Available online at www.medicinescience.org

ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2025;14(2):482-6

Relation between Alzheimer's type dementia and *GDNF-AS1* gene polymorphisms

Ayse Dondu[1], Seda Orenay Boyacioglu[2]

[1]*Aydın Adnan Menderes University, Faculty of Medicine, Department of Psychiatry, Aydın, Türkiye*
[2]*Aydın Adnan Menderes University, Faculty of Medicine, Department of Medical Genetics, Aydın, Türkiye*

Received 14 February 2025; Accepted 11 March 2025
Available online 31 May 2025 with doi: 10.5455/medscience.2025.02.040

Abstract

In patients diagnosed with early-stage Alzheimer's disease (AD), glial cell line-derived neurotrophic factor (*GDNF*) concentrations have been found to be significantly elevated in cerebrospinal fluid while being reduced in serum. This pattern suggests a potential regulatory mechanism contributing to AD pathology. The *GDNF-AS1* gene, a cis-antisense long non-coding RNA (lncRNA) transcribed from the complementary strand of the *GDNF* gene, is differentially expressed in the human brain, and its transcriptional activity may influence *GDNF* expression in cerebral tissue. The present study investigates the association between *GDNF-AS1* polymorphisms and the pathophysiology of AD. The study cohort comprised 70 Alzheimer's-type dementia patients recruited from the Psychiatry Outpatient Clinic of the State Hospital of Aydın and 70 cognitively healthy participants.Peripheral blood samples were analysed for two *GDNF-AS1* polymorphisms (rs62360233 and rs112179570) using the Fluidigm SNP-type method. The data were processed using SNP genotyping analysis software (v.4.1.3) (Fluidigm, San Francisco, CA, USA). The study found no statistically significant differences between the Alzheimer's-type dementia and control groups in genotype distribution or allele frequency for either polymorphism ($p>0.05$).The study concluded that, although no relationship was detected between *GDNF-AS1* polymorphisms and Alzheimer's type dementia, further studies with a larger number of cases are recommended.

Keywords: Alzheimer's type dementia, glial cell line-derived neurotrophic factor gene, glial cell line-derived neurotrophic factor antisense1 gene, Long non-coding RNA, polymorphism

Introduction

Dementia is a multifactorial/complex disorder that presents itself as a significant health issue in advanced societies, leading to the early death of neurons through genetic, metabolic, vascular, and epigenetic factors. The most prevalent type of dementia is Alzheimer's disease (AD), accounting for 50–70% of all dementia cases, followed by vascular and mixed-type dementia, with 30–40% and 15–20% prevalence rates, respectively [1]. AD is characterised as a progressive neurodegenerative disease, typified by the pathological formation of extracellular amyloid plaques and intracellular neurofibrillary tangles, in addition to the loss of synapses and neurons [2,3]. Depending on the age of onset, AD is classified as early-onset (EOAD) or late-onset (LOAD) AD. The prevalence of EOAD ranges from 1% to 6% among all AD cases, typically manifesting between the ages of 30 and 60 or 65. Conversely, the prevalence of LOAD, which accounts for over 90% of AD cases, is defined as AD with an onset age after 60 or 65 years [4,5]. It is noteworthy that both EOAD and LOAD have the potential to manifest in individuals with relatives who are affected by AD. A survey reported that 60% of cases of EOAD had family members with AD, and 13% of these cases showed an autosomal dominant pattern of inheritance affecting at least three generations [6,7]. Apart from a few autosomal dominant families with single gene mutations, most EOAD cases appear to be complex diseases involving multiple susceptibility genes (such as *APP, PSEN1, PSEN2*) and environmental factors [6,8]. Although the likelihood of developing the condition is approximately two times higher among first-degree relatives of LOAD patients, the mode of inheritance is rarely consistent with that predicted by Mendel [7,9,10]. Genome-wide association (GWAS) and genetic sequencing studies have identified multiple susceptibility genes linked to LOAD. Notwithstanding, Apolipoprotein E gene variations have emerged in recent years as a potential target for both the identification of individuals with a predisposition to developing AD, and for the formulation of novel therapeutic

CITATION
Dondu A, Orenay Boyacioglu S. Relation between Alzheimer's type dementia and *GDNF-AS1* gene polymorphisms. Med Science. 2025;14(2):482-6.

Corresponding Author: Ayse Dondu, Aydın Adnan Menderes University, Faculty of Medicine, Department of Psychiatry, Aydın, Türkiye
Email: ayse.dondu@adu.edu.tr

DOI: 10.5455/medscience.2025.02.040

strategies [11,12].

Glial cell line-derived neurotrophic factor (*GDNF*) is a group of neurotrophic factors that have been shown to promote neuronal survival, synaptic plasticity, neurite branching and neural stem cell migration and differentiation [13-17]. It has been demonstrated that *GDNF* is expressed by developing neurons as well as neurons in the adult nervous system. Given the crucial role of neurotrophic factors in the development and maintenance of overall central nervous system function *GDNF*, widely recognized for its neuroprotective effects, particularly on catecholaminergic neurons, may also be significantly involved in neurodegenerative disorders primarily affecting cholinergic CNS neurons, such as AD. In patients with EOAD, a significant increase in *GDNF* concentration in cerebrospinal fluid (CSF) has been observed, accompanied by a decrease in serum concentrations. These findings suggest that *GDNF* regulation may be implicated in the pathological processes underlying AD. The glial cell line-derived neurotrophic factor-antisense 1 (*GDNF-AS1*) gene is a cis-antisense long non-coding RNA (lncRNA) gene copied from the complementary strand of the *GDNF* gene.The *GDNF-AS1* gene is expressed differently in the human brain, and its transcriptional levels may contribute to the increased *GDNF* levels observed in brain tissue. Currently, few studies provide evidence for the posttranscriptional regulation of *GDNF* mediated by *GDNF-AS1*. These studies have led researchers to speculate that the *GDNF-AS1* antisense gene may also play a role in synaptic plasticity, and further investigation is needed in this regard [18]. Therefore, in this study, *GDNF-AS1* polymorphisms in Alzheimer's-type dementia patients were examined to investigate the role of these polymorphisms in the pathophysiology of Alzheimer's-type dementia.

Material and Methods

Ethical Approval and Cases

Ethical approval for this study was obtained from the Noninterventional Ethics Committee (#2023/173, date: 16.11.2023) of the Faculty of Medicine at Aydın Adnan Menderes University. Informed voluntary consent forms were obtained from all participants before the study. The study was performed in accordance with the Helsinki Declaration. Clinical trial numbers do not applicable to this article.

A total of 140 individuals were included in the study. Of these, 70 patients were diagnosed with Alzheimer's-type dementia by the Psychiatry Outpatient Clinic of Aydın State Hospital, along with 70 healthy control individuals who did not have any major psychopathology. Patients with diagnoses of dementia other than Alzheimer's-type dementia, those with neurological diseases, those diagnosed with schizophrenia or bipolar disorder, and those with a history of mental retardation or substance use disorder were excluded from the study.

DNA isolation

Two millilitres of peripheral blood were collected from both the patient and control groups. Genomic DNA was isolated in accordance with the manufacturer's recommendations using a DNA isolation kit (Roche High Pure DNA Isolation Kit, USA). The genomic DNA concentrations were determined via the Qubit™ 1X dsDNA High-Sensitivity Assay Kit (Thermo Fisher Scientific, USA)

Genotyping and Data Analysis

The Fluidigm SNPType method, utilising the BioMark HD dynamic array system (manufactured by Fluidigm, CA, USA), was employed for the genotyping of the *GDNF-AS1* polymorphisms (rs62360233 A>C and rs112179570 A>C). The procedure followed the manufacturer's instructions strictly. Each test was conducted thrice, using three primers: specific target amplification (STA), locus-specific (LS), and allele-specific (AS). Details of the SNP primers utilised are provided in Table 1. All SNP-type primers and mixtures were prepared in strict accordance with the manufacturer's guidelines.A mixture of 360 µl 2X Fast Probe Master Mix (Biotium, PN31005), 36 µl 20X SNP Type Sample Loading Reagent, 12 µl 60X SNP Type Reagent (Fluidigm, PN 100-3402), 4.3 µl ROX (Biotium, PN29052), and 7.7 µl RNase-free water was made. A total of 3.5 µl of this mixture was pipetted into each well of a clean 96-well plate. Subsequently, 2.5 µl of each diluted STA DNA sample and positive control sample were added, after which the plate was sealed, mixed at 2000 rpm for 3 minutes, and then subjected to centrifugation for 1 minute.Thereafter, 4.00 µl of each assay mixture was added to the dynamic array assay wells, and 5.00 µl of each sample mixture was added to the dynamic array sample wells. Subsequent loading of the dynamic array into the IFC controller initiated the operation, after which it was transferred to the dynamic array BioMark. The array was then subjected to genotyping screening markings and the "SNP Type 96.96 v1" protocol was selected. The program was initiated at this point. Subsequent to this, genotyping data analysis was carried out via Fluidigm SNP genotyping analysis software (Fluidigm, CA, USA), which compared fluorescence intensities to detect the genotype of the samples.

Table 1. SNPtype *GDNF-AS1* primer sequences

***GDNF-AS1* SNP_NAME**	**ASP1_SEQ**	**ASP2_SEQ**	**LSP_SEQ**	**STA_SEQ**
rs112179570	GCTAGGGAGGCCTCACA	GCTAGGGAGGCCTCACC	TTCCCCTGCACAAGCTCTCT	TGACTCACAATTCCACATGGC
rs62360233	AAGTAAGAAAGCGACAGGGTCAT	AGTAAGAAAGCGACAGGGTCAG	AGCCAAACCATATCAGTAGGCCC	GGAGTAAAAGCATTGAAAGTTCCCA

DOI: 10.5455/medscience.2025.02.040

Statistical Analysis

Statistical analyses for the patient-control group were conducted utilising GraphPad InStat software. Data are presented as the means±standard deviations. Genotype distributions and allele frequencies were compared between groups via the chi-squared test or the Fisher exact test. Genetic model analyses were performed via the Cochran-Armitage trend test to evaluate the associations between SNPs and AD. A significance level of 0.05 was adopted to ascertain statistical significance.

Results

The mean age of the Alzheimer's-type dementia patients was determined to be 65.1±16.8 years, whereas in the control group, it was 60.5±12.7 years. The difference between the mean ages of the groups was not statistically significant (p=0.87). In our study group, there were 42 female and 28 male Alzheimer's-type dementia patients, whereas in the control group, there were 40 female and 30 healthy male individuals. The groups were homogeneous, with no significant difference detected in terms of sex distribution (p=0.11).

Table 2 displays the distribution of the genotype and allele frequencies of the *GDNF-AS1* polymorphisms in the groups. For the *GDNF-AS1* rs62360233 A>C polymorphism, the genotype distribution in the Alzheimer's-type dementia group was 91.43% AA, 7.14% AC, and 1.43% CC, whereas in the control group, it was 92.86% AA, 5.71% AC, and 1.43% CC. The allele frequency distribution for the rs62360233 A>C polymorphism was 95% A and 5% C in the Alzheimer's-type dementia group and 95.72% A and 4.28% C in the control group. When the groups were compared for their genotype and allele frequency distribution, statistically no significant difference was detected between the AD and the control groups (p=0.94 and p=0.81, respectively).

Table 2. Comparison of *GDNF-AS1* polymorphism genotype distribution of Alzheimer's type dementia and control groups

GDNF-AS1 genotypes	Alzheimer's type Dementia group	Control group	Df	p	X^2
rs62360233					
AA	64 (91.43%)	65 (92.86%)			
AC	5 (7.14%)	4 (5.71%)	2	0.94	0.75
CC	1 (1.43%)	1 (1.43%)			
rs112179570					
AA	55 (78.57%)	56 (80%)			
AC	14 (20%)	13 (18.57%)	2	0.98	0.05
CC	1 (1.43%)	1 (1.43%)			

For the *GDNF-AS1* rs112179570 A>C polymorphism, the genotype distribution in the Alzheimer's-type dementia group was 78.57% AA, 20% AC, and 1.43% CC, whereas it was 80% AA, 18.57% AC, and 1.43% CC in the control group. Similarly, for the same polymorphism, the allele frequency distribution in the Alzheimer's-type dementia group was 88.57% A and 11.43% C, whereas in the control group, it was 89.29% A and 10.71% C. The genotype and allele frequency distribution comparisons between the two groups revealed no significant difference (p=0.98 and p=0.87, respectively).

According to the hereditary model risk analyses, the *GDNF-AS1* rs62360233 A>C and rs112179570 A>C polymorphisms were not significantly associated with the Alzheimer's type dementia group (p>0.05) (Table 3).

Table 3. Comparison of *GDNF-AS1* allele frequency distribution of Alzheimer's type dementia and control groups

GDNF-AS1 allele frequency	Alzheimer's type Dementia group	Control group	Df	P value	X^2
A	95%	95.72%	1	0.81	0.05
C	5%	4.28%			
A	88.57%	89.29%	1	0.87	0.02
C	11.43%	10.71%			

Discussion

Alzheimer's type dementia is observed at a relatively high rate in women [19]. In our study, similar to the literature, 58.7% of individuals in our Alzheimer's-type dementia group were female.

GDNF plays a crucial role in the formation of synapses and in the development of neuronal plasticity. It is distributed widely throughout the CNS, spanning regions such as the cortex, hippocampus, cerebellum, striatum, hypothalamus, midbrain, and spinal cord [20,21]. Moreover, in vivo studies have demonstrated that recombinant *GDNF* protects motor neurons from undergoing cell death, a process termed apoptosis [21]. During the periods of embryonic and postnatal development, *GDNF* is required for the survival of motor neurons, which are

DOI: 10.5455/medscience.2025.02.040

prone to extensive branching.However, *GDNF* is downregulated in the substantia nigra and putamen in human Parkinson's disease [22-24]. The regulation of *GDNF* in AD is less documented [25]. A reduction in this neurotrophic factor has been linked to the symptoms and disease processes of AD, and it may represent a therapeutic avenue for preventing neurodegeneration [25]. Ghribi et al. (2001) demonstrated notable neuroprotection in a model of AD in rabbits by upregulating bcl-XL and eliminating caspase-3 activity through the administration of *GDNF*, countering aluminium-induced apoptosis [26]. In addition, there are reports indicating that *GDNF* offers protection to both neurons and glial cells against toxicity and oxidative stress [27]. In another study, it was reported that *GDNF* decreased in the plasma while it increased in the CSF of patients with EOAD [28]. Similar results were reported by Straten et al. (2011), who found a decrease in serum *GDNF* in AD patients and an increase in CSF [29]. A further study reported that individuals with mild cognitive impairment and moderate AD displayed downregulated expression of *NGF* and *GDNF* [30]. The downregulation of *GDNF* has been postulated as a causative factor in the disruption of glutamate transporter (GLT-1) regulation, which is responsible for the clearance of glutamate from the synaptic cleft. This has been suggested to result in excessive glutamate release and excitotoxicity, which precedes dopaminergic degeneration [31].

There is a strong link between dopaminergic degeneration and AD. Pharmacological strategies focused on boosting the dopaminergic system have improved synaptic dysfunction and memory impairment in patients [32]. Interestingly, in hypermorphic *GDNF* mice (*GDNF* wt/hyper), the upregulation of endogenous *GDNF* suppressed the gradual decline in cholinergic transmission, one of the distinguishing features of dementia. These results indicate that the overexpression of *GDNF* effectively prevents cognitive decline and memory impairment in AD [33]. Although studies have demonstrated a connection between AD and *GDNF*, only one study [18] has related AD to the antisense gene *GDNF-AS1*, which regulates the *GDNF* gene. Therefore, this study aimed to investigate the link between AD and *GDNF-AS1*. In the literature, the only study that has investigated the relationship between *GDNF-AS1* and AD is the one conducted by Airavaara et al. (2011). In this study, they identified new *GDNF* isoforms, including *GDNF-AS1*, and reported that both mature *GDNF* peptide and *GDNF* isoforms were significantly downregulated in the postmortem brains of AD patients [18].

In the literature, in addition to AD, a novel association between narcolepsy and the *GDNF-AS1* (rs62360233) polymorphism was identified in a study on pandemrix-induced narcolepsy [34]. Furthermore, in a different study, a GWAS investigating whether SNPs are associated with chronic mucus hypersecretion in smokers with and without chronic obstructive pulmonary disease (COPD) found a significant association between *GDNF-AS1* (rs10461985) polymorphism and COPD patients [35].

In our study, contrary to the literature, a connection between *GDNF-AS1* polymorphisms and AD could not be identified. This may be due to the inadequacy of our sample size. Our study should be supported by additional research with a larger sample size and the inclusion of other *GDNF-AS1* polymorphisms along with *GDNF-AS1* gene expression studies.

The limitations of our study include the difficulty we faced in collecting cases, resulting in a small sample size. Additionally, further functional studies, such as mRNA expression analysis, epigenetic studies, and protein interaction assays, are needed to better understand the biological role of *GDNF-AS1* in neurodegeneration.

Conclusion

The identification of peripheral biomarkers for AD remains a crucial research goal. Given the regulatory role of lncRNAs in neurodegeneration, *GDNF-AS1* may serve as a promising biomarker for AD diagnosis and progression. Future research should employ larger sample sizes and explore additional polymorphisms, along with functional studies on *GDNF-AS1* expression.

Conflict of Interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

The authors declare that they have received no financial support for the study.

Ethical Approval

The ethical approval for this study was obtained from the Non-Interventional Ethics Committee (#2023/173, date: 16.11.2023) of Faculty of Medicine in Aydın Adnan Menderes University.

References

1. Khan SS, Khatik GL, Datusalia AK. Strategies for treatment of disease-associated dementia beyond Alzheimer's disease: an update. Curr Neuropharmacol. 2023;21:309-39.
2. Gao S, Burney HN, Callahan CM, et al. Incidence of dementia and Alzheimer disease over time: a meta-analysis. J Am Geriatr Soc. 2019;67:1361-9.
3. Scheltens P, De Strooper B, Kivipelto M, et al. Alzheimer's disease. Lancet. 2021;397:1577-90.
4. Falgàs N, Allen IE, Spina S, et al. The severity of neuropsychiatric symptoms is higher in early-onset than late-onset Alzheimer's disease. Eur J Neurol. 2022;29:957-67.
5. Tort-Merino A, Falgas N, Allen IE, et al. Early-onset Alzheimer's disease shows a distinct neuropsychological profile and more aggressive trajectories of cognitive decline than late-onset. Ann Clin Transl Neurol. 2022;9:1962-73.
6. Rezazadeh M, Hosseinzadeh H, Moradi M, et al. Genetic discoveries and advances in late-onset Alzheimer's disease. J Cell Physiol. 2019;234:16873-84.
7. Brickell KL, Steinbart EJ, Rumbaugh M, et al. Early-onset Alzheimer disease in families with late-onset Alzheimer disease: a potential important subtype of familial Alzheimer disease. Arch Neurol. 2006;63:1307-11
8. Uddin MS, Hasana S, Hossain MF,et al. Molecular genetics of early- and late-onset Alzheimer's disease. Curr Gene Ther. 2021;21:43-52.

DOI: 10.5455/medscience.2025.02.040

9. Bird TD. Genetic aspects of Alzheimer disease. Genet Med. 2008;10:231-9.
10. Campion D, Dumanchin C, Hannequin D, et al. Early-onset autosomal dominant Alzheimer disease: prevalence, genetic heterogeneity, and mutation spectrum. Am J Hum Genet. 1999;65:664-70.
11. Roses AD. On the discovery of the genetic association of apolipoprotein E genotypes and common late-onset Alzheimer disease. J Alzheimers Dis. 2006;9:361-6.
12. Kamboh MI. Molecular genetics of late-onset Alzheimer's disease. Ann Hum Genet. 2004;68:381-404.
13. Hidalgo-Figueroa M, Bonilla S, Gutierrez F, et al. GDNF is predominantly expressed in the PV+ neostriatal interneuronal ensemble in normal mouse and after injury of the nigrostriatal pathway. J Neurosci. 2012;32:864-72.
14. Pochon NA, Menoud A, Tseng JL, et al. Neuronal GDNF expression in the adult rat nervous system identified by in situ hybridization. Eur J Neurosci. 1997;9:463-71.
15. Trupp M, Belluardo N, Funakoshi H, et al. Complementary and overlapping expression of glial cell line-derived neurotrophic factor (GDNF), c-ret proto-oncogene, and GDNF receptor-α indicates multiple mechanisms of trophic actions in the adult rat CNS. J Neurosci. 1997;17:3554-67.
16. Nosrat CA, Tomac A, Lindqvist E, et al. Cellular expression of GDNF mRNA suggests multiple functions inside and outside the nervous system. Cell Tissue Res. 1996;286:191-207.
17. Golden JP, DeMaro JA, Osborne PA, Expression of neurturin, GDNF, and GDNF family-receptor mRNA in the developing and mature mouse. Exp Neurol. 1999;158:504-28.
18. Airavaara M, Pletnikova O, Doyle ME, et al. Identification of novel GDNF isoforms and cis-antisense GDNFOS gene and their regulation in human middle temporal gyrus of Alzheimer disease. J Biol Chem. 2011;286:45093-102.
19. Alzheimer's Association report. 2017 Alzheimer's disease facts and figures. Alzheimer's & Dementia. 2017;13:325-73.
20. Ledda F, Paratcha G, Sandoval-Guzman T, et al. GDNF and GFRalpha1 promote formation of neuronal synapses by ligand-induced cell adhesion. Nat Neurosci. 2007;10:293-300.
21. Duarte Azevedo M, Sander S, Tenenbaum L. GDNF a neuron-derived factor upregulated in glial cells during disease. J Clin Med. 2020;9:456.
22. Mahato AK. Targeting GDNF receptors with small molecules for the treatment of Parkinson's disease. Doctoral dissertation, University of Helsinki. Finland, 2022.
23. Chauhan NB, Siegel GJ, Lee JM. Depletion of glial cell line-derived neurotrophic factor in substantia nigra neurons of Parkinson's disease brain. J Chem Neuroanat. 2001;21:277-88.
24. Hunot S, Bernard V, Faucheux B, et al. Glial cell line-derived neurotrophic factor (GDNF) gene expression in the human brain: a post mortem in situ hybridization study with special reference to Parkinson's disease. J Neural Transm. 1996;103:1043-52.
25. Siegel GJ, Chauhan NB. Neurotrophic factors in Alzheimer's and Parkinson's disease brain. Brain Res Rev. 2000;33:199-227.
26. Ghribi O, Herman MM, Forbes MS, et al. GDNF protects against aluminum-induced apoptosis in rabbits by upregulating Bcl-2 and Bcl-XL and inhibiting mitochondrial Bax translocation. Neurobiol Dis. 2001;8:764-73.
27. Cheng H, Fu YS, Guo JW. Ability of GDNF to diminish free radical production leads to protection against kainate-induced excitotoxicity in hippocampus. Hippocampus. 2004;14:77-86.
28. Marksteiner J, Kemmler G, Weiss EM, et al. Five out of 16 plasma signaling proteins are enhanced in plasma of patients with mild cognitive impairment and Alzheimer's disease. Neurobiol Aging. 2011;32:539-40.
29. Straten G, Saur R, Laske C, et al. Influence of lithium treatment on GDNF serum and CSF concentrations in patients with early Alzheimer's disease. Curr Alzheimer Res. 2011;8:853-9.
30. Forlenza OV, Miranda AS, Guimarães I, et al. Decreased neurotrophic support is associated with cognitive decline in non-demented subjects. J Alzheimers Dis. 2015;46:423-9.
31. Farrand AQ, Gregory RA, Scofield MD, et al. Effects of aging on glutamate neurotransmission in the substantia nigra of GDNF heterozygous mice. Neurobiol Aging. 2015;36:1569-76.
32. Krashia P, Nobili A, D'Amelio M. Unifying hypothesis of dopamine neuron loss in neurodegenerative diseases: focusing on Alzheimer's disease. Front Mol Neurosci. 2019;12:123.
33. Mitra S, Turconi G, Darreh-Shori T, et al. Increased endogenous GDNF in mice protects against age-related decline in neuronal cholinergic markers. Front Aging Neurosci. 2021;13:714186.
34. Hallberg P, Smedje H, Eriksson N, et al. Pandemrix-induced narcolepsy is associated with genes related to immunity and neuronal survival. EBioMedicine. 2019;40:595-604.
35. Dijkstra AE, Boezen HM, Van Den Berge M, et al; Lifelines Cohort Study Group. Dissecting the genetics of chronic mucus hypersecretion in smokers with and without COPD. Eur Respir J. 2015;45:60-75.