

Tumor necrosis factor alpha gene rs1799724 polymorphism in Alzheimer's disease

Belkis Atasever-Arslan^{1,2}, Yagmur Ozer³, Fatih Ozen⁴, Yunus Diler⁵ and Ayse D. Yalcin⁵

¹ Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Üsküdar University, Istanbul, Turkey

² Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago Illinois, USA

³ Neuroscience Program, Health Sciences Institute, Uskudar University, Istanbul, Turkey

⁴ Department of Bioengineering, Faculty of Engineering and Natural Sciences, Uskudar University, Istanbul, Turkey

⁵ Neurology Clinic, Ümraniye Research and Training Hospital, Istanbul, Turkey

Abstract. Neurofibrillar tangles formed by the accumulation of hyperphosphorylated tau proteins in the intracellular space and the senile plaques formed by amyloid β ($A\beta$) accumulating in extracellular environment are shown as two main elements of Alzheimer's disease (AD). In our study, the relationship between the risk of Alzheimer's disease and *TNF α* rs1799724 polymorphism in the Turkish population was investigated. Our study is the first report investigating the relationship between the risk of Alzheimer's disease and *TNF α* rs1799724 gene polymorphism in Turkish population. No significant relation was found for rs1799724 polymorphism in AD patients. Since *TNF α* rs1799724 gene polymorphism was also associated with type 2 diabetes mellitus (T2DM), the polymorphism also was evaluated in T2DM within the AD patients group. According to our results rs1799724 polymorphism was found to be a significant relationship with T2DM within AD patients group. On the other hand, there was no significant difference between fasting blood glucose values of AD patients and -857C>T (rs1799724) polymorphism. According to our results, -857C>T rs1799724 polymorphism may have a relationship with T2DM as independent from AD.

Key words: *TNF α* — rs1799724 — Alzheimer's disease — T2DM

Introduction

Neurodegenerative diseases are hereditary or age-related diseases associated with many pathological changes in the brain, including extracellular and intracellular protein deposits (Quinlan et al. 2017). Neurofibrillar tangles formed by the accumulation of hyperphosphorylated tau proteins in the intracellular space and the senile plaques formed by amyloid β ($A\beta$) accumulating in extracellular environment

are shown as two main elements of Alzheimer's disease (AD) (Hardy and Selkoe 2002; Uzunoglu and Atasever Arslan 2019). However, the mechanistic link between these two hallmarks as well as the role of $A\beta$ plaques in the pathobiology of AD remains elusive (Ovsepian et al. 2019).

Inflammation is an immunological defense reaction to various foreign pathogens and systemic injuries. Not only activation of leukocytes, but also many inflammatory mediators secreted by various active cells play a role in this process (Arslan et al. 2019). Inhibition of peripheral *TNF α* synthesis by the efferent vagus nerve implicates a mechanism for signaling from the vagus nerve to *TNF α* producing cells. Nicotinic acetylcholine receptor alpha 7 subunit is an essential regulator of inflammation and is required for acetylcholine inhibition of macrophage *TNF α* release (Pavlov et al. 2003; Wang et al. 2003). Acetylcholine and cholinergic innervations play a role in regulation of amyloid precursor protein (APP) processing with production of $A\beta$ and other

Correspondence to: Belkis Atasever-Arslan, Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Uskudar University, Istanbul, Turkey
E-mail: belkisatasever.arslan@uskudar.edu.tr

Belkis Atasever-Arslan, Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago Illinois, United States of America
E-mail: batase2@uic.edu

APP fragments and control of the phosphorylation of microtubule-associated protein (MAP) tau (Ovsepian et al. 2016).

Regarding the apolipoprotein E epsilon-4 allele (APOE- ϵ 4), inheritance of the rs1799724-T allele of the *tumor necrosis factor α* (*TNF α*) polymorphism, a proinflammatory cytokine, has been shown to increase the risk of AD in APOE- ϵ 4 carriers (Albani et al. 2011). On the other hand, Pšemeneckienė et al. (2019) found that there is no a significant relationship rs1799724-T polymorphism with APOE ϵ 4 carriers in Lithuanian population. Also, it was demonstrated that there is higher *TNF α* expression in *TNF α* SNP-857T allele carriers (Kimura et al. 2016; Oki et al. 2017).

Overproduction of inflammatory factors can contribute to age-related diseases such as neurodegenerative disorders, autoimmunity and cancer (Caruso et al. 2004; Van Bodegom et al. 2007; Albani et al. 2012). Therefore, considering the effect of rs1799724-T on *TNF α* expression, it suggests that more detailed scans should be studied on this issue.

Diabetes mellitus (DM) is a chronic carbohydrate, fat and protein metabolism disorder characterized by high glucose levels in the blood due to insulin secretion or insulin insensitivity from β cells (Vardi et al. 2003; Altinoz et al. 2015). Several studies have shown that Type 2 DM (T2DM) is associated with increases in acute phase proteins such as C-reactive protein (CRP), fibrinogen, plasminogen activator inhibitor, cytokines, and chemokines (Pickup et al. 1997; Spranger et al. 2003; Herder et al. 2009; Salcini et al. 2016).

In our study, the relationship between the risk of Alzheimer's disease and *TNF α* rs1799724 gene polymorphism in the Turkish population was investigated.

Materials and Methods

Participants

Twenty-one patients with Alzheimer's disease, who were diagnosed as Alzheimer's disease according to NINCDS-ADRDA criteria applied to the Umraniye Education and Research Hospital, Department of Neurology, Istanbul.

Fifteen control participants who were admitted to the same hospital without any neurological, oncological and rheumatologic disease were included in this study. The study methods conformed to the ethical guidelines of the Declaration of Helsinki and were approved by the Umraniye Education and Research Hospital Ethics Committee and all patients and controls gave written informed consent to participate in the study.

Genotyping

The genotyping of *TNF α* gene rs1799724 polymorphism were performed by using TIB MolBiol LightCycler® FastStart DNA Master HybProbe PCR kit, according to the manufacturer's instructions.

Statistical evaluation

IBM Statistical Product and Service Solutions (SPSS) Statistic 23 program was used for statistical analysis of results. T-test was used to compare the ages of the patients and controls. The relationship between genotype and allele distribution of *TNF α* rs1799724 polymorphism was obtained using Fisher's Exact Test. Compliance of patient and control groups to the Hardy-Weinberg Balance (HWE) test Michael H. Court's (2005–2008) Excel-based HWE online calculator was used. Logistic regression analysis of *TNF α* polymorphism in AD patients and controls was calculated from binary logistic regression. All *p* values < 0.05 were considered significant. The mean of fasting blood glucose levels of AD patients and their *TNF α* rs1799724 genotypes were compared by Kruskal-Wallis test.

Results

The mean ages of Alzheimer patients and controls were 71.95 ± 6.05 and 67.07 ± 9.02 , respectively. According to the *t*-test results, *p* value was calculated as 0.600 and there was no statistically significant difference between the patient and control groups as age.

Number of AD patients was 9 women and 12 men; control group consisted of 8 female and 7 men individuals. There was no a statistical difference between the patient and controls. The *p* value was found to be 0.535 for gender distribution in the study. The results were shown in Table 1.

The suitability and adequacy of controls and AD patients were tested by the Hardy-Weinberg Balance Test (HWE). Values of Hardy-Weinberg X^2 for AD patients was 2.378 (*p* = 0.0979) and for control 0.150 (*p* = 0.6985). According to the calculated results, genotype distribution in both patients and controls was in Hardy-Weinberg balance (*p* > 0.05). The distribution of genotype and allele frequencies

Table 1. Distribution of AD patients and Control group by sex

	AD patients <i>n</i> (%)	Control <i>n</i> (%)
Female	9 (57.1)	8 (53.3)
Male	12 (42.9)	7 (46.7)
Total	21	15

chi-square (X^2) test; *p* = 0.535 male vs. female.

Table 2. Distribution of the TNF α rs1799724 genotypes and alleles in AD patients and Control group

	AD patients <i>n</i> (%)	Control <i>n</i> (%)	<i>p</i> *
<i>Genotype</i>			
CC	5 (23.8)	7 (46.7)	0.304
CT	14 (66.7)	6 (40)	
TT	2 (9.5)	2 (13.3)	
<i>Allele</i>			
C	24 (57.1)	20 (66.7)	0.469
T	18 (42.9)	10 (33.3)	

* Fisher's Exact Test.

of the genotyping study related to rs1799724 C/T polymorphism in the TNF α gene is shown in Table 2. Genotype frequencies of TNF α gene rs1799724 C/T for CC, CT and TT were compared statistically by using Fisher Exact test, there was no significant difference between the control and patient groups. When C and T allele frequencies were statistically compared using the Fisher Exact test, there was no significant difference between the control and patient groups ($p > 0.05$) (Table 2).

Multivariate logistic regression analysis was performed to evaluate effect of TNF α gene rs1799724 on Alzheimer's disease. Dominant, recessive and additive models of rs1799724 were analyzed. In dominant model, AD patients with variant homozygous genotype (TT) and with heterozygous genotype (CT) were included in the same group and compared to wild genotype (CC) group. According to the model, there was no a significant difference between TT+CT genotypes and CC genotype for AD patients in the study ($p = 0.133$; OR = 2.975; 95%CI = 0.717–12.338) (Table 3).

On the other hand, when the patients with homozygous wild genotype CC and with heterozygous genotype CT were included in the same group and compared with individuals with variant homozygous genotype TT, there was no any significant difference. According to the results, it was shown that TT genotype did not increase AD risk ($p = 0.721$; OR = 1.462; 95%CI = 0.182–11.735).

In additive model, AD patients with variant homozygous genotype TT and with homozygous wild genotype CC were

Table 3. Logistic regression analysis of TNF α rs1799724 polymorphism in AD patients and Control group

Model	OR	95% CI	<i>p</i> *
Dominant	2.975	(0.717–12.338)	0.133
Recessive	1.462	(0.182–11.735)	0.721
Additive	1.400	(0.144–13.568)	0.772

* value calculated from binary logistic regression.

Table 4. Comparison of TNF α rs1799724 genotype with the presence of T2DM in AD patients

T2DM	Genotype <i>n</i> (%)			<i>p</i> *
	CC	CT	TT	
+	0 (100)	7 (50)	2 (100)	0.031
-	5 (0)	7 (50)	0 (0)	

* Fisher's Exact test.

compared each other. It was found that T allele did not increase AD risk in the study ($p = 0,772$ OR = 1.400 %95CI = 0.144–13.568).

To understand whether there is a relationship between patients with Alzheimer's disease and with Type 2 DM, their distributions of TNF α rs1799724 genotypes were analyzed by using Fisher's Exact test. It was shown that there is a significant relationship between Type 2 DM and TNF α gene rs1799724 ($p < 0.05$) (Table 4).

The mean of fasting blood glucose levels of AD patients and their TNF α rs1799724 genotypes were compared by Kruskal-Wallis test. Although fasting blood glucose values of AD patients with variant homozygous genotype TT (18.50 mg/dl; $n = 2$) were higher than genotypes CC (8.20 mg/dl; $n = 5$) and CT (10.93 mg/dl; $n = 14$), the difference was not statistically significant ($p > 0.05$).

Discussion

The study is the first report investigating the relationship between the risk of Alzheimer's disease and TNF α rs1799724 gene polymorphism in the Turkish population. Albani et al. (2011) found that the inheritance of the rs1799724-T allele of TNF α polymorphism increased the risk of AD in APOE- ϵ 4 carriers. In addition, it was demonstrated that there was no significant relationship with rs1799724-T polymorphism in APOE ϵ 4 carriers in Lithuanian population (Pšemenekienė et al. 2019). According to the studies, TNF α rs1799724 polymorphism is not independent but it can act on APOE- ϵ 4 allele carriers or it is ineffective. No significant relation was found for rs1799724 polymorphism in Turkish population in our study.

Since TNF α rs1799724 gene polymorphism was also associated with T2DM, the polymorphism also was evaluated in T2DM within the AD patients group. According to our results rs1799724 polymorphism was found to be a significant relationship with T2DM within AD patients group. On the other hand, there was no significant difference between fasting blood glucose values of AD patients and -857C>T (rs1799724) polymorphism.

In Japanese population, -857C>T (rs1799724) polymorphism is associated with insulin resistance, T2DM, and

carotid plaque formation in T2DM patients (Kamizono et al. 2000; Buraczynska et al. 2004; Wang et al. 2005; Yamashina et al. 2007; Ohara et al. 2012). However, Sesti et al. (2015) found an independent association of the 308G>A polymorphism in the *TNFA* gene with proliferative diabetic retinopathy PDR in Caucasian Brazilians with T2DM, not -857C>T (rs1799724) polymorphism. According to the results, the relation of T2DM and -857C>T (rs1799724) polymorphism is controversial.

There were some limitations in the study. These are the sample size and also TNF α plasma levels could not be measured in plasma in the study group. According to our results, -857C>T rs1799724 polymorphism may have a relationship with T2DM as independent from AD. Although there is no significant relationship between AD and -857C>T (rs1799724), investigation of genetic variants of TNF α and other proinflammatory cytokines may contribute to understanding underlying mechanisms of neuroinflammation in the disease.

Conflict of interest. The authors have no conflict of interest.

References

- Albani D, Tettamanti M, Batelli S, Polito L, Dusi S, Ateri E, Forloni G, Lucca U (2012): Interleukin-1 α , interleukin-1 β and Tumor necrosis factor- α genetic variants and risk of dementia in the very old: Evidence from the „Monzino 80-plus“ prospective study. *Age* **34**, 519-526
<https://doi.org/10.1007/s11357-011-9249-x>
- Altinoz E, Taskin E, Oner Z, Elbe H, Atasever Arslan B (2015): The effect of saffron (its active constituent, crocin) on the cardiovascular complication and dyslipidemia in streptozotocin induced diabetic rats. *African Journal of Traditional, Complementary and Alternative Medicines* **12**, 1-7
<https://doi.org/10.4314/ajtcam.v12i5.1>
- Arslan BA, Ozen F, Catal T, Akalin E (2019): Resin extract obtained from cilician fir (*Abies Cilicica*) inhibits glucose dependent inflammation in vitro. *J. Exp. Ther. Oncol.* **13**, 23-31
- Buraczynska K, Koziol-Montewka M, Majdan M, Tokarz A, Ksiazek A (2004): Genetic determination of TNF and myeloperoxidase production in dialyzed patients with diabetic nephropathy. *Ren. Fail.* **26**, 633-639
<https://doi.org/10.1081/JDI-200037165>
- Caruso C, Lio D, Cavallone L, Franceschi C (2004): Aging, longevity, inflammation, and cancer. *Ann. N. Y. Acad. Sci.* **1028**, 1-13
<https://doi.org/10.1196/annals.1322.001>
- Hardy J, Selkoe DJ (2002): The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297**, 353-356
<https://doi.org/10.1126/science.1072994>
- Herder C, Brunner EJ, Rathmann W, Strassburger K, Tabak AG, Schloot NC, Witte DR (2009): Elevated levels of the anti-inflammatory interleukin-1 receptor antagonist precede the onset of type 2 diabetes: the Whitehall II study. *Diabetes Care* **32**, 421-423
<https://doi.org/10.2337/dc08-1161>
- Kamizono S, Yamada K, Seki N, Higuchi T, Kimura A, Nonaka K, Itoh K (2000): Susceptible locus for obese type 2 diabetes mellitus in the 50-flanking region of the tumor necrosis factor- α gene. *Tissue Antigens* **55**, 449-452
<https://doi.org/10.1034/j.1399-0039.2000.550508.x>
- Kimura K, Takayanagi R, Yokoyama H, Yamada Y (2016): Effects of tumor necrosis factor α -857C/T Polymorphism on the expression of tumor necrosis factor α . *APMIS* **124**, 669-674
<https://doi.org/10.1111/apm.12559>
- Ohara M, Maesawa C, Takebe N, Takahashi T, Yamashina M, Ono M, Matsui M, Sasai T, Honma H, Nagasawa K, et al. (2012): Different susceptibility to insulin resistance and fatty liver depending on the combination of TNF- α C-857T and adiponectin Gp276T gene polymorphisms in Japanese subjects with type 2 diabetes. *Tohoku J. Exp. Med.* **226**, 161-169
<https://doi.org/10.1620/tjem.226.161>
- Oki E, Norde MN, Carioca AAF, Souza JMP, Castro IA, Marchioni DML, Fisberg RM, Rogero MM (2017): Polymorphisms of the TNF- α gene interact with plasma fatty acids on inflammatory biomarker profile: A population-based, cross-sectional study in São Paulo, Brazil. *Br. J. Nutr.* **117**, 1663-1673
<https://doi.org/10.1017/S0007114517001416>
- Ovsepian SV, O'Leary VB, Zaborszky L (2016): Cholinergic mechanisms in the cerebral cortex: beyond synaptic transmission. *Neuroscientist* **22**, 238-251
<https://doi.org/10.1177/1073858415588264>
- Ovsepian SV, O'Leary VB, Zaborszky L, Ntzachristos V, Dolly JO (2019): Amyloid plaques of Alzheimer's disease as hotspots of glutamatergic activity. *Neuroscientist* **25**, 288-297
<https://doi.org/10.1177/1073858418791128>
- Pavlov VA, Wang H, Czura CJ, Friedman SG, Tracey KJ (2003): The cholinergic anti-inflammatory pathway: a missing link in neuroimmunomodulation. *Mol. Med.* **9**, 125-134
<https://doi.org/10.1007/BF03402177>
- Pšemeneckienė G, Petrikonis K, Rastenytė AD (2019): Polymorphisms of proinflammatory cytokines in relation to APOE epsilon 4 and risk of Alzheimer's disease in the Lithuanian population. *Medicina (Kaunas)* **55**, 689
<https://doi.org/10.3390/medicina55100689>
- Pickup JC, Mattock MB, Chusney GD, Burt D (1997): NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. *Diabetologia* **40**, 1286-1292
<https://doi.org/10.1007/s001250050822>
- Quinlan S, Kenny A, Medina M, Engel T, Jimenez-Mateos EM (2017): MicroRNAs in neurodegenerative diseases. *Int. Rev. Cell Mol. Biol.* **334**, 309-343
<https://doi.org/10.1016/bs.ircmb.2017.04.002>
- Salcini C, Atasever-Arslan B, Sunter G, Gur H, Isik FB, Saylan CC, Yalcin AD (2016): High plasma pentraxin 3 levels in diabetic polyneuropathy patients with nociceptive pain. *Tohoku J. Exp. Med.* **239**, 73-79
<https://doi.org/10.1620/tjem.239.73>
- Sesti LF, Crispim D, Canani LH, Polina ER, Rheinheimer J, Carvalho PS, Gross JL, Santos KG (2015): The -308G>a polymor-

- phism of the TNF gene is associated with proliferative diabetic retinopathy in Caucasian Brazilians with type 2 diabetes. *Invest. Ophthalmol. Vis. Sci.* **56**, 1184-1190
<https://doi.org/10.1167/iops.14-15758>
- Spranger J, Kroke A, Mohlig M, Hoffmann K, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF (2003): Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study. *Diabetes* **52**, 812-817
<https://doi.org/10.2337/diabetes.52.3.812>
- Uzunoglu T, Atasever Arslan B (2019): Possible microRNAs participating in Alzheimer's pathology via signaling pathways affecting axonal transport. *J. Neuro. Behav. Sci.* **6**, 54-61 (in Turkish)
- Van Bodegom D, May L, Meij HJ, Westendorp RG (2007): Regulation of human life histories: the role of the inflammatory host response. *Ann. N. Y. Acad. Sci.* **1100**, 84-97
<https://doi.org/10.1196/annals.1395.007>
- Vardi N, Muharrem U, Oztürk F (2003): Morphological changes of rat endocrine pancreas in experimental diabetes. *Turkiye Klinikleri J. Med. Sci.* **23**, 27-32 (in Turkish)
- Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, Li JH, Wang H, Yang H, Ulloa L, et al. (2003): Nicotinic acetylcholine receptor $\alpha 7$ subunit is an essential regulator of inflammation. *Nature* **421**, 384-388
<https://doi.org/10.1038/nature01339>
- Wang Y, Ng MC, So WY, Ma R, Ko GT, Tong PC, Chan JC (2005): Association between tumour necrosis factor- α G-308A polymorphism and risk of nephropathy in obese Chinese type 2 diabetic patients. *Nephrol. Dial. Transplant.* **20**, 2733-2738
<https://doi.org/10.1093/ndt/gfi101>
- Yamashina M, Kaneko Y, Maesawa C, Kajiwara T, Ishii M, Fujiwara F, Taneichi H, Takebe N, Ishida W, Takahashi K, et al. (2007): Association of TNF α gene promoter C-857T polymorphism with higher serum LDL cholesterol levels and carotid plaque formation in Japanese patients with type 2 diabetes. *Tohoku J. Exp. Med.* **211**, 251-258
<https://doi.org/10.1620/tjem.211.251>

Received: May 17, 2020

Final version accepted: August 21, 2020