T.C. YEDİTEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF MOLECULAR MEDICINE

INVESTIGATION OF ANGIOTENSIN–CONVERTING ENZYME I/D POLYMORPHISM IN PATIENTS WITH ALZHEIMER'S DISEASE

MASTER OF MOLECULAR MEDICINE THESIS

EZGİ YALÇINKAYA

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated……..and numbered .....

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DECLARATION

I declare that this thesis is my own work and it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

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LIST OF SYMBOLS AND ABBREVATIONS

Al	Aluminum
AD	Alzheimer’ s Disease
ACE	Angiotensin-converting enzyme
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
Aβ	Amyloid beta protein
CSF	Cerebrospinal fluid
FAD	Familial Alzheimer’s disease
NFTs	Neurofibrillary tangles
NMDA	N-Methyl-d-aspartate
PSEN1	Presenilin 1
PSEN2	Presenilin 2
PiB	Pittsburgh compound

ABSTRACT

Yalçınkaya, E. (2023). Investigation of Angiotensin – Converting Enzyme I/D Polymorphism in Patients with Alzheimer's Disease. Yeditepe University, Institute of Health Sciences, Department of Molecular Medicine, MSc Thesis, Istanbul.

Dementia is a common clinical syndrome that causes cognitive disorders and affects daily life significantly, and the risk of being seen increases significantly with aging. Although dementia is not a single disease type, it is the general name of the findings characterized by deterioration in mental abilities. Alzheimer's disease, which is a common type of dementia in the population, is a progressive neurological disease that occurs in individuals with the destruction of brain cells. Most patients who suffer from Alzheimer's Disease show the symptoms of this disease in their later ages, whereas the earliest sign being memory loss. Although recurring forgetfulness is observed in sick individuals, past memory is preserved. As the disease progresses, cognitive abilities decline as well as loss of aphasia and apraxia. Although the exact causes of Alzheimer's disease are not known, it is thought that genetic factors may play limited role in the occurrence of this pathophysiology. The angiotensin-converting enzyme (ACE) hydrolyzes peptide molecules and proteins by removing a dipeptide from the C terminus of polypeptides such as in the case of Angiotensin. As a result of previous studies, it has been observed that ACE can cleave amyloid beta peptides in vitro. It is known that amyloid beta plaques are an important factor in AD. Therefore, in previous studies it was asserted that ACE and ACE variants could be associated with Alzheimer's Disease. In this study, we aimed to elucidate the effect of ACE polymorphisms on the risk of developing late-onset Alzheimer's in Turkish and Mediterranean populations in samples taken from Alzheimer's patients and in conjunction with meta-analysis including samples from previous data sets. PCR studies revealed the presence of single nucleotide polymorphisms. Genetic variations were determined by Sanger sequencing from the samples with polymorphism. As a result of meta-analysis, it was determined that rs1799752 ACE polymorphisms were not associated with late-onset Alzheimer's disease in Turkish and Mediterranean populations.

Key words: Angiotensin Converting Enzyme, Alzheimer Disease, Polymorphism

ÖZET

Yalçınkaya, E. (2023). Alzheimer Hastalarında Anjiyotensin Dönüştürücü Enzim I/D Polimorfizminin İncelenmesi. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Moleküler Tıp ABD., Yüksek Lisans Tezi. İstanbul.

Demans, bilişsel bozukluklara sebep olan ve günlük yaşamı önemli ölçüde olumsuz etkileyen, yaşlanma ile görülme riski belirgin olarak artış gösteren, dünya genelinde sık rastlanan klinik bir sendromdur**.** Demans esasında tek bir hastalık tipi olmamakla beraber zihinsel yeteneklerdeki bozulmalarla karakterize edilen bulguların genel adıdır. Popülasyon içerisinde yaygın olarak görülen demans türü olan Alzheimer hastalığı beyin hücrelerinin yok olmasıyla bireylerde ilerleyici bir durumda ortaya çıkan nörolojik hastalıktır. Özellikle hasta bireylerin çoğunluğunun ilerleyen yaşlarda bu hastalığın bulgularına sahip olduğu görülmektedir ve bu bireyler en erken olarak hafıza kaybı belirtileri göstermektedir. Hasta bireylerde yinelenen unutkanlıklar görülmekle beraber geçmiş hafıza korunmaktadır. Hastalığın ilerlemesiyle kognitif yeteneklerde gerilemenin yanı sıra afazi ve apraksi gibi yitimler görülmektedir. Alzheimer hastalığının kesin nedenleri bilinmemekle beraber genetik faktörlerin bu patofizyolojinin oluşmasında düşük düzeyde rol oynadığı düşünülmektedir. Angiotensin-converting enzim (ACE) Angiotensin v.b. bazı polipeptidlerin C terminusundan bir dipeptid çıkararak proteinler ve diğer peptid moleküllerini hidrolize edebilir. Yapılan çalışmalar sonucu ACE'nin amiloid beta peptidlerini in vitroda ayırabileceği gözlemlenmiştir. Amiloid beta plaklarının Alzheimer hastalığında önemli bir etken olduğu bilinmektedir. Dolayısıyla daha önceki bazı çalışmalarda ACE ve ACE varyantlarının Alzheimer hastalığı ile ilişkili olabileceği ileri sürülmüştür. Bu çalışmamızda Alzheimer hastalarından alınan örneklerde ACE polimorfizmlerin çalışılması ve iş bu deneysel çalışmanın daha önceki çalışmalarda elde edilen veri seti ile kombine edilmesi ile gerçekleştirilen meta-analizler ile, Türk ve Akdeniz toplumlarında geç başlangıçlı Alzheimer'ın gelişme riskine etkisini aydınlatılmaya çalışılmıştır. PCR çalışmaları sonucu tek nükleotid polimorfizmlerin varlığını saptanmıştır. Polimorfizm saptanan örneklerden Sanger sekanlama ile genetik varyasyonları belirlenmiştir. Meta analizleri sonucu Türk ve Akdeniz toplumlarında rs1799752 ACE polimorfizminin geç başlangıçlı Alzheimer hastalığı ile ilişkisinin olmadığı saptanmıştır.

Anahtar Kelimeler: Anjiyotensin Dönüştürücü Enzim, Alzheimer, Polimorfizm

1. INTRODUCTION AND PURPOSE

Dementia is a big health problem all around the world [1]. Dementia is an aging-related syndrome that manifests itself with a decrease in cognitive abilities, which seriously affects daily life [2]. 35 million people worldwide are diagnosed with dementia and this number is expected to reach 65 million in 2030 and 113 million in 2050 [1]. The number of patients diagnosed with dementia is expected to increase in developing and developing countries, and it is thought that by 2040, 71% of the 81 million dementia cases in the developing world will be diagnosed in these countries [1]. In order to lower disease risk or delay onset of dementia, more emphasis is being placed on psychosocial risk factors that make disease onset and progression more likely. A number of studies suggest stressful life events may have adverse effects on cognitive function, especially in old age. Moreover, Dementia is essentially the general name for findings characterized by deterioration in mental abilities.

Although Alzheimer's disease (AD) is a type of dementia, it is a disease that shows a progressive behavior with the destruction of brain cells. AD is manifested by a decrease in cognitive abilities, neuropsychiatric and behavioral disorders, as well as negatively affecting the daily lives of diagnosed patients and causing physical and physiological negativities in individuals. Alzheimer's disease consists of three stages [3], [4]. While repetition of questions, difficulty in writing and using tools, and irritability are seen in the early stage, problems such as gradual loss of past experiences and learned information and difficulty in recognizing relatives are seen in the middle stage. In the advanced stage, we see that the past and present time are mixed, communication is severely impaired, and the patient is in need of care [5]. Many mechanisms and neurotransmitter changes make the pathophysiology of AD very complex. The 3 main components of the mechanism are β amyloid plaques, neurofibrillary tangles and neuronal cell death [6]. The first component of the amyloid cycle to show itself is Aβ production and plaques. After that process continues with a series of simultaneous excitotoxicity, inflammation, oxidation and tau hyperphosphorylation, results in neurotransmitter disorder and evokes programmed cell death [7], [8]. The clinical equivalent of this pathophysiological cycle is progressive cognitive loss and deterioration in activities of daily living with psychiatric symptoms.

There are two sets of genes that determine whether people will develop AD, and these are risk genes and descriptive genes, respectively. Risk genes indicate the probability of developing a AD, but it certainly does not mean that AD will. Researchers have found several genes that increase probability of developing AD. APOE-e4 is the first risk gene identified and has the strongest probability of developing AD. Genetic predisposition is seen in 40% of AD cases. The blood tests can identify the Apo alleles a person has, but cannot predict whether they will develop Alzheimer's [9], [10]. The APOE test helps detect early brain changes and compare the effectiveness of possible treatments for people with different APOE profiles.

Genetic testing is used to help diagnose early- onset Alzheimer's disease and to test people with a family history of Alzheimer's or a related brain disease. Descriptive genes cause disease directly. There are three gene mutations related to the early-onset Alzheimer's disease. These are amyloid protein precursor (APP), Presenilin 1 (PSEN1) and Presenilin 2 (PSEN2) mutations. They are located on chromosome 14 and chromosome 1, respectively. Mutations in these genes lead to the formation of abnormal proteins that can cause diseases. Each of these mutations is involved in the degradation of the APP protein. The function of the APP protein is unknown completely. This deterioration is part of a process that creates the harmful amyloid plaques that are the hallmark of Alzheimer's disease. Inherited genes caused familial Alzheimer's are rare, but their discovery provides cluesto help us understand the mechanism of Alzheimer's. These genes affect the processing or production of beta-amyloid, this protein is the main component of plaques. Beta-amyloid plays a major role in the reduction and death of brain cells. Genetic studies on Alzheimer's disease continue and new findings are being obtained.

Nowadays ACE and mutations related with this protein have been associated with Alzheimer's disease. It has also been reported that the ACE DD genotype is more common in stroke and AD cases. In addition, it has been reported that ACE activity in the cerebrovascular system increases with aging and Alzheimer's. [11]. In addition, more studies are needed to explain the association of ACE polymorphism with AD. In addition, although we have findings on genetic markers for early-onset AD, we have limited information about late-onset AD. However, in our opinion there have not been a sufficient

number of studies in the literature, on the relationship between ACE rs1799752 polymorphism and late-onset AD specifically in the in the Turkish and Mediterranean Populations.

In this thesis, it was aimed to assess an association between incidence of late-onset AD risk and ACE rs1799752 polymorphism in Turkish and Mediterranean populations.

2. GENERAL INFORMATION

2.1. Dementia

Dementia is a chronic and progressive memory disorder, interrelated with the decrease of speech, perception, calculation, memory and intellectual capacity, in at least two of cognitive functions such as problem solving, judgment and abstract thinking [12]. Impairment in daily life activities of the individual, in social and professional life consists of depending on the cognitive impairment restriction. To diagnose dementia clinically, multiple losses and disruption in daily living activities, subsequent deterioration in mental functions, acquired mental functions such as memory, attention, language of the patient expected [13]. Dementia, with its insidious onset lasts for months or years and with its progressive continuation, should be distinguished from simple forgetfulness, depression and non- progressive mild cognitive impairment which does not affect daily life. Dementia, which is a major health problem in aging societies and develops due to vascular or degenerative changes, is seen in 5% of individuals aged 65 and over, and at a rate of 20% around the age of 80 [13]. The number of dementia patients, which was estimated to be 24 million in 2001 in the world, is expected to increase to 81 million in 2050 [14]. In parallel with the rapid increase in the elderly population, diseases common in old age such as dementia become a major problem for the society. Dementia occurs as a result of various causes including degenerative, vascular, traumatic, demyelinating, neoplastic, infectious, inflammatory, systemic and toxic causes [15]. It can be classified according to their etiology as primary and secondary, localizationas cortical and subcortical and its clinical features [16] (Figure 1). Symptoms that occur in dementia can be discussed under the headings of cognitive, behavioral, functional, motor, autonomous and sleep. Memory problems from recent and distant past, lack of concentration and inattention, difficulties on word finding, comprehension, reading, writing and calculation, change in typeface, experience a difficulty to use tools, dressing, sitting, and walking, recognizing objects are related to cognitive area [17]. Personality changes, apathy, disinhibition, social inappropriateness, sorrow, reluctance, restlessness, irritability, inappropriate joy, theft, infidelity, visual and other hallucinations, and related behavior changes as behavioral symptoms decreased interest in social relationships, hobbies, news, shopping, lack of self-care like eating, bathing, dressing, make-up, shaving, impairment in sphincter control as functional symptoms can be observed.

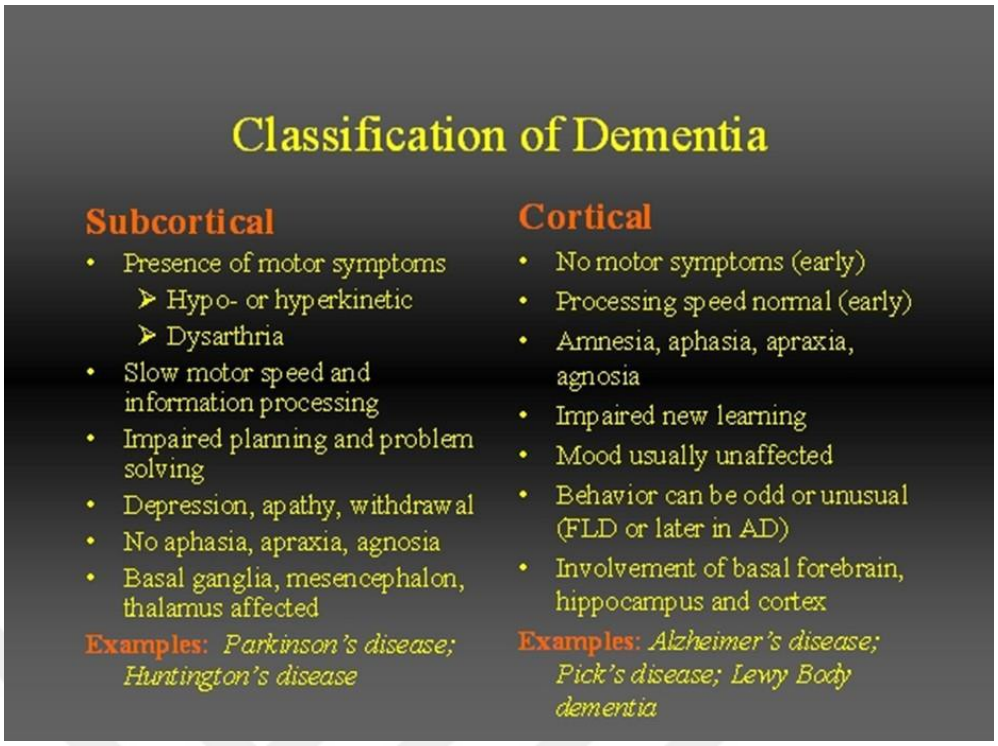

Figure 1. Classification of Dementia [18]. Alzheimer disease develops from cortical region disorders.

Gait disturbance, falls, freezing, imbalance, slowness of movement, muscle weakness as motor skill struggles, autonomic dysfunction such as incontinence, impotence, constipation, sweating and sleeping disorders such as excessive daytime sleepiness, sleep apnea are the common symptoms of dementia [18]. The development of cognitive disorders takes places in three main stages. The first stage of the disease is slow, and it is the amnestic period that starts and develops insidiously. During this period, which corresponds to the pre-clinical period, the medial temporal region of the brain is affected, defined as a single-functioning cognitive syndrome. The next stage is the early clinical period in which dementia as well as mental disorders are reflected in daily life. This is also defined as multifunctional cognitive syndrome. The last stage of the disease is the global syndrome period in which common disorders are seen. Neuropathological changes start from the mesial temporal areas of the brain, especially the hippocampus and amygdala, during this period, synapse loss is particularly pronounced in the entorhinal region, hippocampus and amygdala. The disease progresses to the parietotemporal areas and prefrontal cortex, in the last stage it also affects the primary areas of the cortex. Changes in the brain structure also lead to changes in the psychological field. The main observed disorders are anxiety and depression. In addition to these, behavioral disorders such as apathy, personality changes, restlessness, hyperactivity, and agitation are also

observed. Paranoid delusions and suicidal thoughts can also be observed in patients with Alzheimer's disease. Living creatures can make sense of and interpret what is happening in their environment and reveal appropriate behaviors if they have sufficient cognitive processes such as attention, memory, perception and language. Neuropathological changes starting from the hippocampus in Alzheimer's primarily manifest themselves with memory impairment. Parallel to the progression of the disease, impairments are observed in other cognitive areas such as attention, executive functions, language, and visual-spatial domain. In later periods, disease related impairment is observed in short-term memory and some tasks related to sensory-motor performance and apraxia and agnosia are observed in the advanced stages of the disease. These impairments in the cognitive and affective domains lead to workplace mistakes, forgetfulness, disturbances in focusing attention, language functions, time-space orientation, exploration and abstract thinking. At the onset of the disease, Alzheimer's patients who try to hide recall disorders, behaviors such as repetitive movements, losing by putting things in strange places, messing up, going around naked, and getting lost by not being able to adapt to the place may occur over time. Important types of dementia are Vascular Dementia, Lewy Body Dementia, Frontotemporal Dementia and Alzheimer Dementia.

2.2. Vascular Dementia

Vascular dementia is one of the most common forms of dementia that is caused by damage to blood vessels and reduced blood flow in the brain. Vascular dementia MRI imaging was shown in Figure 2. Dementia can be caused by a single stroke, as well as many mini strokes known as transient ischemic attacks that occur over time. Vascular dementia patients can be examined in 3 main groups as multi-infarct dementia, post stroke dementia and Binswanger's disease (subcortical vascular dementia). Focal neurological signs such as partial paralysis, numbness sensations, impaired gait coordination, speech or swallowing disorders occur in the early stage or immediately after an infarction.

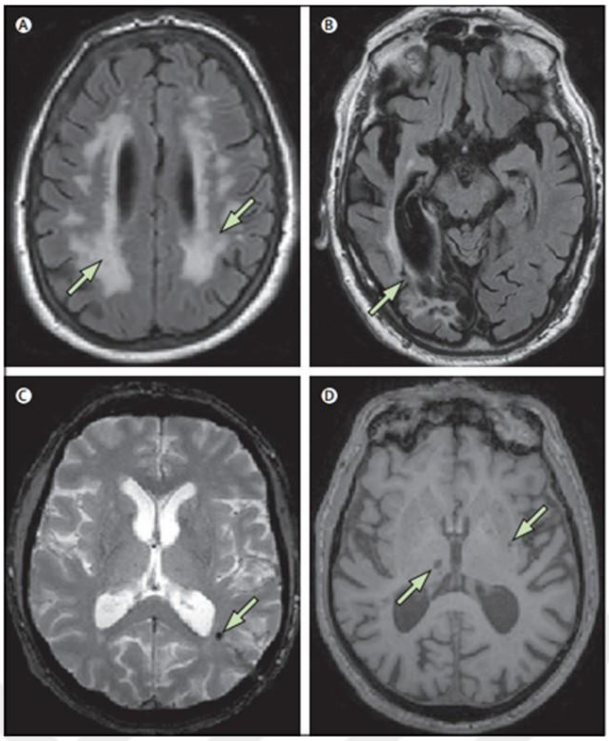

Figure 2. Vascular dementia MRI imaging was demonstrated [19].

White arrows show **A)** white matter lesions, **B)** cortical infarctions, **C)** microbleed and **D)** lacunar infarcts respectively.

It is not possible to repair brain regions damaged by infarctions. With a targeted treatment, in most cases, the disease process can be positively affected, and the independence of the patients can be preserved for a long time.

2.3. Lewy Body Dementia

Lewy Body Dementia is a common form of dementia that cognitive involvement, extrapyramidal system involvement, and psychiatric symptoms may occur simultaneously or at different stages of the disease. According to their degenerative dementia and neuropsychological features, Parkinson's disease and Parkinson's disease dementia can be included in this form. Slowness in movements, falling out of bed, falls or tendency to fall, having vivid dreams while asleep at night, mobility during sleep, syncope, having hallucinations, sphincter control disorder are main symptoms in terms of diagnosis. Acetylcholinesterase inhibitors are used in Lewy body dementia patients who have significant cholinergic deficiency in their brains. Lewy body dementia histological data was shown in Figure 3.

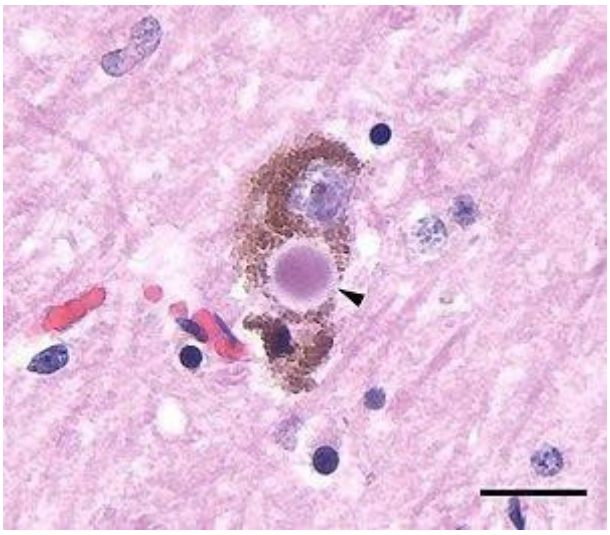

Figure 3. Immunohistochemical data demonstrates Lewy body dementia [20].

Black arrow shows Lewy body in a substantia nigra.

2.4. Frontotemporal Dementia

Frontotemporal dementia is defined as asymmetrical or symmetrical hypometabolism and atrophy in the anterior temporal and frontal region. The registration of objects can be done better multimodally and can be recalled. Utilization behavior, depression, decrease in speech and reasoning, agrammatism, difficulty understanding things they hear, behave childishly are indications that distinguish them from the other forms. There is no accepted treatment approach for cognitive symptoms for frontotemporal dementia, however serotonin reuptake inhibitors may benefit behavioral symptoms based on the studies.

For the diagnosis of dementia, a detailed history and neurological and mental examination are required. Complete blood count, biochemistry, thyroid, liver-kidney function tests, vitamin B12 examination, cranial magnetic resonance imaging should be viewed. Neuropsychological tests can be used for staging, monitoring development and distinguishing the disease from other diseases. There is no effective treatment for dementia, the disease-modifying, symptomatic and preventive treatments are used, and studies are ongoing on new treatment options. Currently available treatments are focused to slow the progression of the disease and manage symptoms. Stopping or slowing down cognitive and functional impairment, treating behavioral and psychiatric symptoms,

reducing the caregiver burden and delaying nursing home and hospital admissions are expected from the treatment of dementia.

The risk factors of the most common type of dementia, AD, and dementia are including advanced age, female gender, low education level, family history, head trauma history, vascular events, aluminum and electromagnetic fields. Alzheimer's disease is the most common form of dementia, approximately 70% of all people with dementia [21]. Therefore, in this thesis, we focused on Alzheimer Disease.

2.5. Alzheimer's Disease (AD)

Alzheimer's disease (AD) is a chronic neurodegenerative disease known since 1907 and is the most common type of dementia. It is estimated that 4.6 million new AD cases develop worldwide each year. It was first described in 1907 by the German physician Alois Alzheimer with its clinical and neuropathological features [21]. However, the exact physio pathogenesis has not been revealed yet. The risk of developing the disease increases logarithmically with age. The incidence in the population between 60-65 years of age is approximately 0.1%, while the incidence increases up to 47% over the age of 85. It is predicted that more than 25% of the world population will be over 65 years old in 2050 [22]. These figures give the impression that AD, which increases logarithmically with the increasing elderly population, will be one of the most important health problems in the future. In a study reported from our country in 1999, it is estimated that the number of Alzheimer's patients in Turkey is over 200,000 [23]. In this review, the factors suggested in the physiopathology of AD, which is now becoming a serious public health problem, the histopathological findings of AD and the risk factors thought to be associated with AD will be reviewed.

In a histopathological manner, there are senile amyloid plaques, neurofibrillary tangle (NFTs) formation, synapse-neuron loss, and significant atrophy in the brain [24]. The key component of NFTs is hyperphosphorylated Tau protein. In the pathogenesis of AD, hyperactive kinases and / or hypoactive phosphatases cause hyperphosphorylation of the tau protein, impairing its ability to bind to microtubules. Unbound phosphorylated tau polymerizes into insoluble double stranded filaments. These become intraneuronal NFTs over time. NFTs cause cell death by disrupting the integrity of the cytoskeleton and

axonal transport. The second major neuropathological change in AD is amyloid plaques, the key component of which is amyloid beta protein (Aβ). Amyloid β aggregates into diffuse plaques and these become dense neuritic plaques. It is not clear how loose plaques containing harmless amyloid develop into harmful dense plaques with folds. Amyloid β formation is shown in Figure 4. Amyloid β accumulation in loose plaques may lead to the formation of oxidative stress and free radicals, and these factors may lead to physical change of plaques. After the neuritic plaque is formed, the secondary stage of excitotoxicity, inflammation and possibly apoptosis mediates additional damage.

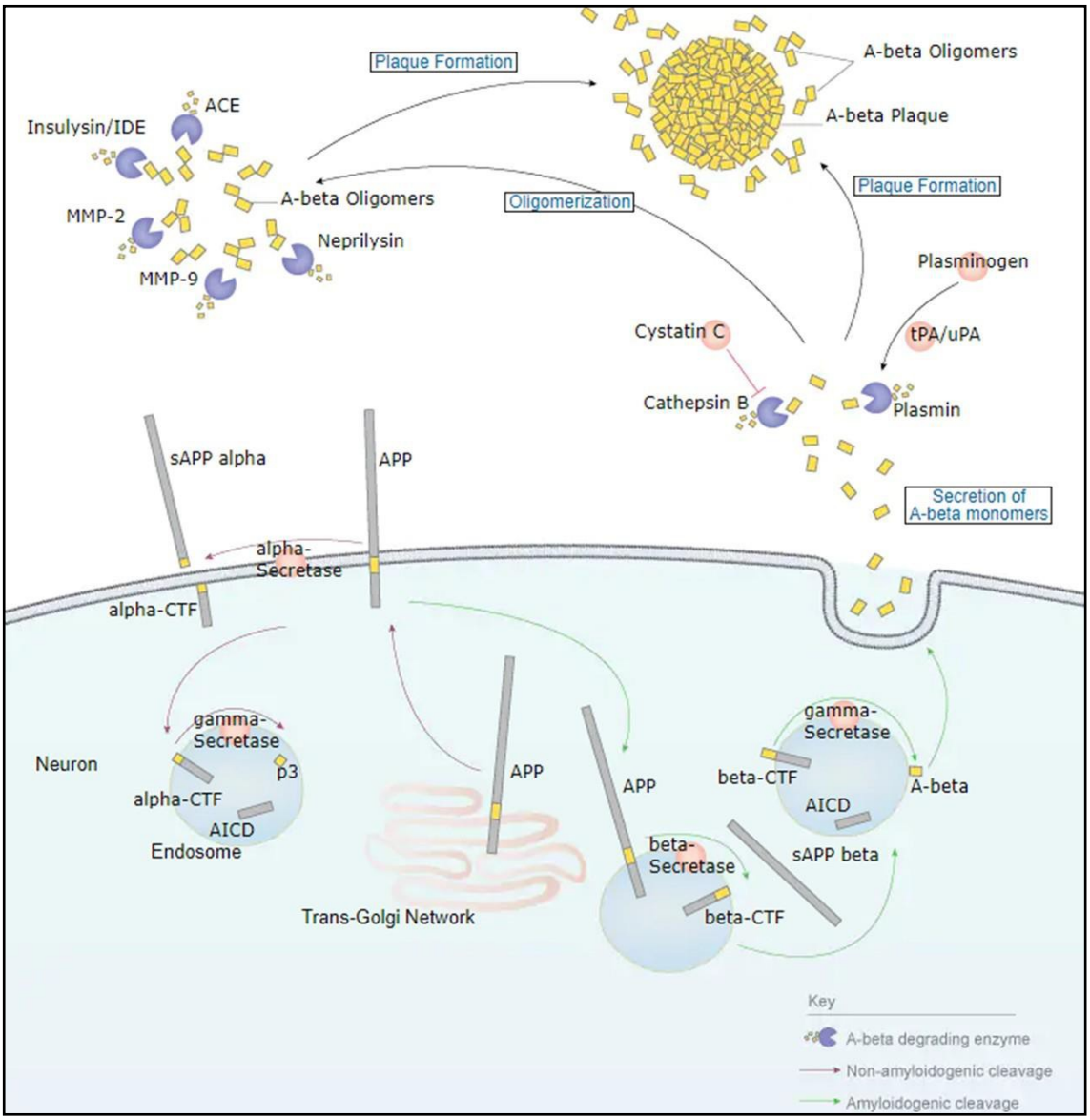

Figure 4. Amyloid ß plaque formation [24]

Increased amyloid β42 and amyloid β oligomers are found in brain tissues and cerebrospinal fluid (CSF) of patients with early dementia, and these levels are correlated with cognitive decline [25]. Lipid peroxidation may also have a significant role in AD. The severity of cognitive deterioration in AD is correlated with the amount of NFTs rather than amyloid deposition. Some studies suggest that the primary neurotoxic formation is mutant tau protein rather than NFTs. On the other hand, both NFTs and amyloid plaques may not be mediators of AD pathogenesis, but also end products. All risk factors and pathological products associated with AD may impair neuroplasticity. If plasticity cannot be achieved, NFT formation in free tau polymers and accumulation of Aβ in loose plaques and subsequent transformation into solid plaques will increase.

Neurotoxins, glutamate, organic solvents, industrial dyes, some metals such as aluminum (Al), iron, copper, zinc and lead have been suggested to be associated with the risk of AD. In addition, AD was detected more frequently in areas with high Al drinking water. However, subsequent controlled studies did not detect an increase in Al in the brains of Alzheimer's patients, and aluminum-reducing treatments were unsuccessful in the treatment of AD. Glutamate is an excitotoxin that acts on *N*-Methyl-d-aspartic acid or *N*-Methyl-d-aspartate (NMDA) and increases intracellular free radicals. Glutamate is an excitatory toxic amino acid. It is shown among the risk factors for AD and is accused in the pathogenesis. It has been suggested that glutamate regulates the intracellular calcium transfer via NMDA and keinetin receptors, and that excess glutamate causes more calcium and water to enter the cell and has a toxic effect on the neuron. There are studies that organic solvents can also facilitate glutamatergic toxicity.

Cholesterol is closely related to the formation of amyloid plaques in AD. A relationship has been shown between cholesterol level and excess APP formation. On the other hand, there are studies suggesting that besides their lipid -lowering properties, antioxidant, nitric oxide-mediated effects, antiaggregant and anti-inflammatory effects of statins may be effective in preventing AD and suggesting that lowering cholesterol level with statins reduces the risk of AD by 70%. The relationship between vascular factors and AD has become increasingly important in recent years. Different results have been reported in terms of the relationship between blood pressure and AD. It has also been suggested that mutations and polymorphisms in the angiotensin converting enzyme gene

may be risk factors for the development of AD. High homocysteine significantly affects arterioles. It is associated with endothelial dysfunction, oxidative damage, nitric oxide level changes, and cerebral microangiopathy. In a sub-branch of the Framingham study, high homocysteine was found to be an important risk factor in patients without dementia who developed AD after 8 years of follow-up. Increased homocysteine-induced endothelial toxicity and NMDA receptor inhibition are observed in vitamin B12 deficiency. Also, the presence of stroke is an important risk factor for AD as well as vascular dementia. On the other hand, there are studies suggesting that diabetes is not a risk or a low risk for AD. The risk of AD was found to be higher in type 1 diabetes than in type 2. The relationship between diabetes and AD has been tried to be explained by findings such as hyperglycemia may increase amyloid deposition and aggregation, and the presence of glycosylated end products in NFTs. Forward glycosylated proteins may increase amyloid toxicity and facilitate NFT formation. Hyperglycemia suppresses the production of acetylcholine by affecting the use of glucose in the brain. On the other hand, it is thought that neuronal damage due to hypoglycemia, which can develop frequently in diabetics, may facilitate the development of dementia. It is not yet known whether diabetes is a real risk factor or indirectly increases the risk of AD with the vascular problems it causes and accompanying hypertension and dyslipidemia. Vitamin E and C, carotene deficiency may be associated with AD.

In the Rotterdam Study, it was observed that the development of dementia was less in individuals who received high vitamin E and C and carotene at a five-year follow-up. Oxidative damage mediated by free oxygen radicals is one of the main neurodegenerative mechanisms in AD. The intense oxidative stress of the whole brain, especially NFTs, is involved in the pathogenesis of AD. In AD, there are free radical sources, iron ions that have +2 charge (Fe^{2+}), activated microglia, amyloid β, advanced glycosylation products, and mitochondrial anomalies in the brain. In addition to the publications that advocate the use of many antioxidants to be protective and therapeutic in AD, a 2003 study found that dietary or external intake of vitamins A, C and E did not reduce the risk of AD. There are studies showing that the development of AD in smokers increases, decreases and does not change. Although it is said that nicotine can increase cognitive functions in experimental studies, clinical improvement with nicotine has not been seen in controlled studies. It is thought that nicotine may inhibit amyloid β aggregation. The nicotinic receptor modulation of galantamine used in the treatment also

supports this pathogenesis. However, the EuroDEM study, which is a prospective study, reported that smokers had more AD.

The presence of a long pre-symptomatic phase of AD seems very appropriate for the implementation of prevention interventions in this period. In order to evaluate the efficacy of such treatments, it will be necessary to detect neuropathological anomalies that develop before cognitive changes occur. To assist in this regard, the use of Pittsburgh compound B (PiB), a thioflavin T derivative, has been reported to provide imaging of amyloid β in persons with AD and in some elderly persons without dementia. Accordingly, AD can be detected preclinically and clinically by imaging with PiB. In the future, it may be possible to visualize tau deposits in the living human brain. In the treatment of AD, the inhibition of the toxicity of plaque-Aβ protofibrils or Aβ oligomers outside/inside neurons using vaccine or ligand, reduction of secretases that mediate the cleavage of APP in amyloidogenic direction and stimulation of non-amyloidogenic secretases, modulation of secretases, modulation of kinases involved in possible tau aggregation and phosphatases are investigated.

There are many factors suggested for the etiology, pathogenesis, physiopathology, biochemistry and histopathology of Alzheimer's disease, but none of them have been clearly revealed yet. In the brains of patients with AD, extracellular amyloid-β, intracellular neurofibrillary tangle accumulation and concomitant neuronal loss are in question. However, the cause of neuronal death in the brain in AD is unknown. Abnormal processing of the amyloid-β protein probably plays a central role in the pathogenesis of AD. However, the severity of cognitive deterioration in AD correlates with the amount of NFT rather than amyloid deposition. Understanding the link between amyloid and NFTs is absolutely essential to understanding the pathophysiology of AD. Both NFTs and amyloid plaques may be outcome products, not mediators, of AD pathogenesis. Many studies on this subject are still in progress.

2.6. The Effect of Genetic Factors on The Development of Alzheimer's Disease

It has been demonstrated by epidemiological studies that Alzheimer's disease is not dependent on a single factor. Neuronal and central vascular disorders have a very important role in the development of the disease due to aging. Factors such as head trauma, metabolic lesions and infections also increase the risk of AD. Recently, it has been suggested that feeding with a high amount of saturated fat increases the risk of developing Alzheimer's. On the other hand, in some cases, genetic predisposition has a significant contribution. Genetic factors are shown in Figure 5 to develop AD.

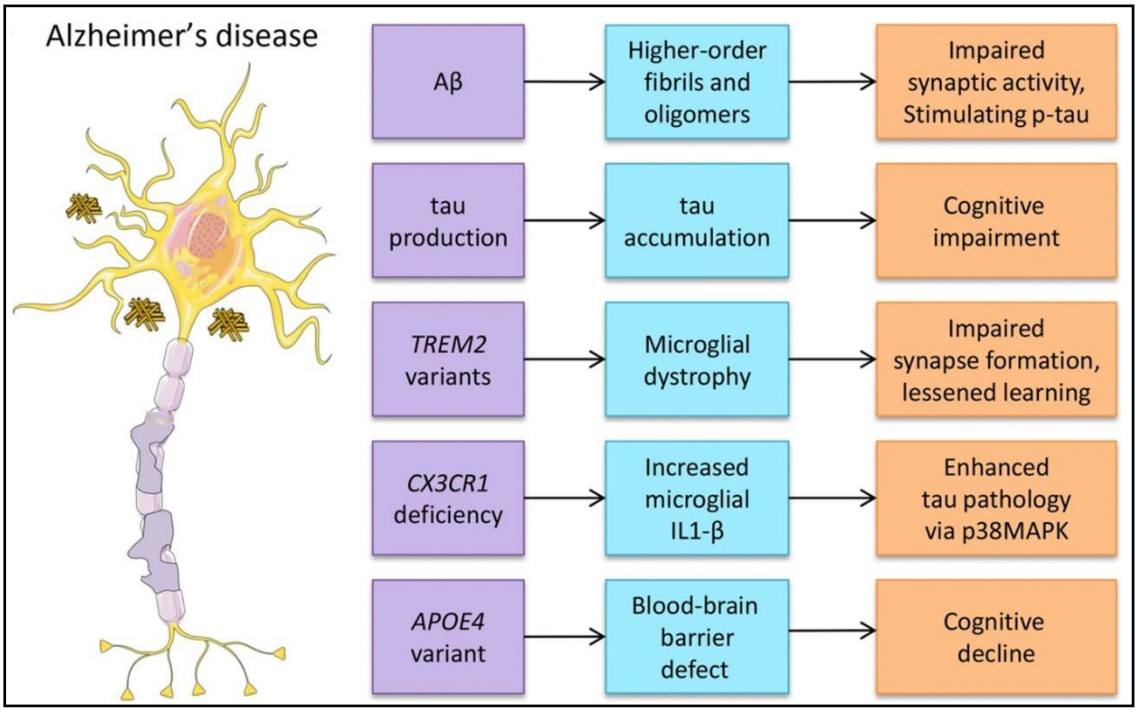

Figure 5. Effect of gene expression on AD [26].

Identifying the familial forms of AD and learning the responsible genes has enabled a better understanding of the pathophysiology of AD. Genetic factors in AD are mostly risk factors that constitute a predisposition to environmental factors for the development of the disease. In other words, AD occurs with a complex interaction of genetic and environmental factors. Most of the cases with AD are sporadic. However, less than 1% of all Alzheimer's cases have the feature of being hereditary (simple Mendelian autosomal dominant inheritance) [27].

Autosomal dominant AD typically begins early (presenile), before the age of 65 [28]. The age of onset can go down to the 20s. AD is a polygenic and multiallelic disease that also shows heterogeneity in terms of Mendelian transmission. It means that numerous different mutations of gene loci in more than one chromosome lead to the same disease. Three distinct genes responsible for autosomal dominant inheritance have so far been found: amyloid precursor protein (APP) gene (21st chromosome), presenilin 1 (PS1) gene (14th chromosome) and presenilin 2 (PS2) gene (1st chromosome) [29]. All three proteins encoded by these genes are transmembrane proteins whose normal functions are not well known, and they are suggested to play a role in neuronal plasticity. All the mutations in the genes mentioned lead to an increase in the production of the longer form (Aβ 42) accumulated in the amyloid plaques by not being discarded of the Aβ protein metabolized from APP. Although families carrying APP gene mutations are very few, they shed light on the pathogenesis of the disease through amyloid metabolism and also illuminated the relationship between Down syndrome and AD. As it is known, people with Down syndrome carry three copies of the 21st chromosome (Trisomy 21). These patients almost always begin to show the neuropathological changes of AD from the age of 30. People with Down syndrome produce a lot of APPs due to the presence of an extra copy of chromosome 21. Mutations in the APP gene are responsible for a minority of familial Alzheimer's disease (FAD). Mutations in PS1 are the most common cause of familial AD. More than 160 mutations have been identified in the PS1 and PS2 genes. Presenilins are central components of atypical aspartyl protease complexes responsible for cleavage of APP by γ-secretase (gamma secretase). Presenilin mutations increase the amyloid β 42/amyloid β 40 ratios. This change is probably due to the change in function. Amyloid β 42 deposition is an early finding in the preclinical stage of PS1 mutations. Studies in transgenic animal models suggested that decreased γ-secretase activity may induce hyperphosphorylation of tau without amyloid β deposits.

Accordingly, studies in familial AD cases form the basis of the amyloid cascade hypothesis, suggesting that the increase in amyloid β 42 is responsible for all AD cases, NFT formation, neuron degeneration, and dementia are related to and following amyloid β 42 formation. The mutations identified so far constitute up to 50% of familial AD. For the remaining 50%, genes that are not yet found and forms of autosomal non-dominant inheritance are suggested.

In addition to classical risk factors such as age, female gender, family history and Down Syndrome, many minor risk factors such as hypertension, diabetes mellitus, dyslipidemia, low education level, low socioeconomic level, exposure to neurotoxins such as aluminum, iron, copper, lead, estrogen deficiency, menopause, inflammation, oxidative damage, nutritional deficiencies, high homocysteine, vitamin B12 deficiency, hypothyroidism, stroke, and infections have begun to be defined in recent years. In a study conducted in Turkey, the risk factors for the development of AD were examined in our country and it was determined that female gender, age, vitamin use and presence of depression were independent risk factors in the logistic regression analysis. The accepted and established risk factors for AD are age, female gender, and family history. It has been reported in many studies that AD is more common in women. Although it has been suggested that women live longer or generally have lower levels of education and it increases this rate, it has been confirmed that more women are affected by dementia than men according to logistic regression analysis models where age and education factors are eliminated. Factors such as estrogen deficiency and age at menopause have been investigated, but no clear relationship has been demonstrated. The role of estrogen deficiency and menopause in AD is unclear. It has been reported that estrogen deficiency can affect cerebral blood flow, stimulation of neurons, development of glial cells and expression of Apolipoprotein E (ApoE). ApoE' s effects on brain for AD development and therapy options are shown in Figure 6. It has been suggested that estrogen may delay the age of onset of dementia by inhibiting the expression of E4 allele in ApoE4 heterozygotes.

In the Women's Health Initiative study published in 2003, it was determined that the risk of dementia increased in individuals using estrogen, and it is not recommended for the treatment or prevention of AD. Family history is very significant as the risk of AD. The presence of AD in a first-degree relative increases the risk of developing this type of dementia by 3 times. Additionally, low education level, has been reported in many studies as one of the possible risk factors in AD. Also, there are studies that both support and do not support the connection between head trauma and AD.

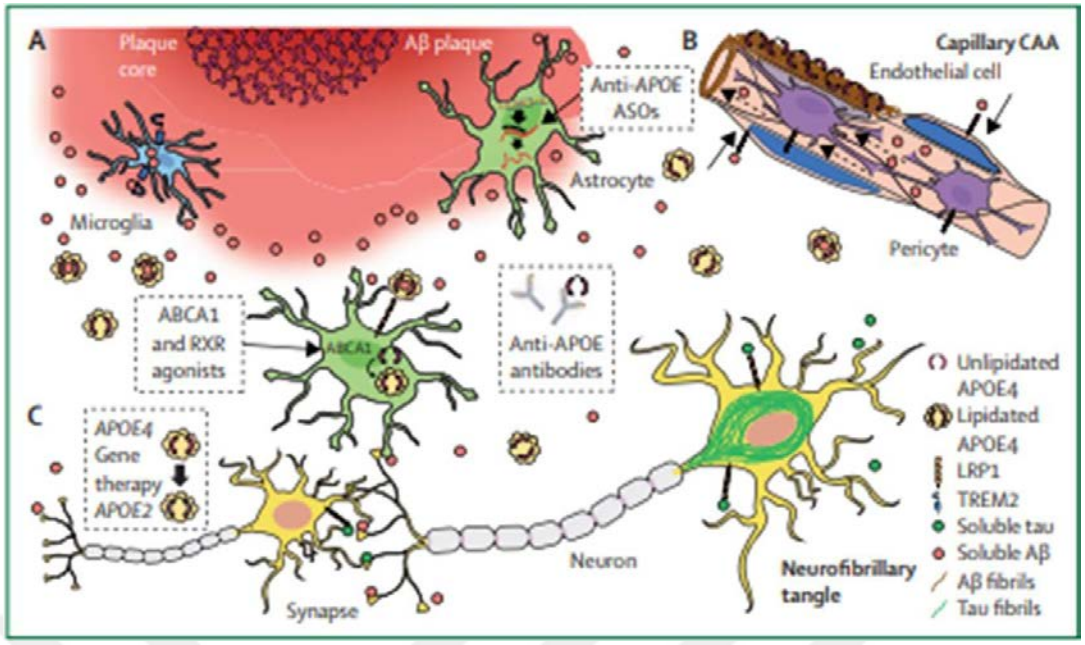

Figure 6. Effects of ApoE on brain for AD development and potential strategies to decrease ApoE4 [30]

In addition to all these, It has been shown in recent years that ACE polymorphisms may be associated with the development of AD.

2.7. Angiotensin-Converting Enzyme (ACE)

Angiotensin converting enzyme (ACE) is a zinc metalloprotease widely distributed on the surfaces of epithelial and endothelial cells. Angiotensin converting enzyme is involved in the atherosclerotic process by converting angiotensin I to angiotensin II (in Figure 7), causing vascular hypertrophy and vasoconstriction.

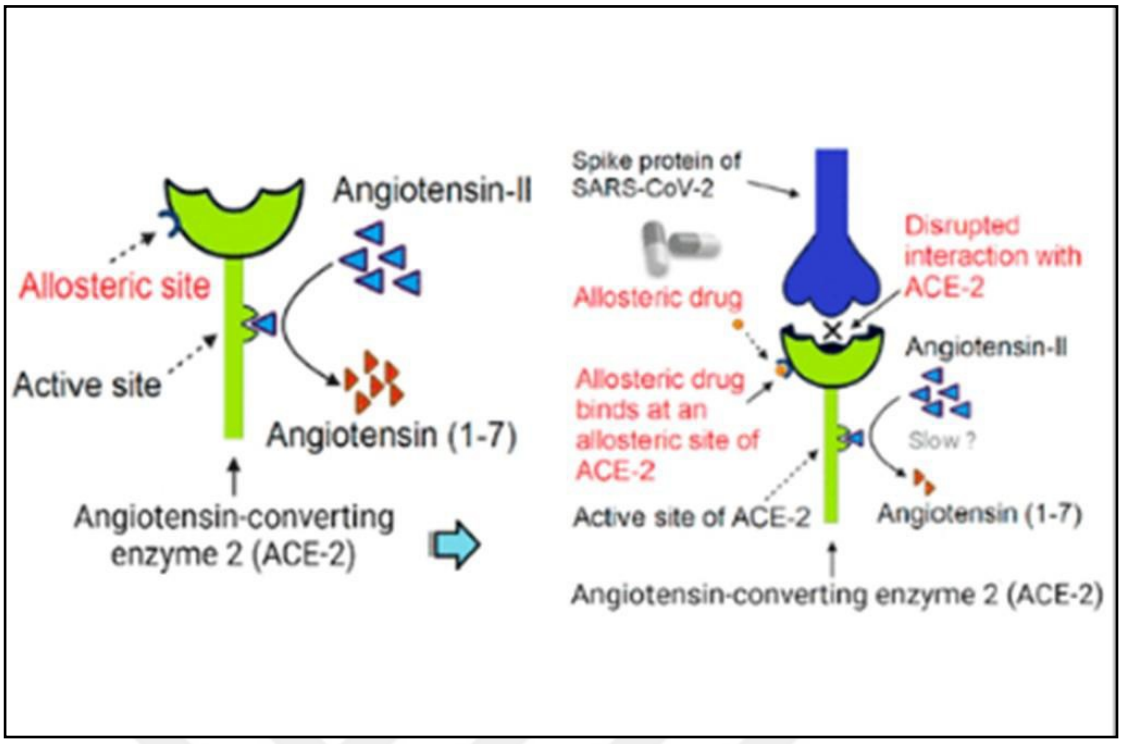

Figure 7. Allosteric site of ACE [31].

ACE is responsible for the reduction of bradykinin, which is considered to have a vasodilator effect by stimulating nitric oxide production. The angiotensin converting enzyme gene consists of 26 exons, occupies 21 kb on chromosome 17, and encodes a protein of 1306 amino acids (in Figure 8).

Figure 8. ACE genomic regions, transcripts, and products [32].

The polymorphism that occurs with the presence (I allele) or absence (D allele) of the repeat sequence at the 287th base pairing in intron 16, which is common in this sequence, is mentioned. The genetic polymorphism of the angiotensin converting enzyme plays a decisive role in ACE activity. For instance, when individuals homozygous for the D allele are compared with those homozygous for the I allele, ACE activity has been shown to increase by 56% in those carrying the ACE D/D gene. Individuals with the angiotensin converting enzyme I/D genotype show moderately increased ACE activity. It has been suggested that the I/D gene polymorphism of the angiotensin converting enzyme is a risk factor for a number of diseases such as myocardial infarction, stroke, diabetic nephropathy and hypertension in different ethnic groups. Effects of ACE polymorphisms on clinical results are shown in Figure 9.

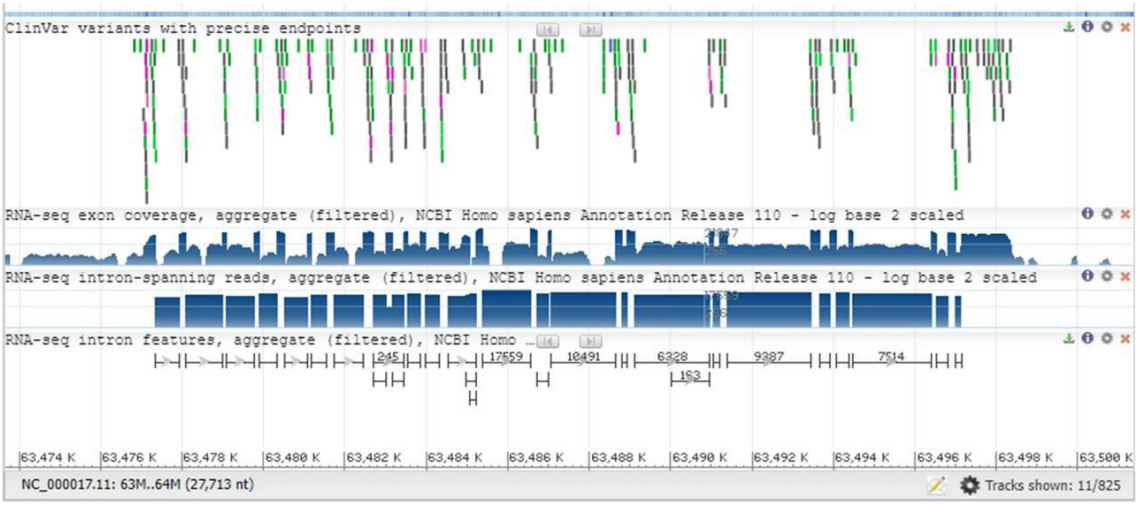

Figure 9. ACE variants show clinical effects with precise endpoints. https://www.ncbi.nlm.nih.gov/gene/1636 [33].

Although hypertension is a very prominent risk factor for vascular dementia, studies on the effect of hypertension in ATD bring different observations. While hypertension did not appear to be a risk factor in some of these studies, it was reported that a history of high systolic blood pressure and low diastolic blood pressure increased the risk in some studies. In the Rotterdam study, there was no significant reduction in the incidence of ATD, while the risk of vascular-type dementia was reduced in those taking antihypertensive drugs from baseline. It has also been reported that ACE I/D gene polymorphism poses a risk for ATD.

2.8. The Relationship of ACE with Alzheimer's Disease

The insertion and deletion polymorphism of the ACE gene was determined by the presence or absence of a DNA fragment of 287 base pairs in size. The presence of the D allele means higher levels of ACE and therefore more destruction of neurotransmitters such as dopamine. The expression of the ACE gene is still largely unknown, although it is emphasized that it may be tissue specific. The ACE gene is localized on chromosome 17 and 287 base pairs in the intron 16 of the gene.

This polymorphism occurs by repeating some of it. In this polymorphism, ACE D/D and I/I are homozygous, while ACE I/D is heterozygous. This insertion in the ACE gene reduces the expression of ACE, so D/D homozygotes have 65% more ACE and I/D heterozygotes 31% more than I/I homozygotes. Low ACE activity observed in I alleles,

the increase in the half-life of bradykinin and the decrease in angiotensin II production emphasize the physiological importance of the ACE genotype due to the increased vasodilation due to the endothelium. ACE substrates are catalyzed by ACE are shown in Figure 10.

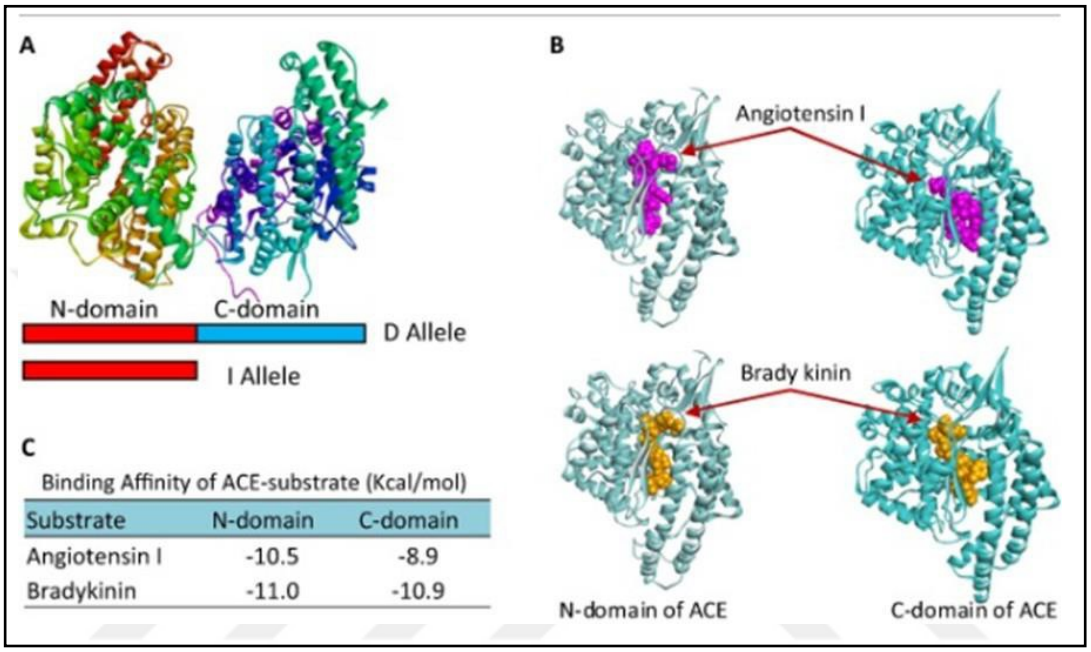

Figure 10. ACE substrates are that Angiotensin I and Bradykinin are interacted by the same domain of ACE [34].

It has been reported that there is a relationship between the ACE I / D polymorphism and the development of many pathological conditions such as coronary heart disease, ventricular hypertrophy, myocardial infarction, restenosis after coronary angioplasty, cardiomyopathy, and sudden cardiac death. It has been found that there may be a relationship between coronary artery disease and the D allele in the Turkish population. It has been reported that in individuals with DD genotype, there is an increase in the decrease in left ventricular systolic performance, progressive left ventricular dilatation, and mortality in patients with heart failure compared to those with the II genotype. However, there are obvious ethnic differences. Studies have shown that plasma ACE activity is higher in subjects with ACE D alleles than in persons with ACE I alleles. In a study conducted in the Turkish population, it was concluded that DD genotype is a predisposing factor and ACE I/D polymorphism may act as one of the independent factors on hypertension in patients with severe hypertension with a positive family history. In

addition to studies reporting an association between ACE genotype and diastolic blood pressure in men, there are also studies reporting that there is no relationship between ACE genotype and blood pressure. In another study conducted in Turkish population, no relationship was found between hypertension and ACE I/D polymorphism in Type II diabetic patients. There are studies reporting a role for the ACE D allele in cases of acute cerebral ischemia and sarcoidosis. It is also reported that there are structural changes in the vascular wall according to the ACE genotype. There are studies reporting a relationship between serum ACE activity and myocardial infarction and coronary artery disease. Another study reported that hemodialysis patients with ACE DD genotype are at high risk for hemodialysis.

Angiotensin converting enzyme has a significant place in the renin-angiotensin system catalyzes the formation of angiotensin II from angiotensin I. The role of angiotensin II in the circulatory system has been extensively studied, but it has been revealed that each organ has its own renin-angiotensin system like paracrine system and organ-specific functions of this system. It has also been indicated that it suppresses acetylcholine release in the brain and catalyzes the destruction of neurokinins, a family of neurotransmitters involved in central nervous system functions such as pain transmission and emotion regulation.

When there is a loss of water and salt or in the event of sympathetic activation, renin release from the juxtaglomerular apparatus of the kidney occurs. This converts the angiotensinogen synthesized in the liver to angiotensin I. Angiotensin I, on the other hand, turns into angiotensin II with ACE, which is present in the endothelium of many vessels and in high concentrations in pulmonary vessels. Angiotensin II stimulates aldosterone secretion from the adrenal cortex and is a powerful vasoconstrictor. Aldosterone causes retention of sodium ion. Angiotensin II is converted to angiotensin III by aminopeptidase A (Angiotensinase). Angiotensin III is a powerful stimulator in adrenal production of aldosterone. The endothelial cells of all mammalian tissues express ACE on their luminal faces and secrete in soluble form. Lung and brain microvascular and capillary cells and intestinal, choroid plexus and renal tubule cells are rich in ACE.

In the male reproductive system, ACE was found in seminiferous and epididymal tubules and sperm secretion was found. ACE is a metallopeptidase enzyme with zinc in its active center, which, in addition to converting angiotensin I to angiotensin II, provides degradation of bradykinin, a vasodilator on the other endothelial surface and consequently important in vasoactive peptide metabolism. In addition, bradykinin inhibits the proliferation of vascular smooth muscle cells and inhibits the release of vasodilators such as nitric oxide and prostacyclin from the endothelium. The success of ACE inhibition in the treatment of hypertension and congestive heart failure is mentioned, because the vasodilator plays a primary role in the inactivation of bradykinin and the importance of this event in blood pressure and electrolyte homeostasis. Consequently, ACE plays an important role in the regulation of the cardiovascular system and blood pressure.

ACE causes thromboembolism as a result of its potent vasoconstrictive effects, decreased fibrinolysis, platelet activation and aggregation. Although a direct functional relationship with ACE concentrations could not be determined, those with DD genotype had the highest plasma ACE activity and those with II genotype had the lowest plasma ACE activity. Moderate levels are seen in those with the ID genotype. ACE gene polymorphism is also associated with an increase in vascular tone, a decrease in fibrinolysis, and an increase in platelet aggregation.

3. MATERIAL AND METHODS

Angiotensin converting enzyme insertion (I) and deletion (D) polymorphism rs 1799752 of the ACE gene is associated with a significant genetic effect with the inter-individual variability of plasma ACE concentration. ACE activity in the cerebrovascular system increases due to aging and Alzheimer's disease. This study aims to evaluate the effect of ACE gene polymorphism on the risk of developing late onset dementia in the Turkish and Mediterranean populations. According to this purpose, the procedures were followed.

3.1. Participants and Data Collection

Collecting blood sample period from patients with dementia and healthy volunteers as control group at İstanbul University, Medicine Faculty Department of Neurology. The collected blood samples were stored at + 4^{o}C.

3.2. qPCR and SNP Analysis

Approximately 350 ml of peripheral blood was drawn into 5 ml EDTA tubes from patients with Alzheimer’s Disease. For DNA isolation, iPrep DNA (iPrep Pure link, invitrogen) isolation robot was used. Concentrations of DNA obtained was determined by NanoDrop (Thermoscientific) device. For purity measurements, measurements were made spectrophotometrically with the NanoDrop device. Isolated DNA samples were kept at + 4^{o}C until the experiment day. In order to detect ACE mutations, Applied Bioscience 7500 Fast Real Time device was used for Real Time PCR (polymerase chain reaction) and special primer sequences were used to identify possible mutations in target variations. The test is based on the multiplex mini sequencing, which is also called SnapShot sequencing principle.

Genetic regions in the CVD Panel are amplified by 3 separate multiplex PCRs. Following the purification of the PCR products, the CVD mutation primers in the test system were taken into 3 separate multiplex mini sequencing reactions. Fluorescently labeled ddNTPs in the environment were added to the specific mutation primer designed separately for each mutation, thanks to the polymerase enzyme, and the primer elongation

reaction ends. The ddNTPs added to the mutation primers designed to detect each CVD mutation in the test system give the fluorescence signal of the Normal and / or Mutant nucleotide, depending on the presence or absence of the mutation. The imaging and analysis of the signals were done in the Capillary Electrophoresis device. After the PCR Mix and Primer Mix are thawed, they should be vortexed and spun. The PCR Mix, ddH2O, DMSO, CVD Primer Mix, Taq DNA Polymerase and DNA were combined in 0.2 ml PCR tubes at the following ratios, vortex and short spin.

Table 1. PCR reaction conditions

Component	**Amount (µl)**
ddH2O	15,6
PCR Master Mix	4,2
DMSO	2
CVD Primer Mix	1
Taq DNA Polymerase	0,2
DNA	2

Table 2. Prepared tubes were placed in the PCR device and the following program was run

95°C	3 min	
95°C	30 sec	X 15
55°C	45 sec	
72°C	60 sec	
95°C	30 sec	X 20
58°C	45 sec	
72°C	60 sec	
72°C	10 min	
4°C	∞	

3.3. Exo-Sap PCR Purification

It is recommended to use Exonuclease I + SAP (Shrimp Alkaline Phosphatase) for purification of products after PCR reaction. For each sample, 0.5µl Exonuclease I, 1 µl rAPid Alkaline Phosphatase and 8 µl PCR product were mixed in separate PCR tubes, vortexed and short-spun. The tubes were placed in the PCR device and the program below was run:

Table 3. Exo-Sap protocol in PCR device

37°C	70 dk.
72°C	20 dk.
4°C	∞

3.4. Sanger Sequencing

Purified PCR products were taken to the Sanger Sequencing Reaction. For the reaction, 1.5 µl ddH_2O, 0.5 µl ABI PRISM® SNaPshotTM Mix, 1.1 µl CVD MSQ Primer Mix and 1.1 µl purified PCR product were placed in individual PCR tubes for each sample, vortexed and short-spun.

Table 4. Sanger sequencing reaction conditions.

ddH2O	1.5 µl
MSQ Mix	0.5 µl
MSQ Primer Mix	1.1 µl
Purified PCR product	1.1 µl

Table 5. Cycle sequencing program conditions in PCR device (to be used for further Analysis using Sanger Sequencer)

96°C	10 sec x 25
50°C	5 sec x 25
60°C	30 sec x 25
4°C	∞

Table 6. Cycle Sequencing reaction products were loaded into Applied Biosystems 3500xL under the following conditions

HI-DI Formamide	10 µl
Mini Sequencing Product	1.2 µl

DS-01 Matrix Standard is defined in the device. It was studied in the Applied Biosystems 3500xL 24-Capillary electrophoresis device. The signals obtained as the result of the capillary electrophoresis were analyzed in accordance with the Table 7.

Table 7. The reference for order of mutations in the electropherogram

Region	Nucleotide changes	Observed on the device	Evaluation
ACE	**G>A**	**G>A**	**G: Deletion A: Insertion**

G: the blue luminous peak observed

A: the greenluminous peak observed

3.5. Biostatistics and Meta Analysis

Our experimental results for the II, ID and DD genotypes in patients vs healthy control groups were analyzed using Fisher's exact test with SPSS 25.0.

Since the samples used in our study are few in number, we considered the possibility that they do not reflect the Turkish population. In order to reflect the Turkish population, two similar studies have been identified in the literature with samples obtained from the Turkish community. Meta-analysis was performed by combining the data in these studies with our own data. Moreover, data available in the literature were used to determine the association of ACE polymorphism with late-onset Alzheimer's disease in Mediterranean populations. With these data, two scenarios, heterozygous / homozygous and allele-specific mutations, were analyzed with the chi-square test. For meta analysis of II, ID and DD genotypes in patients vs healthy control groups, Chi Square tests were utilized. As a result of the analysis, the chi-square statistics p value and values were calculated with the standard formula.

The data from scientific literature has been collected considering case/control studies, particularly considering cohorts from Mediterranean cohorts with sufficiently high number of participants, typically with 50 or more samples. For meta-analysis, the data from individual cohorts has been aggregated usingthe same groups as well as similar variation profiles.

Considering the discrete nature of the data (number of individuals with specific variant or allele for each group), the collected data both from literature as well as experiments has been formulated as contingency table (rows being groups (AD/control) and columns being variants or alleles) from which Chi2 test has been carried against the null hypothesis that there is no association between groups and variants (Observed = Expected). Cases with p Values less than 0,05 has been considered as statistically significant. Degrees of freedom (DoF) are the measurements of the number of the values in the statistic explain the differences between observed and expected values by using formula (numbers of columns – 1) x (numbers of rows – 1).

For binary outcomes (e.g., with allele counts) the odds ratio (OR) of AD with respect to a specific allele against control has also been calculated using the contingency table. The odds ratios are measurements of the association between occurrence of the outcome of patients and control groups. The OR is calculated as (odds that a case was exposed) / (odds that a control was exposed). An odds ratio equals to 1.0 or close to 1.0 mean that the exposure of patients and control groups are same or similar to each other. Finally, 95% confidence interval for the OR is calculated to estimate standard error.

3.6. Biostatistics

Statistical Analysis: Fisher's Exact test Excel 2018 was used for the statistical analysis of the I and D alleles in patients vs healthy control groups. The Chi square test was used to compare allele fractions and genotypes in the comparison of patient group and control group participants for the metal analysis Chi square test was used to measure the significance of patient and control groups. Significance level was considered as $p < 0,05$.

3.7. Ethics

This thesis working is performed under the Ethics Committee Permission dated 22/04/2021 and Ref: 37068608-6100-15-2133 issued by Yeditepe University Ethics Committee.

4. RESULTS

The patient blood tissues used in this thesis study were obtained from Istanbul University Medical Faculty within the scope of another study. Only Turkish origin patient and volunteer samples were used in this study. Within the scope of the study, 25 Alzheimer's patient samples and 25 healthy volunteer control patient samples were used. However, since epicrisis information about some patients could not be reached in the future, five patients were excluded from each patient and control group. For this reason, our study continued on 18 Alzheimer's patient samples and 20 volunteer control samples. Genomic DNA was isolated from these patient and volunteer blood samples. Then, DNA samples were used for ACE single nucleotide polymorphism (SNP) analyzes in qPCR studies.

Sanger sequencing was performed with these samples in the following process and ACE polymorphisms were determined. Sanger sequencing results were analyzed in terms of different scenarios such as insertion, deletion homozygosity, and heterozygosity, by performing the Fisher’s exact test, to analyze whether ACE polymorphism is associated with late-onset Alzheimer's disease in the Turkish population. Then, using the data in the literature, our findings were compared with the data obtained from the Mediterranean societies genetically and culturally close to the Turkish population by meta-analysis by using Excel. As a result of meta-analysis, it was determined that ACE polymorphisms were not associated with late-onset Alzheimer's in Turkish and Mediterranean populations.

4.1. SNP Analysis Shows ACE Polymorphisms

In our study, 5 ml peripheral blood samples were collected in EDTA tubes from Alzheimer's patients and volunteers. DNA was isolated from 350 ml of peripheral blood using the iPrep DNA isolation robot (iPrep Pure link, invitrogen). The obtained DNA concentrations were determined with the help of Nanodrop. It has been observed that the isolated DNAs are suitable for use in further studies in terms of purity and concentration.

In order to detect ACE mutations, Applied Bioscience 7500 Fast Real Time device was used with Real Time PCR (polymerase chain reaction) and special primer sequences was used to identify possible mutations in target variations.

The test is based on the multiplex mini sequencing principle. Genetic regions in the CVD Panel were amplified by 3 separate multiplex PCRs. Following the purification of the PCR products, the CVD mutation primers in the test system were taken into 3 separate multiplex sanger sequencing reactions. Fluorescently labeled ddNTPs in the environment were added to the specific mutation primer designed separately for each mutation, thanks to the polymerase enzyme, and the primer elongation reaction ends. The ddNTPs added to the mutation primers designed to detect each CVD mutation in the test system will give the fluorescence signal of the Normal and / or Mutant nucleotide, depending on the presence or absence of the mutation. The imaging and analysis of the signals were done in the Capillary Electrophoresis device.

4.2. ACE Polymorphisms No Associated Late On-Set Alzheimer Disease at Turkish Population

When the studies in the literature were examined, it has been found that ACE polymorphisms may be related to the symptoms of Alzheimer's disease. In the light of these findings, it has been observed that the peptide cleavage feature of ACE may cause amyloid beta plaques. In the samples collected in our study, ACE polymorphisms were determined by Sanger sequencing method. Sanger sequencing findings were classified as insertion, / deletion, and heterozygous / homozygous. Thus, it was aimed to clarify whether the association of ACE polymorphisms with late-onset Alzheimer's disease occurs on the basis of mutation and in case of single / double allele. Two patient samples used in the study were excluded from the experimental group because they did not work in the SNP analysis. In this context, further studies were carried out with samples obtained from 18 Alzheimer's patients and 20 volunteers. The polymorphism obtained as a result of Sanger sequencing of the patients we used in our study was evaluated in terms of insertion and deletion. Fisher’s exact test was used for these evaluations. The Fisher’s exact test was used to determine whether there is dependence between quantitative or qualitative variables, whether the sample results fit a certain theoretical probability distribution, whether two or more samples come from the same population, whether more than two population ratios are equal to each other, and whether various population ratios are equal to a certain value. used in research. In this context, it was an ideal analysis method for our study. As a result of chi-square analysis, it was determined that ACE polymorphisms had 3.79 chi-square statistical data in our data. Moreover, the p value was

found to be 0.14 p=>0,05, and these polymorphisms were found to have weak significance in late-onset Alzheimer's patients. The analysis is shown in Table 8.

Table 8. The analysis of Data from Sanger sequencing in our study shows that ACE polymorphism is not associated with late-onset Alzheimer's disease.

	II	**ID**	**DD**	**Total Sample**
Alz	4	11	3	18
Ctrl	4	7	9	20
	8	18	12	38
Alz	3,79	8,53	5,68	18
Ctrl	4,21	9,47	6,32	20
	8	18	12	38
				Chi Square
Alz	0,011	0,72	1,27	**3,79**
Ctrl	0,010	0,64	1,14	**P value**
				0,14

The result of the chi-square statistical test analysis was determined as 3,79 and p=>0,05.

Later, we wanted to make our study more specific and examine the relationship of ACE polymorphisms with late-onset Alzheimer's disease in Turkish populations. Because it is known that the relations of societies with environmental factors, cultures can cause changes, albeit small, in their genetic infrastructure. Moreover, two-thirds of dementia cases in the Turkish population are diagnosed with Alzheimer's. In this context, in the next part of our study, it was aimed to determine the relationship between ACE polymorphisms and Alzheimer's disease in the Turkish population. Since the data we used in our study were not sufficient to reflect the Turkish population, it was aimed to elucidate the relationship between ACE polymorphisms and late-onset Alzheimer's disease in the

Turkish population by obtaining data in the literature from similar studies on the Turkish population. In the literature search conducted in this context, it was determined that there were only two studies with similar characteristics [31], [32]. Then, our own data were added to these groups and analyzed again. Likewise, when our data was combined with the data set from the previous studies Durmaz and İsbir. Thus, 153 Alzheimer's patients and 149 control samples were analyzed. As a result of the analysis, the chi-square statistical data was calculated as 0.41 p=0.81. Therefore, the p value was determined as greater than 0.05 and it was determined that ACE polymorphisms were not associated with late-onset Alzheimer's disease in the Turkish population. Analysis results are shown in Table 9.

Table 9. The data obtained as a result of the addition of our data in the study to the data of Durmaz and İsbir show that ACE polymorphism is not associated with late-onset Alzheimer's disease.

	II	**ID**	**DD**	**Total Sample**
Alz	29	82	42	153
Ctrl	32	75	42	149
	61	157	84	302
Alz	30,9	79,5	42,5	153
Ctrl	30,09	77,5	41,4	149
	81	208	120	302
				Chi Square
Alz	0,12	0,08	0,01	**0,41**
Ctrl	0,12	0,08	0,01	**P value**
				0,81

This meta-analysis was carried out on the data of a total of 302 individuals. As a result of statistical analysis, it was determined that ACE polymorphism was not associated with late-onset Alzheimer's disease in the Turkish population. II: Indicates Insertion / Insertion, ID: Insertion / Deletion, DD: Deletion / Deletion. The result of the chi-square statistical test analysis was determined as 0,41 p=>0,05

The mentioned findings include an evaluation result in terms of heterozygous / homozygosity of ACE polymorphisms and their relationship with Alzheimer's disease in the Turkish population. Therefore, it does not provide any information about the relationship between ACE insertion deletions and Alzheimer's patients. Moreover, the number of data increases in the analyzes performed on the allele-specific study scenario. This increases the accuracy of our analyzes by providing the opportunity to analyze with larger data. To elaborate our study, this time our analyzes were allele-specific. In this context, our groups were arranged as showing insertion or deletion in only one allele. Afterwards, an arrangement was made in the form of groups in which the examples we used in our study were mentioned. When we separated the samples in our study as allele-specific insertions and deletions, it was determined that the chi-square statistical value was 1.78 and the p value was 0.18. Therefore, it was determined that ACE polymorphisms in the samples used in our study were not associated with late-onset Alzheimer's on level of allele-specific are shown in Table 10.

Table 10. ACE polymorphisms in the samples used in our study were not associated with late-onset Alzheimer's.

ACE I/D	Patients N=18 n=%	Control N=20 n=%	X^2	OR [CI 95%]	p value
II	4 (%22,2)	4 (%20)	3,79	-	0,15
ID	11 (%61,1)	7 (%35)			
DD	3 (%16,6)	9 (%45)			
I	19 (%52,7)	15 (%37,5)	1,78	1,86 (0,8603-2,2156)	0,18
D	17 (%47,3)	25 (%62,5)			

Homozygotes / heterozygotes or one-specific allele mutations don’t show association with late-onset Alzheimer’s. II: Indicates Insertion / Insertion, ID: Insertion / Deletion, DD: Deletion / Deletion, I: one allele insertion, D: one allele deletion. p=>0,05

Then, these two data including the Turkish population and the data obtained from the samples collected in our study were combined and analyzed. Thus, 153 Alzheimer's patients and 149 control samples were analyzed. When we separated the samples in our study as allele-specific insertions and deletions. Thus, 604 ACE polymorphisms were detected as insertions / deletions. It was determined that the chi- square statistical value was 0,04 and the p value was 0,82. Therefore, it was determined that ACE polymorphisms in the samples used in our study were not associated with late- onset Alzheimer's in the Turkish population and are shown in Table 11.

Table 11. The data obtained as a result of the addition of our data in the study to the data of Durmaz and İsbir show that ACE polymorphism is not associated with late-onset Alzheimer's disease in Turkish population.

ACE I/D	Patients N=153 n=%	Control N=149 n=%	X^2	OR [CI 95%]	p value
II	29 (%18,9)	32 (%21,47)	0,40	-	0,81
ID	82 (%53,5)	75 (%50,33)			
DD	42 (%27,4)	42 (%28,18)			
I	140 (%45,75)	139 (%46,6)	0,04	0,96 (0,8388-1,1507)	0,82
D	166 (%54,25)	159 (%53,4)			

Homozygotes / heterozygotes or one- specific allele mutations don’t show association with late-onset Alzheimer’s in Turkish population. II: Indicates Insertion / Insertion, ID: Insertion / Deletion, DD: Deletion / Deletion, I: one allele insertion, D: one allele deletion. p=>0,05

4.3. ACE Polymorphisms No Associated Late On-Set Alzheimer Disease at Mediterranean Population

In the next part of our study, it was investigated whether ACE polymorphisms were associated with late-onset Alzheimer's disease in Mediterranean populations due to the similarity of cultural and environmental factors. In this context, two scenarios were created again. In the first scenario, we wanted to clarify the relationship between ACE polymorphisms in terms of heterozygosity / homozygosity and late-onset Alzheimer's.

In the second scenario, it was desired to determine the relationship between ACE insertions and deletions specific to the allele and late-onset Alzheimer's. As a result of our research on the literature, data from 12 different studies (Chapman 1998, Keikhaee 2006, Monastero 2002, Nacmias 2007, Palumbo 1999, Panza 2002, Scacchi 1998, Seripa 2003, Zuiliani 2001, Alvarez 1999, Achouri-Rassas 2015, Fekih-Mrissa 2017) on the Mediterranean populations, where ACE polymorphisms in Alzheimer's Disease were studied, were extracted to be used in meta-analysis in conjunction with our data set and other data obtained from the Turkish population by the previous studies undertaken by İsbir and Durmaz. As a result of our first meta-analysis, ACE rs 1799752 polymorphisms were not found to be associated with the occurrence of late onset AD in the Turkish population, so we investigated whether the same polymorphism is associated with late-onset Alzheimer's in regions culturally close to the Turkish population. Thus, ACE polymorphism data in a total of 2041 Alzheimer's and 2244 control samples were analyzed. As a result of the analysis, the chi-square statistical data was calculated as 0.13 and the p value as 0.93. Therefore, as a result of this meta-analysis, it was determined that ACE rs1799752 polymorphism was not associated with late-onset Alzheimer's in the Mediterranean population. Then, allele-specific insertion and deletion analyzes were performed. Thus, it was seen that the data set included 7070 insertion and deletion alleles. As a result of these analyzes, the chi-square statistical value was calculated as 0.104 and the p value as 0.74. In the light of the findings, it was determined that the different ACE alleles at rs1799752 did not have a statistically significant relationship with late-onset Alzheimer's disease in Mediterranean populations, and it is shown in Table 12.

Table 12. The data obtained as a result of the combination of our experimental data with the data of Durmaz and İsbir and other previous studies of Mediterranean populations show that ACE polymorphism is not associated with late-onset Alzheimer's disease in Turk

ACE I/D	Patients N=2041 n=%	Control N=2244 n=%	X^2	OR [CI 95%]	p value
II	327 (%16,02)	345 (%15,37)	0,13	-	0,93
ID	952 (%46,64)	1042 (%46,43)			
DD	762 (%37,33)	857 (%38,19)			
I	1606 (%39,34)	1732 (%38,59)	0,104	0,98 (0,8943-1.0834	0,746
D	2476 (%60,65)	2756 (%61,40)			

Homozygosity / heterozygosity or the presence of one-specific allele do not show association with late- onset Alzheimer's in Mediterranean population. II: Indicates Insertion / Insertion, ID: Insertion / Deletion, DD: Deletion / Deletion, I: one allele insertion, D: one allele deletion. p=>0,05

5. DISCUSSION

This study was conducted on the analysis of prevalence of the Angiotensin Converting Enzyme (ACE) I/D polymorphism in patients with Alzheimer's disease (AD) in comparison with a control group.

The known role of ACE is in the renin-angiotensin system (RAS), whereas the conversion of Angiotensin I to Angiotensin II is catalyzed by ACE and Angiotensin II is a potent vasoconstrictor.

The enzyme ACE is the catalyzer for the conversion of Angiotensin I to Angiotensin II and is localized primarily in the vascular endothelium of the kidneys and lungs. It affects the kidney, adrenal cortex, arterioles, and brain by binding to angiotensin II type I (AT1) and type II (AT2) receptors as an outcome of the conversion of Angiotensin I to Angiotensin II [33]. The contribution of AT receptors is still under investigation, however it has been recognized that vasodilation occurs by nitric oxide generation. Angiotensin II has a half-life of 1 to 2 minutes in the plasma where peptidases degrade to angiotensin III and IV [34].

Angiotensin II has three impacts on the brain. It binds to the hypothalamus, stimulates thirst and increases the water intake. The stimulation of the release of ADH (antidiuretic hormone) by the posterior pituitary is the second effect. ADH or vasopressin effects to increase water reabsorption in the kidney by introducing aquaporin channels into the collecting duct. As the third effect, angiotensin II reduces the sensitivity of the baroreceptor reflex. This reduces the baroreceptor response to a crease in blood pressure, which could counter the purpose of the RAAS(the renin-angiotensin-aldosterone system) [34], [35]. Angiotensin IV, one of the degradation products of Angiotensin II, is a hexapeptide with a wide spectrum of activity in the central nervous system [36]. The precise identity of AT4 receptors has not been created. The AT4 region may be involved in memory acquisition and retrieval, as well as in the regulation of blood flow [36]. Angiotensin IV and its analogs may benefit object recognition and avoidance (conditioned and passive avoidance as spatial memory tasks [37].

Due to the fact that Angiotensin variants such as Angiotensin II and Angiotensin IV interact with the CNS (Central Nervous System) and possibly play roles in the memory

formation, the interaction between the main Enzyme (ACE) which act in the conversion of Angiotensin I into Angiotensin II and the occurrence of Dementia and Alzheimer's Disease which is one of the main subtypes of late onset dementias has been closely studied in the past two decades.

In a few studies, it was shown that the I/D polymorphism has a recognizable effect on ACE quantity that was the highest in the DD genotype, followed by the ID genotype and lowest in the II genotype [38], [39]. In accordance with a recent meta-analysis, it was asserted that ACE I/D polymorphism is unlikely to be a major determining factor in the development of Sporadic AD (SAD) [40].

Earlier studies focus on a possible association of ACE gene polymorphisms and risk of AD. The association between polymorphisms and risk of AD may depend on the population differences and case numbers. The aim of this study is to investigate the association of ACE insertion/deletion (I/D) polymorphisms in patients with AD.

Some studies have shown that I/D polymorphism in the ACE gene could be associated with dementia. A study in a Tunisian population [41], a study in a Spanish population [42], and a study in a Tunisian population [43] found an association of the ACE gene I/D polymorphism with AD. However, the results are inconclusive and there is no clear evidence between ACEI/D polymorphism and AD. The results of meta-analysis of recent years' studies indicate that insertion allele has an impact on increase of the risk for AD [44]. Our results also confirmed this correlation since our results also indicated that I allele has higher frequency in AD patients. It was expected that D allele may be defined as protection against Alzheimer's disease where I allele may be the risk allele, since it deduces the reduction in ACE levels [45].

Based on our results, there is no statistically significant association between D and I allele frequencies of the ACE and /or I/D polymorphism with the occurrence Alzheimer's Disease in our experimental group. As a second scenario, we focused on the three studies to observe the results in the Turkish population. The combined results of Isbir [31], Durmaz [39] and our study also came up with similar findings.

We also combined the results from 12 previously published studies from countries of the Mediterranean region [31], [38], [39], [46],[50] and Isbir and Durmaz studies with our experimental data in order to implement a meta-analysis. The results of 2041 combined cases and 2244 controls from these studies and our study were investigated. As a result of the combination of the several datasets from previous studies with our own experimental data, the meta-analysis of these different studies that involve patients from the Mediterranean region was implemented. The final results show no significant association between the occurrence of AD and rs 1799752 ACE polymorphism in either Turkish or Mediterranean Populations. It is important to highlight that the difference of our results from other studies implemented on different populations may be due to differences in the genomic makeup and environmental factor that affect Mediterranean populations from the populations, on which the other studies were previously implemented.

6. REFERENCES

[1] M. Prince, R. Bryce, E. Albanese, A. Wimo, W. Ribeiro, and C. P. Ferri, "The global prevalence of dementia: A systematic review and metaanalysis," *Alzheimer's Dement.*, vol. 9, no. 1, pp. 63-75.e2, 2013.

[2] H. Chertkow, H. H. Feldman, C. Jacova, and F. Massoud, "Definitions of dementia and predementia states in Alzheimer's disease and vascular cognitive impairment: Consensus from the Canadian conference on diagnosis of dementia," *Alzheimer's Res. Ther.*, vol. 5, no. SUPPL. 1, pp. 1–8, 2013.

[3] E. Reeve, P. Molin, A. Hui, and K. Rockwood, "Exploration of verbal repetition in people with dementia using an online symptom-tracking tool," *Int. Psychogeriatrics*, vol. 29, no. 6, pp. 959–966, 2017.

[4] R. Tarawneh and D. M. Holtzman, "The clinical problem of symptomatic Alzheimer disease and mild cognitive impairment," *Cold Spring Harb. Perspect. Med.*, vol. 2, no. 5, pp. 1–16, 2012.

[5] D. S. Knopman and R. C. Petersen, "Mild cognitive impairment and mild dementia: A clinical perspective," *Mayo Clin. Proc.*, vol. 89, no. 10, pp. 1452– 1459, 2014.

[6] C. M. Med and P. M. Pubmed, "Beta-amyloid , neuronal death and Alzheimer ' s," *Search*, pp. 11–12, 2001.

[7] S. Turgut, "Anjiotensin dönüştürücü enzim ve I/D polimorfizmi," *SDÜ Tıp Fakültesi Derg.*, vol. 12, no. 4, pp. 53–57, 2009.

[8] M. Rodríguez-Giraldo, R. E. González-Reyes, S. Ramírez-Guerrero, C. E. Bonilla-Trilleras, S. Guardo-Maya, and M. O. Nava-Mesa, "Astrocytes as a Therapeutic Target in Alzheimer's Disease–Comprehensive Review and Recent Developments," *Int. J. Mol. Sci.*, vol. 23, no. 21, p. 13630, 2022.

[9] H. N. Yassine and C. E. Finch, "APOE Alleles and Diet in Brain Aging and Alzheimer's Disease," *Front. Aging Neurosci.*, vol. 12, no. June, pp. 1–18, 2020.

[10] S. Garg and C. Editor, "Alzheimer Disease and APOE e4," pp. 5–8, 2023.

[11] S. Love and J. S. Miners, "Cerebrovascular disease in ageing and Alzheimer's disease," *Acta Neuropathol.*, vol. 131, no. 5, pp. 645–658, 2016.

[12] G. Cipriani, S. Danti, L. Picchi, A. Nuti, and M. Di Fiorino, "Daily functioning and dementia," *Dement. e Neuropsychol.*, vol. 14, no. 2, pp. 93–102, 2020.

[13] S. Duong, T. Patel, and F. Chang, "Dementia: What pharmacists need to know,"

Can. Pharm. J., vol. 150, no. 2, pp. 118–129, 2017.

[14] E. Nichols *et al.*, "Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019," *Lancet Public Heal.*, vol. 7, no. 2, pp. e105–e125, 2022.

[15] D. M. Molinares, D. R. Gater, S. Daniel, and N. L. Pontee, "Nontraumatic Spinal Cord Injury: Epidemiology, Etiology and Management," *J. Pers. Med.*, vol. 12, no. 11, p. 1872, 2022.

[16] S. T. Sarmast, A. M. Abdullahi, and N. Jahan, "Current Classification of Seizures and Epilepsies: Scope, Limitations and Recommendations for Future Action," *Cureus*, vol. 12, no. 9, 2020.

[17] J. D. Rohrer, W. D. Knight, J. E. Warren, N. C. Fox, M. N. Rossor, and J. D. Warren, "Word-finding difficulty: A clinical analysis of the progressive aphasias," *Brain*, vol. 131, no. 1, pp. 8–38, 2008.

[18] S. Sveinbjornsdottir, "The clinical symptoms of Parkinson's disease," *J. Neurochem.*, vol. 139, pp. 318–324, 2016.

[19] J. T. O'Brien and A. Thomas, "Vascular dementia," *Lancet*, vol. 386, no. 10004, pp. 1698–1706, 2015.

[20] L. A. Hershey and R. Coleman-Jackson, "Pharmacological Management of Dementia with Lewy Bodies," *Drugs and Aging*, vol. 36,no. 4, pp. 309–319, 2019.

[21] L. Zhagn and Z. Li, "Alzheimer and the discovery of Alzheimer's disease," *Zhonghua Yi Shi Za Zhi*, vol. 44, no. 5, pp. 288–290, 2014.

[22] A. P. Porsteinsson, R. S. Isaacson, S. Knox, M. N. Sabbagh, and I. Rubino, "Diagnosis of Early Alzheimer's Disease: Clinical Practice in 2021," *J. Prev. Alzheimer's Dis.*, vol. 8, no. 3, pp. 371–386, 2021.

[23] S. Taşci, P. T. Kartin, Ö. Ceyhan, G. Sungur, and S. Göriş, "Living with an alzheimer patient in Turkey," *J. Neurosci. Nurs.*, vol. 44, no. 4, pp. 228–234, 2012.

[24] K. A. Jellinger, "The neuropathological diagnosis of Alzheimer disease," *J. Neural Transm. Suppl.*, vol. 5, no. 53, pp. 97–118, 1998.

[25] A. Ritter and J. Cummings, "Fluid biomarkers in clinical trials of Alzheimer'sdisease therapeutics," *Front. Neurol.*, vol. 6, no. Aug, 2015.

[26] H. Wang, F. Yang, S. Zhang, R. Xin, and Y. Sun, "Genetic and environmental

factors in Alzheimer's and Parkinson's diseases and promising therapeutic intervention via fecal microbiota transplantation," *npj Park. Dis.*, vol. 7, no. 1, pp. 1–10, 2021.

[27] R. E. Tanzi, "The genetics of Alzheimer disease," *Cold Spring Harb. Perspect. Med.*, vol. 2, no. 10, 2012.

[28] J. Williamson, J. Goldman, and K. S. Marder, "Genetic aspects of alzheimer disease," *Neurologist*, vol. 15, no. 2, pp. 80–86, 2009.

[29] M. H. Dai, H. Zheng, L. D. Zeng, and Y. Zhang, "The genes associated with early-onset Alzheimer's disease," *Oncotarget*, vol. 9, no. 19, pp. 15132–15143, 2018.

[30] A. Serrano-Pozo, S. Das, and B. T. Hyman, "APOE and Alzheimer's disease: advances in genetics, pathophysiology, and therapeutic approaches," *Lancet Neurol.*, vol. 20, no. 1, pp. 68–80, 2021.

[31] T. Isbir *et al.*, "Apolipoprotein-E gene polymorphism and lipid profiles in Alzheimer's disease," *Am. J. Alzheimers. Dis. Other Demen.*, vol. 16, no. 2, pp. 77–81, 2001.

[32] A. Durmaz *et al.*, "Genetic factors associated with the predisposition to late onset Alzheimer's disease," *Gene*, vol. 707, no. January, pp. 212–215, 2019.

[33] L. Wallace, O. Theou, K. Rockwood, and M. K. Andrew, "Relationship between frailty and Alzheimer's disease biomarkers: A scoping review," *Alzheimer's Dement. Diagnosis, Assess. Dis. Monit.*, vol. 10, pp. 394–401, 2018.

[34] K. E. Bernstein, Z. Khan, J. F. Giani, D. Y. Cao, E. A. Bernstein, and X. Z. Shen, "Angiotensin-converting enzyme in innate and adaptive immunity," *Nat. Rev. Nephrol.*, vol. 14, no. 5, pp. 325–336, 2018.

[35] J. P. Arroyo, C. Ronzaud, D. Lagnaz, O. Staub, and G. Gamba, "Aldosterone Paradox : Differential Regulation of Ion Transport in Distal," pp. 115–123, 2023.

[36] C. A. Wagner *et al.*, "Effect of mineralocorticoids on acid-base balance," *Front. Neuroendocrinol.*, vol. 9, no. March, pp. 2728–2737, 2008.

[37] S. Shantanam and MUELLER, "乳鼠心肌提取 HHS Public Access," *Physiol. Behav.*, vol. 176, no. 1, pp. 139–148, 2018.

[38] B. Nacmias *et al.*, "Angiotensin converting enzyme insertion/deletion polymorphism in sporadic and familial Alzheimer's disease and longevity," *Arch.*

Gerontol. Geriatr., vol. 45, no. 2, pp. 201–206, 2007.

[39] A. Durmaz *et al.*, "Genetic factors associated with the predisposition to late onset Alzheimer's disease," *Gene*, vol. 707, pp. 212–215, 2019.

[40] C. Bettegowda *et al.*, "Detection of circulatingtumor DNA in early- and late-stage human malignancies," *Sci. Transl. Med.*, vol. 6, no. 224, 2014.

[41] N. Fekih-Mrissa *et al.*, "Association between an angiotensin-converting enzyme gene polymorphism and Alzheimer's disease in a Tunisian population," *Ann. Gen.Psychiatry*, vol. 16, no. 1, pp. 4–11, 2017.

[42] R. Alvarez *et al.*, "Genetic variation in the renin-angiotensin system and athletic performance," *Eur. J. Appl. Physiol.*, vol. 82, no. 1–2, pp. 117–120, 2000.

[43] A. Achouri-Rassas *et al.*, "Association between ACE polymorphism, cognitive phenotype and APOE E4 allele in a Tunisian population with Alzheimer disease," *J. Neural Transm.*, vol. 123, no. 3, pp. 317–321, 2016.

[44] P. G. Kehoe *et al.*, "Haplotypes extending across ACE are associated with Alzheimer's disease," *Hum. Mol. Genet.*, vol. 12, no. 8, pp. 859–867, 2003.

[45] J. F. Lucatelli *et al.*, "Genetic influences on Alzheimer's disease: Evidence of interactions between the genes APOE, APOC1 and ACE in a sample population from the South of Brazil," *Neurochem. Res.*, vol. 36, no. 8, pp. 1533–1539, 2011.

[46] S. B. Chapman, A. P. Highley, and J. L. Thompson, "Discourse in fluent aphasia and Alzheimer's disease: Linguistic and pragmatic considerations," *J. Neurolinguistics*, vol. 11, no. 1–2, pp. 55–78, 1998.

[47] M. R. Keikhaee, S. B. Hashemi, H. Najmabadi, and M. Noroozian, "C677T methylentetrahydrofulate reductase and angiotensin converting enzyme gene polymorphisms in patients with Alzheimer's disease in Iranian population," *Neurochem. Res.*, vol. 31, no. 8, pp. 1079–1083, 2006.

[48] R. Monastero *et al.*, "Lack of association between angiotensin converting enzyme polymorphism and sporadic Alzheimer's disease," *Neurosci. Lett.*, vol. 335, no. 2, pp. 147–149, 2002.

[49] B. Palumbo, D. Cadini, G. Nocentini, E. Filipponi, F. M., and U. Senin, "'Angiotensin Converting Enzyme Deletion allele in different kinds of dementia disorders', Neuroscience Letters. 267 (1999), pp. 97-100, Elsevier Science Ireland,

ISSN: 03043940.,” *Neurosci. Lett.*, vol. 267, pp. 97–100, 1999.

[50] D. Seripa *et al.*, “Methylenetetrahydrofolate reductase and angiotensin converting enzyme gene polymorphisms in two genetically and diagnostically distinct cohort of Alzheimer patients,” *Neurobiol. Aging*, vol. 24, no. 7, pp. 933–939, 2003.

7. APPENDIX

7.1 Volunteer Form

Sayın Hastamız,

· Bu belge bilgilendirilme ve aydınlatılmış onam haklarınızdan yararlanabilmenizi amaçlamaktadır.

· Size gerçekleştirilebilecek klinik araştırmalar amaçlı girişimler konusunda, tüm seçenekler ile bu girişimlerin yarar ve muhtemel zararları konusunda anlayabileceğiniz şekilde **bilgi alma hakkınız ve bir kopyasını isteme hakkınız** vardır.

· Yasal ve tıbbi zorunluluk taşıyan durumlar dışında **bilgilendirmeyi reddedebilirsiniz**. Yazılı bildirmek koşulu ile bilgi almama veya yerinize güvendiğiniz bir kimsenin bilgilendirilmesini talep etme hakkına sahipsiniz.

· Klinik araştırmalara katılım konusunda bilgilendirildikten sonra bunu kabul edebilirsiniz. Ya da k**arar verebilmek için uygun zaman talep edebilirsiniz.**

· Hayatınız veya hayati organlarınız tehlikede olmadığı sürece onamınızı (yazılı talep etme koşulu ile) **dilediğiniz zaman geri alabilir** ya da önceden kabul etmediğiniz herhangi bir tanı/tedavi amaçlı girişimi **tekrar talep edebilirsiniz**.

· Hastanemizde verilen hizmetleri **Hastane Tanıtım Broşürü**nden edinebilirsiniz. Ayrıca Hastane personeli hakkında http://www.yeditepehastanesi.com.tr, web sayfasından daha detaylı bilgilere ulaşabilirsiniz.

· Burada belirtilenlerden başka sorularınız varsa bunları yanıtlamak görevimizdir.

TANIMLAMA

1. *Araştırmanın Adı:* Angiotensin – Converting Enzyme Genotipi ile Demans Hastaları Arasındaki Bağlantının Araştırılması

Araştırmaya Katılımcı Sayısı: 65

Bu araştırmanın Amacı: Demans, bilişsel bozukluklara sebep olan ve günlük yaşamı önemli ölçüde olumsuz etkileyen, yaşlanma ile görülme riski belirgin olarak artış gösteren, dünya genelinde sık rastlanan klinik bir sendromdur. Dünyada yaklaşık 350 milyon kişiye demans tanısı konmuşolup, bu sayının 2030'da 65 milyona ve 2050'de 113 milyona ulaşacağı ön görülmektedir. Sayılarının giderek artacağı tahmin edilen demans tanılarının 2040 yılına kadar 81,1 milyon tanıya ulaşacağı ve bu tanıların %71inin az gelişmiş veya gelişmekte olan ülkelerden olacağı tahmin edilmektedir. Hastalık riskini azaltmak veya demans başlangıcını geciktirmek için, hastalığın başlangıcını ve ilerlemesini daha olası kılan psikososyal risk faktörlerine dikkat çekilmektedir. Birçok araştırma stresli bir yaşamın, özellikle yaşlılıkta, bilişsel işlev üzerinde olumsuz etki yaratabileceğini öne sürmektedir.

ACE genindeki anjiyotensin dönüştürücü enzim insersiyon (I) ve delesyon (D) polimorfizminin, plazma ACE konsantrasyonunun bireyler arası değişkenliği ile önemli ölçüde bir genetik etki ile ilişkili olduğu ve delesyon polimorfizminin, miyokardiyal enfarktüs ve kardiyomiyopati için bir risk faktörü olduğu tanımlanmıştır. Kişisel veya ailevi inme öyküsü ve Alzheimer hastalığı olan kişilerde ACE DD genotipinin daha yaygın olduğu da bildirilmiştir. Serebrovasküler sistemdeki ACE aktivitesi, yaşlanma ve Alzheimer hastalığına bağlı olarak artış göstermektedir. Bu araştırma, ACE geni polimorfizminin geç başlangıçlı demansın gelişme riskine etkisini Türk popülasyonunda değerlendirmeyi amaçlamaktadır.

Süresi: 12 ay

İzlenecek Yöntem/Yöntemler:

DNA ELDESİ:

Demans hastası olduğu tespit edilen hastalardan 5ml EDTA ‘ lı tüplere yaklaşık 350 ml ‘ lik periferik kan alınacaktır. DNA izolasyonu için iPrep DNA (iPrep Pure link , invitrogen) izolasyon robotu kullanılacaktır. Elde edilen DNA ‘ ların konsantrasyonları nanodrop (Thermoscientific) cihazı ile belirlenecektir. Saflık ölçümleri için Nano Drop cihazı ile spektrofotometrik olarak ölçüm yapılacaktır. DNA ‘ lar çalışma gününe kadar -+4^{o}C ‘ de bekletálecektár.

GENOTİP ANALİZİ:

ACE mutasyonlarının belirlenebilmesi için Applied bioscience 7500 Fast Real Time cihazı kullanılarak Real Time PCR (polimeraz zincir reaksiyonu) ile yapılacak ve hedef varyasyonlarındaki olası mutasyonların belirlenebilmesi için özel primer dizileri kullanılacaktır.

İSTATİKSEL ANALİZ:

Çalışmanın istatistiki analizi için SPSS 25.0 sürümü kullanılacak. Hasta grubu ve kontrol grubu katılımcıların karşılaştırılmalarında allel fraksiyonlarının ve genotiplerin karşılaştırılması için ki kare testi kullanılacaktır. Hasta ve kontrol grubu anlamlılık ölçümü için student 's t test kullanılacaktır. Anlamlılık seviyesi $p < 0.05$ kabul edilecektir.

Araştırma Sonunda Beklenen Fayda

Angiotensin – Converting Enzyme Genotipi ile Demans Hastaları Arasındaki Bağlantının Belirlenmesi

Bu Çalışmada Herhangi Bir Alternatif Tedavi ya da Girişimde Bulunulmayacaktır.

Bu Araştırma Gönüllüler İçin Hiçbir Risk Teşkil Etmemekte ve Hiçbir Rahatsızlığa Sebep olmamaktadır.

ONAM (RIZA)

Bilgilendirilmiş Gönüllü Olur Formundaki tüm açıklamaları okudum. Bana, yukarıda konusu ve amacı belirtilen araştırma ile ilgili yazılı ve sözlü açıklama aşağıda adı belirtilen hekim tarafından yapıldı. Araştırmaya gönüllü olarak katıldığımı, istediğim zaman gerekçeli veya gerekçesiz olarak araştırmadan ayrılabileceğimi ve kendi isteğime bakılmaksızın araştırmacı tarafından araştırma dışı bırakılabileceğimi biliyorum. Bu durumda hastanenin çalışma düzeni ve hastalara verilen bakımda aksaklık olmayacağı konusunda bilgilendirildim. Bu araştırmaya katılırken zorlama, maddi çıkar ve ast üst ilişkisine dayalı herhangi bir baskı olmaksızın bu çalışmaya katıldığımı beyan ederim. Bu bilimsel çalışmanın devamı esnasındaki süreçle ilgili olarak ayrıca eklenen çalışma protokolü ile bilgilendirildim. Tarafımdan alınan kan ve doku örneklerinin daha sonra başka araştırma çalışmalarında kullanılmasında herhangi bir sakınca yoktur.

Söz konusu araştırmaya, hiçbir baskı ve zorlama olmaksızın kendi rızamla katılmayı kabul ediyorum.

Gönüllünün:

Adı Soyadı:

İmzası:

Tarih:

Açıklamaları Yapan Kişinin:

Adı Soyadı:

İmzası:

Tarih:

Gerekiyorsa Olur İşlemine Tanık Olan Kişinin:

Adı Soyadı:

İmzası:

Tarih:

Gerekiyorsa Yasal Temsilcinin :

Adı Soyadı:

İmzası:

Tarih:

Klinik Araştırma Proje Koordinatörü İletişim Bilgileri:

Adı Soyadı: Uzmanlık alanı: Kurumu:
E-posta adresi:

Telefon numarası:

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15- 2133 **22/04/2021**
Konu: Klinik Araştırmalar
Etik Kurul Başvurusu hk.

İlgili Makama (Ezgi Yalçınkaya)

Yeditepe Üniversitesi Tıbbi Biyoloji AD. Prof. Dr. Altay Burak Dalan'ın proje koordinatörü olduğu, Nöroloji AD. Dr. Öğr. Üyesi Hakan Şilek'in sorumlu araştırmacı olduğu ve Moleküler Tıp AD. Prof. Dr. Turgay İsbir'in yardımcı araştırmacı olduğu **"Angiotensin – Converting Enzyme Genotipi İle Demans Hastaları Arasındaki Bağlantının Araştırılması"** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası **(2162)** kayıtlı Numaralı KAEK Başvuru Dosyası, Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **21.04.2021** tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu, yukarıdaki isim belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir **(KAEK Karar No: 1429)**

Prof. Dr. Hasan AYDIN

Yeditepe Üniversitesi
Klinik Araştırmalar Etik Kurul Başkan Yrd.