

T.R.
GEBZE TECHNICAL UNIVERSITY
GRADUATE SCHOOL

**METABOLITE BIOMARKERS FOR THE SUBTYPES OF
ALZHEIMER'S DISEASE FROM TRANSCRIPTOME DATA: A
GENOME-SCALE METABOLIC MODELING APPROACH**

KADER SARIBULAK

**A THESIS OF MASTER OF SCIENCE
DEPARTMENT OF BIOENGINEERING
BIOINFORMATICS AND SYSTEMS BIOLOGY PROGRAM**

ADVISOR: PROF. DR. TUNAHAN ÇAKIR

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GEBZE TEKNİK ÜNİVERSİTESİ
LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

**ALZHEIMER HASTALIĞININ ALT TİPLERİ İÇİN TRANSKRİPTOM
VERİLERİNDEN METABOLİT BİYOBELİRTEÇ TAHMİNİ: GENOM
ÖLÇEKLİ METABOLİK MODELLEME YAKLAŞIMI**

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YÜKSEK LİSANS TEZİ
BİYOMÜHENDİSLİK ANABİLİM DALI
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*To those whose love, support, and
wisdom have shaped both my heart and
my mind*

ABSTRACT

Alzheimer's disease (AD) is the most common neurodegenerative disease, characterized by progressive memory impairment and changes in brain physiology. The presence of brain abnormalities long before clinical symptoms underscore the urgent need for early diagnosis. However, the heterogeneous and complex pathology of AD limits the effectiveness of single-type diagnostic strategies. In this thesis study, transcriptome data of 295 AD patients from the Religious Orders Study and Memory and Aging Project (ROSMAP) and Mount Sinai Brain Bank (MSBB) cohorts were used to predict extracellular metabolite production profiles for each patient using the TrAnscriptome-based Metabolite Biomarkers by On-Off Reactions (TAMBOOR) algorithm. The algorithm works by integrating RNA-seq data into a human genome-scale metabolic model. In this study, analyses were conducted under two main sections: (i) general metabolite secretion prediction by pooling all AD patients, termed the subtype-unaware approach, and (ii) metabolite secretion prediction specific to three previously defined AD molecular subtypes. The predictions revealed that acylcarnitine, HDL, and sulfolactate showed abnormal activity in the subtype-unaware approach, and previous experimental studies supported these findings. Then, biomarker candidates specific to the three main subtypes of AD were determined; betaine, PGD2, 5-HETE and proline in one of the subtypes; creatine phosphate, CDCA and phosphoethanolamine in another subtype; and glycogen, hyaluronate and succinic semialdehyde in the last subtype. In addition, metabolite activities predicted by TAMBOOR were analyzed with a feature selection algorithm to identify the metabolites showing significant differences between subtype-dominant clusters. In this context, elaidate, corticosterone and its derivatives, and coenzyme Q₁₀ were determined as important metabolites, emphasizing differences between subtypes. In light of the findings, it has been demonstrated that although some common metabolic changes are observed in AD, different subtypes have their own metabolic profiles. Moreover, the biomarkers successfully predicted with TAMBOOR are of critical importance in terms of distinguishing AD patients according to their subtypes. This integrative approach holds significant potential for the establishment of personalized diagnostic frameworks and the development of targeted treatment strategies in AD.

Keywords: Alzheimer's Disease, Genome-scale Metabolic Model, Transcriptome, Biomarker, Disease Subtypes

ÖZET

Alzheimer hastalığı (AH), ileri düzey bilişsel gerileme ve nöronların ölümüyle karakterize olan en yaygın nörodejeneratif hastalıktır. Klinik semptomların başlamasından çok önce beyin anomalilerinin varlığı, erken tanıya olan kritik ihtiyacı vurgulamaktadır. Bununla birlikte, AH'nin heterojen ve karmaşık bir patolojiye sahip olması, tek tip tanı stratejilerinin etkinliğini sınırlayabilmektedir. Bu çalışmada, Religious Orders Study and Memory and Aging Project (ROSMAP) ve Mount Sinai Brain Bank (MSBB) kohortlarında yer alan 295 hastadan elde edilen transkriptom verileri, TrAnscriptome-based Metabolite Biomarkers by On-Off Reactions (TAMBOOR) algoritması ile işlendi ve hücre dışına salgılanan metabolit salınım profilleri her hastaya özgü olarak tahmin edildi. Bunu yapabilmek için RNA-seq verileri insana özgü genom ölçekli bir metabolik modele haritalandı. Bu çalışmada analizler, iki ana başlık altında yürütüldü: 1) tüm AH hastalarını kapsayan genel metabolit salınım tahminleri ve 2) önceden tanımlanmış üç AH moleküler alt tipine özgü metabolit salınım tahminleri. Elde edilen tahminler sonucunda AH'de genel olarak asilkarnitin, HDL ve sulfolaktat metabolitlerinin sağlıklılara göre farklı aktivite gösterdikleri belirlendi ve bu bulgular önceki deneysel çalışmalarla desteklendi. Ardından, AH'nin üç ana alt tipine özgü biyobelirteç adayları belirlendi; alt tiplerin birinde betain, PGD2, 5-HETE ve prolin; bir diğer alt tipte kreatin fosfat, CDCA ve fosfoetanolamin; ve son alt tipte glikojen, hiyalüronat ve süksinik semialdehit ön plana çıktı. Ayrıca, önceki alt tip etiketleri, TAMBOOR tarafından tahmin edilen metabolit aktivitelerinin davranışları ile karşılaştırıldı ve öznelilik seçimi yaklaşımı kullanılarak alt tiplerin baskın olduğu sınıflar arasında anlamlı farklılık gösteren metabolitler belirlendi. Bu kapsamda elaidik asit, kortikosteron ve türevleri ile koenzim Q₁₀, alt tipler arası farklılıkları vurgulayan önemli metabolitler olarak öne çıktı. Elde edilen bulgular ışığında, genel olarak AH'de bazı ortak metabolik değişiklikler görülmesine rağmen, farklı alt tiplerin kendilerine özgü metabolik profilleri olduğu ortaya konmuştur. Bununla birlikte, AH hastalarının alt tiplerine göre ayrımının yapılabilmesi açısından TAMBOOR ile başarılı tahmin edilen biyobelirteçlerin kritik bir öneme sahip olduğu anlaşılmıştır. Bu bütünleştirici yaklaşım, kişiselleştirilmiş tanı yaklaşımlarının oluşturulması ve AH'de hedefe yönelik tedavi stratejilerinin geliştirilmesi için önemli bir potansiyel oluşturmaktadır.

Anahtar Kelimeler: Alzheimer Hastalığı, Genome-ölçekli Metabolik Model, Transkriptom, Biyobelirteç, Hastalık Altıpleri

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LIST OF SYMBOLS AND ABBREVIATIONS

A β	: amyloid-beta
AChEI	: Acetylcholinesterase Inhibitor
AD	: Alzheimer's Disease
ALOX5	: Arachidonate 5-Lipoxygenase
APOE	: Apolipoprotein E
APP	: Amyloid Precursor Protein
BBB	: Blood-Brain Barrier
BCAA	: Branched-Chain Amino Acids
CD	: Canavan Disease
CDCA	: Chenodeoxycholic Acid
CDR	: Clinical Dementia Rating
CERAD	: The Consortium to Establish a Registry for Alzheimer's Disease
CO	: Carbon Monoxide
COBRA	: Constraint-Based Reconstruction and Analysis
Cogdx	: Final Consensus Cognitive Diagnosis
CoQ10	: Coenzyme Q10
CSF	: Cerebrospinal Fluid
CT	: Computed Tomography
DEG	: Differentially Expressed Gene
FBA	: Flux Balance Analysis
FC	: Fold Change
FDA	: Food and Drug Administration
FDR	: False Discovery Rate
FTD	: Frontotemporal Dementia
FVA	: Flux Variability Analysis
GABA	: Gamma-Aminobutyric Acid
GEM	: Genome-Scale Metabolic Model
GHB	: Gamma-Hydroxybutyrate
GPR	: Gene-Protein-Reaction
H ₂ S	: Hydrogen sulfide
HA	: Hyaluronic Acid
HMR	: Human Metabolic Reactions
LBD	: Lewy Body Dementia
MAP	: Memory and Aging Project
MCI	: Mild Cognitive Impairment
MRI	: Magnetic Resonance Imaging
MSBB	: Mount Sinai Brain Bank
NFT	: Neurofibrillary Tangles
NMDA	: N-Methyl-D-Aspartate
PC	: Phosphatidylcholine
PCA	: Principal Component Analysis
PCr	: Phosphocreatine
PD	: Parkinson Disease
PE	: Phosphatidylethanolamine

PET	: Positron Emission Tomography
PGD2	: Prostaglandin D2
PHG	: Parahippocampal Gyrus
PMI	: Post-Mortem Interval
PSEN1	: Presenilin 1
PSEN2	: Presenilin 2
PUFA	: Polyunsaturated Fatty Acid
RF	: Random Forest
ROS	: Religious Orders Study
ROS	: Reactive Oxygen Species
ROSMAP	: Religious Orders Study and Memory and Aging Project
RNA-seq	: RNA-sequencing
SSA	: Succinic Semialdehyde
SSADH	: Succinic Semialdehyde Dehydrogenase
TAMBOOR	: TrAnscriptome-based Metabolite Biomarkers by On-Off Reactions
TCA	: Tricarboxylic Acid Cycle
TIMBR	: Transcriptionally Inferred Metabolic Biomarker Response
TMM	: Trimmed Mean of the M-values
WSCNA	: Weighted Sample Gene Network Analysis

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1. INTRODUCTION

Most people in the world suffer from diseases and the consequences they bring to life as they grow older. Alzheimer's Disease (AD) is one of the most common diseases in the elderly, occurring through different mechanisms due to brain deterioration and restructuring (**Castellani et al., 2010**). AD has become an urgent public health concern due to its global prevalence. Today, more than 50 million people live with AD, and this number is estimated to double every five years (**"2024 Alzheimer's Disease Facts and Figures," 2024; Breijyeh & Karaman, 2020**). Moreover, AD begins with subtle molecular changes in the brain years before clinical symptoms appear, causing the disease to be diagnosed much later than it should be (**Ballard et al., 2011; Duara & Barker, 2022; Ewers et al., 2011; Jellinger et al., 2008**).

AD is known with heterogeneous biology and multiple causalities, indicating that each patient may respond differently to AD (**Ballard et al., 2011; Duara & Barker, 2022; Ewers et al., 2011**). To overcome this problem, researchers clustered patients who showed the same pattern using different approaches (**P. Chen et al., 2023; Ferreira et al., 2017; Lian et al., 2023; Neff et al., 2021; Tijms et al., 2024**). Personalized predictions on these clusters pave the way for individual assessment of each patient and analysis of subtypes with similar patterns of metabolic changes. The main goal of this thesis study is to gain a deeper understanding of the complex mechanisms underlying AD by identifying subtype-specific biomarkers, uncovering disease-specific molecular patterns, and comprehensively investigating metabolic changes.

In the first section within this chapter, the known symptoms associated with AD and the neuropathological, genetic, and metabolic mechanisms involved in the course of the disease are highlighted. In the second section, current approaches to the diagnosis of AD are evaluated together with the treatment methods currently available on the market. The heterogeneous nature of the disease and the importance of its separation into subtypes are also emphasized.

In the third section, omics technologies and genome-scale metabolic models (GEMs) are reviewed. Specifically, the importance of systems biology and various approaches that can provide early diagnosis of complex diseases such as AD is emphasized. In this

context, the mechanism of a metabolic modelling-based biomarker prediction algorithm, TrAnscriptome-based Metabolite Biomarkers by On-Off Reactions (TAMBOOR) (Abdik & Çakır, 2024), is detailed, and the role of this algorithm in biomarker prediction is explained.

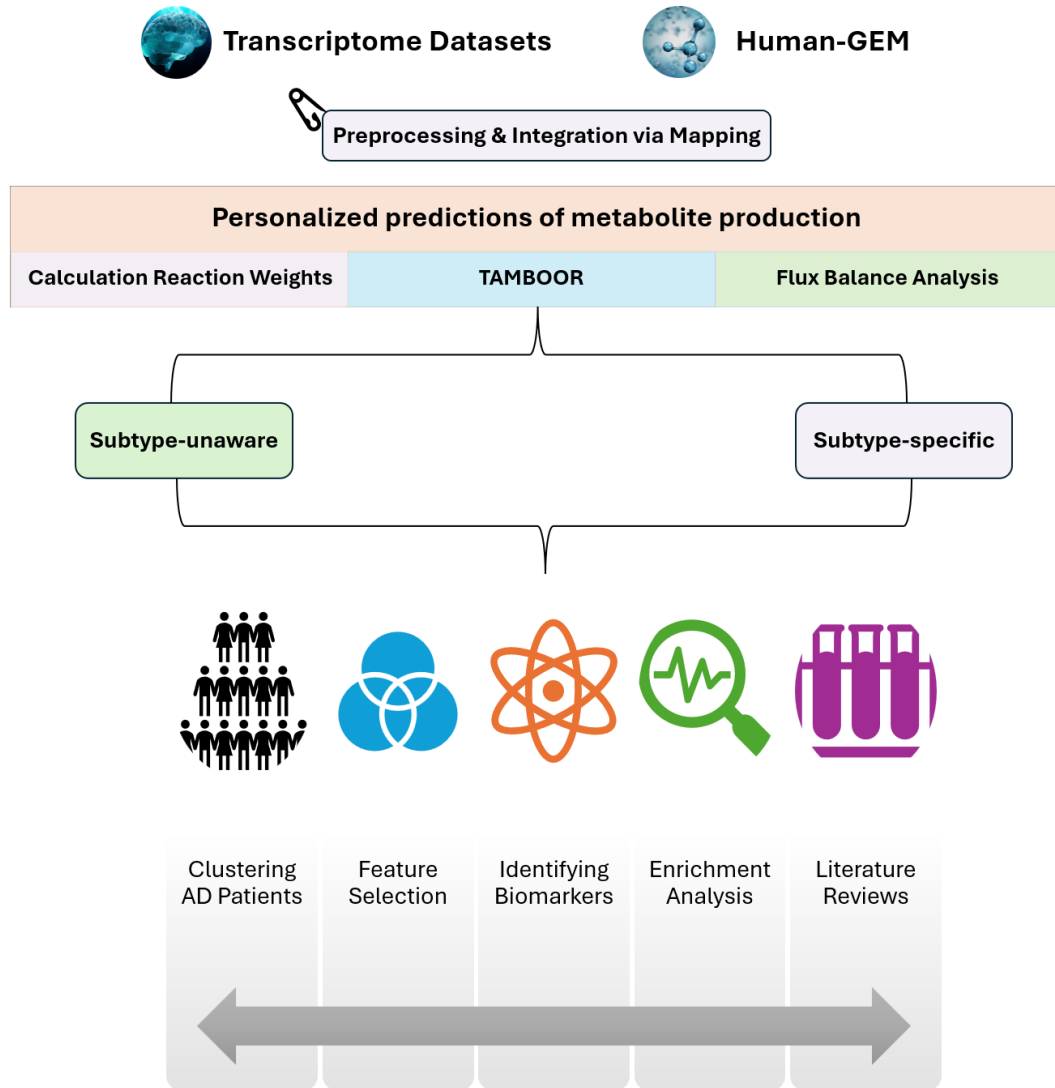


Figure 1.1: The workflow of the analyses performed in the thesis.

The analyses performed in the thesis are schematized in **Figure 1.1** within the framework of a general workflow. Within the scope of the thesis, personalized and subtype-specific metabolite biomarker predictions for AD were carried out using the TAMBOOR algorithm and a human genome-scale metabolic model. For this purpose, transcriptome data obtained from two different large-scale cohort studies were used.

In order to better understand the heterogeneous structure of AD, three different subtypes previously defined in the literature were separately considered, and biomarker recommendations specific to each subtype were made. In this direction, comprehensive analyses were performed on both general AD (subtype-unaware) and subtype-specific biomarker groups. Clustering was applied based on the prediction of the changes in metabolite secretion patterns in AD samples. Then, the compatibility between the predefined subtypes and the predictions from the clusters was reported. To assess the reliability of the algorithm, a comprehensive literature review was conducted, and a list of reliable biomarkers based specifically on metabolomics studies was formed. As a result, the complex and heterogeneous structure of AD was investigated in this thesis study in depth, with a significant potential to contribute to the early diagnosis of the disease.

1.1. Alzheimer's Disease

Dementia is a serious medical disorder that deeply affects older individuals. It is characterized by progressive deterioration in brain functions, leading to fatal consequences with increasing neurodegeneration (**Apostolova, 2016; Breijyeh & Karaman, 2020**). AD is the most common type of dementia and causes a rapid deterioration in thinking skills and the inability to perform even simple tasks. AD gradually destroys memory, changes behavior and leads to the death of neuron cells in the brain, causing communication between neurons to be disrupted (**Kriebel-Gasparro, 2020; Larson et al., 1992**). In addition to memory loss, symptoms such as behavioral disorders, apathy, and difficulty in speaking and thinking are also observed. As the disease progresses, damage spreads to different areas of the brain, including those responsible for reasoning, social behavior, and visual skills (**Apostolova, 2016; Merriam et al., 1988**).

There are also several risk factors that make these symptoms worse (**Figure 1.2**). Lifestyle is the critical point for AD (**Killin et al., 2016; Praag, 2018**). Unhealthy habits such as consumption of fast foods, alcohol use, and smoking significantly increase the risk of developing dementia. Additionally, several medical conditions—including obesity (**Flores-Cordero et al., 2022**), diabetes (**Sims-Robinson et al., 2010**), and cardiovascular diseases (**Leszek et al., 2021**)—have been closely linked to AD. Therefore, a healthy lifestyle, a supportive environment, and maintaining general

physical well-being play an important role in both the prevention and progression of the disease (**Dhana et al., 2020**).

Diagnosing AD can be challenging due to the variability in symptoms across patients. Therefore, genetic, pathological, neuropsychiatric, psychological, and clinical impacts need to be considered (**Figure 1.2**). Clinical and pathological findings may not always align, as not all patients with confirmed pathological changes show the expected clinical symptoms (**Galton et al., 2000; B. Lam et al., 2013; Sirkis et al., 2022; Vogel & Hansson, 2022**). Therefore, making a diagnosis with a single approach will not be sufficient. Two main scoring approaches are generally used to aid diagnosis. The first is pathology, which evaluates brain abnormalities through imaging technologies. The second is clinical, where doctors assess the presence and severity of dementia through behavioral changes, physical examination, and other symptoms that may indicate AD. These approaches, in which the level of pathological change and the level of cognitive impairment are evaluated together, help to make a more accurate diagnosis.

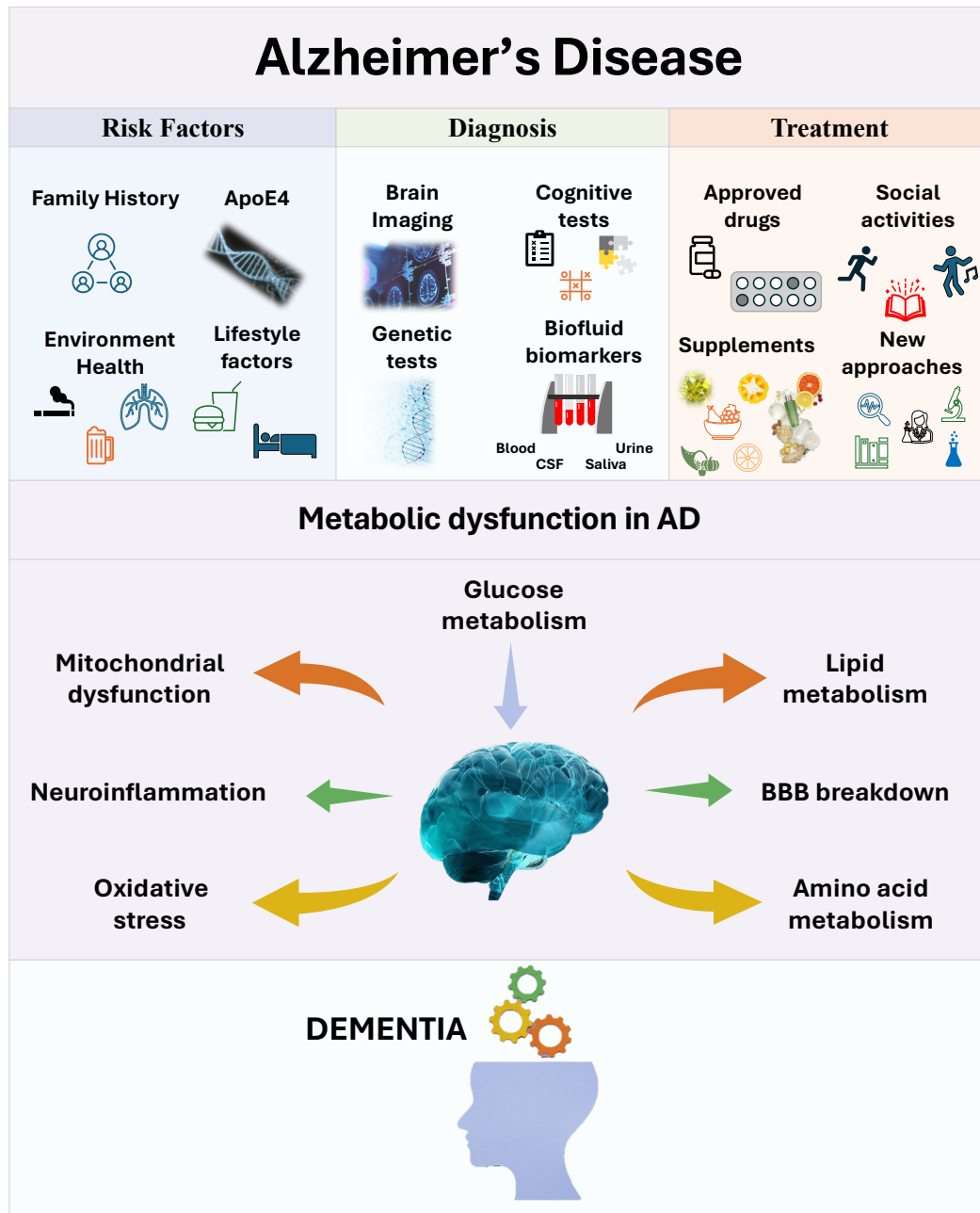


Figure 1.2: Diagnosis, treatment, cellular metabolic changes, and risk factors that are related to AD are summarized.

There are scores commonly used for clinical and pathological evaluation. One of the most commonly used metrics for neuropathological assessments is The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) (Fillenbaum et al., 2008) score, developed in 1991. The CERAD score is a classification system for evaluating general AD pathology by measuring the presence and density of neuritic plaques in postmortem brain tissue. One of the important assessment tools used in the clinical

framework is the Clinical Dementia Rating (CDR) (**J. C. Morris, 1997**) score. This method is applied to individuals by evaluating the functionality in the areas of memory, orientation, judgment, problem-solving, community affairs, home and hobbies, and personal care. Another commonly used scoring system in the clinical diagnosis of AD is the Final Consensus Cognitive Diagnosis (cogdx) (**Schneider et al., 2007**) score. This score provides a classification for evaluating the patient's dementia diagnosis at the time of death.

AD is driven by multiple mechanisms that contribute to its neurodegenerative nature, making it heterogeneous. The pathobiology of AD is enriched by numerous hypotheses proposed to explain its onset and progression. In some cases, AD is genetically inherited due to mutations in critical genes (**Van Cauwenberghe et al., 2016**). To examine the course of the disease, metabolism is a key point. Metabolic dysfunctions affecting various cellular mechanisms have been identified by researchers, leading to severe dysregulation that disrupts brain homeostasis and contributes to cognitive decline (**L. Xu et al., 2023; Yan et al., 2020; Yuan et al., 2024**).

1.1.1 Neuropathological Mechanism of AD

AD is characterized by brain damage with a complex pathology that will provide evidence of disease progression and symptoms (**Long & Holtzman, 2019**). The primary neuropathological indicators supporting the presence of AD include the deposition of amyloid beta ($A\beta$), serving as the principal constituent of extracellular amyloid plaques. The second indicator is intracellular accumulation of hyperphosphorylated tau protein, the primary component of neurofibrillary tangles (**Plascencia-Villa & Perry, 2022; Trejo-Lopez et al., 2022**). AD neuropathological changes are divided into two types: positive lesions resulting from the accumulation of peptides in the brain, which indicate inflammation, and negative lesions resulting from neural, neuropil, and synaptic loss, which indicate death (**Serrano-Pozo et al., 2011**).

$A\beta$ accumulation is thought to be the primary starting point of AD by disrupting information transmission in $A\beta$ -impaired neurons (**Sadigh-Eteghad et al., 2014**). These plaques are a hallmark of AD, and this is a major concern for researchers aiming to find a cure for AD (**Yiannopoulou & Papageorgiou, 2013**). $A\beta$ forms dense senile

plaques in key brain regions such as the hippocampus, amygdala, and cerebral cortex, contributing significantly to neurotoxicity and neural dysfunction. Beyond cognitive decline, A β causes axonal, dendritic, and synaptic damage in neurons by activating astrocytes and microglia **(Breijyeh & Karaman, 2020; Spires-Jones & Hyman, 2014)**. Neurofibrillary tangles (NFTs) replace primary lesions of AD alongside amyloid plaques. NFTs are abnormal filaments of hyperphosphorylated tau proteins **(Brion, 1998)**. Tau protein is a microtubule-associated protein produced from the MAPT gene, which is responsible for binding microtubules, the structures that make up the cytoskeleton, to facilitate the neuronal transport system **(Goedert, 1993)**. Neurofibrillary tangles can twist around each other and fold into intraneuronal tangles to form insoluble fibrils at some morphological stages **(Brion, 1992)**.

1.1.2 Genetic Mechanism of AD

In the pursuit of unravelling AD, attention has turned towards exploring genetic factors. The identification of these factors has shed light on their significant contributions to the effects and outcomes of the disease. An astounding 70% of individuals diagnosed with AD are linked to hereditary factors **(Breijyeh & Karaman, 2020; Van Cauwenberghe et al., 2016)**. Cases of early-onset AD predominantly arise from mutations in Amyloid precursor protein (APP), Presenilin 1 (PSEN1), and Presenilin 2 (PSEN2) genes are major causes of early-onset AD, and apolipoprotein E (ApoE) is the strongest genetic risk factor for late-onset AD.

APP is a transmembrane protein of the type I variety, which undergoes enzymatic processing to release A β and other proteins. The APP gene is responsible for encoding APP protein **(Tew & Goate, 2017)**. Remarkably, several mutations in the APP gene have been identified as the potential cause of AD. These mutations lead to A β formation and affect other brain cell activity - astrocyte-, microglia- and synapse toxicity **(N. M. Li et al., 2019)**.

PSEN1 proteins are presenilin proteins encoded by the PSEN1 gene, and PSEN2 proteins are encoded by the PSEN2 gene. PSEN1 and PSEN2 are autosomal dominant forms of early onset AD **(Xiao et al., 2021)**. Mutations in the PSEN1 gene play a larger role in the formation of A β compared to the mutations in the PSEN2 gene. Some of the mutations in PSEN2 are not known pathogenic mutations, but any mutation in the

gene can cause familial AD in the presence of normal PSEN1 (Cai et al., 2015; Lanoiselée et al., 2017; Xiao et al., 2021).

The ApoE protein is involved in lipoprotein-mediated lipid transport in the mammalian body, encoded by the APOE gene (J. Kim et al., 2009). This glycoprotein is primarily expressed in astrocytes and microglia cells in the brain (Lanfranco et al., 2021). ApoE gene has three major isoforms, *APOE-ε4*, *APOE-ε3*, and *APOE-ε2*. The *APOE-ε4* isoform has been identified as a major risk factor for AD and plays a major role in Aβ accumulation relative to other alleles (Narasimhan et al., 2024; Raulin et al., 2022).

1.1.3 Metabolic Mechanism of AD

AD is a highly heterogeneous disease that occurs due to multiple pathological factors. In addition to the obvious pathological changes, metabolic pathways also contribute significantly to the course of the disease (Figure 1.2). These metabolic disruptions affect critical biochemical reactions in the brain. The brain is a lipid-rich organ with high energy demands and is highly sensitive to metabolic imbalances (Aureli et al., 2015; Corraliza-Gomez et al., 2019). It is protected by the blood-brain barrier (BBB), a selective and semi-permeable barrier that prevents the entry of harmful substances from outside of the cell (Wu et al., 2023). Glucose, a primary energy source of the brain, is essential for survival in neuronal homeostasis. Due to its role in the brain, glucose/energy metabolism has been observed in many studies to be the first thing to be disrupted in AD (An et al., 2018; Bayraktar et al., 2021; Dewanjee et al., 2022; Q. Wang et al., 2022; L. Yu et al., 2022; Yuan et al., 2024). This metabolic dysfunction can be one of the earliest detectable changes in the disease process.

Given the high lipid content of the brain, lipid metabolism is also profoundly affected in AD (Kang & Rivest, 2012; J. A. Lee et al., 2021; Matsuzaki et al., 2011; Yin, 2023). Lipids are essential for cell membrane structure and function, and their dysregulation alters membrane integrity and signaling (Aureli et al., 2015; Corraliza-Gomez et al., 2019). These changes are closely associated with APOE, a lipoprotein that is an important genetic risk factor for AD. Oxidative stress, triggered by the overproduction of reactive oxygen species (ROS), is another critical contributor. ROS are highly reactive molecules that are abundant in AD brains and lead to oxidative phosphorylation in mitochondria (Dhapola et al., 2024; Huang et al., 2016;

Tamagno et al., 2021). Mitochondrial dysfunction (**Ashleigh et al., 2023**), combined with altered glucose consumption (**An et al., 2018**) and lipid alteration (**Wong et al., 2017**), highlights the metabolic component of AD.

Carbohydrate metabolism, particularly glycolysis and the tricarboxylic acid (TCA) cycle, is also dysregulated in AD (**Buendia et al., 2023; L. Wood et al., 2021**). These disruptions lead to abnormal accumulation or depletion of key metabolites that affect cellular energy balance. Similarly, amino acid metabolism is disrupted, in part, by abnormal accumulation of proteins and impaired ubiquitination processes that inhibit protein degradation and synthesis (**Griffin & Bradshaw, 2017; Gueli & Taibi, 2013; W. Yu et al., 2023**). Moreover, insulin signaling (**Sędzikowska & Szablewski, 2021**), a pathway closely linked to glucose homeostasis and diabetes, has been identified as a risk factor for AD. Other metabolic conditions such as hypercholesterolemia (elevated LDL levels) (**Digital et al., 2013; W. G. Wood et al., 2005**) and obesity (**Flores-Cordero et al., 2022**) are also considered risk factors for the disease. The pathways altered in these diseases may directly or indirectly play a role in AD.

Given these underlying metabolic disturbances, metabolic profiling holds promise for early diagnosis of AD, potentially before the onset of clinical symptoms. Since significant neuronal loss often occurs before AD becomes clinically evident, early detection through metabolic signatures may enable timely intervention. However, metabolic changes can vary significantly among individuals, reflecting the heterogeneity of symptoms and disease progression. Therefore, classifying patients according to similar patterns of metabolic dysregulation and analyzing these subtypes may help identify robust metabolic biomarkers. Such biomarkers may facilitate earlier and more accurate diagnosis and may also provide insight into personalized treatment strategies for AD.

1.2. Characterization of AD

AD is an age-related disease that causes both memory loss and rapidly progressive changes within the brain (**Apostolova, 2016**). Since these impairments can also be observed in healthy aging, early diagnosis of AD has become a challenge (**Fjell et al., 2014**). There are treatments available to reduce the effects of symptoms in AD, but unfortunately there is still no permanent or neuroprotective solution. AD begins with

subtle molecular changes in the brain years before clinical symptoms; therefore, timely identification through novel biomarkers is essential for the diagnosis of AD. (Ballard et al., 2011; Duara & Barker, 2022; Ewers et al., 2011; Jellinger et al., 2008).

1.2.1. Diagnosis of AD

Diagnosing AD is a complex and challenging process that involves multiple types of assessments. These assessments can be categorized into four main fields (**Figure 1.2**). Genetic testing is generally not recommended by the physicians and is not used to determine cognitive or any pathological level. However, if there is a family background of the disease, doctor may request genetic test. Genetic tests can be valuable for younger patients with early onset AD (Roses, 1997). In such cases, genetic analysis may focus on the presence of the APOE gene allele and mutations in three significant genes (APP, PSEN1, PSEN2) associated with the AD risk (Juganavar et al., 2023).

There are many tests that can be used to evaluate the patient's behavioral and mental functions from a cognitive, physical, neurological, and psychiatric perspective (Bomasang-Layno & Bronsther, 2021; Jack et al., 2024). These may include tasks such as puzzles and problem-solving activities that help assess memory, attention, visual & hearing and reasoning skills that are often affected in AD (Schultz et al., 2015).

Brain imaging techniques play a critical role in the diagnosis of AD. These techniques include MRI (Magnetic Resonance Imaging) and CT (Computed Tomography), which are used to detect brain injuries, strokes, or tumors that may explain brain deformation (Jack et al., 2024). The other structure imaging technique is PET (Positron Emission Tomography) (Juganavar et al., 2023). It is used to visualize amyloid and tau protein accumulations, and glucose metabolism (using fluorodeoxyglucose) (Duan et al., 2023), providing information about the activity and function of the brain. Additionally, physicians order laboratory tests to check vitamin, lipid, and hormone levels to catch any levels that indicate disease progression. Due to the lack of powerful biomarkers for diagnosis, there are many studies investigating biofluid biomarkers that can be detected in urine, saliva, blood and cerebrospinal fluid (CSF) (Blennow et al., 2010; Ibáñez et al., 2013; Jellinger et al., 2008; Zetterberg & Blennow, 2018).

1.2.2. Treatment of AD

Although the symptoms in AD patients may differ from person to person, there are some medications that can reduce the symptoms (**Peng et al., 2023; Vaz & Silvestre, 2020**). In order to prevent further progression of cognitive impairment in patients, appropriate treatments other than medication should be given under the supervision of a physician (**Figure 1.2**). If patients are found to have functional impairment in social activities and judgement, appropriate supervision and safety should be provided (**Apostolova, 2016**) are two classes of medications approved by the The US Food and Drug Administration (FDA) for AD: acetylcholinesterase inhibitors (AChEIs) and the N-methyl-D-aspartate (NMDA) receptor antagonist memantine. Three agents known and sold in the USA are donepezil, rivastigmine, and galantamine (**Peng et al., 2023; Varadharajan et al., 2023; Vaz & Silvestre, 2020**). It has been reported that these three agents have positive effects on functional and cognitive disorders and can improve memory and concentration problems throughout the progression of the disease. In 2021, the drug "Aducanumab" (**Dunn et al., 2021**) was approved by the FDA for the treatment of AD, followed by the drug "Lecanemab" in 2023 (**Mahase, 2023**). Even though the pharmaceutical industry is so advanced today, a completely effective treatment for this disease is not available in the market.

Dietary supplements are considered an additional treatment method for reducing symptoms of AD (**Chimakurthy et al., 2023; Shah et al., 2023**). Certain nutrients, vitamins, and hormones may act as antioxidants or anti-inflammatory agents or support the regulation of neuronal autophagy, all of which play important roles in brain health (**Kamaljeet et al., 2024**). The potential benefits of vitamins such as folate, B12, and D on dementia are particularly highlighted (**M. C. Morris et al., 2006**). Additionally, ongoing studies are investigating novel therapeutic agents that may impact the progression of AD. Additionally, making healthier lifestyle changes (better diet, regular physical/social, and mental activities) is a critical factor in slowing the progression of the disease.

1.2.3. Heterogeneity and Subtypes of AD

Recent studies have revealed that each patient responds differently to AD and the disease has a heterogeneous structure (**Duara & Barker, 2022; Ferreira et al., 2018;**

Jellinger, 2022; Neff et al., 2021). The factors that may cause the heterogeneity of AD have not yet been determined. AD is a multifactorial disease; therefore, its point of onset is still unclear. Various profiles, including metabolism, genetics, pathogenic implications, and demographic factors, may play a role in shaping the variation of symptoms and disease progression among individuals. Symptoms and response to treatments vary among patients, making the course of the disease unpredictable. On the other hand, dementia can also be observed in other neurodegenerative diseases, so accurately distinguishing AD from other types of dementia remains a significant clinical challenge. To navigate the complexity and heterogeneity of the disease, researchers have classified AD patients into specific subtypes using different approaches (**P. Chen et al., 2023; Ferreira et al., 2017; Hanyu et al., 2022; Lian et al., 2023; Neff et al., 2021; Tijms et al., 2024**). In these studies, AD patients were divided into subtypes that showed similar profiles by focusing on resting state connectivity (**P. Chen et al., 2023**), imaging finding by MRI and CT (**Hanyu et al., 2022**), brain atrophy patterns (**Ferreira et al., 2017**), metabolic (**Lian et al., 2023**) and transcriptome signatures (**Neff et al., 2021**). In the study conducted by Neff et al., three main subtypes of AD were identified using gene expression profiles through network-based clustering approaches.

Neff and colleagues used a large-scale cohort dataset as the main dataset and preprocessed the RNA sequencing data (**Neff et al., 2021**). Although the cohort dataset included data from multiple brain regions, the parahippocampal gyrus (PHG) region was selected as the focal point for determining the subtypes of AD patients. The reason for this selection is that the PHG region carries the strongest molecular signals in terms of AD-associated differentially expressed genes (DEGs). The researchers analyzed different clustering methods to separate patients into subtypes. It was decided to use the weighted sample gene network analysis (WSCNA) method, which provides the most stable clustering results. As a result of this analysis, three main subtypes (A, B, and C) were identified. They obtained DEGs for each subtype and then compared them to Blalock transcriptomic signatures (**Blalock et al., 2011**) based on their up and down-regulation. They stated that subtype A is the atypical form of AD, which shows the opposite direction of signatures; subtype B matches the AD profile moderately; and subtype C represents the group with typical features of the disease, which shows consistent regulation direction with the signatures. Following the molecular subtyping,

each group was analyzed in detail, and subtype-specific genetic, molecular, and pathological signatures were defined. They analyzed the amount of plaque and NFT level across the subtypes and, they showed that subtypes A, B, and C reflect features of tau-NFT resistant, tau-NFT predominant, and A β -dominant patterns, respectively.

Personalized investigation of disease effects on each patient's metabolism enables defining patients having similar patterns of metabolic changes and showing the impact of subtypes on metabolism. Therefore, identifying subtype-specific biomarkers will enhance uncovering of disease-specific patterns and better understanding of subtype-specific metabolic changes.

1.3. Systems Biology and Omics Technologies

Every event in biology is like a series of puzzle pieces that follow one another. Focusing on just one piece prevents us from understanding the full scope and meaning of the biological system. Therefore, if an analysis focuses on functional disorders that occur only in one-part, other problems that include large-scale issues will be ignored. To make accurate assessments and derive meaningful inferences, advanced technologies are needed to bring the pieces of this puzzle together into a coherent whole.

With the developing technology, DNA and RNA sequencing has become a reality, but the question of what to do with the obtained data has come to light. It has become clear that the complexity of these data requires computational models to interpret them. Systems biology was brought forward by the concept of high-dimensional data and computational interpretation models (**Angione, 2019**). The fact that biological networks are so interconnected brings about high-throughput approaches, namely 'omics' platforms, such as genomics, transcriptomics, proteomics, metabolomics (**Edwards, 2017**). Today, genome and transcriptome data are widely used in the studies because they are low-cost and have high coverage.

Transcriptome data includes expressions of genes that can be used in various analyses to get information about the biological system (**Brown, 2002**). The data provides the function of the RNA molecules derived from protein-coding genes. Today, many RNA-sequencing (RNA-seq) technologies are widely used (**Stark et al., 2019**). The activity may vary depending on the organism, cell, or environmental conditions in

which they are found (**Angione, 2019**). These differences can also be associated with diseases through the metabolic pathways that genes play a role in and affect. The best indicator for the physiology of the cell is metabolism (**Carthew, 2020**).

One of the main areas of use of transcriptome data is to compare gene expression values of disease and control states (**Kukurba & Montgomery, 2015**). For this purpose, the gene activity of patients is compared with healthy individuals to determine which genes differ. In this way, researchers can determine which genes are more or less active in the disease state (**Costa-Silva et al., 2017**). Transcriptome data make it possible to analyze not only at the gene level but also on changes in metabolism by considering the gene-enzyme relations, which affect reaction activity. To understand the expression of genes in the context of metabolic reactions, all biochemical reactions occurring within the cell must be known and integrated. In response to this need, metabolic models at genome scale were generated. Genome-scale metabolic models (GEMs) are platforms to investigate metabolism from gene to reaction levels (**Passi et al., 2022**). These models provide powerful tools that aim to predict the phenotype at the cellular level in the most accurate way (**Bernstein et al., 2021; S. Lam et al., 2020**).

1.3.1. Genome-Scale Metabolic Models

Genome-scale metabolic models (GEMs) are a network-based tool that collects all previously explored metabolic reactions in a biological system, including genes, enzymes, reactions, and metabolites in the associated organism (**Passi et al., 2022**). GEMs attempt to investigate cell metabolism in terms of both gene expression level and reactions by considering gene-protein-reaction (GPR) rules. GPR rules are used by the GEMs to analyze data at the gene expression level based on the relationships between genes and reactions (**Grausa et al., 2022**). GPR rules express a relationship extending from gene to enzyme, from enzyme to reaction. In this way, it becomes possible to map transcriptome data into biochemical network modeling by considering the expression levels of genes that code for enzymes in reactions. These rules are crucial to map transcriptome data on GEMs so that disease and individual-specific metabolic models can be created (**Agren et al., 2012; Zur et al., 2010**). These tools can estimate reaction rates, termed fluxes, to provide insight into the desired disease metabolism, and several studies have been conducted on the distribution of metabolic

fluxes (Ambikan et al., 2023; Gatto et al., 2014; Hyötyläinen et al., 2016; Lüleci et al., 2023, 2024; Uzuner Odongo et al., 2025; Varma et al., 2021). In some cases, known reaction rates of the working organism are introduced to the metabolic model as inputs, and a prediction is made within these constraints. Flux balance analysis (FBA), the most common method used in constraint-based analysis, estimates flux distributions in GEMs at steady state (Machado & Herrgård, 2014; Mahadevan et al., 2002).

Different GEMs have been reconstructed for humans such as Recon (Swainston et al., 2016), Human Metabolic Reactions (HMR) (Pornputtapong et al., 2015), and Human1 (Robinson et al., 2020). Human1, also known as Human-GEM, is the most recent and most comprehensive human GEM, and is still regularly updated.

Human-GEM is a comprehensive human metabolism model generated by Robinson and colleagues in 2020 (Robinson et al., 2020) by combining previously developed models. The model includes nine cellular components, such as cytosol, extracellular, and nucleus. In addition, there are metabolic reactions, metabolites that participate in these reactions, and genes that catalyze reactions. The most recent version includes 12971 reactions controlled by 2887 genes. The relationships between genes and reactions are defined by the GPR rules, as described before; These rules are written with logical expressions "and" (&) or "or" (|) to indicate whether the enzymes must work together (enzyme complex) or whether anyone alone is sufficient (isoenzyme) to catalyze corresponding reactions.

1.3.2. Role of GEMs in Biomarker Prediction

The integration of GEMs with multi-omics data enables biomarker prediction and brings in the likelihood of metabolites being released outside the cell (Ozerov et al., 2016; Stempler et al., 2014). There are different approaches focusing on metabolite release to identify potential disease-related metabolite biomarkers (Abdik & Çakır, 2024; Blais et al., 2017; Cooke et al., 2024; Lewis & Kemp, 2021; Moolamalla & Vinod, 2020). Most studies in the field of biomarker prediction are based on Flux Variability Analysis (FVA) (Kenefake et al., 2022). FVA is a method used for metabolite-based biomarker prediction by determining the minimum and maximum values of metabolic reaction rates that define secretion of a metabolite from the cell

(Asgari et al., 2018; Salazar et al., 2016; Stempler et al., 2014). This method calculates the range of secretion rates that metabolites can take. Then, it helps to determine the biomarker potential of the excreted metabolite by comparing these rate ranges between health and disease. Among the GEM studies where biomarker identification was the focus, Blais et al. (Blais et al., 2017) developed an algorithm called The Transcriptionally Inferred Metabolic Biomarker Response (TIMBR) based on constraint-based analysis to predict metabolite biomarkers. TIMBR compares total reaction rates required for maximum secretion of a metabolite using the change in gene expression between two states. TIMBR has been used previously in different conditions, including neurodegenerative diseases (Abdik & Çakır, 2021; Pannala et al., 2018; Rawls et al., 2019). A recent study predicted potential biomarker candidates in Parkinson's mouse models with TIMBR (Abdik & Çakır, 2021).

1.3.3. TAMBOOR Algorithm

TAMBOOR (TrAnscriptome-based Metabolite Biomarkers by On–Off Reactions) (Abdik & Çakır, 2024), is an algorithm that was inspired by TIMBR, predicts metabolite biomarkers by considering the flux demand required to produce the desired external metabolite. The algorithm compares the number of active reactions required to produce high levels of metabolites in control and disease states based on changes in the level of gene expression. The key point of the algorithm is to predict potential production of external metabolites that could be metabolite biomarkers. It makes these predictions by integrating transcriptomic data with Human-GEM. This approach was applied to 13 human Parkinson's disease (PD) transcriptome datasets and was shown to be effective in identifying disease-associated metabolic changes (Abdik & Çakır, 2024). This algorithm, which is an effective tool in revealing disease-specific metabolic changes, enables the identification of metabolite-based biomarkers. In this way, it is suitable for biomarker prediction for diseases such as AD, which are quite difficult to diagnose early. At the same time, considering the heterogeneous structure of the disease, it is possible to identify subtype-specific biomarkers to show that AD progresses differently in patient groups and to go beyond approaching each patient in the same way. This approach holds promise for identifying subtype-unaware biomarkers that reflect AD in general and AD subtype specific biomarkers.

2. METHODS

2.1. Transcriptome Datasets and Preprocessing

The first bulk RNA-seq dataset used was obtained from the Religious Orders Study and Memory and Aging Project (ROSMAP) (**Bennett et al., 2018**). The project provides transcriptomic data from 636 individuals in the Religious Orders Study (ROS) and the Memory and Aging Project (MAP). The raw RNA-seq files in the form of fastq were downloaded from the Synapse database (<https://synapse.org/>) (accession ID: syn17024112) previously in our research group. Then, the raw files were processed to obtain raw counts and metadata with age, sex and post-mortem interval (PMI) of the patients as described in two different studies (**CEYLAN et al., 2024; Uzuner Odongo et al., 2025**). The condition of the samples was determined using the CERAD (**Fillenbaum et al., 2008**) and cogdx (**Schneider et al., 2007**) scores, following the ROSMAP clinical codebook. CERAD measures neuritic plaque burden, while cogdx indicates the last clinical diagnostic level of cognition at the time of death. To eliminate the possibility of false positives in diagnosis, clinical and pathological scoring were used to identify patients and controls, by eliminating samples that have contradicting classifications (AD vs. control) by these scores. Based on these metrics, the dataset includes 192 AD patients (CERAD: 1 & 2 & cogdx: 4) and 79 control samples (CERAD: 4 & cogdx: 1). First, lowly-expressed genes (CPM < 0.1) were filtered out, and then gene expression data were normalized using the Trimmed Mean of the M-values (TMM) method in the R package edgeR, followed by log2 transformation. Principal Component Analysis (PCA) was applied to detect outliers within the samples. As a result, 4 AD (367_120502, 500_120515, 769_130523, 492_120515) and 1 Control (380_120503) samples were removed. Covariate adjustment analysis to eliminate the effects of confounding variables, including age, sex, and post-mortem interval (PMI), was performed using the removeBatchEffect function from the limma package in R.

A second bulk RNA-seq dataset was used for validation in this thesis study, and it was obtained from the Mount Sinai Brain Bank (MSBB) (**M. Wang et al., 2018**). The study provides transcriptomic data from 242 individuals collected from the PHG brain

region. The raw counts and metadata, including age, sex and PMI, were obtained from the Synapse database (accession ID: syn7416949). Unlike the ROSMAP dataset, the CDR (J. C. Morris, 1997) score was used instead of cogdx in the MSBB dataset, where CDR is a metric for measuring the clinical level of cognitive impairment. Based on both criteria, 109 AD patients (CERAD: (1 & 2), & CDR: ≥ 1) and 6 control samples (CERAD: 4 & CDR: 0) were identified. Normalization, PCA, and covariate adjustment analysis were performed following the same procedures as in ROSMAP, resulting in the removal of 2 AD patients (hb_RNA_10742_resequenced, BM_36_324) identified as outliers.

After completing all preprocessing steps, the ROSMAP dataset consisted of 188 AD patients and 78 control samples, with a total of 34,258 genes, while the MSBB dataset consisted of 107 AD patients and 6 control samples, with a total of 46,178 genes.

2.2. Prediction of Potential Biomarkers via TAMBOOR

The TAMBOOR algorithm was used to predict the biomarker potential of metabolites for each patient in the datasets. The focus was metabolites that are known to be secreted outside the cell since they can be measured from body fluids, making them potential biomarkers. The patients were then grouped and analyzed according to pre-defined subtypes and independently of the subtypes. The approach described in Abdik et al. (Abdik & Çakır, 2024) was followed at each step. The algorithm consists of three interconnected processes to generate predictions: (i) Calculation of maximum production/release capacities of the metabolites using Human-GEM, ii) Fold-change calculations to determine reaction weights based on gene expression values, and iii) Computing and comparing active reaction demands for the maximum release rates of metabolites using the TAMBOOR algorithm for two conditions using reaction score fold changes as weights in the objective function of the optimization problem.

Flux Balance Analysis (FBA) was the first step in the metabolite biomarker prediction approach used in this thesis study. FBA was applied to identify maximum rates of metabolite secretions outside of the cell. The analysis was conducted using the generic Human-GEM, version 1.18.0, including 12,995 biochemical reactions, 8,456 metabolites, and 2,889 genes. A total of 1,665 metabolites were defined in the GEM to be exchanged between the cell and the outside environment. These metabolites were

used as inputs to the biomarker prediction approach. In the FBA simulations, metabolites present in the Ham's medium were allowed to be taken up by the cell to ensure cell viability. Ham's medium comprises 44 metabolites including glucose, amino acids, organic & inorganic molecules, vitamins & coenzymes and nucleotides (Robinson et al., 2020). Additionally, three metabolites, NH_3 , taurine and ornithine, were included (Table 2.1). The maximum glucose uptake rate was set to $0.32 \mu\text{mol g}^{-1} \text{min}^{-1}$, while the oxygen uptake rate was defined as $1.76 \mu\text{mol g}^{-1} \text{min}^{-1}$, approximately 5.5 times of glucose uptake rate; in accordance with the values reported in healthy brain (Çakir et al., 2007). The uptake rates for 14 amino acids, NH_3 , and other carbon sources in Ham's medium were constrained to not to exceed 1/10 of the glucose uptake rate. Additionally, the biomass production rate was set to be at least $0.01 \mu\text{mol g}^{-1} \text{min}^{-1}$ to ensure sufficient macromolecule synthesis. Except for these predefined metabolites, the lower bounds of all other exchange reactions were set to zero to prevent the uptake of any additional metabolites from outside of the cell. Each exchange reaction within Human-GEM ($n=1,665$) was individually maximized by FBA under the given constraints, and a total of 1,043 metabolites were identified as potentially secreted outside the cell. In other words, the maximum secretion rate was predicted to be zero by FBA for the remaining metabolites. The maximum secretion rate of each metabolite for both datasets was obtained through FBA optimization via Gurobi Optimizer (Gurobi Optimization LLC, 2024) in MATLAB environment (version 2023b).

Table 2.1: The given constraints of metabolites included in Ham's medium.

Metabolites	Maximum Uptake Rates ($\mu\text{mol/g/min}$)	Metabolites	Maximum Uptake Rates ($\mu\text{mol/g/min}$)
alanine	0.032	methionine	0.032
alpha-tocopherol	0.032	NH_3	0.032
aquacob(III)alamin	0.032	nicotinamide	0.032
arginine	0.032	ornithnine	0.032
asparagine	0.032	pantothenate	0.032
aspartate	0.032	phenylalanine	0.032
biotin	0.032	proline	0.032
choline	0.032	pyridoxine	0.032
cysteine	0.032	retinoate	0.032
folate	0.032	riboflavin	0.032
gamma-tocopherol	0.032	serine	0.032

Table 2.1 Continued: The given constraints of metabolites included in Ham's medium.			
glutamate	0.032	taurine	0.032
glutamine	0.032	thiamin	0.032
glycine	0.032	threonine	0.032
histidine	0.032	thymidine	0.032
hypoxanthine	0.032	tryptophan	0.032
inositol	0.032	tyrosine	0.032
isoleucine	0.032	valine	0.032
leucine	0.032	glucose	0.32
linoleate	0.032	O₂	1.76
linolenate	0.032	Fe²⁺	1000
lipoic acid	0.032	H₂O	1000
lysine	0.032	Pi	1000
sulfate	1000		

Fold-change (FC) calculation using gene expression values was the second step in the biomarker prediction process using TAMBOOR since they are used as weights in the objective function of the optimization. Firstly, since the normalized gene expression data was in log2 scale, the log2 inverse transformation was applied to each dataset to restore the original scale of gene expression values and to obtain absolute fold changes for prediction simulations. The mean gene expression values of all control samples were calculated to serve as a reference for comparison with each AD patient. Subsequently, the gene expression levels of each AD patient and the mean of control samples were separately mapped to reaction scores using the GPR rules defined in Human-GEM. The GPR rules specify which gene(s) code for the enzyme(s) that catalyze each reaction, and this information was integrated with the gene expressions in the transcriptome data using mapExpressionToReactions function in the COBRA Toolbox. In the reaction rules, if a reaction is catalyzed by isoenzymes, which can catalyze the reaction independently, the gene with the highest expression among the genes that code for these enzymes is selected and assigned as the reaction score for the relevant reaction. Conversely, if a reaction is catalyzed by an enzyme complex, which requires multiple enzymes working together, the gene with the lowest expression among the genes encoding all the components of the complex is assigned as the reaction score.

Fold changes were then computed by dividing the mapped reaction scores of the control condition by those of the AD patients, referring control/disease. To prevent extreme values from affecting optimization, fold changes exceeding 10 were set at 10,

while values between 0 and 0.1 were set to 0.1. This tuning ensured computational stability and prevented undesirable outputs caused by extreme FC values. Adjusted fold change results in the form of control to disease ratios indicate the reaction weight used in the disease-case simulations. FCs calculated after manipulation were reversed to disease/control, indicating the reaction weight used in the healthy case simulation. The same tuning was applied to both datasets before making predictions. When applying the TAMBOOR algorithm, reaction fold changes were used as reaction weights for each reaction. Before optimization, exchange reactions were assigned a default weight of 2 and internal reactions were assigned a weight of 1 for reactions that do not have GPR rules within Human-GEM.

The TAMBOOR algorithm was applied for personalized biomarker predictions by computing differences in the number of active reactions required to secrete a desired metabolite between disease and healthy conditions, following the approach described in Abdik et al. (Abdik & Çakır, 2024). The optimization aimed to minimize the weighted sum of fluxes through equation (2.1), where W represents reaction weights and V denotes fluxes. In addition to the given constraints outlined in the FBA section, a key constraint was included to ensure that the secretion flux of the target metabolite remained at least 90% of its maximum value determined by FBA, as shown in equation (2.2). The network demand was defined as in equation (2.3), and it was computed separately for disease and control conditions, considering the inverse relationship between reaction weights and fluxes—where higher reaction weights led to lower flux values since this is a minimization problem, as indicated in equation (2.1). In this way, the minimum network demand necessary for metabolite secretion could be determined while incorporating fold changes derived from gene expression data. The optimization problem was solved under these constraints for each metabolite secretion.

$$\text{objective function} = \min \sum W|V| \quad (2.1)$$

$$V_{\text{metabolite}} \geq 0.9 V_{\text{metabolite(max)}} \quad (2.2)$$

$$X_{\text{metabolite}} = \text{number of active reactions} \quad (2.3)$$

The number of active reactions required to secrete a desired metabolite under these constraints was counted for both conditions (AD and control) by assuming reactions with absolute flux values lower than 10^{-5} to be inactive. The secretion scores are

defined in equation (2.4), and they were calculated and standardized for 1,043 metabolites based on the differences between the network demand of the disease and control conditions, using z-score transformation.

$$X_{production\ score} = \frac{X_{control} - X_{disease}}{X_{control} + X_{disease}} \quad (2.4)$$

Secretion rates of 1,043 external metabolites were predicted for each patient in the ROSMAP and MSBB datasets as described, respectively. Standardized metabolite secretion scores were then ranked at the individual patient level, and the top 15% and the bottom 15% were assumed to be metabolite biomarkers. The top 15% represent metabolites that have higher secretion rates in the AD patients while the bottom 15% identified metabolites that have lower secretion rates in the AD patients.

The predictions of metabolite secretion tendencies were made for each AD patient within both datasets. AD samples were also analyzed regardless of subtype status (subtype-unaware) to identify general AD biomarkers. Subtypes of AD patients were determined based on the clusters identified by Neff et al. Three main subtypes (A, B, and C) defined in their study were matched with AD patients selected based on our criteria. The subtypes of patients in the ROSMAP and MSBB datasets were labelled after matching the pre-defined labels. Based on the pairing, the ROSMAP dataset (n=166) included 45 patients in Subtype A, 38 in Subtype B, and 83 in Subtype C, while the MSBB dataset (n=107) had 31 patients in Subtype A, 35 in Subtype B, and 41 in Subtype C. Subsequently, the metabolite secretion predictions were analyzed separately for patients in Subtype A, B, and C.

All the calculations and simulations were performed in MATLAB 2023b. Constraint-Based Reconstruction and Analysis (COBRA) Toolbox (**Heirendt et al., 2019**) was used in MATLAB for all needed functions for genome scale metabolic model analysis. All simulations were optimized with the Gurobi Optimizer version of 11.0.0. Throughout the study, data manipulations and figure generations were performed in R version of 4.2.1.

2.3. Assessment of the Predictions

All patients were first evaluated independently of subtypes (subtype-unaware) based on metabolites identified as either increased or decreased secretion in the patients. Following this, analyses were conducted on the three main subtypes (A, B and C) defined in the study by Neff et al. Metabolite occurrences in the increase and decrease lists were counted across all AD patients to identify potential biomarkers independent of subtypes. Similarly, occurrences in each cluster (A, B, and C) were counted to identify subtype-specific potential biomarkers. In the subtype-unaware group, metabolites that showed an oversecretion or undersecretion profile in at least 30% of patients were identified. In the subtype-specific groups, metabolites predicted to be oversecreted or undersecreted secretions in at least 50% of the patients were selected as potential biomarkers. The metabolic activities of the predicted biomarker candidates in biological samples were examined through metabolomic studies and pathways related to AD in the literature.

2.3.1. Clustering AD Patients Based on Metabolite Patterns

Each subtype (A, B, and C) was characterized according to the TAMBOOR predictions. The patients were further clustered to assess consistency among the patients in the same subtype based on the predicted metabolite activity (increase, decrease, or no prediction). Each metabolite was assigned a value of 1 if it was predicted to have increased secretion, -1 if it was predicted to have decreased secretion, and 0 if no prediction was made across 1,043 metabolites, generating matrices for each cluster. To ensure robustness, metabolites that were not predicted to be biomarkers in at least 90% of the patients within the dataset were removed. Heatmaps of the three combined clusters, along with their metabolite predictions, were generated using the pheatmap package in R. Clustering was performed with Manhattan distance and the Ward.D2 method. Additionally, the proportions of previously labelled subtypes of patients within clusters were calculated to evaluate the relationship between biomarker prediction-based clusters and pre-defined subtypes.

2.3.2. Feature Selection

Metabolites representing significant changes in the direction of their secretion among the three main clusters were identified using the Kruskal-Wallis ANOVA test (cutoff $p < 0.01$). Additionally, the Random Forest (RF) algorithm was employed to determine important metabolites/features across clusters based on two key metrics: Gini and Accuracy. The top 100 significant metabolites were selected for each metric, and metabolites were considered reliable if they were identified by both metrics. Finally, the statistically significant metabolites from the ANOVA test (p-value cut-off 0.01) were overlapped with the Random Forest results to generate the final list of important metabolites across the clusters. Additionally, pairwise Wilcoxon test (p-values adjusted by Benjamini-Hochberg correction) was applied to determine which clusters showed significant differences.

2.3.3. Enrichment Analysis

Metabolites determined as potential biomarkers for subtype-unaware and subtype-specific analyses were used to find associated pathways and diseases. The KEGG IDs of these metabolites were identified using Human GEM 1.18.0. These metabolites were used for pathway and disease enrichment analysis using MBRole3 (**Lopez-Ibanez et al., 2023**) without making distinction on their metabolite secretion direction (increase & decrease). Bubble plots via ggplot2 and Sankey plot via SRplot (**Tang et al., 2023**) were implemented and visualized.

2.3.4. Validation by MSBB

All analysis steps performed with the ROSMAP dataset were repeated for the MSBB dataset, and metabolite biomarker candidates were determined at both subtype-unaware and subtype-specific levels. The overlap between the potential biomarkers predicted by both approaches (subtype-unaware and subtype-specific) and the candidate metabolites identified from the ROSMAP dataset were evaluated using a hypergeometric test, and statistical significance was assessed. Additionally, metabolites identified as the most important features were compared with the ROSMAP results to identify overlapping candidates. These metabolites, which differentiate between classes, were further evaluated in light of literature.

3. RESULTS

The main objective of this study is to identify subtype-specific and subtype-unaware metabolite biomarkers via personalized predictions of metabolites with altered secretion rates in the AD patients from the ROSMAP and MSBB cohorts, using the TAMBOOR algorithm. All analyses for pre- and post-prediction in the study are summarized in a workflow shown in **Figure 3.1**. The predictions were analyzed with different approaches to uncover potential and novel biomarkers specific to AD and AD-subtypes.

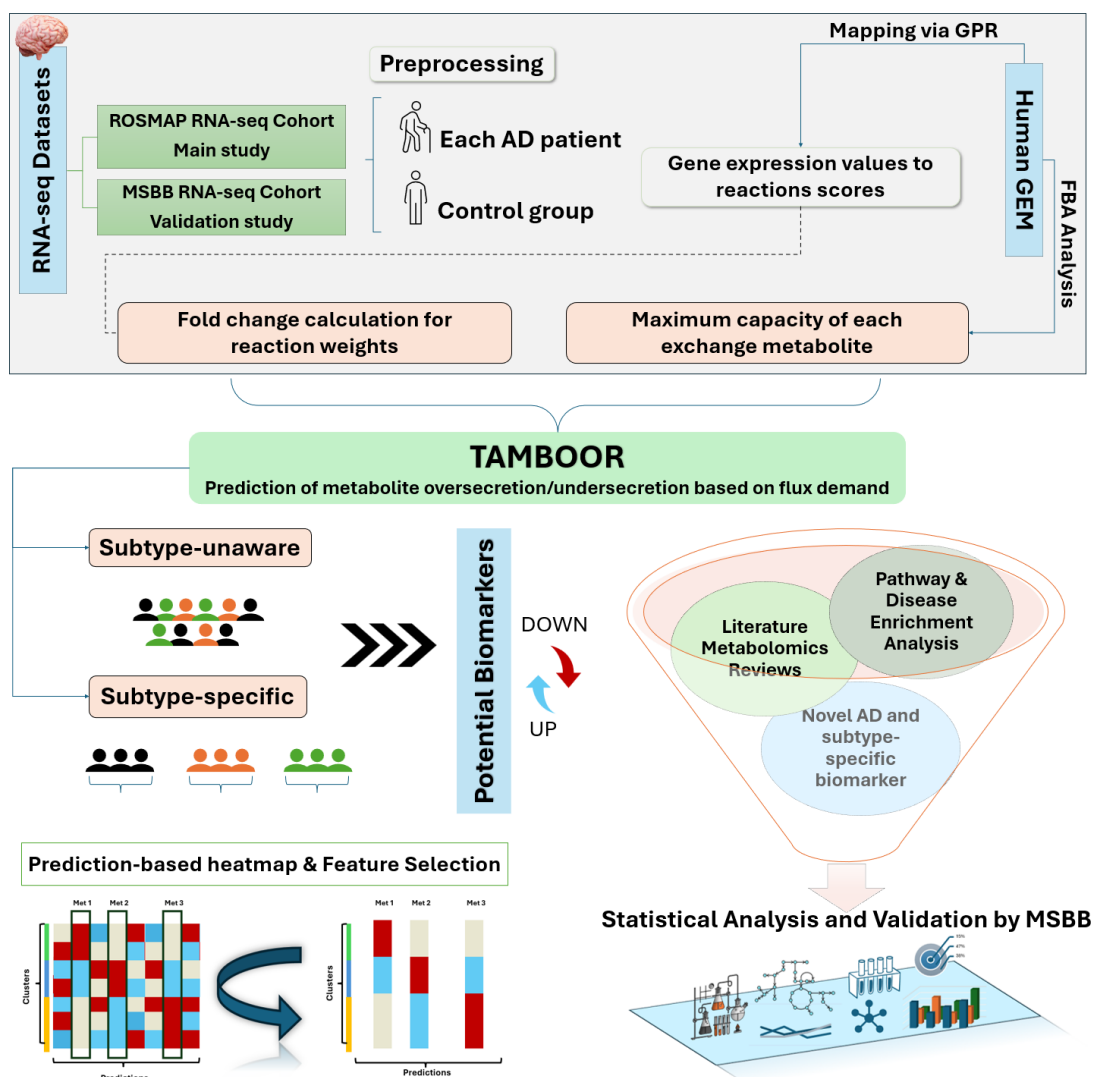


Figure 3.1: Workflow of the study. The ROSMAP dataset was used as the main study and the MSBB dataset was used as the validation study. The same preprocessing steps were performed on each dataset. Gene expression values were converted to reaction scores to obtain fold change-based reaction weights to be used in TAMBOOR. For the second part, FBA analysis was applied to Human-GEM to identify the maximum secretion capacities of the metabolites and use them as constraints in TAMBOOR. Then, the TAMBOOR algorithm was applied using as inputs FC-based reaction weights and maximum secretion capacity of metabolites secreted out of the cell for each AD patient in both datasets. Predictions were analyzed under two concepts: subtype-specific and subtype-unaware approaches. The predictions were interpreted by applying a variety of analyses such as feature selection, literature review, enrichment analysis, and statistical analysis, including validation with the MSBB dataset.

3.1. Assessment of the Subtype-Unaware Predictions

Potential biomarkers were identified for each AD patient in ROSMAP (n=188) using TAMBOOR. Metabolites predicted to have higher secretion rates in AD were termed “oversecreted metabolites” while those with lower predicted secretion rates in AD were referred to as “undersecreted metabolites” in this thesis. Subtype-unaware results were assessed by counting the number of times each external metabolite was predicted as oversecreted or undersecreted in AD samples. For a given sample, metabolites were ordered based on the production scores, and the top/bottom 15% were assumed to be predicted as oversecreted/undersecreted in that sample. Then, the number of samples in which each metabolite was identified as a biomarker was calculated, while also retaining the oversecretion/undersecretion status. A metabolite was considered to be a biomarker for the subtype-unaware analysis if (i) it showed the same secretion pattern (oversecreted/undersecreted) in at least 50% of the AD patients, or (ii) if observed in 30% to 50% of the patients with contradicting secretion patterns, i.e. oversecreted in some and undersecreted in some others, one pattern occurred at least twice as often as the other. A total of 24 metabolites were identified and classified as oversecreted potential biomarkers while 26 metabolites were classified as undersecreted potential biomarkers. A total of 50 metabolites were predicted as general AD biomarkers. Among the 50 predicted potential metabolite biomarkers, numerous metabolites associated with lipid subfamilies were prominent, including unsaturated fatty acids, arachidonic acid derivatives, and choline. Additionally, TCA cycle intermediates associated with energy metabolism, including fumarate and glycerate derivatives, were also identified. Furthermore, it is important to note the observed undersecretion pattern in many amino acids such as serine and aspartate.

The 50 predicted metabolites were categorized according to associated metabolic pathways, as summarized in **Figure 3.2**. These metabolites were grouped into six different categories: lipid metabolism, amino acid metabolism, nucleic acid metabolism, carbohydrate metabolism, one-carbon metabolism and other metabolic pathways under “Others.”. Out of the 50 predicted potential biomarkers, 36 metabolites had KEGG IDs to apply for pathway and disease enrichment analysis. Pathways with a false discovery rate (FDR) below 0.05 were considered significant. There are 42 pathway-enriched terms; several pathways found to be associated with

AD were selected and visualized, as shown in **Figure 3.3A**. Enriched pathways primarily include pyruvate metabolism and signaling processes, including the TCA, glycolysis, AMP-cGMP signaling pathways, amino acid biosynthesis, lipid metabolism, and pathways associated with neurodegeneration. On the other hand, there are also pathways related to vitamins and minerals involved in the biosynthesis of cofactors.

Based on the disease enrichment analysis results, several diseases exhibited strong associations with AD through disruptions in neurological function and energy metabolism, as shown in **Figure 3.3B**. Notably, Frontotemporal dementia, Lewy body disease, and Alzheimer's disease were enriched with the overlapping metabolite signatures, including aspartate, serine, PEP, creatinine, AMP, and hypoxanthine. In addition to these tightly connected diseases, the analysis also identified other conditions such as organ-specific diseases -particularly gastrointestinal ones like Crohn's disease, Canavan disease, and Irritable bowel syndrome- and mitochondrial-based disorders. Besides them, kidney and liver diseases are among the significantly enriched diseases. Moreover, inflammation-related diseases, some types of cancer, and rare diseases were also among the enriched diseases.

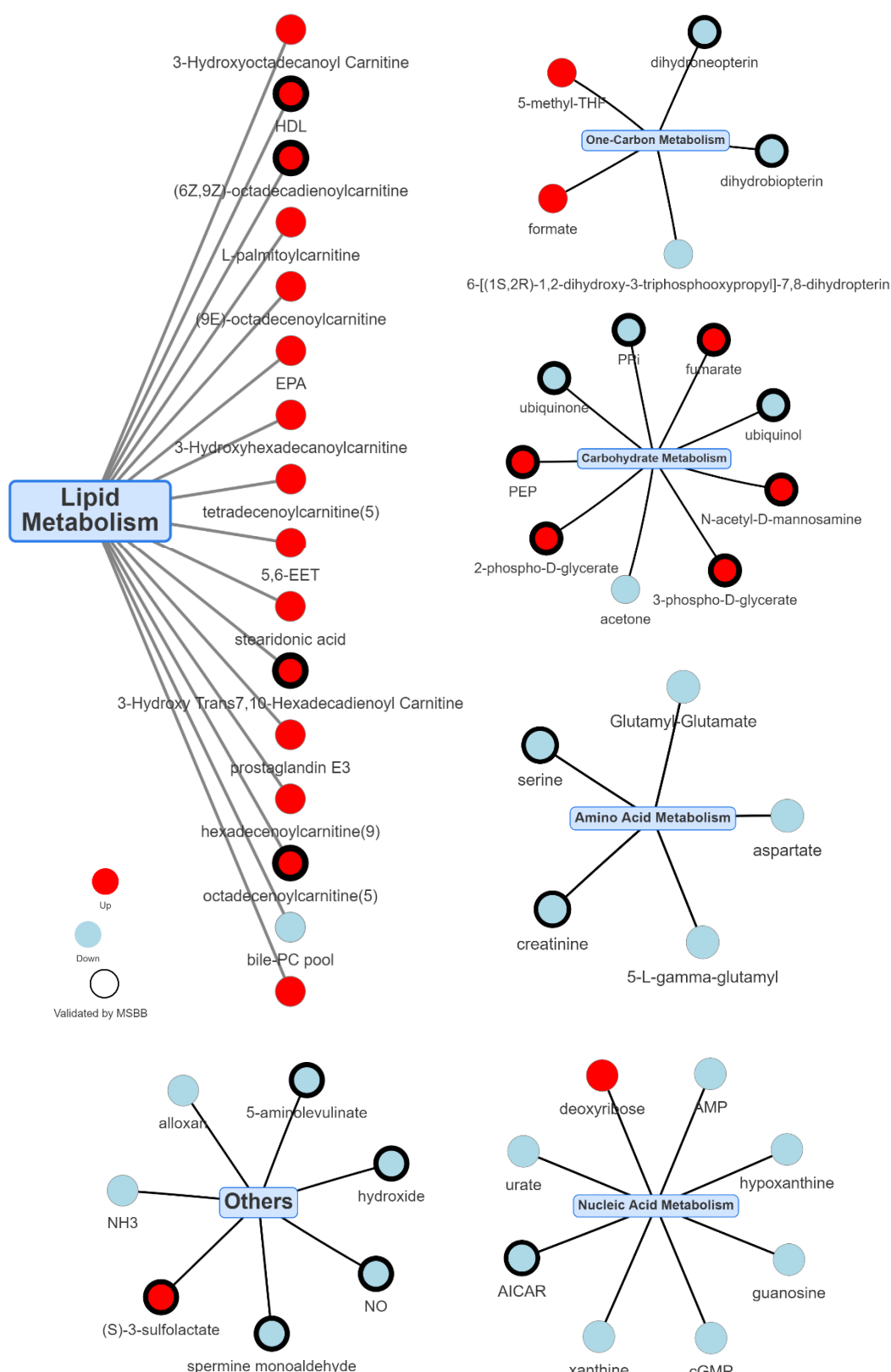


Figure 3.2: Analysis of subtype-unaware ROSMAP prediction results. The network based on general metabolic pathways associated with 50 metabolites predicted as potential biomarkers (oversecreted/undersecreted) across AD patients (n = 188) in the ROSMAP dataset.

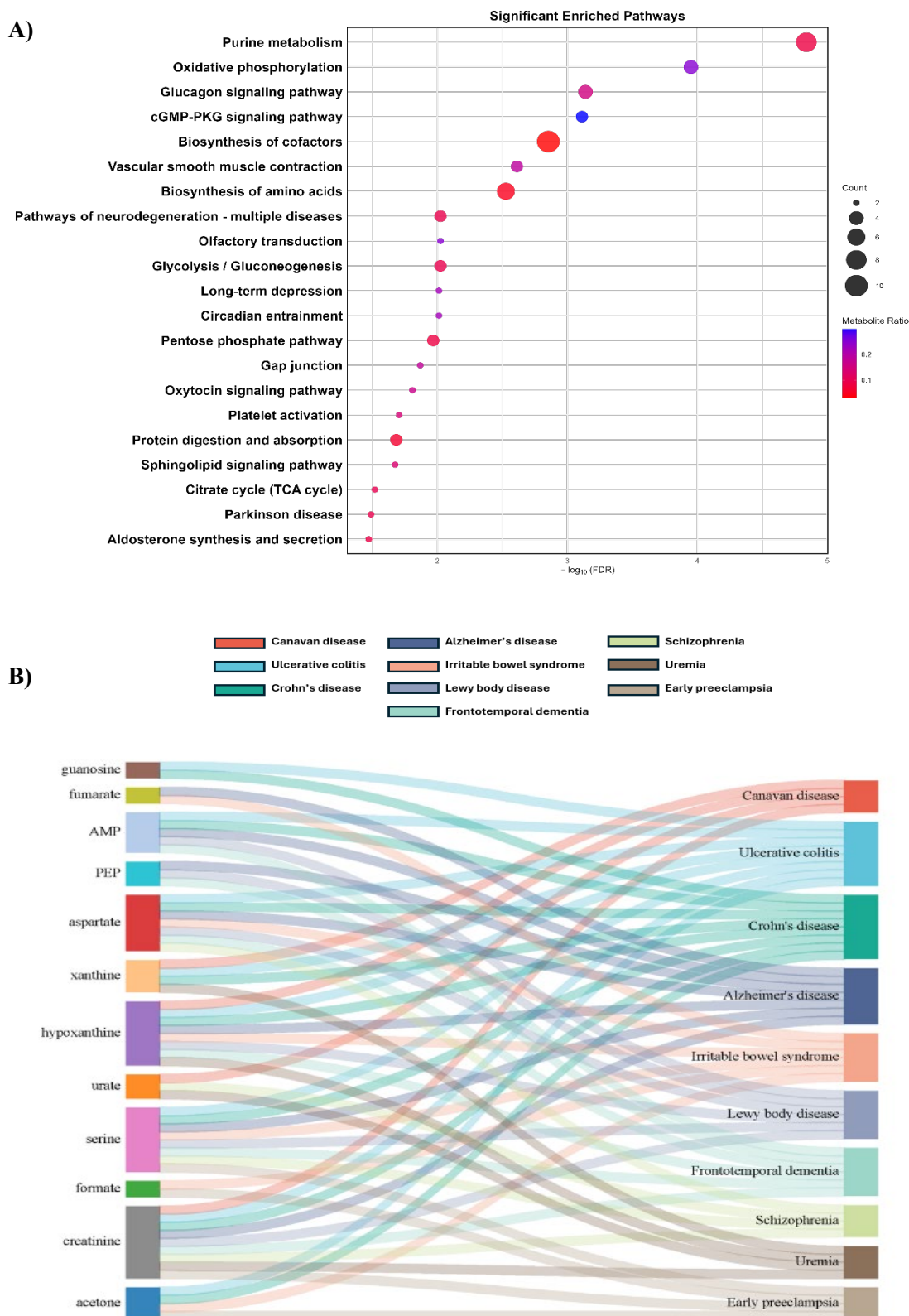


Figure 3.3: Pathway and disease enrichment analysis on subtype-unaware ROSMAP prediction results. **A)** Pathway enrichment analysis. 21 significantly enriched terms were selected and visualized ($\text{FDR} < 0.05$). **B)** Sankey plot of disease enrichment analysis results for the 36 annotated metabolites. The figure displays selected AD-related enriched disease terms with an $\text{FDR} < 0.05$ & $\text{Count} > 3$.

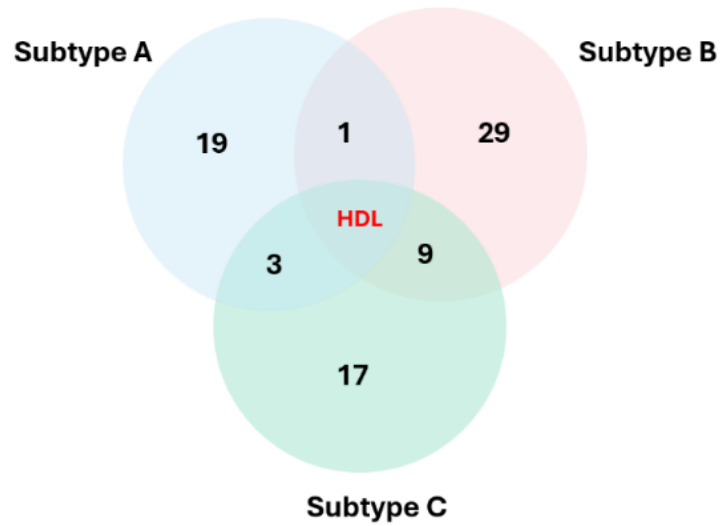
Subtype-unaware results from the MSBB cohort were used to validate the ROSMAP predictions. As a result, 22 of the 50 metabolites predicted by ROSMAP were validated by MSBB (**Figure 3.2**). 10 of the validated metabolites were in the increased metabolite secretion category and 12 were in the decreased metabolite secretion category. Most of the oversecreted metabolites were associated with lipid and carbohydrate metabolism, including metabolites such as HDL, fumarate, glycerate, and carnitine derivatives. On the other hand, the undersecreted metabolites included members of the pterin family, creatinine, serine, and ubiquinone, suggesting that pathways beyond lipid metabolism are also affected in AD. Assessment of the overlap of the ROSMAP predictions with the MSBB predictions via the hypergeometric test showed that oversecreted metabolites had $p = 8 \times 10^{-5}$ and undersecreted metabolites had $p = 6.6 \times 10^{-6}$. Considering a total of 22 validated metabolites, the p-value was calculated as 3×10^{-4} .

3.2. Assessment of the Subtype-Specific Predictions

Based on the subtypes defined by Neff et al. (Neff et al., 2021), AD patients in the ROSMAP dataset were grouped according to molecular subtype labels. As a result, 45 patients were assigned to Subtype A, 38 to Subtype B, and 83 to Subtype C. Potential biomarkers for each AD-subtype were identified by selecting metabolites predicted in at least 50% of the patients within the same AD subtype. According to results of prediction based on metabolite secretion changes, 24 metabolites were predicted as potential biomarkers, with 10 classified as oversecreted and 14 as undersecreted for Subtype A. For Subtype B, 30 metabolites were identified, of which 15 were oversecreted and 25 were undersecreted. For Subtype C, 30 metabolites were identified, of which 24 were oversecreted and 6 were undersecreted. In terms of the overlap between the subtypes, Subtypes A and B shared 1 common metabolite, Subtypes A and C shared 3, and Subtypes B and C shared 9 common metabolites, as summarized in **Figure 3.4A**. Notably, one metabolite, HDL, was identified as a common potential biomarker across all three subtypes. While HDL was oversecreted in Subtypes B and C, it was undersecreted in Subtype A. To sum up, 19 metabolites were identified as Subtype A-specific potential biomarkers while it was 29 metabolites for Subtype B and 17 metabolites for Subtype C.

Following the identification of subtype-specific potential biomarkers in the ROSMAP dataset, the predicted metabolite biomarkers across subtypes were visualized in **Figure 3.5**. Among these metabolites, those predicted by ROSMAP are shown in red, and those confirmed by MSBB (matching both subtype and direction of change) are highlighted in blue. Particularly for Subtype C, more than half of the metabolites predicted from ROSMAP AD samples were successfully validated in the MSBB dataset, as clearly shown in **Figure 3.4B**. Validation across all subtypes was assessed using a hypergeometric test. For Subtype A, 1 out of 24 predicted metabolites was confirmed, yielding a p-value of 0.37, which was not significant. For Subtype B, 10 out of 40 predicted metabolites were validated, producing a significant p-value of 1.69×10^{-3} . For Subtype C, 23 out of 30 predicted metabolites were confirmed, generating a highly significant p-value of 1.83×10^{-17} .

A)



B)

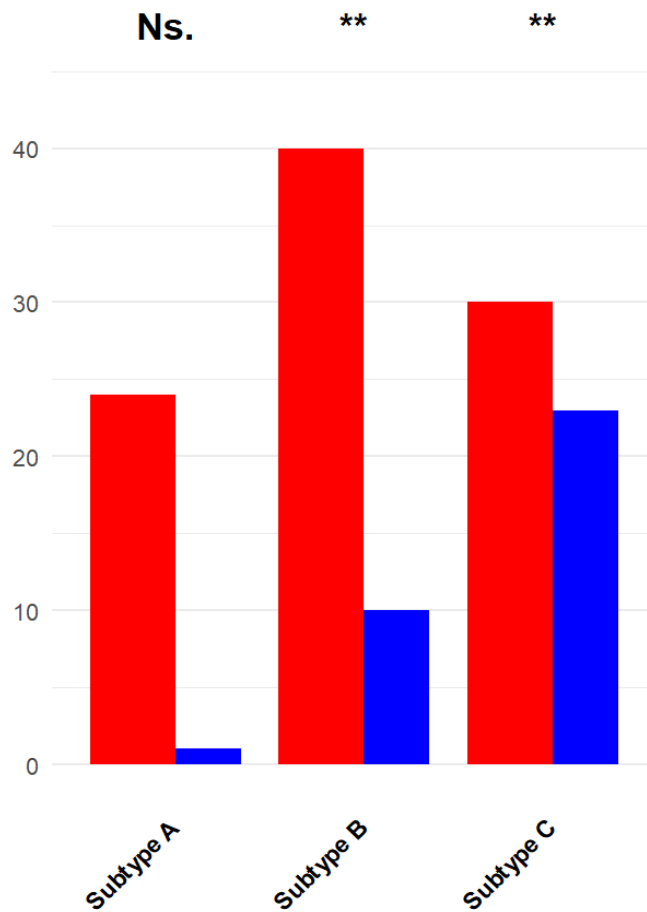


Figure 3.4: Evaluation of subtype-specific potential biomarkers. **A)** Venn diagram of metabolites predicted as potential biomarkers for each AD subtype in the ROSMAP dataset. **B)** Bar chart for summarizing the number of metabolites predicted from the ROSMAP dataset and confirmed by the MSBB dataset for each AD subtype (red: ROSMAP predictions; blue: MSBB predictions). Statistical significance of the validation was computed using the hypergeometric test. Ns: not significant; **: $p < 0.01$.

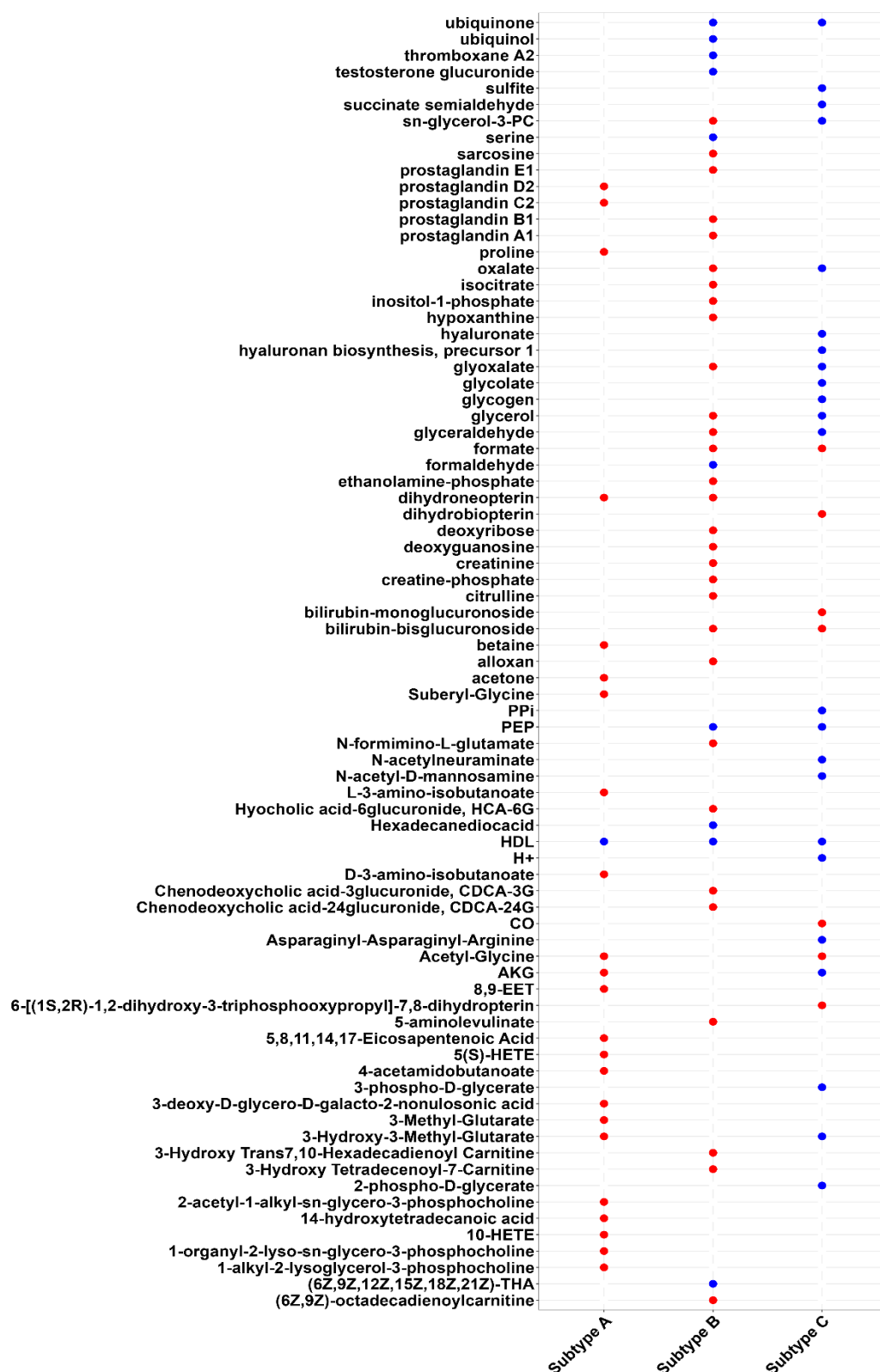


Figure 3.5: Subtype-specific potential biomarkers. Bubble plot of the subtype-specific predicted metabolite biomarkers, with validated ones by the MSBB dataset highlighted. Metabolites predicted by ROSMAP are shown as red dots. Metabolites validated by MSBB and matching both subtype and direction of change are highlighted in blue dots.

To identify key biological and clinical signatures among AD subtypes, pathway and disease enrichment analysis (**Figure 3.6**) was performed using metabolites identified as potential biomarkers for the subtypes. In terms of pathway enrichment, lipid metabolism-related pathways (including arachidonic acid metabolism, sphingolipid metabolism, and ether lipid metabolism) were among the key differentiating features among subtypes. Amino acid synthesis and related metabolic pathways were found to be enriched in almost all subtypes. Subtype A showed enrichment in pathways such as vascular smooth muscle contraction, lipid metabolism and serotonergic synapse activity. Interestingly, “Asthma” was uniquely enriched in Subtype A based on metabolism and disease-associations. Subtype B is specifically enriched in pathways involved in the purine metabolism and ABC transporters. In contrast, results based on Subtype C potential biomarkers were characterized by significant enrichment in energy metabolism pathways, such as TCA cycle and glycolysis, thermogenesis and proximal tubule bicarbonate reclamation.

Regarding disease enrichment, a wide range of diseases were significantly associated with the subtype-specific metabolite biomarkers (**Figure 3.6B**). A total of 23 diseases were commonly enriched in the three main AD subtypes, including Alzheimer's disease, Lewy body disease, and Frontotemporal dementia. Besides common terms, cerebral vasospasm and asthma for Subtype A, cystic fibrosis, phenylketonuria and cerebral creatine deficiency syndrome 2 for Subtype B, hepatitis, salla disease and biliary atresia for Subtype C were some of those uniquely associated with certain subtypes.

A)

Pathway Enrichment Analysis

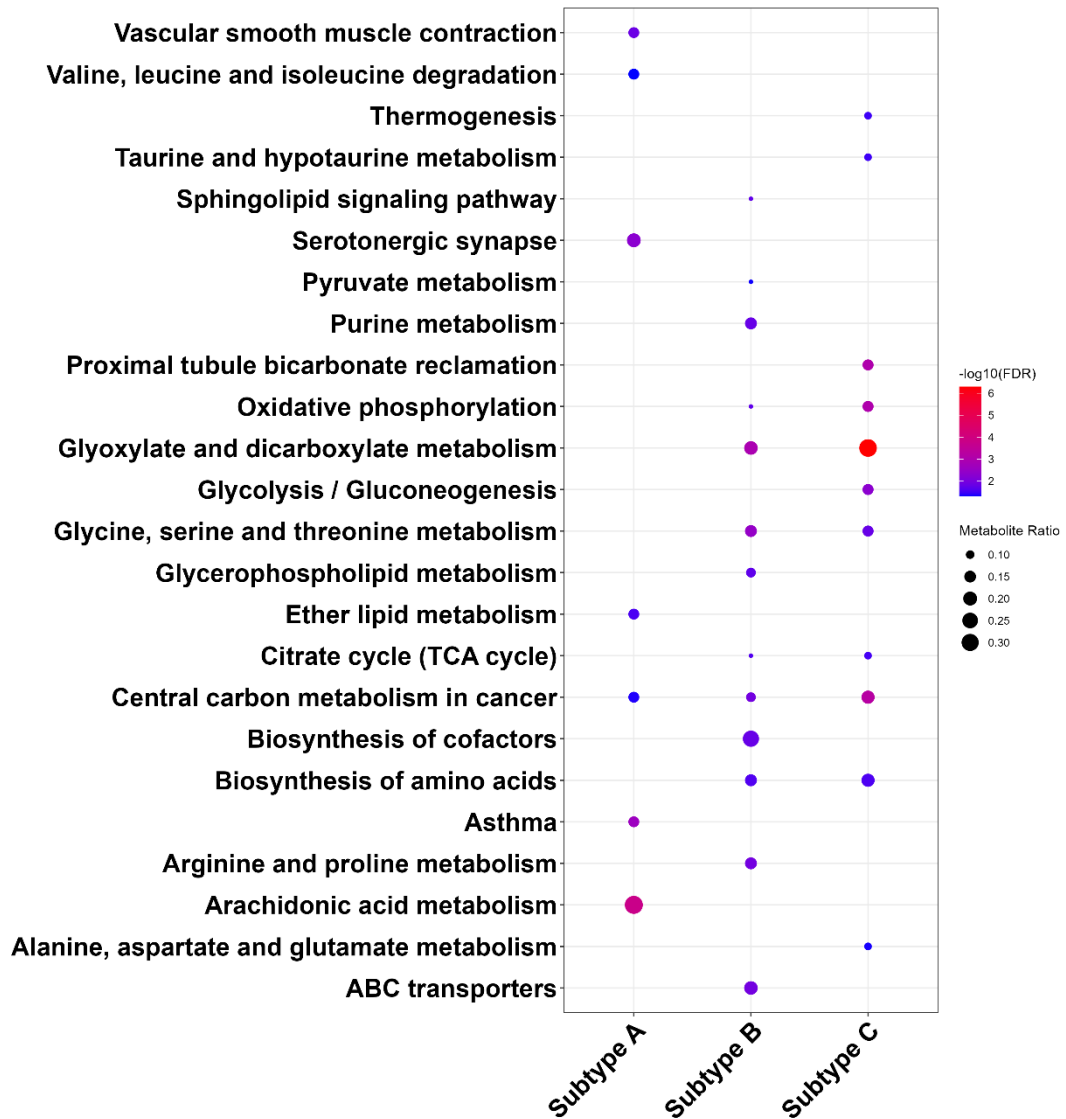


Figure 3.6: The pathway and disease enrichment analysis results. **A)** Pathway enrichment analysis and **B)** Disease enrichment analysis based on identified potential metabolite biomarkers for three main AD subtypes (FDR < 0.05).

B)

Disease Enrichment Analysis

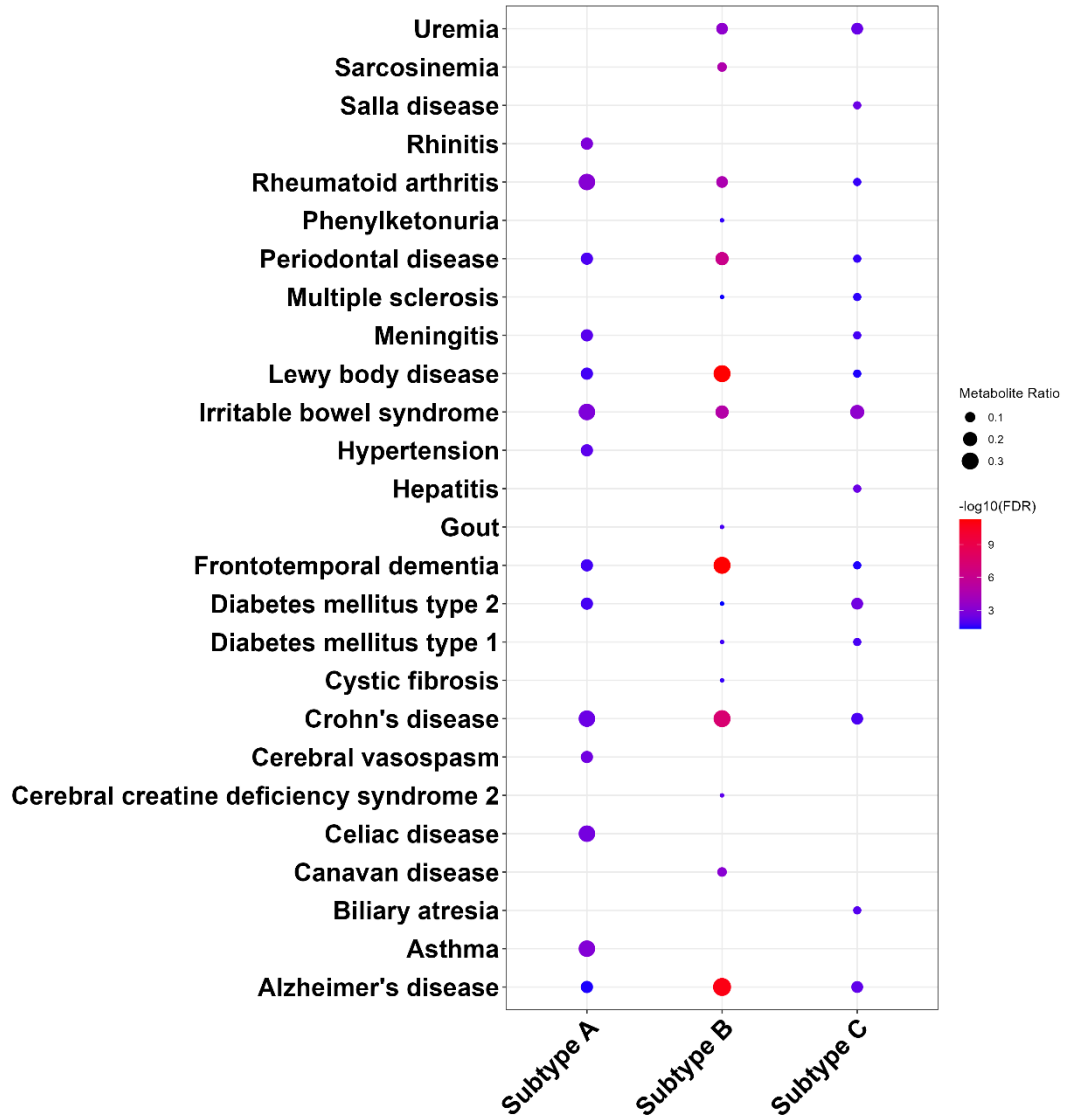


Figure 3.6 Continued: The pathway and disease enrichment analysis results. **A)** Pathway enrichment analysis and **B)** Disease enrichment analysis based on identified potential metabolite biomarkers for three main AD subtypes (FDR < 0.05).

3.3. Clustering of AD Samples & Feature Selection

Differences in the predicted metabolite biomarkers across the subtypes are in line with the known molecular heterogeneity of AD. This suggests that metabolite secretion

patterns can vary across the subtypes. A given metabolite might exhibit undersecretion in one subtype while showing oversecretion or no secretion change in another. Clustering was used to uncover such patterns across the subtypes. Predicted biomarkers for each AD sample were visualized using a hierarchical clustering approach, described in Methods, Section 2.3.1, based on the predicted direction of metabolite secretion alterations, categorized as 1 (oversecretion), -1 (undersecretion), or 0 (no change). During clustering, samples were also labelled with the pre-defined subtypes. Analysis revealed three major clusters, as highlighted in **Figure 3.7A**. The first cluster was predominantly associated with Subtype A from the Neff et al. study, the second cluster was associated with Subtype C, and the third cluster showed enrichment for Subtype B. The proportions of these pre-defined subtypes within our clusters derived from metabolite secretion predictions were calculated, as shown in **Figure 3.7B**, and statistically analyzed via the hypergeometric test. The first cluster had 49% overlap with Subtype A (p-value of 4.54×10^{-6}), the second cluster had 87% overlap with Subtype C (p-value of 8.62×10^{-12}), and the third cluster had 44% overlap with Subtype B (p-value of 4.64×10^{-7}).

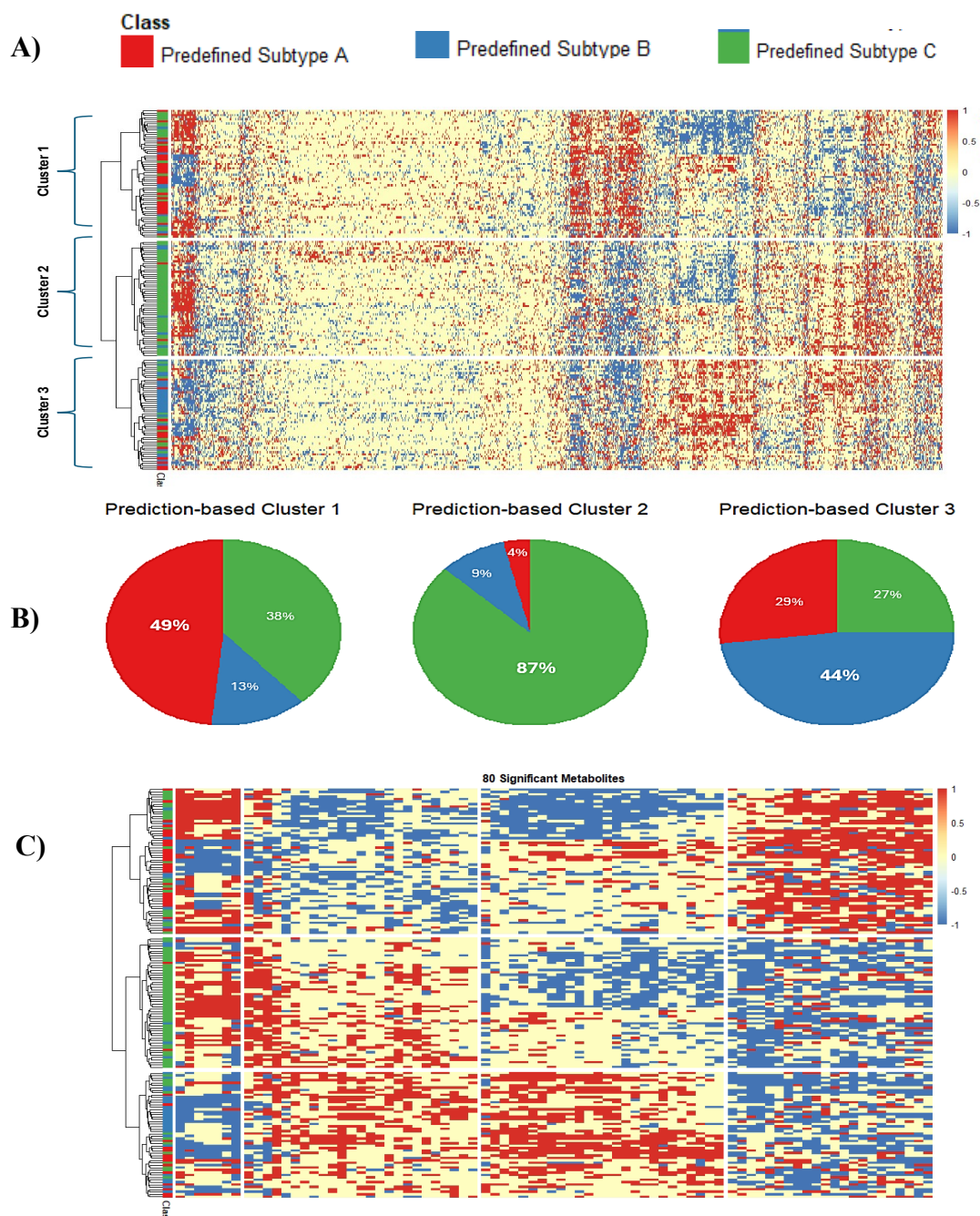


Figure 3.7: Analysis of subtype-specific predictions for metabolite oversecretion/undersecretion. **A)** Heatmap of the AD samples from the ROSMAP dataset ($n = 166$), clustered using hierarchical clustering based on metabolite secretion predictions. Three major clusters were revealed. Red highlights metabolites predicted to be oversecreted (potential biomarkers), blue highlights undersecretion predictions, and cream highlights predictions of no change. Samples were also annotated with the pre-defined AD subtypes. **B)** Pie charts showing the proportion of the pre-defined AD subtypes within each of the three dominant clusters identified in section A. **C)** Heatmap representing 80 significantly altered metabolites across clusters. These metabolites were selected based on the intersection of ANOVA ($p < 0.01$) and Random Forest results using both Gini importance and accuracy metrics.

After characterizing personalized metabolite secretion profiles and clustering them based on their predicted directional changes (oversecretion/undersecretion), metabolites with high power of separating the subtypes were identified through feature selection. Following the steps mentioned in Methods, Section 2.3.2, 80 metabolites were selected based on the overlap between ANOVA and Random Forest, depicted in **Figure 3.7C**. The resulting heatmap generated with metabolic directional change with the same approach as in **Figure 3.7A** was divided into four sections to uncover differences in metabolite directional changes across clusters, each predominantly associated with a specific AD subtype.

The first quadrant of the metabolite cluster in the heatmap shows metabolite changes that distinguish between the subtype C and subtype B dominant clusters. The second quadrant of the heatmap shows metabolites that are undersecreted in the subtype A dominant cluster but oversecreted in clusters associated with the other subtypes. The third quadrant presents a group of metabolites that do not show a clear subtype-specific pattern, although the majority of the samples from the subtype B cluster exhibit oversecreted levels in this group. The fourth quadrant contains a metabolite set that is particularly oversecreted in the cluster enriched in subtype A, while their levels are undersecreted in subtypes B and C. Of the 80 metabolites identified, the activity profiles of the three metabolites that display significant differences across clusters are shown in **Figure 3.8**.

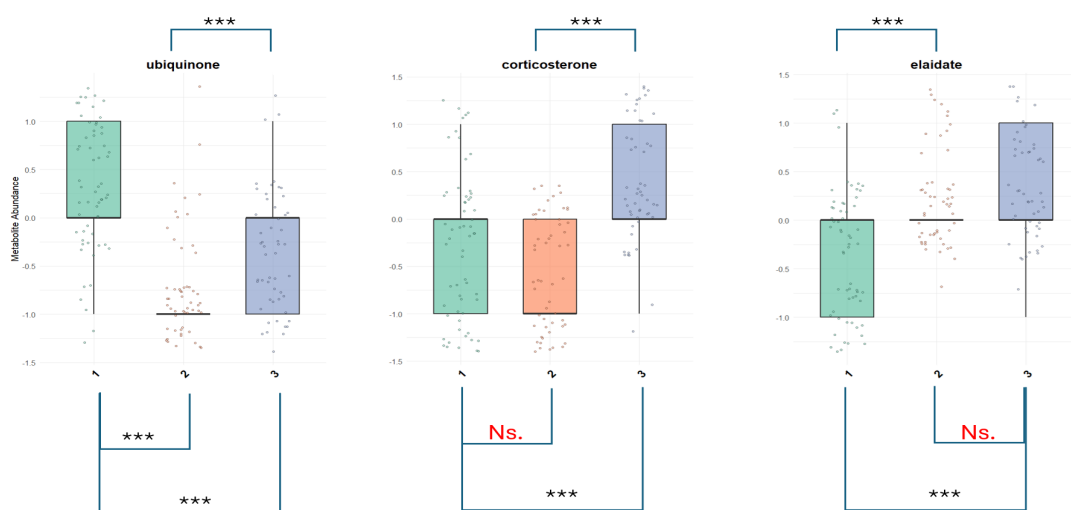


Figure 3.8: Activity of three metabolites across subtype-dominant clusters. While the x-axis represents the cluster label, the y-axis represents the predicted metabolite secretion levels across samples within the specified cluster. ***: $p < 0.001$. Ns.: Not significant.

4. DISCUSSION

To shed light on the diagnosis of AD, the TAMBOOR prediction algorithm was applied to two independent RNA-seq datasets to identify both general AD (subtype-unaware) and AD subtype-specific biomarkers. The results highlight novel biomarkers that could be applicable to all AD patients, while the identification of subtype-specific biomarkers represents a critical point of this thesis study.

4.1. Subtype-Unaware Potential Metabolite Biomarkers

Subtype-unaware AD metabolic biomarkers were identified without taking into account the disease subtypes. Biomarkers were identified such that they had the same secretion direction (oversecretion or undersecretion) in at least 30% of the patients. This approach enabled the identification of 50 metabolites as potential biomarkers (**Figure 3.2**) which were categorized according to their associated general metabolic pathways.

The metabolic group with the highest number of predicted biomarker metabolites was lipid metabolism, with 16 metabolites. Lipid dysregulation is widely recognized as the most common metabolic alteration in AD, affecting membrane structure, energy storage, blood–brain barrier (BBB) integrity, and inflammation (**Akyol et al., 2021; Chew et al., 2020; He et al., 2025; Kang & Rivest, 2012; Marques et al., 2013; Zhao et al., 2023**). Therefore, the finding that nearly one-third of the potential biomarkers are lipid-related is not unexpected. Recent lipidomic studies have shown that plasma and CSF lipid levels are dysregulated, and they can form metabolite biomarker signatures in the early stages of AD (**Barupal et al., 2019; Ferré-González et al., 2024; Shao et al., 2020; Wilkins & Trushina, 2018; Wong et al., 2017; J. Xu et al., 2020**). Among 16 metabolites, a significant proportion of lipid-associated metabolites were predicted to be oversecreted in AD, with an exception that the bile-phosphatidylcholine (PC) pool was undersecreted. These predicted potential biomarkers can be classified into five lipid subclasses: acylcarnitine (characterized by the carnitine suffix), polyunsaturated fatty acid (PUFA), eicosanoids, HDL and bile-PC pool. The eight metabolites predicted to be oversecreted in AD are from the acylcarnitine family. Acylcarnitines play a role in the transport of fatty acids into the

mitochondria, are necessary for the brain's energy demands, and maintain synaptic integrity (Jones et al., 2010; Nalecz et al., 2007; Virmani & Binienda, 2004). Due to their role in energy metabolism, alterations in the levels of metabolites in the acylcarnitine family have been a major concern for AD by researchers (Arnold et al., 2025; Baloni et al., 2021; Huguenard et al., 2023). Recent metabolomics studies on palmitoyl carnitine (C16), a family of acylcarnitines, have revealed important findings associated with AD. In this scope, predicted potentially elevated metabolites in this study; L-palmitoylcarnitine (C16:0), hexadecenoyl-L-carnitine (C16:1), 3-hydroxyhexadecenoyl-L-carnitine (C16-OH), 3-hydroxyhexadecenoyl-L-carnitine (C16:1-OH) and 3-Hydroxy Trans7,10-Hexadecadienoyl Carnitine (C16:2-OH) can be evaluated together since they have a long-chain C16 carbon structure (Cristofano et al., 2016). Several experimental studies on biological samples have found increased (Berezhnov et al., 2020; Ferraretto et al., 2018; Fonteh et al., 2014; Fraser et al., 2010; Nasaruddin et al., 2016; Xiang et al., 2025; Yoon et al., 2024), and decreased levels of palmitoyl-carnitine (Cristofano et al., 2016; González-Domínguez et al., 2015b; G. Wang et al., 2014) associated with AD and its pathology. The long-chain acylcarnitines C18:0 (octadecanoyl-L-carnitine), C18:1 (octadecenoyl-L-carnitine), C18:2 (octadecadienoyl-L-carnitine), and C18-OH (3-hydroxyoctadecanoyl-L-carnitine) were predicted to have increased levels in AD. Studies on C18:0 levels have reported both increased (Nasaruddin et al., 2016) and decreased (Fonteh et al., 2014; Yoon et al., 2024) levels in AD patients. On the other hand, especially C18:1 showed a positive correlation with age and had increased levels in different diseases (Ahmad et al., 2016; Cao et al., 2020; K. H. Chang et al., 2018; Horgusluoglu et al., 2022; Jarrell et al., 2020; Mitchell et al., 2018), including AD. C14:1 levels have been associated with mitochondrial dysfunction (Abu Bakar & Sarmidi, 2017; A. M. Das et al., 2006), insulin resistance and type 2 diabetes (Mai et al., 2013) and are significantly associated with cognitive decline and brain atrophy (Toledo et al., 2017).

Three biomarkers that were predicted to be oversecreted in AD (eicosapentaenoic acid, stearidonic acid, and omega-3-arachidonic acid) were PUFAs and reported to be altered in AD in the literature (Snowden et al., 2017). Many studies have been conducted on the use of ω -3 fatty acids as supplements, and their association with cognitive impairment has been investigated (Daiello et al., 2015; Lopez et al., 2011; M. C. Morris et al., 2003; Quinn et al., 2010; Shahidi & Ambigaipalan, 2018;

Tomata et al., 2020; Vakhapova et al., 2014). Interestingly, while these metabolites have been specifically studied as supplements for AD, they were predicted in the list of oversecreted potential biomarkers in this thesis study. Increased secretion rates of eicosanoids (prostaglandin E3, 5,6-EET) and HDL metabolites and a decrease in the bile-PC pool secretion rate have been predicted. Experimental studies on these metabolites suggest that they may disrupt lipid homeostasis and impair HDL function, promoting neuroinflammation, cholesterol transport and potentially contribute to AD pathology (**Biringer, 2019; Furman et al., 2018; Ghosh et al., 2020; González-Domínguez et al., 2014; Hussain et al., 2024; Kjeldsen et al., 2022; Whiley et al., 2014).** Interestingly, while these metabolites have been specifically studied as supplements for AD, they were predicted in the list of oversecreted potential biomarkers in this study.

All the predicted biomarkers grouped within the amino acid metabolism were found to be undersecreted in AD in this study. In this scope, glutamate (**Berezhnoy et al., 2023; González-Domínguez et al., 2014; G. Wang et al., 2014**), and glutamine levels (**González-Domínguez et al., 2014; Redjems-Bennani et al., 1998; G. Wang et al., 2014**) were generally found to be decreased in the biofluid samples. The metabolites creatinine, serine, and aspartate, which were predicted as biomarkers in this study, have been shown to be significantly altered in AD and affect neurodevelopment processes in several studies (**Ibáñez et al., 2013; Jukarainen et al., 2008; Kalhan & Hanson, 2012; Trushina et al., 2013; Van Der Velpen et al., 2019; J. Xu et al., 2016**). Moreover, hypoxanthine, xanthine, and urate show differential changes in AD among biological systems (**Hatanaka et al., 2015; Ibáñez et al., 2013; T. S. Kim et al., 2006; Q. Liang et al., 2015; Trushina et al., 2013; Van Der Velpen et al., 2019**). The thesis study also reveals that metabolite secretion behavior was altered in the metabolites associated with the pathways such as one-carbon and carbohydrate metabolism. Glycolytic intermediates (2-phospho-D-glycerate, 3-phospho-D-glycerate) were reported to have decreased levels in AD, associated with glycation and its end products accumulation (**Bergau et al., 2019; Gil et al., 2002; Lüth et al., 2005**). Another metabolite categorized in carbohydrate metabolism, fumarate, has been predicted to be oversecreted in AD. Fumarate, which has a role in energy metabolism, had decreased levels in CSF (**Redjems-Bennani et al., 1998**) and increased levels in brain tissue (**J. Xu et al., 2016**). Oversecretion was predicted for

(S)-3-sulfolactate, which is a microbial intermediate converted to hydrogen sulfide (H₂S) by gut bacteria (**Frommeyer et al., 2020; Hanson et al., 2021**). As part of this pathway, it is closely linked to sulfur metabolism in the gut microbiota (**Paul & Pieper, 2024**). Increased H₂S production in the gut can disrupt intestinal barrier integrity and lead to its release into the bloodstream also potentially binding to the brain and contributing to neuroinflammation (**Munteanu et al., 2024**). Increased H₂S in plasma has been associated with dementia and memory impairment (**Disbrow et al., 2021**). High levels of bound sulfites in the plasma have been associated with decreased grey matter in the brain (**Reekes et al., 2023**). A separate study has indicated that amyloid production is reduced after sulfur amino acid restriction, which may protect cognitive function (**Sambamurti et al., 2023**). As a sulfonated analog of lactate, sulfolactate accumulation may indicate disturbances in energy metabolism and redox imbalance, potentially contributing to neurodegeneration in AD.

Pathway enrichment analysis showed that predicted potential biomarker metabolites were generally associated with known dysregulated pathways in AD (**Figure 3.3A**). Particularly, the presence of PD (**Mattson et al., 1999**) and depression (**Sáiz-Vázquez et al., 2021**) in the pathway enrichment results implies that metabolic changes observed in AD are also common in these diseases. Other notable pathways significantly associated with AD include platelet activation (**Sevush et al., 1998**), oxytocin signaling (**Takahashi et al., 2023**), and gap junction (**Takeuchi et al., 2011**), uncovering their potential roles in AD-related higher basal activation, memory loss, and cognitive impairment. Disease enrichment analysis revealed that several conditions share overlapping metabolic or genetic mechanisms with AD. Notably, Lewy body dementia (LBD) (**Prasad et al., 2023**) and schizophrenia (**Ohi et al., 2024**) exhibit shared genetic underpinnings with AD, while Frontotemporal dementia (FTD) shows overlapping metabolite profiles (**Mendez et al., 1996; Ulugut & Pijnenburg, 2023**). Systemic inflammatory diseases such as Crohn's disease, ulcerative colitis, and uremia were also identified in the enrichment analysis, and they are all associated with increased dementia risk (**C. H. Chen et al., 2016; Daulatzai, 2014; Kuźniar et al., 2024**). The relationship between gut microbiota and the brain appears to lead to inflammation and contribute to AD through neurotransmitter pathways and various pathological mechanisms (**Kowalski & Mulak, 2019**).

All these findings from the existing literature suggest that the predicted metabolites may also be common features of other systemic and neurological diseases with AD. Predictions of metabolite biomarkers with potential for oversecretion/undersecretion in AD in the ROSMAP dataset were validated by comparing the results with the MSBB dataset. This validation increased the reliability of the predicted potential metabolites.

4.2. Subtype-Specific Potential Metabolite Biomarkers

The potential metabolite biomarkers were determined for each pre-defined subtype (A, B and C) such that at least half of the patients in the same subtype group showed the same metabolite secretion direction. The metabolites that met this criterion were evaluated as potential biomarkers for the relevant subtype. According to Neff et al., Subtype A shows molecular features largely opposite to those typically seen in AD. Subtype B presents an intermediate molecular profile that partially resembles classical AD, while Subtype C has been associated with typical AD.

Subtype B showed the highest number of biomarkers (n=40) and overlapped more with Subtype C than with A, suggesting that Subtype B moderately reflects the molecular signature of AD (Neff et al., 2021). HDL was a common biomarker across all subtypes and was confirmed by MSBB, but the direction of change varied—oversecreted in Subtypes B and C (as in the subtype-unaware prediction) and undersecreted in Subtype A. These findings reveal that HDL may be a key biomarker in understanding general AD patterns and subtype-based differences. From other perspectives, the overlap with the subtype-unaware AD biomarkers was lowest in Subtype A (13%), while Subtypes B (35%) and C (33%) showed higher and directionally consistent overlap. These findings suggest that Subtype A reflects the AD profile in the opposite way as found by Neff et al. The concordance between metabolic and transcriptomic-based subtype behaviors supports integrated analyses of personalized transcriptome data and metabolic modelling.

Validation with the MSBB dataset confirmed the subtypes B and C with strong significance, but failed to confirm the Subtype A. This suggests that metabolite activity of the Subtype A is not stable and varies from data to data. The prediction approach applied with the TAMBOOR algorithm allowed the detection of consistent biomarker patterns despite the heterogeneity present in AD patients and between datasets.

Validation of ROSMAP-based subtype-specific biomarkers obtained in the MSBB dataset increased confidence in the algorithm's outputs and strengthened the generalizability and biological significance of the findings. These results prove that the used prediction algorithm will contribute not only to biomarker detection but also to revealing metabolic alterations between AD subtypes and identifying personalized diagnosis strategies and targeted therapies. In this section, selected metabolites predicted as subtype-specific biomarker candidates are reviewed and high-confidence metabolite biomarkers specific to each subtype are critiqued.

4.2.1. Subtype A-specific Biomarker Candidates

Several metabolite biomarker candidates that play a role in amino acid metabolism in AD were predicted. Two of them involved in valine metabolism (L-3-amino-isobutanoate and D-3-amino-isobutanoate) are predicted to be oversecreted in Subtype A, and this is supported by the increased branched-chain amino acid (BCAA) levels in AD patients, indicating that this amino acid family is important in the disease (**Pan et al., 2016; Polis & Samson, 2020**). The betaine metabolite, an amino acid derivative, was predicted to be undersecreted solely in Subtype A. The betaine molecule plays roles in cellular processes and mechanisms including methylation and carnitine synthesis (**Lever & Slow, 2010**). Betaine deficiency has been associated with various diseases, including glucose intolerance (**Esfahani et al., 2023**), liver disorder (**Y. Zhang et al., 2020**), metabolic dysfunction (**Marino et al., 2025**), inflammation (**Medici et al., 2013**), and memory impairment (**Miwa et al., 2011**). Many studies using betaine as a supplement have shown various benefits, such as reducing brain damage, increasing synaptic function, strengthening antioxidant defenses, and regulating cytokine release from glial cells (**Adresi et al., 2020; Chai et al., 2013; Ibi et al., 2021; Leiteritz et al., 2018; Z. J. Yang et al., 2024**). Using a high-potency molecule as a supplement suggests that betaine levels decrease and the effects of betaine are reversed; therefore, betaine may be a potential biomarker for subtype-A. Proline was predicted to be oversecreted in Subtype A. Given its role in neural function, it has been the focus of research on psychiatric and neurodegenerative disorders (**Yao & Han, 2022**). While no relationship was found between the CSF proline levels and AD in initial studies (**Baxter et al., 1984**), a relationship was later reported between proline substitutions in PSEN1 and mitochondrial dysfunction

(Ben-Gedalya et al., 2015). Furthermore, increased plasma proline levels (Molina et al., 1998; G. Wang et al., 2014) in AD indicated decreased white matter density (Steinlin et al., 1989).

Different metabolites involved in arachidonic acid metabolism, which is impaired in AD, have been identified as biomarker candidates. Prostaglandin D2 (PGD2), predicted to be oversecreted in subtype A, is linked to inflammation (Maesaka et al., 2013; Thomas et al., 2016) and is elevated in AD brains (Biringer, 2019). PGD2 receptor interactions play dual roles in neuronal loss and protection (Mohri et al., 2007), having impact on astrogliosis, demyelination and proinflammatory responses (X. Liang et al., 2005; Mohri et al., 2006, 2007). 5-HETE is one of the metabolites predicted to be oversecreted in the Subtype A group. This molecule is one of the products of the ALOX5 (arachidonate 5-lipoxygenase) enzyme and has been studied in different studies to play a role in various pathological processes associated with AD (Biringer, 2019; Ikonovic et al., 2008; Q. Y. Sun et al., 2019). There are several studies showing that 5-HETE and ALOX5 influence AD pathology, brain glia cells, and synaptic dysfunction (Biringer, 2019; Chu & Praticò, 2011; Firuzi et al., 2008; Joshi et al., 2014). The other predicted biomarker candidate specific to Subtype-A is the undersecretion of 3-deoxy-D-glycero-D-galacto-2-nonulosonic acid, which is sialic acid (Nakata et al., 2001). Sialic acid is the most abundant glycan in the brain and enables the interaction of axons with the myelin sheath on nerve cell surfaces (Rawal & Zhao, 2021). In the light of these findings, oversecretion of proline, PGD2 and 5-HETE and undersecretion of betaine may provide a clue to neurotransmitter dysregulation within brain, potentially being a biomarker for Subtype A.

4.2.2. Subtype B-specific Biomarker Candidates

Creatine phosphate (PCr), phosphate-bound high-energy form of creatine, was predicted to be undersecreted in Subtype-C, which indicates that creatine levels may have decreased in the context of metabolic reactions (Bessman & Carpenter, 1985). In this way, many studies have focused on the role of the creatine system in AD and published findings regarding the decrease in creatine levels (González-Domínguez et al., 2014, 2015a; Smith et al., 2023; Trushina et al., 2013). Two metabolites of Chenodeoxycholic acid (CDCA) were predicted to be oversecreted in Subtype-B. CDCA, a primary bile acid, has been associated with various neurodegenerative

diseases and the metabolic pathways associated with these diseases (Baloni et al., 2020; Ehtezazi et al., 2023; Grant & Demorrow, 2020; Mehreen et al., 2025). In some studies, the levels of CDCA and its derivatives were reported to increase (Rolo et al., 2000; Shao et al., 2020) or decrease (T. Chen et al., 2024) in AD. In the subtype-B prediction results, the metabolites isocitrate (Jaramillo-Jimenez et al., 2023; Tsuruoka et al., 2013) and citrulline (Fonteh et al., 2007) were found to be oversecreted. A decrease was predicted in ethanolamine-phosphate (phosphoethanolamine (PE)) secretion rate in Subtype-B. The role of PE against gamma-aminobutyric acid (GABA) receptors is linked to neurotransmission (Klunk et al., 1995), while decreased PE and ethanolamine-derived plasmalogens levels are associated with neuronal survival (Ellison et al., 1987), cognitive impairment and tau pathology (Kling et al., 2020). Overall, undersecretion of PCr, PE and oversecretion of CDCA can highlight distinct biomarker profiles for Subtype B.

4.2.3. Subtype C-specific Biomarker Candidates

Glycogen was predicted to be oversecreted in Subtype C. Glycogen is predominantly stored in astrocytes, and it supports brain energy metabolism and neuron–astrocyte communication. One of its most prominent functions is to support synaptic activity through lactate transport (Rich et al., 2019). Studies have shown that glycogen accumulation continues for a long time in AD and therefore the increase begins before clinical symptoms (Gentry & Gentry, 2023) and deterioration of axonal transport (Mann et al., 1987). In addition, it is known that the GSK-3 β enzyme disrupts glycogen metabolism, increases tau hyperphosphorylation, and triggers neuronal death (Avila et al., 2010; Lauretti et al., 2020).

Recent studies highlight the role of extracellular matrix components such as hyaluronate (HA) in AD (Y. Sun et al., 2021; Zakusilo et al., 2021). In this thesis study, HA was predicted to be oversecreted in Subtype-C, consistent with previous findings showing that increased HA levels are associated with amyloid and tau accumulation, BBB disruption, oligodendrocyte maturation, and synaptic dysfunction (Back et al., 2011; Hayashi et al., 2010; Nägga et al., 2014; Reed et al., 2019). Bilirubin monoglucuronide, predicted to be oversecreted in Subtype-C, is a bilirubin derivative formed in the liver that becomes water-soluble for excretion (Chowdhury et al., 1981; Kalakonda et al., 2022). While metabolomics studies specifically on

bilirubin monoglucuronide in AD are limited, studies on total bilirubin levels in AD patients show conflicting results: some studies report increased levels (**Ramdane & Ramdane, 2021; H. Wang et al., 2022**), while others find decreased levels (**Hatanaka et al., 2015; T. S. Kim et al., 2006**) or no direct association (**Ahmed et al., 2014; H. Wang et al., 2022**). Succinic semialdehyde (SSA) is an intermediate in GABA metabolism, was predicted to be oversecreted for the Subtype C. SSA is oxidized to succinic acid by enzyme succinic semialdehyde dehydrogenase (SSADH) and participates in the TCA cycle (**Didiasova et al., 2020**). Its deficiency may lead to inadequate metabolism of SSA and its accumulation. SSA accumulation increases (**Struys et al., 2005**) the production of gamma-hydroxybutyrate (GHB) via the alternative pathway. While GHB may be neuroprotective in the short term, chronic accumulation can cause neurotoxic effects (**Didiasova et al., 2020**). Elevated levels of SSA and related metabolites (GHB, 2,4-dihydroxybutyric acid) have been linked to oxidative stress, mitochondrial dysfunction, and age-related neurotoxicity- all related to AD pathophysiology (**Picklo et al., 2001; Salminen et al., 2016**). N-acetylneuraminic acid, a sialic acid predicted to be secreted at a higher rate in Subtype-C, plays a role in AD through oxidative stress, impaired antioxidant defenses, and synaptic dysfunction (**Terracina et al., 2022; Yadav et al., 2020; K. Yang et al., 2021**). Mutations affecting its biosynthesis and elevated levels in the obesity-associated AD models (**Suzzi et al., 2023**) highlight its role in neurodegeneration and memory impairment (**Bu et al., 2023**).

Sulfite, predicted to be undersecreted in Subtype-C, reflects a dysregulation in sulfur metabolism. Studies have shown that reduced sulfatides are linked to ventricular enlargement in the early stage (**Palavicini et al., 2022**), impaired H₂S signaling leads to tau hyperphosphorylation (**Giovinazzo et al., 2021**), and elevated 3-O-sulfated heparan sulfates may accelerate tau uptake and disease progression (**Z. Wang et al., 2023**). Carbon monoxide (CO), predicted to have higher release rates in Subtype-C, has been associated with elevated AD risk through its role in oxidative stress (**Bagheri & Rashedi, 2020**), cognitive impairments (**Amitai et al., 1998**), and dementia (**M. Zhang et al., 2024**).

Among the candidates identified for Subtype-C, oversecretion of glycogen, HA and SSA (as a novel biomarker) were predicted to be specific biomarkers for this subtype. Based on predicted metabolites for each subtype, potential biomarkers were identified

through literature evaluation. Notably, sialic acid derivatives emerged as the main metabolites prevalent and affected in all subtypes, considering that further studies are needed.

4.2.4. Enrichment Analysis

Pathway enrichment analyses of predicted metabolite biomarkers reveal common dysregulation in carbon and amino acid metabolism across all AD subtypes, underlining their central role in disease pathology. Subtype A is particularly marked by disruptions in vascular function (**Aguilar-Pineda et al., 2021**), BCAA metabolism (**Siddik et al., 2022**), and serotonergic signaling (**Chakraborty et al., 2019**), aligning with the disruption of brain and neurotransmitter paths. Subtype B shows distinct alterations in ABC transporters (**Pereira et al., 2017**), signaling pathways (**T. K. Das et al., 2023**), and purine/pyruvate metabolism (**Ansoleaga et al., 2015; Isopi et al., 2015**) reflecting nervous system and BBB impairment. Subtype C is distinguished by impaired glycolysis (**X. Zhang et al., 2021**), taurine metabolism (**Santa-María et al., 2007**), and kidney-associated pathways such as thermogenesis (**Serrano et al., 2023**), and proximal tubule bicarbonate reclamation (**Kwon et al., 2023**), suggesting a metabolic signature involving oxidative stress and renal-inflammatory links to AD.

Disease enrichment results revealed significant enrichment of “Alzheimer's Disease” term from HMDB in all three subtypes. Additionally, certain diseases such as periodontal disease (**Bouziane et al., 2023**), rheumatoid arthritis (**Sangha et al., 2020**), and both type 1 and type 2 diabetes (**Sims-Robinson et al., 2010**) were common across all subtypes, uncovering systemic risk factors that may overall influence AD progression. While disease enrichment analysis revealed diseases specific to each subtype, the association of these diseases with AD was also supported by previous studies. Subtype A was associated with inflammation-related diseases such as asthma (**Bartels et al., 2024; Nair et al., 2022; X. Wang et al., 2024**), cerebral vasospasm (**X. Wang et al., 2023**), celiac disease (**Ford et al., 2022**), suggesting an inflammatory or allergic response profile (**Joh et al., 2023**). Subtype B showed enrichment in genetically linked diseases such as gout (**X. Li et al., 2023; Lu et al., 2016; Sung et al., 2023**), phenylketonuria (**Tufekcioglu et al., 2016; van Spronsen et al., 2021**), cystic fibrosis (**Lahousse et al., 2003; Patton-Parfyonov et al., 2025**) and cerebral creatine deficiency (**Schütz & Stöckler, 2022**), suggesting a stronger link to genetic

predisposition. Subtype C was characterized by liver and metabolism-related diseases such as salla disease (Alajoki et al., 2004; Sonninen et al., 1999), biliary atresia (Babu et al., 2020; Kamp et al., 2024; Tian et al., 2022), and hepatitis (Choi et al., 2021; Polukchi & Slavko, 2023; Su et al., 2024), highlighting systemic and peripheral contributions to AD pathology.

4.3. Feature Selection

In this section, first the AD patients were clustered based on their predicted secretion directions (oversecretion/undersecretion/no change) of 1,043 external metabolites (Figure 3.7A). As a result of the clustering, Subtype A patients were predominantly enriched in Cluster 1, Subtype C in Cluster 2, and Subtype B in Cluster 3. As seen in the pie charts (Figure 3.7B), the subtype proportions within each cluster were found to be statistically significant. The AD patients in the Subtype C group showed largely consistent metabolite activities and were selected as the most stable subtype with respect to both Neff et al. and this study. These findings reveal that TAMBOOR's metabolite production predictions effectively differentiate AD subtypes based on distinct metabolic patterns—complementing transcriptome-based subtyping. The metabolites that exhibited significantly different behavior among clusters were selected. Among the 80 important and significant metabolites, several corticosteroid derivatives exhibited consistent metabolite production alterations across subtypes. One of the main metabolites, corticosterone, predicted to be undersecreted in the Subtype A and Subtype C dominant clusters, while oversecreted in the Subtype B dominant cluster.

Cortisol and corticosterone are commonly used as biomarkers for depression, with cortisol often preferred due to its practicality. However, studies show that corticosterone is correlated with cortisol, and, in chronic stress, corticosterone may better reflect the adaptive response of body (Gong et al., 2015). This suggests that corticosterone levels could vary with stress in AD patients and differ across disease subtypes. Previous studies have shown that elevated cortisol levels are associated with AD (Armanini et al., 2003; Dronse et al., 2023; Holleman et al., 2022; Joshi et al., 2012; Ouanes & Popp, 2019; Zheng et al., 2020). These findings suggest that oversecretion of corticosterone in Subtype B and undersecretion in other subtypes may reflect different stress responses.

Among the subtype-unaware AD biomarkers, ubiquinone (Coenzyme Q10, CoQ10), was predicted to be undersecreted while it was predicted to be oversecreted in the Subtype A dominant cluster and undersecreted in the Subtype B and C dominant clusters. These findings show that Subtype B and C overlap with the subtype-unaware profile and that different regulations in CoQ10 metabolism may be at the forefront for each subtype. CoQ10 has been reported to have important roles in mitochondrial function and energy production in AD (**Fišar & Hroudová, 2024; Jiménez-Jiménez et al., 2023; Rauchová, 2021**). Significant benefits of CoQ10 in experimental models have been shown (**Dumont et al., 2011; Kumar et al., 2023; X. Yang et al., 2010**). In addition to the multifaceted effects of these metabolites, one study found no effect on dementia (**Momiyama, 2014**), while another study showed that low levels lead to amyloid beta accumulation (**P. S. Chang et al., 2022**). The findings obtained from TAMBOOR reveal that each subtype exhibits different metabolic changes and that these differences are supported by findings in the literature, underlying this heterogeneity of AD.

Elaidate (synonym of oleic acid and elaidic acid in HMDB) was predicted to be undersecreted in the Subtype A dominant cluster, oversecreted in the Subtype B dominant cluster, and no change significantly (seems neutral) was predicted in the Subtype C dominant cluster. Elaidate has been associated with an increased risk of dementia (**Honda et al., 2019**), suppressing of insulin signaling and reduction of glucose uptake (**Ishibashi et al., 2020**), which are critical for synaptic function and energy metabolism. While high plasma oleic acid may reduce mild cognitive impairment (MCI)-to-AD progression (**Dakterzada et al., 2024**), studies show that oleic (**González-Domínguez et al., 2015b**) and elaidic acid (**Nasaruddin et al., 2016**) levels increase but may decrease in plasma (**G. Wang et al., 2014**). These conflicting findings reflect subtype-specific metabolic behaviors in AD and emphasize the need for interpretation at the subtype level.

In the analysis in which AD patients were clustered into subgroups according to the metabolic patterns they showed, ubiquinone, elaidate, corticosterone, and its derivatives were selected as potential biomarkers that can distinguish these subtype groups from each other. All predicted metabolite biomarker candidates should be evaluated not only as biomarkers but also as potential targets for future studies.

5. CONCLUSION

As life expectancy increases worldwide, the prevalence of AD is also increasing significantly. The most critical problem here is that a large portion of brain cells have been irreversibly lost by the time the disease is clinically diagnosed. Therefore, early diagnosis has become more than a necessity. In this way, the heterogeneous nature of AD clearly demonstrates that a universal diagnostic approach will be inadequate. Prediction of metabolite secretion activity specific to AD and its subtypes has been an important step towards a better understanding of the biodiversity of the disease and the development of specific diagnostic strategies. Some key metabolites reflecting the general AD metabolic profile - acylcarnitine levels (C16, C18 and C14), sulfolactate, and HDL - have emerged as potential biomarkers that can be used in diagnosis. In terms of subtypes, betaine, PGD2, 5-HETE, and proline for Subtype A; PCr, CDCA, and PE for Subtype B; and glycogen, HA, and SSA for Subtype C were identified as prominent candidate metabolites. Sialic acid derivatives, which were commonly affected in all groups, stand out as one of the metabolic traces of AD. Additionally, the analysis of metabolic secretion activity largely overlaps with previously defined transcriptome-based AD subtypes. In conclusion, evaluating AD not only from a single profile but also with a multidimensional approach focusing on its subtypes is of critical importance in terms of drawing a more accurate picture of the disease and developing personalized and targeted diagnostic strategies.

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BIOGRAPHY

Kader SARIBULAK received her bachelor's degree from Gebze Technical University, Department of Bioengineering, as an honor student in 2023. During her undergraduate education, she worked as an intern in the TUBITAK 2247-C project, and her bachelor's thesis was supported by the TUBITAK 2209-A program and successfully completed. In the fall semester of 2023, she began her master's degree in Bioinformatics and Systems Biology at Gebze Technical University, Department of Bioengineering. During this period, she worked as a scholar on two separate TUSEB projects, one of which was directly related to her own thesis. She is currently working as an Assistant R&D Engineer at Idea Technology Solutions.

PUBLICATIONS AND PRESENTATIONS FROM THE THESIS

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