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Association of Alzheimer's Disease with Promoter Variations in NPY2R Gene

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Abstract

Background: Alzheimer's disease (AD) is a widespread neurodegenerative disorder among the elderly, characterized by dementia. The development of AD is significantly influenced by genetic risk factors.
Methods: in this study, we have investigated the impact of rs2234759 and rs12507396 polymorphisms in the neuropeptide Y receptor Y2 (NPY2R) gene on AD. Nineteen AD patients and nineteen healthy controls were enlisted in our research. and the DNA samples of all participants were genotyped using amplification refractory mutation system-polymerase chain reaction (ARMS-PCR).
Results: The results revealed a noteworthy association between rs2234759 and AD, with a noticeable difference observed in the frequency of genotypes and alleles of this polymorphism between patients and healthy controls ($P< 0.001$ for both). However, no significant difference was detected in the genotype distribution concerning the rs12507396 polymorphism between the two groups.
Conclusion: Our findings provide compelling evidence of an association between the rs2234759 polymorphism in NPY2R and Alzheimer's disease. Given the significant role of NPY2R in brain tissue, this particular polymorphism may result in strengthened presynaptic inhibition of glutamate release.

Keywords: Alzheimer's Disease, Genes, NPY2 receptor, Single Nucleotide Polymorphism.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline and memory impairment due to the death of brain neurons (1, 2). As a progressive condition, AD's symptoms develop gradually over the years, eventually becoming more severe. It impacts various brain functions, with memory impairment being the most common manifestation. Additionally, patients with AD may experience illusions, delusions, pessimism, depression, confusion, and speech loss (3-5).

In AD, abnormal levels of beta-amyloid (Aβ) peptide led to the formation of amyloid plaques. Overexpression of amyloid precursor protein (APP) affects Bcl- 2 mediated pro-apoptotic pathways in neuronal cells, promoting neuronal survival during the preliminary stage of Alzheimer's disease (6). Studies indicate that age and genetic factors are the primary risk factors influencing the progression of AD, with an elevated rate of micronuclei originating from chromosome 21 observed in these patients (7).

Alzheimer's disease (AD) is typically categorized into two groups based on the age of onset: a) early-onset AD, which is a rare form of dementia affecting individuals younger than 65 years old. Early-onset AD follows an autosomal dominant inheritance pattern and has been associated with three genes (APP, PSEN 1, and PSEN 2)

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responsible for autosomal dominant familial AD. Collectively, these three genes account for 60%-70% of early-onset AD cases and less than 1% of all AD patients (8, 9). b) late-onset AD, the most common form of AD, affects people aged 65 and older. While a particular gene has not been identified as the sole cause of late-onset AD, genetic factors are believed to play a role in its development. Some studies have linked an increased risk of developing late-onset AD to the apolipoprotein E (APOE) gene found on chromosome 19 (10, 11).

The hippocampus represents one of the earliest brain regions affected by AD (12). Studies indicate a reduction in the concentration of the glutamate neurotransmitter in the hippocampus of individuals with AD (13). In this context, the neuropeptide Y (NPY) system assumes a role in neuron protection and modulation of the glutamatergic system (14, 15). The human neuropeptide Y receptor type 2 (Y2), encoded by the NPY2R gene (Gene ID: 4887), spans 8.5 kilobase pairs and is located on chromosome 4 (4q31) (16). The resulting receptor, encoded by the NPY2R gene, comprises 381 amino acids with a mass of 42 kDa, and possesses 7 transmembrane domains typical of G-Protein coupled receptors (17). NPY2R is most commonly found on neuropeptide Y neurons in the hypothalamus and other brain regions, including the hippocampus (18, 19). Hence, variations in the NPY2R sequence may be linked to AD.

The NPY gene exhibits extensive expression in brain tissue, exerting its effects through five receptors: Y1, Y2, Y4, Y5, and Y6. In the human brain, the Y1, Y2, and Y5 subtypes are prevalent. These subtypes of NPY receptors are most commonly localized on neuropeptide Y neurons in the hypothalamus and other brain regions, such as the hippocampus (20, 21).

The rs2234759 and rs12507396 polymorphisms represent main variants of NPY2R, These polymorphisms are located at the promoter and rs2234759 influences the expression of the gene (22).

Materials and Methods

Subjects

Peripheral blood samples were collected from 90 patients diagnosed with AD and 90 healthy controls without any cognitive impairment or known relatives with AD. The patients' mean age was 73.81 years (±8.36), while the healthy controls had an average age of 72.06 years (±8.65) (Table 1).

The study received ethical approval from the Medical University of Kerman Ethics Committee, and the study objectives were thoroughly explained to all participants, who provided informed consent. All participating subjects completed a comprehensive questionnaire, which recorded disease-related data, MMSE score, as well as other essential details such as age, age of onset, and sex.

Table 1. Distribution of age and sex in patients and healthy controls.

Factor	Patients (n=90)	Healthy controls (n=90)	p-value
Age (means*±SD*)	8.36±73.81	8.65±72.06	0.171
Sex (M, F)	57(63%), 33(37%)	44(48%), 46(52%)	0.097

DNA extraction

DNA was isolated using the standard salting-out method. The quantity and quality of isolated DNA were assessed using a Nanodrop spectrophotometer and regarding the 260 nm/280 nm ratio.

Genotyping

The genotypes of rs2234759 A/G and rs12507396 A/T polymorphisms were determined through the amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) with the following

primers: For rs2234759 polymorphism - Reverse primer: CAGTCCGTCTGTCCGATGATTA, for A allele: GTGAAGTCGGCCTCAAGTCCA, for G allele: GTGAAGTCGGCCTCAAGTCAG. For rs12507396 polymorphism - Reverse primer: GAATCTCTCCCTTTGCTCTCTA, for A allele: CTATCCCTATCCTAGCTTTTAA, for T allele: CTATCCCTATCCTAGCTTTAAT.

The PCR cycling for rs2237459 and rs12507396 was performed in three steps: a) initial denaturation at 95 °C for 5 min, b) 37 cycles of 95 °C for 1 minute, 64°C for 1 minute, and 72 °C for 1 minute, c) final incubation at 95 °C for 10 min. The PCR products were then electrophoresed on 2% agarose gels.

Additionally, direct Sanger sequencing of 6 samples for each polymorphism was performed using an ABI automated DNA sequencer (model: 3730XL).

Statistical analysis

Statistical analysis was performed using SPSS version 20. The Chi-square test was utilized to compare expected genotype frequencies with those observed under Hardy-Weinberg equilibrium. Furthermore, the Chi-square test and T-test were employed to evaluate the frequency distribution of alleles, genotypes, age, and sex between the patient and control groups. Logistic regression was used to calculate odds ratios and confidence intervals (95%), and to predict the impact of rs2234759 and rs12507396 polymorphisms on AD susceptibility. A significance level of $P< 0.05$ was considered statistically significant.

Results

Demographic Characteristics

The demographic characteristics of the study participants are summarized in Table 1. There were no statistically significant differences in age ($p = 0.171$) or sex distribution ($p = 0.097$) between the Alzheimer's disease (AD) group and the control group, indicating that the two groups were well-matched for these variables.

The genotypes of rs2234759 A/G and rs12507396 A/T polymorphisms were determined through the (ARMS-PCR) and the PCR products were then electrophoresed (Figs. 1 & 2).

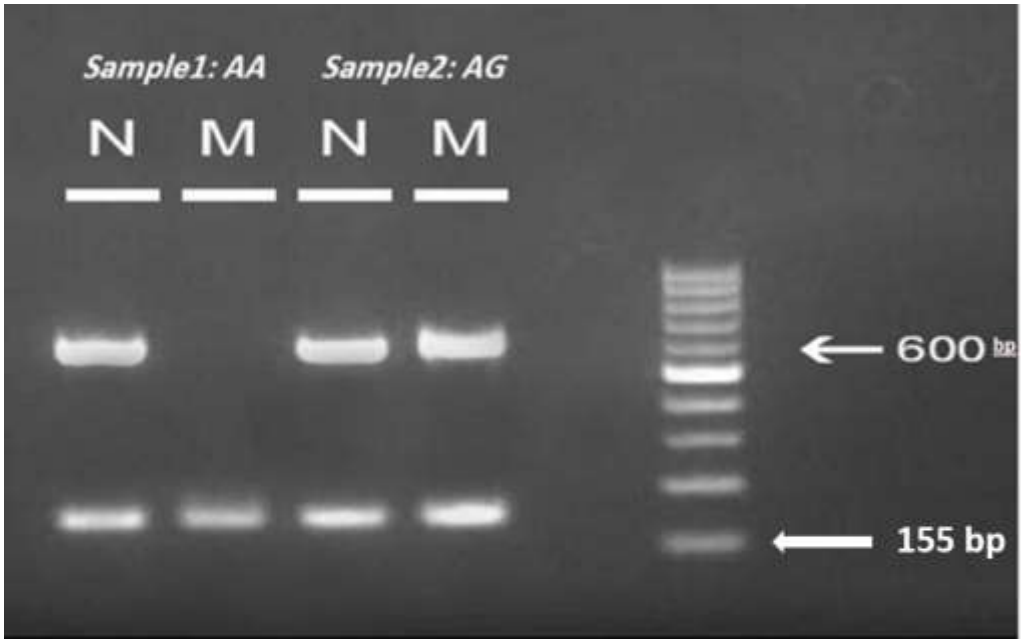

Fig. 1. ARMS-PCR amplification of the *rs2234759* SNP in the *NPY2R* gene. "N" indicates the normal allele-specific reaction (A allele); "M" indicates the mutant allele-specific reaction (G allele). Sample 2 shows only the N band, corresponding to the AA genotype. Sample 2 shows both N and M bands (~600 bp), consistent with the AG genotype. A constant lower band (155bp) in all lanes corresponds to the internal control (β-actin gene). A 100 bp DNA ladder was used as molecular size marker. (DNA ladders were purchased from Yekta tajhiz Azma. Iran. YT8503).

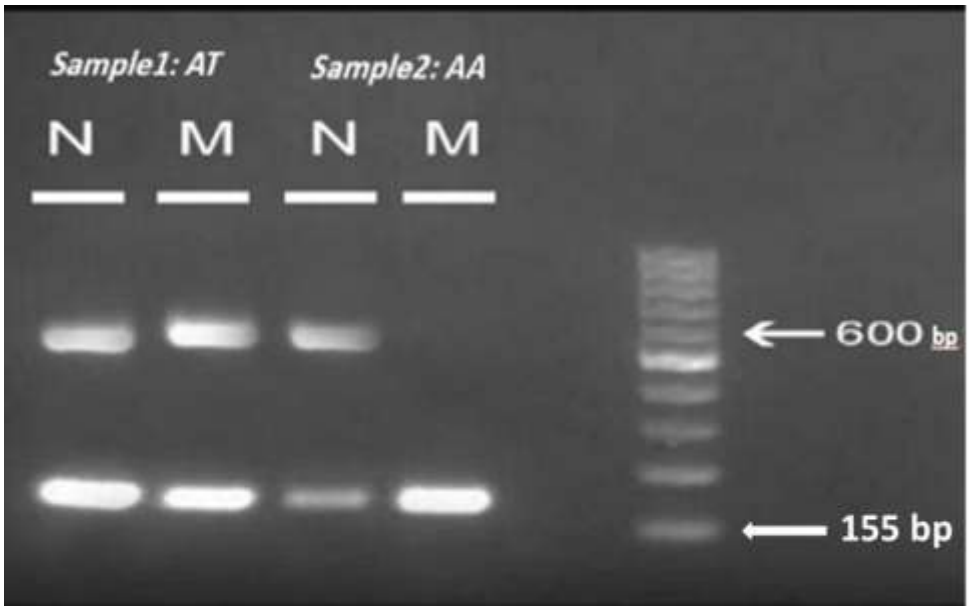

Fig. 2. ARMS-PCR amplification of the *rs12507396* SNP in the *NPY2R* gene. "N" indicates the normal allele-specific reaction (A allele); "M" indicates the mutant allele-specific reaction (T allele). Sample 1 shows both N and M bands (~600 bp), representing the AT genotype. Sample 2 shows only the N band, indicating the AA genotype. The lower band (155bp) in all lanes corresponds to the internal control (β-actin gene). A 100 bp DNA ladder was used as molecular size marker. (DNA ladders were purchased from Yekta Tajhiz Azma, Iran).

Sequencing Analysis

direct Sanger sequencing of 6 samples for each polymorphism was carried out. The Chromatogram of Sanger sequencing showing the heterozygous genotype (AG) at the *rs2234759* SNP position in the *NPY2R* gene (Fig. 3) and the Chromatogram of Sanger sequencing showing the heterozygous genotype (AT) at the rs12507396 SNP position in the *NPY2R* gene (Fig. 4).

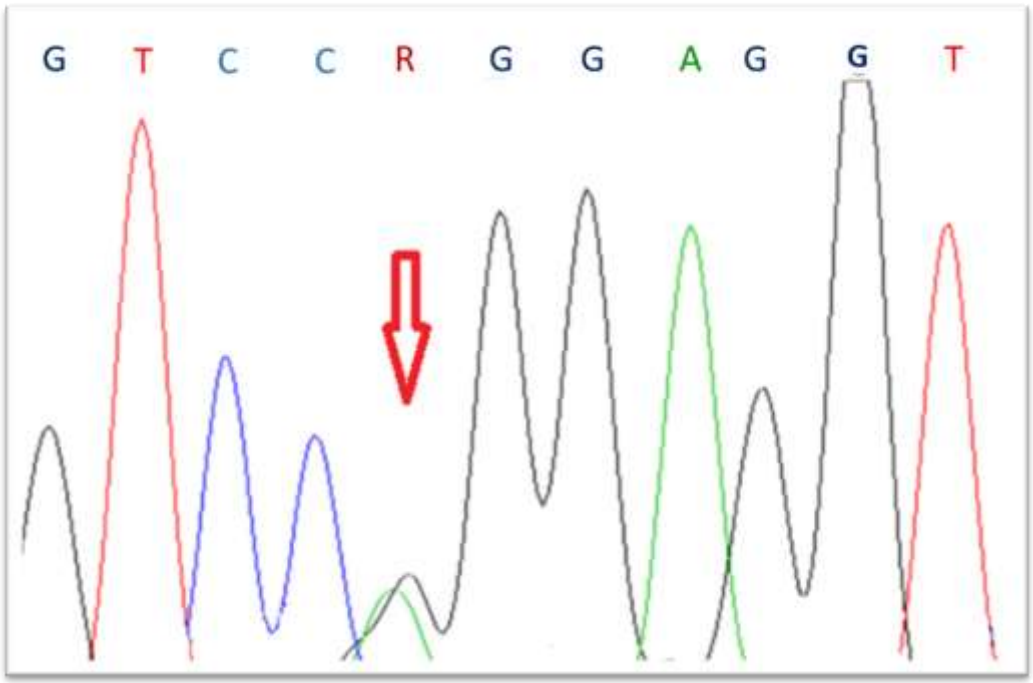

Fig. 3. Chromatogram of Sanger sequencing showing the heterozygous genotype (AG) at the *rs2234759* SNP position in the *NPY2R* gene. Overlapping peaks for adenine (A) and guanine (G) at the polymorphic site indicate the presence of both alleles, confirming a heterozygous genotype. The SNP position is marked with a red arrow.

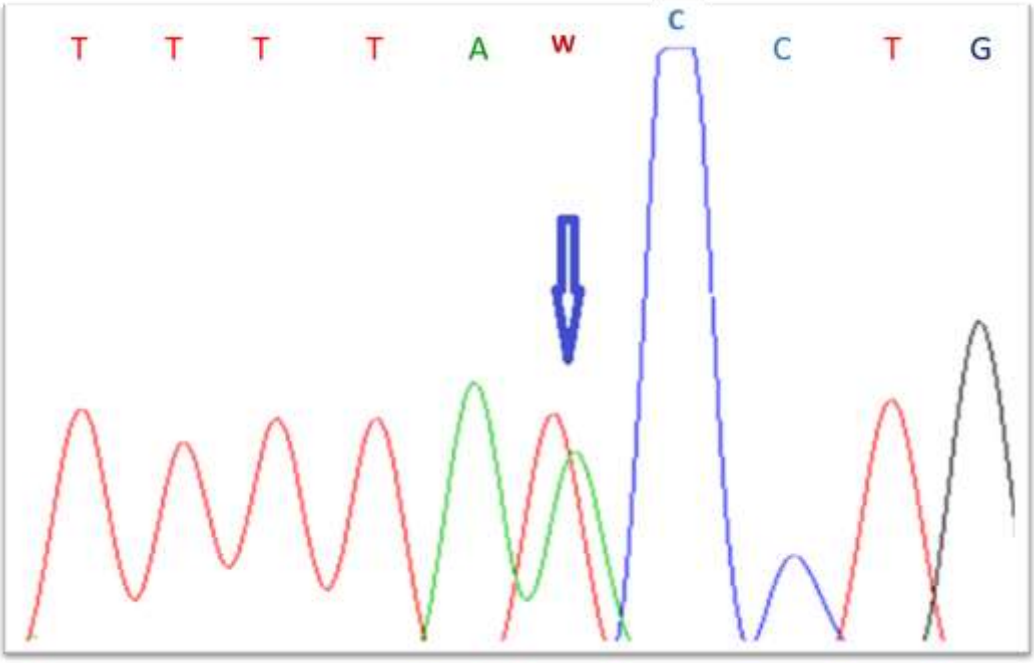

Fig. 4. Chromatogram shows heterozygote AT genotype for rs12507396. Chromatogram of Sanger sequencing showing the heterozygous genotype (AT) at the rs12507396 SNP position in the *NPY2R* gene. Overlapping peaks for adenine (A) and thymine (T) at the polymorphic site indicate the presence of both alleles, confirming a heterozygous genotype. The SNP position is marked with a blue arrow.

Association of rs2234759 Polymorphism with Alzheimer's Disease

Genotypic and allelic distributions of the rs2234759 SNP in the NPY2R gene are shown in Table 2. Among AD patients, the AA and AG genotypes were observed in 55.6% and 44.4% of individuals, respectively, while the GG genotype was absent. In contrast, in the control group, 87.8% had the AA genotype and 12.2% had the AG genotype, with no individuals carrying the GG genotype. These differences in genotype frequencies were statistically significant ($p< 0.001$). The analysis of allele frequencies also revealed a significant association with AD. The A allele was observed in 77.8% of alleles in the patient group and 93.9% in controls, while the G allele was found in 22.2% of patients and 6.1% of controls ($p< 0.001$). These findings suggest that the G allele of rs2234759 may be associated with an increased risk of AD.

Table 2. Genotype and allele frequencies of rs2234759 in healthy controls and patients.

Genotype and allele	Patients (n=90)	Healthy controls (n=90)	p-value	OR
AA	50(55.6)	79(87.8)	<0.001***	0.174(0.08-0.37)
AG	40(44.4)	11(12.2)		5.75
Allele A	140(77.8)	169(93.9)	<0.001***	0.3(0.11-0.82)
Allele G	40(22.2)	11(6.1)		4.39

OR= odds ratio.

The genotypic and allelic distribution analysis of rs2234759 revealed a significant association with Alzheimer's disease. Individuals with the AG genotype exhibited a markedly increased risk compared to AA carriers, with an odds ratio (OR) of 5.75. Similarly, the G allele was significantly more frequent among patients than controls, with an OR of 4.39, indicating that carriers of the G allele are over four times more likely to develop the disease compared to A allele carriers. These findings suggest that rs2234759 may contribute to genetic susceptibility to Alzheimer's disease.

Association of rs12507396 Polymorphism with Alzheimer's Disease

As presented in Table 3, the distribution of genotypes for rs12507396 was as follows: among AD patients, 72.2% were AA, 27.8% were AT, and none were TT. In the control group, 82.2% were AA and 17.8% were AT, with no TT genotypes observed. The difference in genotype frequencies did not reach statistical significance (p = 0.110).

However, a statistically significant difference was found in the allelic distribution. The A allele was more frequent in the control group (91.1%) than in patients (86.1%), whereas the T allele was more frequent among patients (13.9%) than controls (8.9%) (p = 0.009), suggesting a potential role of the T allele in AD susceptibility.

Table 3. Genotype and allele frequencies of rs12507396 in healthy controls and patients.

Genotype and allele	Patients (n=90)	Healthy controls (n=90)	p-value	OR
AA	65(72.2)	74(82.2)	0.110	0.562(0.297-1.14)
AT	25(27.8)	16(17.8)		
Allele A	155(86.1)	164(91.1)	0.009	0.27(0.090.78)
Allele T	25(13.9)	16(8.9)		

OR= odds ratio.

Genotypic distribution of rs12507396 did not differ significantly between Alzheimer's patients and healthy controls (AT vs. AA: OR = 0.562; 95%; p = 0.110). In contrast, the T allele was significantly underrepresented in cases compared to controls (13.9% vs. 8.9%), yielding an OR of 0.27 (p = 0.009). These findings indicate that the T allele at rs12507396 may exert a protective effect against Alzheimer's disease.

Sex-Stratified Genotypic Analyses

To assess potential sex-specific genetic effects, genotype frequencies of rs2234759 and rs12507396 were analyzed separately by sex within each group (Tables 4). The analyses revealed no statistically significant differences in genotype distributions between male and female participants in either the case or control groups for both SNPs (all p > 0.05).

Association Between SNPs and Age at Disease Onset

The mean age at disease onset among AD patients was 70.97 ± 8.53 years. As shown in Table 5, there was no statistically significant associations were found between age at onset and the genotypes of rs2234759 (p = 0.300) or rs12507396 (p = 0.944), indicating that these polymorphisms do not influence the age of disease onset (Table 6).

Table 4. Stratification analysis of rs2234759 A/G genotypes frequency in patients and healthy control groups.

Group	Sex	AA	AG	p-value
Patients	**Men**	32(64)	25(62.5)	0.883
	Women	18(36)	15(37.5)	
Healthy controls	**Men**	40(50.6)	6(54.5)	0.808

Table 5. Stratification analysis of rs12507396 A/T genotypes frequency in patients and healthy control groups.

Group	Sex	AA	AT	p-value
Patients	**Men**	40(61.5)	17(68)	0.569
	Women	25(38.5)	8(32)	
Healthy controls	**Men**	39(52.7)	7(43.8)	0.516
	Women	35(47.3)	9(56.2)	

Table 6. Stratification Analysis age onset in patients.

Rs12507396	**AA**	**AT**	**p-value**
	6.58±70.93	8.05±71.08	0.944
Rs2234759	**AA**	**AG**	**p-value**
	8.35±70.14	8.73±72.05	0.300

Discussion

In this study, we researched the association of rs2234759 A/G and rs12507396 A/T polymorphisms with Alzheimer's disease. Our findings reveal that the presence of G alleles in the rs2234759 polymorphism of the NPY2R gene is linked to AD. Notably, the hippocampus, one of the first brain regions affected in AD, experiences reduced glutamate levels in AD patients. Given the NPY system's role in protecting neurons and modulating glutamatergic neural transmission, we explored the effects of sequence variations in the NPY2R gene, specifically focusing on the rs2234759 and rs12507396 polymorphisms in relation to AD.

The rs2234759 and rs12507396 polymorphisms in the NPY2R promoter are common SNP variants in this gene, with rs2234759 influencing the expression level of the gene. Our study revealed a significant association between the rs2234759 polymorphism and AD, while no such association was observed for the rs12507396 polymorphism.

The presence of the rare G allele in rs2234759 within the NPY2R promoter enhances gene expression compared to the A allele. It has been suggested that high-expression NPY2R genotypes may lead to more robust presynaptic inhibition of glutamate release (23). This hypothesis is further supported by the association of the NPY2R G allele with Iconic memory, as studies have linked diminished iconic memory to an increased risk of AD (24, 25). Therefore, increased NPY2R gene expression in AD patients may contribute to reduced glutamate levels in the brain. Additionally, it has been demonstrated that treatment with Galantamine can elevate glutamate in the hippocampus, leading to symptom reduction in AD patients (26).

The results of our study revealed the significance of the rs2234759 polymorphism in NPY2R in AD. The presence of G alleles in the rs2234759 polymorphism of the NPY2R gene among AD patients may lead to increased gene expression. Consequently, individuals with high expression of NPY2R genotypes may experience stronger presynaptic inhibition of glutamate release. Numerous studies have corroborated the reduction of this excitatory neurotransmitter in the brains of AD patients, particularly in the hippocampal region.

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Conflict of interest

The authors declared no conflict of interest.

Ethics

This study was validated by the Ethics Committee of the University of Kerman Ethics Committee (IR.KMU.REC.1394.494).

References

1. Buckner RL, Snyder AZ, Shannon BJ, LaRossa G, Sachs R, Fotenos AF, et al. Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. J Neurosci. 2005;25(34):7709-17.
2. Jessen F, Amariglio RE, van Boxtel M, Breteler M, Ceccaldi M, Chételat G, et al. A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. Alzheimers Dement. 2014;10(6):844-52.
3. Dubois B, Feldman HH, Jacova C, DeKosky ST, Barberger-Gateau P, Cummings J, et al. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS–ADRDA criteria. Lancet Neurol. 2007;6(8):734-46.
4. Gauthier S, Loft H, Cummings J. Improvement in behavioural symptoms in patients with moderate to severe Alzheimer's disease by memantine: a pooled data analysis. Int J Geriatr Psychiatry. 2008;23(5):537-45.
5. Goedert M, Spillantini MG. A century of Alzheimer's disease. Science. 2006;314(5800):777-81.
6. Chan HH, Leong YQ, Voon SM, Pan ML, Leong CO, Lim CL, Koh RY. Effects of Amyloid Precursor Protein Overexpression on NF-κB, Rho-GTPase and Pro-Apoptosis Bcl-2 Pathways in Neuronal Cells. Rep Biochem Mol Biol. 2021;9(4):417-425.
7. Biglari S, Kamali K, Banihashemi S, Faal Sezavari A, Aghajanpour-Mir SM, Behjati F. Investigation of Aneusomy of Chromosome 21 in the Micronuclei of 13 Patients with Early Onset Alzheimer's Disease Using Fluorescence in Situ Hybridization: A Pilot Study. Rep Biochem Mol Biol. 2020;8(4):446-453.
8. Raux G, Guyant-Maréchal L, Martin C, Bou J, Penet C, Brice A, et al. Molecular diagnosis of autosomal dominant early onset Alzheimer's disease: an update. J Med Genet. 2005;42(10):793-5.
9. Rovelet-Lecrux A, Hannequin D, Raux G, Le Meur N, Laquerrière A, Vital A, et al. APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy. Nat Genet. 2006;38(1):24-6.
10. Coon KD, Myers AJ, Craig DW, Webster JA, Pearson JV, Lince DH, et al. A high-density whole-genome association study reveals that APOE is the major susceptibility gene for sporadic late-onset Alzheimer's disease. J Clin Psychiatry. 2007;68(4):613-8.
11. Zhang B, Gaiteri C, Bodea LG, Wang Z, McElwee J, Podtelezhnikov AA, et al. Integrated systems approach identifies genetic nodes and networks in late-onset Alzheimer's disease. Cell. 2013;153(3):707-20.
12. Scheff SW, Price DA, Schmitt FA, Mufson EJ. Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. Neurobiol Aging. 2006;27(10):1372-84.
13. Rupsingh R, Borrie M, Smith M, Wells JL, Bartha R. Reduced hippocampal glutamate in Alzheimer disease. Neurobiol Aging. 2011;32(5):802-10.
14. Vezzani A, Sperk G. Overexpression of NPY and Y2 receptors in epileptic brain tissue: an endogenous neuroprotective mechanism in temporal lobe epilepsy? Neuropeptides. 2004;38(4):245-52.
15. Xapelli S, Silva AP, Ferreira R, Malva JO. Neuropeptide Y can rescue neurons from cell death following the application of an excitotoxic insult with kainate in rat organotypic hippocampal slice cultures. Peptides. 2007;28(2):288-94.
16. Ammar DA, Eadie DM, Wong DJ, Ma YY, Kolakowski LF Jr, Yang-Feng TL, Thompson DA. Characterization of the human type 2

neuropeptide Y receptor gene (NPY2R) and localization to the chromosome 4q region containing the type 1 neuropeptide Y receptor gene. Genomics. 1996;38(3):392-8.
17. Rose PM, Fernandes P, Lynch JS, Frazier ST, Fisher SM, Kodukula K, et al. Cloning and functional expression of a cDNA encoding a human type 2 neuropeptide Y receptor. J Biol Chem. 1995;270(39):22661-4.
18. Broberger C, Landry M, Wong H, Walsh JN, Hökfelt T. Subtypes Y1 and Y2 of the neuropeptide Y receptor are respectively expressed in pro-opiomelanocortin- and neuropeptide-Y-containing neurons of the rat hypothalamic arcuate nucleus. Neuroendocrinology. 1997;66(6):393-408.
19. Gerald C, Walker MW, Vaysse PJ, He C, Branchek TA, Weinshank RL. Expression cloning and pharmacological characterization of a human hippocampal neuropeptide Y/peptide YY Y2 receptor subtype. J Biol Chem. 1995;270(45):26758-61.
20. Morales-Medina JC, Dumont Y, Quirion R. A possible role of neuropeptide Y in depression and stress. Brain Res. 2010;1314:194-205.
21. Rose JB, Crews L, Rockenstein E, Adame A, Mante M, Hersh LB, et al. Neuropeptide Y fragments derived from neprilysin processing are neuroprotective in a transgenic model of Alzheimer's disease. J Neurosci. 2009;29(4):1115-25.
22. Kloster E, Saft C, Akkad DA, Epplen JT, Arning L. Association of age at onset in Huntington disease with functional promoter variations in NPY and NPY2R. J Mol Med (Berl). 2014;92(2):177-84.
23. Silva AP, Pinheiro PS, Carvalho AP, Carvalho CM, Jakobsen B, Zimmer J, Malva JO. Activation of neuropeptide Y receptors is neuroprotective against excitotoxicity in organotypic hippocampal slice cultures. FASEB J. 2003;17(9):1118-20.
24. Arning L, Stock AK, Kloster E, Epplen JT, Beste C. NPY2-receptor variation modulates iconic memory processes. Eur Neuropsychopharmacol. 2014;24(8):1298-302.
25. Lu ZL, Neuse J, Madigan S, Dosher BA. Fast decay of iconic memory in observers with mild cognitive impairments. Proc Natl Acad Sci U S A. 2005;102(5):1797-802.
26. Penner J, Rupsingh R, Smith M, Wells JL, Borrie MJ, Bartha R. Increased glutamate in the hippocampus after galantamine treatment for Alzheimer disease. Prog Neuropsychopharmacol Biol Psychiatry. 2010;34(1):104-10.