T.R.

USKUDAR UNIVERSITY

INSTITUTE OF SCIENCE

DEPARTMENT OF MOLECULAR BIOLOGY

MOLECULAR BIOLOGY PROGRAM

MASTER THESIS

"EXAMINATION OF BDNF RS6265 POLYMORPHISM IN ALZHEIMER'S PATIENTS"

Djouhaina Maarouk

Thesis Advisor

Professor Muhsin Konuk, Phd

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DECLARATION

I declare that this study is my thesis study, that I have committed no unethical behavior at any stage from planning to writing, that I have obtained all the information in the thesis within academic and ethical rules, and that I resource all the information and comments that are not obtained through the thesis study.

Date: 21/8/2024.

Djouhaina Maarouk

FOREWORD

I am deeply grateful to Professor Muhsin Konuk and Dr. Tayfun for their unwavering support and guidance throughout the development of this research. I am sincerely thankful for their dedication and generosity in sharing their knowledge. I look forward to learning from their example and contributing to our field.

I would also like to extend my deepest gratitude to my dear parents, Abdulhamid and Akila, for their unconditional love and dedication; to my siblings, Djihad, Adem, Hanadi, and Yakin, for their love and encouragement; and to my grandmother OumElKhir, my aunts Bahdja and Rachida, and my cousins Rayane, Romaissa, Amina, Nirmine, Chaima, and Aya. I offer my heartfelt thanks to my entire family for their emotional and practical support.

I must not forget my wonderful friends, Chawahed, and Rawnek, whose role in motivating and supporting me through challenging times has been remarkable.

SUMMARY

The brain-derived neurotrophic factor is a gene encoded by the BDNF rs6265 gene, which plays an important role in neuronal growth and cognitive function. Genetic analysis of BDNF rs6265 polymorphism is essential to understanding its role in Alzheimer's disease (AD). Variations in this gene can influence the risk of Alzheimer's disease and its progression, which can guide treatment strategies for AD.

In this research, a collection of 25 whole blood samples were collected in 2% EDTA from Alzheimer's patients between the ages of 54-84, who applied to the neurology outpatient clinic of Istanbul NP Brain Hospital, were admitted to the ward or followed up as outpatients and were independently diagnosed with Alzheimer's disease.

In our study, different methods (DNA isolation, CLOCK gene polymorphism genotyping with Thermo Fisher Quantstudio 5 Real Time PCR kit) were used to investigate the BDNF rs6265 polymorphisms in 25 Alzheimer's disease patients using pharmacogenomics test (PGx).

Individual characteristics of the 25 patients diagnosed with Alzheimer's disease included in the study, such as age, gender, disease use status, and BDNF rs6265 genetic polymorphisms, were taken into consideration.

This study evaluated BDNF rs6265 genetic polymorphisms and predicted phenotypes in patients suffering from Alzheimer's disease. The results showed that most patients had the risk phenotype (72%) associated with the TT genotype, while a lower percentage had the normal phenotype (4%) related to the CC genotype. In 24% of patients, an intermediate CT genotype was discovered that corresponds to the observed allele frequencies.

However, further research with a big group of samples is essential to validate these results and to explore the mechanisms underlying the observed associations.

Keywords: BDNF rs6265, Alzheimer's Disease, Polymorphism, Pharmacogenomics test, Genotype, Phenotype, Allele.

ÖZET

Beyin kaynaklı nörotropik faktör (BDNF), nöronal büyüme ve bilişsel fonksiyonda önemli bir rol oynayan BDNF rs6265 geniyle kodlanan bir gendir. BDNF rs6265 polimorfizminin genetik analizi Alzheimer hastalığındaki rolünü anlamak için gereklidir. (AD). Bu genin değişiklikleri Alzheimer hastalığı riskini ve ilerlemesini etkileyebilir, bu da AD için tedavi stratejilerini yönlendirebilir.

Bu çalışmada, İstanbul NP Beyin Hastanesi'nin nörolojisi ambulans kliniğine başvurmuş, bölgeye kabul edilmiş veya ambulans olarak takip edilen ve bağımsız olarak Alzheimer hastalığı teşhisi edilen 54-84 yaşları arasındaki Alzheimer Hastalığı hastalarından %2 EDTA'da toplam 25 adet tam kan örnekleri toplandı.

Çalışmamızda, 25 Alzheimer hastasındaki BDNF rs6265 polimorfizmlerini farmakogenomik test kullanılarak incelemek için farklı yöntemler (DNA izolasyonu, CLOCK gen polimorfizmi genotipleme ile Thermo Fisher Quantstudio 5 gerçek zamanlı PCR kit) kullanıldı. (PGx).

Çalışmada Alzheimer hastalığı teşhisi konan 25 hastanın, yaş, cinsiyet, hastalık kullanım durumu ve BDNF rs6265 genetik polimorfizmleri gibi bireysel özellikleri dikkate alındı.

Bu çalışmada BDNF rs6265 genetik polimorfizmleri değerlendirildi ve Alzheimer hastalığı olan bireylerde fenotipler öngörüldü. Sonuçlar, hastaların çoğunluğunun TT genotipi ile ilişkili risk fenotipi (72%), daha düşük bir yüzdesi CC genotipiyle ilişkili normal fenotip (4%) olduğunu gösterdi. Hastaların% 24'ünde, gözlemlenen alel frekanslarına karşılık gelen bir ortalama CT genotipi keşfedildi.

Ancak bu bulguları doğrulamak ve gözlemlenen ilişkilerin altındaki mekanizmaları araştırmak için büyük örnek boyutları ile daha fazla araştırma gereklidir.

Anahtar Kelimeler: BDNF rs6265, Alzheimer Hastalığı, Polimorfizm, Farmakogenetik test, Genotip, Fenotip ve Allel.

ABSTRACT

Examination of BDNF rs6265 polymorphism in Alzheimer's patients

Background: The BDNF rs6265 gene encodes the brain-derived neurotrophic factor that is crucial for promoting neuronal growth and function. This research aims to examine the individual characteristics of the BDNF rs6265 genetic variation and its predicted phenotypes concerning Alzheimer's Disease through a pharmacogenomics test.

Methods: Blood samples were collected from 25 patients with Alzheimer's Disease. Invitrogen's BDNF rs6265 a genomic DNA kit was utilized to obtain genomic DNA from blood samples (Invitrogen, USA). Genotyping analyses were conducted using TaqMan Genotyping Assays (provided by Applied Biosystems, Foster City, CA, USA) on the Thermo Fisher Quant Studio 5 Real-Time PCR system (Thermo Scientific, Waltham, MA, USA).

Results: Results of 25 patients with Alzheimer's Disease showed the genotype distribution belonging to the BDNF rs6265 polymorphism was found to be 4% (n=1) TT, 24% (n=6) CT, and 72% (n=18) CC genotype. When the allele distributions were examined, 16% (n=8) T and 84% (n=42) C alleles were determined. Additionally, the phenotype distributions of the BDNF rs6265 polymorphism were found to be 4% (n=1) normal, 24% (n=6), and 72% (n=18) risk phenotype.

Conclusions: We found that 72% of patients exhibited the risk phenotype associated with the TT genotype, while only 4% had the normal phenotype linked to the CC genotype. Additionally, 24% of patients had the intermediate CT genotype. Further research with larger sample sizes and comprehensive allele profiling will enhance our understanding of how BDNF polymorphism impacts Alzheimer's Disease progression and inform more personalized treatment strategies.

Keywords: BDNF rs6265, Alzheimer's Disease, Polymorphism, Pharmacogenetics test, Genotype, Phenotype, Allele.

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INDEX OF SYMBOLS AND ABBREVIATIONS

AD: Alzheimer's Disease

BDNF: Brain-Derived Neurotrophic Factor

mRNA: Messenger RNA

Aβ: β-Amyloid

NINCDS-ADRDA: National Institute of Neurological and Communicative Disorders and Stroke– Alzheimer's Disease and Related Disorders Association

CVD: Cardiovascular Diseases

A-beta: Beta-Amyloid

APOE e4: Apolipoprotein E epsilon 4

APP: Amyloid Precursor Protein

PSEN1: Presenilin-1

PSEN2: Presenilin-2

SORL1: Sortilin-Related Receptor 1

NFT: Neurofibrillary Tangles

ApoE: Apolipoprotein E

PET: Positron Emission Tomography

MCI: Mild Cognitive Impairment

-synuclein: Alpha-synuclein

ACh: Acetylcholine

Aβ42: Amyloid Beta Peptide with 42 Amino Acids

SNP: Single Nucleotide Polymorphism

Val66Met: Substitution of Valine with Methionine at Position 66

NGF: Nerve Growth Factor

NT-3: Neurotrophin-3

GFP: Green Fluorescent Protein

ProBDNF: Precursor Brain-Derived Neurotrophic Factor

MRI: Magnetic Resonance Imaging

GMV: Gray Matter Volume

aMCI: Amnestic Mild Cognitive Impairment

MTL: Medial Temporal Lobe

rs6265: Reference SNP ID for the BDNF Val66Met Polymorphism

LD: Linkage Disequilibrium

MCI: Mild Cognitive Impairment

CSF: Cerebrospinal Fluid

MG: Milligram

KDa: Kilo Dalton

HPA: Hypothalamic-Pituitary-Adrenal

NFB: Neurofibrillary Tangles

TBI: Traumatic Brain Injury

BACE1: Beta-Secretase 1

PCR: Polymerase Chain Reaction

LDL: Low-Density Lipoprotein

HDL: High-Density Lipoprotein

MMSE: Mini-Mental State Examination

TLR: Toll-Like Receptor

VLDL: Very Low-Density Lipoprotein

SNV: Single Nucleotide Variant

NFTs: Neurofibrillary Tangles

FAD: Familial Alzheimer's Disease

aa: Amino Acid

PS-1: Presenilin 1 (also referred to as S182/PS-1)

PS-2: Presenilin 2 (also referred to as STM-2/PS-2)

VGS: Volga Germans

PGx: Pharmacogenomics

Met: Methionine

Val: Valine

TT: Genotype with two T alleles

CT: Genotype with one C allele and one T allele

CC: Genotype with two C alleles

C allele: Relates to the allele associated with the Methionine (Met) variant

T allele: Relates to the allele associated with the Valine (Val) variant

EDTA: Ethylenediaminetetraacetic Acid

DNA: Deoxyribonucleic Acid

TaqMan®: A trademark of Applied Biosystems for their Genotyping Assays

1. INTRODUCTION

The swift advancement of innovative medical techniques proves advantageous for public health by notably decreasing the occurrence of neurodegenerative diseases and overall mortality rates, consequently enhancing life expectancy. Despite all these medical advances and developments as well as the improvement of different living conditions, we are currently seeing a considerable increase in chronic neurodegenerative diseases, including Alzheimer's disease (Hane, Francis T. et al. 2017).

Alzheimer's disease is categorized as a gradual neurological condition causing a major disturbance in the natural composition and function of the brain. Cellularly, AD is distinguished by a progressive decline in cortical nerve cells, particularly pyramidal cells, which play a crucial function in advanced cognitive processes (Mann DM. 1996; Norfray JF, Provenzale JM. 2004).

Recently, numerous countries have dealt with this disease as a significant obstacle, almost 15 million people around the world are affected by Alzheimer's disease, with a steady increase in new cases, from 0.5 percent per year at age 65 to about 8 percent annually after age 85 (Evans D.A., et al. 1989). In America, AD represents an important risk to public health; it is classified as the sixth principal factor in death broadly and the fifth for persons 65 years and older (Alzheimer's Association, 2022). Research using postmortem brain tissue from Alzheimer's disease patients exhibits decreased levels of brain-derived neurotrophic factor in both mRNA and protein forms. (Phillips et al., 1991; Narisawa- Saito et al., 1996; Connor et al., 1997; Ferrer et al., 1999; Holsinger et al., 2000; Hock et al., 2000; Garzon et al., 2002; Fahnestock et al., 2002).

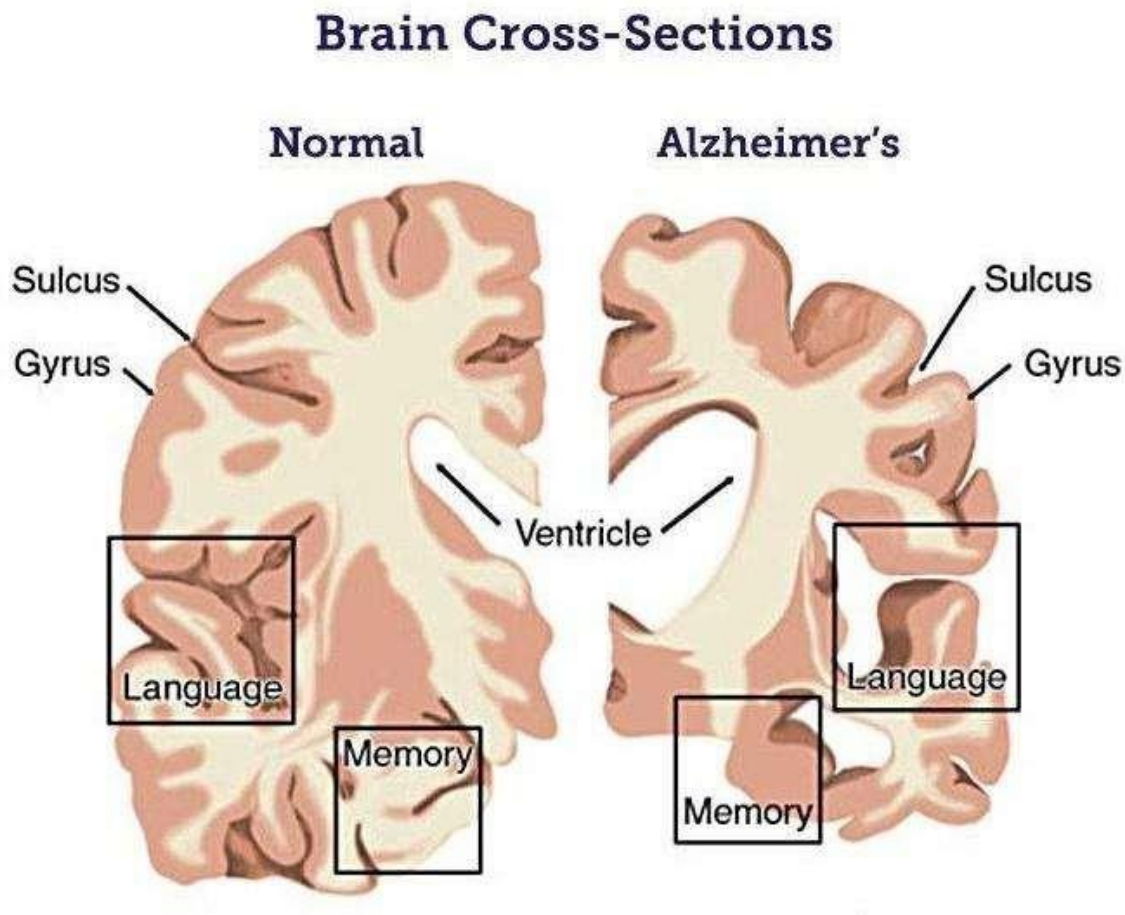
These findings lead to an inquiry into whether the related decrease in serum levels of BDNF would serve as a biomarker for Alzheimer's disease. BDNF levels declined among individuals with advanced Alzheimer's Disorder in a preliminary study compared to controls in the elderly (Yasutake et al., 2006).

1.1. Alzheimer’s disease:

Alzheimer’s disorder is an age-related neurodegenerative disease that appears itself as a progressive decrease in cognitive capacity, usually beginning with memory impairment (Hashimoto et al., 2009). According to Knopman et al. (2021), Beta-amyloid plaques are presented in the extracellular space, and intracellular neurofibrillary tangles, including tau protein, are observed as defining features of this disorder.

The first symptoms of Alzheimer’s disease are neurological modifications that the person can't notice. It takes years for patients to show visible signs, such as memory impairment and language problems, resulting from brain alterations (Alzheimer’s Association, 2021).

Figure 1: Brain cross-sections in Alzheimer’s disease.



(BrightFocus Foundation, 2022)

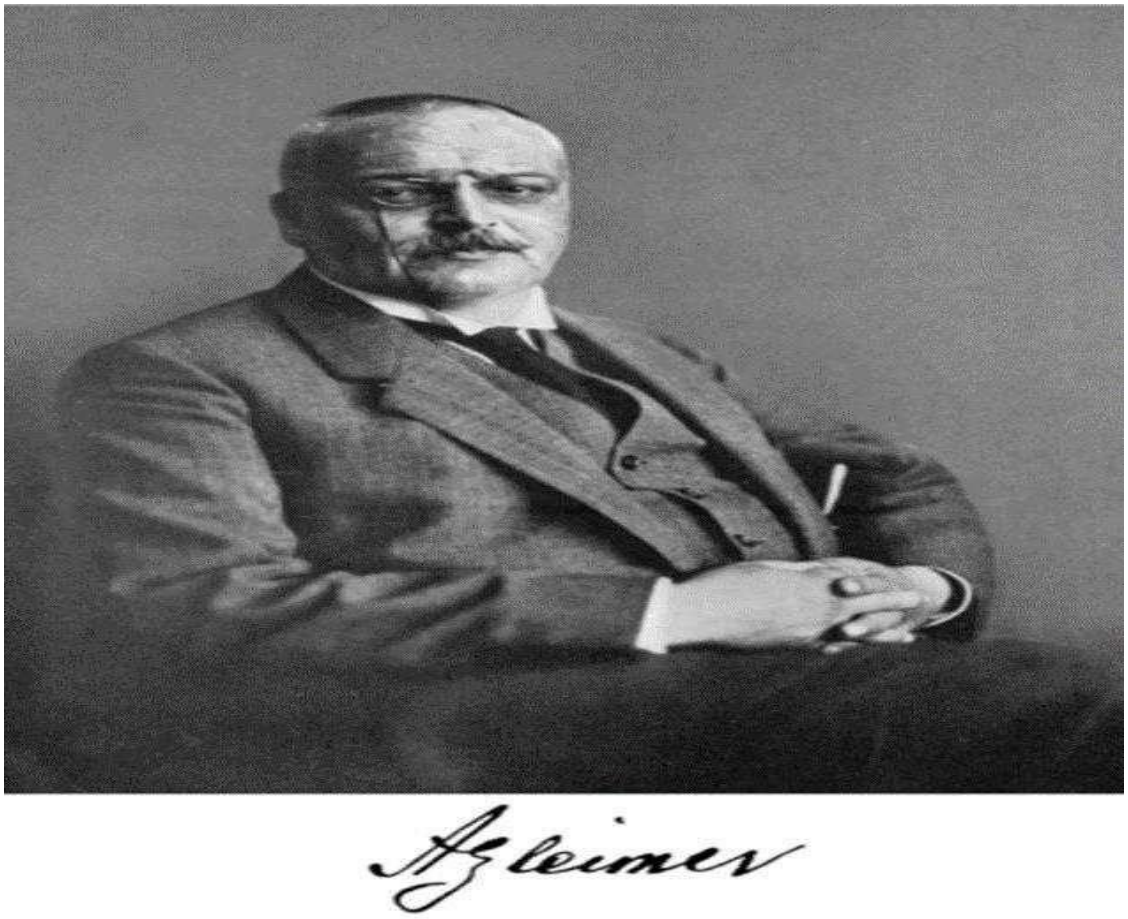
While the disorder evolves, the affected individual could suffer from confusion, changes in personality and manners, altered judgment, and struggles with finding words, finalizing thoughts or following guidance. The rate of these changes differs considerably between individuals, and not all individuals experience these changes, however the result remains the same (Cutler, 2010).

1.1.1. History of Alzheimer disease:

Alzheimer's disease received its name from Emil Kraepelin, who gave it the name of his pupil, Alois Alzheimer. Nevertheless, there is limited information available concerning the life of the individual to whom this significant and widely recognized disease is named (Tagarelli et al., 2006). The German psychiatrist Alois Alzheimer was one of the early users of emerging advanced instruments for studying the human brain, specifically histology, as his area of interest was Advancing psychiatry with microscopic insights (Maurer et al., 1997; Maurer and Maurer, 1999; Spielmeyer, 1916).

Even a century after it was first described, the disorder—named for the psychiatrist Alois' discovery—remains one of the most common neurodegenerative diseases globally. This disorder is responsible for more than 65% of late-onset dementia cases and associated diseases that relentlessly result in significant health deterioration and, ultimately, death.

Figure 2: Aloys Alzheimer (1864–1915).



(Jellinger, 2006)

The centenary of the first clinical and histological description of AD by Alois Alzheimer was commemorated on September 21, 2006, which is celebrated as World Alzheimer's Day (Jellinger, 2006). Almost simultaneously with advances in neuroanatomy, histology, and other biological fields, the clinical and histological characteristics began to be systematically documented only by the end of the 19th century, even though different types of dementia had long been recognized (Beach, 1987; Berchtold and Cotman, 1998). In the eighth edition of Kraepelin's psychiatry reference book, following the release of the section titled (Senile and Presenile Dementia), the designation Alzheimer's disease (AD) began to receive broad recognition, despite not being widely accepted before (Kraepelin, 1910).

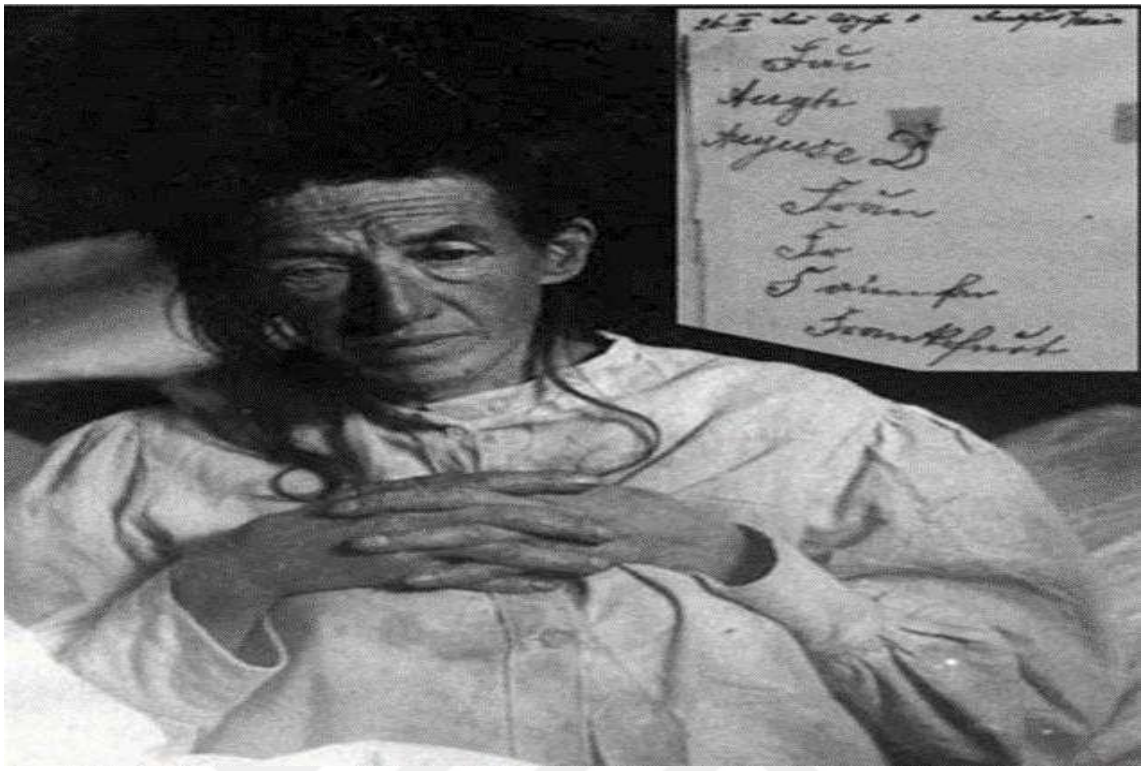
Before the 1970s, AD was classified as a type of early-onset dementia, similar to the condition observed in Alzheimer's initial patients (Amaducci, Rocca, and Schoenberg, 1986).

In the 1990s, doctors became more proficient at diagnosing prominent cognitive decline before it impacted daily activities. As a result, issues related to the effectiveness of the term "probable AD" in identifying neurotic plaques and neurofibrillary tangles began to emerge (Flicker, Ferris, and Reisberg, 1991; Morris et al., 1991).

This word "Alzheimer's," which was formerly used to refer to all forms of dementia, has come to symbolize a well-known illness in the twenty-first century (Jellinger, 2006). The diagnostic phrase "Alzheimer's disease" contains numerous meanings, which presents a difficulty to the study of AD. There is a significant amount of variance in the underlying knowledge related to the condition, since each interpretation has a distinct viewpoint on it. (Knopman, Petersen, and Jack Jr, 2019).

Alois Alzheimer evaluated Auguste D, a 51-year-old lady accompanied by substantial social and behavioral problems, apraxia, hallucinations, delusions, paranoia, and progressive memory loss for almost five years, in the psychiatric facility located in Frankfurt/Main. When Alzheimer analyzed her brain, he found extensive atrophy and, using Bielschowsky's silver impregnation method, he found miliary deposits across the cerebral cortex that correlated with amyloid plaques and neurofibrillary tangles in nerve cells (Graeber and Mehraein, 1999; Maurer and Maurer, 1999, 2003). In November 1906, during the 37th conference of psychiatrists from Southwest Germany in Tübingen, he presented this "unique cerebral cortex disease." His concise description, which lacked illustrations (Alzheimer, 1907), became the defining reference for Alzheimer's disease.

Figure 3: An image from 1902 featuring a handwritten sample of Auguste D.



(Jellinger, 2006)

1.1.2. Etiology:

Alzheimer's disorder is defined by advancing neuronal degeneration, which leads to cell death. This process usually begins in the entorhinal cortex inside the hippocampal region. Genetic factors have been recognized as affecting early and late-onset versions of the condition. For example, trisomy 21 is a known risk determinant for early-onset dementia. AD represents a complex disorder impacted by numerous identified risk factors, where age represents the key contributing factor. Once reaching 65 years, the rate of AD increases twofold every five years (Kumar, Sidhu, Goyal, and Tsao, 2023). Cardiovascular disease (CVD) recognized as a main factor of AD increased; In addition to this, it also exacerbates dementia from stroke or circulatory disorders (Santos et al., 2017).

Figure 4: Risk factors associated with Alzheimer's disease.

(Thompson, 2023)

Obesity and diabetes are two crucial modifiable risk factors linked to Alzheimer's disease. Being overweight can promotes to rise in the risk of type II diabetes and harm glucose tolerance. Continuously high blood sugar levels may affect mental abilities by promoting the accumulation of amyloid beta (A-beta) and causing nerve inflammation; these two factors are detrimental to mental performance. Obesity can also raise the risk by promoting the production of pro-inflammatory cytokines and fostering insulin sensitivity problems (Santos et al., 2017).

Among other risk factors for Alzheimer's disease, we mention:

- Depression
- Advanced parental age at birth
- Traumatic brain injuries
- Cardiovascular and cerebrovascular diseases
- Tobacco use
- Genetic history of dementia
- Increased homocysteine levels
- APOE e4 allele.

If one has a first-degree family member with Alzheimer's disorder, the risk of advancing the disease increases by 10 to 30%. Those whose siblings have late-onset AD have a three-times greater chance than the average population (Nicolas et al., 2018).

Moreover, various factors impact the occurrence of AD, including (Guo et al., 2020):

- Non-coding ribonucleic acids
- Neurovascular barrier
- Increased systolic blood pressure
- Educational background and gender

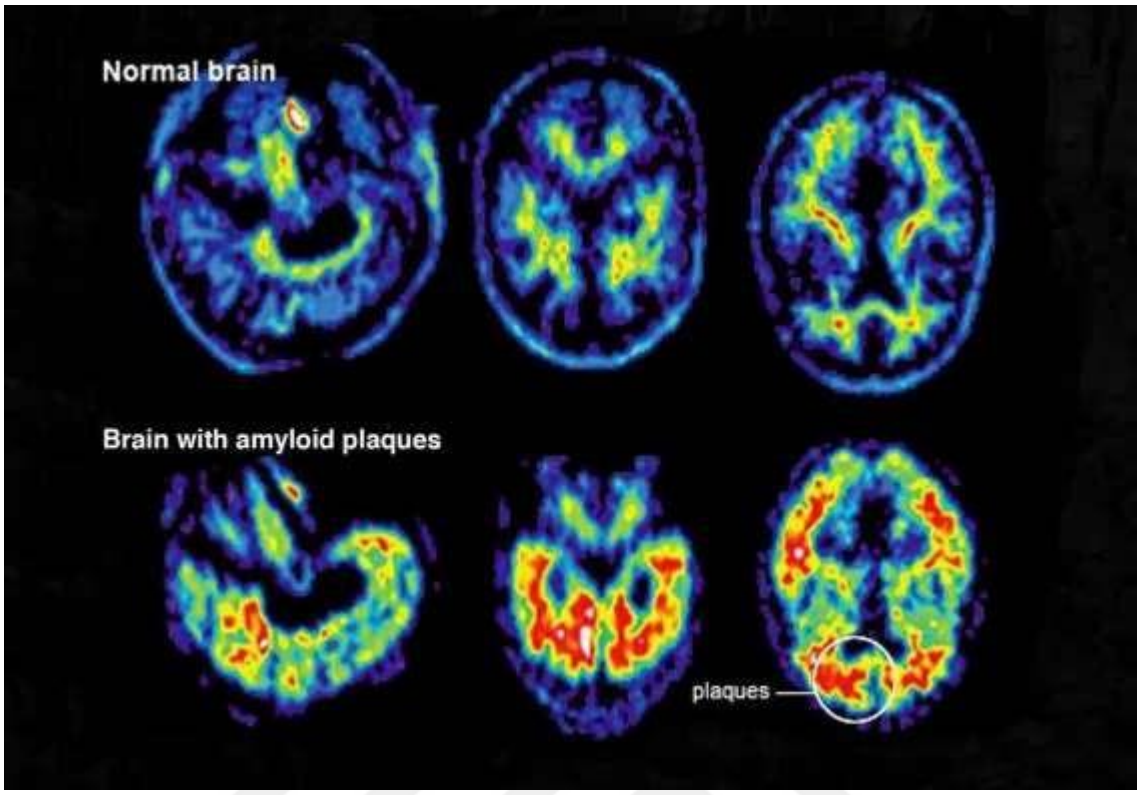
The neuropathological origins of Alzheimer's disease are still unclear, although they are likely associated to the interplay between genetic variations and environmental influences. Amyloidogenic precursor protein (APP), Presenilin-1 protein (PSEN1), Presenilin-2 protein (PSEN2), and Sortilin-related receptor 1 (SORL1) are genetic polymorphism related to Alzheimer's disorder (Campion et al., 1999; Saunders et al., 1993).

1.1.3. Diagnosis:

Alzheimer's disorder is a progressively advancing neurodegenerative disorder most linked to memory loss and cognitive impairment, even though uncommon clinical manifestations are more frequently recognized. Although the fundamental pathological characteristics of the disease have been understood for over a century, the existence of amyloid plaques and neurofibrillary tangles is still necessary for diagnostic pathology today (DeTure & Dickson, 2019).

The diagnosis of this disease can be produced from the initiation of symptoms, which could have started 20 years ago (Serrano-Pozo, Frosch, Masliah, and Hyman, 2011).

Figure 5: Alzheimer's diagnosis.



(Wang, 2017)

The main cause of cognitive decline symptoms is AD, subsequently cerebrovascular dementia and other neurodegenerative disorders. Although the research and use of modern brain imaging technologies have progressed for more than a century, it is still challenging to distinguish AD from other neurological disorders associated with dementia. The symptom overlap across different neurological conditions and the subtle initiation of AD creates significant challenges in achieving accurate early-stage clinical assessment and diagnosis. Recent literature has sought to distinguish between the symptom-based diagnosis of "Alzheimer's disease" and the pathological characteristics typically associated with it. This difference is due to the finding in numerous studies that many individuals have neuropathological changes associated with AD after death without having shown any significant symptoms of dementia before that. Despite the ability of modern PET imaging techniques to detect amyloid deposits in living people, it is difficult to evaluate before death several histopathological aspects of Alzheimer's disease, including loss of synapses, presence of Lewy bodies, neuronal degeneration, gliosis, granulovascular changes, and cerebral amyloid angiopathy (Hyman et al., 2012).

It is crucial to differentiate the symptoms of AD, such as the pseudo- diaphragm or depression, the symptoms of the Lewy corps, the frontal vertebral column and the vascular area for diagnosis. Additionally, it is important to consider and rule out additional factors such as age-related memory loss, substance abuse (including alcohol and drugs), vitamin B12 deficiency, individuals undergoing dialysis, thyroid issues, and the potential effects of taking multiple medications (Geldmacher & Whitehouse, 1997).

Comorbidities including Lewy's body disorder and vascular insults can occur with AD and can result in a reduced capacity to think (Montine and Larson, 2009). According to the research by Selnes and Vinters (2006) and Nelson et al. (2007), Lewy's body disease is a type of neurological disorder in which the α-synuclein protein accumulates and aggregates, forming amyloid-β-like fibrils. (A). Parkinson's disease and Lewy's body-related dementia belong to this type. Besides that, autopsies of individuals with Alzheimer's disease often reveal signs of cerebral vascular disease and brain vascular impairment. The current diagnostic recommendations distinguish three initial stages of Alzheimer's disease depending on the clinical course (McKhann et al., 2011; Sperling et al., 2011):

1. Preclinical.

2. Mild Cognitive Impairment (LCD).

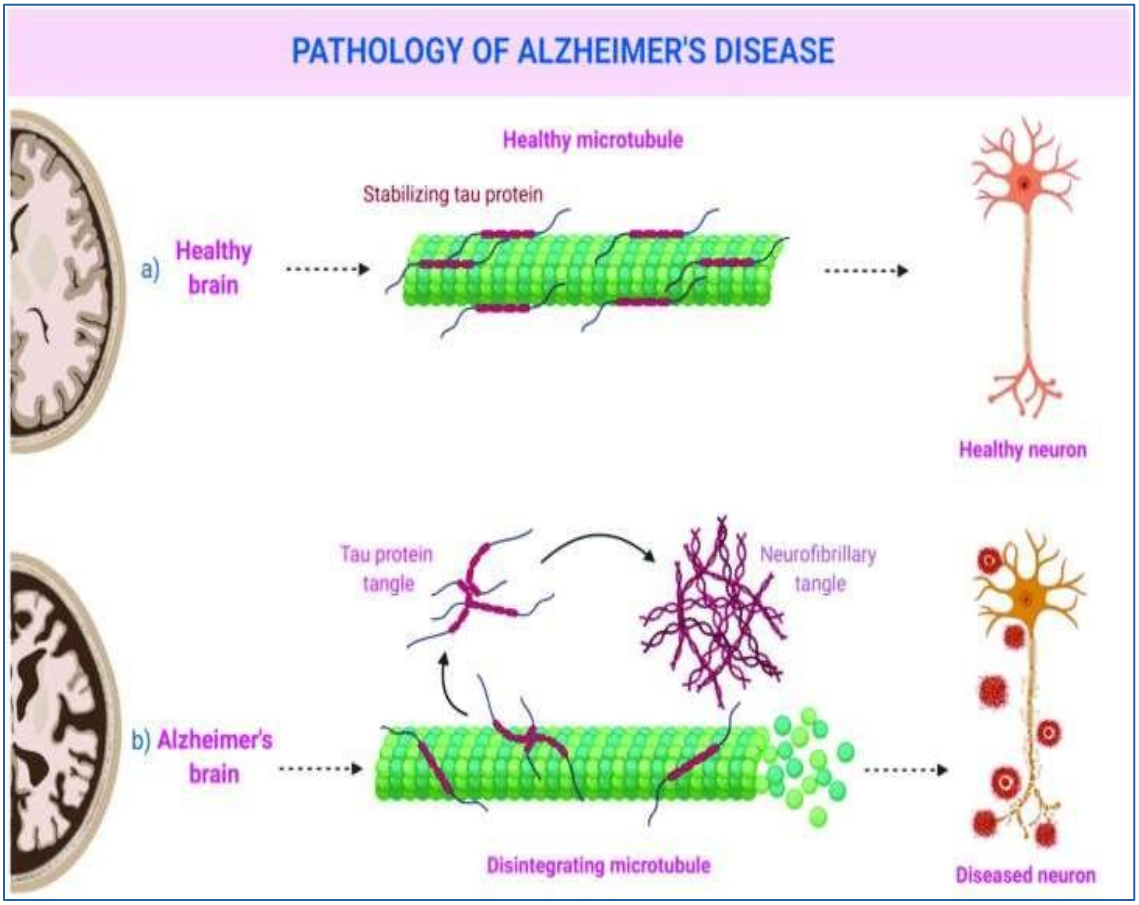
3. Alzheimer's disease-related dementia.

The various stages of the disease typically span a duration extending over many years (Morris, 2005).

1.1.4. Pathophysiology:

The accumulation of abnormal neuritic plaques and neurofibrillary tangles is considered a pathological cause of Alzheimer's disease. Following these alterations, a dysfunction in neural cells can be observed, specifically defined by the depletion of cholinergic neurons in the basal forebrain region and the neocortex (Kumar, Sidhu, Goyal, and Tsao, 2023).

Figure 6: Pathology of Alzheimer's Disease.



(Aloizou et al., 2021)

Founded on these pathological changes, two major hypotheses have been suggested in the field of pathology (Breijyeh and Karaman, 2020):

1. The Cholinergic Hypothesis: According to this hypothesis, the progression of AD is strongly influenced by a decrease in acetylcholine (ACh) concentrations in the brain, resulting from neuronal degeneration in Meynert's Nucleus Basalis. This is confirmed by the premature decrease of cholinergic neurons in AD, which emphasizes the role of AC in cognitive functions.

The cholinergic function is considered to be disrupted by beta-amyloid, which reduces cholinergic synapses and disrupts the release of acetylcholine. (ACh). In addition, clinical studies show that anticholinergic drugs may harm memory in older people (Hampel et al., 2018).

2. The Amyloid Hypothesis: This hypothesis, considered to be the main pathophysiological cause of Alzheimer's disease, in genetic cases, suggests that the beta-amyloid peptide (Aβ) is obtained from the proteins precursor to the amyloid (APP) by the function of the enzymes beta-secretase and gamma-secretase**.** The AAP is usually separated by alpha or beta-secretase, which generates small non-toxic fragments.

Nevertheless, during the sequential cracking of the HAP by beta-secretase and then gamma- secretase, a formation of 42-amino-acid peptides (Aβ42) is observed. High concentrations of Aβ42 lead to the accumulation of amyloids, which negatively affects neurons. The preferred form of accumulated fibrillary amyloid proteins is Aβ42, rather than degrading them normally (Paroni, Bisceglia, and Seripa, 2019).

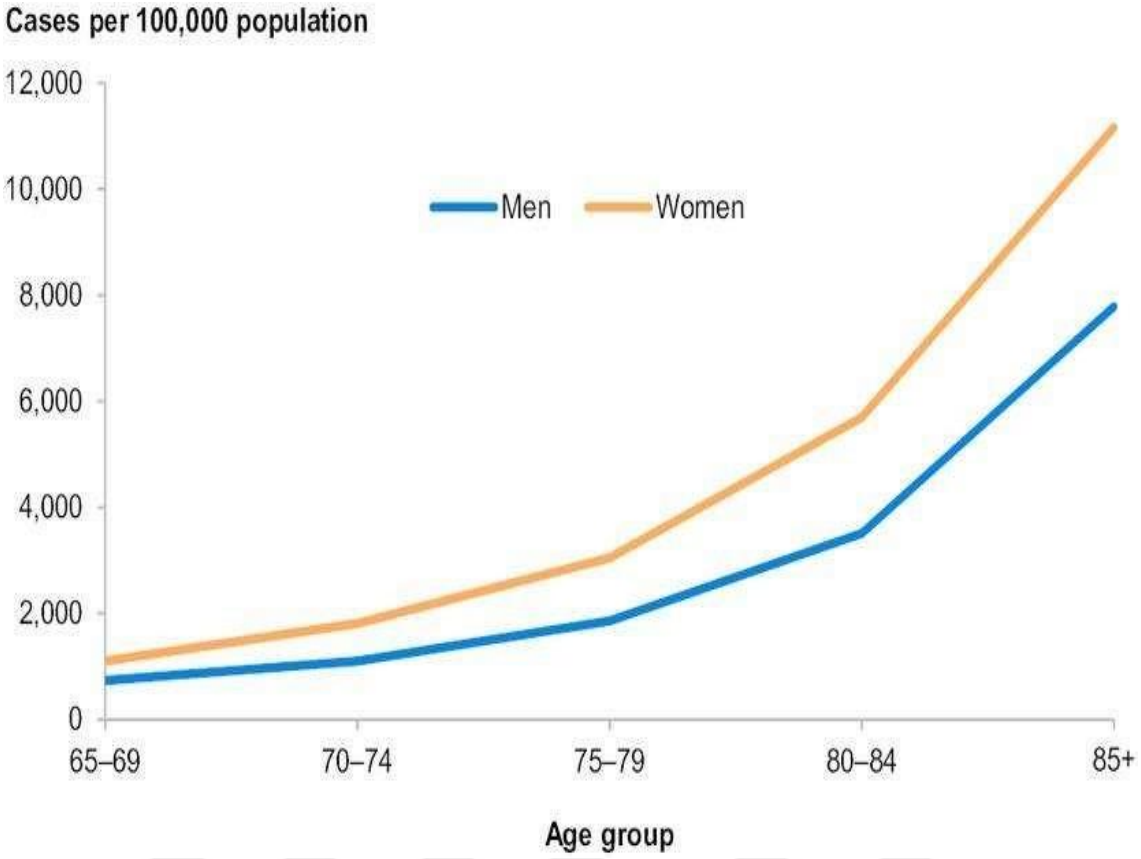
Thus, it is possible to conclude that the main pathological manifestations observed in the nerve tissue of Alzheimer's disorder are the buildup of extra-cellular neuritic amyloid plates and intracellular neurofibrillary tangles (Reitz, Brayne, and Mayeux, 2011).

1.1.5. Epidemiology:

The epidemiological study of AD is closely related to the study of dementia from various causes (Schneider, Arvanitakis, Leurgans, and Bennett, 2009), various brain vascular conditions or neurodegenerative disorders can cause dementia. Especially in elderly individuals (Kapasi, DeCarli, and Schneider, 2017).

At five-year intervals after reaching the age of 65, the rate of Alzheimer's disorder has doubled. Age-related rates of occurrence rise markedly from below 10% beyond age 65 to 40% after reaching age 85 (Qiu, Kivipelto, and von Strauss, 2009).

Figure 7: Fatal fall rates by age and sex group.



(Tovell, Harrison, and Pointer, 2014)

1.1.6. Alzheimer's disease and genetic factor:

Diagnosed dementia and aggregation of Aβ amyloid plaques and neurofibrillary structures holes (NFTs) are the main characteristics of AD. However, AD is expected to show a large diversity in its origins and genetic factors, with the interactions between genetic and environmental elements being very important (Levy-Lahad and Bird, 1996).

Alterations in the amyloid precursor protein, presenilin 1, and presenilin 2, genes have been related to three distinct types of early family Alzheimer's disease, inherited autosomal dominantly. Moreover, the APOE gene is more frequently related to late-onset forms associated with Alzheimer's risk (Bird, 2008).

1. **The APP Gene:** This APP gene was the first identified gene with mutations linked to FAD (Goate, Chartier-Harlin, Mullan, et al., 1991).

It was identified as the gene encoding AP (Kang, Lemaire, Unterbeck, et al., 1987). The primary component of the amyloid plaque, which is the unique neuropathological characteristic of Alzheimer's disease, is the AP peptide composed of 39 to 43 amino acids (aa) (Glenner and Wong, 1984). This gene encodes a large number of precursor proteins (from 695 to 770 aa), which are proteolytically cleaved to form AP. Exons 16 and 17 are part of the group of 19 exons of the APP gene, which encodes Alzheimer's disease (Lemaire, Salbaum, Multhaup, et al., 1989). In addition to the fact of these characteristics, APP identifies chromosome 21 (Tanzi, Gusella, Watkins, et al., 1987).

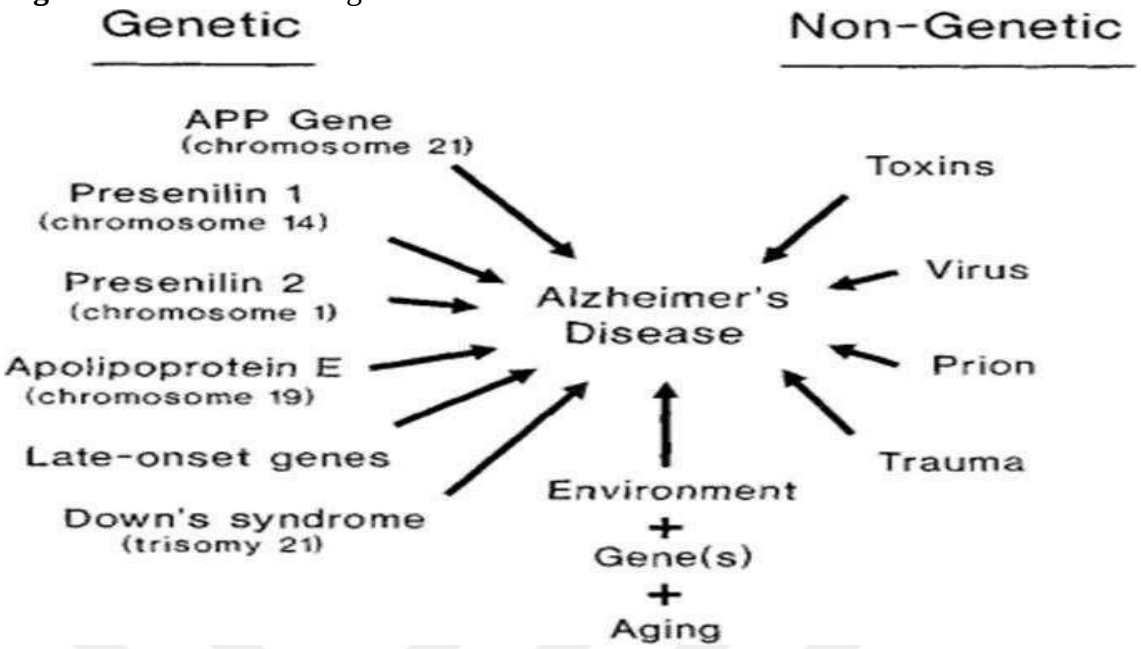
2. **The S182/PS-1 Gene:** This gene was found using only genetic methods. The link between early-onset FAD and a part of chromosome 14 was first found in 9 families (Schellenberg, Bird, Wijsman, et al., 1992), and quickly verified in most published early-onset FAD family studies (St. George- Hyslop, Haines, Rogaev, et al., 1992). The gene, called S182, was duplicated and is also known as presenilin (PS-1) (Sherrington, Rogaev, Liang, et al., 1995).

3. **The STM-2/PS-2 Gene:** Studies of Volga Germans (VGs) shows the identification of the STM-2 and PS-2 gene. FAD represents due to a founder genetic effect in a group of eight pedigrees. Using genome-wide association analysis, the VG FAD region was identified at the level of the first chromosome (Levy-Lahad, Wijsman, Nemens, et al., 1995). Genetic heterogeneity complicated this analysis (VG families with no link to chromosome 1).

4. **APOE Gene:** This gene acts as a cause of Alzheimer's disorder in line with genetic study of late-onset FAD families (Pericak-Vance, Rebout, Gaskell, et al., 1991). In 1891, this risk factor was determined to be within amyloid plaques and neurofibrillary tangles in the brain with Alzheimer's disease, and in cerebrospinal fluid, ApoE was found to attach to fixed AP (Sherrington, Rogaev, Liang, et al., 1995). These results made ApoE a likely gene for the chromosome 19 FAD region, which was tested by checking people from late-onset families for the ApoE e2/e3/e4 gene change. This change involves differences at specific spots 112 and 158 of the ApoE gene and results in three types of the ApoE protein.

APOEε4 is the major genetic contributor to sporadic AD. It advances the formation of senile plaques; however, its mechanism is not yet known (Ott et al., 1999).

Figure 8: Genetic and nongenetic factors related to AD.



(Levy-Lahad and Bird, 1996)

Due to the high similarity between S182/PS-1 and STM-2/PS-2, it is believed that the presenilins may serve similar roles. Both genes are widely expressed throughout the body, including in all areas of the brain (Van Hoorckhoven, Rackhovens, & Guts, 1994).

In the brain, expression is primarily found in neurons. (Kovacs, Fausett, Page, et al., 1996). These rare families with mutations in APP, PS-1, and PS-2 pose challenges for genetic counseling, like those faced with other autosomal dominant neurogenetic conditions where direct DNA mutation testing can be done (Bird and Bennett, 1995).

1.2. Brain-Derived Neurotrophic Factor (BDNF) genes:

Brain-derived neurotrophic factor, a substance crucial for supporting neuron preservation and adaptability, is abundantly found in the brains of both growing and mature mammals (Huang and Reichardt 2001, Chao 2003).

This BDNF present in both the neural system and peripheral areas (Huang and Reichardt, 2001). This neurotrophic factor is a 27-kilodalton polypeptide known for its crucial involvement in the preservation, specialization, and growth of specific outer and inner nerve cells both during growth and throughout adult life (Schinder and Poo, 2000). Moreover, BDNF is recognized for its role in adaptive processes driven by activity, including extended potentiation, along with processes associated to knowledge and memory (Malcangio and Lessmann, 2003).

In recent years, it has been discovered a frequently occurring SNP within the BDNF gene, which results in a change from valine to methionine at position 66 within the prodomain (Val66Met). It has also been shown that this polymorphism affects the volume of the hippocampus and brain functions (Egan et al, 2003). This SNP in the BDNF gene is exclusive to humans and has been linked to increased vulnerability to a variety of mental health conditions (Momose et al 2002, Neves-Pereira et al 2002, Sklar et al 2002, Ventriglia et al 2002, Sen et al 2003).

Considering BDNF's recognized involvement in facilitating processes associated with cognitive development and memory (Korte et al 1995, Patterson et al 1996, Desai et al 1999), this heightened vulnerability to mental decline implies that BDNF genetic polymorphism could contribute to the onset of neuropsychiatric conditions and impact the functioning of the neural system.

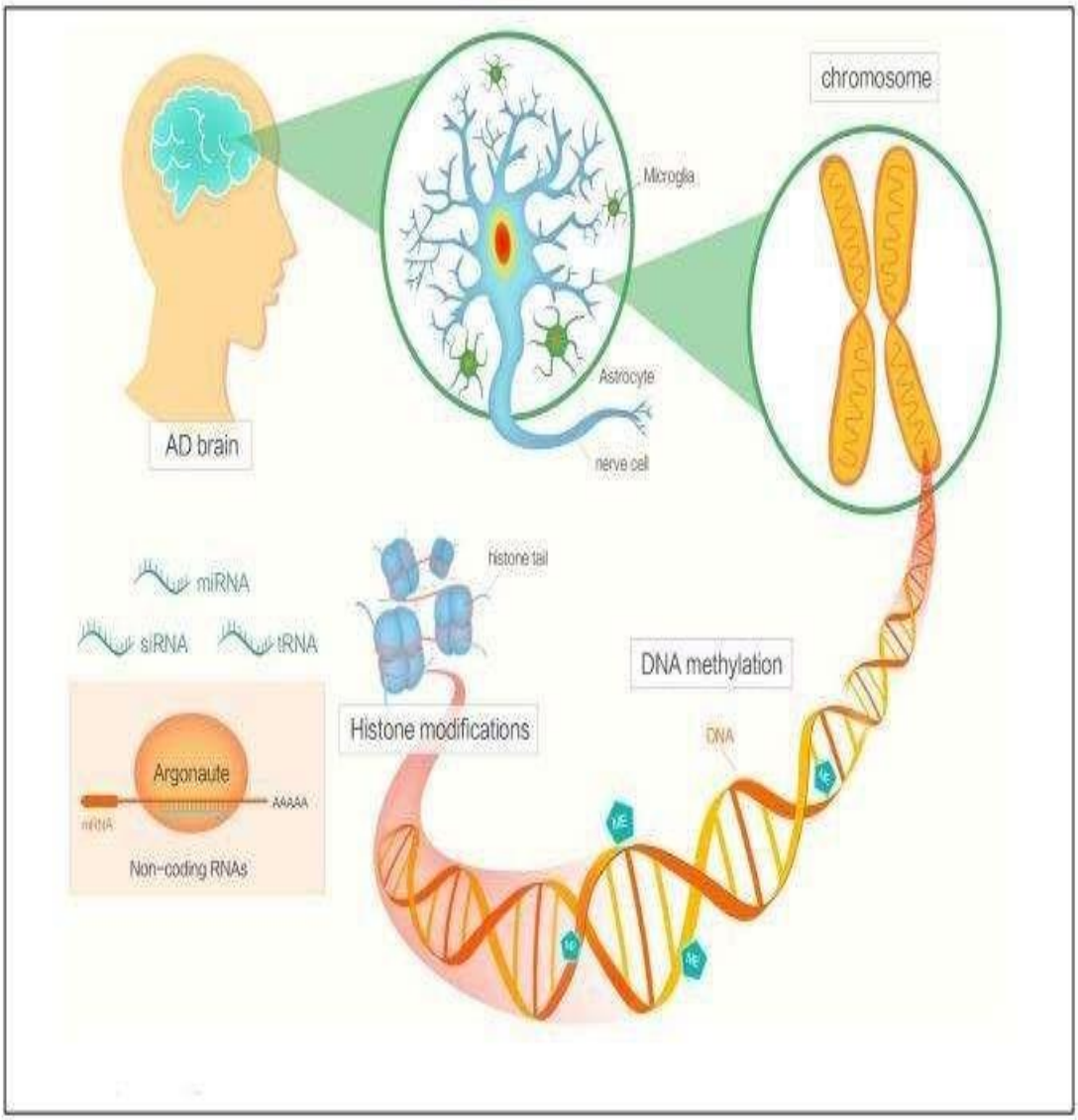
Therefore, we can deduce that this BDNF gene polymorphism serves as an important early instance of how neurotrophins influence human behavioral functions (Chen et al., 2008).

Lately, it has been demonstrated that sortilin, a trafficking protein, is essential for the effective processing of BDNF into the controlled secretion process. Sortilin specifically works with BDNF in the area around the Met change (Chen et al., 2005).

Alzheimer's disease (AD) remains incompletely comprehended. Present knowledge about the impact of epigenetics in AD emphasizes chromatin restructuring, DNA modification, histone alterations, and control by non-coding RNAs (Liu, Jiao, and Shen, 2018).

Inherited gene activity modifications which don't include alterations in DNA strands, known as epigenetic factors, have additionally been recognized. These factors might significantly influence the expression of various genes related to Alzheimer's disease (Kwok, 2010).

Figure 9: Epigenetics factors in Alzheimer's disease.

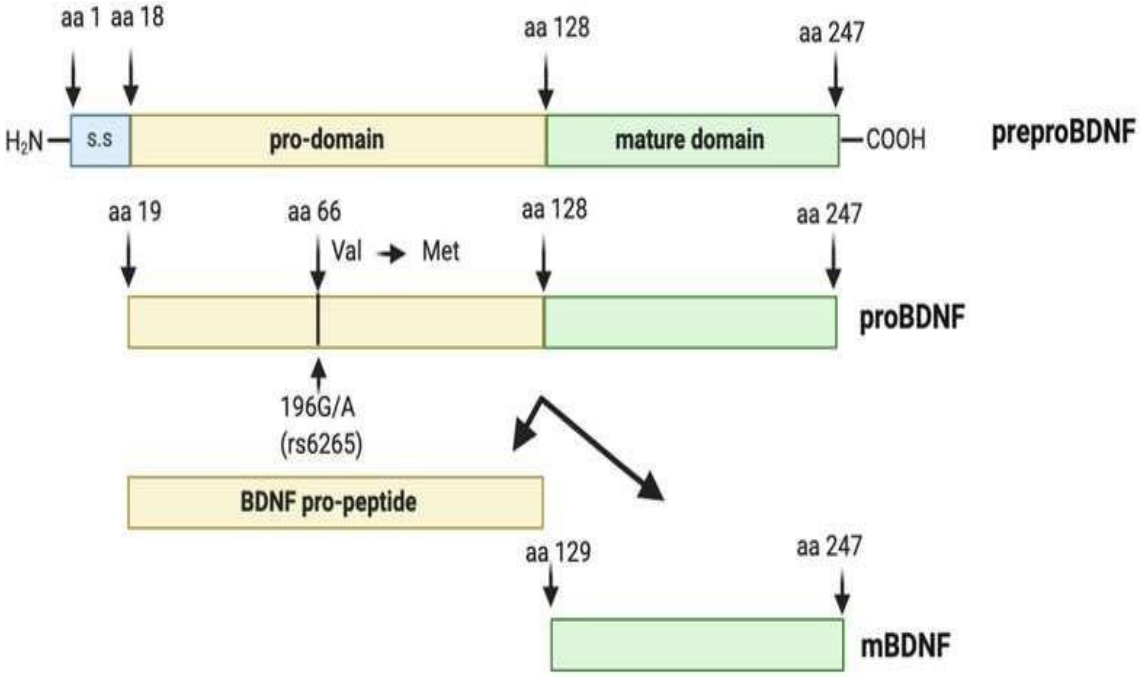


(Liu, Jiao, and Shen, 2018).

1.2.1. ProBDNF precursor:

The human central nervous system contains two BDNF variants: ProBDNF, and the mature BDNF. Research has found that in the brains of people with late- stage Alzheimer's disorder, levels of BDNF messenger RNA and protein, such as proBDNF, are significantly decreased (Peng, Wuu, Mufson, and Fahnestock, 2005).

Figure 10: Pro-brain-derived neurotrophic factor protein structure.



(Colucci-D'Amato, Speranza, and Volpicelli, 2020)

The BDNF gene directs the making of a starting peptide form called proBDNF. At first, BDNF is formed in the form of preproBDNF within the cell's internal network. After the signal peptide is taken away, proBDNF is transferred to the Golgi apparatus, where it is organized into constitutive or regulated vesicles involved in secretion. Inside the cell, proBDNF can be transformed into fully formed BDNF either within the trans-Golgi network by endoproteases from the subtilisin–kexin group, this can involve enzymes like furin, or occur in developing secretory granules through the activity of proprotein convertases (Greenberg, Xu, Lu, & Hempstead, 2009).

Extracellular enzymes, including matrix metalloproteinase-7 and plasmin, might transform proBDNF into mature BDNF (Lee, Kermani, Teng, and Hempstead, 2001).

It was previously believed that just the secreted form of mature BDNF was biologically effective, with proBDNF being confined to intracellular locations and acting solely as a dormant precursor. Nevertheless, emerging evidence now indicates that proBDNF might also have biological activity (Woo et al., 2005). Therefore, we conclude that ProBDNF and BDNF are crucial for various physiological processes (Hashimoto, 2007).

The decline in mature BDNF and proBDNF occurs before the reduction in the function of the enzyme called choline acetyltransferase, that is observed at a later stage in AD. Levels of both mature BDNF and proBDNF show a positive relationship with cognitive evaluations including the MMSE score and the overall cognitive score.

These results highlight that the decrease in these two types of BDNF happens early in AD and correlates with mental decline, this indicates that BDNF and proBDNF are involved in synaptic decline and cellular damage, which play a role in cognitive deficits associated with AD (Peng, Wuu, Mufson, and Fahnestock, 2005).

Extensive research has recorded significant reductions in the enzyme choline acetyltransferase, crucial for acetylcholine production, within the cortex and hippocampus during late-stage Alzheimer's disorder. This depletion is accompanied by synaptic damage, cellular shrinkage, and a dramatic loss of nerve cells that use acetylcholine—up to 90%-within the basal forebrain (Bartus et al. 1982; Whitehouse et al. 1982; Terry et al. 1991).

1.3. BDNF Genes, Brain-Derived Neurotrophic Factor (BDNF), and its role in neurology:

Brain-derived neurotrophic factor is classified as a neurotrophic protein group, and it has crucial functions in the maturation, specialization, and restoration of many forms of neurons in the brain (Fahnestock, Garzon, Holsinger, and Michalski, 2002).

BDNF, neurotrophin-3 (NT-3), and nerve growth factor (NGF) help support neuroprotection (Maisonpierre et al., 1990), like AD, these protein gene polymorphisms lead to neurodegenerative alterations. Inside the temporal lobe and postmortem hippocampus of people with Alzheimer's disorder, a decrease in mRNA production has been noticed (Connor et al., 1997). On human chromosome 11p13, the BDNF genes are situated and formed of six 5′ exons, which are linked differentially with exon 7, the single 3′ terminal exon. This form codes the mature BDNF sequence.

The BDNF 196G/A gene variation leads to a change from valine (Val) to methionine (Met) at position 66 in the 5′ pro-region of the human BDNF gene. Neurons expressing BDNF-66Met with a GFP tag exhibited reduced secretion upon depolarization, although their constant secretion levels were unaffected. Moreover, the GFP-tagged BDNF-66Met did not properly Target secretory granules or synaptic sites (Egan et al., 2003).

1.3.1. The Val66Met gene variant:

The BDNF (Val66Met) genetic variant is the initial instance among neurotrophic factors to be associated with specific structural and behavioral characteristics in people (Chen et al., 2008). This variant is thought to impact the intracellular movement and release of BDNF and has been associated with changes in hippocampal size and episodic memory. Notably, studies have found that the levels of BDNF in the fluid around the fetus of people with proBDNF-66Met variant (Met/Met and Met/Val) are considerably decreased compared to those in individuals without the variant (Val/Val) (Cattaneo et al., 2010).

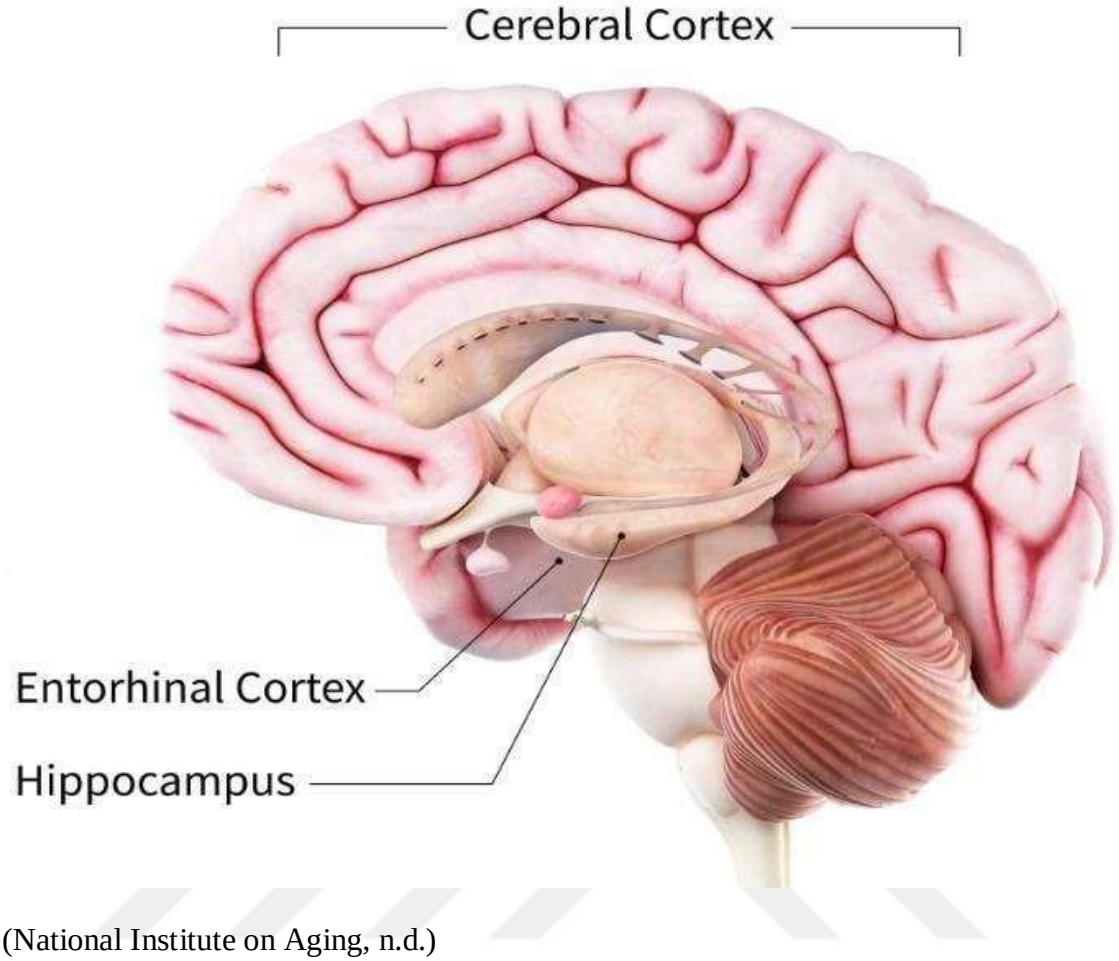
This genetic variation of Val66Met might affect modifications in how variant levels of coding or neural function sites influence memory, attention, and perception. The prefrontal cortex is thought to have an internal clock that helps synchronize time perception (Marinho et al. 2018a). This genetic variant can impact the activity of the parietal and prefrontal cortices, thereby reducing the efficiency of time interval encoding (Marinho, Silva, Oliveira, and Santos, 2019).

1.4. BDNF rs6265 Polymorphism in Alzheimer's Disease

1.4.1. Impact on Hippocampal Volume and Cognitive Decline:

According to Smith (2002), several years before people with Alzheimer's disease (AD) display any medical symptoms, they could have appeared in neuropathological lesions. Lately, there has been a significant focus on advancing biological markers for the prematurely identification of AD, especially during preclinical phase known as aMCI. Researchers have suggested MRI-based volumetry as a valuable technique for identifying structural modifications and biomarkers present in cerebrospinal fluid, such as reductions in gray matter volume (GMV). Most of the research has concentrated on evaluating the volume of medial temporal lobe (MTL) structures to assess their diagnostic utility in AD and aMCI (Chetelat and Baron, 2003; Apostolova et al., 2010).

Figure 11: Critical brain regions for memory.



(National Institute on Aging, n.d.)

The hippocampus represents a main element of the MTL in individuals with AD, which is necessary for memory and cognition (Raji et al., 2009). This hippocampus has a special structure consisting of atrophy that starts from entorhinal cortex regions and transentorhinal and then expands to all others. According to MRI examinations, the advancement of hippocampal volume loss at the Mild Cognitive Impairment (MCI) phase before dementia is regarded as a specific indicator of Alzheimer's disease development (Dubois, Picard, and Sarazin, 2009).

Early genetic investigations into the BDNF Val66Met polymorphism showed that the Val/Val genotype was linked to AD (Harris et al., 2006).

Variations in cortical and hippocampal structure, a significant diagnostic feature of AD according to advanced neuroimaging research, also contributed to the impact observed in individuals with the allele (Val/Met).

Patients with Val/Met have small hippocampal volume regarding the homozygous samples for the Val (Val/Val); this is what brain morphometry research using anatomical MRI scans has shown (Pezawas et al., 2004; Szeszko et al., 2005). This is attributed to the significant role that BDN and its receptors play in the evolution of neuroplasticity (Huang and Reichardt, 2001; Lu, Pang, and Woo, 2005).

1.4.2. Rs6265 polymorphism and AD pathophysiology:

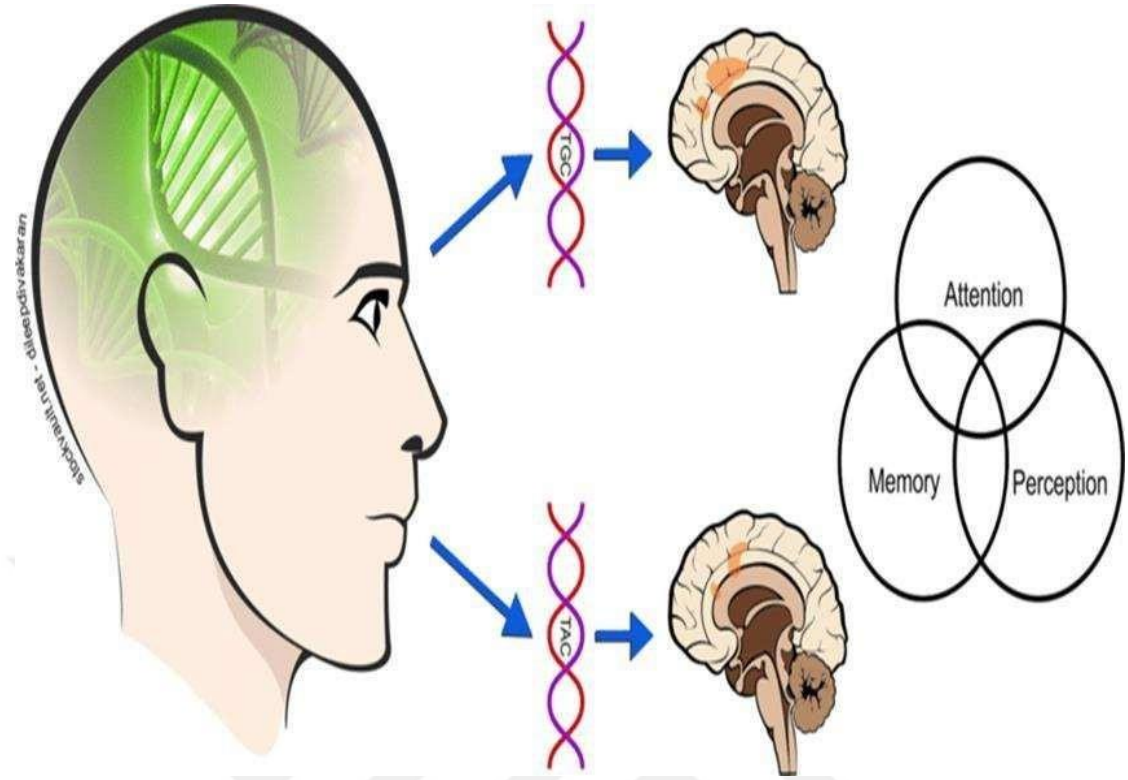
Several genetic variations have been examined in brain-driven neurotrophic genes (rs2030324, rs16917204, rs7103411, rs6265, and rs11030104). Nevertheless, just rs2030324 and rs6265 were extensively examined in the absence of linkage disequilibrium (LD) among them (Matsushita et al., 2005).

BDNF gene in humans situated on chromosome 11 at 13 sites (11p13) has several genetic variations. The rs6265 variant in the BDNF involves a switch from valine to methionine at the 66th position in this protein, so that it greatly affects the response of BDNF functions (Wang et al., 2003).

The deterioration of mental functions has been linked to a single frequent behavioral trait related to the BDNFMet variant. As noted by Egan et al. (2003) and Hariri et al. (2003), there is a difference in memory functions within the hippocampus between patients with the Val/Met allele and those with the Val/Val allele, such that the first type shows negative activity compared to the second type who carries the Val/Val allele.

The function of brain-derived neurotrophic factors in the human mature brain is represented in the protection of cholinergic neural cells from the basal forebrain region and those in the hippocampal region from destruction (Morse et al., 1993).

Figure 12: The BDNF Val66Met polymorphism.



(Marinho et al., 2019)

In summary, BDNF participates in the progression of hippocampal regions associated with enduring memories (Hall, Thomas, and Everitt, 2000). Elevated BDNF levels are also connected with reduced risk of cognitive deterioration in individuals with AD (Laske et al., 2011). Based on these functional this BDNF might be a promising prospective gene, as its polymorphism could elevate the risk of AD. Indeed, a single nucleotide polymorphism has been identified in a non-coding region of the BDNF gene at 11p13, which involves a C→T change located at position 270 (Kunugi et al., 2001).

In addition, this gene influences the risk to neural structures and mental functions in a way that depends on age, corresponding with the neural pathways that are most susceptible in the initial stages of AD (Voineskos et al., 2011).

As mentioned before in Alzheimer's disorder, there is a reduction in BDNF concentrations in the hippocampus and entorhinal cortex (Narisawa-Saito et al., 1996); neurons with neurofibrillary aggregates, which are a defining feature of AD, lack detectable levels of BDNF. Conversely, neurons that show higher labeling with BDNF-specific antibodies do not display these tangles (Murer et al., 1999).

Changes in BDNF concentrations in blood serum and cerebrospinal fluid have been observed in individuals with AD and are associated with the extent of the condition and autobiographical memory function (Peng, Wuu, Mufson, and Fahnestock, 2005).

The Val66Met polymorphism (rs6265) within the BDNF gene, situated in the 5' section, impacts the intracellular handling and BDNF secretion (Egan et al., 2003). The BDNF gene impacts brain structures and cognitive abilities in an age-dependent way, matching the neural pathways that are most susceptible in the initial phase stages of Alzheimer's disorder (Voineskos et al., 2011).

The relationship between the Val66Met polymorphism and age was found to forecast variations in cortical thickness, particularly in the temporal gyri region and entorhinal region, as well as anisotropy fraction in white matter pathways, especially those linked to the medial temporal area, and episodic memory effectiveness (Voineskos et al., 2011).

Additionally, low BDNF levels are associated with aging and appear especially in women, the elderly, and individuals with increased body weight (Komulainen et al., 2008).

So, we conclude that BDNF Val66Met polymorphism influences age; this is achieved by identifying the differences in the neural tissues at risk and the cognitive abilities associated with AD in healthy individuals using an approach that aligns with biological convergence (Gomez-Isla et al., 1996)

2. MATERIAL AND METHODS

2.1. Patient Selection

This study is a prospective cross-sectional study. Patients with Alzheimer's disease between the ages of 54-84, who applied to the neurology outpatient clinic of Istanbul NP Brain Hospital, were admitted to the ward or followed up as outpatients and were independently diagnosed with Alzheimer's disease.

2.2 Ethics Statement

Üsküdar University Unitential Research of Ethics Assembly 27/03/2020 The meeting of 03 on the date of 03 "Genotype-phenotype in Alzheimer's diseases Assessment of the Relationship "decided that this study research project is ethically eligible.

2.3. Sample Collection

A total of 25 whole blood samples were collected in 2% EDTA from Alzheimer's patients either admitted to the neurology ward or followed up as outpatients. These samples were processed at the NP Istanbul Beyin Hospital, Faculty of Medicine, Molecular Genetics and Molecular Diagnostics Laboratory, Üsküdar University. The focus of the study was on BDNF gene polymorphisms. Before their participation, all patients were fully briefed about the study and gave their written consent.

2.4. Equipment

- 20 °C freezer Arçelik (Türkiye).
- Refrigerator Vestel (Türkiye) 3.1.2. Chemical Substances Used.
- Inolab WTW pH Meter (Germany).
- Thermo Scientific Automatic Micropipettes, Eppendorf Research Plus (USA).
- Radway AS 220 / C / 2 Precision Scale (Poland).
- SBH130 Water Bath, Block Heater (UK).
- Stuart Vortex (England).
- Microfuge 16 Microcentrifuge, Beckman Coulter (USA).
- Thermo Scientific Smart 2 Pure 3 Distilled Water Device (U.S.A.).

- Quant Studio 3 (Thermofisher, USA).

2.5. Material

- Fast 96-well Reacton plate.
- TaqMan SNP Genotyping Assays, Human, SM (Thermofisher, USA).
- TaqMan Universal Master Mix Version II, UNG (Uracil-DNA glycosylase) (USA).
- Eppendorf Tube.
- Micropipette and Micropipette Tips.
- Optical adhesive covers.
- Swab DNA Isolation Kit (Thermofisher Scientific Invitrogen, USA).
- Distilled Water (dH2O).

2.6. DNA isolation

Invitrogen's BDNF rs6265 genomic DNA kit was utilized to obtain genomic DNA from blood samples (Invitrogen, USA). Following these steps:

- 200 µl of blood was taken and 200 µl of binding buffer was incorporated to the first tube.
- Then, 20 µl of proteinase and 20 µl of RNAse were incorporated into the mixture.
- The mixture was vortexed completely, and the tube was placed in a heater set to 55°C for 10 minutes to inhibit.
- After heating, 200 µl of ethanol was included in the mixture, and it was mixed again.
- The mixture was moved to the yellow filter tube and centrifuged at 11,000 rpm for 3 minutes, making sure the tubes were placed evenly.
- The filter tube was moved to a new empty tube, and the bottom tube was removed.
- 500 µl of wash buffer was included to the filter tube for the first wash.
- It was centrifuged at 14,000 rpm for 3 minutes.
- The filter tube was then moved to a new empty tube, and the bottom tube was removed again.
- 500 µl of wash buffer was included in the filter tube for the second wash, and it was

centrifuged at 14,000 rpm for another 3 minutes.

- Finally, the filter tube was moved to a new empty tube, 100 µl of elution buffer was added, and it was centrifuged at 14,000 rpm for 2 minutes to elute the sample.

2.7. BDNF rs6265 gene polymorphism genotyping with Thermo Fisher Quanti study 5 Real-Time PCR kit: kit.

Genotyping analyses were conducted using TaqMan Genotyping Assays (Applied Biosystems, Foster City, CA, USA) on the Thermo Fisher QuantStudio 5 Real-Time PCR system (Thermo Scientific, Waltham, MA, USA). DNA samples were collected from Alzheimer's patients. A total of 25 Alzheimer's DNA samples were analyzed by fluorescence, using specific probes that hybridize during the annealing step of the PCR amplification cycle.

2.8. Preparation of Samples for Real-Time PCR

The Thermo Fisher Quant Studio 5 Real-Time PCR device was turned on, and a self-test was performed to ensure proper functioning. The BDNF rs6265 gene polymorphism analysis protocol was loaded into the system, and the patient list was entered. Reaction kits were removed from -20°C and thawed.

The genotyping protocol for the BDNF gene rs6265 SNP, as outlined in the research by Dimitra-Weglarz et al. (2015), was followed. The PCR mix was prepared by combining 5 µL of master mix (Applied Biosystems, USA), 3.75 µL of distilled water, 0.50 µL of TaqMan Genotyping Assays, and 1 µL of DNA (10 ng), resulting in a total volume of 10 µL.

The prepared mixture was added to a 96-well, 0.1 mL reaction plate (Applied Biosystems, MicroAmp Fast 96-Well Reaction Plate). Positive and negative controls were included on the plate. For the negative control sample, distilled water was used in place of DNA. For the positive control, a heterozygous DNA sample with a known genotype was used.

The plate was then tightly covered with optical adhesive covers (Applied Biosystems, USA). Air bubbles in the wells were checked for and removed by gently tapping the plate. After ensuring that no bubbles were present, the necessary checks were completed, and the PCR program was initiated.

2.9. Statistical analysis

The IBM SPSS Statistics for Windows, Version 25.0 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA) package was utilized for the statistical analysis of the genotyping results. Categorical data (n and %) and numerical data for socio- demographic, clinical and BDNF polymorphism of patients were given as Average±SS. Kruskall Wallis test was used to compare genotypes and age. Fisher's Exact test was applied to compare genotypes and gender, with $p<0.05$ regarded as statistically significant.

3. RESULTS

Table 1: Characteristic features of Alzheimer's patients.

No	Gender	Age
ALZ-1	Female	68
ALZ-2	Male	63
ALZ-3	Female	60
ALZ-4	Female	74
ALZ-5	Male	66
ALZ-6	Male	62
ALZ-7	Male	79
ALZ-8	Male	82
ALZ-9	Female	75
ALZ-10	Male	57
ALZ-11	Female	81
ALZ-12	Female	61
ALZ-13	Male	83
ALZ-14	Male	84
ALZ-15	Female	67
ALZ-16	Male	54
ALZ-17	Male	65
ALZ-18	Female	77
ALZ-19	Female	61
ALZ-20	Male	79
ALZ-21	Female	65
ALZ-22	Female	71
ALZ-23	Female	69
ALZ-24	Male	71
ALZ-25	Female	71

As seen in Table 1, the characteristics of 25 Alzheimer's patients (identified as ALZ-1 to ALZ-25) are listed, including their genders and ages, which range from 54 to 89 years.

Table 2: Distributions of patient characteristics.

Variables	**N**	**%**
Age		
Ort ± SD	69.80 ± 8.57	
Median (min-max)	69.0 (54-84)	
Gender		
Male	12	48.0
Female	13	52.0

As seen in Table 2, the median age of the patients was 69.80 ± 8.57, and the median age was

69.0. The minimum age for the patients was 54, and the maximum age was 84. 48.0% of patients (n=12) were male and 52.0% (n = 13) were female.

Table 3: BDNF genotype and Allele distribution of patients.

Variables	**N**	**%**
rs6265		
Genotype		
TT	1	4.0
CT	6	24.0
CC	18	72.0
Allele		
T	8	16.0
C	42	84.0

As seen in Table 3, the spread of genotypes associated with BDNF rs6265 polymorphism was identified as 4% (n=1) TT, 24% (n=6) CT, and 72% (n=18) CC genotype. When the allele distributions were examined, 16% (n=8) T and 84% (n=42) C alleles were determined.

Table 4: BDNF Phenotype Results of Patients.

Genotype		Phenotype	N	%
CC		Normal	1	4.0
CT	Risk		6	24.0
TT	Risk		18	72.0

As seen in Table 4, the phenotype distributions of the BDNF rs6265 polymorphism were determined as 4% (n=1) normal, 24% (n=6), and 72% (n=18) risk phenotype.

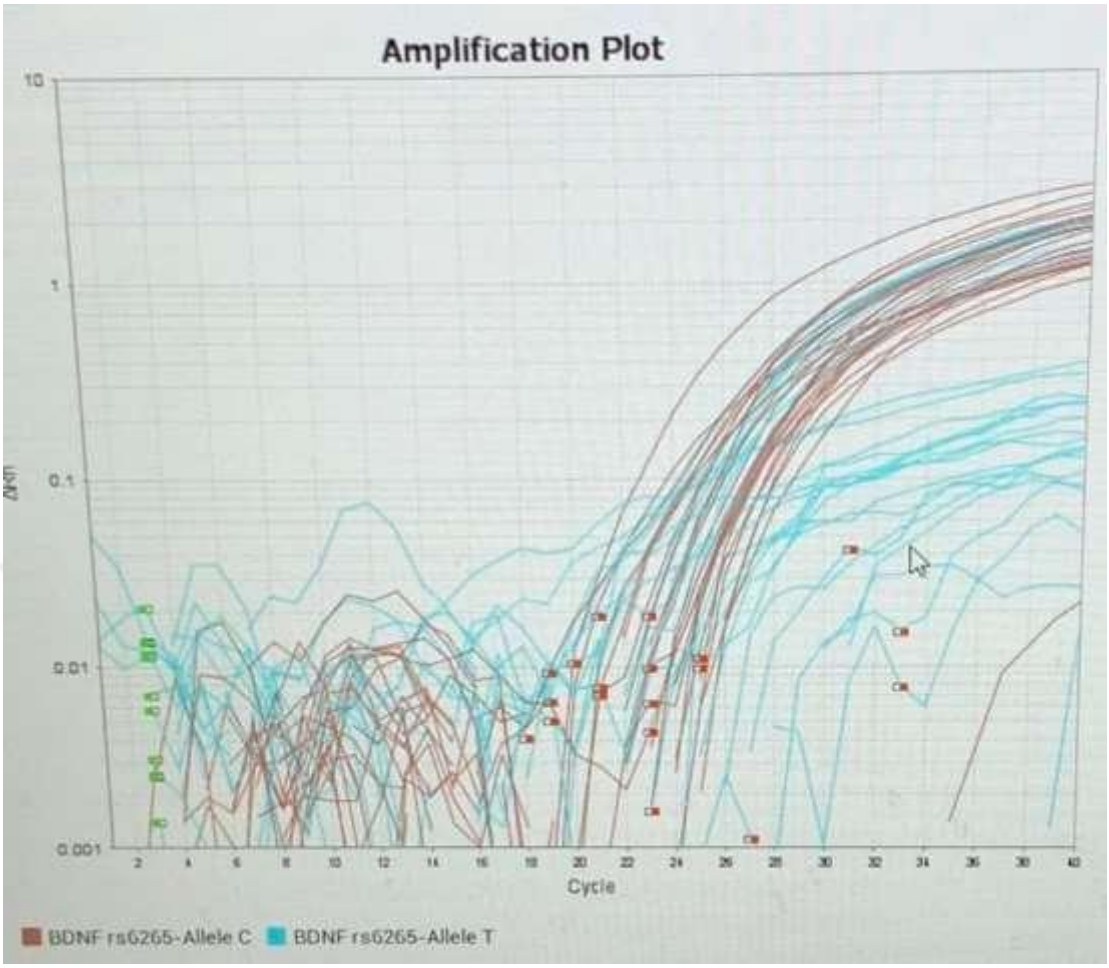
Table 5: Comparison of BDNF genotypes with age and gender.

	BDNF rs6265			
Variables	**TT**	**CT**	**CC**	**p**
Age Ort ± SD	68.0 ± 7.51	67.3 ± 7.52	70.7 ± 9.15	0.706[a]
Gender, n (%)				
Female	1 (%100.0)	2 (%33.3)	10 (%55.6)	0.504[b]
Male	0 (%0.0)	4 (%66.7)	8 (%44.4)	

a: Kruskal Wallis test, b: Fisher's Exact test, $p<0.05$ statistically significant

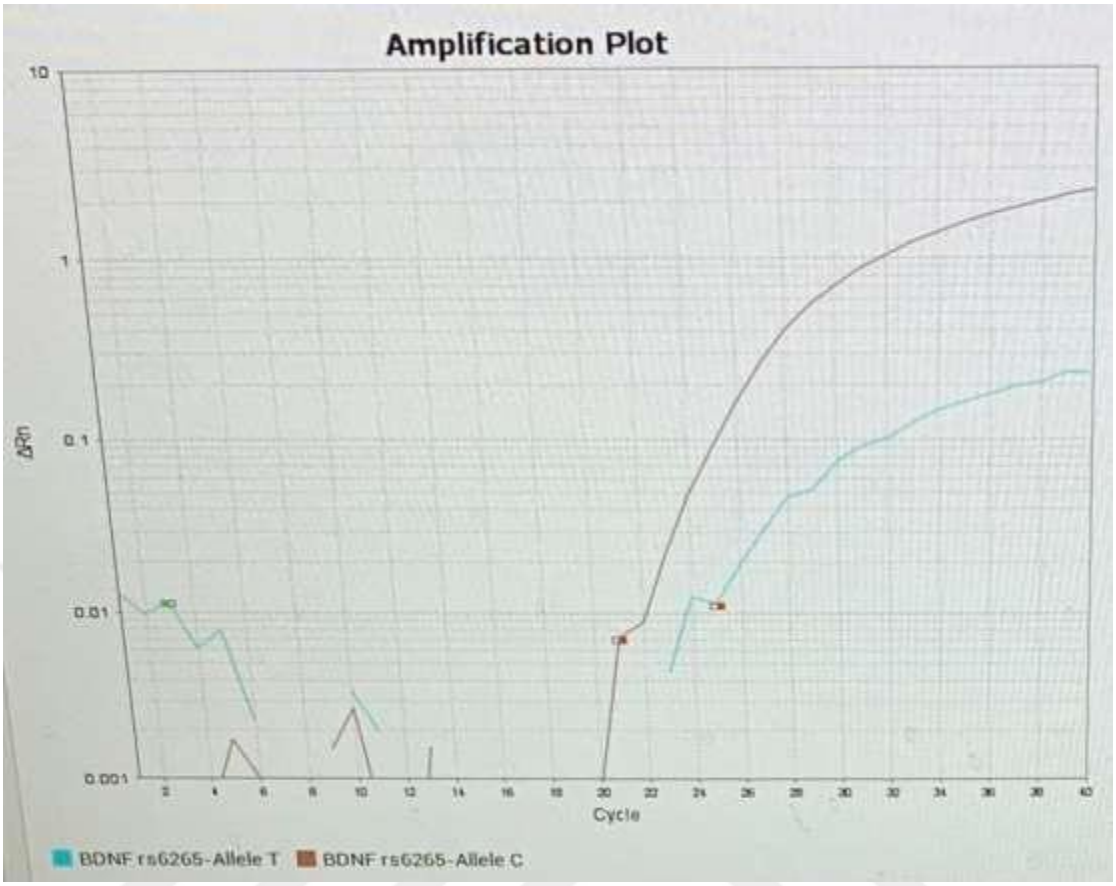
As seen in Table 5, BDNF genotypes did not show any notable difference regarding age (p=0.706) and gender (p=0.504).

Figure 13: The real-time PCR Amplification plot 1.



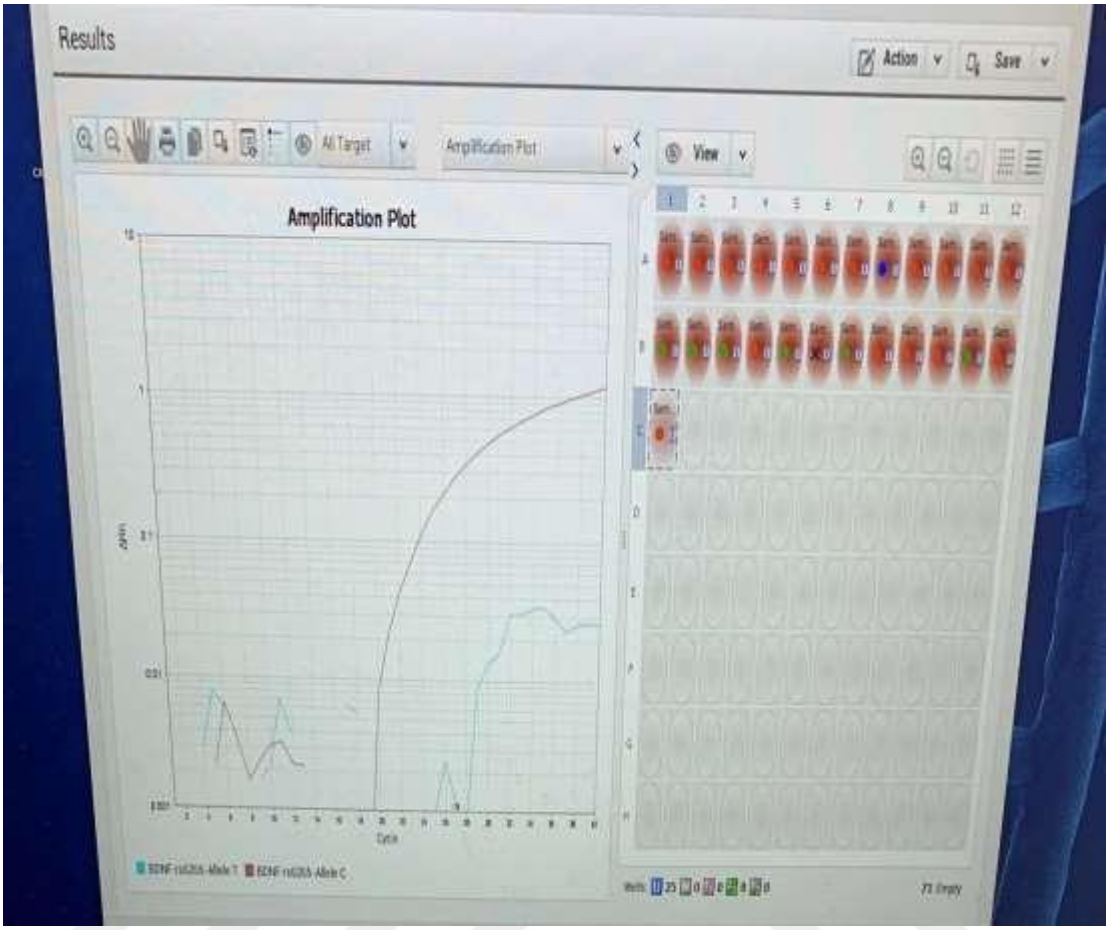
This figure represents an amplification plot from real-time PCR experiments focusing on the amplification of two alleles: BDNF rs6265 Allele T shown in red and BDNF rs6265 Allele C shown in bleu.

Figure 14: The real-time PCR Amplification plot 2.



According to this figure, this amplification plot reveals that the BDNF allele rs6265 C (red) amplifies later than the T allele (blue), with different curves indicating disparities in the amount of DNA or the effectiveness of amplification between the two alleles.

Figure 15: Thermo Fisher Cloud results.



The figure represents amplification curves for two alleles (BDNF rs6265 Allele T and Allele C) and a grid of wells showing the results for 25 different samples.

4. DISCUSSION

The examination of the BDNF rs6265 polymorphism in Alzheimer's disease (AD) provides information on the genetic elements that may vary the risk and progression of this disease. In our research, we looked at the spread of the BDNF rs6265 genotypes and their link with Alzheimer's disease, focusing on the frequency of C and T alleles and their influence on phenotypic outcomes.

In this study, the C variant of the BDNF rs6265 polymorphism is used to represent the methionine variant (Met), while the T allele is used for the valine. (Val). In this study, most patients with AD had the genotype CC (72%), followed by CT (24%) and TT (4%). In allele distribution, allele C dominates (84 %) against allele T (16 %). These results, where the methionine (Met) variant is dominant, are consistent with previous studies that often report the C allele as more common in different populations. Consistency with wider research highlights the importance of the methionine (Met) variant in understanding the genetic predisposition to disease. On the other hand, it was observed that most patients had the risk phenotype (72%) associated with the TT genotype, while a lower percentage had the normal phenotype (4%) related to the CC genotype. In 24% of patients, an intermediate CT genotype was discovered that corresponds to the observed allele frequencies.

According to the results, BDNF rs6265 polymorphism may be associated with the risk of Alzheimer's disorder. In particular, the T allele (particularly in the TT genotype) appears to be associated with a riskier phenotype. This finding confirms previous studies have indicated that the Met (T) variety of BDNF is linked to impaired neuroplasticity and cognitive disorders, which can result in a higher risk of AD. In addition, we can infer that, several studies show the Val66Met BDNF SNP is related to brain structure (Tanzi, 2012).

The contrasting findings across different populations highlight the complexity of genetic associations with Alzheimer's Disorder. In Italy, the BDNF 11757 G/C polymorphism was significantly linked to AD, indicating a possible genetic marker for risk in this population (Boiocchi et al., 2013). In China, however, no significant link was found between BDNF rs6265 and AD, suggesting that this polymorphism may not be a significant risk factor in the Chinese Han population (He et al., 2007). Conversely, among the Arab population, the BDNF Val66Met polymorphism showed an association

with cognitive performance, rather than directly with AD susceptibility (Abanmy et al., 2021). These disparities highlight the importance of continuing studies on the interaction between genetic factors, such as BDNF rs6265 polymorphism, and environmental and ethnic factors to affect the risk and advancement of AD. These divergent results could be influenced by regional genetic diversity, population-specific genetic backgrounds and different methodological approaches.

Even if our study provides valuable information, its limitations must be taken into account. The sample of 25 patients has a relatively small size, which may have an impact on the generalization of the results. In addition, the BDNF rs6265 polymorphism has been studied in particular, and other genetic factors and polymorphisms may also influence the risk of Alzheimer's disorder. Future studies must include wider samples and a wider variety of genetic markers to better understand the function of BDNF and other genes in AD. Furthermore, analysis of the functional mechanisms by which BDNF rs6265 polymorphism affects neuroplasticity and cognitive function could provide more precise insight into its role in Alzheimer's disorder.

5. CONCLUSION

In conclusion, this study has been an important step towards the examination of BDNF rs6265 polymorphisms and their predicted phenotypes in individuals with Alzheimer's disease through the use of pharmacogenomics (PGx). However, the obtained findings ought to be evaluated with caution because of their restrictions and deficiencies. Future research with larger sample sizes and other clinical factors will provide a clearer understanding of the effects of BDNF rs6265 genetic polymorphisms on treatment responses and drug metabolism in Alzheimer's patients. Such studies will contribute to the improvement of Alzheimer's disorder treatment processes and the progression of personalized treatment approaches.

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