Analysis of the BACE1 and Clusterin Genes Expression Levels in Alzheimer's Disease

Hani Alsaadoni[1], Burcu Çaykara[1], Sadrettin Pençe[1], Saffet Tüzgen[2], Halime Hanım Pençe[3]

[1]Department of Molecular Medicine, İstanbul University Aziz Sancar Institute of Experimental Medicine (ASDETAE), İstanbul, Turkey
[2]Department of Neurosurgery, İstanbul University-Cerrahpaşa, Cerrahpaşa School of Medicine, İstanbul, Turkey
[3]Department of Medical Biochemistry, University of Health Sciences, School of Medicine, İstanbul, Turkey

Cite this article as: Alsaadoni H, Çaykara B, Pençe S, Tüzgen S, Pençe HH. Analysis of the BACE1 and Clusterin Genes Expression Levels in Alzheimer's Disease. JAREM 2019; 9(1): 45-9.

ABSTRACT

Objective: This study aimed to explore the mRNA expressions of the β-site amyloid precursor protein-cleaving enzyme 1 (BACE1) and clusterin (CLU) genes in blood samples of patients with Alzheimer's disease (AD) and healthy subjects.

Methods: The expression levels of CLU and BACE1 in blood samples of subjects were examined using the real-time polymerase chain reaction (RT-PCR). The expression levels of patient and control groups were calculated using the 2-ΔΔCt method. The Statistical Package for Social Sciences (SPSS) version 22 (IBM Corp., Armonk, NY, USA) was used for statistical analysis, and a p of <0.05 was accepted as statistically significant.

Results: The BACE1 and CLU genes were found 14-fold and 13-fold more expressed in the blood of the AD group than on the control group, respectively.

Conclusion: Our study showed that the blood mRNA levels of the BACE1 and CLU genes were associated with AD.

Keywords: Alzheimer's disease, BACE1, clusterin, gene expression

INTRODUCTION

Alzheimer's disease (AD) currently affects 44.4 million people worldwide (1), including 5.3 million Americans, and this number is estimated to triple by the year 2050 (2). As the prevalence of AD increases, the AD-associated economic burden will also increase (2). Early-onset AD (EOAD) occurs before the age of 65, and late-onset Alzheimer disease (LOAD) appears after the age of 65 (3). Alzheimer's prevalence is found to be 6.4% in individuals aged 65 years and older in Turkey (4).

The accumulation of amyloid beta peptide (Aβ) and fibrillar plaques in brain regions characterize AD (5). Moreover, intracellular neurofibrillary tangles, neuronal dysfunction, neuroinflammation, and death further characterize AD (6). The actual neurodegenerative process begins 20 to 30 years before first clinical symptoms (7). Amelioration of these pathological alterations before their expansion is crucial for an actual cure. Defining of a marker present in blood will facilitate discovery of a therapeutic procedure at the onset of the neurodegenerative process.

Amyloid beta peptide (Aβ) fragments are produced through amyloid precursor protein (APP), which is a type I transmembrane glycoprotein (8). The proteolysis of the APP by enzymes including the β- and γ-secretase activity is called amyloidogenesis. The beta-site APP-cleaving enzyme 1 (BACE1) has been identified as an important β-secretase in that process (9). Many studies suggested that BACE1 is essential for the amyloid β protein production, causing AD (10, 11). BACE1 is also found in several tissues including blood, and for this reason, BACE1 could a good candidate for biomarker analysis (12, 13).

Another AD risk gene is clusterin (CLU) known as apolipoprotein J (APOJ) (14), and it directly binds Aβ and regulates the Aβ pathology (15). It has been shown that CLU also regulates the inflammation and oxidative stress in the brain (16). Therefore, CLU causes neuronal dysfunction via the accumulation of fibrillar amyloid plaque (17). The CLU protein levels were found elevated in the frontal cortex and hippocampus in AD (18).

ORCID IDs of the authors: H.A. 0000-0001-9943-3364; B.Ç. 0000-0001-7063-2140; S.P. 0000-0001-9453-4166; H.H.P. 0000-0002-8346-1018.

Corresponding Author: Sadrettin Pençe,
E-mail: sadrettin.pence@istanbul.edu.tr

Received Date: 06.12.2018 **Accepted Date:** 15.01.2019

DOI: 10.5152/jarem.2019.2671

These data strongly suggested that BACE1 and CLU may play an important role in the pathogenesis of AD via regulation of the brain amyloid burden. This study was conducted to show the relation between the CLU and BACE1 mRNA levels and AD.

METHODS

The study protocol was approved by the Ethical Committee of Clinical Research of Istanbul University School of Medicine. Fifty patients with AD were included in the patient group, and 50 healthy volunteers were included in the control group. Written informed consent was obtained from patients who participated in this study.

RNA Isolation and Quantitative Real-Time Polymerase Chain Reaction

The peripheral blood at the amount of 3 mL was collected from the patient and control groups and transported in an Ethylenediaminetetraacetic acid (EDTA) anticoagulant solution. The RNA was extracted from blood using the TRIzol reagent (Qiagen Inc., Germany) according to the manufacturer's protocol. The concentration of the RNA solution was tested, with the ratio of OD260/OD280>1.80. The reverse transcriptase reactions were performed with the SensiFAST CDNA Synthesis kit (Bioline USA Inc.). The polymerase chain reactions (PCR) were carried out with a total volume of 20 µL containing 2 µL of Total RNA, 4 µL of 5x TransAmp Buffer, 1 µL of reverse transcriptase and 13 µL of DNase/RNase free-water. The reactions were subjected to 25°C for 10 min, 42°C for 15 min, 85°C for 5 min, and 4°C hold.

For quantitative real-time PCR (qPCR), the GAPDH gene was used as an endogenous reference gene for the RNA expression detection. The qPCR reactions were performed with the SensiFAST SYBR No-ROX Kit (Bioline USA Inc.). The PCR primers were 5'-AAC CTG CCA AAT ATG ATG AC-3' (forward) and 5'-TTG AAG TCA GAG GAG ACC AC-3' (reverse) for GAPDH, 5'-TGA TCC CAT CAC TGT GAC GG-3' (forward) and 5'-GCT TTT TGC GGT ATT CCT GC-3' (reverse) for the CLU gene, 5'-TGG AGG GCT TCT ACG TTG TC-3' (forward), and 5'-CAG AGT GGC AGC AGC ATG AAG AG-3' (reverse) for the BACE-1 gene. The qPCR reactions were carried out with a total volume of 20 µL containing 10 µL of 2x SensiFAST SYBR No-ROX Mix, 1.6 µL of f primers, 5 µL of template, and 8.4 µL of DNase/RNase free-water. The qPCR reactions were subjected to hot start at 95°C for 2 min, followed by denaturation at 95°C for 5 s, annealing 60°C for 10 s, and extension at 52°C for 10 s using the real-time detection system. The expression of genes was quantified by measuring the cycle threshold (Ct) values and normalized using the $2^{-\Delta\Delta CT}$ method relative to the GAPDH RNA.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences version 22.0 (IBM Corp., Armonk, NY, USA) package program. The Kolmogorov-Smirnov, analysis of variance, and the Mann–Whitney U and Spearman's rho tests were used in the statistical analysis of the data. A $p<0.05$ was considered as statistically significant.

RESULTS

A total of 100 samples were included in the study: 50 samples in the AD group and 50 samples in the control group. The AD group consisted of 33 males and 17 females, while the control group consisted of 24 males and 26 females. The number of patients with dementia was 42 in the AD group, and the mean

Table 1. Clinical parameters of Alzheimer's disease group

	Alzheimer's disease group			Control group			
Parameters	Min	Max	Mean±SD	Min	Max	Mean±SD	p
Age (year)	58	102	81.64±8.39	24	66	37.4±10.06	<0.001
Height (cm)	150	187	165±9	150	189	169±9.6	0.022
Weight (kg)	48	98	70.78±13.7	50	108	76.9±16.05	0.040
Glucose (mg/dL)	64	366	124.7±52.68	74	125	92.15±10.5	<0.001
Total cholesterol (mg/dL)	92	264	197.28±35.89	109	311	195.1±42.3	0.28
LDL (mg/dL)	53	176	109.94±21.67	48	224	114.65±34.1	0.694
HDL (mg/dL)	21	89	45±12.58	30	87	51±12.62	0.015
Triglycerides (mg/dL)	48	475	209.54±104.54	45	278	111.17±60.1	<0.001
B12 (pg/mL)	94	580	287.5±80.74				
Folate (µg/dL)	1.69	60	10.03±9.21				
TSH (mIU/l)	0.01	8.33	2.5±2.2				
T3 (ng/dL)	0.98	4.8	2.6±1				
T4 (µg/dL)	0.47	4.11	2.03±0.86				

LDL: low-density lipoprotein; HDL: high-density lipoprotein; TSH: thyroid-stimulating hormone; T3: triiyodotironin; T4: tetraiodothyronine; SD: standard deviation

Table 2. Correlation analysis of BACE1, CLU, and other parameters

Spearman's rho correlation	CLU expression in AD group	BACE1 expression in AD group	Total cholesterol	HDL	T4
CLU expression in AD group	-	0.000*	0.46	0.14	0.46
BACE1 expression in AD group	0.000*	-	0.9	0.17	0.94
Total cholesterol	0.46	0.9	-	0.000*	0.002*
HDL	0.14	0.17	0.000*	-	0.13
T4	0.46	0.94	0.002*	0.13	-

BACE1: beta-site APP-cleaving enzyme 1; CLU: clusterin; AD: Alzheimer's disease; HDL: high-density lipoprotein; T4: tetraiodothyronine
*p<0.002

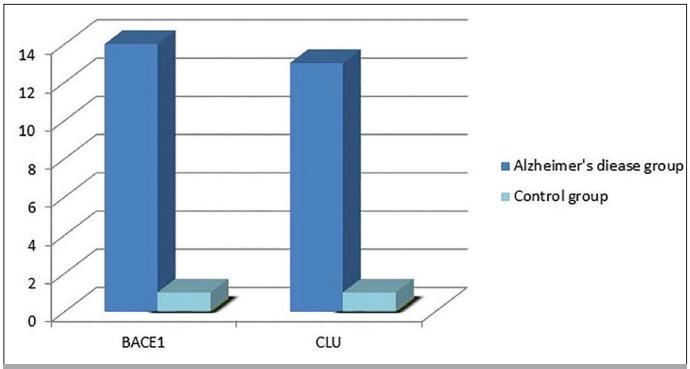

Figure 1. BACE1 and CLU gene expression changes in groups
BACE1: β-site amyloid precursor protein-cleaving enzyme 1; CLU: clusterin

duration of dementia was 5.71±3.4 years. The mean age of the AD group was 81.64±8.39, and the mean age of the control group was 37.4±10.06 (p<0.001). Only 1 patient had EOAD, while others had LOAD. The clinical parameters of the AD group are given in Table 1. Vitamin B12, folate, thyroid-stimulating hormone (TSH), triiodothyronine (T3), and tetraiodothyronine (T4) values were not found in the control group of the clinical parameters given in Table 1. When the parameters in the patient and control groups were compared, they were significantly different between the groups (p<0.05), except for the total cholesterol and low-density lipoprotein (LDL). There was no statistically significant difference in the expression of BACE1 and CLU genes in the patient and control groups (p=0.64 and p=0.53, respectively). However, BACE1 was 14-fold overexpressed in the AD group. Also, CLU was 13-fold overexpressed in the patient group compared to the control group (Figure 1). A positive correlation was found between the BACE1 and CLU gene expressions in the AD group (p<0.001). Furthermore, total cholesterol was positively correlated with the high-density lipoprotein and T4 levels (p=0.039) in the control group (Table 2).

DISCUSSION

The BACE1 and CLU genes were found to be overexpressed in the AD group. Although our results were not statistically significant between the groups, we observed the 13- and 14-fold expression changes. Moreover, the expression of BACE1 and CLU was positively correlated in the AD group (p<0.001).

Alzheimer's disease (AD), the most common encountered neurodegenerative disease, is often characterized by progressive dementia affecting the episodic memory (3). A number of novel risk genes were identified for LOAD, the most common type of AD, and CLU is one of these genes (19). Normally, CLU released from cells is a stress-activated adenosine triphosphate-independent molecular chaperone, and it plays a role in the inhibition of apoptosis and protein homeostasis/proteostasis (20). Chen et al. (20) showed that CLU interacted with β-amyloid peptide (21) and was associated with AD. Furthermore, CLU variants have been shown to increase the AD risk in association with Tau protein (22). Yu et al. (23) reported that elevated CLU expression levels in AD affected brain tissues. Modestly increased CLU expression levels in the frontal and temporal cortex were observed in another study (24). Plasma CLU levels were also found to be positively associated with a rapid clinical progression in AD (25). Thus, researchers suggested that CLU might be evaluated as a potential biomarker in the diagnosis of AD (14, 23). However, some studies did not find a diagnostic value of CLU in AD (26, 27). We found elevated CLU expression levels (13-fold higher) in the peripheral blood of AD patients, but our result was not statistically significant (p>0.05).

One of the early pathogeneses of AD is Aβ, and the BACE1 regulates the production of Aβ. BACE1 is mostly presented within late endosomes at the presynaptic terminals of patient brains, and it increases synaptic Aβ levels (28). The role in the formation of Aβ peptides has made BACE1 a therapeutic target for AD (29). BACE1 showed an increased activity in the brain tissue and cerebrospinal fluid of subjects with mild cognitive impairment and probable AD (30). Studies showed the upregulation of BACE1 in the brain tissues (29) and plasma of patients with AD (31). High plasma levels of BACE1 were suggested as a possible biomarker for the AD risk (30). When we compared the AD group to the control group, we observed the overexpression of BACE1 (14-fold higher). Moreover, we found positively correlated BACE1 and CLU expression levels in the AD group. This may be derived from the effects each protein has on Aβ peptides.

CONCLUSION

Our results show that BACE1 and CLU play a role in the pathology of AD. High expression levels of these genes in plasma could make possible biomarkers for AD. Furthermore, these genes may be evaluated as potential therapeutic targets. More studies are required to elucidate the role of these genes in the mechanism of AD pathology.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethical Committee of Clinical Research of İstanbul University School of Medicine.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - H.A., B.Ç., S.P., S.T., H.H.P.; Design - H.A., B.Ç., S.P., S.T., H.H.P.; Supervision - H.A., B.Ç., S.P., S.T., H.H.P.; Resources - H.A., B.Ç., S.P., S.T., H.H.P.; Materials - H.A., S.T.; Data Collection and/or Processing - B.Ç., S.P.; Analysis and/or Interpretation- H.A., B.Ç., S.P., S.T., H.H.P.; Literature Search - H.A., B.Ç., S.P., S.T., H.H.P.; Writing Manuscript - H.A., B.Ç., S.P., S.T., H.H.P.; Critical Review - H.A., B.Ç., S.P., S.T., H.H.P.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: This study was supported by İstanbul University Scientific Research Project Unit (Project No: 29034).

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