCA GAG TAG TGA GCT AAC-3’ (forward, rs2030324), 5’-CAG CAG GGG AAT ATA AAG GTC-3’ (reverse, rs2030324) were used in the polymerase chain reaction (PCR)–restriction fragment length polymorphism method to amplify and genotype the three variants of BDNF. The PCR reaction was carried out with a thermal cycler (Techne Genius, Stone, Staffordshire, UK) by using 4 μl of DNA (20–100 ng), 3 μl of PCR buffer (1×), 1.8 μl of MgCl\(_2\) (1.5 mM), 0.3 μl of deoxyribonucleoside triphosphate (dNTP) mix (0.2 mM each), 0.45 μl of forward primer(0.3 μM), 0.45 μl of reverse primer (0.3 μM), 0.44 μl Hot Taq DNA Polymerase (2.2 U), and 19.56 μl of nuclease-free water in a final volume of 30 μl. PCR conditions were set up as the first denaturation for 13 min at 95°C, followed by 38 cycles of denaturation for 30 s at 95°C, annealing for 30 s at 57°C, and extension for 33 s at 72°C with a final extension for 7 min at 72°C. At the end of the reaction, a 198-bp-long PCR product was obtained for rs6265, 153 bp for rs1491850, and 92 bp for rs2030324.

**PmII restriction enzyme cutting was applied to the rs6265 variant, AfiIII restriction enzyme cutting to the rs1491850 variant, and AcII restriction enzyme cutting to the rs2030324 variant. The cut PCR products were imaged in 2 to 4% agarose gel electrophoresis and sized by comparing a 50 to 1000 bp DNA size marker. Accordingly, a single 198 bp result was evaluated as GG(Val/Val) genotype for rs6265, 198, 128, and 70 bp results as GA(Val/Met) genotype, 128 and 70 bp results as mutAA(Met/Met) genotype. For rs1491850, a single 153 bp result was evaluated as TT genotype, 153, 103, and 50 bp results as TC genotype, 103
and 50 bp results as mutated CC genotype. Finally, for rs2030324, a single 92 bp result was evaluated as TT genotype, 92, 52, and 40 bp results as TC genotype, 52 and 40 bp results as mutated CC genotype.

### Statistical Assessment

The data were transferred to SPSS-WINDOWS 15 packet software to perform One-way analysis of variance (ANOVA) and Chi-squared ($\chi^2$) tests.

### RESULTS

The age range of the patient group was 22 to 55 years, whereas it was 23 to 55 years for the control group. Of the patient group, 36.5% (19) were female and 63.5% (33) were male, whereas 31.0% (13) of the control group were female and 69.0% (29) were male. The distribution of gender between the patient and control groups were comparable and no statistically significant difference was present between the groups ($P < 0.05$). The average age and standard deviation were 43.6 ± 10.7 and 39.3 ± 9.8 years for the patient and control groups, respectively [Table 2].

Twenty-six patients had localized bilateral tinnitus, 21 of them on the left ear and five of them on the right ear. According to the distribution of tinnitus frequency, majority of the patients had tinnitus at 4000 and 8000 Hz [Figure 1], and the tinnitus intensity was mostly between 45 and 55 dB [Figure 2]. About 9.6% ($n = 5$) of the patients in the study group suffered from tinnitus for less than a year, 46.2% ($n = 24$) for 1 to 2 years, 25.0% ($n = 13$) for 2 to 5 years, and 19.2% ($n = 10$) for 5 to 10 years. The mean THI and SCID-I scores of the patients were 37.4 ± 20.0 and 47 ± 10, respectively, whereas the mean VAS scores were as follows: VAS-1 6.1 ± 2.3, VAS-2 7.9 ± 2.7, VAS-3 5.8 ± 2.8, VAS-4 3.2 ± 1.7, and VAS-5 3.7 ± 1.9.

The average serum BDNF level was 1374 ± 326 pg/ml in the patient group, whereas it was 1640 ± 193 pg/ml in the control group, which revealed a statistically significant difference ($P < 0.0001$) after one-way ANOVA [Table 3].

Based on the genotype analysis, the genotype distribution of BDNF gene polymorphisms was 84.6% GA and 15.4% AA in the patient group for rs6265, whereas it was 81% GA and 19% AA in the control group. For rs1491850, it was 32.7% CC, 49.9% CT, and 17.4% TT in the patient group and 30.9% CC, 40.5% CT, and 28.6% TT in the control group.

![Figure 1: Tinnitus frequency distribution of the patients](image1.png)

![Figure 2: Tinnitus intensity distribution of the patients](image2.png)

Table 2: The age, gender distribution, and gender average of tinnitus patient and control groups

| Group     | Gender | N  | Minimum age | Maximum age | Average age | S.D. |
|-----------|--------|----|-------------|-------------|-------------|------|
| Tinnitus  | Female | 19 | 22          | 55          | 42.7        | 10.2 |
|           | Male   | 33 | 23          | 55          | 44.1        | 11.0 |
|           | Total  | 52 | 22          | 55          | 43.6        | 10.7 |
| Control   | Female | 13 | 24          | 55          | 38.5        | 7.5  |
|           | Male   | 29 | 23          | 55          | 39.7        | 10.7 |
|           | Total  | 42 | 23          | 55          | 39.3        | 9.8  |

Table 3: Serum BDNF levels in tinnitus patient and control groups

| Group     | Gender | N   | Minimum (pg/ml) | Maximum (pg/ml) | Average (pg/ml) | S.D. | P       |
|-----------|--------|-----|-----------------|-----------------|-----------------|------|---------|
| Patient   | Female | 19  | 644.9           | 1832.8          | 1398.6          | 358  | <0.0001 |
|           | Male   | 33  | 699.8           | 2110.0          | 1360.2          | 312  |         |
|           | Total  | 52  | 644.9           | 2110.0          | 1374.2          | 326  |         |
| Control   | Female | 13  | 1394.5          | 1844.1          | 1673.6          | 117  |         |
|           | Male   | 29  | 1178.7          | 2224.1          | 1625.4          | 219  |         |
|           | Total  | 42  | 1178.7          | 2224.1          | 1640.0          | 193  |         |
The distribution for rs2030324 was 23.4% CC, 42.0% CT, and 34.6% TT in the patient group and 23.8% CC, 42.8% CT, and 33.4% TT in the control group. As for allele frequencies of the patient group, G allele frequency was 57.7% for rs6265 and A allele frequency was 42.3%. For rs1491850, T allele frequency was 42.3% and C allele frequency was 57.7%. For rs2030324, T allele frequency was 55.6% and C allele frequency was 44.4%. In the control group, G allele frequency was 59.5% for rs6265 and A allele frequency was 40.5%. For rs1491850, T allele frequency was 42.3% and C allele frequency was 57.7%. For rs2030324, T allele frequency was 55.6% and C allele frequency was 44.4%. No significant difference was found with \( \chi^2 \) test between the patient group and the control group in terms of the genotype and allele distributions (P = 0.23) [Table 4]. The genotype and allele distributions showed no statistical significance in terms of gender (ANOVA) (P = 0.35) [Table 5]. In the tinnitus group, there was no statistically significant difference between rs6265, rs1491850, and rs2030324 and serum BDNF levels (ANOVA) (P = 0.22) [Table 6]. In the control group, there was no statistically significant difference between rs6265, rs1491850, and rs2030324 and serum BDNF levels (ANOVA) (P = 0.22) [Table 7].

No statistically significant relationship could be found between THI and BDNF gene polymorphisms and also between THI and BDNF serum levels (P = 0.30). The relationships between tinnitus frequencies and BDNF gene polymorphisms and between tinnitus frequencies and BDNF serum levels were not statistically significant (P = 0.55 and 0.38, respectively). Also there was no statistically significant relationship between tinnitus frequency, tinnitus severity, and BDNF gene polymorphisms and between tinnitus intensity and BDNF serum levels (P = 0.32 and 0.40, respectively). No statistically significant relationship could be found between tinnitus VAS values and BDNF gene polymorphisms, and between tinnitus VAS values and BDNF serum levels (P = 0.47 and 0.25, respectively).

**DISCUSSION**

There was a close relationship between tinnitus and mood disorders such as depression with a rate of coexistence up to 30%. For that reason, patients, whose etiology is not dependent on a mood disorder, were selected in the current study and patients diagnosed with MDD based on DSM-IV criteria were excluded from the study.

Looking at the gender effect on the tinnitus frequency, in spite of the studies indicating no difference between males and females it is known that tinnitus frequency was slightly higher in females than in males due to spontaneous autoacoustic emissions, a case more frequently encountered in women, and the effect of sex hormones and neurotransmitters on the auditory canal under the age of 50 years, which is equalized after the age of 50 years. Tinnitus frequency increases to 12% in males especially above the age of 65 years due to effects of various environmental factors (working at noisy workplaces etc.) as opposed to 7% in females. In our study, the patient group was composed of individuals between the ages of 22 and 55 years, and 36.5% of the patients were female, whereas 63.5% of them were male.

Several studies have been carried out on the localization of tinnitus, and although the studies indicate no difference between right and left ears, some studies report a higher localization in the left ear. In the current study, 26 tinnitus patients have bilateral tinnitus with 21 of them in the left ear and five of them in the right ear.

Tinnitus frequency is known to occur over 2000 Hz, mostly at 4000 Hz. However, with the inclusion of

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**Table 4: Allele and genotype distribution for rs6265 (Val66Met), rs2030324, and rs1491850 in tinnitus patient and control groups**

| SNP          | Groups  | Distribution | AA  | GA  | GG  | Total | Allele (%) |
|--------------|---------|--------------|-----|-----|-----|-------|------------|
| Rs6265       | Patient | n            | 8.0 | 44.0| –   | 52.0  | 57.7       | 42.3       |
|              |         | %            | 15.4| 84.6| –   | 100.0 |            |            |
|              | Control | n            | 8.0 | 34.0| –   | 42.0  | 59.5       | 40.5       |
|              |         | %            | 18.7| 81.3| –   | 100.0 |            |            |
| Rs1491850    | Patient | n            | 9.0 | 26.0| 17.0| 52.0  | 42.3       | 57.7       |
|              |         | %            | 17.4| 49.9| 32.7| 100.0 |            |            |
|              | Control | n            | 12.0| 17.0| 13.0| 42.0  | 48.8       | 51.2       |
|              |         | %            | 28.6| 40.5| 30.9| 100.0 |            |            |
| Rs2030324    | Patient | n            | 18.0| 22.0| 12.0| 52.0  | 55.6       | 44.4       |
|              |         | %            | 34.6| 42.0| 23.4| 100.0 |            |            |
|              | Control | n            | 14.0| 18.0| 10.0| 42.0  | 54.8       | 45.2       |
|              |         | %            | 33.4| 42.8| 23.8| 100.0 |            |            |
### Table 5: Genotype and allele distribution based on gender in tinnitus patient and control groups

| rs6265 | GG | N  | %  | GA | N  | %  | AA | N  | %  | Total | N  | %  |
|--------|----|----|----|----|----|----|----|----|----|-------|----|----|
|        |    |    |    |    |    |    |    |    |    |       |    |    |
| Female |    |    |    |    |    |    |    |    |    |       |    |    |
| Patient| 5  | 9.8| 14 | 26.1| –  | –  | 19 | 35.9|
| Control| 2  | 5.2| 11 | 26.2| –  | –  | 13 | 31.4|
| Male   |    |    |    |    |    |    |    |    |    |       |    |    |
| Patient| 3  | 5.6| 30 | 58.5| –  | –  | 33 | 64.1|
| Control| 6  | 14.0| 23 | 55.0| –  | –  | 29 | 69.0|

| rs1491850 | TT | N  | %  | TC | N  | %  | CC | N  | %  | Total | N  | %  |
|-----------|----|----|----|----|----|----|----|----|----|-------|----|----|
|           |    |    |    |    |    |    |    |    |    |       |    |    |
| Female    |    |    |    |    |    |    |    |    |    |       |    |    |
| Patient   | 4  | 7.8| 9  | 17.3| 6  | 11.6| 19 | 36.7|
| Control   | 2  | 4.7| 8  | 19.2| 3  | 7.1 | 13 | 31.0|
| Male      |    |    |    |    |    |    |    |    |    |       |    |    |
| Patient   | 5  | 9.6| 17 | 32.6| 11 | 21.1| 33 | 63.3|
| Control   | 10 | 23.9| 9 | 21.3| 10 | 23.8| 29 | 69.0|

| Female    |    |    |    |    |    |    |    |    |    |       |    |    |
| Control   | 3  | 7.2| 8  | 19.0| 2  | 4.8 | 13 | 31.0|
| Male      |    |    |    |    |    |    |    |    |    |       |    |    |
| Patient   | 11 | 21.4| 15 | 28.8| 7  | 13.3| 33 | 63.5|
| Control   | 11 | 26.2| 10 | 23.8| 8  | 19.0| 29 | 69.0|

| rs2030324 | TT | N  | %  | TC | N  | %  | CC | N  | %  | Total | N  | %  |
|-----------|----|----|----|----|----|----|----|----|----|-------|----|----|
|           |    |    |    |    |    |    |    |    |    |       |    |    |

| Female    |    |    |    |    |    |    |    |    |    |       |    |    |
| Control   | 7  | 13.2| 7  | 13.2| 5  | 10.1| 19 | 36.5|
| Male      |    |    |    |    |    |    |    |    |    |       |    |    |
| Patient   | 11 | 21.4| 15 | 28.8| 7  | 13.3| 33 | 63.5|
| Control   | 11 | 26.2| 10 | 23.8| 8  | 19.0| 29 | 69.0|

| N  | %  | N  | %  | N  | %  | N  | %  |
|----|----|----|----|----|----|----|----|
According to Pan et al., the tinnitus pitch is often associated with the frequency where the highest magnitude of loss occurs audiometrically,[43] though some researchers favor using a range of pitches to describe tinnitus pitch-match data.[13] The average pitch according to Pan et al. was 4968 Hz,[43] whereas almost half of the patients in our study have tinnitus pitch at 8000 Hz or above. Yilmaz et al. reported the initial average tinnitus frequency as $5796.67 \pm 3017.89$ Hz.[44]

Patient statements in our study indicated that 9.6% of the patients suffered from tinnitus for less than a year, whereas 46.2% for 1 to 2 years, 25.0% for 2 to 5 years, and 19.2% for 5 to 10 years. In a larger study by American Tinnitus Association (ATA) conducted on 13,000 patients, 4.1% of the patients suffered from tinnitus for less than a year, 11.7% for 1 to 2 years, 23.1% for 2 to 5 years, 23.3% for 5 to 10 years, 20.7% for 10 to 20 years, and 17.2% for more than 20 years.[45] The amount of time the patients in the current study suffered from tinnitus is considerably shorter compared to that of ATA’s report in 1986. This shorter duration that the patients of the current study suffered from tinnitus could be partly due to the overall development of technology since 1986, the increasing number of drugs available for tinnitus therapy, the advancements in electrophysiological studies, the relatively higher level of awareness of today’s patients in seeking treatment, and also the fact that the subjects in the current study were under the age of 55 years.

In this study investigating the relationship between tinnitus and BDNF gene polymorphisms as well as BDNF serum levels, BDNF serum levels were examined by the ELISA method, and a statistically significant difference was observed between the BDNF serum levels of patient and control groups ($P < 0.0001$). The serum BDNF level average was observed to be $1374 \pm 326$ pg/ml in the patient group and $1640 \pm 193$ pg/ml in the control group. Many studies mention a decrease in BDNF serum and plasma levels in MDD.[46-48] Also rate of coexistence of mood disorders such as depression and tinnitus was reported to be high.[36,49] Goto et al. indicated that plasma BDNF levels changed with the tinnitus intensity and for that reason, it could be used to examine tinnitus objectively, and also to express the existence of a coexistent mood disorder in a study carried out with 43 tinnitus patients composed of 14 males and 29 females and 30 healthy people composed of 15 males and 15 females.[22] The tinnitus cases in our study were selected from individuals who were not diagnosed with MDD or any other mood disorders according to the SCID-I assessment structured for DSM-IV axis I disorders and who were not exposed to an antidepressant treatment. Higher BDNF protein values were found in the patient group as opposed to the control group.

There are numerous SNPs on the BDNF gene. Also the effects of BDNF gene SNPs on BDNF serum level changes and chronic tinnitus as well as the relationship between [rs6265 (Val66Met), rs2030324, and rs1491850] polymorphisms and changes in the scores of BAER tests were studied.[17,22,23,50] In the current report, the highest frequency distribution was at 4000 and 8000 Hz.

### Table 6: Comparison of the BDNF polymorphisms and serum BDNF levels in tinnitus patient group

| SNP    | Genotype | Gender | N   | BDNF-average | S.D. |
|--------|----------|--------|-----|--------------|------|
| rs6265 | AA       | Female | 5   | 1123.1       | 297  |
|        |          | Male   | 3   | 13330.6      | 212  |
|        | GA       | Female | 14  | 1496.9       | 332  |
|        |          | Male   | 30  | 1363.1       | 323  |
|        | GG       | Female | –   | –            | –    |
|        |          | Male   | –   | –            | –    |
|        | Total    |        | 52  |              |      |
| rs1491850 | TT     | Female | 4   | 1166.1       | 201  |
|         |          | Male   | 5   | 1216.0       | 388  |
|         | CT       | Female | 9   | 1581.1       | 170  |
|         |          | Male   | 17  | 1377.0       | 345  |
|         | CC       | Female | 6   | 1279.6       | 519  |
|         |          | Male   | 11  | 1399.8       | 218  |
|         | Total    |        | 52  |              |      |
| rs2030324 | TT     | Female | 7   | 1127.9       | 367  |
|          |          | Male   | 11  | 1231.7       | 403  |
|          | CT       | Female | 7   | 1542.3       | 182  |
|          |          | Male   | 15  | 1408.5       | 266  |
|          | CC       | Female | 5   | 1576.0       | 346  |
|          |          | Male   | 7   | 1458.6       | 187  |
|          | Total    |        | 52  |              |      |

### Table 7: Comparison of the BDNF polymorphisms and serum BDNF levels in the control group

| SNP    | Genotype | Gender | N   | BDNF-average | S.D. |
|--------|----------|--------|-----|--------------|------|
| rs6265 | GG       | Female | 2   | 1776.0       | 7    |
|        |          | Male   | 6   | 1647.5       | 290  |
|        | GA       | Female | 11  | 1653.7       | 118  |
|        |          | Male   | 23  | 1619.5       | 205  |
|        | AA       | Female | 0   | 0            | 0    |
|        |          | Male   | 0   | 0            | 0    |
|        | Total    |        | 42  |              |      |
| rs1491850 | TT     | Female | 2   | 1485.2       | 128  |
|          |          | Male   | 10  | 1600.6       | 143  |
|          | CT       | Female | 8   | 1731.0       | 78   |
|          |          | Male   | 9   | 1659.9       | 165  |
|          | CC       | Female | 3   | 1641.5       | 50   |
|          |          | Male   | 10  | 1600.9       | 321  |
|          | Total    |        | 42  |              |      |
| rs2030324 | TT     | Female | 3   | 1617.2       | 197  |
|          |          | Male   | 11  | 1604.8       | 208  |
|          | CT       | Female | 8   | 1697.2       | 81   |
|          |          | Male   | 10  | 1620.9       | 171  |
|          | CC       | Female | 2   | 1618.4       | 42   |
|          |          | Male   | 8   | 1668.5       | 325  |
|          | Total    |        | 42  |              |      |
study carried out with family-based 136 people by Sklar et al., the Met allele frequency was indicated to be lower in the bipolar disorder. In a study carried out by Hünnerkopf et al., it was suggested that there is a lower relationship between Met/Met genotype and neurotic structure; thus, the Val allele might be related with a higher risk for mood and anxiety disorders. In the study of Myajima et al., it was indicated that cognitive performance decreases with the existence of Met allele in all cognitive tests, and Met alleles have no protective role in non-demensive individuals. In another study carried out on 100 healthy individuals with BDNF Val66Met polymorphism by Bhang et al., it was found that Met allele carriers have lower serum BDNF levels compared to those carrying Val alleles. In the in vitro and in vivo studies, Egan et al. indicated that Met alleles affect evoke-dependent emissions of BDNF and its intracellular functions; thus, it might lead to abnormal hippocampal functions and a weaker, verbal episodic memory. In our study, the Met allele frequency was found to be 42.3% in the patient group, which was lower than the Val allele frequency. Neither in the patient group nor in the control group was a meaningful relationship found between the existence of Met alleles and BDNF serum levels.

There are several studies conducted about mood disorders and responses to antidepressant treatments in MDD patients in reference to the BDNF rs1491850 polymorphism. The BDNF rs2030324 polymorphism was also examined in terms of its relationship with the tendencies toward schizophrenia, asthma, and nicotine addicted individuals. In the study carried out by Juckkel et al. to examine the relationship among rs6265, rs1491850, and rs2030324 polymorphisms and changes in LDAEP, they stronger LDAEP values were observed in G(Val)-C-T haplotype carriers. LDAEP is an indicator for the central serotonergic neurotransmission. The correlation between LDAEP and decreased serotonin activity is also emphasized in the studies of Hegerl et al. indicating weak LDAEP response in the serotonin syndrome patients taking SSRI treatment. In the study carried out by Lang et al. and Park et al. to examine the relationship between BDNF and serotonin activity, they shed light on the relationship between low serotonin activity as well as low serum BDNF levels and BDNF G-C-T (for rs6265, rs2030324, and rs1491850) carriers. In this study, it was observed that there was no statistically significant relationship between rs1491850 and rs2030324 polymorphisms and serum BDNF levels in the patient and control group.

**Conclusion**

Based on the results of this study, the serum BDNF protein level has been found to be lower in tinnitus patients compared to the control subjects. However, no statistically significant relationship was observed between the polymorphisms of the BDNF gene and the tinnitus disease. This might be the result of the fact that the BDNF gene has different expression patterns in various tissues and pathologies due to the dynamic functional regulation. For that reason, the study may be improved by including a larger number of patients and analyzing BDNF expression patterns in various tissues, thus extending its scope.

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**Conflicts of interest**

There are no conflicts of interest.

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