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Research Article

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Brain Derived Neurotropic Factor Gene Val66Met Polymorphism in Alzheimer's Patients in Northern Turkey

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Abstract

Objective: The present study aimed to determine the frequency of Brain Derived Neurotropic Factor (*BDNF*) gene 196 A/G polymorphism in Alzheimer Disease (AD) patients in northern Turkey.

Materials and Methods: 184 probable AD patients (according to NINCDS and ADRDA criteria) and 133 healthy controls were included to this study. The genotyping of *BDNF* gene rs6265 polymorphism were evaluated in the laboratories of our University Medical Faculty Medical Biology department, in the StepOnePlus Real-Time PCR system using by TaqMan SNP genotyping assay according to the manufacturer's instructions.

Results: In all, 54 (29.35%) patients had mild dementia, 72 (39.13%) had moderate dementia, and 58 (31.52%) had advanced-stage dementia. Mean duration of AD was 4.33 years. There was not a significant difference if the frequency of *BDNF* gene Val66Met polymorphism between the patient and control groups. The patient group included 88 (47.83%) males and 96 (52.17%) females, versus 72 (54.14%) males and 61 (45.86%) females in the control group. Moreover, there was not a significant association between the frequency of *BDNF* gene Val66Met polymorphism and gender distribution in the patient group. Mean age in the patient group was 75.83 years, versus 74.29 years in the control group; however, there were not any significant changes when correlating *BDNF* gene Val66Met polymorphism with age. Similarly, there was not a significant association between *BDNF* gene Val66Met polymorphism and AD stage in the patient group.

Conclusion: In conclusion, there was not an association between AD and *BDNF* gene Val66Met polymorphism in the northern Turkish population.

Keywords

Brain derived neurotropic factor, Polymorphism, Alzheimer disease, Northern Turkey

Introduction

Sporadic Alzheimer's disease (SAD) is a progressive condition characterized by deficits in short-term episodic memory, language, and visual-spatial-executive functions, and is frequently accompanied by late-onset neurobehavioral abnormalities. When all causes of dementia are considered, Alzheimer's disease (AD) alone constitutes approximately 90% of dementias. On the other hand, the most important risk factor for the development of AD is age. The pathological

features of AD include the presence of neurofibrillary tangles and senile plaques [1]. Although the onset of AD is typically at age 65 years, AD has also been reported in patients aged under 45 years [1].

Genetic predisposition (often exhibiting autosomal dominant inheritance) was proposed to be a factor in early-onset AD. Various mutations were identified in genes encoding amyloid precursor protein (APP), presenilin 1 (PS-1), and presenilin 2 (PS-2) in patients with autosomal dominant inheritance. Moreover, genetic risk factors were also suggested to be important in the development of late-onset familial AD. In particular, inheritance of the *ApoEε4* allele is among the most important risk factors for AD [2, 3]. When considering the genetic basis of the disease as currently known, it remains unclear if there is a genetic predisposition for AD.

Recently, a number of studies evaluated the association between the presence of Val66Met polymorphism in the *BDNF* gene and AD. *BDNF* is a member of the neurotrophin gene family that encodes neurotrophic growth factors [4] and is very important for neuronal development and plasticity; this hormone is produced in multiple parts of the central nervous system (the hippocampus, cortex, and basal forebrain) (CNS) and peripheral nervous system (PNS) [5]. *BDNF* promotes the survival of existing neurons, and aids the growth and differentiation of new neurons and synapses associated with long-term memory. *BDNF* is produced in the endoplasmic reticulum, is released into the vesicles, and finally binds to the carboxypeptidase enzyme [6]. Some studies have shown that knock out of the *BDNF* gene in mice causes loss of sensory and autonomic neurons, and eventually death [7]. *BDNF* binds to ≥ 2 receptors on the surface of cells that are capable of responding to this growth factor, Tropomyosin receptor kinase B (TrkB), and LNGFR (low-affinity nerve growth factor receptor, also known as p75) [8].

Moreover, a number of earlier studies reported a decrease in *BDNF* gene expression in multiple regions of the brain in AD patients. It was also suggested that there might be an association between attenuated *BDNF* gene expression and the development of neurofibrillary tangles and senile plaques [9, 10]. A *BDNF* gene polymorphism 196 A/G; (dbSNP number: rs6265) results in an addition of a misallocated valine amino acid instead of methionine at the 66th position of the amino acid sequence of polypeptide that may eventually lead to changes in intracellular protein synthesis [11]. Additionally, a number of researchers that studied *BDNF* gene 196 A/G polymorphism in various neuropsychiatric disorders, such as schizophrenia, reported that this polymorphism impairs cognitive function due to changes in the hippocampal region [11]. This polymorphism was also shown to be associated with reduced cerebral cortical thickness and memory functions, even in healthy individuals [12].

Following these studies, researchers in numerous countries conducted novel studies on the relationship between this *BDNF* gene polymorphism and the development of AD. Only a few of these studies that included AD patients of varying ethnic origin suggested there is an association between the polymorphism and AD [13, 14]. In contrast, the majority of the studies indicated that there was not an association between the polymorphism and AD [15, 16].

The inconsistent findings reported to date are insufficient to definitely conclude that there is a relationship between *BDNF* gene 196 A/G polymorphism and AD. As such, the present study aimed to determine the frequency of this gene polymorphism in AD patients in northern Turkey and to determine if there is an association between the polymorphism and AD, based on such parameters as age, gender distribution, and stage of disease.

Materials and Methods

This study included 184 AD patients and 133 healthy controls. Patients that presented to our clinic between 2013 and 2015 that were diagnosed as AD based on National Institutes of Neurological and Communicative Disorders and Stroke (NINCDS) and Alzheimer's Disease and Related Disorders Association (ADRDA) criteria. NINCDS and ADRDA criteria were first defined by McKhann et al. [17] as a diagnosis method in 1984 and were revised in 2011 by the same group [18]; diagnosis of the disease has become more practical using the revised criteria. AD is diagnosed according to NINCDS and ADRDA criteria, and is categorized into the following 3 categories: 1. Probable AD dementia; 2. possible AD dementia; 3. probable or possible AD dementia with any evidence of the AD pathophysiological processes. The study protocol was approved by the University local Ethics Committee and all of the participants (or family members, if required) provided written informed consent.

AD categories 1 and 2 are intended for use in all clinical settings, whereas category 3 is currently intended for research purposes only. These are the including criteria of the study; 1.to be diagnosed as only Probable AD dementia (category 1), 2. Should not have any other additional neurodegenerative disease like Parkinson's disease, Multiple sclerosis, epilepsy etc. 3. To be live in northern Turkey (northern sites of Turkey; Tokat, Samsun, Sivas). AD stage was determined using a standard mini mental state test (MMT); scores of 19-23 were defined as mild dementia, 10-18 as moderate dementia, and ≤9 as advanced-stage dementia [19]. Distribution of age and gender, AD stage, medications used, and sociodemographic factors, including history of an additional illness, were recorded. Patients that presented to the neurology clinic without any known neurological or genetic disease were included in the control group. All the controls had an MMT score > 24. All of the patients and controls lived in northern Turkey.

Peripheral venous blood samples were collected and preserved in EDTA tubes. Genomic DNA (gDNA) was extracted from blood samples using an Invitrogen Genomic DNA Isolation Mini Kit (K1820-02, Invitrogen Life Technologies, Carlsbad, CA, USA). Genetic analysis was then performed using the TaqMan allelic discrimination assay. The genotyping of *BDNF* gene rs6265 polymorphism were performed by StepOnePlus Real-Time polymerase chain reaction (PCR) system (Applied Biosystems, Foster city, CA, USA), according to the manufacturer's instructions.

Statistical Analysis

Data were analyzed using SPSS for Windows v.15.0 (SPSS Inc., Chicago, IL, USA). Genotype distributions and allele frequencies of the *BDNF* gene rs6265 polymorphism were compared using Fisher's exact chi-square (χ^2) test. The level of statistical significance was set at $p < 0.05$. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to determine the association between *BDNF* allelic and genotypic variants, and the occurrence of AD.

Results

In all, 54 (29.35%) patients had mild dementia, 72 (39.13%) had moderate dementia, and 58 (31.52%) had advanced-stage dementia. In addition, mean duration of AD was 4.33 years. The participants' general features are shown in Table 1. There was not a significant difference if the frequency of *BDNF* gene Val66Met polymorphism between the patient and control groups ($p > 0.05$) (Table 2). The patient group included 88 (47.83%) males and 96 (52.17%) females (Table 1), versus 72 (54.14%) males and 61 (45.86%) females in the control group (Table 1). Moreover, there was not a significant correlation between the frequency of *BDNF* gene Val66Met polymorphism and gender distribution in the patient group ($p > 0.05$) (Table 3).

Table 1: Participant general characteristics.

		Patient group n (%)	Control group n (%)
Gender	Female	88 (47.83%)	61 (45.86%)
	Male	96 (52.17%)	72 (54.14%)
Age (years)		75.83	74.29
Mean duration of AD (years)		4.33	-
AD Stage	Mild	54 (29.35%)	-
	Moderate	72 (39.13%)	-
	Advanced	58 (31.52%)	-

AD: Alzheimer Disease

Table 2: Genotypic and allelic findings in the patient and control groups.

Genotype	Patient Group n (%)	Control Group n (%)	P	OR/ 95% CI
GG	130 (70.65%)	90 (67.67%)	0.622	1.15/ 0.71-1.86
GA	50 (27.17%)	41 (30.83%)	0.530	0.84/ 0.51-1.37
AA	4 (2.17%)	2 (1.50%)	1.000	1.46/ 0.26-8.02
Allele				
G	310 (84.24%)	221 (83.08%)	0.744	1.09/ 0.71-1.66
A	58 (15.76%)	45 (16.92%)		

G: Guanine; GG: guanine-guanine; A: adenine; AA: adenine-adenine; GA: guanine-adenine. OR: Odd's ratio; CI: confidence intervals

Mean age in the patient group was 75.83 years, versus 74.29 years in the control group (Table 1); however, there were not any significant changes when correlating *BDNF* gene Val66Met polymorphism with age ($p > 0.05$) (Table 3). Similarly, there was not a significant association between *BDNF* gene Val66Met polymorphism and AD stage in the patient group ($p > 0.05$) (Table 3).

Table 3: Genotypic distributions and allelic frequencies in the patient group, according to gender and Alzheimer Disease stage.

		GG n (%)	GA n (%)	AA n (%)	G n (%)	A n (%)
Gender (n)	Female (88)	59 (67.05%)	26 (29.55%)	3 (3.41%)	144 (81.82%)	32 (18.18%)
	Male (96)	71 (73.96%)	24 (25.00%)	1 (%1.04)	166 (86.46%)	26 (13.54%)
P		0.334	0.511	0.350	0.253	
AD Stage (n)	Mild (54)	39 (72.22%)	14 (25.93%)	1 (1.85%)	92 (85.19%)	16 (14.81%)
	Moderate (72)	48 (66.67%)	22 (30.56%)	2 (2.78%)	118 (81.94%)	26 (18.06%)
	Advanced (58)	43 (74.14%)	14 (24.14%)	1 (1.72%)	100 (86.21%)	16 (13.79%)
P		0.860	0.857	1.000	0.875	

G: Guanine; GG: guanine-guanine; A: adenine; AA: adenine-adenine; GA: guanine-adenine. AD: Alzheimer Disease

Discussion

This is the first study conducted in Turkey on the association between the frequency of *BDNF* gene Val66Met polymorphism and AD predisposition. The presenting findings show that there isn't an association between *BDNF* gene Val66Met polymorphism and AD in the population of northern Turkey. Furthermore, there isn't an association between AD and *BDNF* gene Val66Met polymorphism according to age, gender, or AD stage. Following reports of the relationship between *BDNF*, and memory and cognition, researchers sought to determine if there was any association between AD and *BDNF* gene Val66Met polymorphism in patients of varied ethnicity; however, the findings were inconsistent.

Combarros et al. [20] reported that there isn't a relationship between *BDNF* gene Val66Met polymorphism and AD in their total Spanish cohort or in their subgroups (early-onset and late-onset). They split their data based on *ApoE* carrier status and observed that there was not a significant correlation between the Val/Val or Met/Met genotypes, and the risk of AD in *ApoEε4* carriers and non-*ApoEε4* carriers. He et al. [21] studied *BDNF* gene Val66Met polymorphism in 513 Chinese SAD patients and 575 controls, and did not observe a correlation between AD and Val66Met polymorphism. Additionally, they observed the same when their patients and controls were compared in terms of distribution of age and gender, and age at disease onset. Similarly, Taiwanese researchers noted that there isn't a significant difference in the

frequency of *BDNF* gene Val66Met polymorphism in either all groups or subgroups according to age, gender, or age at disease onset [16]. Furthermore, in the US Desai et al. [15] studied the frequency of *BDNF* gene Val66Met polymorphism in Negro AD patients (n = 995) and Caucasian AD patients (n = 64). They reported that there was not a significant association between *BDNF* gene Val66Met polymorphism and AD, as subsequently reported in another study from the US [22].

In contrast, some studies reported that there is an association between AD and *BDNF* gene Val66Met polymorphism. Ventriglia et al.'s [14] 2002 study on the frequency of *BDNF* gene Val66Met polymorphism in AD included 130 Caucasian AD patients and 110 healthy controls in northern Italy. PCR amplification was used to evaluate the polymorphism and the frequency of A/G alleles, and genotype distribution differed between the AD patients and controls. Bian et al. [23] reported that the frequency of *BDNF* gene Val66Met polymorphism was similar in Chinese patients with AD (n = 203) and controls (n = 239); however, the polymorphism was more common in the female AD patients than in the control group. In addition, the frequency of *BDNF* gene Val66Met polymorphism was higher in carriers of *ApoEε4*, which is in contrast to the findings reported by Ventriglia et al. [14]. On the other hand, regarding the gender distribution of patients included in all of the above-mentioned studies, there were far more female than males patients, whereas there were more male than female patients in the present study; however, it remains difficult to make a clear conclusion about the association between *BDNF* gene Val66Met polymorphism and AD according to gender and additional larger scale research is necessary for clarification.

As the method of analysis of peripheral venous blood samples was the same in all earlier studies and in the present study, it is difficult to implicate the analysis methodology in the observed differences in findings. Nonetheless, there might be several plausible reasons for the literature's inconsistent findings. Firstly, although great effort was made in these studies to recruit volunteers in such a way as to generate ethnically homogenous groups, it may be considered impossible to fully homogenize patients in all groups. As such, although all of the present study's patients were from the same region of northern Turkey, this population is ethnically diverse and the required homogeneity may not have been achieved. Secondly, the present study's patients were diagnosed as probable AD. To make a definite diagnosis of AD histopathological assessment of brain tissue samples is necessary, but was not performed in the present study; therefore, none of the patients were definitively diagnosed as AD. Moreover, creation of the control group was affected by the same issues. Although a clear association was not observed between the *BDNF* gene polymorphism and AD, it may have been because only the frequency of Val66Met polymorphism was analyzed and no other mutation/polymorphism located on the *BDNF* gene was investigated. Lastly, the lack of comparison of the functional level of the *BDNF* protein makes it impossible to conclude that there isn't an association between *BDNF* gene Val66Met polymorphism and AD; only additional research can clarify the association.

However, this study had some limitations. Firstly, the number of control group samples was relatively lower than the patient group. Secondly, in this study we were evaluated the association between *BDNF* val66met gene polymorphism and Alzheimer disease, but we could not examined the *BDNF* gene expression and protein levels in our study group.

In conclusion, there was not an association between AD and *BDNF* gene Val66Met polymorphism in the present study's total population, or according to age, gender, or AD stage; however, regarding above mentioned plausible reasons, it is also difficult to make a conclusion suggesting a certain irrelevance situation. Additional prospective studies are required to investigate additional mutations in larger and more homogenized cohorts.

Conflict of Interest

All the authors declares that there is no conflict of interests.

References

1. Balin BJ, Hudson AP. 2014. Etiology and pathogenesis of late-onset Alzheimer's disease. *Curr Allergy Asthma Rep* 14(3): 417. https://doi.org/10.1007/s11882-013-0417-1
2. Mazzotti DR, Singulane CC, Ota VK, Rodrigues TP, Furuya TK, et al. 2014. Association of APOE, GCPII and MMP9 polymorphisms with common diseases and lipid levels in an older adult/elderly cohort. *Gene* 535(2): 370-375. https://doi.org/10.1016/j.gene.2013.11.040
3. Fabelo N, Martin V, Marin R, Moreno D, Ferrer I, et al. 2014. Altered lipid composition in cortical lipid rafts occurs at early stages of sporadic Alzheimer's disease and facilitates APP/BACE1 interactions. *Neurobiol Aging* 35(8): 1801-1812. https://doi.org/10.1016/j.neurobiolaging.2014.02.005
4. Pencea V, Bingaman KD, Wiegand SJ, Luskin MB. 2001. Infusion of brain-derived neurotrophic factor into the lateral ventricle of the adult rat leads to new neurons in the parenchyma of the striatum, septum, thalamus, and hypothalamus. *J Neurosci* 21(17): 6706-6717.
5. Zigova T, Pencea V, Wiegand SJ, Luskin MB. 1998. Intraventricular administration of *BDNF* increases the number of newly generated neurons in the adult olfactory bulb. *Mol Cell Neurosci* 11(4): 234-245. https://doi.org/10.1006/mcne.1998.0684
6. Benraiss A, Chmielnicki E, Lerner K, Roh D, Goldman SA. 2001. Adenoviral brain-derived neurotrophic factor induces both neostriatal and olfactory neuronal recruitment from endogenous progenitor cells in the adult forebrain. *J Neurosci* 21(17): 6718-6731.
7. Lonetti G, Angelucci A, Morando L, Boggio EM, Giustetto M, et al. 2010. Early environmental enrichment moderates the behavioral and synaptic phenotype of MeCP2 null mice. *Biol Psychiatry* 67(7): 657-665. https://doi.org/10.1016/j.biopsych.2009.12.022
8. Michaelsen K, Zagrebelsky M, Berndt-Huch J, Polack M, Buschler A, et al. 2010. Neurotrophin receptors TrkB.T1 and p75NTR cooperate in modulating both functional and structural plasticity in mature hippocampal neurons. *Eur J Neurosci* 32(11): 1854-1865. https://doi.org/10.1111/j.1460-9568.2010.07460.x
9. Fahnestock M, Garzon D, Holsinger RM, Michalski B. 2002. Neurotrophic factors and Alzheimer's disease: are we focusing on the wrong molecule? *J Neural Transm Suppl* (62): 241-252. https://doi.org/10.1007/978-3-7091-6139-5_22
10. Murer MG, Yan Q, Raisman-Vozari R. 2001. Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease. *Prog Neurobiol* 63(1): 71-124. https://

doi.org/10.1016/S0301-0082(00)00014-9

11. Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, et al. 2003. The BDNF Val66Met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* 112(2): 257-269. https://doi.org/10.1016/S0092-8674(03)00035-7
12. Voineskos AN, Lerch JP, Felsky D, Shaikh S, Rajji TK, et al. 2011. The brain-derived neurotrophic factor Val66Met polymorphism and prediction of neural risk for Alzheimer disease. *Arch Gen Psychiatry* 68(2): 198-206. https://doi.org/10.1001/archgenpsychiatry.2010.194
13. Matsushita S, Arai H, Matsui T, Yuzuriha T, Urakami K, et al. 2005. Brain-derived neurotrophic factor gene polymorphisms and Alzheimer's disease. *J Neural Transm (Vienna)* 112(5): 703-711. https://doi.org/10.1007/s00702-004-0210-3
14. Ventriglia M, Bocchio Chiavetto L, Benussi L, Binetti G, Zanetti O, et al. 2002. Association between the BDNF 196 A/G polymorphism and sporadic Alzheimer's disease. *Molecular psychiatry* 7(2): 136-137. https://doi.org/10.1038/sj/mp/4000952
15. Desai P, Nebes R, DeKosky ST, Kamboh MI. 2005. Investigation of the effect of brain-derived neurotrophic factor (BDNF) polymorphisms on the risk of late-onset Alzheimer's disease (AD) and quantitative measures of AD progression. *Neurosci Lett* 379(3): 229-234. https://doi.org/10.1016/j.neulet.2005.01.008
16. Tsai SJ, Hong CJ, Liu HC, Liu TY, Hsu LE, et al. 2004. Association analysis of brain-derived neurotrophic factor Val66Met polymorphisms with Alzheimer's disease and age of onset. *Neuropsychobiology* 49(1): 10-12. https://doi.org/10.1159/000075332
17. McKhann G, Drachman D, Folstein M, Katzman R, Price D, et al. 1984. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 34(7): 939-944. https://doi.org/10.1212/WNL.34.7.939
18. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, et al. 2011. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 7(3): 263-269. https://doi.org/10.1016/j.jalz.2011.03.005
19. Mungas D. 1991. In-office mental status testing: a practical guide. *Geriatrics* 46(7): 54-58, 63, 66.
20. Combarros O, Infante J, Llorca J, Berciano J. 2004. Polymorphism at codon 66 of the brain-derived neurotrophic factor gene is not associated with sporadic Alzheimer's disease. *Dement Geriatr Cogn Disord* 18(1): 55-58. https://doi.org/10.1159/000077736
21. He XM, Zhang ZX, Zhang JW, Zhou YT, Tang MN, et al. 2007. Lack of association between the BDNF gene Val66Met polymorphism and Alzheimer disease in a Chinese Han population. *Neuropsychobiology* 55(3-4): 151-155. https://doi.org/10.1159/000106473
22. Zhang H, Ozbay F, Lappalainen J, Kranzler HR, van Dyck CH, et al. 2006. Brain derived neurotrophic factor (BDNF) gene variants and Alzheimer's disease, affective disorders, posttraumatic stress disorder, schizophrenia, and substance dependence. *Am J Med Genet B Neuropsychiatr Genet* 141B(4): 387-393. https://doi.org/10.1002/ajmg.b.30332
23. Bian JT, Zhang JW, Zhang ZX, Zhao HL. 2005. Association analysis of brain-derived neurotrophic factor (BDNF) gene 196 A/G polymorphism with Alzheimer's disease (AD) in mainland Chinese. *Neurosci Lett* 387(1): 11-16. https://doi.org/10.1016/j.neulet.2005.07.009