T.R.
ÜSKÜDAR UNIVERSITY
INSTITUTE OF SCIENCE

DEPARTMENT OF MOLECULAR BIOLOGY

MOLECULAR BIOLOGY PROGRAM

MASTER THESIS

INVESTIGATION OF CYP2D6*4 RS3892097 POLYMORPHISM IN ALZHEIMER'S PATIENTS

Ayşenur ÇİÇEKLİOĞLU

Thesis Advisor

Professor Dr. Muhsin KONUK

İSTANBUL-2026

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ÖZET

ALZHEIMER HASTALARINDA CYP2D6*4 RS3892097 POLİMORFİZMİNİN İNCELENMESİ

Alzheimer hastalığı, tipik olarak, insan beyninde yer alan tau nörofibriller yumaklar ve Aβ birikimiyle karakterize bir nörodejeneratif hastalıktır. Bu tez çalışması, belirli örneklemdeki Alzheimer hastalarında CYP2D6*4 polimorfizminin metabolik profilini belirlemeyi amaçlamaktadır. DNA izolasyonu, PCR ve genotipleme methodları kullanılarak istatiksel veriler elde edilmiştir. Bu istatiksel verilere göre, CYP2D6*4 (rs3892097), G (%55) ve A (%45) alel dağılımına; AG (%60), GG (%25) ve AA (%15) genotip dağılımına sahiptir. Demografik açıdan ise, yaş özelliğinde anlamlı bir fark (p = 0.460) bulunmazken; cinsiyet özelliğinde anlamlı bir fark (p = 0.001) tespit edilmiştir. Ayrıca, CYP2D6*4 polimorfizmi, EM (%85) ve PM (%15) metabolizer fenotipleriyle ilişkili bulunmuştur. Elde edilen bulgulara rağmen, kontrol grubunun eksikliği ve örneklemin sınırlılığı kesin bir çıkarımı zorlaştırmaktadır. İlerleyen zamanlarda, daha kapsamlı çalışmalarında gerçekleştirilmesiyle birlikte kişiselleştirilmiş tedavi (farmako/genetik) alanında nitelikli avantajlara yol açacaktır.

Anahtar Kelimeler: Alzheimer Hastalığı, Nörodejeneratif, CYP2D6*4, rs3892097, Polimorfizm.

ABSTRACT

INVESTIGATION OF CYP2D6*4 RS3892097 POLYMORPHISM IN ALZHEIMER'S PATIENTS

Alzheimer's disease is a neurodegenerative disease typically characterized by tau neurofibrillary tangles and Aβ accumulation in the human brain. This thesis aims to determine the metabolic profile of CYP2D6*4 polymorphism in Alzheimer's patients in a specific sample. Statistical data were obtained using DNA isolation, PCR, and genotyping methods. According to these statistical data, CYP2D6*4 (rs3892097) has an allele distribution of G (55%) and A (45%); and a genotype distribution of AG (60%), GG (25%), and AA (15%). Demographically, no significant difference was found in age (p = 0.460), while a significant difference was found in gender (p = 0.001). Furthermore, CYP2D6*4 polymorphism was found to be associated with EM (85%) and PM (15%) metabolizer phenotypes. Despite the findings, the lack of a control group and the limited sample size make it difficult to draw definitive conclusions. Further, more comprehensive studies will lead to significant advantages in the field of personalized treatment (pharmaco/genetics).

Keywords: Alzheimer's Disease, Neurodegenerative, CYP2D6*4, rs3892097, Polymorphism.

ACKNOWLEDGEMENTS

Throughout my master's journey, I am grateful to my esteemed thesis advisor, Prof. Dr. Muhsin KONUK, for his valuable knowledge, guidance and support at every stage.

I thank Medical Genetics and Molecular Diagnostics Laboratory specialist Tayfun GÖZLER, for his support during experimental phase of my thesis period.

I also thank my family, who supported me all kinds during my entire education and especially my mother, Leyla ÇİÇEKLİOĞLU, for giving me the strength to achieve my goals.

I offer my deepest gratitude.

Ayşenur ÇİÇEKLİOĞLU

DECLARATION FORM

I declare that I have obtained all the information and documents in this study within the framework of academic rules, that I have presented all visual, audio and written information and results in accordance with scientific ethics, that I have not made any falsifications in the data I have used, that I have cited the sources I have used in accordance with scientific norms, that my thesis is original except for the cases cited, that it was produced by me and that it was written in accordance with the Thesis Writing Guide of the Üsküdar University Institute of Science.

.04.2026

Ayşenur ÇİÇEKLİOĞLU

İmzası

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INDEX OF SYMBOLS AND ABBREVIATIONS

SYMBOLS

≥ : and above

dH_2O: distilled water

Pb: Lead

Al: Aluminum

Cd: Cadmium

Zn^{+2} : Zinc

Cu^{+2} : Copper

Fe^{+2} : Iron

ABBREVATIONS

ACh: Acetylcholine

AD: Alzheimer Disease

aa: Amino Acid

Aβ: Amyloid-β

APP: Amyloid Precursor Protein

ApoE: Apolipoprotein

ApoE-ε4: Apolipoprotein E-ε4

BBB: Blood-Brain Barrier

CYP2D6: Cytochrome P450 2D6

DNA: Deoxyribonucleic Acid

EM: Extensive Metabolizer

EOAD: Early Onset Alzheimer Disease

FRs: Free Radicals

IM: Intermediate Metabolizer

LOAD: Late Onset Alzheimer Disease

MM: Master Mix

NBM: Meynert Basal Nucleus

NFTs: Neurofibrillary Tangles

PCR: Polymerase Chain Reaction

PM: Poor Metabolizer

PSEN1: Presenilin-1

PSEN2: Presenilin-2

RM: Rapid Metabolizer

ROS: Reactive Oxygen Species

SNP: Single Nucleotide Polymorphism

1. INTRODUCTION

1.1. Alzheimer's Disease

Alzheimer's disease (AD) is a neurodegenerative disorder with a multifactorial structure (e.g., oxidative stress, neuroinflammation) (Rostagno, 2022; Twarowski & Herbet, 2023). As shown in Figure 1, amyloid-β (Aβ) is found pathologically in the human brain as plaques and neurofibrillary tangles. (NFTs) (Breijyeh & Karaman, 2020). It also has a neurotoxic effect due to the formation of tangles and plaques.

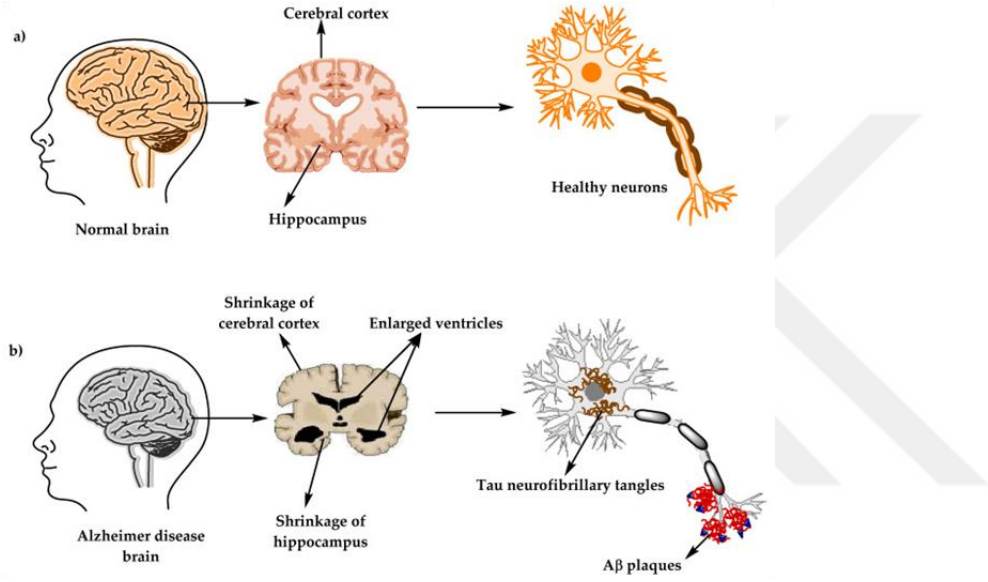

Figure 1. The physiological structure of the brain and neurons in (**a**) healthy brain and (**b**) Alzheimer's disease (AD) brain (Breijyeh & Karaman, 2020).

AD cases are divided into two categories based on age of onset: late-onset (LOAD) and early-onset (EOAD) (Breijyeh & Karaman, 2020). LOAD constitutes the majority of cases (99%), while EOAD accounts for a smaller proportion (1%) (Zheng & Wang, 2025). EOAD is mostly characterized by onset before the age of 65, while LOAD is characterized by the apolipoprotein E-ε4 (ApoE-ε4) risk allele (Rostagno, 2022).

AD primarily involves storage and encoding disorders; however, over time, metabolic, behavioral, and (due to brain atrophy) cognitive symptoms also develop (Breijyeh & Karaman, 2020; Rostagno, 2022; Soria Lopez et al., 2019; Twarowski & Herbet, 2023). Despite the identification of symptoms, there is no definitive cure for AD, and it can be fatal due to neurodegeneration (Twarowski & Herbet, 2023).

1.2. Brief History of Alzheimer's Disease

The discovery of AD began in 1906 when psychiatrist Alois Alzheimer examined the seizure symptoms of a patient (Breijyeh & Karaman, 2020; Twarowski & Herbet, 2023). As a result of his investigations, Alzheimer published a clinical report in 1907 (Bondi et al., 2017). This report stated that the patient exhibited rapid forgetfulness of object names or functions and nonsensical reading (skipping spellings or sentences).

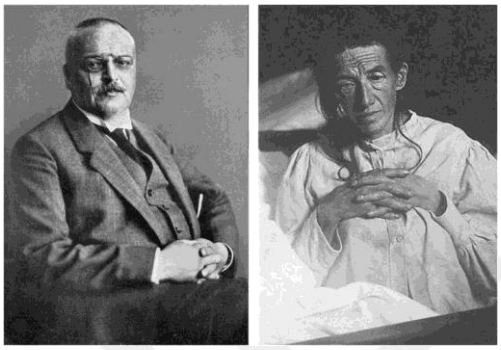

Figure 2. Photographs of Alois Alzheimer (left) and his patient Auguste Deter (right). (Bondi et al., 2017)

Following the death of the female patient shown in Figure 2, a pathomorphological autopsy was performed using histological and dissection methods (Bondi et al., 2017; Twarowski & Herbet, 2023). During the autopsy, Alzheimer made the drawings shown in Figure 3. These drawings are basic images of the main AD morphological structures, now called neurofibrillary tangles (NFTs) and amyloid (angiopathy) (Bondi et al., 2017; Twarowski & Herbet, 2023).

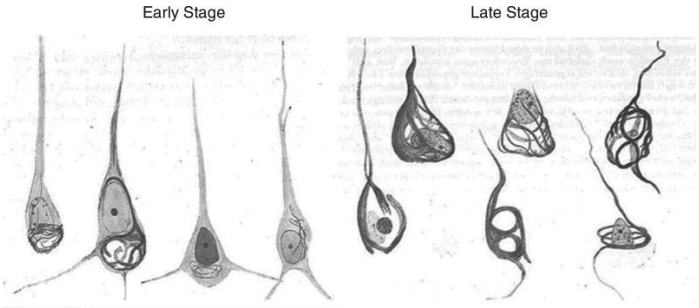

Figure 3. Sketches of Auguste Deter's histopathologic preparations of early and late stage neurofibrillary tangle pathology as drawn by Alzheimer from his 1911 paper entitled "Über eigenartige Krankheitsfälle des späteren Alters." (Bondi et al., 2017).

Later, research on early-onset AD conducted by Edgar Miller argued that the duration of storage (long or short) and subsequent erroneous states in memory could contribute to

memory deficits. (Bondi et al., 2017). Furthermore, in 1976, Robert Katzman's work on AD's claim of a "fourth cause of death" for older individuals intensified (Katzman, 1976).

The real turning point began in the 2000s (Bondi et al., 2017). The genetic risks of AD, which began to be examined from a heredity perspective, were scrutinized. Among the types of AD, apolipoprotein E-ε4 (ApoE-ε4), a risk factor especially for sporadic AD, was identified. This allele has been reported to be present in 50-60% of individuals with AD (Katzman & Kawas, 1994).

1.3. Epidemiology of Alzheimer's Disease

Globally, the prevalence of AD, which is proportional to the elderly population, is high in countries such as America, China and Japan (Zhang et al., 2021). For example, according to a 2020 review of AD in America, it is expected that there will be 13.8 million people with AD in 2050 (Alzheimer’s Association, 2020). This could significantly increase the number of deaths due to AD (Zheng & Wang, 2025). In addition, there are various risk factors that affect the prevalence and incidence of AD epidemiology.

1.3.1. Risk Factors

Risk factors include many modifiable and non-modifiable factors such as environmental, obesity, smoking, depression, gender, genetic, age and stress, as shown in Figure 4 (Zhang et al., 2021). Among these, age is the most fundamental risk factor, while ApoE-ε4 is the most influential factor in AD (Zheng & Wang, 2025).

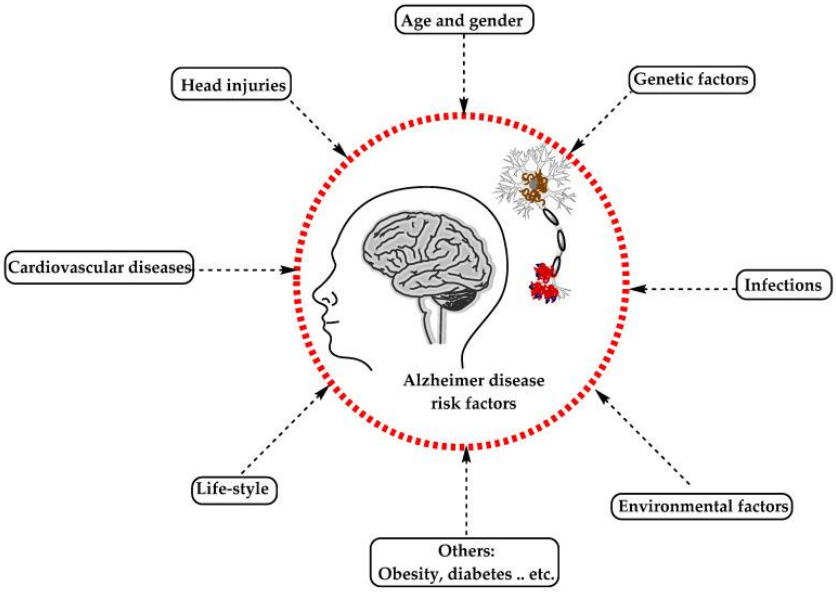

Figure 4. The risk factors for Alzheimer's disease. (Breijyeh & Karaman, 2020)

1.3.1.1. Age

Age is the primary and unmodifiable risk factor for AD (Breijyeh & Karaman, 2020; Hong et al., 2024). Age of onset is a critical determinant that can differentiate AD into types (such as LOAD and EOAD). Generally, the prevalence of AD increases from the age of 60 onwards (Masters et al., 2015). In particular, individuals 85 years or older are more likely to have AD than individuals 65 years or older (Alzheimer's Association, 2023). This may be due to age-related brain changes (e.g., synapse loss, brain volume reduction, NFTs) (Breijyeh & Karaman, 2020).

1.3.1.2. Gender

Gender is an important risk factor for AD. Female have a higher probability of developing AD than male in terms of neurofibrillary tangle/tau pathology, menopause, and biological lifespan (Buckley et al., 2018; Riedel et al., 2016; Scheltens et al., 2021). Some data suggest that age, particularly 80 years (as in Figure 5), may be a critical AD prevalence threshold for female (Scheltens et al., 2021). In male, the probability of developing EOAD is higher (Ruitenberg et al., 2021).

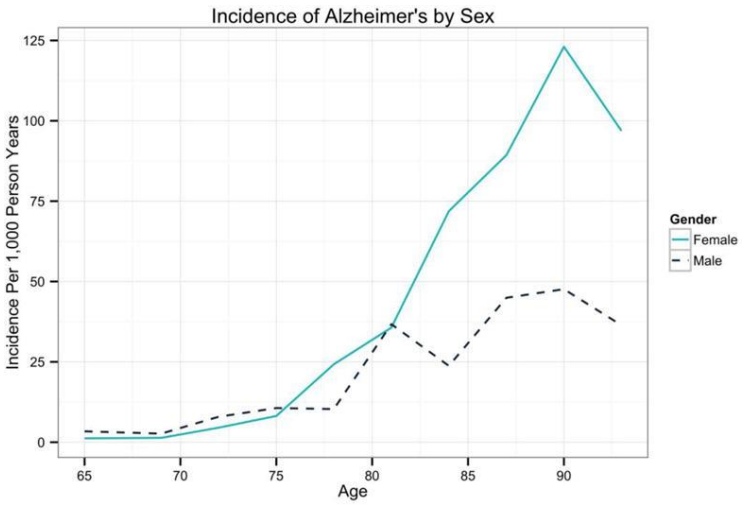

Figure 5. Sex-specific incidence estimates of Alzheimer's per 1,000 person years (Riedel et al., 2016).

1.3.1.3. Apolipoprotein - ε4

Apolipoprotein (ApoE) is secreted from brain astrocyte cells located in the nervous system (Breijyeh & Karaman, 2020; Climacosa et al., 2025). This glycoprotein, which plays an active role in cholesterol and/or lipid metabolism in the human brain, has three gene variants: ε2, ε3, and ε4 (Breijyeh & Karaman, 2020; Climacosa et al., 2025). The ε4 allele, in particular, is a significant genetic (risk) factor for AD (Kenaan & Alshehabi, 2025). The high Aβ load associated with ApoE-ε4 supports cognitive impairment (Climacosa et al., 2025; Karagas et al., 2025). Furthermore, it can closely interact with drug metabolisms of genes such as CYP2D6 (Climacosa et al., 2025).

1.3.1.4. Other Factors

In addition to key risk factors (age, gender, ApoE-ε4) contributing to AD pathogenesis, environmental and medical factors are also present (Breijyeh & Karaman, 2020; Scheltens et al., 2021). These factors trigger the risk of AD development by activating mechanisms such as neuro-inflammatory and oxidative stress (Breijyeh & Karaman, 2020). This is summarized in Tables 1 and 2.

Table 1. Contribution of Environmental Factors to AD Risk (Adapted from Breijyeh & Karaman, 2020).

Environmental Factors	Contribution of AD Risk
Diet	Nutritional supplements, polyphenols, antioxidants and vitamins reduce the risk of AD; intake of (high) calorie, saturated fatty acids and food processing increase the risk of AD.
Metals	Aluminum (Al) accumulates in the cerebellum and cortex, promoting protein degredation (such as phosphorylation and aggregation) in (tau) proteins; Lead (Pb), can alter neural differentiation, leading to an increase

	in Aβ accumulation and β-secretase levels; Cadmium (Cd) contributes to aggregation of Aβ and tau in the brain (of individuals with AD).
Air Pollution	Excessive stages of air pollutants (e.g., Sulfur dioxide, Ozone, Carbon monoxide, Nitrogen oxides, Lead, Particule matter) predispose to changes (such as formation of Aβ plaques and dysfunction of cognitive) in the frontal cortex, olfactory bulb (and mucosa).
Infections	Chronic central nervous system infections (as viral and bacterial) activate the accumulation of Aβ plaques and inflammatory reaction in the brain. The bacteria *Treponema pallidum* contributes to syphilitic dementia; the bacterium *Chlamydia Pneumonia* contributes to LOAD, enhancing the risk of developing AD.

Table 2. Contribution of Medical Factors to AD Risk (Adapted from Breijyeh & Karaman, 2020)

Medical Factors	**Contribution of AD Risk**
Obesity	Cytokines secreted by excess fat (adipose) tissue accumulated in the body due to obesity initiate inflammation. Inflammation, whether widespread or focal, is related to decreased synaptic plasticity, Aβ accummulation and tau pathology. Diabetes (e.g., type 2 diabetes) and (chronic) hyperglycemia enhance the risk of dementia and AD inducing brain damage (e.g., cerebral edema) or loss of memory.
Cardiovascular Diseases	Cardiovascular diseases such as hypertension, atrial fibrillation and heart failure are changeable factor and a significant impact on AD risk. They may bring about cerebral and nerve damage (or loss) through pathways such as stroke and embolism. This can promote impairment of cognitive function, raising the risk of AD (and dementia).

1.4. Pathology of Alzheimer's Disease

The pathological mechanism of AD is a cycle triggered by several factors, such as hyperphosphorylation of tau protein and oligomer or fibrillar accumulation of Aβ, as shown in Figure 6 (Ivanova et al., 2025).

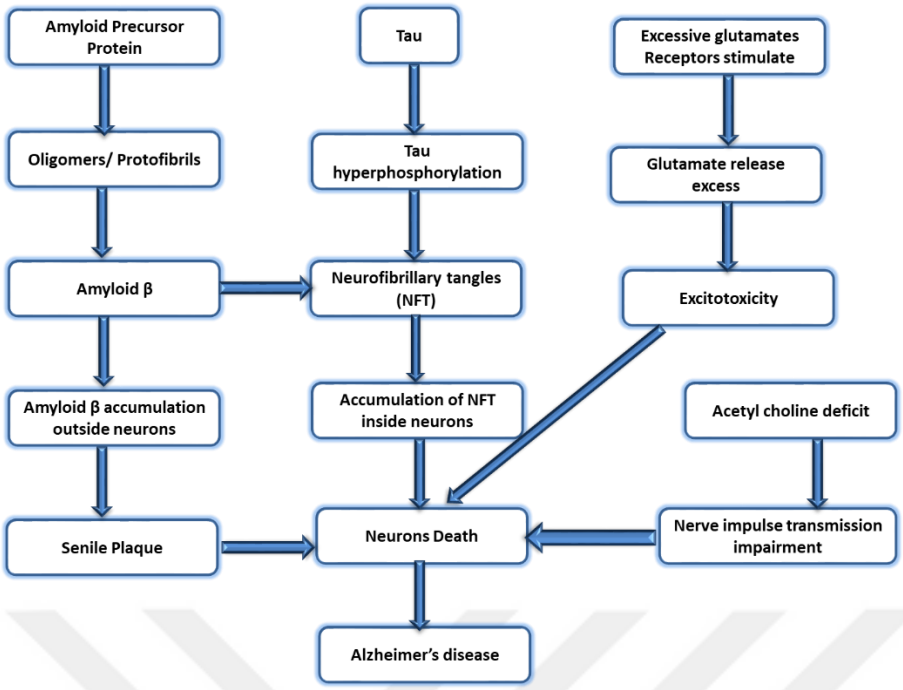

Figure 6. Different Phases of the Pathophysiology of AD (Maisam et al., 2023).

1.4.1. Causal Genes

Mutations in the Amyloid Precursor Protein (APP), Presenilin-1 (PSEN1), and Presenilin-2 (PSEN2) genes contribute to the exacerbation of AD (specifically familial/EOAD) pathogenesis (Bekris et al., 2010; Breijyeh & Karaman, 2020).

1.4.1.1. Amyloid Precursor Protein

APP is a transmembrane glycoprotein (type I) with an extracellular domain, encoded by the APP gene located on chromosome 21 of humans (Breijyeh & Karaman, 2020; Tiwari et al., 2019). This glycoprotein, 770 amino acids (aa) long, is involved in neurite and cellular growth functions (Tiwari et al., 2019). Furthermore, there are approximately 30 mutations in the APP gene locus, 25 of which are closely associated with AD (Breijyeh & Karaman, 2020).

APP is cleaved at specific sites via secretase enzymes (i.e., β, γ, and α) (Breijyeh & Karaman, 2020). Depending on which enzyme cleaves it, it follows two different pathways. The amyloidogenic pathway contributes to the formation of Aβ40 and Aβ42 peptides. Specifically, Aβ42, with its high neurotoxicity and insoluble structure, can be described as the "toxic building block" of aggregates (Tiwari et al., 2019). It causes severe neuronal deterioration and death in AD by promoting energy metabolism, inhibition of

ion channels, and increases/decreases in (mitochondrial) oxidative stress. The non-amyloidogenic pathway, on the other hand, is linked to synaptic plasticity and signaling based on its neuroprotective effect (Tiwari et al., 2019).

1.4.1.2. Presenilins: Presenilin-1 and Presenilin-2

Presenilins (PSEN1 and PSEN2) have a homologous structure at a rate of 67% (Breijyeh & Karaman, 2020). PSEN1 is located at 14q24.2 and encodes a polytopic core protein (Bekris et al., 2010; Breijyeh & Karaman, 2020). Mutations in this gene (missense) impair the functionality of γ-secretase (Bekris et al., 2010). The inability of secretase to perform its function promotes the accumulation of toxic Aβ, leading to synaptic dysfunction (Breijyeh & Karaman, 2020; Tiwari et al., 2019). This results in changes in cognitive memory (Bekris et al., 2010; Breijyeh & Karaman, 2020). Furthermore, 70% of AD cases are associated with PSEN1 variants (Karagas et al., 2025). In contrast, PSEN2, located at 1q42.13, contains monogenic and rare mutations (Bekris et al., 2010; Breijyeh & Karaman, 2020).

1.4.2. Neuropathology

Microscopic findings of plaques and tangles, distinctive neuronal markers of AD pathology, are shown in Figure 7 (Armstrong, 2009). These findings include lesion types related to accumulation (positive) and loss (negative) (Breijyeh & Karaman, 2020).

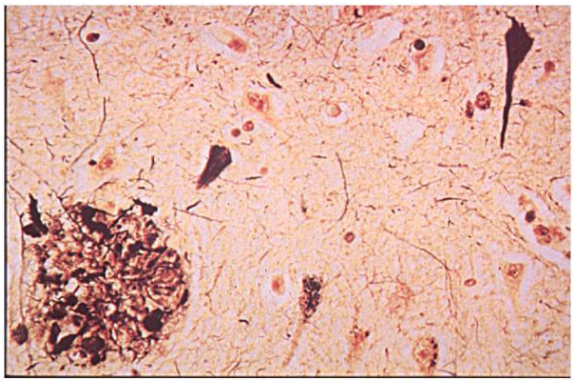

Figure 7. Microscopic neuropathology of Alzheimer's disease showing a neuritic plaque (lower left hand corner) and neurofibrillary tangles (upper right hand corner) (Bird, 2008).

1.4.2.1. Amyloid Plaques

Amyloid plaques are defined as anomalous aggregates formed extracellularly by Aβ40, Aβ42 molecules released as a result of the breakdown of APP by secretase enzymes

(Breijyeh & Karaman, 2020; Liu et al., 2024). The accumulation of these plaques in the hippocampus and cerebral cortex regions triggers conditions such as oxidative/axonal damage, inflammation, and synaptic dysfunction through "synaptic pruning" (Ahmed et al., 2026; Liu et al., 2024). These conditions can accelerate the development of AD by causing cognitive impairments.

1.4.2.2. Neurofibrillary Tangles

NFTs are abnormally dense filaments formed as a result of excessive phosphorylation of tau protein. They mostly form in the dendrite and axon regions of neurons, as shown in Figure 8, leading to cytoskeletal disruption and neuronal damage (Breijyeh & Karaman, 2020; Orobets & Karamyshev, 2023). Furthermore, the structural locations of intracellular accumulation tangles and extracellular accumulation plaques are shown in Figure 8 (Abdul Manap et al., 2024; Breijyeh & Karaman, 2020).

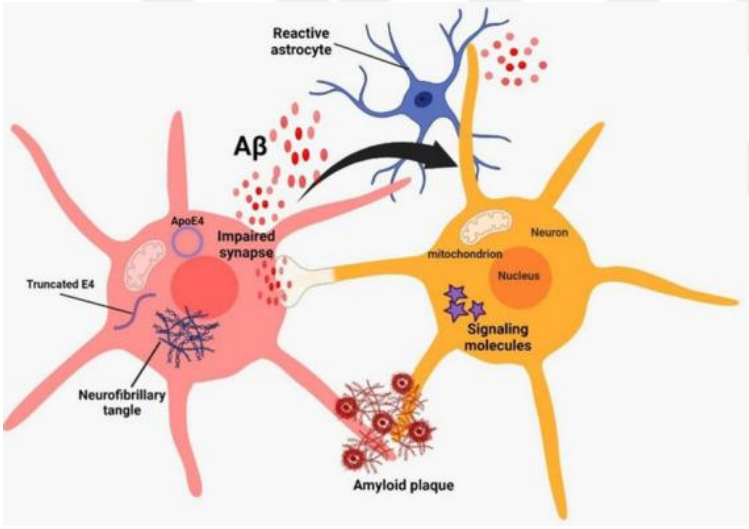

Figure 8. Alzheimer's pathogenesis based on two classical hallmarks on amyloid beta and neurofibrillary tangles (Abdul Manap et al., 2024).

1.5. Hypotheses of Alzheimer's Disease

1.5.1. Pathological Hypothesis

1.5.1.1. Tau Hypothesis

Tau is a protein typically composed of two terminals: an amino (N-) and a carboxy (C-) terminal (Hong et al., 2025). It is located in axons and interacts with tubulin (a building block of microtubules) (Ti, 2022). It maintains the stability of microtubules by regulating the phosphorylation mechanism (phosphatase-kinase cycle) within the neuronal system

(Hong et al., 2025). However, any disruption in this mechanism can reduce tau's binding affinity, leading to the active process of "downstream pathology" (Hong et al., 2025; Ivanoa et al., 2025). Specifically, damage occurring within neurons accumulates to form NFTs. NFTs trigger hyperphosphorylation, a type of post-translational modification (e.g., glycation, acetylation, ubiquitination, truncation, glycosylation), leading to aggregate formation. (Hong et al., 2025; Ivanoa et al., 2025). These aggregate formations (especially the insoluble oligomer form of tau) can contribute to the toxicity severity of AD and neuronal apoptosis by triggering synaptic dysfunction (e.g., plasticity, signaling) and mitochondrial damage (as morphological and functional). This can impair cognitive function and result in neurodegeneration (Sarkar et al., 2023). In summary, the tau hypothesis explains that abnormal changes in phosphorylated-tau are part of the underlying mechanism of AD neurodegeneration (Figure 9).

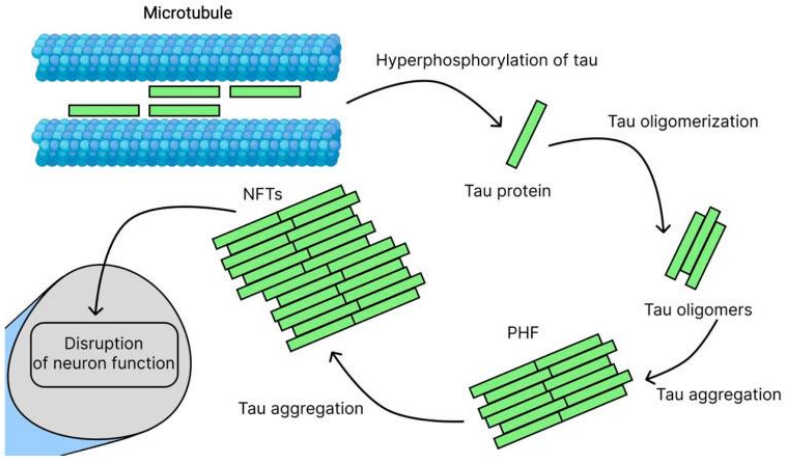

Figure 9. Molecular mechanisms of tau protein hyperphosphorylation and NFT formation (Ivanoa et al., 2025).

1.5.1.2. Amyloid Hypothesis

Within the context of AD pathogenesis, the classical amyloid hypothesis suggests that Aβ is the most important cause of AD and dementia (Breijyeh & Karaman, 2020). According to this hypothesis, amyloid precursor protein (APP) is proteolytically cleaved by two different methods: ‘amyloidogenic and non-amyloidogenic’. In the amyloidogenic method, as shown in Figure 10, APP (β- and γ-) is cleaved by secretases, resulting in Aβ peptides (Aβ40 and Aβ42) of length 39-42 amino acids (Ivanoa et al., 2025).

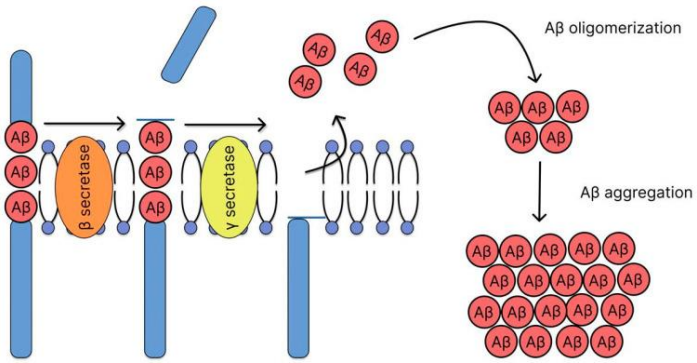

Figure 10. Amyloidogenic pathway of APP processing and Aβ formation (Ivanoa et al., 2025)

According to the theory, if Aβ peptides are not sufficiently broken down due to certain conditions (e.g., aging), a tendency to clump together (in oligomeric or fibril form) can be triggered, leading to plaque formation (Breijyeh & Karaman, 2020; Ivanoa et al., 2025). This can then lead to neurotoxicity and neuroinflammation activation (Breijyeh & Karaman, 2020), thus supporting numerous pathological processes, including neurodegeneration.

Despite all the advances, the amyloid cascade theory remains insufficient to comprehensively explain some mechanisms in AD. It still has limitations in identifying the link between amyloid levels in the brain and cognitive dysfunction, and in defining the pathological chain of non-amyloidgenic factors (Ivanoa et al., 2025).

1.5.2. Non-Pathological (Symptomatic) Hypothesis

Alternative theories have been developed to explain the multifactorial nature of AD and the mechanisms at play in the disease's later stages. Table 3 summarizes current hypotheses that contribute to AD pathogenesis, mostly symptomatically (such as acceleration, deceleration).

Table 3. Alternative Hypotheses of AD Development Mechanism (Compiled from Breijyeh & Karaman, 2020; Ivanoa et al., 2025; Scarano et al., 2025).

Types of Hypotheses	Role of Hypotheses in AD Mechanism
Cholinergic	The basal forebrain contains the Meynert Basal Nucleus (NBM). Damage to cholinergic neurons in the NBM can lead to a deficiency in the production of the neurotransmitter acetylcholine (ACh). This contributes to impairment in cognitive functions such as attention, memory (retention), learning and in (synaptic) signal transmission. As a result of this impairment, synaptic and neuronal loss may occur.
Neuroinflammation	Microglia are the brain's natural immune cells. In normal conditions (protective function), they eliminate Aβ aggregates through phagocytosis. In AD conditions, they biochemically interact with "proteinopathies (β-amyloid and tau)" inducing in pathways of pro-inflammatory signaling

	(e.g., NLRP3/ASC). This induction initiates chronic neuroinflammation, resulting in an active response (i.e., pro-inflammatory cytokine secrete). This situation enhances neuronal damage and neurodegeneration.
Metal Ion	Trace elements (copper, zinc and iron) found existing as metal ions are responsible for sustaining 'neuronal homeostasis'. In the AD brain, Aβ aggregates form that are not easily broken down owing to metal ion imbalance. Zinc (Zn^{+2}), in its presence, regulates glutamate excitotoxicity; in its inadequacy, can lead to synaptic dysfunction. Copper (Cu^{+2}) and iron (Fe^{+2}) cause important events such as lipid peroxidation, fenton reaction and ROS production in predominantly Aβ aggregates. Therefore, it affects cognitive functions giving rise to neurodegeneration, oxidative stress and neuronal death. In summary, this hypothesis explains the integrative upstream occurrence for AD.
Oxidative Stress	Aβ oligomers accumulate in the AD brain. This accumulation leads to mitochondrial dysfunction. Mitochondrial dysfunction promotes the production of reactive oxygen species (ROS) and an increase in free radicals (FRs) (e.g., $OH\cdot$, $NO\cdot$). This affects the antioxidant system responsible for protecting the human brain. In (AD) patients, when antioxidant are insufficient despite increased ROS levels, energy metabolism becomes unbalanced, giving rise to oxidative stress/damage in structures such as DNA and proteins. As this damage becomes chronic, the process is characterized by neuronal degeneration and death.
Microbiota-Gut-Brain	The gut microbiata optimizes physiological conditions. This microbiata contributes to the endocrine (systemic), neural and immune systems as well as the nervous system. However, triggering factors such as antibiotics and functional changes in the immune system can increase susceptibility to dysbiosis of the gut microbiata. Dysbiosis causes damage to the intestinal wall and results in leakage (i.e., gut of leaky). This condition can initiate systemic and neuronal inflammation in AD. Specifically, cytokines can penetrate the blood-brain barrier (BBB) and interact with astroyctes and microglia. Thus, as response to the disrupted neurotransmission process, stress of proteotoxic and autophagy can begin. In the ongoing process, AD neurodegeneration and neuronal damage occur.

1.6. Cytochrome P450 2D6 Gene

The human genome theoretically contains 57 (functional) CYP genes. These active CYP genes, found in both prokaryotic and eukaryotic organisms, 12 in particular (e.g., CYP1A1, CYP2B6, CYP2C19, CYP3A5) stand out in terms of (microsomal) drug metabolism. One of these 12 genes is cytochrome P450 2D6 (CYP2D6). CYP2D6 is an autosomal gene discovered in the 1970s (Table 4) (Climacosa et al., 2025; Nofziger et al., 2020; Taylor et al., 2020). This gene is located at the chromosomal locus "22q13.2" (Taylor et al., 2020). It has a DNA length of approximately 4,382 bp. It contains 9 exons (Nofziger et al., 2020).

Table 4. The Historical Development of CYP2D6 between 1970 and 1990 (Adapted from Nofziger et al., 2020).

Years	Contribution to CYP2D6 Gene
1970s	Identification
1984	Purification (for human CYP2D6 protein)
1987	Chromosome Mapping (22q13)
1989	Cloning and Sequencing
1990	Alleles (*3 and 4*) Identification

Other hand, the CYP2D6 gene undergoes a central dogma process to become a protein. This CYP2D6 protein is localized to mitochondria or the endoplasmic reticulum and encodes the production of the CYP2D6 enzyme (Taylor et al., 2020). It actively metabolizes in tissues such as the liver, intestine and brain, as well as in cells such as lymphocytes (Nofziger et al., 2020; Taylor et al., 2020).

Morever, the CYP2D6 gene has a pharmacogenomic role in relation to AD (Taylor et al., 2020). With the contribution of its alleles and variations, it is a critical factor that can guide the response to classic drugs used in AD treatment. Therefore, it can also be defined as a pharmacogene in a medical context (Taylor et al., 2020).

1.6.1. Genomic Variation

Genomic variation of CYP2D6 includes various (small or large-scale) changes such as (chromosomal) deletions, duplications, single nucleotide polymorphisms, and structural hybridization (with pseudogenes) (Taylor et al., 2020).

1.6.2. Allele Types

Alleles of the CYP2D6 gene are divided into two main categories: functional and non-functional. Alleles *9, *17, *29, and *41 are functional, while alleles *3, *4, *5, *6, *7, *8, *12, and *14 are non-functional (Climacosa et al., 2025).

1.6.3. CYP2D6*4 (rs3892097)

In a population (e.g., humans, mammals), allele variation with a frequency of ≥ 1% is defined as polymorphism (Brookes, 1999; Karki et al., 2015). One of the most studied

polymorphic variants is CYP2D6*4 (rs3892097) (Taylor et al., 2020). It is characterized by a nucleotide change called "g.1847G>A" among 28 sub-variates (Nofziger et al., 2020). This single nucleotide polymorphism (SNP) (i.e., rs3892097) has a "splice defect" (Maulana et al., 2024). Table 5 contains specific information about CYP2D6*4 (rs3892097).

Table 5. Specifications of CYP2D6*4 (rs3892097) (This data compiled from NCBI, 2024, 2025).

Organism	*Homo sapiens*
Position (genomic)	chr22:42128945
Location (chromosomal)	22q13.2
Canonical SPDI	NC_000022.11:42128944:C:T
Gene: Consequence	CYP2D6: Splice Acceptor Variant
Variation ID	16889
Variation Type	SNV (Single Nucleotide Variation)
Length	1 bp (base pair)

The frequency of CYP2D6*4 (rs3892097) varies in different population groups. According to the table in Taylor et al. (2020), it was detected in 12% (0.12) of the African population, 15% (0.15) of the European population, and 9% (0.09) of the South Asian population (Taylor et al., 2020). In short, CYP2D6*4 (rs3892097) is common in populations of European origin.

2. MATERIALS AND METHODS

2.1. Patient Population and Ethical Approval

This study was conducted with a total of 20 participants (10 female and 10 male) aged 60-82 years who were diagnosed with Alzheimer's disease after clinical evaluation by neurology specialists at the NPISTANBUL Brain Hospital neurology medical unit. This sample group was informed about the study, and written informed consent was obtained from the participants on a voluntary basis.

This thesis study was conducted at the Multidisciplinary-I Laboratory of the Faculty of Medicine, Üsküdar University. Ethical approval for the study was granted by the Üsküdar University Non-Interventional Research Ethics Committee (Approval No: 61351342/2020-169).

2.2. Materials

- Vortex Mixer, Stuart (UK)
- Microcentrifuge, Microfuge 16, Beckman Coulter (USA)
- Real-Time PCR System, QuantStudio 3, Thermo Fisher (USA)
- Refrigerator, Vestel (Türkiye)
- -20º C Freezer, Arçelik (Türkiye)
- Distilled Water System, Smart 2 Pure 3, Thermo Scientific (USA)
- Automatic Micropipettes, Eppendorf Research Plus, Thermo Scientific (USA)
- Computer
- Eppendorf Tubes
- Filter (Spin Column) Tubes
- Collection Tubes
- MicroAmp Fast Reaction Plate, 96-well, Applied Biosystems (USA)
- Sterile Pipette Tips (20 µl, 200 µl, 1000 µl), Eppendorf (Germany)
- Tube Racks
- Disposable Gloves
- Multimark Permanent Marker, Faber – Castell (Germany)
- Optical Adhesive Cover, Applied Biosystems (USA)
- Patient DNA
- Distilled Water (dH_2O), Thermo Fisher Scientific (USA)

- TaqPath Genotyping Master Mix
- TaqMan SNP Genotyping Assay, Human, Applied Biosystems (USA)

2.3. Methods

2.3.1. DNA Isolation

This section describes the isolation procedures performed to obtain genomic DNA from peripheral blood samples taken from patients, respectively:

The blood sample is vortexed for 4-5 seconds. Using a micropipette, 200 µl of blood is took and transferred to an Eppendorf tube. To the same Eppendorf tube, 20 µl of proteinase, 20 µl of RNase and 200 µl of binding buffer are added. The resulting mixture is vortexed. The Eppendorf tube is placed in a heating block and incubated at 55°C for 10 minutes. 200 µl of ethanol is added to the same tube and vortexed. Then, the mixture is transferred to the filter tube. The filter tube is placed evenly in the microcentrifuge. Centrifuged at 11,000 rpm for 3 minutes. The filter tube is placed into the collection tube and the liquid at the top (supernatant) is discarded. Add 500 µl of washing buffer-1 to the filter tube. It is centrifuged at 14,000 rpm for 3 minutes. The supernatant is discarded after transferring the filter tube to a new collection tube. 500 µl of washing buffer-2 is added to the filter tube and centrifuged at 14,000 rpm for 3 minutes. One last time, the filter tube is transferred to a new Eppendorf tube. 100 µl of elution buffer is added and centrifuged at 14,000 rpm for 2 minutes. Finally, the resulting purified genomic DNA is stored at -20°C for further processing.

2.3.2. Genotyping Analysis

Before the reaction stage, a master mix is prepared. 100 µl MM and 70 µl dH_2O are added to an empty Eppendorf tube. The mixture (MM and dH_2O) is vortexed for 3-4 seconds. Then, 8.5 µl MM and dH_2O is loaded to into each well. Then, 0.5µl of assay is loaded to into each well. Finally, 1 µl of patient deoxyribonucleic acid (DNA) is added and all the necessary components for the polymerase chain reaction (PCR) pre-preparation step are prepared in a single eppendorf tube. The prepared mixture (MM, dH_2O, assay and DNA) is transferred to a 96-well PCR plate. If there are air bubles in this plate, they are removed and a optical cover is glued to the plate surface. This prepared plate is placed in the PCR device and the thermal cycling program is run. After the reaction is complete, the

amplified DNA, ready for analysis (optionally after being stored at -20ºC for a period of time), can be read.

2.3.3. Statistical Analysis

Statistical analyses were performed using “IBM SPSS Statistics for Windows. Version 25.0 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA)”. Descriptive statistics are presented as n and % for categorical variables, and Mean±SD and Median (min-max) for continuous variables. Independent t-test was used for pairwise group comparisons. Pearson Chi-Square test was used for comparison of categorical variables. $p<0.05$ was considered statistically significant.

3. RESULTS

Data regarding the characteristics features of the 20 patients with AD who participated in the thesis study are presented in Table 6.

Table 6. Characteristic Features of the Patients

No	Age	Gender	Disease
1	68	Male	ALZ
2	63	Male	ALZ
3	60	Male	ALZ
4	74	Female	ALZ
5	66	Female	ALZ
6	79	Female	ALZ
7	82	Female	ALZ
8	75	Female	ALZ
9	81	Male	ALZ
10	67	Male	ALZ
11	65	Female	ALZ
12	69	Female	ALZ
13	71	Male	ALZ
14	71	Female	ALZ
15	67	Male	ALZ
16	66	Female	ALZ
17	60	Male	ALZ
18	63	Male	ALZ
19	70	Male	ALZ
20	64	Female	ALZ

Table 7. Distribution of Characteristics Features of the Patients

Variables	N	%
Age		
Mean ± SD	69.05 ± 6.42	

Median (minmax)	67.5 (60-82)	
Gender		
Male	10	50.0
Female	10	50.0

As seen in Table 2, the average age of the patients was 69.05 ± 6.42 years, and the median age was 67.5 (60-82). The minimum age of the patients was 60 and the maximum age was 82. Also, 50% (n = 10) of the patients were male and 50% (n = 10) were female.

Table 8. CYP2D6 *4 Genotype and Allele Distributions of Patients

Variables	N	%
Genotype		
GG	5	25.0
AG	12	60.0
AA	3	15.0
Allele		
G	22	55.0
A	18	45.0

As seen in Table 3, the distribution of the genotypes belonging to the CYP2D6 *4 polymorphism was determined as 25% (n = 5) GG, 60% (n = 12) AG and %15 (n=3) as AA. When the allele distributions were examined, 55% (n = 22) were determined as the G allele, and 45% (n = 18) were defined as the A allele.

Table 9. CYP2D6 *4 Metabolisms of Patients

Variables	N	%
EM	17	85.0
IM	0	0.0
RM	0	0.0
PM	3	15.0

(EM: Extensive Metabolizer, IM: Intermediate Metabolizer, RM: Rapid Metabolizer, PM: Poor Metabolizer)

As seen in Table 4, the distribution of metabolisms belonging to CYP2D6 *4 polymorphism was determined as 85% (n = 17) EM-normal metabolism and 15% (n = 3) as PM-Poor metabolism.

Table 10. Distribution of Characteristic Features of Patients

	CYP2D6*4 metabolism		
Variables	**EM**	**PM**	**p**
Age, Mean ± SD	63.00 ± 10.42	66.11 ± 11.14	0.586[b]
Gender, n (%)			
Men	8 (%57.1)	2 (%33.4)	0.001[a]
Women	6 (%42.9)	4 (66.6)	

a: Fisher's Exact Test, b: Mann Whitney U Test, p<0.05

As seen in Table 5, CYP2D6 *4 metabolism groups did not show a significant difference in terms of age (p = 0.460). However, a significant difference was found in terms of gender (p = 0.001).

4. DISCUSSION

This thesis study investigated the CYP2D6*4 (rs3892097) polymorphism in a small sample group (10 men and 10 women) with AD. When the CYP2D6*4 metabolic distribution results were evaluated in terms of age and gender, which are among the classic risk factors for AD, two independent results were obtained. From the age factor perspective, (individuals aged 60-82 years) did not show a significant difference ($p = 0.460$). Regarding the gender factor, a statistically significant difference ($p = 0.001$) was observed. However, due to the small sample size and the absence of a control group, a definitive conclusion can not be reached.

The genotype distribution of the sample was AG (60%), followed by GG (25%) and AA (15%). The allele distribution was determined as G (55%) and A (45%). These data show that the genotype profile associated with AD is likely AG, and the gene variant likely G.

According to the data obtained, the CYP2D6*4 metabolizer phenotype was EM in 85% of cases and PM in 15%. This suggests that the CYP2D6 polymorphic enzyme may provide clues about drug response in AD patients. Thus, determining the patient's genetic predisposition (to a large extent) could enable more personalized drug dosage or selection.

5. CONCLUSION AND FUTURE PERSPECTIVES

This thesis study investigated the allele, genotype, metabolic, and characteristic (age, gender) distributions of the CYP2D6*4 (rs3892097) polymorphism in AD patients. Based on these distribution results, it was concluded that EM metabolic type, AG genotype, and the G allele are closely associated with the rs3892097 SNP.

Metabolism groups (EM and PM) showed significant differences for gender, but the same was not true for age. Furthermore, the small population size and lack of a control group limited the power of the statistical data.

Future studies examining polymorphisms at the allele and AD level will significantly contribute to elucidating the heterogeneous nature of AD and to the widespread adoption of personalized (pharmacogenetic) therapies.

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