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Risk factors associated with Alzheimer's disease in Kazakhstan

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NORMATIVE REFERENCES

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GOST 7.9-95 (ISO 214-76) System of Standards on Information,

Library, and Publishing. Abstract and Annotation. General Requirements;

GOST 7.32-2001 (amended 2006). Research Report. Structure and Design Rules;

GOST 7.1-2003. Bibliographic Entry. Bibliographic Description. General Requirements and Compilation Rules

DESIGNATIONS AND ABBREVIATIONS

AD	–	Alzheimer's disease
ADIPOQ	–	Adiponectin gene
AdipoR1	–	Adiponectin receptor type 1
AdipoR2	–	Adiponectin receptor type 2
AIC	–	Akaike information criterion
ALT	–	Alanine aminotransferase
ANOSIM	–	Analysis of similarities
APOE	–	Apolipoprotein E
APP	–	Amyloid precursor protein
AST	–	Aspartate aminotransferase
AUC	–	Area under the curve
Aβ	–	Amyloid-beta
BDNF	–	Brain-derived neurotrophic factor
BMAL1	–	Brain and Muscle ARNT-Like 1
BMI	–	Body mass index
CAD	–	Coronary artery disease
CDT	–	Clock Drawing Test
CI	–	Confidence interval
CpG	–	Cytosine-phosphate-guanine
CRP	–	C-reactive protein
CSF	–	Cerebrospinal fluid
DALY	–	Disability-adjusted life year
DASH	–	Dietary Approaches to Stop Hypertension
DNA	–	Deoxyribonucleic acid
EDTA	–	Ethylenediaminetetraacetic acid
ELISA	–	Enzyme-linked immunosorbent assay
F/B	–	Firmicutes-to-Bacteroidetes ratio
FDR	–	False discovery rate
FETA	–	FFQ-EPIC Tool for Analysis
FFQ	–	Food frequency questionnaire
FINGER	–	Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability
GABA	–	Gamma-aminobutyric acid
GBD	–	Global Burden of Disease
GFAP	–	Glial fibrillary acidic protein
GWAS	–	Genome-wide association study
HDL	–	High-density lipoprotein
HDL-C	–	High-density lipoprotein cholesterol
IL	–	Interleukin
IQR	–	Interquartile range
L2FC	–	Log2-fold change
LDA	–	Linear discriminant analysis

LDL	–	Low-density lipoprotein
LDL-C	–	Low-density lipoprotein cholesterol
LEfSe	–	Linear discriminant analysis effect size
LMICs	–	Low- and middle-income countries
LOO	–	Leave-one-out
MAF	–	Minor allele frequency
MCI	–	Mild cognitive impairment
MIND	–	Mediterranean-DASH Intervention for Neurodegenerative Delay
MMSE	–	Mini-Mental State Examination
MRI	–	Magnetic resonance imaging
mRNA	–	Messenger ribonucleic acid
MSP	–	Methylation-specific PCR
MUFA	–	Monounsaturated fatty acids
NCDs	–	Non-communicable diseases
NFTs	–	Neurofibrillary tangles
NIA-AA	–	National Institute on Aging–Alzheimer's Association
NINCDS-ADRDA	–	National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association
NSP	–	Non-starch polysaccharide
OR	–	Odds ratio
OTU	–	Operational taxonomic unit
PBMC	–	Peripheral blood mononuclear cells
PCA	–	Principal component analysis
PCR	–	Polymerase chain reaction
PERMANOVA	–	Permutational multivariate analysis of variance
PET	–	Positron emission tomography
PUFA	–	Polyunsaturated fatty acids
QC	–	Quality control
RCT	–	Randomized controlled trial
RDA	–	Recommended dietary allowance
RIPA	–	Radioimmunoprecipitation assay
RNA	–	Ribonucleic acid
rRNA	–	Ribosomal ribonucleic acid
SAT	–	Subcutaneous adipose tissue
SCFA	–	Short-chain fatty acids
SD	–	Standard deviation
SFA	–	Saturated fatty acids
SNI	–	Social Network Index (Berkman–Syme)
SNP	–	Single-nucleotide polymorphism
SOPs	–	Standard operating procedures
TBI	–	Traumatic brain injury
TIA	–	Transient ischemic attack

TNF	–	Tumor necrosis factor
VD	–	Vascular dementia
VLDL	–	Very low-density lipoprotein
WHO	–	World Health Organization

INTRODUCTION

General characteristics of the work. This dissertation conducts a comprehensive, multi-domain investigation of risk factors and emerging biomarkers of Alzheimer's disease (AD) in the Kazakhstani population. The study employs a case-control design with 177 patients with clinically diagnosed AD and 241 age-matched cognitively healthy controls from Astana and Almaty, the two largest cities in Kazakhstan. The research integrates sociodemographic, lifestyle, genetic, biochemical, epigenetic, dietary, and microbiome approaches to characterize, for the first time, the multifactorial risk architecture of AD in a Central Asian population.

Relevance of the research topic. Alzheimer's disease is the leading cause of dementia worldwide, accounting for 60–70% of all dementia cases. The Global Burden of Disease Study 2021 estimated that the number of people living with dementia has reached 57.4 million globally and is projected to increase to over 152 million by 2050, with the fastest increases expected in low- and middle-income countries. The population aged 65 years and older is increasing. However, there is no systematic epidemiological data on AD and its determinants in the country.

The Lancet Commission on dementia prevention, intervention and care, 2024, identified 14 potentially modifiable risk factors that together account for some 45% of global dementia cases. But most of the estimates are based on populations from North America, Western Europe and East Asia. Central Asian populations, which are characterized by admixed genetic ancestry, traditional meat- and dairy-heavy diets, distinctive patterns of education and literacy shaped by Soviet and post-Soviet transitions, and unique environmental exposures, have been virtually absent from the global dementia epidemiology literature. The few existing studies from the region are limited to small-scale cognitive screening surveys and address the full spectrum of genetic, metabolic, dietary, and microbiome-related risk factors.

Understanding the population-specific determinants of AD in Kazakhstan is essential for several reasons: first, to determine whether established risk factors from Western populations apply in a local context or whether novel, region-specific factors predominate; second, to provide an evidence base for national dementia prevention policy; and third, to contribute to the still-limited global evidence on AD risk factors in ethnically and geographically diverse populations. This dissertation addresses these needs by providing the first comprehensive, multi-domain characterization of AD risk factors in a Central Asian population.

The purpose and objectives of the study. The purpose of this study was to investigate risk factors and emerging biomarkers of Alzheimer's disease in the Kazakhstani population through a comprehensive, multi-domain case-control approach.

To achieve this goal, the following objectives were set

1. To compare sociodemographic, psychosocial, clinical, and lifestyle factors of AD and healthy controls and to identify independent predictors of AD using hierarchical multivariable logistic regression modeling

2. To assess genetic factors associated with Alzheimer's disease in the Kazakh population, including apolipoprotein E genotype distribution and a pilot genome-wide association study.

3. To assess biochemical biomarkers and metabolic profiles associated with AD, including glucose metabolism, lipid profiles, and liver function parameters.

4. To evaluate blood cytokine profiles in patients with Alzheimer's disease and cognitively healthy controls.

5. To determine the relationship between AD and levels of adiponectin, promoter methylation of the adiponectin gene and expression of adiponectin receptors.

6. To evaluate dietary patterns and nutritional risk factors associated with Alzheimer's disease based on data from a food frequency questionnaire.

7. To characterize gut and oral microbiome composition in AD patients and controls and identify microbial taxa and metabolic pathways associated with cognitive status.

Object of study. Individuals aged 55 years and older with clinically diagnosed Alzheimer's disease and age-matched cognitively healthy controls residing in Astana and Almaty, Republic of Kazakhstan.

Subject of study. Sociodemographic, psychosocial, lifestyle, genetic, metabolic, dietary, and microbiome-related risk factors as well as emerging molecular and biochemical biomarkers associated with Alzheimer's disease in the Kazakhstani population

Research methods: The study employed a case-control design with comprehensive multi-domain data collection. Cognitive assessment was performed using the Mini-Mental State Examination (MMSE) and the Clock Drawing Test (CDT). Sociodemographic and lifestyle data were collected via a structured questionnaire, with social integration assessed using the Berkman–Syme Social Network Index. Dietary intake was evaluated using a validated Food Frequency Questionnaire processed through the FETA nutrient analysis tool; dietary patterns were assessed using the MIND diet score and principal component analysis. PCR-based methods were used for APOE genotyping; a pilot genome-wide genotyping study was conducted on Illumina SNP arrays, with bioinformatic analysis using PLINK and BCFtools. Biochemical profiling included fasting glucose, insulin, lipid panel, liver function markers (ALT, AST, bilirubin), C-reactive protein, and adiponectin (ELISA). ADIPOQ promoter methylation was assessed by methylation-specific PCR. Adiponectin receptor (AdipoR1, AdipoR2) expression in peripheral blood mononuclear cells was measured by ELISA. Gut and oral microbiome composition was characterized by 16S rRNA gene sequencing and bioinformatic analysis using QIIME2 , DESeq2 and LEfSe. Statistical analyses included descriptive statistics, Mann–Whitney U tests, chi-square tests, hierarchical multivariable logistic regression, principal component analysis with varimax rotation, and Spearman correlation analyses.

Scientific novelty of the dissertation. This dissertation provides several novel contributions to the field:

For the first time in Central Asia, a comprehensive multi-domain case-control study was conducted, simultaneously analyzing sociodemographic, psychosocial, genetic, biochemical, epigenetic, dietary, and microbiome risk factors for AD within a single cohort. Functional illiteracy, rather than formal educational attainment, was identified as the strongest independent predictor of AD, a finding that reframes how educational variables should be operationalized in dementia research across low- and middle-income countries. Pronounced regional (Astana vs. Almaty) and sex-specific heterogeneity in the APOE ε4–AD association was demonstrated for the first time in a Central Asian population, with the ε4 allele conferring significant risk among women but not men, and in Astana but not Almaty. The first pilot genome-wide association study in Central Asia was conducted, identifying suggestive signals in biologically plausible candidate genes (ADARB2, MAST4, ADAM23, LINC01122). PCA-derived culturally specific dietary patterns substantially outperformed the Western-derived MIND diet score in discriminating AD cases from controls, underscoring the need for culturally adapted dietary assessment tools. Elevated serum adiponectin was identified as the most powerful biochemical discriminator of AD, and a novel association between increased ADIPOQ promoter methylation at CpG −74 nt and AD was described. The first characterization of both gut and oral microbiome composition in AD patients from Central Asia was performed, revealing population-specific microbial signatures and regional variation within the same ethnic group.

Theoretical and practical significance of the work. From a theoretical perspective, this dissertation advances understanding of the architecture of AD risk factors in a previously unstudied Central Asian population. The finding of functional illiteracy as a better predictor of AD than formal education refines the cognitive reserve hypothesis and has implications for the conceptualization of educational variables in global dementia epidemiology. Context-sensitive approaches to genetic epidemiology are important to demonstrate region- and sex-specific heterogeneity in genetic associations. Locally derived dietary patterns were superior to Western indices, which emphasizes the limitations of using culturally non-adapted assessment tools across different populations.

From a practical point of view, the results provide a population-specific evidence base for developing targeted dementia prevention strategies in Kazakhstan. The identification of illiteracy, depression, social isolation, physical inactivity and dietary patterns as the main modifiable risk factors directly guides the development of multidomain intervention programs. The metabolic biomarker profile (adiponectin, glucose, ALT, and triglycerides) provides candidate markers for early risk stratification in clinical settings. The microbiome findings open new avenues for prevention through dietary modification and probiotic interventions. The results are directly relevant to the implementation of Kazakhstan's national health policy and to the WHO Global Action Plan on Dementia 2017–2025.

Key points of the dissertation to be defended

1. Hierarchical logistic regression identified functional illiteracy, depression, social isolation, and rural birthplace as independent predictors of AD. Functional illiteracy was the strongest predictor, with an effect size substantially exceeding that

reported for low formal education in Western cohorts, confirming that socio-educational disadvantage accumulated over the life course represents the dominant modifiable risk burden in this population.

2. APOE ε4 was confirmed as a significant genetic risk factor for AD, with a pronounced dose effect among ε4/ε4 homozygotes. The association showed marked regional and sex-specific heterogeneity, being significant in Astana and among women but absent in Almaty and among men, indicating that the population-level impact of this genetic variant is shaped by environmental and demographic context. A pilot genome-wide association study identified suggestive novel loci, contributing some of the first genome-wide data from a Central Asian population and establishing a foundation for future genetic epidemiology work in the region.

3. A distinctive metabolic profile was identified in AD patients, characterized by hepatic and lipid metabolic dysregulation that diverges from the cardiovascular-predominant patterns typical of Western cohorts. The independence of these biochemical alterations from APOE ε4 genotype suggests that metabolic dysfunction represents a pathophysiological axis of public health relevance that operates separately from genetic predisposition and may be amenable to population-level intervention through cardiometabolic risk management programs.

4. Serum adiponectin emerged as the strongest single biochemical discriminator of AD status in this cohort. Epigenetic and transcriptional dysregulation of the adiponectin signaling pathway was observed alongside a positive correlation between adiponectin levels and cognitive performance within the AD group, suggesting a compensatory neuroprotective response. These findings position adiponectin as a candidate population biomarker for AD risk stratification, though longitudinal evidence is needed to establish its predictive utility in public health screening contexts.

5. Cytokine profiling using multiplex technology indicated a predominantly pro-inflammatory profile of the systemic immune system in AD patients. IL-1α and IL-17A remained significant following correction for multiple comparisons. The pattern of innate and adaptive immune co-activation, mechanistically coherent with the observed adiponectin dysregulation, supports chronic low-grade systemic inflammation as a measurable and potentially modifiable feature of AD in this population. From a public health perspective, inflammatory burden may serve as a biological mediator linking known upstream risk exposures such as poor diet, physical inactivity, and social isolation to downstream neurodegeneration.

6. Three complementary approaches to diet analysis revealed broad nutritional compromise in AD patients, with deficits concentrated in neuroprotective nutrients and beneficial fatty acids. The consistent inability of the MIND diet score to differentiate between groups in the whole cohort indicates a fundamental mismatch between dietary assessment tools developed in the West and the Central Asian food environment, with direct methodological implications for nutritional epidemiology in the region. Dietary patterns derived from PCA were more contextually appropriate and more discriminative in identifying independently significant risk exposures of low vegetable intake and high red meat consumption that persisted after genetic adjustment.

7. Both gut and oral microbiome compartments of AD patients were characterized by significant compositional changes including loss of beneficial commensal taxa and enrichment of pro-inflammatory organisms. Preliminary correlations between microbial metabolic pathways and clinical parameters suggest mechanistic links between dysbiosis and cognitive impairment. Taken together with the dietary findings, these results highlight the gut-brain axis as an emerging and underexplored area for dementia prevention research in Central Asia, warranting dedicated prospective investigation

Connection of work with research programs. The dissertation work was completed within the framework of grant funding from the Ministry of Science and Higher Education (AP14871338, AP09259730) and as part of the Nazarbayev University Collaborative Research Program grant (No. 091019CRP2112).

Approval of work. The main results of the dissertation were presented and discussed at the following international conferences: Alzheimer's Association International Conference (AAIC) 2020 (online), AAIC 2021 (online), AAIC 2022 (online), AAIC 2023 (Amsterdam, Netherlands), International Brain Research Organization World Congress 2023 (Granda, Spain), 36th Global Conference of Alzheimer's Disease International 2024 (Krakow, Poland), and AAIC 2025 (Toronto, Canada).

Publications. The main findings of the research on the dissertation topic were published in 16 scientific papers. It includes five articles in an international peer-reviewed journal listed in the Scopus and Web of Science databases, which have an impact factor; ten abstracts in journals that appear in the proceedings of international conferences; and one article in a journal recommended by the Committee for Control in the Sphere of Education and Science of the Ministry of Higher Education of the Republic of Kazakhstan.

Volume and structure of the dissertation. The dissertation is presented on 125 pages of typewritten text and consists of a list of notations and abbreviations, an introduction, a literature review, materials and methods, results and discussion, conclusions, and a list of references comprising 244 entries. The work contains 21 tables and 21 figures.

1 LITERATURE REVIEW

1.1 Alzheimer's disease - an overview

Alzheimer's disease (AD) is a chronic, progressive neurodegenerative disorder that accounts for an estimated 60–80% of all dementia cases globally [1]. It represents one of the most pressing public health challenges of the twenty-first century. First described by Alois Alzheimer in 1906, the disease is characterized neuropathologically by extracellular amyloid-beta (Aβ) plaques and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein [2]. Clinically, AD manifests as a progressive decline in memory, cognitive function, and the capacity for independent living. It is the seventh leading cause of death globally and imposes enormous economic costs, estimated at over $1.3 trillion annually in societal costs worldwide [3, 4].

The clinical hallmark of AD is a gradual, insidious onset of cognitive impairment, typically beginning with episodic memory deficits and progressing over a period of years to encompass language disturbances, visuospatial dysfunction, impaired executive function, and ultimately the loss of basic activities of daily living. The disease duration from diagnosis until death is generally between 4 and 8 years, but some individuals can survive for much longer [5].

Up to 5-10% of all AD cases are early-onset, with symptoms beginning before age 65, and are frequently linked to autosomal dominant mutations in the amyloid precursor protein (APP), presenilin 1 (PSEN1) or presenilin 2 (PSEN2) genes [6]. Conversely, late-onset AD, the more common sporadic form of the disease, is the result of complex interactions between genetic susceptibility (notably, the APOE ε4 allele) and cumulative environmental and lifestyle exposures over the life course [4,p. 18]. It is important to recognize that AD occurs in a biological continuum that starts with a prolonged preclinical phase of 15–25 years, during which neuropathological changes accumulate silently before the appearance of clinical symptoms [7].

Population aging is a global phenomenon that is rapidly emerging as a demographic challenge across all regions. The combination of declining mortality and fertility rates, improved health care, and longer life expectancy has significantly increased the proportion of older people. The trend is expected to continue and the United Nations estimates that the number of people aged 65 years and older will more than double by 2050 (figure 1) [8]. This rapid population aging is especially sharp in developing countries like Kazakhstan. The country is undergoing health transition [9], with a significant increase in life expectancy and a decline in mortality. This is of course a positive development, but it has also led to a major demographic shift, with an increasing proportion of the population now aged 65 and over [4,p. 18]. Because age is the single strongest risk factor for AD, this demographic transformation directly underpins the anticipated surge in dementia cases across the region and globally.

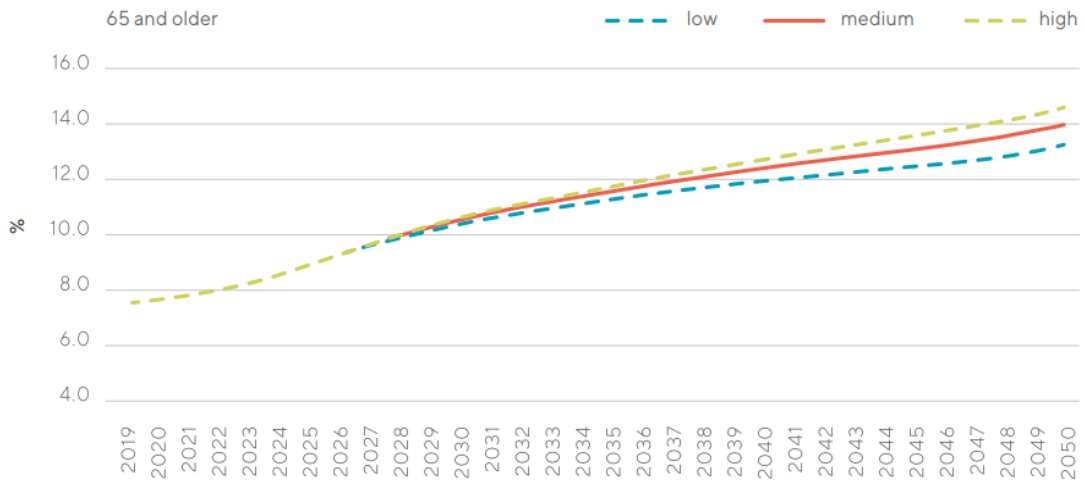


Figure 1 - Projected proportions of people aged 65 and older in Kazakhstan by 2050

Note – [8,p. 2]

The scale of the global dementia epidemic is staggering. The GBD 2019 Dementia Forecasting Collaborators estimated that around 57 million people were living with dementia in 2019, and this number is expected to nearly triple by 2050 [10]. AD accounts for 60–70% of these cases, making it the dominant subtype across nearly all populations studied [11]. The global macroeconomic burden of AD and other dementias is estimated to have been around 2,331 billion US dollars during the study period, according to a recent analysis published in The Lancet Global Health [12].

Dementia is an emerging global priority but there are significant geographic inequalities in the burden of disease and the availability of epidemiological data. High-income countries in North America, Europe and parts of East Asia have well-established surveillance systems and large-scale cohort studies, whereas low- and middle-income countries (LMICs), particularly in Central Asia, are critically underrepresented in the global literature [13]. Once again, the World Alzheimer's Reports have pointed to this data gap over and over again, indicating a continued lack of research on dementia in Central Asia [14]. This inequity in research coverage has direct implications for policy, prevention and care, as risk factor profiles may differ substantially between populations.

1.1.1 Epidemiology of Alzheimer's disease

The global prevalence of dementia has increased dramatically in the last three decades, primarily because of population aging, rather than an increase in age-specific rates. From 1991 to 2021, the prevalence of AD and other dementias worldwide increased by 160% (from 21.8 million to nearly 50 million prevalent cases) according to a 2021 Global Burden of Disease (GBD) study [15]. Projections indicate that this burden will continue to rise sharply, particularly in LMICs where over 60% of dementia cases currently occur [10,p. 105]. The 2025 Alzheimer's Association Facts and Figures report estimates that 6.9 million Americans aged 65 and older are currently

living with Alzheimer's dementia, with deaths attributed to the disease having increased by more than 142% between 2000 and 2022 [1,p. 702].

The epidemiology of AD varies significantly across regions of the world. Age-standardized prevalence is highest in high-income countries, but the fastest growth in absolute case numbers is occurring in sub-Saharan Africa, North Africa, the Middle East, and parts of East and Central Asia, where rapid population ageing is converging with high rates of modifiable risk factors and limited healthcare infrastructure [10,p. 105].

In 2019, there were an estimated 51.6 million prevalent cases of AD and other dementias worldwide, with an age-standardized prevalence rate of 682.5 per 100,000, a 5.7% increase since 1990. East Asia (781.4 per 100,000) and North Africa/Middle East (777.6) had the highest regional rates, while South Asia (428.4) and Western Sub-Saharan Africa (469.8) had the lowest (figure 2) [4,p. 18].

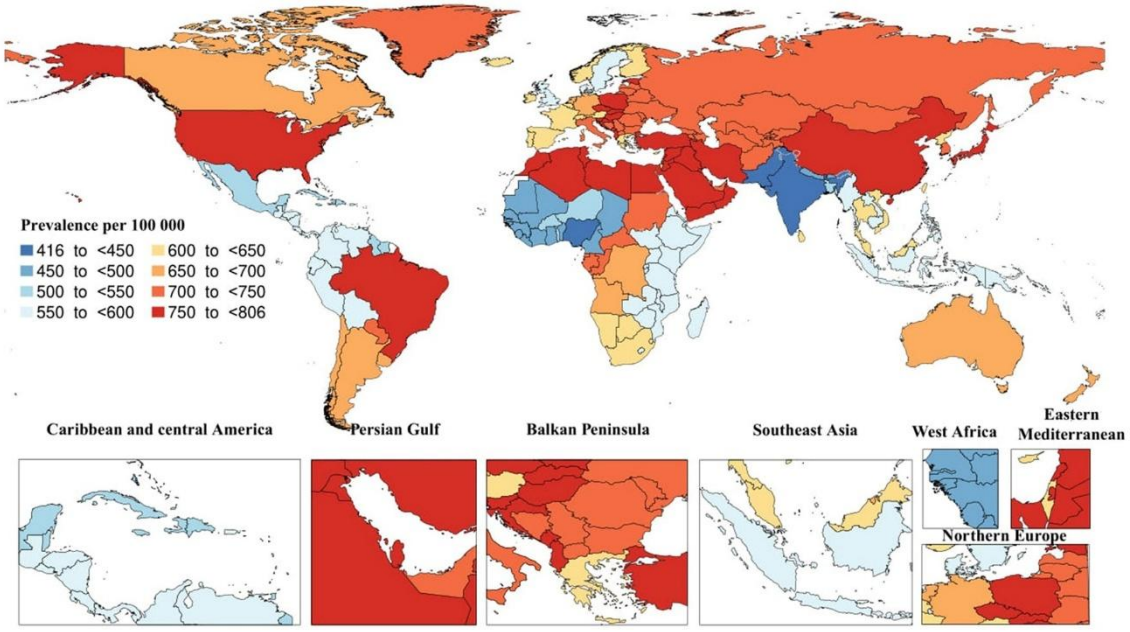


Figure 2 - Age-standardized prevalence of Alzheimer's disease per 100.000 population in 2016 by country

Note – [4,p. 27]

Analysis of data from GDB 2021 estimated that East Asia experienced the most pronounced increase in age-standardized incidence (Estimated annual percent change (EAPC): 0.4; 95% CI: 0.33–0.48), while Australasia reported the steepest decline (EAPC: −0.56; 95% CI: −0.60 to −0.52) [16]. In 2021, the highest age-standardized incidence rates were observed in East Asia (149.61 per 100,000), North Africa and the Middle East (132.19 per 100,000), and high-income North America (131.39 per 100,000) [16,p. 701]. These patterns reflect a complex interplay of population aging, changing risk factor profiles, improvements in diagnostic ascertainment, and socioeconomic development.

There also has been a large increase in mortality from AD and other dementias (ADRD). The GBD 2021 analysis showed that the global mortality for ADRD among adults aged 65 and older increased by 115% from 6.5 to 14.0 per 100,000 population between 1991 and 2021. In this age group, ADRD was estimated to cause 8.02 million deaths and 25.38 million disability-adjusted life years (DALYs) worldwide in 2021 [17].

According to GBD 2021 projections, AD will reach the 8th position as most important cause of death worldwide in 2050, up from 22nd in terms of disability-adjusted life years [16,p. 701]. In the US, Alzheimer's Disease (AD) was the seventh leading cause of death, with 119,399 deaths from AD listed on official death certificates in 2021 [18]. This projected growth makes it all the more urgent to develop both prevention strategies and care infrastructure, especially in areas where there is a lack of dementia services.

Dementia epidemiology is one of the least studied areas in Central Asia, including Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan and Uzbekistan. A systematic review of trends in cognitive impairment and dementia in Asia [13,p. 4233] identified 39 eligible studies from 2019 to 2023, of which 29 were from East Asia, 7 from South Asia, 2 from Southeast Asia and 1 from Central Asia. Thus, lower rates of dementia in Central Asia may reflect poor data collection and lack of population-based studies rather than true lower prevalence.

The GBD 2021 reported ~400,000 prevalent cases of ADOD in Central Asia with an age-standardized prevalence rate (ASPR) of 626.8 per 100,000 (95% CI: 541.9–713.5), which is a slight decrease of 1.8% from 1990. The region recorded roughly 69,800 new cases annually, corresponding to an age-standardized incidence rate of 109.8 per 100,000 (95% CI: 95.9–125.3), also slightly lower than the 1990 baseline. Mortality attributable to ADOD reached approximately 11,000 deaths, with an age-standardized death rate of 20.1 per 100,000 (95% CI: 5.0–55.7), and the total DALY burden was estimated at 232,300 (age-standardized rate: 379.2 per 100,000) [19]. However, these relatively low absolute numbers are likely attributable to the region's younger age structure rather than lower age-specific risk. As populations in Central Asia age rapidly, the burden is expected to increase substantially.

Tsoy et al. [20] conducted the first epidemiological study of cognitive impairment in Kazakhstan. They screened 668 randomly selected community-dwelling people aged 60 years and over from polyclinic registers in 15 clinics in Almaty. The median Montreal Cognitive Assessment (MoCA) score was 22 and the prevalence of mild cognitive impairment (MCI) was 30%. In a separate comparative study, Turuspekova et al. [21] screened 300 respondents (150 Kazakhs and 150 Kyrgyz) aged 60 to 90 years in Almaty and Bishkek using the MoCA. This cross-national comparison of cognitive impairment risk factors in Kazakh and Kyrgyz populations provided preliminary evidence that the burden of cognitive impairment is substantial across Central Asian populations, though sample sizes remained small.

Given that up to 30% of MCI cases may progress to AD, these prevalence figures suggest that the burden of AD in Kazakhstan is likely much higher than official statistics indicate. A striking feature of the dementia landscape in Kazakhstan is the

enormous gap between estimated and registered cases. According to official Ministry of Health statistics, approximately 10,000 patients were registered as having dementia in the country [22]. But experts suggest the real number could be 14 to 20 times higher, ranging from 100,000 to 200,000 people [11,p. 155].

The post-Soviet health care system in Central Asia has left a legacy of both problems and opportunities. There is a network of primary healthcare facilities (polyclinics) that could in theory be used for cognitive screening. However, dementia awareness among the general public and healthcare professionals is low, diagnostic capacity is limited and stigma around cognitive decline is high. With the aging of the world's population, the prevalence of Alzheimer's disease will increase, presenting major challenges to health care systems and societies. Recognizing the intricate interplay of genetic, molecular and environmental factors in Alzheimer's disease is crucial for the development of preventive strategies and novel therapeutic approaches to fight this devastating disorder [23].

1.1.2 Pathophysiology of Alzheimer's disease

The neuropathological hallmarks of AD are extracellular senile plaques composed of aggregated amyloid beta (Aβ) peptides and intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein. These two proteinopathies, together with neuroinflammation, synaptic dysfunction, and neuronal loss, constitute the core pathological substrate of the disease [24,25].

The amyloid cascade

The amyloid cascade hypothesis has dominated the AD field for over three decades. In this model, sequential cleavage of APP by β-secretase and γ-secretase results in the generation of misfolded and aggregating Aβ42 peptides. These initially form soluble oligomers, now increasingly appreciated as the most neurotoxic species, before assembling into insoluble fibrils and diffuse plaques, and finally dense-core neuritic plaques [7,p. 456]. Neuritic plaques are defined by the presence of tau-positive dystrophic neurites in and around the amyloid deposit, linking the two hallmark proteinopathies at the histological level. Aβ oligomers directly impair synapses, disrupting long-term potentiation, promoting calcium dysregulation and compromising synaptic plasticity and stability [26]. The recent validation of the amyloid cascade has been bolstered by the clinical efficacy, albeit modest, of anti-amyloid monoclonal antibodies such as lecanemab and donanemab, which reduce brain amyloid burden and slow cognitive decline [27,28].

However, the hypothesis has also been challenged. A cross-sectional analysis applying the 2024 Alzheimer's Association revised criteria to the ADNI cohort found that only 31–36% of amyloid-positive individuals followed the expected cascade trajectory of progressive amyloid, tau, and then cognitive decline, with the remainder classified as either resilient or as having co-pathology-driven impairment [29]. This underscores that the amyloid cascade can be substantially modulated by non-amyloid factors, including vascular copathology, cognitive reserve, and APOE genotype.

Tau pathology

Tau is a microtubule associated protein that normally stabilizes the neuronal cytoskeleton and promotes axonal transport. In AD, tau is hyperphosphorylated, dissociates from microtubules and forms paired helical filaments, which are the major component of NFTs. The number and distribution of NFTs correlate better with severity of cognitive impairment than amyloid plaque burden, suggesting tau pathology is a more distant driver of clinical symptoms [30]. Tau pathology propagates through the brain in a stereotypic pattern first described by Braak [30,p. 50], beginning in the transentorhinal cortex and spreading into the hippocampus and eventually into the neocortex. Emerging evidence indicates that this spread happens through a prion-like mechanism whereby pathological tau seeds are transmitted trans-synaptically from neuron to neuron and through exosomes released by microglia [31,32]. The dominance of the amyloid cascade hypothesis has been increasingly questioned following the limited clinical benefits of amyloid-clearing therapies, and tau-directed therapeutic strategies are now under active investigation [25,p. 34].

Neuroinflammation

Chronic neuroinflammation has been identified as a third component of AD pathophysiology, along with amyloid and tau. Microglia are the brain's resident immune cells. In response to Aβ plaques and tau aggregates, microglia are activated to mount an initial protective phagocytic response. However, prolonged activation leads to the release of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), reactive oxygen species and nitric oxide, which contributes to a self-perpetuating inflammatory cycle that damages neurons and enhances further protein aggregation [33]. Astrocytes are also recruited into this inflammatory milieu, initially attempting to clear tau and maintain homeostasis, but ultimately succumbing to the pressure, resulting in impaired synaptic pruning and deranged cholesterol metabolism. The genetic evidence for neuroinflammation's importance is compelling: many of the AD risk loci identified by GWAS, including TREM2, CD33, PLCG2, and INPP5D, encode proteins expressed predominantly in microglia and involved in innate immune signaling [34]. A systematic review by Greer and Anderton identified 30 distinct risk factors for AD [35]. It demonstrated that dysfunction of the neuroimmune–neuroinflammation axis was the only mechanism shared by all 30 factors, highlighting neuroinflammation as a unifying pathogenic pathway [35,p. 41].

Synaptic dysfunction and neuronal loss

In addition, both Aβ oligomers and tau tangles individually interfere with synaptic signaling, neurotransmitter release and structural integrity of dendritic spines. The cholinergic system is particularly vulnerable, and degeneration of cholinergic neurons in the basal forebrain nucleus of Meynert accounts for the characteristic memory deficits of AD and provides the basis for cholinesterase inhibitor therapy [33,p. 14]. Mitochondrial dysfunction, impaired autophagy, oxidative stress and disrupted calcium homeostasis further contribute to progressive loss of neuronal viability [7,p. 456].

1.1.3 Current diagnostic criteria and treatment

The current criteria for diagnosing Alzheimer's disease include a full medical history, cognitive and functional testing, and laboratory tests to rule out other causes of dementia. The most common diagnostic tools used are the Mini-Mental State Examination and the Clinical Dementia Rating scale, which assess the severity of cognitive and functional impairment and provide important information on a patient's cognitive ability including memory, language, attention and problem-solving skills. Neuroimaging tools such as magnetic resonance imaging (MRI) and positron emission tomography (PET) also can identify structural and functional brain changes associated with Alzheimer's disease. The imaging methods allow clinicians to see and analyze the brain's structure and activity, which can help in diagnosing and monitoring the disease. Moreover, the National Institute on Aging and the Alzheimer's Association have developed criteria that incorporate clinical, cognitive, and neuroimaging assessments to offer a comprehensive framework for diagnosing Alzheimer's disease [36-38].

Evolution of diagnostic criteria

The diagnostic framework for AD has undergone a fundamental transformation over the past four decades. The original 1984 NINCDS-ADRDA criteria relied exclusively on clinical presentation and the exclusion of other causes of dementia, as stated explicitly at the time that the diagnosis could not be determined by laboratory tests. The 2011 NIA-AA guidelines introduced biomarkers as supportive diagnostic tools, while the 2018 NIA-AA research framework took the landmark step of defining AD biologically through the AT(N) system: Amyloid (A), Tau (T), and Neurodegeneration (N) [38,p. 535].

In June 2024, the Alzheimer's Association Workgroup published the most significant revision to date: the Revised Criteria for Diagnosis and Staging of Alzheimer's Disease [39]. These criteria are founded on several core principles. First, AD is defined as a biological process, not a clinical syndrome. It begins with the asymptomatic accumulation of AD neuropathologic change (ADNPC). It progresses to clinical symptoms as neuropathologic burden increases. Second, biomarker evidence of ADNPC is both necessary and sufficient for diagnosis. Third, the disease exists on a continuum, and clinical symptoms are not required for a biological diagnosis.

The 2024 criteria also introduced a new system of classifying biomarkers, replacing the AT(N) framework with a more complex system. Biomarkers are now grouped into three categories: core biomarkers of ADNPC, non-specific biomarkers of processes also involved in AD (such as neurodegeneration and inflammation), and biomarkers of common non-AD co-pathologies (such as cerebrovascular disease and α-synuclein pathology). Core biomarkers are further subdivided into Core 1 (early-changing) and Core 2 (later-changing). Core 1 biomarkers are amyloid PET, CSF Aβ42/40, CSF p-tau181/Aβ42 and validated plasma assays, including plasma p-tau217, which has shown diagnostic accuracy comparable to CSF measures [40]. A single abnormal Core 1 biomarker is now sufficient to establish a diagnosis of AD and inform clinical decision-making. Core 2 biomarkers, including tau PET and certain biofluid markers, provide prognostic staging information and increase confidence that AD is contributing to any observed symptoms. The full biomarker profile is described

as AT1T2NISV (A = amyloid, T1 = secreted phospho-tau, T2 = tau neurofibrillary pathology, N = neurodegeneration, I = inflammation, S = α-synuclein, V = vascular) [39,p. 5143].

A major catalyst for the 2024 revision was the development of blood-based biomarkers. Plasma p-tau217, p-tau181 and Aβ42/40 ratios have shown high concordance with CSF and PET measures, providing a more accessible, scalable and affordable route to biological diagnosis. In May 2025, the US FDA approved the first blood test for early detection of amyloid plaques in symptomatic adults aged 55 years and older, a revolutionary step forward for a broader clinical uptake of AD biomarkers [41].

Current treatment landscape

The treatment of AD has historically relied on symptomatic pharmacotherapy. Cholinesterase inhibitors, such as donepezil, rivastigmine and galantamine, are the standard of care for mild to moderate AD and can provide modest, but clinically meaningful improvements in cognition and activities of daily living by enhancing the availability of acetylcholine at the synapse. Memantine (an NMDA receptor antagonist) is approved for moderate to severe AD, as monotherapy or in combination with a cholinesterase inhibitor. These agents do not change the disease course per se but are an important part of symptom management and quality of life [42].

The treatment paradigm changed in the 2023–2024 period with the FDA approval of two disease-modifying anti-amyloid monoclonal antibodies. Lecanemab (Leqembi) received full FDA approval in July 2023 [27,p. 9] based on the CLARITY AD trial showing 27% slowing of cognitive decline over 18 months in patients with early-stage AD; it also targets Aβ protofibrils. In January 2025, the FDA approved a maintenance dosing regimen (intravenous once every 4 weeks), and in August 2025, a weekly subcutaneous self-injection option was approved for maintenance therapy. Donanemab (Kisunla) targeting pyroglutamate-modified Aβ was approved in July 2024 based on the results of the TRAILBLAZER-ALZ 2 trial which showed a 35% reduction in the risk of disease progression over 76 weeks [28,p. 512]. Crucially, donanemab can be discontinued once amyloid has been cleared, providing a finite treatment approach.

Both therapies carry a risk of amyloid-related imaging abnormalities (ARIA), including cerebral edema and microhemorrhages, in approximately 20–25% of patients treated. The risk is increased in APOE ε4 carriers, especially homozygotes and should be periodically surveiled by MRI. As of 2025, 138 drugs are being tested in 182 clinical trials for AD targeting different mechanisms such as tau aggregation, neuroinflammation, synaptic function and metabolic dysfunction [42]. There is an increasing trend in the field toward the use of precision medicine approaches, where treatment approaches are tailored to the biomarker and genetic profiles of patients.

Nonetheless, nonpharmacologic interventions are an important part of comprehensive management of AD. Suggested components of holistic care include cognitive stimulation therapy, physical exercise programs, music therapy, occupational therapy, and psychosocial support for patients and care givers. The WHO 2019 guidelines on risk reduction of cognitive decline and dementia support a multi-

component approach including both pharmacological and lifestyle interventions [3,p. 5].

Diagnostic biomarkers

The diagnostic landscape of AD is being reshaped by the emergence of novel fluid-based and digital biomarkers that complement the established Core 1 and Core 2 markers described in the 2024 revised criteria [39,p. 5143]. Beyond the now-validated plasma p-tau217 and Aβ42/40 assays, a range of additional blood-based biomarkers are under active investigation. These include glial fibrillary acidic protein (GFAP), a marker of astrocytic activation that reflects neuroinflammatory processes and has demonstrated strong associations with amyloid positivity and cognitive decline; neurofilament light chain (NfL), a non-specific but sensitive indicator of neurodegeneration and axonal injury; and synaptic biomarkers such as neurogranin and SNAP-25, which may capture the synaptic dysfunction that most closely correlates with clinical symptoms [40,p. 1085]. Alpha-synuclein amplification tests in cerebrospinal fluid are also emerging as tools for identifying co-pathology with Lewy body disease, which commonly co-occurs with AD. Another promising area is the development of retinal biomarkers including retinal nerve fiber layer thinning, retinal amyloid deposits and retinal vascular abnormalities detectable by optical coherence tomography and retinal imaging. The retina shares a common embryological origin with the brain and may serve as an accessible window onto cerebral neuropathology.

Digital biomarkers, including speech and language analysis, gait monitoring, eye-tracking, and smartphone-based cognitive assessments, represent a further frontier that could enable continuous, non-invasive, and risk monitoring in community settings. While most of these emerging biomarkers are still at the research or early clinical validation stage, they collectively point toward a future in which AD diagnosis and risk stratification can be achieved earlier, more accurately, and more affordably than is currently possible.

1.2 Risk factors of Alzheimer’s disease

Both modifiable and non-modifiable risk factors influence Alzheimer's disease. Understanding the risk factors associated with age-related neurodegenerative disorders is crucial for early detection, prevention, and effective management. Aging is well accepted as the major risk factor for these conditions, with deleterious biochemical processes in the brain leading to neurodegeneration with aging [43].

Genes also have an important role in the development of AD. Some genetic variants may increase an individual’s risk for AD, indicating the importance of understanding family history and genetic background [44]. In addition, many health-related factors such as cardiovascular disease, hypertension, diabetes and stroke are associated with a higher risk of developing dementia and Alzheimer’s disease as they cause a deterioration in brain health and cognitive functioning [44,p. 74].

Research has also pointed to a role for diet and lifestyle factors as influencers of AD risk. Research has shown that a healthy diet, physical activity and cognitive stimulation may help to reduce the risk of developing these conditions [44,p. 74]. Early identification of high risk individuals and development of effective interventions are

important public health priorities [45]. It is essential to understand the biological pathways that connect risks for cognitive decline and dementia for developing targeted treatments and prevention strategies.

1.2.1 Non-modifiable risk factors

1.2.1.1 Age and gender/sex

Age is the single greatest risk factor for AD and other dementias. After the age of 65, the incidence of dementia doubles roughly every five years, and approximately one-third of people 85 and older have dementia [1,p. 702]. GBD 2021 data confirm that incidence, prevalence, mortality and disability-adjusted life years all steeply increase with age in all world regions [16,p. 701]. However, age-standardized incidence rates have shown modest declines in several high-income countries over recent decades, likely reflecting improvements in the management of vascular risk factors and in educational attainment [46]. These encouraging trends underscore that the relationship between aging and dementia is not entirely immutable.

At the cellular level, aging is associated with a constellation of changes that increase vulnerability to neurodegeneration: accumulation of oxidative damage, mitochondrial dysfunction, impaired proteostasis, declining efficiency of the glymphatic system for clearing toxic metabolites during sleep, telomere shortening, epigenetic drift, and a shift toward a pro-inflammatory microglial phenotype often termed “inflammaging.” The interaction between these age-related processes and disease-specific pathologies (amyloid accumulation, tau hyperphosphorylation) likely explains why age is such a dominant risk factor, even though aging per se does not cause AD.

Women are disproportionately affected by AD, accounting for approximately two-thirds of all cases. But the difference is more than just different longevity. Biological factors include the reduction in estrogen levels at menopause that removes a neuroprotective influence on synaptic plasticity, neuroinflammation and Aβ clearance, and sex-specific differences in brain structure, immune function and APOE genotype interactions [1,p. 702]. A study of the Canadian Longitudinal Study on Aging analyzed and found it was related to estrogen, supporting the hypothesis. It found in 2025 that an earlier age of menopause was associated with poorer cognition. Men, on the other hand, have higher exposure to certain risk factors, such as traumatic brain injury and tend to have a less favorable survival prognosis once dementia develops, possibly due to later diagnosis and different patterns of comorbidity [47].

The social and economic dimensions of gender are equally important. In many parts of the world, women have historically had less access to formal education, fewer economic resources, and more caregiving responsibilities, which can influence cognitive reserve, health care-seeking behavior, and exposure to modifiable risk factors. In the 2024 Lancet Commission, 57% of the modifiable risk factors identified are more common in men. In contrast, the overall disease burden falls disproportionately on women, suggesting that gender-based social determinants substantially shape dementia risk at the population level [48].

1.2.1.2 Genetic factors

APOE genotype

The apolipoprotein E (APOE) gene is the most robust and widely replicated genetic r+isk factor for late-onset AD. Three common alleles exist: ε2, ε3, and ε4. The ε4 allele also confers dose-dependent risk, with heterozygous carriers at 2- to 4-fold increased risk and ε4/ε4 individuals at ∼8- to 12-fold increased risk compared with ε3/ε3 individuals [49,50]. About 25% of individuals of European descent have at least one ε4 allele, compared to 50–60% of AD patients of European descent [51]. However, the ε2 allele is considered to be protective with one neuropathological study of over 5,000 individuals showing that ε2 homozygotes are unlikely to develop AD [52].

The mechanistic role of APOE4 in AD pathogenesis is complex. ApoE is the main lipid transport protein in the brain, mostly produced by astrocytes and microglia. It provides cholesterol and phospholipids for membrane repair, synaptic maintenance and immune regulation. The ε4 isoform exhibits a different conformation of the protein with a decrease in lipid transport efficiency, impaired receptor-mediated Aβ clearance, increased tau hyperphosphorylation, blood-brain barrier breakdown, and persistent neuroinflammation [53]. A landmark study by Fortea et al published in 2024 [54] proposed that APOE4 homozygosity may represent a distinct genetic form of AD since nearly all individuals in this group develop AD biomarker abnormalities and exhibit a predictable trajectory of neuropathological changes. This interpretation is still controversial, but has sparked new interest in therapeutic approaches aimed at apoE, including gene therapy to convert ε4 to ε2 via AAV-mediated delivery, structure correctors to restore normal protein folding, and agents that promote apoE lipidation [54,p. 72].

Importantly, the APOE ε4 effect size is not uniform across ancestral populations. The risk associated with ε4 carriage seems to be highest in populations of European and Japanese origin, somewhat lower in African Americans and Hispanics, and varies across other ethnic groups [55,56]. In the Peruvian population, where the proportion of Amerindian ancestry is high, the APOE e4 allele is strongly associated with the increased risk of AD, suggesting that local Amerindian ancestry contributes to the increased risk [57]. The risk of AD related to APOE e4 is lower in African Americans than in Caucasians and Hispanics, suggesting that other genetic or environmental factors may modify the effect of APOE e4 in African Americans [58]. The relationship between APOE e4 and AD risk is also different in Hispanics, but a significant relationship revealed only in the South American subgroup [59]. However, the APOE e4 allele is associated with an increased risk of different stroke subtypes in the Chinese population, suggesting a more widespread effect on the cerebrovascular system [60]. Variants in the APOE gene region, such as the APOE promoter polymorphism 219 T/G, may account for ethnic differences in the association between AD and APOE e4. This polymorphism changes AD risk in East Asians and Europeans suggesting that increased APOE expression may reduce AD risk in APOE e4 homozygotes [61]. Furthermore, the mortality risk by APOE genotype differs across ethnic groups. In African Americans, the E4/E3 genotype is associated with the lowest mortality risk, but not in Caucasians and Hispanics [62]. These differences could be attributed to

interactions between APOE genotype, genetic modifiers in specific populations, diet intake, vascular risk profiles, and environmental exposures. This variation needs to be understood for the development of risk prediction models applicable globally and prevention strategies appropriate for the ethnicities.

Other genetic risk loci

Genome-wide association studies and whole-genome sequencing projects have identified more than 75 genetic loci, in addition to APOE, that are associated with late onset AD risk [34,p. 412]. These include variants in genes involved in innate immune function (TREM2, CD33, PLCG2, INPP5D), endosomal trafficking (BIN1, PICALM, SORL1), lipid metabolism (CLU, ABCA7) and complement activation (CR1). Rare variants with large effect sizes, such as R47H in TREM2, which approximately triples AD risk, have been identified alongside common variants of individually small effect that together explain a meaningful proportion of genetic susceptibility [63]. Autosomal dominant mutations in APP, PSEN1, and PSEN2 cause familial early-onset AD but account for less than 1% of all cases [6,p. 795]. Polygenic risk scores that combine many common variants are being used more often to measure overall genetic risk beyond just APOE. A systematic review by Stocker and colleagues [64] evaluated multiple polygenic risk scores. The report concluded that while they significantly improve prediction over APOE alone, their clinical utility as stand-alone diagnostic tools is still limited and is under further refinement.

1.2.2 Modifiable risk factors

Modifiable risk factors provide concrete opportunities for prevention and intervention, as opposed to genetic and demographic characteristics. The idea that a significant percentage of dementia cases may be preventable by controlling modifiable risk factors has gained considerable scientific traction in recent years. The 2024 Lancet Commission report represents the most comprehensive assessment to date, identifying 14 modifiable factors distributed across early, mid, and later life and estimating that their collective elimination could prevent approximately 45% of all dementia cases globally [65]. A subsequent analysis by Mostert and colleagues [48,p. 103] proposed the addition of four further factors, such as poverty, income inequality, and HIV, that could raise the proportion of preventable cases to approximately 65%. In the following sections this dissertation will examine each major category of modifiable risk factors in detail.

1.2.2.1 Cardiovascular and metabolic risk factors

The relationship between cardiovascular health and brain health is now firmly established. The concept of “what is good for the heart is good for the brain” is supported by extensive epidemiological, neuropathological, and interventional evidence. Cardiovascular risk factors collectively contribute to a substantial proportion of dementia cases through interrelated mechanisms, including cerebral small vessel disease, white matter lesions, blood–brain barrier disruption, chronic systemic inflammation, and direct acceleration of AD-specific neuropathology. Vascular and metabolic factors: Hypertension, type 2 diabetes mellitus, dyslipidemia, obesity. AD is

almost twice as likely to occur in people with diabetes, due to sustained hyperglycemia, insulin resistance and their effects on cerebral vasculature and amyloid metabolism [66]. Abnormal cholesterol and triglyceride levels, and midlife obesity, further increase risk, likely via effects on neuronal health and cerebrovascular integrity. However, late-life weight loss may reflect disease progression rather than risk [67,68].

Hypertension

Midlife hypertension is consistently associated with an elevated risk of dementia in later life. In the region, studies show that Mongolia reports high rates of impaired glucose metabolism and hypertension among AD patients [69]. At the same time, studies in Russia and Uzbekistan strongly implicate atherosclerosis, diabetes, and dyslipidemia as contributors to both AD and broader cardiovascular burden [70,71].

Chronic high blood pressure accelerates cerebral small-vessel disease, promotes white matter lesions and lacunar infarcts, and may exacerbate amyloid deposition by impairing perivascular clearance mechanisms [65,p. 572]. Large-scale observational studies and meta-analyses confirm that sustained blood pressure elevation during the ages of 40–65 years is particularly harmful to long-term cognitive health, while the relationship becomes more complex in older age, where low blood pressure may also be associated with cognitive decline, possibly reflecting reverse causation or cerebral hypoperfusion [4,p. 18]. The SPRINT-MIND trial provided interventional evidence that intensive blood pressure control (systolic target below 120 mmHg) significantly reduced the incidence of mild cognitive impairment. However, the effect on dementia itself did not reach statistical significance, likely because the trial was stopped early for benefits on its primary cardiovascular endpoint [72]. The Dutch preDIVA trial similarly demonstrated that intensive vascular care reduced dementia incidence in a subgroup of patients with untreated hypertension at baseline [73]. Taken together, these findings support the idea that treating hypertension in midlife is a key strategy for preventing dementia.

Diabetes mellitus

Type 2 diabetes mellitus is a known risk factor for both AD and vascular dementia. Meta-analyses have reported a 50–65% increased risk of dementia in diabetic patients compared to the general population. Mechanistic links between diabetes and AD are numerous and include cerebral insulin resistance (which impairs Aβ clearance and drives tau phosphorylation), chronic hyperglycaemia-induced formation of advanced glycation end-products, dyslipidemia, microvascular damage, neuroinflammation, oxidative stress and mitochondrial dysfunction [74]. Neuroinflammation seems to be a key connector among these. The diabetic pathology induces an upregulation of activated microglia and a secretion of pro-inflammatory cytokines via the NLRP3 inflammasome pathway [75]. UK Biobank data analyzed by Manuello and colleagues in 2024 confirmed diabetes mellitus as one of the most significant modifiable risk factors for AD [76]. The concept of “type 3 diabetes”, referring to brain-specific insulin resistance as a feature of AD itself, has gained traction, although it remains a subject of active debate. Importantly, several studies suggest that effective glycemic control, particularly in midlife, may attenuate the excess dementia risk associated with diabetes [77].

Obesity

Midlife obesity (typically defined as a body mass index greater than 30 kg/m²) is associated with a significantly increased risk of dementia in later life. Several large cohort studies have demonstrated this association independently of other cardiovascular factors. Obesity promotes chronic systemic inflammation characterised by elevated levels of C-reactive protein, IL-6, and TNF-α; insulin resistance; dyslipidemia; and vascular dysfunction. All of these may accelerate neurodegeneration [1,p. 702]. A pilot neuroimaging study by Dolatshahi and colleagues [78] demonstrated that midlife obesity was associated with increased AD-type neuropathology and neurodegeneration on brain imaging. Paradoxically, weight loss in older age is often observed as a prodromal feature of AD, complicating the interpretation of cross-sectional studies. A prospective study by Beeri et al.showed that BMI stability over time was associated with a better cognitive trajectory in older adults, suggesting that the dynamic pattern of weight change may be more informative than a single measurement [79].

High LDL cholesterol

A particularly noteworthy addition to the 2024 Lancet Commission report is the inclusion of elevated midlife LDL cholesterol as a new modifiable risk factor. Based on evidence from large cohort studies involving over one million participants and a Mendelian randomisation meta-analysis of 27 studies, the Commission estimated that high LDL cholesterol contributes to approximately 7% of the global population attributable fraction of dementia. This is the joint highest single-factor estimate alongside hearing loss [65,p. 572]. The mechanisms linking cholesterol to AD include direct effects on Aβ production and clearance (cholesterol-rich membrane microdomains favor amyloidogenic processing of APP), promotion of cerebrovascular atherosclerosis, and modulation of microglial inflammatory responses. This finding has fueled interest in statin therapy as a dementia prevention strategy. While observational studies have long shown suggestive but inconsistent protective associations, the Mendelian randomisation evidence provides stronger support for a causal relationship. Randomised controlled trial evidence for statins specifically preventing dementia remains limited, however, and is an active area of investigation.

1.2.2.2 Lifestyle factors

Lifestyle risk factors constitute a significant and modifiable component in the development and progression of AD. Unhealthy behaviors like physical inactivity, smoking, excessive alcohol intake, poor eating habits, chronic sleep disturbances and a limited range of cognitive and social activities, have been shown to significantly increase the risk of AD by numerous epidemiological and clinical studies. These factors do not work in isolation but their combined effect often increases the likelihood of cognitive decline, especially in the presence of vascular and metabolic disorders such as hypertension and diabetes [80].

Physical inactivity

Physical inactivity is a well-established, modifiable risk factor for AD. Sedentary lifestyle has been repeatedly shown to be associated with increased risk of developing

AD in numerous studies [81,82]. Regular physical activity is one of the most commonly cited protective factors against cognitive decline and dementia. Exercise stimulates neurogenesis (particularly in the hippocampus), increases cerebral blood flow, decreases neuroinflammation, improves insulin sensitivity, reduces cortisol levels and improves in synaptic plasticity by increasing the release of brain-derived neurotrophic factor [4,p. 18]. Meta-analyses have shown that physically active individuals have a 20–30% lower risk of developing dementia than sedentary peers [77,p. 701]. Both aerobic exercise and resistance training seem to have benefit. The WHO 2019 guidelines strongly encourage physical activity as part of a comprehensive prevention strategy. A multi-domain intervention including supervised physical exercise improved cognitive function in at-risk older adults, as shown in the Finnish FINGER trial [83]. Even modest increases in physical activity may bring significant cognitive benefits for sedentary older adults, and emerging evidence suggests that these benefits also extend to individuals at high genetic risk, such as APOE ε4 carriers [83,p. 2255]. Thus, public health policies and personal lifestyle changes that promote physical activity are critical to lower the incidence and progression of Alzheimer's disease.

Smoking

The association between smoking and dementia is well established. It causes oxidative stress, accelerates cerebrovascular atherosclerosis, damages the blood-brain barrier, triggers neuroinflammation and leads to brain atrophy. Meta-analyses suggest that the risk of dementia is 30–50% higher in current smokers compared to never-smokers [65,p. 572]. The GBD 2019 study identified smoking as one of only three risk factors with sufficiently strong causal evidence for inclusion in global dementia forecasting models [10,p. 105]. Population-level declines in smoking prevalence in high-income countries have contributed to observed decreases in age-specific dementia incidence. However, in many LMICs and in several Central Asian countries, smoking prevalence remains high [84,85], particularly among men, and tobacco control therefore represents a major opportunity for dementia prevention in these settings. Notably, heavy smoking in men is a significant driver of both vascular and dementia risk in the region, with one study showing up to a 62% smoking rate among AD patients in certain age groups [86]. Evidence indicates that smoking cessation at any age reduces the excess risk, though the benefit is greatest when cessation occurs in midlife.

Excessive alcohol consumption

The relationship between alcohol and dementia risk follows a complex, likely J-shaped pattern. While some earlier observational studies suggested that light-to-moderate alcohol intake might be protective, more rigorous analyses accounting for abstainer bias, reverse causation, and confounding factors have questioned this finding [65,p. 572]. Excessive alcohol consumption is unequivocally associated with direct neurotoxicity, thiamine deficiency leading to Wernicke-Korsakoff syndrome, hepatic dysfunction, nutritional deficiencies, and an increased risk of both AD and alcohol-related dementia. Alcohol use disorder is a particularly strong risk factor for young-onset dementia. Heavy episodic (binge) drinking appears to be especially harmful. Analyzes of the UK Biobank showed that the frequency of alcohol intake was a significant modifiable risk factor for AD, with strongest associations found at the

highest levels of intake [76,p. 25]. The majority of current guidelines suggest steering clear of heavy or binge drinking and limiting alcohol consumption as part of a brain-healthy lifestyle.

Diet and nutrition

Dietary patterns have been increasingly studied as modulators of dementia risk . Prospective cohort studies have associated the Mediterranean, DASH and MIND diets with reduced risk of cognitive decline and dementia [87,88]. These dietary patterns have a focus on plant-based foods, fish, olive oil, whole grains, nuts and legumes, and limiting red and processed meat, saturated fats, refined sugars and ultra-processed foods. The protective mechanisms are believed to involve anti-inflammatory and antioxidant effects (from polyphenols, omega 3 fatty acids, and vitamins E and C), improved vascular health, reduction of insulin resistance, and beneficial modulation of the gut microbiome.

A particularly important mediating pathway has been identified as the gut–brain axis Gut microbial diversity is directly influenced by diet composition and there is a growing body of evidence that associates dysbiosis with increased intestinal permeability, systemic inflammation and Aβ deposition [89,90]. The Mediterranean diet has been found to enrich for beneficial microbial genera such as *Prevotella* and reduce pro-inflammatory taxa. Adherence to this dietary pattern is associated with lower AD risk in several prospective cohorts [91]. Observational and some intervention studies have suggested neuroprotective effects of certain dietary components including omega-3 polyunsaturated fatty acids, curcumin, resveratrol and B-vitamins (particularly folate and B12) [88,p. 3053]. However, large-scale RCT evidence for single-nutrient supplementation in dementia prevention remains limited. The overall quality of the dietary pattern appears more important than any individual component.

1.2.2.3 Psychosocial and educational factors

Low educational attainment

Lower educational attainment in early life is one of the most consistent and powerful predictors of later-life dementia risk. Education is thought to contribute to the brain's capacity to tolerate a greater burden of neuropathology before clinical symptoms manifest, also known as cognitive reserve. Cognitive reserve is built through the formation of extensive neural networks and synaptic connections during formative years, and is further reinforced by lifelong cognitive stimulation through occupational complexity and leisure activities. The 2024 Lancet Commission estimates low education is responsible for approximately 5% of the population attributable fraction of dementia globally [1,p. 702]. The declines in age-specific dementia incidence in high-income countries over the past several decades are believed to be driven, in large part, by global improvements in educational access [10,p. 105]. Large educational disparities remain in LMICs, particularly for women and rural populations, making investments in early-life education a strong long-term dementia prevention strategy with benefits beyond brain health.

Depression

While there is a link between depression and increased risk of dementia, the relationship is complex and likely bidirectional. A new meta-analysis by the 2024 Lancet Commission suggested that persistent or recurrent depression, particularly when it manifests in midlife, is likely a causal risk factor [65,p. 572]. Depression, particularly late-life depression, is a risk factor and prodrome for AD. Meta-analyses show that the risk is increased by 82% in people with depression, which indicates the need for prevention and early treatment [92]. Proposed mechanisms include chronic elevation of cortisol (which is neurotoxic to the hippocampus), dysregulation of the hypothalamic-pituitary-adrenal axis, activation of neuroinflammatory cascades, reduced BDNF levels, hippocampal volume loss, and behavioral mediators like social withdrawal, physical inactivity, and medication non-adherence [93]. There is also evidence of a shared genetic susceptibility between depression and AD [94]. Some cases of depressive symptoms that begin in late life, however, may be a prodromal manifestation of dementia rather than an independent risk factor. It is methodologically difficult to separate cause from consequence and it needs long follow-up periods. Pharmacotherapy, psychotherapy and behavioral activation are effective treatments for depression and could have preventive benefits for dementia, although this has not yet been conclusively demonstrated in RCTs.

Social isolation and loneliness

Social isolation and loneliness are more and more acknowledged as important risk factors for cognitive decline and dementia, especially in older adults. The Lancet Commission estimates that rare social contact is responsible for approximately 5% of the global population attributable fraction of dementia [95]. Social interaction enhances cognitive activity, improves psychological well-being, lessens chronic stress and cortisol exposure, and can dampen neuroinflammatory processes. In contrast, social isolation has been linked with increased inflammatory markers, accelerated brain atrophy (particularly in the hippocampus and prefrontal cortex), and behavioral risk factors such as a sedentary lifestyle, poor diet, and excessive alcohol intake [96]. The COVID-19 pandemic has placed an unprecedented spotlight on this issue: social distancing measures imposed to combat the pandemic have had a disproportionate impact on older adults, potentially accelerating cognitive decline in vulnerable individuals. Population-level initiatives to promote social connectedness (community-based programs, intergenerational activities, approaches to digital inclusion, and interventions to reduce loneliness) are increasingly being recognized as vital components of dementia prevention.

1.2.2.4 Sensory impairment

Hearing loss

Hearing loss, particularly in midlife, has emerged as the single largest modifiable risk factor for dementia on a population level, contributing approximately 7% of the population attributable fraction [84,p. 9117]. There are a number of possible mechanisms. For example, impaired hearing increases cognitive load as the brain works harder to process degraded auditory input, diverting resources from other cognitive processes. Decreased auditory stimulation results in structural changes,

including atrophy in the temporal lobe. Hearing loss promotes social withdrawal and isolation, which independently increases the risk for dementia. Yeo et al. [97] conducted a systematic review and meta-analysis and found that those who used hearing aids and cochlear implants had a significantly lower risk of cognitive decline and dementia, providing important evidence for hearing rehabilitation as a preventative strategy. Hypometabolism of glucose in the auditory pathways has also been detected in neuroimaging studies in individuals with hearing loss and may contribute to vulnerability to AD [98]. Making hearing screenings, hearing aids, and cochlear implants accessible and affordable is therefore a high-priority public health recommendation for dementia risk reduction.

Vision loss

Untreated vision loss in later life was added as a new risk factor in the 2024 Lancet Commission report, based on evidence from two large meta-analyses. It is estimated that 2% of all dementia cases worldwide are caused by vision impairment [32,p. 165]. As with hearing loss, poor vision results in reduced sensory input to the brain, reduced social interaction and physical activity, and depression which independently increase the risk of dementia. Specific visual conditions linked to elevated AD risk include cataracts, glaucoma, age-related macular degeneration, and diabetic retinopathy. There is also growing evidence from retinal imaging studies that retinal changes (including thinning of the retinal nerve fibre layer, retinal vascular abnormalities, and the presence of retinal Aβ deposits) may reflect and even precede cerebral AD pathology [99]. Ensuring access to regular eye examinations and corrective interventions, including cataract surgery, provision of spectacles, and treatment of glaucoma and diabetic retinopathy, is recommended as a cost-effective public health measure for dementia risk reduction.

1.2.2.5 Environmental and other factors

Air pollution

Prolonged exposure to particulate matter and traffic-related pollutants accelerates cognitive decline and increases amyloid deposition, especially in urban or industrial regions [82,p. 413]. Occupational or residential exposure to heavy metals (lead, cadmium) and agricultural pesticides contribute to AD risk, presumably via neurotoxicity and inflammation [92,p. 917]. Exposure to ambient air pollution, particularly fine particulate matter (PM2.5) and nitrogen dioxide (NO_2), has been increasingly recognized as a risk factor for dementia. Air pollutants can reach the central nervous system through multiple routes: direct translocation via the olfactory nerve, passage across the blood–brain barrier following pulmonary absorption, and induction of systemic inflammation that propagates to the brain. Once inside the CNS, pollutants induce neuroinflammation (activation of microglia and release of pro-inflammatory cytokines), oxidative stress, Aβ deposition and microvascular damage [100,101]. The Lancet Commission lists air pollution as one of 14 modifiable risk factors and UK Biobank data identified NO_2 exposure as a significant predictor of AD [76,p. 25]. Epidemiological studies in different countries have shown dose-response relationships between long-term exposure to PM2.5 and the incidence of dementia

[100,p. 716]. Given that air quality is a modifiable environmental factor amenable to regulatory intervention, pollution control policies, including emission standards for vehicles and industry, promotion of clean energy, and urban green space planning, carry significant potential for population-level dementia risk reduction, particularly in rapidly industrialising LMICs.

Traumatic brain injury

Traumatic brain injury (TBI), particularly moderate-to-severe TBI and repetitive subconcussive head impacts, is associated with a substantially increased risk of subsequent dementia. The pathological mechanisms linking TBI to neurodegeneration include diffuse axonal injury, initiation and propagation of tauopathy (including chronic traumatic encephalopathy, or CTE), disruption of the blood–brain barrier, and chronic neuroinflammatory activation [102]. Even a single moderate-to-severe TBI has been shown to increase the risk of subsequent dementia by 1.5–2 fold. TBI is a more prevalent risk factor in men, reflecting higher rates of participation in contact sports, military service, and occupational exposure, and has been specifically suggested as a contributor to the higher dementia mortality observed in men [1,p. 702]. Prevention strategies for road traffic safety, workplace hazards, contact sports rules and falls prevention in older adults are important public health interventions. Helmets, seatbelts, concussion management protocols are practical, evidence-based ways to lower the risk of TBI-related dementia.

Infectious agents

The infectious hypothesis of AD has seen a resurgence as a popular theory. Epidemiological and experimental studies [4,p. 18] have associated human herpesviruses, including HSV-1, HHV-6, and HHV-7, with a higher risk of AD. Mechanisms proposed include direct induction of Aβ deposition as an innate antimicrobial response, induction of neuroinflammation via microglial activation, and disruption of blood–brain barrier integrity. Post-mortem AD brains have found toxic proteases (gingipains) of periodontal pathogens, especially *Porphyromonas gingivalis*, that can promote tau fragmentation and neuroinflammation [103,104]. SARS-CoV-2 infection has also been associated with accelerated cognitive decline in some observational studies, though confounding by social isolation, stress, and comorbidities during the pandemic complicates interpretation [105]. No single infectious agent has been demonstrated to be both necessary and sufficient for AD causation, but microbial infections likely act as contributing risk factors within a multifactorial disease model, particularly by triggering and sustaining neuroinflammatory responses.

1.2.3 Gaps in the literature and future directions

A key insight from the accumulated evidence is that dementia risk is shaped across the entire life span. The 2024 Lancet Commission organizes its 14 modifiable risk factors into three life stages: early life (low education), midlife (hearing loss, high LDL cholesterol, TBI, physical inactivity, diabetes, smoking, excess alcohol, hypertension, obesity, and depression), and later life (social isolation, air pollution, and vision loss). This framework recognizes that cognitive reserve is built during early life, vascular and

metabolic damage accumulates during midlife, and degenerative and social risk factors become increasingly salient in older age [73,p. 797].

Importantly, the life-course model indicates that it is never too early or too late to take preventive action. Even in individuals with high genetic risk, healthy lifestyles involving regular exercise, cognitive stimulation, smoking cessation, and moderate alcohol consumption have been shown to delay the onset of dementia and reduce years lived with the disease. Multi-domain intervention trials, including the Finnish FINGER study, the Dutch preDIVA trial, the German AgeWell.de trial, and the US SMARRT trial have demonstrated that simultaneously targeting multiple risk factors can yield measurable cognitive benefits [106]. However, effects on dementia incidence require longer follow-up and larger samples. These trials also highlight the importance of personalization. Namely, matching interventions to an individual's specific risk profile and socioeconomic context is likely to maximize effectiveness.

Despite considerable progress, several critical gaps remain in our understanding of AD risk factors. First, the vast majority of evidence comes from high-income countries with predominantly European populations. There is a pressing need for large-scale prospective cohort studies in LMICs and among ethnically diverse populations. Second, the causal nature of many observed associations remains uncertain. While Mendelian randomization and multi-domain intervention trials have strengthened causal inference for some factors, others require further mechanistic and interventional evidence. Third, gene-environment interactions are poorly understood and represent a critical point for precision prevention.

The relative contribution of specific risk factors varies significantly across populations and ethnic groups. A 2024 study of nearly 92,000 participants in the US Multiethnic Cohort Study found that modifiable risk factors accounted for 33% of dementia cases among Latinos, 29% among Native Hawaiians, 28% among African Americans, 22% among White Americans, and 14% among Japanese Americans [1,p. 702]. These disparities reflect differences in genetic background, socioeconomic conditions, healthcare access, and exposure to environmental risk factors.

In Kazakhstan and other Central Asian countries where epidemiological data on dementia remain limited, the burden may be shaped by a distinctive combination of risk factors, including high rates of cardiovascular disease and hypertension, dietary patterns rich in animal fats and salt, high prevalence of smoking among men, limited access to quality education (particularly in rural areas), exposure to environmental pollutants from industrial and agricultural activities, and socioeconomic challenges, that are not well captured by models derived primarily from high-income Western populations. Expanding dementia research to include these underrepresented regions, with studies conducted in local languages and culturally appropriate assessment tools, is essential for developing locally relevant prevention strategies and for addressing the growing global equity gap in dementia care and research.

1.3 Emerging biomarkers of Alzheimer's disease

1.3.1 The role of adiponectin, the adiponectin gene, and its receptors in AD

Parts of this section are adapted from our previously published work (Kaiyrlykyzy et al. (2022) [107].

Alzheimer's is increasingly seen as a condition as condition shaped by systemic metabolism, not only by process within the brain. Obesity. Dyslipidemia. Insulin resistance and type 2 diabetes are all recognized risk factors for AD [108,109]. Among the metabolic mediators that may link peripheral physiology to neurodegeneration, adiponectin (ADIPOQ) has attracted considerable interest.

Adiponectin is a protein hormone secreted mainly by white adipose tissue. It makes up roughly 0.01% of all plasma proteins and acts on several metabolic pathways. It improves insulin sensitivity, helps maintain glucose homeostasis, regulates fatty acid metabolism, and exerts anti-inflammatory effects [110,111]. Because of these insulin-sensitizing and anti-inflammatory actions, adiponectin is generally considered protective in peripheral tissues. Low circulating concentrations, known as hypoadiponectinemia, are inversely associated with several age-related cardiometabolic conditions and predict a higher risk of them, including type 2 diabetes, atherosclerosis, and cardiovascular disease [112-116].

The evidence on adiponectin in AD, however, is less straightforward and in some respects points the opposite way [117-123]. Une et al. reported that plasma adiponectin was significantly higher in patients with MCI and AD than in controls, and that plasma levels correlated positively with those in cerebrospinal fluid (CSF), so the peripheral signal appears to be reflected centrally [124]. A more recent cohort study found plasma adiponectin to be independently associated with AD. Levels correlated negatively with MMSE scores and positively with Clinical Dementia Rating (CDR) scores, meaning that higher adiponectin accompanied worse cognition and more advanced disease [125].

Prospective and population-based data point in the same direction and reveal a striking sex specificity. In the Framingham Heart Study, elevated serum adiponectin was an independent risk factor for incident all-cause dementia and AD in women but not in men [121,p. 1018]. The Mayo Clinic Study of Aging produced consistent findings. Among women, especially those with high amyloid burden on PiB-PET, higher plasma adiponectin was linked to smaller hippocampal volume, weaker memory, language, and global cognitive performance, and considerably higher odds of MCI [126]. This sex-specific pattern deserves attention because AD is both more common and more severe in women.

Still, the findings are not uniform. Some studies have detected no meaningful difference in plasma adiponectin in AD [122,p. 213], whereas others have found reduced levels in MCI and AD relative to controls [123,p. 115]. In patients with AD and MCI, higher CSF adiponectin has been associated with lower beta-amyloid deposition and greater hippocampal volume [123,p. 115]. Experimental work also points to a protective role. Chronic adiponectin deficiency in aged mice produces cognitive impairment resembling AD [127], and adiponectin protects against oxidative neurotoxicity induced by amyloid-beta [128]. Overall, raised adiponectin in AD may

not represent a simple beneficial or harmful effect. It could instead reflect a compensatory neuroprotective response, resistance to adiponectin signaling in the brain, or reverse causation driven by ongoing neurodegeneration. Further research is needed to resolve how adiponectin regulation relates to AD.

The biological effects of adiponectin depend on its receptors: adiponectin receptor types 1 and 2 (AdipoR1 and AdipoR2) and T-cadherin. Changes in AdipoR1 and AdipoR2 expression have been described in monocytes from patients with type 2 diabetes and from overweight individuals with coronary artery disease [116,p. 816]. Several studies have also linked single-nucleotide polymorphisms (SNPs) in ADIPOR1 and ADIPOR2 to metabolic syndrome, coronary artery disease, and type 2 diabetes [129-131]. By contrast, little is known about how adiponectin receptor expression changes in AD.

Although adipose tissue was long regarded as the main source of adiponectin, it is now clear that many other cell types can also synthesize it [132-134]. Adiponectin expression has been demonstrated in regulatory T cells within thymic nurse cell complexes, in peripheral blood mononuclear cells, and in macrophages [135-137]. Monocytes appear to contribute in part to the insulin-sensitizing and anti-inflammatory effects of adiponectin [138,139]. Several studies have suggested that changes in ADIPOQ expression and DNA methylation in maternal adipose tissue and blood cells are associated with gestational diabetes mellitus [140]. Methylation patterns of the adiponectin gene also seem to be similar across tissues, including blood [141].

Regarding the regulation of ADIPOQ, the promoter region of the human gene contains three enhancer boxes (E-boxes), which are well-characterized promoter elements. Although E-boxes are widespread and potentially important for transcriptional control, their role in regulating the human ADIPOQ gene has received little study. In the mouse gene, the class I basic helix-loop-helix (bHLH) proteins E47 and inhibitor of differentiation 3 (Id3) suppress adiponectin expression by binding to E-boxes [142]. E-box-mediated regulation, however, appears to differ between mouse and human cells. In human cells, only a single published report has identified an E-box-binding transcriptional regulator, Snail [143]. The E-boxes in the proximal promoter of ADIPOQ also contain cytosine-phosphate-guanine (CpG) dinucleotides, which suggests that DNA methylation may provide a further mechanism for controlling adiponectin expression. García-Cardona et al. showed that methylation at a CpG site located at −74 nt in the ADIPOQ promoter was less frequent in obese and severely obese adolescents than in lean or overweight adolescents [144]. Given the important role of obesity in AD, this −74 nt CpG site is of particular interest.

1.3.2 The role of microbiomes in Alzheimer's disease

Parts of this section are adapted from our previously published work (Askarova et al. (2020) [145], Kaiyrlykyzy et al. (2022) [146], Issilbayeva et al. (2024) [147] and Kozhakhmetov et al. (2024) [148].

Over the past decade, microbiome research has shown that the gut microbiota and the central nervous system are closely linked through what is now called the gut-brain axis. This two-way communication network operates through neural, immune, and

endocrine pathways and affects both gastrointestinal function and cognition [149]. Disruption of the normal composition of the gut microbiota, known as dysbiosis, has been associated with a range of neurological conditions, AD among them. Changes in the gut microbiome may alter brain function in several ways: microbes generate metabolites with neuroactive effects, shape immune responses, and interact directly with the nervous system [150].

Numerous studies have examined the potential role of the gut and oral microbiome as significant risk factors for the development and progression of Alzheimer's disease from an epidemiological perspective. The gut microbiota, a collective term, refers to the trillions of diverse microbial organisms that inhabit the human gastrointestinal tract. These microbes play a crucial and multifaceted role in regulating various aspects of host physiology, including immune system function, inflammatory processes, and brain development and function [151]. Disturbances in the composition and functionality of the gut microbiota, a condition known as dysbiosis, have been consistently associated with the pathogenesis of various neurological disorders, including AD [152]. These dysbiosis in the gut microbiome can trigger inflammatory cascades, influence neurotransmitter production, and potentially contribute to the development of characteristic neuropathological hallmarks of Alzheimer's disease, such as neuroinflammation and neural degeneration.

The intestinal microbiota can shape brain function and behavior through several interconnected routes of communication. One of the most studied is the production of microbial metabolites, such as short-chain fatty acids (SCFAs) and neurotransmitters, which can influence neuronal signaling and activity [151,p. 145]. SCFAs in particular are able to cross the blood-brain barrier and act on the brain directly, whereas microbially derived neurotransmitters are thought to act mainly through indirect routes, including the vagus nerve and the enteric nervous system. The gut microbiome can also modulate the host immune system, releasing inflammatory mediators that can trigger neuroinflammation and alter neuronal activity [152,p. 12]. Furthermore, the gut microbiome can indirectly influence brain function and behavior by modulating the vagus nerve, a bidirectional communication pathway between the gut and brain. These multifaceted interactions between the gut microbiome and the central nervous system can ultimately impact neurotransmission, neuroinflammation, and overall brain health and behavior.

Among the mechanisms proposed to link the gut microbiome to AD immune and inflammatory signaling, gut-barrier and blood-brain-barrier dysfunction, microbial metabolites, microglial activation, and vagal and neuroendocrine pathways epigenetic modification represents one potential downstream mechanism. Microbial metabolites can also act at the epigenetic level. Short-chain fatty acids, butyrate in particular, inhibit histone deacetylases and have been shown to alter histone acetylation and DNA methylation in host cells [153]. These changes may in turn regulate genes that support neurogenesis, synaptic plasticity, and cognitive function. This microbiome-epigenome interaction is a plausible but still preliminary pathway requiring confirmation in human studies.

The intestinal microbiome, which is composed of trillions of microorganisms, including bacteria, viruses, fungi, and archaea, is crucial for the preservation of host homeostasis. Research has demonstrated that the gut microbiota's composition and diversity are considerably altered in Alzheimer's disease patients in comparison to healthy individuals. These changes encompass a reduction in beneficial bacteria, including *Bifidobacterium* and *Lactobacillaceae*, and an increase in potentially harmful taxa, such as *Acidobacteriota* and *Verrucomicrobiota* [95,p. 135]. The composition of the gut microbiome is also sensitive to lifestyle and environmental factors, including diet, physical inactivity, sleep deprivation, circadian disruption, and chronic noise exposure. Many of these same factors are recognized as risk factors for sporadic AD [154]. This suggests that the microbiome may be one pathway through which modifiable lifestyle exposures influence AD risk.

In addition to the gut microbiome, the oral microbiome has also been implicated in the development of Alzheimer's disease. The oral cavity hosts a diverse community of microorganisms, collectively known as the oral microbiome, which is crucial in maintaining oral and overall health. Imbalances or dysbiosis within this microbial ecosystem have been consistently linked to the development of systemic inflammation and neuroinflammation [155]. The release of inflammatory mediators can be triggered by chronic inflammation in the oral cavity, which is frequently caused by dysregulation of the oral microbiome. These mediators have the potential to disseminate throughout the body and cross the blood-brain barrier, thereby contributing to neuroinflammation, a hallmark pathological process in Alzheimer's disease. Additionally, certain oral bacterial species have been demonstrated to generate amyloid proteins that are structurally similar to the amyloid-beta peptides found in the brains of Alzheimer's patients. This has the potential to seed and propagate the formation of the disease's characteristic neuropathological hallmarks. Although these findings do not yet establish causality, the accumulating epidemiological and experimental evidence points to the oral microbiome as a potential contributor to AD onset and progression, and as a possible target for prevention and treatment [147,p. 12].

Like the gut microbiome, the oral microbiome appears to influence brain health. Periodontal disease has been associated with neurodegeneration and cognitive decline, and chronic periodontitis lasting ten years or more has been linked to a substantially higher risk of AD [156]. Experimental studies support these observations. In mice, repeated oral infection with *Porphyromonas gingivalis*, a keystone pathogen in periodontitis, led to the formation of amyloid-beta plaques and neurofibrillary tangles [157]. In humans, *P. gingivalis* and its proteolytic enzymes, gingipains, have been detected in the brains of patients with AD [158].

Interestingly, a recent randomized clinical trial examined the impact of *Lactobacillus rhamnosus GG* probiotic supplementation on psychological status and cognitive function in individuals at risk for Alzheimer's disease [155,p. 192]. The results of this study suggest that modulating the gut microbiome through probiotic interventions may potentially improve cognitive and psychological outcomes in individuals with Alzheimer's disease or at risk of developing the condition. Specifically, the study found that daily supplementation with the *Lactobacillus*

rhamnosus GG probiotic strain over 12 weeks significantly improved memory, attention, and overall cognitive performance among the study participants. Moreover, the probiotic intervention was also associated with reductions in depressive symptoms and enhancements in overall psychological well-being. These findings underscore the intricate gut-brain axis and highlight the potential of targeted microbiome-based therapies to mitigate the cognitive and neuropsychiatric manifestations of Alzheimer's disease. As microbiome research continues to evolve, further investigations into the precise mechanisms by which gut microbiome modulation can influence brain health and function will be crucial in developing effective preventive and therapeutic strategies for this devastating neurodegenerative disorder.

The interaction between the oral and gut microbiomes and Alzheimer's disease highlights the complexity of this neurodegenerative disorder and opens new pathways for research and treatment. Epidemiological evidence suggests that the composition and function of these microbiomes may significantly influence the development and progression of Alzheimer's disease. Ongoing research could lead to innovative therapeutic and preventive strategies aimed at the microbiome to address this devastating condition. In addition, the microbiome and brain health are significantly influenced by contemporary lifestyle factors, including diet, sleep patterns, circadian rhythms, and stress. As we deepen our understanding of the links between microbiota and brain health, it becomes increasingly evident that the microbiome may hold the key to understanding and ultimately combating this disease.

2 MATERIALS AND METHODS

2.1 Study design

The research underlying this dissertation was conducted as a multicenter, case-control study to identify and characterize risk factors for Alzheimer's disease in Kazakhstan. This study design was utilized to make a systematic comparison between individuals diagnosed with AD (cases) and cognitively normal controls, thereby enabling the identification of both independent and interacting factors that contribute to disease risk. The case-control approach is particularly suitable for diseases such as AD, where onset is gradual and multifactorial, enabling the efficient exploration of diverse biological and environmental exposures that may precede clinical manifestation.

The study's primary settings were two major cities, Astana (northern Kazakhstan) and Almaty (southern Kazakhstan), representing contrasting climates, ethnic compositions, and sociodemographic characteristics within the country.

A harmonized methodology was developed and implemented across all participating sites to ensure data comparability. Standardized diagnostic criteria for AD were applied, and all participants underwent a consistent set of clinical, cognitive, and laboratory assessments. Biological samples, including blood for genetic and biochemical analysis, were collected in accordance with standardized protocols. Environmental and lifestyle data were collected using validated questionnaires that addressed diet, physical activity, smoking history, alcohol usage, occupational exposure and psychosocial stress. By capturing the interplay among genetic predisposition, metabolic dysfunction, lifestyle habits, inflammation stamps and sociocultural determinants, this integrated framework facilitated the evaluation risk profiles of AD in local context.

2.2 Participant recruitment and eligibility criteria

AD cases were defined as individuals aged 55 years or older who met established clinical criteria for probable Alzheimer's disease. Diagnoses were ascertained by board-certified neurologists following the National Institute on Aging–Alzheimer's Association (NIA-AA 2011) guidelines or the NINCDS-ADRDA criteria for AD [159]. The diagnostic process consisted of a formal cognitive assessment using standardized screening tools, such as the Mini-Mental State Examination (MMSE) and the Clock Drawing Test, a detailed neurological examination, and a comprehensive clinical interview with the patient and primary caregiver. These instruments were selected for their established validity in detecting cognitive decline across diverse educational backgrounds. Where clinically warranted, brain MRI scans were obtained to exclude alternative causes of dementia such as cerebrovascular disease, normal pressure hydrocephalus, tumors, or structural brain lesions.

The control group consisted of cognitively normal, community-dwelling seniors with no history or symptoms of dementia or major psychiatric, neurological, or somatic illnesses that could confound cognition. Controls were frequency-matched to cases on age, sex, and predominant ethnicity (primarily Kazakh and Russian). Education and socioeconomic status were not used as matching criteria. They were recruited from

hospital outpatient departments, primary care centers, and senior community organizations between 2020 and 2024.

Exclusion criteria for both groups included severe somatic comorbidities (end-stage renal, hepatic, cardiac, or malignant disease), severe psychiatric illness, traumatic brain injury with long-term sequelae, and inability to provide informed consent.

A total of 181 individuals with clinically diagnosed AD and 244 cognitively healthy controls were enrolled in the study. After exclusion of participants with incomplete core covariate data, 177 AD cases and 241 controls (N = 418) constituted the main case-control analytic sample. The hypothesis-driven sociodemographic, clinical, and APOE analyses were conducted on this sample, whereas the pilot GWAS, biochemical and adiponectin/epigenetic profiling, the 41-plex cytokine panel, dietary pattern analysis, and gut and oral microbiome sequencing were performed on domain-specific samples whose sizes varied with biospecimen and data availability, and are therefore interpreted as exploratory and hypothesis-generating. Participant flow from screening to each domain-specific analytic sample is summarized in figure 3.

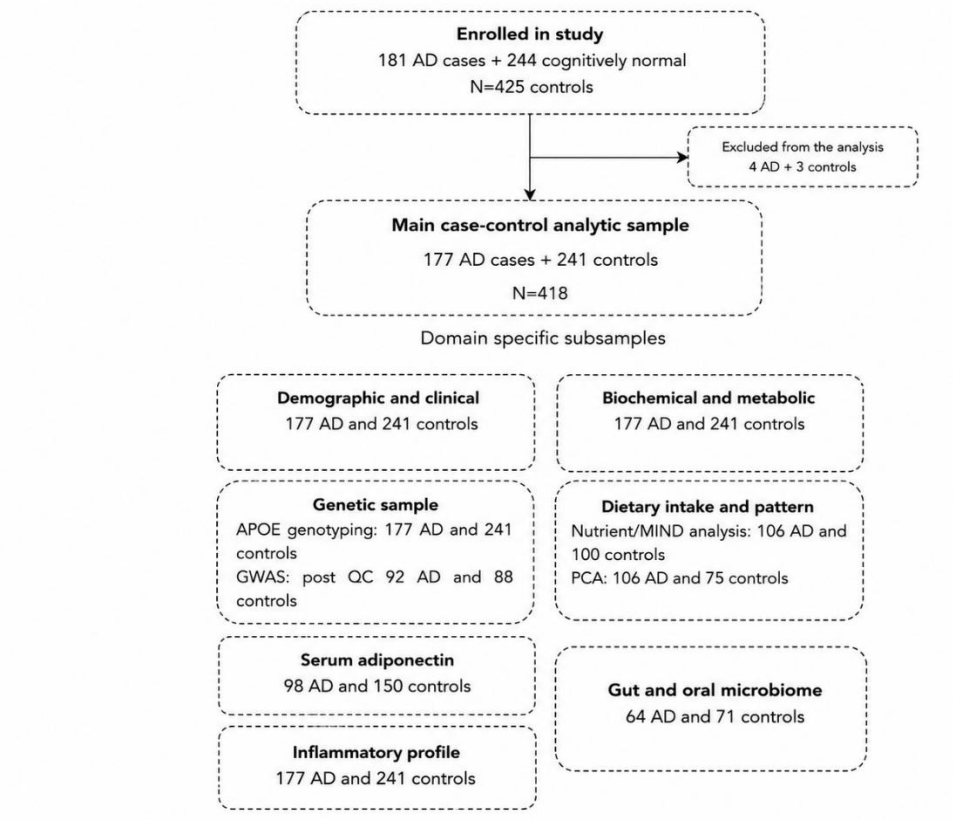


Figure 3 – Study population flowchart and availability of domain-specific datasets

2.3 Data collection

Recognizing the complexity of AD etiology, the study took a multimodal approach, collecting data across several domains as described below.

2.3.1 Sociodemographic and background questionnaire

A detailed, structured questionnaire was administered either in person or with the assistance of informants for participants with cognitive impairments. The questionnaire was designed to allow for regional and ethnic analyses and was piloted in both Russian and Kazakh populations. The questionnaire captured demographic, social, and familial networks, early life exposures, comorbidities and past medical history, family history of dementia among first-degree relatives, lifestyle factors, self-rated health, functional status, and health service utilization (figure 4). To ensure data reliability in the cognitively impaired cohort, survey responses for AD cases were validated through informant-assisted interviews with primary caregivers or family members, thereby mitigating potential recall bias.

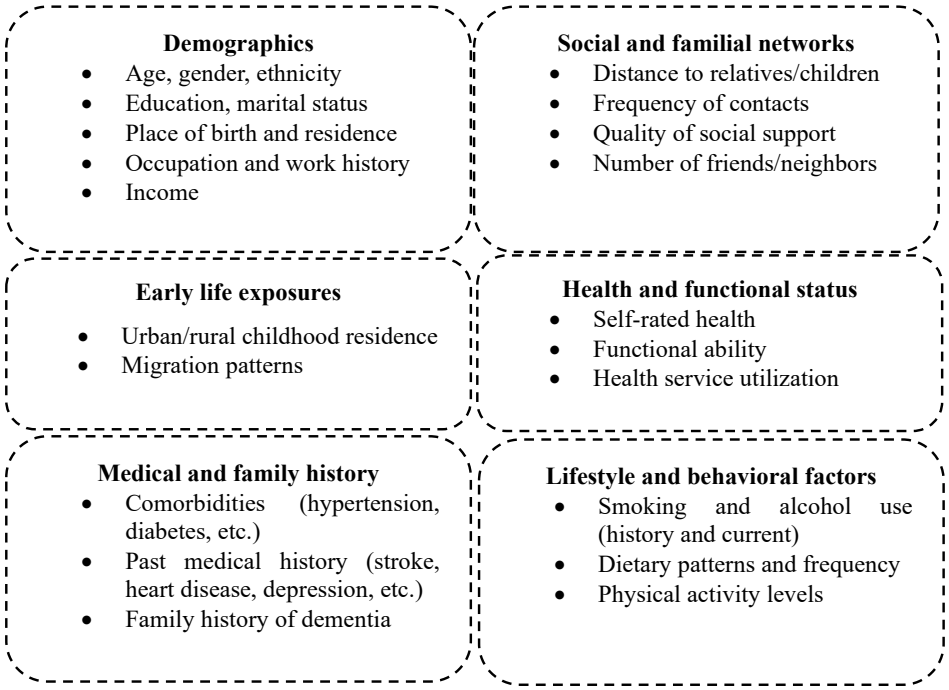


Figure 4 - Domains of the sociodemographic and background questionnaire

Specific emphasis was placed on lifestyle factors and mid-life exposures, including diet and physical activity habits maintained over the preceding decade. Physical activity was evaluated using a multi-dimensional composite index designed to capture both self-perceived exertion and habitual aerobic behavior. Participants were first asked to categorize their overall activity level, including both work and leisure, on a four-point Likert scale, where 1 indicated "very active" and 4 indicated "inactive." To align this with a growth-oriented scale, the values were reverse-coded during analysis so that higher scores indicated greater intensity. This was supplemented by a binary measure of habitual walking, which identified participants who had engaged in continuous walking for at least 500 meters (approximately 10–15 minutes) during the previous month. From these two items, a Physical Activity Index (PAI) was constructed by summing the reverse-coded intensity points and the walking status, resulting in a scale ranging from 1 (fully sedentary) to 5 (highly active). For

multivariate logistic regression, this index was dichotomized into a binary variable. A threshold of ≥4 was established to define the "active" group, encompassing individuals who reported at least moderate activity levels and a consistent walking habit. Participants with a score of ≤3 were classified as "inactive" and served as the reference category in all statistical models. This categorization allowed for a clear quantification of the increased risk of AD associated with a sedentary lifestyle compared to those meeting a baseline threshold of physical engagement.

For the analysis, the Social Network Index (SNI) was calculated to quantitatively assess each participant's level of social Integration across key areas of social contact and participation. SNI was developed following the widely used Berkman-Syme method, which combines information on marital or partnership status, frequency of contact with friends and relatives, and neighbors into a single composite score. Participants answered a standardized set of questions about these areas, and their responses were coded according to established criteria to reflect both the number and frequency of social connections. The resulting index is an ordinal scale in which higher scores indicate greater social Integration and lower scores indicate social isolation. Individuals were then grouped into "socially integrated" or "socially isolated" categories based on predefined SNI thresholds, allowing further Analysis of the relationship between social connectedness and AD status in the cohort.

2.3.2 Cognitive and functional assessment

Standardized cognitive testing was conducted using the Mini-Mental State Examination (MMSE) and the Clock Drawing Test (CDT). MMSE is an internationally recognized tool for cognitive screening, with cut-off scores often adjusted to account for differences in educational background and language proficiency, as these factors significantly affect performance. The CDT complements the MMSE by assessing executive function and visuospatial skills. Cognitive impairment was classified based on MMSE cut-offs adjusted for educational level and language. Functional status and disability were assessed through self-reported and informant-reported functional questionnaires. Diagnostic and Statistical Manual of Mental Disorders [160] and the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders (NINCDS–ADRDA) was used to establish diagnosis in participants [161].

2.3.3 Genetic analyses

Utilizing the QIAGEN DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany), DNA was extracted from peripheral blood samples of patients with dementia and controls in accordance with the manufacturer's guidelines for APOE genotyping. The quality and quantity of DNA were assessed using spectrophotometry with a NanoDrop 2000 and fluorometry with a Qubit 2.0. Genotyping of the SNPs rs429358 and rs7412 in the APOE gene was performed using a 5′-nuclease assay based on PCR with specific TaqMan assays for each SNP (7500HT Real-Time, Life Technologies, Applied Biosystems, USA).

For the multi-SNP dataset, genotyping was performed using high-density SNP arrays. Rigorous quality control steps included sample and SNP call-rate thresholds, Hardy-Weinberg equilibrium testing, relatedness and ancestry checks (principal component analysis), and imputation using global reference panels. Known AD-risk loci and new associations were evaluated.

2.3.4 Biochemical assessment

Fasting venous blood was collected from all participants at recruitment by trained medical staff, using disposable vacuum tubes containing K2-EDTA and tubes with clot activator and separator gel. For serum preparation, samples were allowed to clot for 30 minutes at room temperature and then centrifuged at 4000 rpm for 10 minutes. The following parameters were measured: lipid profile (total cholesterol, high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C], and triglycerides); glucose metabolism (fasting glucose and insulin); liver function (alanine aminotransferase [ALT], aspartate aminotransferase [AST], and total and direct bilirubin); and C-reactive protein (CRP). Assays were conducted at the commercial clinical diagnostic laboratory "Olymp" to ensure standardized, reliable measurements, using both internal and external quality controls.

2.3.5 Biomarker and epigenetic analysis

In a further subset, blood adiponectin, adiponectin receptor expression, and methylation status of the ADIPOQ gene promoter were measured via ELISA, qPCR, and methylation-specific PCR/restriction analysis. The detailed protocol can be found in our published work [107,p. 262].

The manufacturer's instructions were followed to measure serum adiponectin concentrations using ELISA (Sigma-Aldrich, RAB0005). The QIAamp DNA Micro Kit (Qiagen, 56304) was employed to extract genomic DNA from peripheral bloods. Agarose gel electrophoresis and spectrophotometry were employed to assess the quality and quantity of DNA. The EpiJET kit (Thermo Scientific, K1461) was employed to perform bisulfite conversion with two µg of DNA per reaction, adhering to the manufacturer's thermal cycling protocol. The modified DNA was purified and stored at a temperature of -20°C. The methylation of the ADIPOQ promoter CpG site at −74 nt (E-box) was analyzed by MSP using primers that are specific for bisulfite-converted DNA (SibEnzyme). PCR was performed on a Mastercycler (Eppendorf) with PCR Master Mix (Sigma-Aldrich, 4351376) in 50 µL reactions (0.4 µM primers) under the following cycling conditions: 95 °C for 4 min; 40 cycles of 94 °C for 1 min, 53 °C for 15 s, 72 °C for 1 min; and a final extension at 72 °C for 10 min. For methylation-sensitive restriction analysis, 20 U of HspAI (SibEnzyme, E069) was applied to 50 µg/mL DNA under 37 °C conditions for three hours. Primers were developed to include at least one HspA I site within the amplicon. Beta-globin functioned as an internal control for amplification. The PCR products were separated on a 1% agarose gel (TopVision, Fermentas) in TAE buffer (5 V/cm for 1 hour), stained with ethidium bromide, and visualized under UV light using a Gel Doc XR+. HspAI cleaved unmethylated DNA, thereby preventing amplification, while methylated DNA

remained unaltered. The methylation frequency was determined by the percentage of samples that exhibited detectable promoter methylation. ELISA (MyBioSource, MBS2701389; MBS764392) was employed to quantify the protein levels of AdipoR1 and AdipoR2 in PBMC lysates. The PBMC lysates were prepared in RIPA buffer, clarified by centrifugation at 10,000 × g for 10 minutes, and analyzed in accordance with the kit's procedures. Protein concentrations were determined from standard curves and normalized to total protein content, and absorbance at 450 nm was measured using a Synergy H1 microplate reader.

2.3.6 Multiplex cytokine analysis

Multiplex cytokine analysis using Milliplex (MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel – Premixed 41 Plex, immunological multiplex analysis HCYTMAG-60K-PX41, Merck) was employed to assess levels of interferon-gamma (IFN-gamma), interferon-alpha (IFN-α2), interleukins (IL) IL-1α IL-1β IL-1ra IL-2 IL-3 IL-4 IL-5 IL-6 IL-7 IL-8 IL-9 IL-10 IL-12 (p40) IL-12 (p70) IL-13 IL-15 IL-17A, tumor necrosis factor-alpha (TNF-alpha), beta (TNF-β), monocyte chemoattractant protein-1 (MCP-1), protein-3 (MCP-3), IP-10, MDC (CCL22), MIP-1α, MIP-1β, PDGF-AA, PDGF-AB/BB, RANTES, sCD40L, EGF, Eotaxin/CCL11, FGF-2, Flt-3 ligand, Fractalkine, G-CSF, GM-CSF, GRO. Before the study, plasma blood samples were collected from each participant, centrifuged at 3000 rpm for 20 minutes, and stored at -80 °C. The analysis setup procedure was conducted following the manufacturer's protocols. Specifically, 25 µg of plasma (diluted 1:2) were incubated with conjugated antibody-coated magnetic beads for 12 hours at 4°C. After washing, bead complexes were incubated with 50 µL of biotinylated detection antibody for 30 minutes, with the plates being shaken at room temperature. Then, 50 µL of streptavidin-phycoerythrin was added and incubated for 30 minutes on a plate shaker at (20–25 °C). After three washes, 100 µL of sheath fluid was added to all examined samples. The bound bead complexes were counted using the FLEXMAP 3D System with the xPONENT 4.0 software.

2.3.7 Dietary assessment

A semi-quantitative food frequency questionnaire (FFQ) was administered to all participants or their caregivers. It assessed the frequency and portion size of major food groups (meat, fish, dairy, fruits and vegetables, cereals, fats) over the previous year.

FFQ is a tool designed to measure a participant's typical food intake over the previous year. It consists of two parts: the main part, which asks about the frequency of consumption of 130 commonly and less commonly consumed foods; and the second part, which asks additional questions about specific types of food. In the first part, participants are instructed to indicate how often they typically consume each item on the list, selecting from 9 frequency categories ranging from "never or less than once per month" to "6 or more times per day." The questionnaire specifies serving sizes in units or commonly used portions, such as one apple or a slice of bread, as well as household measures, including a glass, cup, or spoon. Each food item on the list has been assigned an average portion size that is the same for all participants, regardless of

age or gender. The second section is dedicated to questions regarding the specific brand and type of breakfast cereal, the quantity and type of milk and cooking fat consumed. These questions relate to the food products in the first section and help estimate overall product consumption.

Adherence to neuroprotective dietary patterns was quantified using the MIND (Mediterranean-DASH Intervention for Neurodegenerative Delay) scoring system. FFQ items were mapped to the 15 MIND components (10 brain-healthy and 5 brain-unhealthy groups). For each component, participants were assigned a score of 0, 0.5, or 1 based on their reported consumption frequency. The total MIND score was calculated as the sum of these components, ranging from 0 to 15, with higher scores indicating higher adherence to the MIND diet.

To facilitate the case-control comparison, the study population was divided into tertiles based on the distribution of total MIND scores. This approach allowed for the categorization of participants into "low," "moderate," and "high" adherence groups relative to the cohort's specific dietary baseline. Multivariable logistic regression was employed to estimate Odds Ratios (ORs) and 95% Confidence Intervals (CIs), with the "Low Adherence" tertile as the reference group. Models were adjusted for potential confounders, including age, sex, and educational attainment.

The FETA system (University of Cambridge) converts the consumption frequency into a multiplier representing a portion size, such as once a week being equal to 1/7 or 0.14. The average daily weight of 130 items in the FFQ can be obtained by multiplying each item's portion size by this value. The nutrient composition per gram is then used to determine the actual nutrient intake based on the weight calculated. Nutrient intake is calculated for each participant by summing the values for all items in the FFQ. The data enabled the calculation of dietary patterns (such as the MIND diet) and nutrient intakes, and allowed for categorization of diet quality.

2.3.8 Gut and oral microbiome analysis

Participants self-collected fecal and oral samples using validated home kits (Human Microbiome Project Core Protocol, ZymoBIOMICS RNA/DNA Shield Collection kits); DNA was extracted, and bacterial communities were characterized via 16S rRNA sequencing (Illumina platform; V3–V4 hypervariable regions). Bioinformatic pipelines (LotuS2, QIIME) were used for OTU assignment, taxonomic classification, and diversity analyses. Swabs were collected from multiple oral regions (saliva, gingiva, mucosa, and dental plaque) according to standardized protocols. DNA extraction and 16S rRNA sequencing were carried out similarly for gut samples.

Bacterial DNA was isolated using the QIAamp DNA stool Mini Kit protocol (Qiagen, 51504): 180–220 mg of stool was weighed into a 2-mL microcentrifuge tube and placed on ice. Then, 1.4 ml of ASL buffer was added to each stool sample, and the mixture was vortexed continuously for 1 minute or until the sample was thoroughly homogenized. The resulting suspension was incubated for 5 min at 70°C. The sample was then vortexed for 15 seconds and centrifuged at full speed for 1 minute. Then, transfer 1.2 ml of supernatant to a new 2 ml microcentrifuge tube, add one tablet of InhibitEX to each sample, and immediately vortex. The sample was centrifuged at full

speed for 3 minutes. Then, the entire supernatant was transferred to a new 1.5 ml tube, and the beads were discarded. Centrifuge the sample again at full speed for 3 minutes. Next, add 15 µl of proteinase K to a new 1.5 ml microcentrifuge tube and 200 µl of supernatant to the 1.5 ml microcentrifuge tube containing proteinase K. Then, add 200 µl of buffer AL and vortex for 15 seconds. Incubate the mixture at 70°C for 10 minutes. Add 200 µl ethanol (96-100%) to the lysate and mix by vortexing. Place the spin column in a 2 ml collection tube. Apply the entire lysate to the column and centrifuge at full speed for 1 minute. Add 500 µl AW1 buffer and centrifuge for 1 minute. Add 500 µl AW2 buffer and centrifuge for 3 minutes. Then transfer to a new 1.5 µL tube, add 200 µL AE buffer, and centrifuge for 1 minute.

2.4 Statistical Analysis

2.4.1 Descriptive statistics and regression analysis

The medians with interquartile ranges (IQR) were employed to summarize quantitative variables, which included demographic, clinical, and biochemical variables. The chi-squared test was employed to compare categorical variables, and Fisher's exact test was employed when the expected cell count was less than 5. Correlations were evaluated using Spearman's rank correlation coefficient, and differences in group medians were assessed using the Wilcoxon rank-sum test. The threshold for statistical significance was established at $p<0.05$. Given the large number of comparisons across multiple domains, analyses were organized into hypothesis-driven primary analyses and exploratory analyses. Primary analyses comprised the pre-specified sociodemographic, psychosocial, clinical, and APOE associations. The high-dimensional analyses, such as the 41-plex cytokine panel, dietary nutrients and food groups, gut and oral microbiome taxa, and the pilot GWAS, were treated as exploratory. Within each family, the false discovery rate was controlled using the Benjamini–Hochberg procedure, with adjusted q-values reported alongside nominal p-values. Associations significant only at nominal $p<0.05$ are described as exploratory and are not interpreted as confirmatory. Analyses were conducted using R/RStudio. Univariable and multivariable logistic regression models were built to identify independent associations of sociodemographic, medical, biochemical, genetic, and lifestyle factors with AD. Models were adjusted for potential confounders, including age, gender, education, region, and ethnic group.

2.4.2 GWAS and genetic analysis

Genotyping was performed using a high-density Illumina microarray (Infinium ImmunoArray-24 Bead Chip Kit), generating raw IDAT files for each sample. The Illumina platform was chosen for its wide genomic coverage and proven reliability in large-scale association studies.

Data Processing

Genotype calls were generated using Illumina's Array Analysis CLI software (version 2.1) and its built-in auto-calling algorithm [1,p. 702]. The IDAT files, accompanied by a sample sheet and chip-specific manifest files, were processed to generate GTC files. These files were then converted into VCF format using

ILLUMINA's Array Analysis CLI GTC-to-VCF function [162]. This step required a reference genome, manifest files in BPM and CSV formats, and specific output parameters to produce compressed VCF files for downstream use. Once the individual VCF files were generated, they were merged into a single file using BCFtools [163]. Following this, a custom sample name conversion file was used to update the VCF header to ensure accurate sample identifiers. The merged VCF file was converted into a binary PLINK format, which is required for downstream GWAS analyses. During this conversion, sample and phenotype information were updated, and non-Kazakh samples were excluded based on metadata.

Principal component analysis (PCA) was conducted using PLINK 2.0 [164] to adjust for population stratification. The top ten eigenvectors were retained and used as covariates in subsequent association analyses to control for potential population structure effects.

Statistical association analyses were carried out using logistic regression under various genetic models, including additive, dominant, and recessive models, using PLINK 1.9 and PLINK2 GLM models [164,p. 137]. Covariates derived from PCA were incorporated to ensure robust results. Outputs from these analyses were stored for further visualization and interpretation, including Manhattan plots and other graphical representations.

Quality Control

Quality control measures were applied at both the variant and sample levels using PLINK 2.0 [164,p. 137]. Variants with non-standard names were updated using a curated SNP dictionary. SNPs with custom or ambiguous identifiers were excluded, as were variants with a missing genotype rate exceeding 5%. Additionally, minor allele frequency (MAF) filtering was performed to remove rare variants with a frequency below 0.001. At the sample level, individuals with a call rate < 90% and more than 2% missing genotype data were excluded. Furthermore, related individuals with a PI-HAT value greater than 0.2 were removed to eliminate potential confounding effects due to relatedness.

2.4.3 Microbiome analysis

Microbiome samples were sequenced at Novogene (Beijing, China) according to standard Illumina protocols. Non-bacterial domain OTUs and OTUs with a total read count less than 0.001% of the overall reads across all samples were excluded. All statistical analyses were conducted in R v. 4.1.2 [127,p. 71], while graphics were created using the ggplot2 package in R [128,p. 523]. The feature table, taxonomic assignments, and tree files for the OTU datasets were imported into phyloseq [129,p. 856] for downstream analysis. Alpha diversity metrics (Shannon, Chao1) and beta diversity metrics (UniFrac, Bray-Curtis) were used to evaluate microbiome profiles within and between groups. LefSe, DESeq2, and other differential abundance tests identified taxa that were significantly enriched or depleted in AD cases versus controls and across regions.

2.5 Ethical considerations and data management

All research procedures were conducted in accordance with the Declaration of Helsinki and were approved by the appropriate local institutional review boards (Local Ethics Committee of National Laboratory Astana, protocol No. 05-2020). Prior to enrollment in the study, written informed consent was obtained from all participants or their caregivers, which included consent for the collection of biological samples and subsequent genetic analyzes. All questionnaires used back-translation and local pilot testing for cultural and language adaptation. Cognitive assessments, biosample collection, and laboratory procedures were conducted in accordance with standardized SOPs and were harmonized across sites and years. Field teams received centralized training. Missing data were tracked and managed through double-entry and coded in accordance with international conventions. The data were anonymized and stored securely in accordance with data protection standards. Results have been shared with the scientific community through peer-reviewed publications and data repositories.

3 STUDY RESULTS AND DISCUSSION

3.1 Demographic and clinical characteristics of the study cohort

3.1.1 Comparative analysis of AD and control groups

In total, 177 participants with Alzheimer's disease and 241 cognitively healthy controls were included in the descriptive analysis. The results of the comparison between the Alzheimer's group (n=177) and the control group (n=241) are summarized in tables 1 and 2.

Table 1 – Sociodemographic characteristics of the study cohort

	AD (n=177)	Controls (N=241)	p-value
Continuous, Median (IQR)			
Age (years)	67 (62; 72)	67 (61; 72)	0.966
MMSE score	20 (12; 23)	29 (28; 30)	<0.001
Fasting glucose	5.15 (4.58; 5.87)	4.76 (4.16; 5.5)	<0.001
Categorical variables, n (%)			
Gender, Female	51 (28.8)	81 (33.6)	0.349
Ethnicity			0.654
Kazakh	135 (76.3)	178 (73.9)	
Non-Kazakh	42 (23.7)	63 (26.1)	
Current residency			0.024
Astana	71 (40.1)	70 (29)	
Almaty	106 (59.9)	171 (71)	
Education			0.015
<12 years	39 (23.2)	78 (35.1)	
≥12 years	129 (76.8)	144 (64.9)	
Marital status, Married	92 (54.1)	96 (49.7)	0.466
Place of birth, Urban	105 (62.1)	134 (59.8)	<0.001
Cannot read	52 (30.8)	15 (6.8)	<0.001
Cannot write	68 (40)	8 (4.3)	<0.001
Socially isolated	120 (67)	119 (49)	0.001
Note – Data presented as median (IQR) for continuous variables and n (%) for categorical variables. p-values from Mann-Whitney U test or Fisher's exact test as appropriate			

Table 2 – Clinical and lifestyle characteristics of the study cohort

	AD (n=177)	Controls (N=241)	p-value
1	2	3	4
Continuous, Median (IQR)			
BMI (kg/m^2)	24.6 (22-28.5)	27.1 (24.2; 29.9)	<0.001
Categorical variables, n (%)			
Hypertension	88 (51.8)	147 (65.9)	0.006
Heart disease	37 (21.9)	90 (42.3)	<0.001
Stroke	13 (7.8)	40 (18.2)	0.005

Continuation of table 2

1	2	3	4
Transitory ischemic attack	19 (11.5)	39 (18.8)	0.076
Traumatic brain injury	25 (15)	31 (15)	1.0
Diabetes	14 (8.3)	37 (17.5)	0.013
Depression	78 (46.4)	45 (20.4)	<0.001
Family history of dementia	13 (7.6)	9 (6)	0.731
Past smoking history	43 (25.3)	42 (19.4)	0.210
History of heavy drinking	9 (8.3)	11 (8.5)	1.0
Physically inactive	113 (67.7)	82 (37.6)	< 0.001
APOE ε4 carrier	67 (41.6)	52 (25.5)	0.001
Note – Data presented as median (IQR) for continuous variables and n (%) for categorical variables. p-values from Mann-Whitney U test or Fisher's exact test as appropriate			

The median age was identical in both groups (67 years), confirming successful age-matching. Cognitive performance differed markedly: median MMSE scores were 20 (IQR 12–23) in the AD group compared with 29 (IQR 28–30) in controls ($p < 0.001$), validating the clinical separation between cases and controls. The sample was predominantly female (71.2% in AD, 66.4% in controls), with no significant difference between groups ($p = 0.297$). Marital status was also evenly distributed ($p = 0.405$).

BMI was lower among AD participants (median 24.6 kg/m²) than controls (27.1 kg/m²; $p < 0.001$). While obesity in midlife is a well-established risk factor for dementia, several longitudinal cohorts have shown that weight loss and low BMI in late life may accompany or precede neurodegenerative processes, potentially reflecting sarcopenia, altered metabolism, or prodromal disease rather than protection from dementia. This pattern is consistent with life-course models in which cardiometabolic risk in midlife and catabolic changes in late life exert opposing influences on dementia risk and clinical presentation [82,p. 413].

Education and literacy emerged as prominent social determinants. Notably, education below 12 years was more prevalent among controls (35.1%) than AD cases (23.2%; $p = 0.011$). This apparently paradoxical finding likely reflects the recruitment of controls from clinical settings where individuals with lower education may be over-represented owing to chronic disease management, as well as selective survival effects in the AD group. However, illiteracy showed a strikingly opposite pattern: 30.8% of AD participants could not read and 40.0% could not write, compared with only 6.8% and 4.3% in controls (both $p<0.001$). These findings support the cognitive reserve hypothesis, suggesting that functional literacy—rather than years of formal schooling alone—may be the more relevant proxy for the cognitive reserve that delays the clinical expression of Alzheimer's pathology [87,p. 4].

The Lancet Commission on dementia prevention estimates that limited education in early life contributes a substantial proportion of potentially preventable dementia cases globally. In this context, the high burden of low education and illiteracy among AD cases in Kazakhstan suggests that historical disparities in schooling and literacy, especially for older birth cohorts, may have translated into reduced cognitive reserve and heightened vulnerability to dementia in later life. In the Kazakhstani context, this

suggests that historical educational infrastructure and early-life literacy programs may have long-term public health implications for the aging population. This has important implications for both the interpretation of the current findings and for long-term policy measures aimed at improving educational access across the life course [65,p. 572].

Social isolation, assessed using the Berkman–Syme Social Network Index, was significantly more prevalent in the AD group (66.7%) than among controls (48.5%; $p < 0.001$), supporting the hypothesis that social integration protects against cognitive decline.

Several traditional vascular risk factors were more prevalent in the control group. Hypertension (65.9% vs 51.8%; $p = 0.005$), heart disease (42.3% vs 21.9%; $p < 0.001$), stroke (18.2% vs 7.8%; $p = 0.003$), and diabetes (17.5% vs 8.3%; $p = 0.009$) were all more frequently reported among controls. This pattern likely reflects a combination of selection bias (controls recruited from internal medicine settings), survival bias, underdiagnosis among AD cases, and the well-documented decline in blood pressure that precedes AD onset [87,p. 4]. Several interpretations can be offered for this finding. For example, biases such as survival and selection bias may play a role. Individuals with severe cardiovascular disease may not survive to the age at which AD is diagnosed, or they may be more likely to be excluded from AD studies if they have vascular dementia instead. The control group might represent a population that is more proactive in seeking medical care for chronic conditions, thus receiving more frequent health screenings and maintaining higher cognitive scores through better general health management. Some global studies have noted that blood pressure often drops in the years preceding an Alzheimer's diagnosis, meaning late-life assessments (like this study) may capture lower hypertension rates in AD patients compared to their healthier counterparts, resulting in a reverse causality issue.

Depression was significantly more prevalent in the AD group (46.4% vs 20.4%; $p<0.001$). The results suggest that depression may be a prodrome or risk factor for our AD cohort. The stark difference in depression rates highlights a profound neuropsychiatric link. Depression is often comorbid with cognitive decline. It remains debated whether depression is a primary risk factor that damages the hippocampus over time or a prodromal symptom (early warning sign) of the neurodegenerative process itself. Given the high significance, screening for depressive symptoms in the elderly population of Kazakhstan could serve as a vital tool for early identification of those at high risk of transitioning to AD.

Fasting glucose was also higher in AD cases (median 5.2 vs 4.8 mmol/L; $p<0.001$), indicating greater metabolic disturbance. Total and LDL cholesterol did not differ between groups. Physical inactivity was markedly more prevalent among AD cases (67.7% vs 37.6%; $p < 0.001$), representing one of the strongest observed associations. APOE ε4 carriage was substantially more common among AD cases (41.6% vs 25.5%; $p = 0.001$). Family history of dementia, past smoking, heavy alcohol use, and traumatic brain injury did not differ significantly between groups [36,p. 255]. Neither past smoking history ($p = 0.210$) nor a history of heavy drinking showed a significant association with Alzheimer's status in this sample. This may be due to the relatively low prevalence of these behaviors among the surviving elderly population

sampled, or perhaps because the cognitive impacts of these habits are overshadowed by the more dominant roles of education and social isolation in this specific demographic.

The findings of this study provide a detailed examination of AD risk factors in Kazakhstan. By comparing a cognitively impaired cohort with an age-matched control group, several critical sociodemographic and clinical determinants have emerged, some aligning with global trends and others presenting unique regional paradoxes. This study indicates that for the population of Kazakhstan, education, literacy, and social connectivity appear to be among the most strongly associated modifiable factors with Alzheimer's disease risk in this population. The paradoxical findings regarding cardiovascular health suggest a need for longitudinal studies that track these factors from midlife to late life to better understand regional disease trajectories. Public health strategies in Kazakhstan should prioritize lifelong learning and reducing social isolation among the elderly as primary pillars of dementia prevention.

3.1.2 Geographic and gender distribution of the study cohort

The study population was drawn from Astana (n=277) and Almaty (n=141), permitting exploration of geographic variation in risk profiles (table 3). Overall, a higher proportion of AD cases resided in Astana compared with controls (40.1% vs 29.0%), whereas a larger share of controls resided in Almaty. Place of birth also differed: although most participants currently lived in urban areas, AD cases were less likely than controls to report an urban birthplace, and this disparity was particularly marked in Almaty, where only about one-third of AD cases versus nearly two-thirds of controls were urban-born.

These results point toward the long-term influence of early-life environment and internal migration. Individuals born in rural areas of Kazakhstan historically had poorer access to education, health care, and cognitively demanding occupations than those born in major cities. The over-representation of rural-born individuals among AD cases, therefore, likely reflects cumulative life-course disadvantage, including lower educational attainment and lower cognitive reserve, rather than a direct biological effect of rural residence. This interpretation is consistent with regional analyses from Eastern Europe and Central Asia showing that older adults with rural origins tend to experience higher burdens of loneliness, economic strain, and health-care barriers, all of which may compound dementia risk [(table 3) 165].

Table 3 – Comparison of risk factors between geographic regions

	AD Astana (n=106)	Control Astana (n=171)	p-value	AD Almaty (n=71)	Control Almaty (n=70)	p-value
Continuous, Median (IQR)						
1	2	3	4	5	6	7
Age (years)	68.0 (62.2; 74.0)	67.0 (62.0; 73.0)	0.623	66.0 (62.0; 69.5)	66.5 (61.0; 70.0)	0.814

Continuation of table 3

1	2	3	4	5	6	7
MMSE score	15.0 (7.0; 21.0)	29.0 (28.0; 30.0)	<0.001	22.0 (21.0; 24.0)	30.0 (29.0; 30.0)	<0.001
BMI (kg/m²)	23.6 (22.0; 25.9)	27.4 (24.8; 29.9)	<0.001	25.6 (22.4; 29.3)	26.4 (22.6; 29.8)	0.466
Dichotomous, n (%)						
Gender, Female	76 (71.7)	109 (63.7)	0.172	50 (70.4)	51 (72.9)	0.748
Ethnicity, Kazakh	80 (75.5)	136 (79.5)	0.428	55 (77.5)	42 (60.0)	0.025
Education <12 years	24 (24.0)	74 (46.5)	<0.001	15 (22.1)	4 (6.3)	0.013
Married	55 (54.5)	65 (50.4)	0.540	37 (53.6)	31 (48.4)	0.550
Place of birth, Urban	42 (42.0)	94 (58.8)	0.009	22 (31.9)	40 (62.5)	<0.001
Midlife, Urban	76 (76.8)	103 (72.5)	0.460	52 (75.4)	58 (92.1)	0.010
Cannot read	45 (44.6)	15 (9.6)	<0.001	7 (10.3)	0 (0.0)	0.013
Cannot write	55 (54.5)	7 (5.6)	<0.001	13 (18.8)	1 (1.6)	0.001
Socially isolated	79 (74.5)	90 (52.6)	<0.001	39 (54.9)	27 (38.6)	0.052
Hypertension	48 (47.5)	107 (67.3)	0.002	40 (58.0)	40 (62.5)	0.594
Heart disease	20 (20.0)	65 (43.3)	<0.001	17 (24.6)	25 (39.7)	0.064
Stroke	11 (11.3)	39 (25.0)	0.008	2 (2.9)	1 (1.6)	1.000
TIA	14 (14.6)	33 (22.8)	0.117	5 (7.2)	6 (9.5)	0.636
TBI	12 (12.1)	17 (11.9)	0.956	13 (19.1)	14 (22.6)	0.627
Diabetes	9 (9.0)	24 (16.3)	0.097	5 (7.2)	13 (20.3)	0.028
Depression	37 (37.0)	28 (17.8)	<0.001	41 (60.3)	17 (26.6)	<0.001
Family history dementia	13 (12.9)	9 (10.6)	0.631	0 (0.0)	0 (0.0)	–
Past smoking	21 (20.8)	24 (15.7)	0.297	22 (31.9)	18 (28.6)	0.679
Heavy drinking	7 (10.8)	11 (12.8)	0.704	2 (4.5)	0 (0.0)	0.494
APOE ε4 carrier	44 (48.9)	32 (23.9)	<0.001	23 (32.4)	20 (28.6)	0.622
Physically inactive	71 (72.4)	60 (39.0)	<0.001	42 (60.9)	22 (34.4)	0.002
Note - Continuous variables reported as median (IQR). Categorical variables as n (%)						

Within cities, the profiles of AD cases in Astana and Almaty showed distinct patterns. In Astana, AD participants were characterised by very low education, high illiteracy (44.6% could not read; 54.5% could not write), and pronounced social isolation (74.5% vs 52.6% in controls). In Almaty, education levels were generally higher, but AD cases showed elevated rates of depression (60.3% vs 26.6%) and physical inactivity (60.9% vs 34.4%). The cardiovascular paradox was most prominent in Astana, where controls showed markedly higher rates of hypertension, heart disease, and stroke. In Almaty, total cholesterol (p = 0.013) and LDL cholesterol (p = 0.008) were significantly lower in AD cases than controls, while fasting glucose did not differ [145,p. 24].

Stratification by gender (table 4) revealed that аьmong women, AD cases had markedly higher rates of illiteracy, social isolation, depression, and APOE ε4 carriage. Nearly half of women with AD could not write (41.3% vs 4.8%), and over 70% were socially isolated. Among men, physical inactivity, past smoking (61.2% vs 35.6%; p =

0.005), and fasting glucose (median 5.2 vs 4.7; p=0.002) were the most distinguishing features [165,p. 156].

These findings mirror global and regional evidence that women bear a disproportionate burden of dementia and are more likely to have experienced cumulative socio-economic disadvantage, including reduced access to education and formal employment, across the life course. A recent analysis of age-sex differences in dementia burden in Eastern Europe and Central Asia similarly highlighted that older women are more likely to be widowed, live alone, and experience loneliness, factors that may interact with biological vulnerability to increase dementia risk. In this Kazakh sample, the clustering of low education, social isolation, depression, and APOE ε4 among women with AD suggests a syndemic pattern in which gendered social inequalities and genetic susceptibility together amplify dementia vulnerability [166].

Among men, case-control differences in education and social isolation were less pronounced, but physically inactive and APOE ε4-positive men were over-represented in the AD group. Male AD participants also showed lower BMI and poorer cognitive performance than male controls. Interestingly, a higher proportion of men with AD remained married compared with women with AD, reflecting gendered survival patterns and caregiving roles observed in many settings. This may partially buffer social isolation among male cases. However, the high prevalence of physical inactivity and metabolic risk factors suggests that behavioral and clinical interventions remain crucial for dementia prevention in men [(table 4) [167].

Table 4 - Comparison of risk factors by gender

	AD Female (n=126)	Control Female (n=160)	p	AD Male (n=51)	Control Male (n=81)	p-value
Continuous, Median (IQR)						
1	2	3	4	5	6	7
Age (years)	67.0 (61.0; 71.8)	67.0 (61.0; 72.0)	0.754	68.0 (64.0; 73.5)	68.0 (63.0; 73.0)	0.602
MMSE score	20.0 (11.2; 22.8)	29.0 (28.0; 30.0)	<0.001	20.0 (14.0; 23.0)	29.0 (28.0; 30.0)	<0.001
BMI (kg/m²)	23.9 (21.2; 28.6)	27.3 (24.2; 30.2)	<0.001	25.1 (23.7; 27.5)	26.9 (24.5; 29.3)	0.021
Dichotomous, n (%)						
Ethnicity, Kazakh	97 (77.0)	118 (73.8)	0.530	38 (74.5)	60 (74.1)	0.956
Education <12 years	30 (24.8)	54 (36.5)	0.040	9 (19.1)	24 (32.4)	0.110
Married	53 (43.8)	55 (42.6)	0.852	39 (79.6)	41 (64.1)	0.072
Place of birth, Urban	48 (40.0)	90 (60.4)	<0.001	16 (32.7)	44 (58.7)	0.005
Midlife, Urban	92 (76.7)	105 (76.6)	0.996	36 (75.0)	56 (82.4)	0.336
Cannot read	36 (29.8)	10 (6.8)	<0.001	16 (33.3)	5 (6.8)	<0.001
Cannot write	50 (41.3)	6 (4.8)	<0.001	18 (36.7)	2 (3.2)	<0.001

Continuation of table 4

1	2	3	4	5	6	7
Socially isolated	89 (70.6)	82 (51.2)	<0.001	29 (56.9)	35 (43.2)	0.126
Hypertension	60 (49.6)	99 (66.9)	0.004	28 (57.1)	48 (64.0)	0.443
Heart disease	27 (22.5)	71 (50.7)	<0.001	10 (20.4)	19 (26.0)	0.475
Stroke	7 (6.0)	20 (13.7)	0.040	6 (12.2)	20 (27.0)	0.049
TIA	12 (10.3)	24 (17.3)	0.108	7 (14.6)	15 (21.7)	0.330
TBI	16 (13.4)	16 (12.0)	0.736	9 (18.8)	15 (20.8)	0.780
Diabetes	6 (5.0)	29 (20.9)	<0.001	8 (16.3)	8 (11.1)	0.406
Depression	60 (50.4)	35 (24.0)	<0.001	18 (36.7)	10 (13.3)	0.002
Family history of dementia	10 (8.3)	5 (5.0)	0.337	3 (6.1)	4 (8.2)	1.000
Past smoking	13 (10.7)	16 (11.2)	0.908	30 (61.2)	26 (35.6)	0.005
APOE ε4 carrier	55 (47.0)	35 (25.4)	<0.001	12 (27.3)	17 (25.8)	0.860
Physically inactive	84 (70.0)	60 (41.4)	<0.001	29 (61.7)	22 (30.1)	<0.001
Total cholesterol	5.0 (4.2; 5.5)	4.9 (4.2; 5.7)	0.631	4.4 (3.9; 4.9)	4.6 (3.9; 5.4)	0.281
LDL cholesterol	3.1 (2.4; 3.8)	3.1 (2.6; 3.7)	0.914	2.9 (2.4; 3.5)	3.0 (2.3; 3.7)	0.975
Fasting glucose	5.1 (4.6; 5.8)	4.8 (4.3; 5.6)	0.044	5.2 (4.7; 6.3)	4.7 (3.9; 5.3)	0.002
Note - Continuous variables reported as median (IQR). Categorical variables as n (%)						

3.2 Genetic risk factors: APOE genotype distribution and GWAS findings

3.2.1 APOE genotype and allele frequencies among AD patients and controls

Apolipoprotein E genotype and allele frequencies were analyzed in 177 AD patients and 241 controls from two major regions of Kazakhstan (Almaty and Astana) (tables 5 and 6, figure 5). The distribution of APOE genotypes differed significantly between the AD and control groups (χ^2=16.38, df =5, p=0.006). The ε3/ε3 genotype was the most prevalent in both groups but was substantially more common in controls (68.6%) than in AD patients (52.2%). Conversely, the ε3/ε4 genotype was found in 31.7% of AD patients compared with 22.5% of controls. Notably, the ε4/ε4 homozygous genotype was 4.7 times more frequent in the AD group (9.3%) than in the control group (2.0%).

Table 5 - APOE Genotype distributions in AD and control Groups

Group	ε2/ε2	ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4	n
AD	2 (1.2%)	8 (5.0%)	1 (0.6%)	84 (52.2%)	51 (31.7%)	15 (9.3%)	161
Control	3 (1.5%)	9 (4.4%)	2 (1.0%)	140 (68.6%)	46 (22.5%)	4 (2.0%)	204
Note - χ^2=16.38, df=5, p=0.006							

Allele frequency analysis revealed that the ε4 allele was nearly twice as prevalent in AD patients (25.5%) compared with controls (13.7%), while the ε3 allele was more common in controls (82.1% vs. 70.5%). The ε2 allele showed comparable frequencies between the two groups (4.0% in AD vs. 4.2% in controls), suggesting a neutral role in this population (figure 5)..

Table 6 - APOE allele frequencies in AD and control groups

Group	ε2	ε3	ε4
AD (n=322)	13 (4.0%)	227 (70.5%)	82 (25.5%)
Control (n=408)	17 (4.2%)	335 (82.1%)	56 (13.7%)

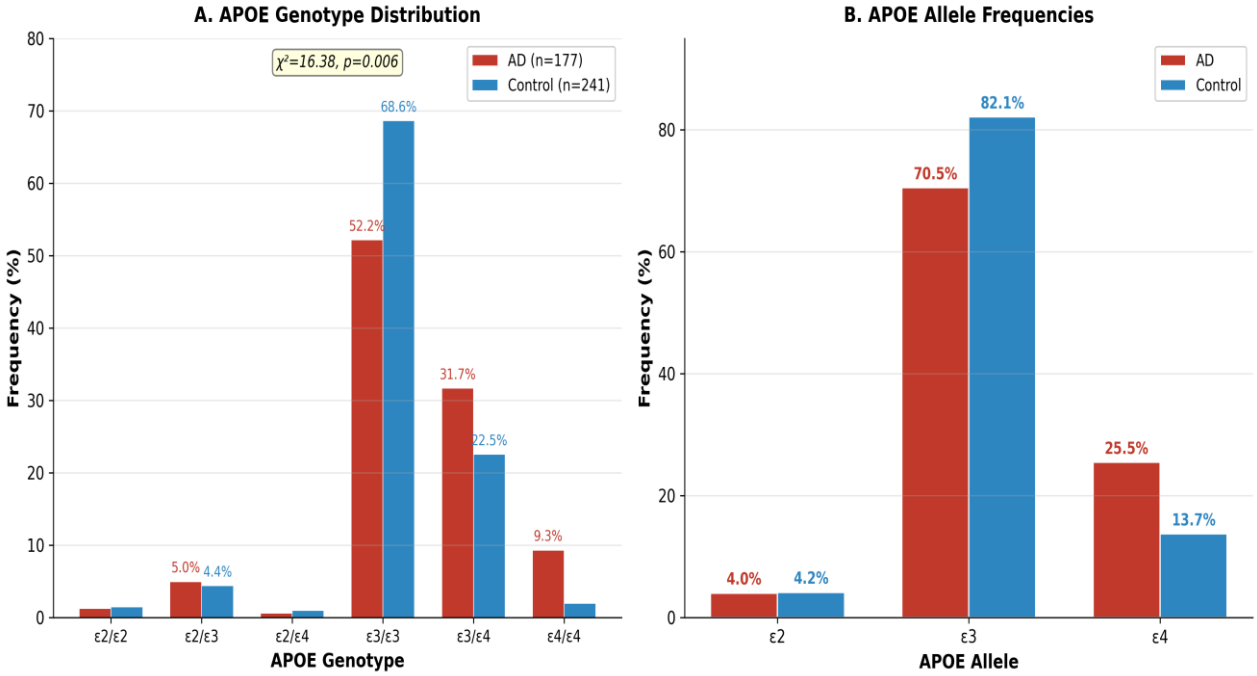


Figure 5 - APOE genotype distribution (A) and allele frequencies (B) in AD and control groups

3.2.2 Regional and gender differences in APOE distribution

Stratified analysis by region revealed a striking geographic heterogeneity in the ε4-AD association. In the Astana (northern) region, the ε4 carrier rate was significantly higher among AD patients (48.9%) than controls (23.9%), yielding an odds ratio of 3.05 (95% CI: 1.72–5.41, p<0.001). In contrast, no significant difference was observed in the Almaty (southern) region (32.4% vs. 28.6%; OR=1.20, p=0.757).

The overall genotype distribution also differed between regions, though not reaching statistical significance (χ^2=8.41, p=0.135). The ε4/ε4 homozygous genotype was more than twice as common in Astana (5.8%) compared with Almaty (4.3%) (figure 6).

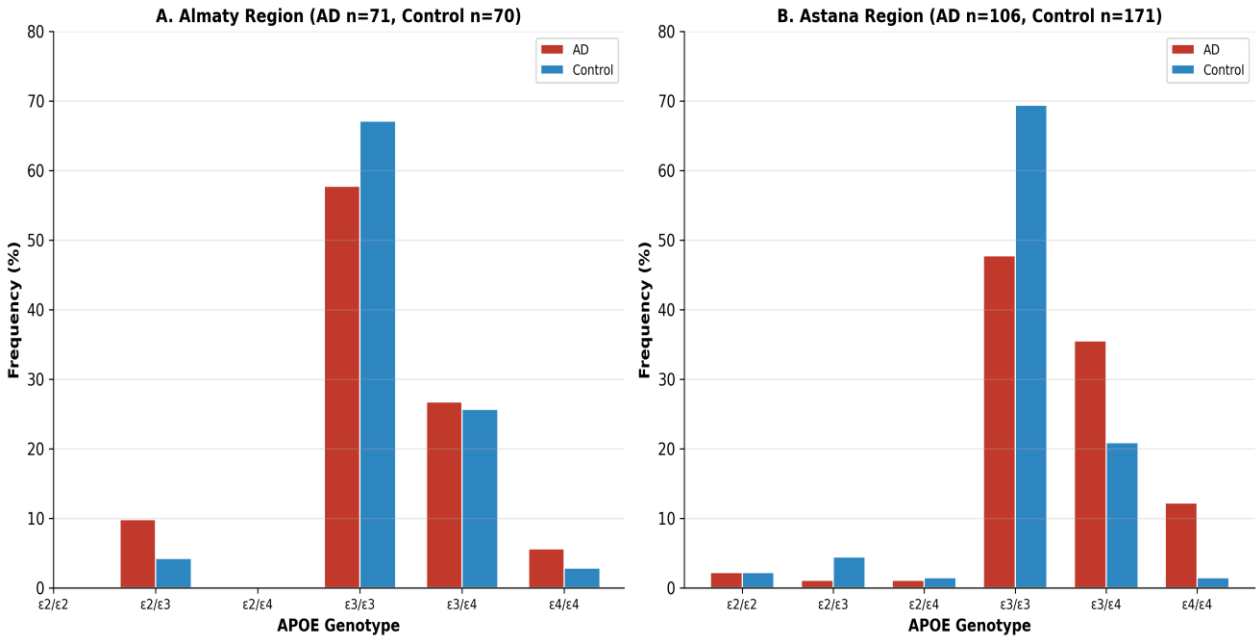


Figure 6 - APOE genotype distribution in the Almaty (A) and Astana (B) regions

The association between ε4 carrier status and AD exhibited a pronounced sex-specific pattern. Among females, ε4 carriers had a significantly elevated AD risk (OR=2.61, 95% CI: 1.54–4.43, p<0.001), with 47.0% of female AD patients being ε4 carriers, compared with 25.4% of female controls. Among males, however, no significant association was detected (OR=1.08, 95% CI: 0.46–2.56, p=1.000), with comparable ε4 carrier rates in both groups (27.3% in AD vs. 25.8% in controls).

The overall genotype distribution did not differ significantly by gender (χ^2=3.90, p=0.565), nor did the ε4 carrier rate (χ^2=2.40, p=0.122). This suggests that the sex difference is specific to the association between ε4 and AD rather than a general difference in ε4 prevalence (figure 7).

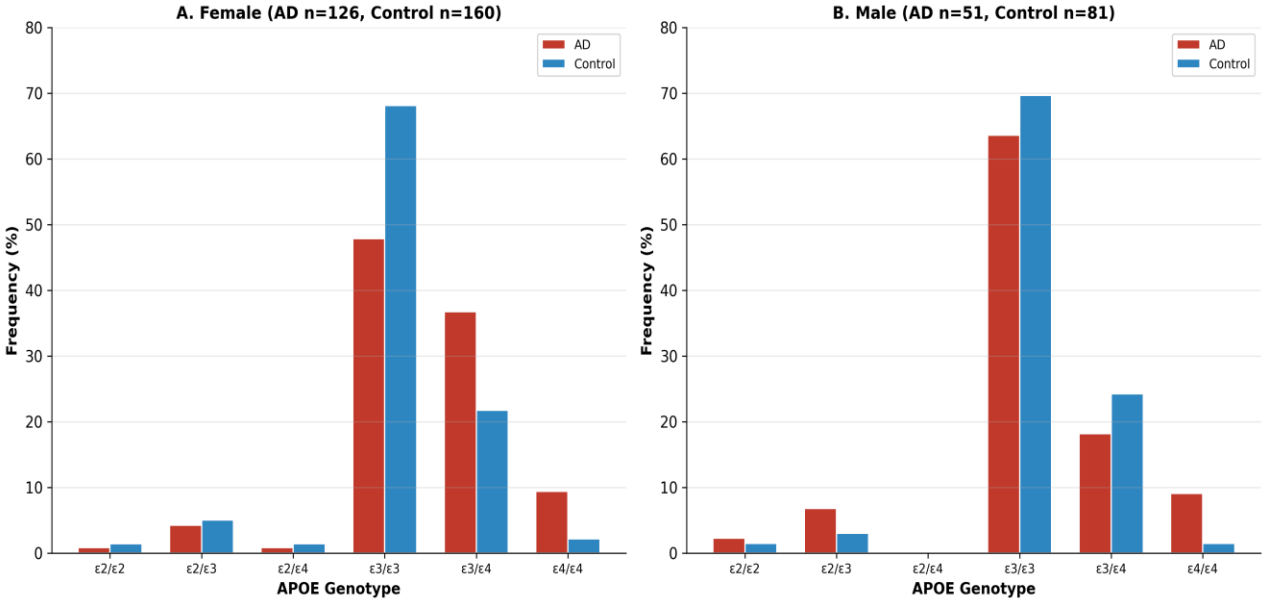


Figure 7 - APOE genotype distribution in females (A) and males (B)

3.2.3 APOE ε4 carrier status and AD risk

Carrying at least one ε4 allele was significantly more common in AD patients (41.6%) than in controls (25.5%), corresponding to an odds ratio of 2.08 (95% CI: 1.34–3.25, p=0.002). Homozygous ε4/ε4 carriers had a markedly elevated risk compared with non-carriers (OR=5.49, 95% CI: 1.79–16.83, p=0.002 (figure 8).

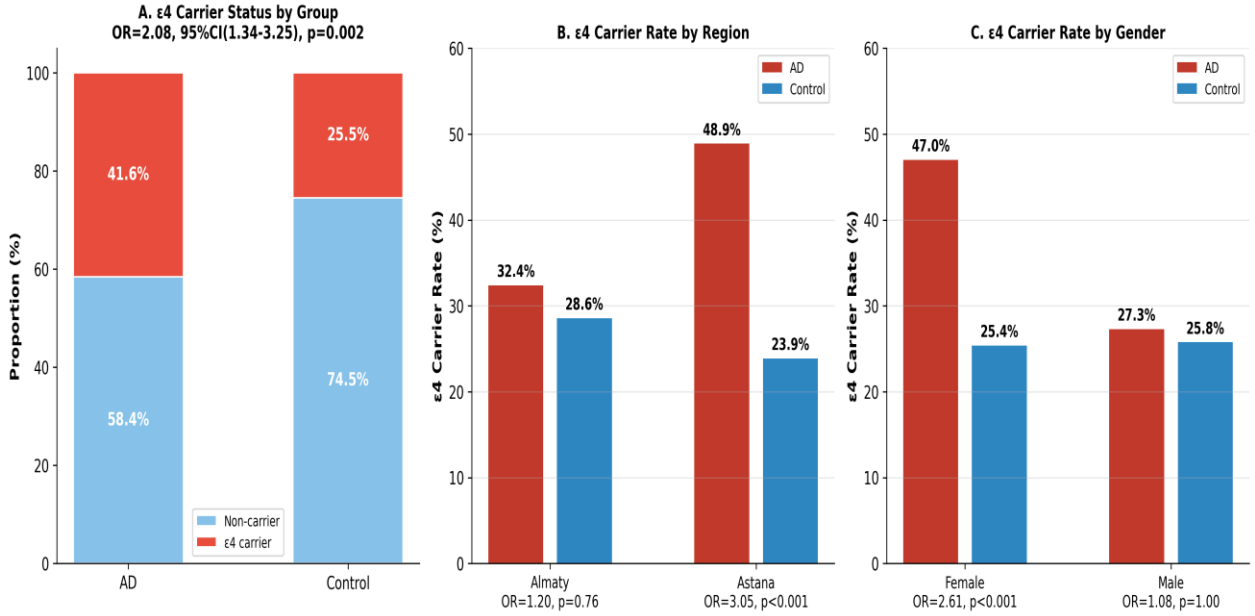


Figure 8 - APOE ε4 carrier rates by group (A), by region (B), and by gender (C)

Table 7 - Association of APOE ε4 carrier status with AD risk by subgroup

Subgroup	AD (ε4+)	Control (ε4+)	OR (95% CI)	χ^2	p
Overall	67/161 (41.6%)	52/204 (25.5%)	2.08 (1.34-3.25)	9.93	0.002
Astana	44/90 (48.9%)	32/134 (23.9%)	3.05 (1.72-5.41)	13.93	<0.001
Almaty	23/71 (32.4%)	20/70 (28.6%)	1.20 (0.58-2.46)	0.10	0.757
Female	55/117 (47.0%)	35/138 (25.4%)	2.61 (1.54-4.43)	12.06	<0.001
Male	12/44 (27.3%)	17/66 (25.8%)	1.08 (0.46-2.56)	0.00	1.000
ε4/ε4 homizygote	15/177 (8.5%)	4/241 (1.7%)	5.49 (1.79-16.83)	9.41	0.002

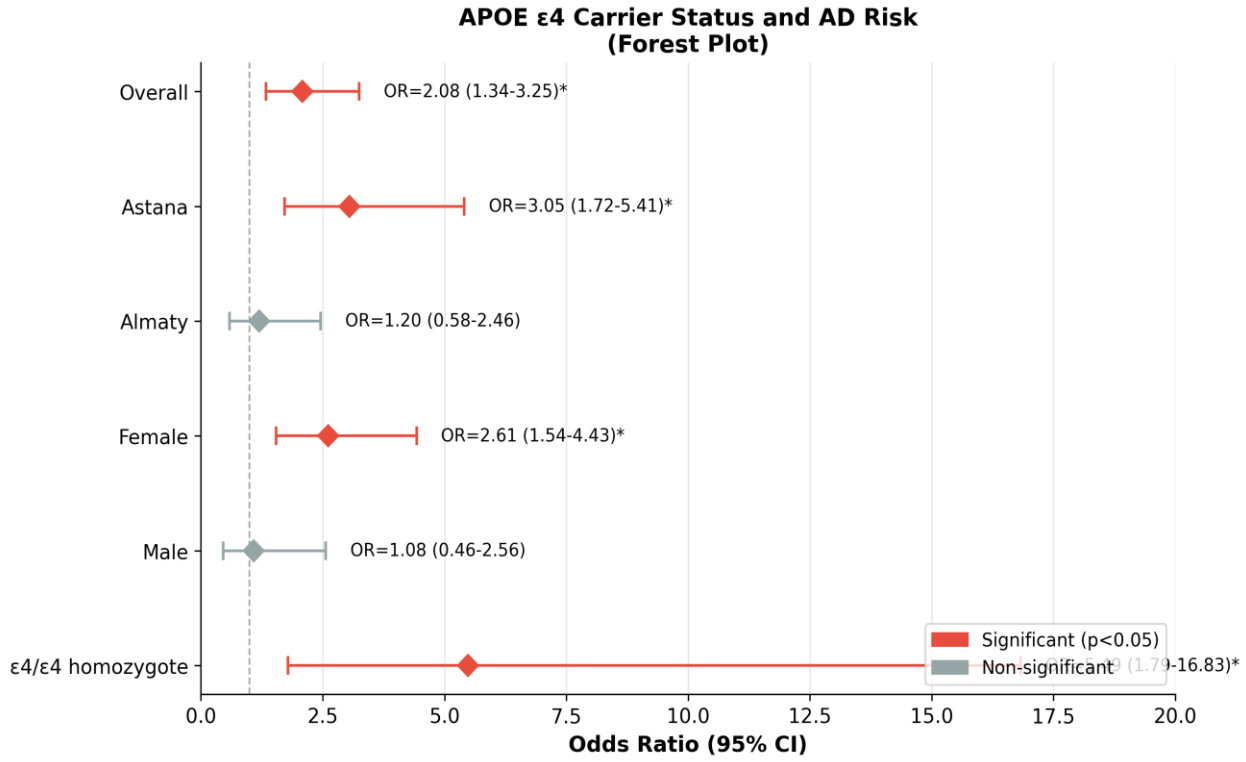


Figure 9 - Forest plot of APOE ε4 carrier status and AD risk across subgroups

APOE4 is a well-studied gene strongly linked to the risk of late-onset AD, and there are worldwide variations in APOE-ε4 carrier rates across regions and ethnicities. The association between APOE genotype and AD has been a consistent topic of interest in genetic epidemiological studies. Present provides a comprehensive analysis of APOE genotype distribution and its association with Alzheimer's disease in a Kazakh cohort from two major cities. The APOE ε4 allele was confirmed as a significant genetic risk factor for AD in this Central Asian population, with an overall odds ratio of 2.08 (95% CI: 1.34–3.25). This finding is consistent with the well-established role of APOE ε4 in AD susceptibility reported globally.

The ε4 allele frequency in our control group (13.7%) falls within the range typically observed in Asian populations (10–25%) and aligns with previously reported estimates for healthy Kazakhs (approximately 16.8%). Among AD patients, the ε4 frequency was 25.5%, consistent with the expectation that ε4 is enriched in AD cohorts worldwide. These frequencies position Kazakhstan between East Asian populations, which tend to have lower ε4 frequencies (approximately 8–15%), and European populations, where ε4 frequencies range from 15–25%. This intermediate position is consistent with the admixed genetic ancestry characteristic of the Kazakh population.

The effect of ε4 was clearly evident: ε4/ε4 homozygotes had a substantially higher risk (OR=5.49) compared with heterozygous ε4 carriers (OR=2.08), supporting a gene-dose model consistent with findings from meta-analyses in European and East Asian populations. The protective or neutral role of the ε2 allele, which showed similar frequencies in both groups (around 4%), is also consistent with prior literature.

A notable finding was pronounced regional heterogeneity in the association between APOE ε4 and AD. The ε4 allele's effect was highly significant in Astana (northern region; OR=3.05, p <0.001) but not in Almaty (southern region; OR=1.20, p=0.757). This pattern is consistent with the well-documented latitudinal gradient in ε4 allele frequency, where ε4 becomes more prevalent at higher latitudes [168]. This gradient has been attributed to evolutionary pressures related to vitamin D metabolism, dietary adaptation, and population history. In China, a similar south-to-north gradient has been reported, with ε4 frequencies positively correlating with latitude [168,p. 489]. Our finding of a stronger association in Astana aligns with previous observations in this cohort. It may reflect both higher baseline ε4 frequency and potentially different environmental or lifestyle factors between the two regions. Astana, located at a higher latitude than Almaty, may present environmental conditions, such as lower ultraviolet exposure and distinct dietary patterns, that interact with the ε4 genotype to modify AD risk. This interaction between genetic susceptibility and environmental context warrants further investigation in prospective studies.

The sex-stratified analysis revealed a striking interaction between gender and the ε4-AD association. The ε4 allele was a strong risk factor in women (OR=2.61, p < 0.001) but showed no significant effect in men (OR=1.08, p=1.000). This sexual dimorphism is consistent with accumulating evidence from worldwide studies demonstrating that women bearing the ε4 allele have a disproportionately higher risk of developing AD compared with men. This disparity has been linked to sex-specific hormonal interactions, particularly the decline of estrogen during menopause, which may exacerbate APOE ε4-mediated pathological mechanisms, including impaired amyloid clearance and increased neuroinflammation.

In East Asian populations specifically, the odds ratio for AD with the ε4 allele has been reported to be higher than in Europeans, and some studies have attributed this to population-specific regulatory variants such as rs405509 in the APOE promoter region, which may reduce APOE expression. Whether similar regulatory mechanisms operate in the Kazakh population remains to be explored through targeted sequencing studies.

3.2.4 Analysis of pilot GWAS data: findings and novel candidate loci

The pilot analysis demonstrates a bioinformatics pipeline that processes Illumina IDAT files from an Alzheimer's study into a format ready for genome-wide association studies. The analysis included steps for genotype calling, quality control, variant annotation, and statistical association testing. Key tools employed in this workflow were Array Analysis CLI software [162,p. 2], BCFtools [163,p. 2987], PLINK1.9 and PLINK 2.0 [164,p. 137], and custom scripts for data manipulation and visualization. This process aims to ensure a high-quality dataset for investigating genetic associations with Alzheimer's disease.

The initial dataset included 288 individuals genotyped using a high-density SNP array. Genotyping quality was assessed through call rates, and samples with low call rates (<90%) or excessive missing data (>2%) were removed to maintain data integrity. Variant-level quality control involved filtering out SNPs with high missingness (>5%), very low minor allele frequencies (MAF <0.001), and ambiguous identifiers. To reduce

confounding from familial relationships, samples with high pairwise kinship coefficients (PI-HAT > 0.2) were also excluded.

Following QC, 180 high-quality, unrelated individuals were retained for GWAS analysis. Association testing was performed using PLINK logistic regression under additive, dominant, recessive, and genotypic models, with the top ten principal components used as covariates to correct for population stratification.

The pilot genome-wide association analysis of 180 post-quality-control samples did not identify any SNPs reaching the conventional genome-wide significance threshold ($p < 5\times10^{-8}$), as expected given the modest sample size. However, several suggestive associations ($p < 1\times10^{-5}$) were identified in biologically plausible candidate genes [169].

Among the 180 post-quality control samples, the AD group consisted of 92 individuals (mean age = 67.77 ± 8.04 years; 73.9% female). The control group included 88 individuals (mean age = 67.15 ± 7.51 years; 67% female). In the overall cohort, 94 participants (52.2%) were from Almaty, and 86 participants (47.8%) were from Astana.

The results of association testing are visualized in a Manhattan plot in figure 10. SNPs with the highest p-values are presented in table 8 with the genes with which they are associated.

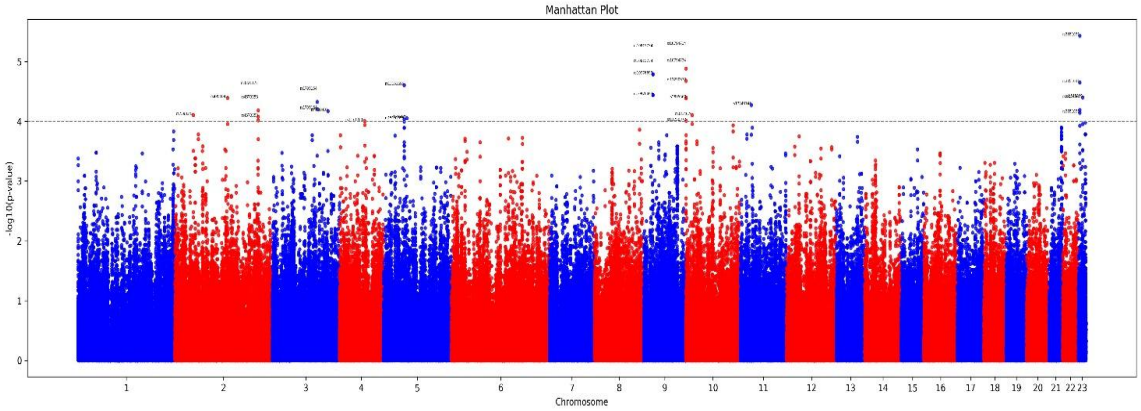


Figure 10 - Manhattan plot displaying the results of the GWAS for AD in Kazakh patients

These SNPs represent the top signals in this Kazakh GWAS for Alzheimer's disease, with several mapping to candidate genes of biological relevance [(table 8).

Table 8 - SNPs with the highest significance level

SNP	CHR	A1	A2	TEST	P-value	Gene name
1	2	3	4	5	6	7
rs10794734	10	C	G	DOM	1.32E-05	ADARB2,LINC00200
rs10794734	10	C	G	GENO	4.05E-05	ADARB2,LINC00200
rs10815734	9	T	G	ALLELIC	3.63E-05	9:7971791
rs10815734	9	T	G	TREND	1.63E-05	9:7971791
rs10976693	9	G	C	ALLELIC	3.63E-05	9:7970004
rs10976693	9	G	C	TREND	1.63E-05	9:7970004

Continuation of table 8

1	2	3	4	5	6	7
rs11250372	10	A	G	DOM	2.11E-05	ADARB2,LINC00200
rs11250372	10	A	G	GENO	9.83E-05	ADARB2,LINC00200
rs11732603	4	T	C	ALLELIC	9.88E-05	4:107750567
rs13161696	5	A	C	ALLELIC	2.48E-05	MAST4
rs13161696	5	A	C	TREND	9.30E-05	MAST4
rs1545469	23	T	G	ALLELIC	3.98E-05	X:44259663
rs17243748	11	T	A	ALLELIC	5.34E-05	11:26862113
rs1795164	3	T	C	ALLELIC	4.74E-05	NEPRO-AS1
rs1795164	3	T	C	TREND	6.24E-05	NEPRO-AS1
rs2805560	10	A	G	REC	4.09E-05	ADARB2,LINC00200
rs3776479	5	T	C	GENO	8.86E-05	ENC1
rs3814676	10	T	C	TREND	7.86E-05	10:8083642
rs3821172	2	G	C	TREND	9.67E-05	ADAM23
rs3851031	23	G	A	ALLELIC	6.46E-05	Y:1803878
rs3851031	23	G	A	DOM	3.72E-06	Y:1803878
rs3851031	23	G	A	TREND	7.22E-05	Y:1803878
rs3851031	23	G	A	GENO	2.25E-05	Y:1803878
rs4673358	2	C	T	ALLELIC	6.57E-05	ADAM23
rs4673358	2	C	T	TREND	8.44E-05	ADAM23
rs4680401	3	A	G	REC	6.78E-05	ENSG00000242536
rs4954095	2	T	C	GENO	4.06E-05	2:133971477
rs7590342	2	A	G	DOM	7.86E-05	LINC01122,ENSG00000285673

Multiple SNPs (rs10794734, rs11250372, rs2805560) mapped to ADARB2, an adenosine deaminase gene, involved in RNA editing. ADARB2 has been previously linked to accelerated cognitive decline during the transition from mild cognitive impairment (MCI) to AD [170]. The identification of this gene in an independent Central Asian population is consistent with a potential role in AD and, if replicated, may indicate relevance across ethnic backgrounds.

The SNP rs13161696 was located in MAST4 (microtubule-associated serine/threonine kinase family member 4), a gene that has recently emerged as a potential neuroprotective factor [171]. Mutations in MAST4 have been associated with structural hippocampal abnormalities and myoclonic epilepsy, both of which are features relevant to AD. The identification of this gene in our GWAS is hypothesis-generating and warrants replication in larger cohorts.

SNPs rs3821172 and rs4673358 were identified in ADAM23 (a disintegrin and metalloproteinase domain 23), a gene previously implicated in AD through its role in synaptic function and cell adhesion [172]. ADAM23 depletion has been shown to increase glutamine synthetase activity in astrocytoma cells, linking it to astrocytic glutamate metabolism, a pathway increasingly recognized as contributing to neurodegeneration. The SNP rs7590342 in LINC01122, a long intergenic non-coding RNA, has previously been reported to be associated with preserved cognitive function,

suggesting that this locus may harbor regulatory variants that influence cognitive resilience [173].

This GWAS was limited by sample size, which reduces statistical power and the ability to detect small effect sizes characteristic of polygenic traits such as AD. The lack of genome-wide significant associations likely reflects this constraint. Although these GWAS findings require replication in larger cohorts, they are noteworthy as some of the first genome-wide association data from a Central Asian population. The suggestive signals in ADARB2, MAST4, ADAM23, and LINC01122 provide candidate loci for targeted follow-up studies and contribute to the growing body of evidence that both shared and population-specific genetic factors underlie AD susceptibility across global populations. Expanding such efforts will not only provide insights into population-specific mechanisms of AD but also contribute to a more inclusive and globally representative understanding of the genetic architecture of neurodegenerative diseases.

3.3 Biochemical biomarkers and metabolic profiles associated with AD

Biochemical and metabolic markers were measured in 418 participants: 177 with Alzheimer's disease (AD) and 241 cognitively healthy controls from Astana (n=277) and Almaty (n=141), and the summary is presented in table 9.

Table 9 - Comparison of key biochemical biomarkers between the AD and control groups

Parameter	AD, median (IQR)	Control, median (IQR)	p-value
Glucose, mmol/L	5.15 (4.58–5.87)	4.76 (4.17–5.50)	<0.001*
Insulin, μU/mL	9.35 (6.90–16.18)	11.30 (7.40–19.90)	0.132
Cholesterol, mmol/L	4.82 (4.08–5.33)	4.81 (4.16–5.70)	0.411
HDL-C, mmol/L	1.29 (1.06–1.60)	1.27 (1.03–1.55)	0.310
LDL-C, mmol/L	3.01 (2.41–3.72)	3.06 (2.50–3.67)	0.995
Triglycerides, mmol/L	1.15 (0.85–1.46)	1.33 (0.93–1.96)	0.003*
Atherogenic Index	2.70 (2.00–3.42)	2.70 (2.00–3.70)	0.393
Adiponectin, μg/mL	19.12 (10.12–30.75)	6.33 (2.85–12.88)	<0.001*
CRP, mg/L	1.30 (0.45–3.35)	1.70 (0.70–4.22)	0.043*
Total Bilirubin, μmol/L	7.60 (5.70–10.00)	6.60 (4.90–9.40)	0.011*
Indirect Bilirubin	6.30 (3.83–9.22)	3.70 (2.20–5.50)	<0.001*
ALT, U/L	12.50 (9.30–17.62)	15.80 (10.97–24.10)	<0.001*
AST, U/L	17.80 (14.90–21.20)	18.60 (15.28–24.57)	0.039*
AST/ALT ratio	1.41 (1.06–1.84)	1.16 (0.92–1.57)	<0.001*
Total Protein, g/L	70.45 (66.57–73.93)	69.90 (66.90–73.70)	0.861
BMI, kg/m²	24.60 (22.04–28.52)	27.14 (24.19–29.94)	<0.001*
Note - Data presented as median (IQR). *Statistically significant ($p < 0.05$, Mann-Whitney U test)			

Glucose Metabolism

Fasting blood glucose was significantly elevated in the AD group, with a median of 5.15 mmol/L (IQR: 4.58–5.87), compared with 4.76 mmol/L (4.17–5.50) in controls ($p<0.001$). This difference persisted in gender-stratified analyses: in females, the

median was 5.08 (4.57–5.78) versus 4.80 (4.28–5.63; p=0.044); in males, 5.19 (4.71–6.34) versus 4.66 (3.87–5.29; p=0.002). In Astana, glucose was significantly higher in AD (4.96 [4.35–5.71] vs. 4.47 [3.98–5.26]; p=0.010), while in Almaty the difference did not reach significance (5.30 vs. 5.21; p=0.223).

Fasting insulin was lower in AD patients (median 9.35 μU/mL [6.90–16.18]) than in controls (11.30 [7.40–19.90]), though not significant overall (p=0.132). A striking regional disparity emerged: in Astana, AD patients had markedly lower insulin (7.95 [6.12–11.85] vs. 12.15 [7.97–20.18]; p<0.001), while no difference was observed in Almaty (p=0.268).

Lipid Profile

Triglycerides were significantly lower in AD: median 1.15 mmol/L (0.85–1.46) versus 1.33 (0.93–1.96) in controls (p=0.003). This was driven by females (1.15 [0.88–1.43] vs. 1.40 [0.91–1.99]; p=0.006) and the Astana subgroup (1.09 [0.82–1.42] vs. 1.38 [0.94–1.96]; p<0.001). The prevalence of hypertriglyceridemia was lower in AD (14.7% vs. 32.8%; p<0.001). Total cholesterol did not differ between AD (4.82 [4.08–5.33]) and controls (4.81 [4.16–5.70]; p=0.411), nor did LDL (3.01 [2.41–3.72] vs. 3.06 [2.50–3.67]; p=0.995) or HDL (1.29 [1.06–1.60] vs. 1.27 [1.03–1.55]; p=0.310). However, in females, HDL was higher in AD (1.42 [1.14–1.71] vs. 1.29 [1.10–1.56]; p=0.039).

Liver Function Markers

ALT was significantly lower in AD: median 12.50 U/L (9.30–17.62) versus 15.80 (10.97–24.10; p<0.001). AST was also lower: 17.80 (14.90–21.20) versus 18.60 (15.28–24.57; p=0.039). The De Ritis ratio (AST/ALT) was elevated in AD: 1.41 (1.06–1.84) versus 1.16 (0.92–1.57; p<0.001). This was significant in females (1.45 [1.06–1.84] vs. 1.16 [0.93–1.64]; p=0.005) but only borderline in males (p=0.075). Total bilirubin was higher in AD: 7.60 μmol/L (5.70–10.00) versus 6.60 (4.90–9.40; p=0.011). Indirect bilirubin was markedly elevated in AD: 6.30 (3.83–9.22) versus 3.70 (2.20–5.50; p<0.001), though based on a smaller subsample (n=20 AD, n=129 controls). Total protein did not differ (p=0.861).

Inflammatory Markers

CRP levels were nominally lower in AD (median 1.30 mg/L [0.45–3.35]) than controls (1.70 [0.70–4.22]; p=0.043 by Mann-Whitney), though both distributions exhibited substantial positive skewness

Within the AD group, MMSE correlated positively with HDL (ρ=0.196, p=0.015), BMI (ρ=0.210, p=0.010), and total protein (ρ=0.217, p=0.030). In females, protein (ρ=0.292, p=0.010) and adiponectin (ρ=0.354, p=0.003) also correlated with MMSE. In males, triglycerides showed the strongest association (ρ=0.365, p=0.015). Within AD, no biochemical biomarker differed significantly between ε4 carriers and non-carriers (all p > 0.05), indicating that the metabolic alterations are independent of APOE ε4 genotype.

3.3.1 Regional and gender disparities in biochemical profiles

In the subgroup analysis, controls differed significantly between cities. Almaty controls had higher glucose (5.21 [4.76–5.86] vs. 4.47 [3.98–5.26]; p<0.001),

cholesterol (5.37 [4.62–6.06] vs. 4.65 [3.99–5.39]; p<0.001), HDL (1.39 vs. 1.25; p=0.011), LDL (3.27 vs. 2.88; p=0.014), and bilirubin (8.00 vs. 6.10; p=0.001). Astana controls had higher ALT (16.50 vs. 13.50; p=0.005) and CRP (2.20 vs. 1.20; p=0.019) [(table 10)

Table 10 - Biochemical profile stratified by regions (median and IQR)

Parameter	AD, median (IQR)	Control, median (IQR)	p-value
Astana			
Glucose, mmol/L	4.96 (4.35–5.71)	4.47 (3.98–5.26)	0.010**
Insulin, μU/mL	7.95 (6.12–11.85)	12.15 (7.97–20.18)	<0.001***
Cholesterol, mmol/L	4.73 (3.83–5.47)	4.65 (3.99–5.39)	0.866
HDL-C, mmol/L	1.22 (0.97–1.46)	1.25 (1.01–1.48)	0.803
LDL-C, mmol/L	3.22 (2.55–3.88)	2.88 (2.46–3.66)	0.066
Triglycerides, mmol/L	1.09 (0.82–1.42)	1.38 (0.94–1.96)	<0.001***
Atherogenic Index	2.80 (2.20–3.50)	2.50 (1.90–3.70)	0.368
ALT, U/L	13.09 (10.50–19.99)	16.50 (11.85–27.00)	0.001**
AST, U/L	17.70 (14.00–20.10)	18.60 (15.25–25.90)	0.020*
AST/ALT ratio	1.41 (0.91–1.65)	1.14 (0.89–1.45)	0.032*
Bilirubin, μmol/L	7.00 (5.30–9.70)	6.10 (4.30–8.80)	0.066
CRP, mg/L	1.10 (0.50–3.50)	2.20 (0.90–4.60)	0.083
Total Protein, g/L	67.10 (63.05–70.50)	68.70 (64.17–70.95)	0.417
BMI, kg/m²	23.62 (22.04–25.88)	27.43 (24.81–29.94)	<0.001***
Adiponectin, μg/mL	19.12 (10.12–30.75)	6.33 (2.85–12.88)	<0.001***
Almaty			
Glucose, mmol/L	5.30 (4.89–6.33)	5.21 (4.76–5.86)	0.223
Insulin, μU/mL	10.25 (8.07–25.12)	9.30 (6.50–18.50)	0.268
Cholesterol, mmol/L	4.89 (4.33–5.26)	5.37 (4.62–6.06)	0.013*
HDL-C, mmol/L	1.42 (1.19–1.67)	1.39 (1.08–1.67)	0.463
LDL-C, mmol/L	2.79 (2.36–3.46)	3.27 (2.84–3.74)	0.008**
Triglycerides, mmol/L	1.17 (0.91–1.50)	1.26 (0.93–1.90)	0.460
Atherogenic Index	2.30 (1.80–3.12)	3.10 (2.20–3.60)	0.011*
ALT, U/L	12.50 (8.12–16.42)	13.50 (9.80–19.90)	0.101
AST, U/L	17.90 (15.78–21.82)	18.50 (15.90–22.60)	0.729
AST/ALT Ratio	1.47 (1.12–2.16)	1.37 (0.98–1.75)	0.081
Bilirubin, μmol/L	8.10 (6.20–10.38)	8.00 (5.67–10.72)	0.544
CRP, mg/L	1.40 (0.40–3.23)	1.20 (0.60–3.30)	0.873
Total Protein, g/L	72.00 (67.88–74.85)	71.50 (67.70–74.50)	0.991
BMI, kg/m²	25.60 (22.35–29.30)	26.43 (22.55–29.83)	0.466
Adiponectin, μg/mL			
Note - *p<0.05, **p<0.01, ***p<0.001. Mann-Whitney U test. Adiponectin data available for Astana cohort only			

In Astana, the metabolic signature of AD was dominated by lower BMI, lower triglycerides, lower insulin, lower transaminases, and elevated glucose. The logistic regression model for Astana achieved 82.1% accuracy. In Almaty, the distinguishing features were lower cholesterol and LDL, with no significant differences in glucose, triglycerides, insulin, or BMI, and model accuracy was only 64.1%.

In females, a broader set of biomarkers differentiated AD from controls: glucose, ALT, HDL, triglycerides, BMI, the ALT/AST ratio, and adiponectin. In males, fewer reached significance: glucose, ALT, BMI, and adiponectin; triglycerides, HDL, and the De Ritis ratio did not. The gender-specific reversal of the adiponectin-MMSE relationship (positive in females, negative in males) is a previously unreported observation that requires replication, given the small male subgroup [(table 11)

Table 11 - Biochemical profile stratified by gender (median and IQR)

Parameter	AD, median (IQR)	Control, median (IQR)	p-value
Females			
Glucose, mmol/L	5.08 (4.57–5.78)	4.80 (4.28–5.63)	0.044*
Insulin, μU/mL	9.25 (6.91–16.08)	11.00 (7.10–20.70)	0.122
Cholesterol, mmol/L	4.97 (4.15–5.52)	4.93 (4.23–5.74)	0.631
HDL-C, mmol/L	1.42 (1.14–1.71)	1.29 (1.10–1.56)	0.039*
LDL-C, mmol/L	3.07 (2.41–3.82)	3.10 (2.56–3.67)	0.914
Triglycerides, mmol/L	1.15 (0.88–1.43)	1.40 (0.91–1.99)	0.006**
Atherogenic Index	2.40 (1.90–3.20)	2.70 (2.00–3.60)	0.078
ALT, U/L	13.09 (9.60–17.50)	15.80 (11.07–23.32)	0.001**
AST, U/L	18.20 (15.30–21.33)	18.65 (15.45–24.02)	0.196
AST/ALT ratio	1.45 (1.06–1.84)	1.16 (0.93–1.64)	0.005**
Bilirubin, μmol/L	7.50 (5.60–9.60)	6.60 (4.80–9.35)	0.052
CRP, mg/L	1.30 (0.40–3.60)	1.75 (0.70–4.10)	0.159
Total Protein, g/L	69.80 (66.30–73.90)	69.90 (66.97–73.95)	0.559
BMI, kg/m²	23.92 (21.17–28.57)	27.34 (24.17–30.21)	<0.001***
Adiponectin, μg/mL	20.19 (10.80–37.80)	7.20 (3.10–13.99)	<0.001***
Males			
Glucose, mmol/L	5.19 (4.71–6.34)	4.66 (3.87–5.29)	0.002**
Insulin, μU/mL	9.85 (7.10–19.48)	11.30 (8.05–17.75)	0.714
Cholesterol, mmol/L	4.45 (3.87–4.95)	4.64 (3.90–5.37)	0.281
HDL-C, mmol/L	1.16 (0.95–1.29)	1.23 (0.98–1.47)	0.186
LDL-C, mmol/L	2.93 (2.43–3.48)	3.01 (2.27–3.67)	0.975
Triglycerides, mmol/L	1.17 (0.79–1.49)	1.22 (0.95–1.77)	0.217
Atherogenic Index	3.05 (2.48–3.60)	2.70 (2.00–4.00)	0.331
ALT, U/L	11.80 (8.45–17.82)	15.85 (10.60–27.18)	0.013*
AST, U/L	17.09 (13.54–20.70)	18.15 (15.07–26.12)	0.051
AST/ALT Ratio	1.41 (0.94–1.80)	1.17 (0.91–1.56)	0.075
Bilirubin, μmol/L	8.31 (6.12–11.80)	6.90 (5.20–9.55)	0.064
CRP, mg/L	1.20 (0.57–2.48)	1.70 (0.70–4.40)	0.138
Total Protein, g/L	71.80 (67.50–73.60)	70.00 (66.90–73.60)	0.584
BMI, kg/m²	25.11 (23.70–27.55)	26.86 (24.53–29.34)	0.021*
Adiponectin, μg/mL	17.98 (10.02–24.13)	5.58 (2.60–12.44)	<0.001***
Note - *p<0.05, **p<0.01, ***p<0.001. Mann-Whitney U test			

3.4 Adiponectin levels, gene methylation, and receptor expression in AD

The results presented in this section were published previously and are adapted from Kaiyrlykyzy et al. (2022) [107,p. 262].

Median serum adiponectin was about three times higher in patients with AD than in cognitively healthy controls (19.1 µg/ml vs. 6.2 µg/ml; $p < 0.001$) (figure 11).

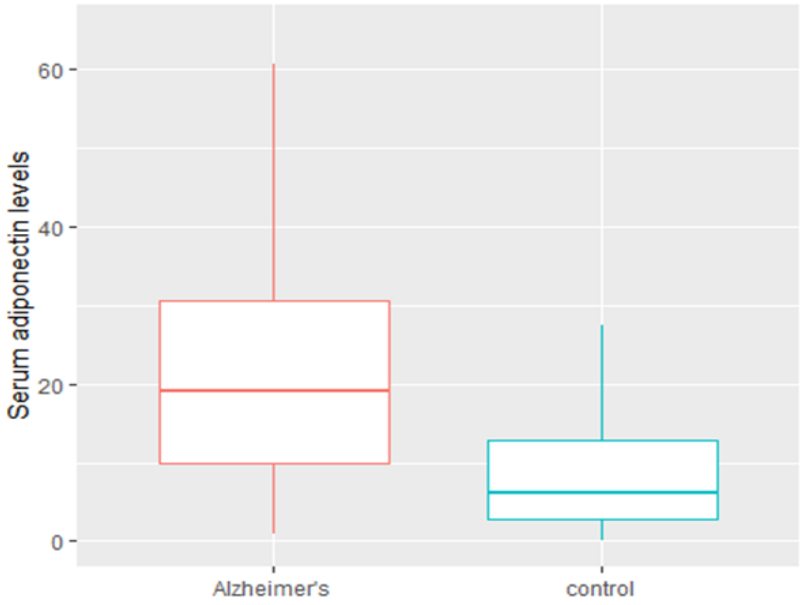


Figure 11 - Serum adiponectin in patients with AD and in controls. Box plots show the median and the lower and upper quartiles ($p < 0.001$)

Note – Adapted from Kaiyrlykyzy et al. (2022) [107,p. 262]

Serum adiponectin was not significantly correlated with fasting glucose, total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), age, or body mass index (BMI). In patients with AD, adiponectin showed weak but statistically significant positive correlations with MMSE score ($r = 0.2$, $p < 0.05$) and with high-density lipoprotein cholesterol (HDL-C) ($r = 0.3$, $p < 0.05$) (figure 12) [107,p. 262].

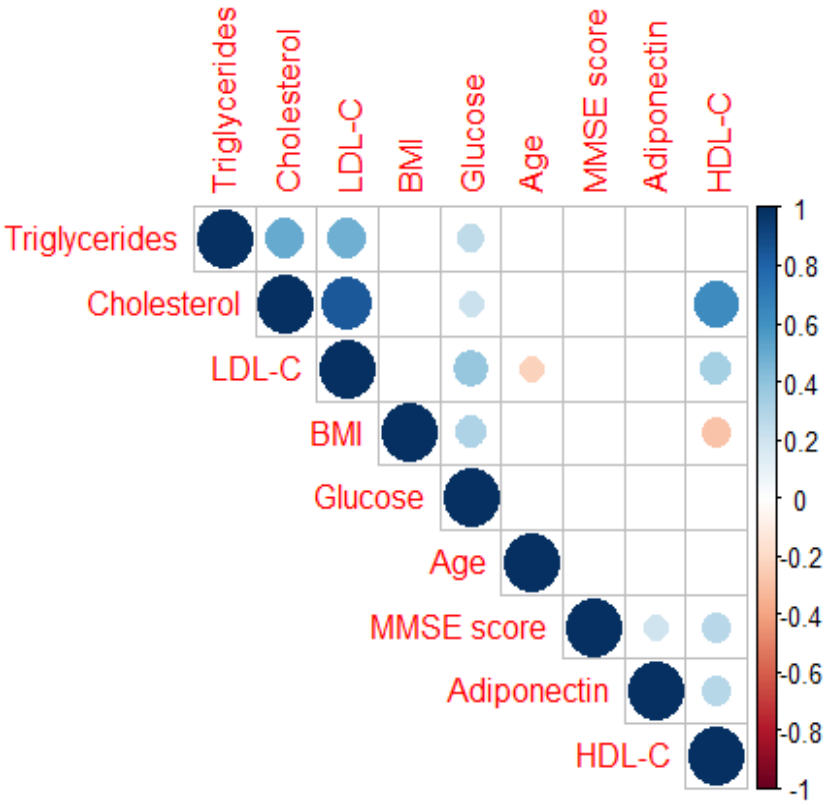


Figure 12 - Spearman correlation heat map of serum adiponectin, total cholesterol, triglycerides, LDL-C, HDL-C, fasting glucose, BMI, and MMSE score in patients with AD. Dark blue cells show self-correlations; significant positive and negative correlations are shown in light blue and orange, respectively; non-significant correlations are omitted

Note - Adapted from Kaiyrlykyzy et al. (2022) [107,p. 262]

Methylation of the ADIPOQ promoter was assessed in 166 participants (62 patients with AD and 104 controls). Methylation of the CpG site at −74 nt was detected in 20.97% of patients with AD (13/62) and in 5.77% of controls (6/104), a frequency about 3.6 times higher in the AD group ($p = 0.006$). Among participants with a methylated promoter, serum adiponectin was significantly higher in patients with AD than in controls ($p = 0.012$). Among participants with an unmethylated promoter, adiponectin did not differ significantly between the groups (figure 13) [107,p. 262].

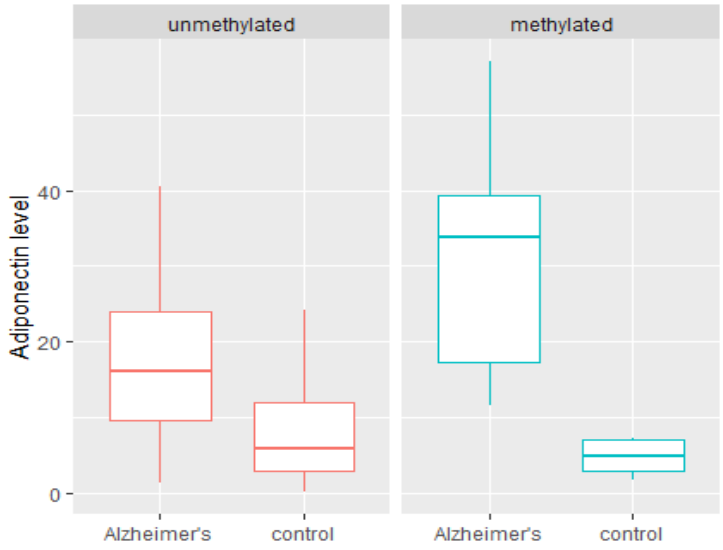


Figure 13 - Serum adiponectin in patients with AD and in controls, stratified by ADIPOQ promoter methylation status. Box plots show the median and the lower and upper quartiles

Note - Adapted from Kaiyrlykyzy et al. (2022) [107,p. 262]

Adiponectin receptor levels were measured by ELISA in lysates of peripheral blood mononuclear cells (PBMCs). In the AD group, median concentrations were 22.8 ng/mg of total protein (IQR 12.6–40.9) for AdipoR1 and 6.17 ng/mg (IQR 4.68–10.6) for AdipoR2; in controls they were 16.6 ng/mg (IQR 7.15–31.5) and 5.61 ng/mg (IQR 3.78–8.75), respectively. AdipoR1 levels were significantly higher in patients with AD ($p = 0.04$), whereas AdipoR2 levels did not differ between the groups ($p = 0.234$) [107,p. 262].

This study examined circulating adiponectin, adiponectin receptor expression in PBMCs, and methylation of the ADIPOQ promoter in relation to AD [107,p. 262]. Findings on adiponectin in AD remain inconsistent. Some studies have reported lower circulating adiponectin in MCI and AD and interpreted this as the loss of a neuroprotective factor [123,p. 115], others have linked higher adiponectin to an increased risk of AD [174], and a recent prospective population-based cohort found no association between serum adiponectin and dementia risk [175]. As we previously published we have observed a higher level of adiponectin in the blood serum of AD subjects compared to controls in our study [107,p. 262]. The higher serum adiponectin we observed in patients with AD agrees with studies that have associated elevated adiponectin with cognitive impairment [176], MCI, and AD [177,178].

Within the AD group, adiponectin correlated positively, though weakly, with MMSE score, so patients with higher adiponectin tended to perform better cognitively. This direction differs from the negative correlation with MMSE reported in another cohort [124,p. 1006]. Because the study was cross-sectional, it cannot show whether the association is causal. Two opposing explanations have been proposed. Elevated adiponectin may be harmful and raise the risk of cognitive impairment; alternatively, adiponectin may rise in people who already carry AD-related neuropathology, even

before symptoms appear, as part of a response against neurodegeneration. The positive correlation with MMSE fits the second explanation better. High serum adiponectin may also reflect adiponectin resistance that develops as the disease progresses, through less efficient receptor signaling, a compensatory response to underlying neurovascular inflammation, or both [179].

At the time of examination, patients with AD had higher serum adiponectin and lower BMI than controls. Because adiponectin has been linked to obesity-associated cognitive decline [180,181], this combination may be relevant to disease pathogenesis. However, weight loss often precedes or accompanies the clinical onset of AD, and midlife body weight was not available for our participants, so firm conclusions about the relationships among adiponectin, BMI, and dementia risk cannot be drawn. The positive correlation between adiponectin and HDL-C agrees with the established role of adiponectin in HDL metabolism [179,p. 153], and with the strong association between the two reported in earlier studies [182,183].

Although the physiological functions of adiponectin are well studied, the mechanisms that control ADIPOQ expression are still not fully understood [184,185]. The transcriptional regulation of the human ADIPOQ promoter region is a multifaceted process that involves numerous regulatory elements, as evidenced by numerous studies [185,p. 41]. Transcription of the human gene depends on many regulatory elements [185,p. 41], and its promoter carries binding sites for PPARG2, LRH, RXR, CEBPA, SREBP1c, TFAP2B, FOXO1, SP1, SIRT1, NCOR1, and NCOR2 [186], The E-box is one of the key promoter regions involved in this regulation [187]. Epigenetic modification of one E-box, the CpG site at −74 nt, has been associated with metabolic disorders in adolescents [144,p. 1457]: methylation at this site was less common in obese and morbidly obese adolescents, whereas its frequency was similar in insulin-resistant and non-insulin-resistant groups. In our study, methylation at the same site was about 3.6 times more frequent in patients with AD than in controls, and among participants with a methylated promoter, patients with AD had higher serum adiponectin. Given the known links between BMI, obesity, adiponectin, and AD [188-190], methylation of the −74 nt CpG site in peripheral blood may be associated with AD.

This result appears to contradict the general view that promoter methylation silences transcription [191]. One possible explanation is that DNA methylation patterns differ between adipose tissue and blood cells, as shown by Ott et al [140,p. 131]. Other studies also indicate that the relationship is not straightforward. In HIV-positive women, greater ADIPOQ promoter methylation was associated with higher adiponectin expression, leading the authors to propose that methylation may in some settings promote gene expression [192]. These authors also propose that the modulation of ADIPOQ mRNA expression is a multifaceted process, and that DNA methylation may even stimulate gene expression [192,p. 248]. In adult offspring of women with diabetes in pregnancy, Houshmand-Oeregaard et al. found reduced ADIPOQ expression together with increased methylation in subcutaneous adipose tissue, but no correlation between this methylation and plasma adiponectin [193]. Similarly, adiponectin secretion by adipose tissue has been shown to relate only weakly to its

serum concentration, which Hoffstedt et al. attributed to the slow turnover and low production rate of the hormone, whose plasma half-life is approximately 2.5 hours [194].

Adiponectin acts through three receptors: AdipoR1 and AdipoR2, which are G protein-coupled receptors, and T-cadherin, a member of the cadherin family [195,196]. AdipoR1 mainly mediates the metabolic effects of adiponectin, while AdipoR2 is additionally involved in its antioxidant and anti-inflammatory actions [179,p. 153]. Several studies have reported direct associations between plasma adiponectin and receptor expression in PBMCs [139,p. 636]. Kollias et al. found reduced plasma adiponectin together with lower AdipoR1 and AdipoR2 expression in monocytes from overweight patients with coronary artery disease [115,p. 14], and Luo et al. showed that overexpression of AdipoR1 in macrophages enhanced adiponectin action [138,p. 124]. In mice, suppression of AdipoR1 caused memory impairment and AD-like pathology [197]. In our study, patients with AD had higher serum adiponectin and higher AdipoR1 levels in PBMCs than controls, whereas AdipoR2 did not differ between the groups [107,p. 262].

These findings may be relevant to the macrophage dysfunction described in AD, particularly the reduced capacity of macrophages to clear Aβ by phagocytosis [198-201]. Polarization of macrophages toward the M1 or M2 phenotype influences how Aβ oligomers are phagocytosed [202,203] and adiponectin itself regulates the polarization of peripheral macrophages [204]. The relationship works in both directions, since the polarization state also determines AdipoR expression and the anti-inflammatory response to adiponectin. In M1 macrophages, adiponectin stimulates the pro-inflammatory cytokines TNF-α, IL-6, and IL-12 and increases AdipoR expression; in M2 macrophages, it induces the anti-inflammatory cytokine IL-10 without affecting AdipoR expression [139,p. 636]. Altered regulation of serum adiponectin and of AdipoR expression in peripheral monocytes may therefore contribute to impaired Aβ phagocytosis and neuroinflammation in AD [107,p. 262].

3.5 Cytokine profiles and systemic inflammation

To characterize the systemic inflammatory environment associated with Alzheimer's disease in this cohort, plasma levels of 41 cytokines, chemokines, and growth factors were measured using a Milliplex MAP Human Cytokine/Chemokine 41-plex magnetic bead panel in 177 AD patients and 241 cognitively healthy controls. Of 41 analytes assessed, 17 showed nominally significant differences between AD patients and controls ($p<0.05$). After FDR correction, two cytokines retained significance at $q<0.05$: IL-1α and IL-17A (table 12). The predominant direction of dysregulation in AD was pro-inflammatory upregulation, with 14 of the 17 nominally significant analytes elevated in the AD group and three reduced (IL-8, EGF, and FGF-2).

Table 12 – Plasma cytokine and chemokine levels in AD patients and cognitively healthy controls

Cytokine	AD median (Q1–Q3)	Control median (Q1–Q3)	p-value
1	2	3	4
Significantly elevated in AD (FDR q<0.05)			
IL-1α	12.96 (6.25–45.77)	6.14 (0.60–24.97)	<0.001*†
IL-17A	1.46 (0.71–2.73)	0.93 (0.00–1.74)	<0.001*†
Nominally elevated in AD (p < 0.05, FDR q > 0.05)			
MCP-1 (CCL2)	644.24 (454.81–985.19)	547.25 (349.01–877.17)	0.0059*
IL-3	0.58 (0.54–0.68)	0.55 (0.13–0.64)	0.0061*
IP-10 (CXCL10)	357.09 (219.99–672.02)	286.59 (141.32–579.61)	0.0121*
IL-15	2.26 (1.13–3.85)	1.74 (0.18–3.89)	0.0136*
TNF-β	4.86 (0.77–26.02)	1.85 (0.00–21.79)	0.0210*
IL-13	2.52 (1.21–25.86)	1.64 (0.00–18.09)	0.0211*
MDC (CCL22)	931.76 (580.33–1421.91)	752.19 (505.93–1220.49)	0.0222*
IFN-α2	25.54 (8.41–44.37)	3.90 (0.00–46.73)	0.0228*
IL-2	1.10 (0.19–2.12)	0.81 (0.00–1.58)	0.0261*
IL-9	1.52 (0.37–2.87)	1.08 (0.07–2.26)	0.0274*
IL-10	3.43 (2.11–14.97)	2.92 (1.68–9.42)	0.0367*
IL-12p40	7.66 (2.20–30.02)	6.03 (1.29–24.13)	0.0473*
Nominally reduced in AD (p<0.05, FDR q > 0.05)			
IL-8	18.80 (10.74–47.64)	33.22 (13.01–92.59)	0.0037*
EGF	110.87 (52.51–230.56)	151.60 (70.74–249.28)	0.0241*
FGF-2	98.98 (51.25–145.54)	108.74 (57.49–235.62)	0.0326*
Not statistically significant (p ≥ 0.05)			
Eotaxin (CCL11)	193.58 (122.85–326.81)	172.57 (114.42–253.61)	0.0577
VEGF	79.11 (10.40–164.03)	60.43 (0.00–139.19)	0.0589
IL-6	2.25 (0.25–10.57)	1.09 (0.00–6.94)	0.0750
IL-12p70	3.01 (1.47–23.45)	2.11 (1.38–13.90)	0.0777
IL-7	6.07 (2.51–15.89)	6.26 (0.81–11.42)	0.0874
MIP-1α (CCL3)	5.09 (0.00–13.93)	6.77 (0.00–23.74)	0.1137
IL-5	1.17 (0.67–3.27)	1.50 (0.87–2.85)	0.1600
MIP-1β (CCL4)	45.74 (28.72–66.52)	50.42 (29.90–76.83)	0.2524
MCP-3 (CCL7)	54.84 (24.20–110.43)	47.03 (20.70–96.59)	0.2769
PDGF-AA	1213.34 (836.17–1979.97)	1377.18 (793.86–2876.19)	0.3175
RANTES (CCL5)	1420.02 (926.67–3703.79)	1569.58 (943.35–7509.58)	0.3206
IL-1β	0.51 (0.24–5.38)	0.55 (0.05–3.43)	0.3296
sCD40L	6216.76 (4596.12–7546.91)	6120.86 (4935.64–8014.99)	0.3441
GRO (CXCL1)	1919.83 (1239.69–2670.72)	1977.43 (1238.75–3474.26)	0.3494
G-CSF	21.55 (7.61–48.27)	18.27 (0.36–52.86)	0.4603
IL-4	17.98 (3.10–100.60)	14.81 (0.87–110.92)	0.4631
Fractalkine (CX3CL1)	77.48 (28.97–189.62)	70.71 (30.63–290.26)	0.4918
TGF-α	5.94 (3.89–8.15)	5.26 (3.15–9.06)	0.5008
IFN-γ	2.73 (1.16–9.75)	2.31 (0.87–19.60)	0.5431

Continuation of table 12

1	2	3	4
GM-CSF	4.11 (1.89–13.81)	4.41 (2.22–11.08)	0.7388
Flt-3L	18.08 (6.58–54.62)	23.02 (5.20–56.17)	0.8278
IL-1RA	12.94 (5.87–27.00)	12.43 (5.44–31.46)	0.8758
TNF-α	14.07 (8.65–30.73)	15.37 (8.57–27.15)	0.9327
PDGF-AB/BB	8747.88 (6028.12–11341.96)	7767.02 (5937.67–11943.75)	0.9972
Note – Data presented as median (Q1–Q3) in pg/mL. Mann-Whitney U test. FDR correction applied across all 41 analytes (Benjamini-Hochberg method). *$p < 0.05$; †$q < 0.05$ after FDR correction. AD: n=181; controls: n=244. Values below the lower limit of detection are treated as missing			

After application of the FDR procedure, IL-1α was elevated approximately 2.1-fold in AD patients compared with controls (median 12.96 pg/mL (IQR 39.52) vs. 6.14 (IQR 24.38); $p = 0.0001$, $q = 0.0015$). IL-1α is a canonical pro-inflammatory cytokine produced by activated monocytes and macrophages, and its elevation in AD is consistent with the documented activation of the NLRP3 inflammasome in the context of amyloid-β deposition and tau pathology. The finding aligns with multiple prior studies demonstrating elevated peripheral IL-1α in AD and mild cognitive impairment cohorts, and suggests that systemic innate immune activation is a feature of AD in the Kazakhstani population.

IL-17A was elevated 1.6-fold in AD (median 1.46 pg/mL [IQR 2.02] vs. 0.93 [IQR 1.74]; $p = 0.0001$, $q = 0.0015$). IL-17A is the signature cytokine of the Th17 lymphocyte lineage and plays a key role in orchestrating chronic inflammation through induction of neutrophil recruitment and amplification of IL-6 and TNF-α signalling. Elevated IL-17A has been reported in AD brain tissue and cerebrospinal fluid, and recent evidence implicates the IL-17 pathway in the disruption of blood–brain barrier integrity and the promotion of neuroinflammatory cascades. The concurrent elevation of both an innate immune mediator (IL-1α) and an adaptive immune effector cytokine (IL-17A) suggests involvement of multiple immunological arms in the systemic inflammatory signature of AD in this cohort.

Among analytes significant at $p < 0.05$ but not surviving FDR correction, several are biologically noteworthy given the broader context of this dissertation. TNF-β (lymphotoxin-α) showed the largest fold-change among nominally significant cytokines (2.6-fold; $p = 0.021$), and IFN-α2 showed a 6.5-fold elevation in AD (median 25.54 vs. 3.90 pg/mL; $p = 0.023$), though both had high inter-individual variability. Elevated MCP-1 (CCL2) in AD patients (median 644.24 vs. 547.25 pg/mL; $p = 0.006$) is consistent with prior reports of increased monocyte chemotaxis in AD and aligns with the microbiome-associated inflammatory pathway identified in section 3.7. MDC (CCL22), IP-10 (CXCL10), and IL-13 were also nominally elevated in AD, consistent with a pattern of mixed Th1/Th2 and chemokine dysregulation. Notably, IL-8 (CXCL8), a potent neutrophil chemoattractant, was nominally lower in AD than controls (median 18.80 vs. 33.22 pg/mL; $p=0.004$), which may reflect neutrophil recruitment to sites of tissue inflammation rather than reduced overall production.

The cytokine profile identified here is mechanistically coherent with the adiponectin findings described in earlier. Adiponectin regulates M1/M2 macrophage polarisation: in M1 macrophages, adiponectin stimulates TNF-α, IL-6, and IL-12 production while increasing adiponectin receptor expression, whereas in M2 macrophages, it induces anti-inflammatory IL-10. The concurrent elevation of adiponectin and pro-inflammatory cytokines (IL-1α, IL-17A, TNF-β) in AD patients is consistent with a model in which elevated adiponectin may reflect a compensatory response to systemic inflammation. The relatively modest but nominally significant elevation of IL-10 in AD (median 3.43 vs. 2.92 pg/mL; p = 0.037) may represent a partially effective counter-regulatory response to the dominant pro-inflammatory signal. Taken together, the cytokine, adiponectin, and microbiome data are consistent with chronic low-grade systemic inflammation as a feature of AD in this population, involving both innate (IL-1α, MCP-1) and adaptive (IL-17A, IFN-α2) immune components.

3.6 Dietary patterns and nutritional risk factors for AD

3.6.1 Nutrient intake profiles in AD and control groups

To characterize the nutritional landscape of AD in Kazakhstan, we first compared daily macro- and micronutrient intake between the AD group (n=106) and controls (n=100) using FFQ data processed with the FETA nutrient analysis tool. The complete results are presented in table 13.

Table 13 - Comparison of daily nutrient intake between AD patients and controls. Values are median (IQR)

Nutrient	AD (n=106)	Control (n=100)	Recommended	p-value
1	2	3	4	5
Energy and Macronutrients				
Energy (kcal)	1696 (1152; 2168)	2019 (1424; 2515)	1600–2400	0.007
Carbohydrate (g)	209 (146; 262)	235 (172; 298)	130	0.022
Protein (g)	74.9 (55.6; 99.2)	90.4 (65.7; 111)	46–56	0.009
Total fat (g)	65.6 (43.3; 92.9)	79.7 (54.2; 101)	44–78	0.021
MUFA (g)	22.1 (15.8; 32.8)	27.9 (18.3; 37.5)	33–44	0.02
PUFA (g)	9.73 (6.41; 15.1)	12.9 (9.32; 17.2)	11–22	0.006
SFA (g)	26.1 (17.5; 35.6)	30.5 (20.9; 39.6)	<22	0.07
Starch (g)	110 (82.8; 141)	130 (96.7; 180)	—	0.006
NSP fiber (g)	13.5 (9.33; 17.7)	15.5 (10.7; 21.9)	—	0.006
Cholesterol (mg)	321 (217; 431)	427 (301; 538)	<300	0.0003
Vitamins				
Folate (mcg)	199 (153; 256)	270 (181; 357)	400	<0.0001
Vitamin A eq (mcg)	1130 (737; 1780)	1470 (957; 2294)	700–900	0.003
Vitamin B1 (mg)	1.20 (0.852; 1.55)	1.46 (0.978; 1.91)	1.1–1.2	0.002
Vitamin B2 (mg)	1.41 (1.00; 1.74)	1.79 (1.22; 2.19)	1.1–1.3	<0.0001
Vitamin B6 (mg)	1.72 (1.36; 2.24)	2.19 (1.40; 2.74)	1.5–1.7	0.005
Vitamin B12 (mcg)	5.76 (3.71; 8.43)	7.55 (5.36; 11.1)	2.4	0.0003

Continuation of table 13

1	2	3	4	5
Vitamin C (mg)	56.3 (37.6; 88.3)	83.0 (50.2; 118)	75–90	0.003
Vitamin D (mcg)	1.92 (1.22; 2.48)	2.31 (1.57; 3.39)	15–20	0.002
Vitamin E (mg)	7.32 (5.50; 11.5)	9.95 (6.55; 13.0)	15	0.006
Niacin (mg)	17.0 (12.5; 22.8)	21.1 (14.8; 28.4)	14–16	0.001
Beta-carotene (mcg)	2329 (969; 4075)	3210 (2089; 4247)	—	0.03
Minerals and Electrolytes				
Calcium (mg)	673 (471; 837)	811 (583; 999)	1000–1200	0.005
Iron (mg)	10.2 (7.56; 12.6)	12.7 (9.33; 17.5)	8	0.0001
Iodine (mcg)	92.0 (60.6; 117)	111 (79.0; 138)	150	0.001
Magnesium (mg)	252 (178; 305)	290 (214; 384)	320–420	0.0015
Selenium (mcg)	56.3 (43.4; 69.6)	71.9 (54.4; 92.7)	55	<0.0001
Zinc (mg)	9.56 (7.02; 12.3)	12.2 (8.37; 15.8)	8–11	0.0004
Potassium (mg)	2609 (1862; 3135)	3175 (2192; 3896)	2600–3400	0.0005
Phosphorus (mg)	1181 (892; 1471)	1464 (1020; 1826)	700	0.0007
Copper (mg)	1.14 (0.828; 1.50)	1.51 (1.03; 1.99)	0.9	0.0004
Manganese (mg)	3.29 (2.43; 4.20)	3.90 (2.94; 5.11)	1.8–2.3	0.002
Sodium (mg)	2849 (1799; 3533)	3224 (2398; 4230)	1500	0.004
Chloride (mg)	4188 (2728; 5283)	4812 (3660; 6254)	1800–2000	0.004
Other				
Alcohol (g)	0 (0; 0)	0 (0; 0.793)	0–28	0.0004

Note - Mann-Whitney U test. Bold p-values indicate statistical significance ($p < 0.05$). Recommended values from RDA (National Academies), 2020–2025 Dietary Guidelines for Americans, and American Heart Association. IQR = interquartile range; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; NSP = non-starch polysaccharide

3.6.1.1 Macronutrient intake

Total daily energy intake was significantly lower in the AD group compared to controls (1696 vs. 2019 kcal/day, p=0.007), a finding consistent with the well-documented decline in appetite and caloric consumption that accompanies cognitive deterioration. This energy deficit was reflected across all three macronutrient classes: carbohydrates (209 vs. 235 g/day, p=0.022), protein (74.9 vs. 90.4 g/day, p=0.009), and total fat (65.6 vs. 79.7 g/day, p=0.021). Sub-analysis of carbohydrate fractions revealed that the groups differed specifically in starch (p=0.006), NSP fiber (p=0.006), and lactose (p=0.03) consumption, while total sugars, fructose, glucose, and sucrose did not differ significantly. Among fats, the most notable disparities were in MUFA (22.1 vs. 27.9 g/day, p=0.02) and PUFA (9.73 vs. 12.9 g/day, p=0.006), both of which have established neuroprotective properties, whereas saturated fat intake did not differ between groups (p=0.07).

3.6.1.2 Micronutrient intake

Micronutrient analysis revealed pervasive differences between groups: of 26 micronutrients assessed, 25 were significantly lower in the AD group ($p<0.05$), spanning B-vitamins (B1, B2, B6, B12, folate, niacin), antioxidant vitamins (C, D, E, beta-carotene), essential minerals (calcium, iron, magnesium, potassium, selenium, zinc, copper, manganese, iodine, phosphorus), and electrolytes (sodium, chloride). The most pronounced deficits were in folate (AD: 199 vs. Control: 270 mcg/day, $p<0.0001$), selenium (56.3 vs. 71.9 mcg/day, $p<0.0001$), vitamin B2 (1.41 vs. 1.79 mg/day, $p<0.0001$), and iron (10.2 vs. 12.7 mg/day, $p=0.0001$). Cholesterol intake was also significantly lower in the AD group (321 vs. 427 mg/day, $p=0.0003$), as was alcohol consumption ($p=0.0004$), with the majority of AD patients reporting no alcohol intake.

3.6.1.3 Regional variation in nutrient intake

To account for potential geographic confounding, a four-group comparison was conducted across study sites (AD Astana, Control Astana, AD Almaty, Control Almaty). This regional stratification revealed an important finding: while macronutrient differences (carbohydrates, protein, fat) were significant in the pooled two-group comparison, they did not remain significant within either city when analyzed separately (all $p>0.05$). This suggests that the macronutrient differences observed in the pooled analysis were partly driven by inter-city variation rather than disease-specific effects.

Micronutrient differences, however, persisted within cities but with distinct regional signatures. In Astana, significant AD vs. control differences were observed for alpha-carotene, chloride, iron, manganese, niacin, potassium, starch, NSP fiber, vitamins B1 and B6, selenium, and zinc. These nutrients are predominantly concentrated in whole grains, potatoes, fish, nuts, and fruits. In Almaty, the significant differences were in copper, iodine, lactose, vitamins B2 and B12, as well as alcohol and cholesterol, which are primarily found in dairy products, fish, organ meats, and vegetables. Folate and selenium showed significant AD vs control differences in both cities, suggesting a more universal association with cognitive status. Importantly, no differences in nutrient intake were found between the two AD groups (AD Astana vs. AD Almaty), suggesting that dietary patterns among individuals with AD are relatively uniform across Kazakhstan, despite regional dietary diversity among cognitively healthy adults.

3.6.1.4 Food group comparison

Comparison of 14 food group categories revealed significant differences in 9 categories in the pooled analysis (table 14). AD patients consumed significantly less meat and meat products (112 vs. 144 g/day, $p=0.001$), fish (19.2 vs. 27.2 g/day, $p=0.002$), vegetables (162 vs. 224 g/day, $p=0.004$), non-alcoholic beverages ($p=0.004$), potatoes ($p=0.04$), eggs ($p=0.005$), fats and oils ($p=0.04$), soups and sauces ($p=0.02$), and alcoholic beverages ($p=0.0003$). No significant differences were observed for fruit, milk products, nuts and seeds, cereals, or sugars and snacks.

Table 14 - Daily food group consumption: AD vs. controls with adequacy assessment

Food Group	AD (n=106)	Control (n=100)	Recommended	p-value	AD status	Ctrl status
Meat/meat products	112 (60; 156)	144 (104; 196)	46–65	0.001	↑	↑
Cereals/cereal products	310 (228; 378)	314 (226; 419)	120–240	0.22	↑	↑
Fish/fish products	19.2 (8.8; 31.4)	27.2 (16.0; 39.6)	30	0.002	↓	↓
Vegetables (g)	162 (84; 258)	224 (140; 324)	250–400	0.004	↓	↓
Fruit (g)	149 (83; 242)	156 (78; 249)	225–300	0.6	↓	↓
Eggs/egg dishes	21.5 (7.0; 32.0)	21.5 (7.0; 50.0)	0–44	0.005	✓	✓
Milk/milk products	196 (138; 270)	208 (165; 284)	3 servings	0.14	✓	✓
Nuts/seeds (g)	3.43 (0; 12.9)	4.2 (1.33; 12.9)	28–30	0.64	↓	↓
Potatoes (g)	43.6 (21; 74.8)	65.7 (26.4; 109)	54–81	0.04	↓	✓
Fats/oils (g)	11.3 (5.7; 16.2)	14.2 (7.5; 21.1)	—	0.04	—	—
Soups/sauces (g)	105 (54; 204)	166 (99; 210)	—	0.02	—	—
Non-alcoholic beverages	523 (475; 859)	668 (503; 900)	1900–2000	0.004	↓	↓
Sugars /snacks (g)	34.8 (17; 58.3)	32.3 (16; 66.5)	0–24	0.85	↑	↑
Alcoholic beverages	0 (0; 0)	0 (0; 9.15)	0–28	0.0003	✓	✓
Note - ↑=above recommended; ↓=below recommended; ✓=within recommended range. Mann-Whitney U test. Bold p-values: $p < 0.05$. Values are median (IQR) in grams/day						

3.6.1.5 Dietary quality assessment against recommended guidelines

When nutrient intakes were evaluated against established dietary guidelines, a pattern of concurrent excess and deficiency emerged in both groups (table 1). Both the AD and control groups exceeded recommended levels for carbohydrates, cholesterol, chloride, copper, iron, manganese, sodium, niacin, phosphorus, protein, vitamins B2 and B12, and saturated fat. Simultaneously, both groups were deficient in calcium, folate, iodine, magnesium, and vitamins D and E, nutrients with documented neuroprotective roles. MUFA intake was below recommended thresholds in both groups. Critically, the AD group was additionally deficient in vitamin C (56.3 vs. recommended 75–90 mg) and PUFA (9.73 vs. recommended 11–22 g), while the control group met or exceeded these thresholds. Selenium intake was borderline deficient in the AD group (56.3 vs. recommended 55 mcg) but comfortably adequate in controls (71.9 mcg).

For food groups, both groups consumed excessive amounts of meat products and cereals, while falling short of recommendations for fish, fruit, vegetables, nuts, and non-alcoholic beverages. Potato consumption was deficient in both AD groups but adequate in both control groups. Sugars and snacks exceeded recommended limits

across all groups except the Astana control group. Vegetable consumption reached adequate levels only in the Almaty control group. This dietary pattern is characterized by overconsumption of meat, refined grains, and sugar alongside underconsumption of fish, fruits, vegetables, and nuts and differ substantially from both the Mediterranean and DASH dietary patterns that have demonstrated cognitive benefits in Western populations.

3.6.2 MIND diet adherence and component analysis

3.6.2.1 Overall MIND Diet Adherence

The MIND diet score was calculated for 179 participants (106 AD, 73 controls) with sufficient FFQ data. The diet was divided into 10 brain-healthy food groups (green leafy vegetables, other vegetables, nuts, berries, beans, whole grains, fish, poultry, olive oil, and wine) and 5 brain-unhealthy food groups (red meats, butter/margarine, cheese, pastries/sweets, and fried/fast food). Each component was scored 0, 0.5, or 1, yielding a total score from 0 to 15.Overall adherence was strikingly low across the entire cohort (figure 14). In the AD group, 93.5% were classified as low adherence, with 6.5% achieving moderate adherence. In the control group, 93.0% had low adherence and 7.0% moderate adherence. No participants in either group met the criteria for high adherence. Fisher's exact test confirmed no significant difference between diagnostic groups ($p=1.000$). The overall MIND diet score did not differ between the AD group (median=4.0, mean=4.63±1.68) and controls (median=4.0, mean=4.75±1.64; $U=3711.5$, $p=0.639$).

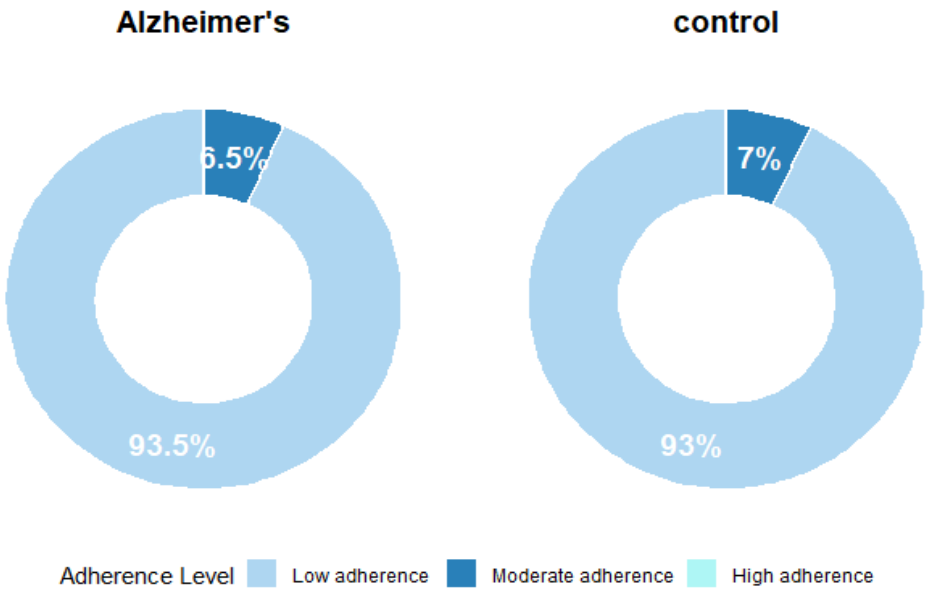


Figure 14 - Distribution of MIND diet adherence levels in AD and control groups. Low (<8), moderate (8–11), high (≥12)

The median score of 4.0 out of 15 is substantially lower than the average of approximately 7.5 reported in the original Rush Memory and Aging Project [205]. Two factors largely account for this gap. First, olive oil and wine (two key MIND components) scored zero for every participant in both groups, reflecting the cultural and dietary reality of Kazakhstan, where these items are rarely consumed. This effectively reduced the functioning scoring range from 15 to 13 points. Second, baseline consumption of berries, fish, nuts, and whole grains was low across the entire sample, reflecting the traditional Central Asian dietary pattern that favors meat, bread, and dairy over plant-based foods. MIND scores did not correlate significantly with either MMSE (ρ=0.085, p=0.256) or Clock Drawing Test (ρ=0.081, p=0.284), and did not differ by sex, education, marital status, ethnicity, or study region.

3.6.2.2 MIND diet component analysis

While the total score failed to differentiate groups, component-level analysis uncovered several significant disparities (table 15). Among brain-healthy food groups, leafy green vegetable consumption was significantly lower in AD patients (0.36 vs. 0.57; p=0.003), as were berries (0.11 vs. 0.23; p=0.035), beans and legumes (0.13 vs. 0.24; p=0.043), and poultry (0.34 vs. 0.49; p=0.023). Fish consumption showed a trend toward lower intake in AD (0.09 vs. 0.18; p=0.085). Among brain-unhealthy components, the red meat score was significantly worse in the AD group (0.38 vs. 0.17; $p < 0.001$), indicating more frequent consumption. Fried/fast food also trended higher in AD (0.44 vs. 0.32; p=0.079). Butter, cheese, and pastry scores were comparable between groups.

Table 15 - MIND diet component scores by groups

Component	AD (mean±SD)	Control (mean±SD)	p-value	Effect	Category
1	2	3	4	5	6
Brain-healthy food groups					
Leafy greens	0.36 ± 0.48	0.57 ± 0.50	0.003*	AD lower	Neuroprotective
Other vegetables	0.86 ± 0.35	0.91 ± 0.29	0.262	–	–
Nuts	0.16 ± 0.37	0.15 ± 0.36	0.837	–	–
Berries	0.11 ± 0.32	0.23 ± 0.42	0.035*	AD lower	Neuroprotective
Beans/legumes	0.13 ± 0.34	0.24 ± 0.43	0.043*	AD lower	Neuroprotective
Whole grains	0.22 ± 0.42	0.33 ± 0.47	0.090	–	–
Fish	0.09 ± 0.29	0.18 ± 0.39	0.085	–	–
Poultry	0.34 ± 0.47	0.49 ± 0.50	0.023*	AD lower	Neuroprotective
Olive oil	0.00	0.00	N/A	Not consumed	–
Wine	0.00	0.00	N/A	Not consumed	–
Brain-unhealthy food groups (higher score=healthier)					
Red / processed meat	0.38 ± 0.49	0.17 ± 0.38	<0.001*	AD higher risk	Harmful
Butter/margarine	0.89 ± 0.32	0.86 ± 0.35	0.548	–	–
Cheese	0.25 ± 0.44	0.24 ± 0.43	0.839	–	–

Continuation of table 15

1	2	3	4	5	6
Pastries/sweets	0.37 ± 0.49	0.40 ± 0.49	0.701	–	–
Fried/fast food	0.44 ± 0.50	0.32 ± 0.47	0.079	–	–
Note - * p < 0.05 (Mann-Whitney U test). For unhealthy groups, lower scores indicate more frequent consumption (less healthy)					

3.6.3 Principal Component Analysis of dietary pattern

To move beyond the Western-derived MIND framework, PCA with varimax rotation was applied to 102 FFQ items among 181 participants (106 AD, 75 controls). Bartlett's test confirmed suitability (χ^2=10,280.5, df=5,151, p<0.001). Six components were retained based on scree plot inspection (figure 15), explaining 33.9% of total dietary variance.

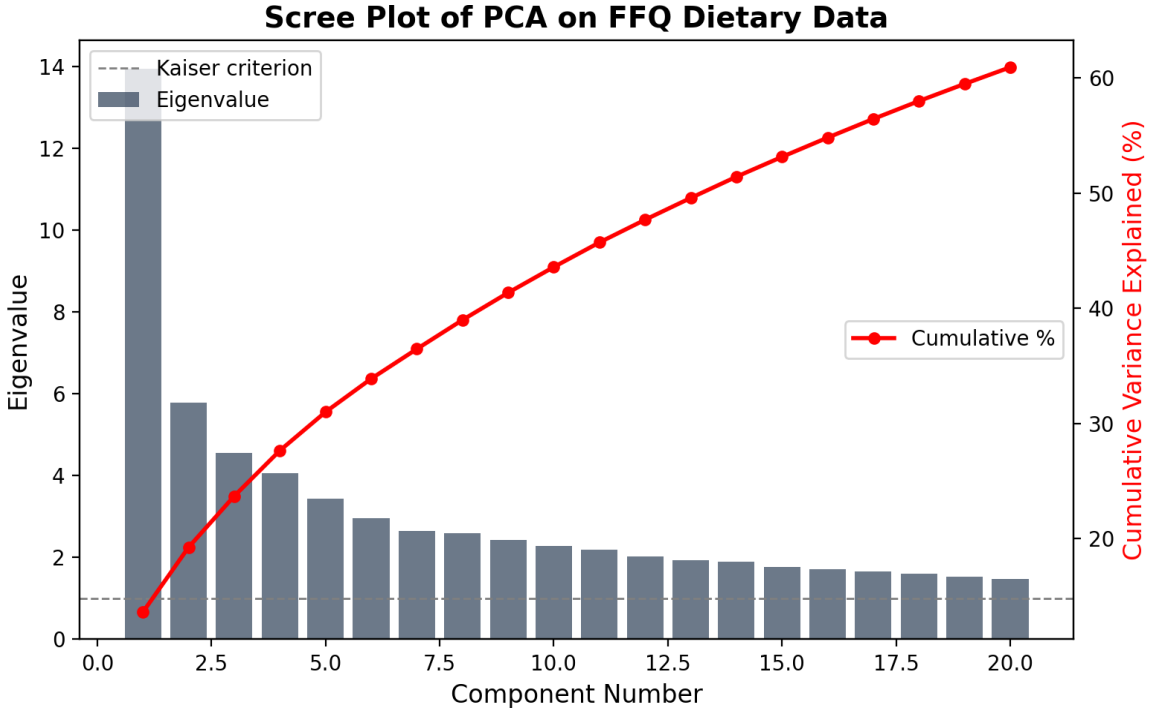


Figure 15 - Scree plot of eigenvalues for the first 20 principal components extracted from FFQ items

Based on which food was loaded most strongly on each component, the following descriptive names to the patterns have been assigned (figure 16):

Pattern 1: Vegetable rich and plant diverse (13.6%): brussels sprouts (+0.73), spinach (+0.72), broccoli (+0.72), avocado (+0.69), lentils (+0.62), tofu (+0.56), bean sprouts (+0.55), beetroot (+0.44). Represents a nutrient-dense, plant-forward dietary pattern rich in leafy greens, cruciferous vegetables, and legumes.

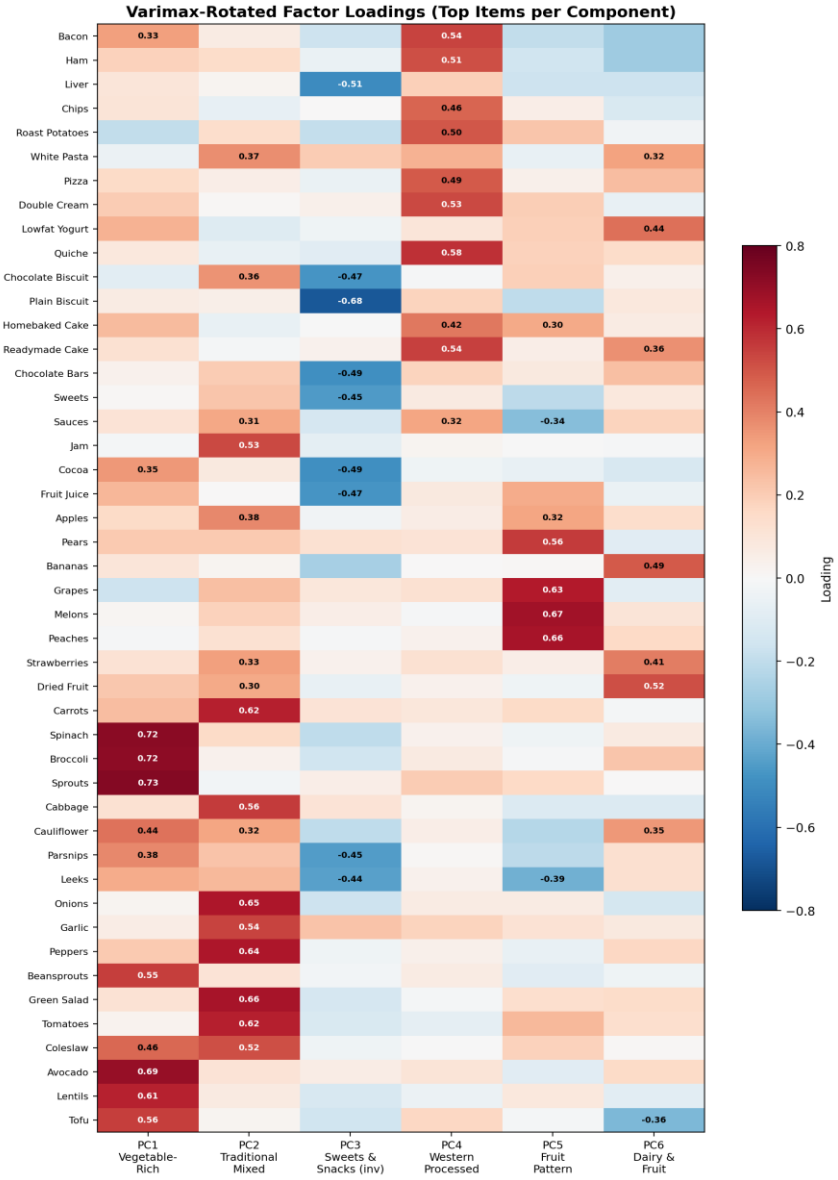
Pattern 2: Traditional mixed and salad (5.6%): green salad (+0.66), onions (+0.65), peppers (+0.65), carrots (+0.62), tomatoes (+0.62), cabbage (+0.56), garlic (+0.54). This pattern reflects everyday local vegetable consumption.

Pattern 3: Sweets and snacks (inverse) (4.5%): biscuits (−0.68), liver (−0.51), cocoa (−0.49), chocolate bars (−0.49), sweets (−0.46).

Pattern 4: Western/processed (4.0%): quiche (+0.58), readymade cake (+0.54), bacon (+0.54), double cream (+0.53), ham (+0.51), pizza (+0.49), chips (+0.47). It captures western-influenced convenience foods.

Pattern 5: Fruit dominant (3.4%): melons (+0.67), peaches (+0.66), grapes (+0.63), pears (+0.56). Seasonal fresh fruit consumption.

Pattern 6: Dairy and dried fruit (2.9%): Dried fruit (+0.52), bananas (+0.49), low-fat yogurt (+0.44), strawberries (+0.41), tea (+0.31) (figure 16).



Values ≥0.30 annotated. Red=positive; blue=negative loadings

Figure 16 - Varimax-rotated factor loading heatmap

Four patterns significantly differentiated AD from controls (figure 17, table 16). The vegetable-rich pattern showed the largest effect (AD: −0.94 ±1.88 vs. Control:

+1.32±3.35; p<0.001, d=−0.87), followed by the sweets and snacks pattern (d=+0.91, p<0.001). In multivariable logistic regression adjusted for age and sex, three patterns independently predicted AD: vegetable-rich (inverse; OR=0.70, 95% CI: 0.58–0.84, p < 0.001), sweets and snacks (positive; OR=1.38, 95% CI: 1.15–1.65, p<0.001), and Fruit (OR=1.33, p=0.004). The full model achieved a pseudo-R^2 of 0.26 (figure 17).

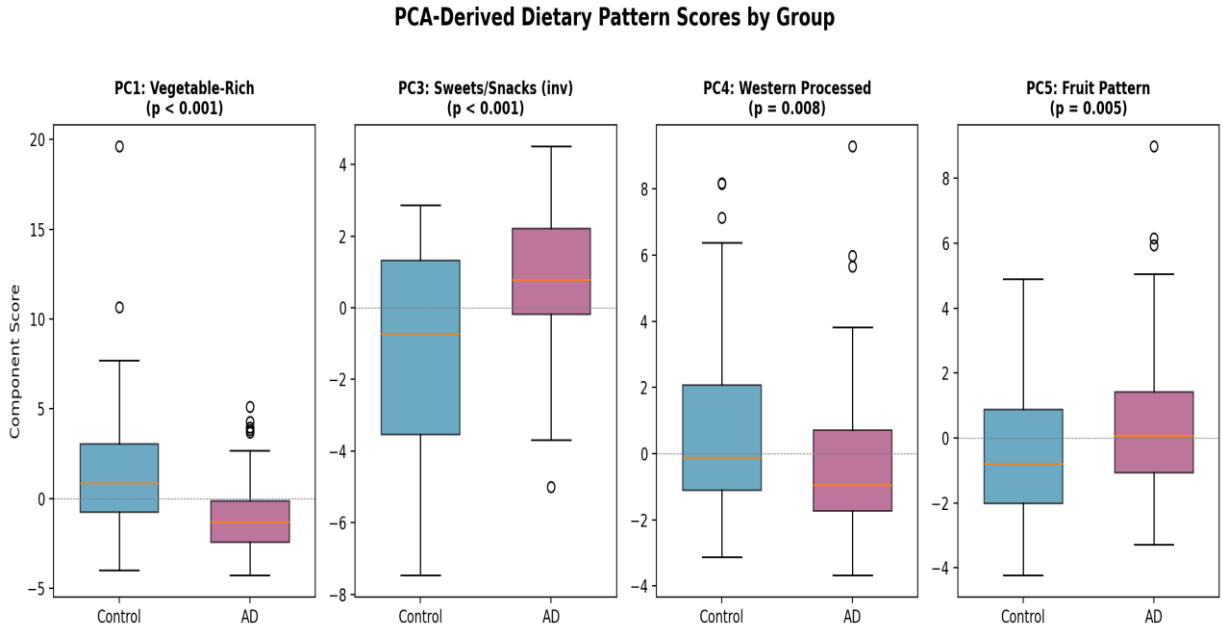


Figure 17 - PCA-derived dietary pattern scores by AD status for four significantly differentiating components

The vegetable-rich pattern also showed the strongest correlations with cognitive test performance (MMSE: ρ=0.41, p<0.001; Clock Drawing: ρ=+0.39, p<0.001) and with the MIND diet score (ρ =0.26, p<0.001). The Sweets/Snacks pattern was negatively correlated with both MMSE (ρ=0.31) and Clock Drawing (ρ=−0.35; both p<0.001). The Western/processed pattern was positively associated with BMI (ρ=0.17, p=0.021) and inversely associated with the MIND diet score (ρ =0.37, p<0.001) [(table 16).

Table 16 - PCA-derived dietary pattern scores by group with logistic regression results (adjusted for age and sex)

Dietary Pattern	AD	Control	p-value	d	OR (95% CI)	p (LR)
1	2	3	4	5	6	7
PC1: Vegetable-rich	−0.94±1.88	+1.32±3.35	<0.001	−0.87	0.70 (0.58–0.84)	<0.001
PC2: Traditional mixed	−0.30±3.14	+0.42±2.23	0.135	−0.26	1.06 (0.92–1.23)	0.415
PC3: Sweets (inv)	+0.84±1.82	−1.19±2.71	<0.001	+0.91	1.38 (1.15–1.65)	<0.001
PC4: Western/processed	−0.39±2.08	+0.55±2.53	0.008	−0.41	1.02 (0.85–1.21)	0.850

Continuation of table 16

1	2	3	4	5	6	7
PC5: Fruit dominant	+0.34±2.02	−0.49±1.88	0.005	+0.42	1.33 (1.10–1.62)	0.004
PC6: Dairy and dried fruit	−0.21±1.74	+0.30±1.88	0.132	−0.28	0.88 (0.71–1.08)	0.215
Note - d=Cohen's d. LR=logistic regression. Pseudo R^2=0.26. inv=inverse scoring (higher score=less consumption)						

Fasting glucose was significantly elevated in the AD group (5.55±2.49 vs. 5.11±1.66 mmol/L; p<0.001, d=0.22), while triglycerides were significantly lower (1.27 ± 0.64 vs. 1.54 ± 0.85 mmol/L; p=0.003, d=−0.34). Total cholesterol, HDL, LDL, and insulin did not differ between groups. BMI was significantly lower in the AD group (25.39±5.83 vs. 27.55±4.99 kg/m²; p<0.001, d =−0.40), and the BMI category distribution also differed significantly (χ^2 =18.75, p<0.001). These metabolic findings, combined with the lower caloric intake (AD: 1696 vs. Control: 2019 kcal/day), are consistent with a pattern of nutritional compromise in AD patients characterized by reduced food intake and metabolic dysregulation.

The analyses presented provide a comprehensive, multi-layered characterization of dietary risk factors for AD in Kazakhstan using four complementary analytical approaches. The nutrient-level analysis established that AD patients have broadly lower intake of both macro- and micronutrients, with particular deficiencies in neuroprotective vitamins (C, D, E, folate) and beneficial fatty acids (MUFA, PUFA), and identified distinct regional signatures in Astana and Almaty. The MIND diet assessment revealed that overall adherence is uniformly low among the elderly population in Kazakhstan. However, individual components, specifically leafy green vegetables (protective, OR=0.42) and red meat (risk, OR=2.52) independently predict AD status even after adjustment for age, sex, and APOE genotype. The PCA analysis confirmed and extended these findings by identifying a vegetable rich dietary pattern as the strongest discriminator of AD status (d=−0.87, OR=0.70), alongside an independent harmful association between sweet/snack consumption and AD (d=+0.91, OR=1.38). The metabolic biomarker profile (3.2.4) revealed elevated fasting glucose and a lower BMI, consistent with the documented nutritional compromise.

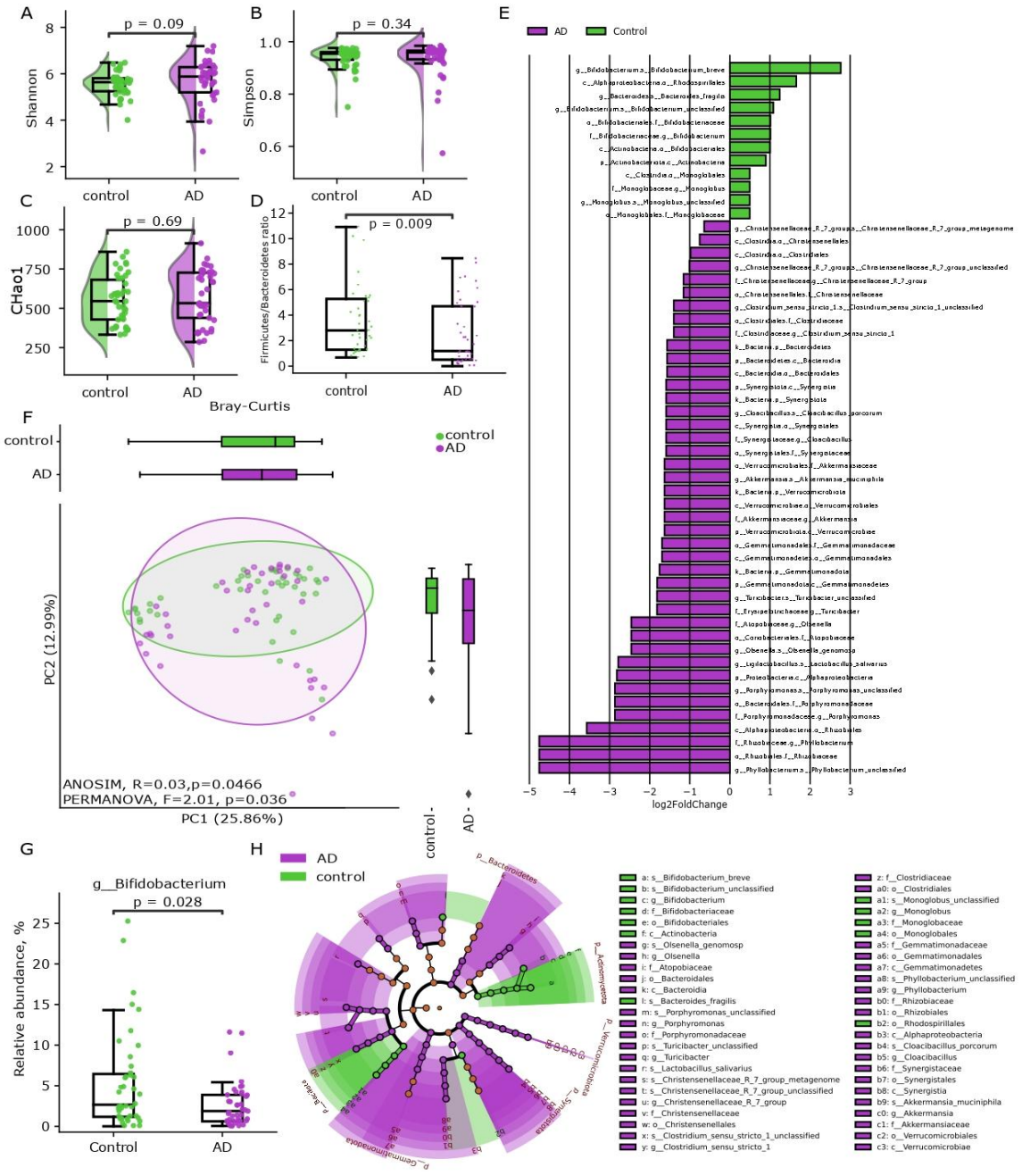
These convergent findings from a priori dietary scoring, data-driven pattern analysis, nutrient-level assessment, and metabolic profiling provide consistent evidence that dietary factors are associated with AD in this Central Asian population. The inability of the total MIND score to discriminate between groups does not indicate that diet is irrelevant; rather, it underscores the need for culturally adapted assessment tools. The PCA-based model (pseudo-R^2=0.26) performed better than the MIND score in this sample, indicating that locally derived dietary patterns may be more informative than Western-centric indices.

3.7 Microbiome composition and inflammatory markers in AD

The results presented in this section were published previously and are adapted from Kaiyrlykyzy et al. (2022) [146,p. 12], Issilbayeva et al. (2024) [147,p. 43] and Kozhakhmetov et al. (2024) [148,p. 974].

3.7.1 Gut microbiome composition in AD

Gut microbiome composition was characterized by 16S rRNA gene sequencing of bacterial DNA isolated from stool samples of patients with AD and healthy controls [146,p. 151]. Within-sample (alpha) diversity was similar in the two groups: the Shannon ($p = 0.09$), Simpson ($p = 0.34$), and Chao1 ($p = 0.69$) indices did not differ significantly (figure 18A–C). Within the two dominant phyla, Firmicutes and Bacteroidetes, diversity was lower in patients with AD. Principal coordinates analysis (PCoA) of Bray-Curtis dissimilarities separated the groups (ANOSIM $R = 0.03$, $p = 0.047$; PERMANOVA $F = 2.01$, $p = 0.036$) (figure 18F), although the low R value indicates that the separation was weak. The position of samples along the PCoA axes was significantly associated (Spearman correlation, $p \leq 0.05$ after FDR correction) with higher abundance of *Butyricicoccus*, *Ligilactobacillus*, *Clostridium sensu stricto 1*, *Turicibacter*, *Enterococcus*, MND1, *Dongia*, RB41, *Skermanella*, *Bryobacter*, *Rhodococcus*, and *Devosia* in the AD group, and with higher abundance of *Eubacterium hallii*, *Collinsella*, *Catenibacterium*, and members of Vicinamibacteraceae in controls.



A–C, alpha diversity (Mann–Whitney U test); D, Firmicutes/Bacteroidetes (F/B) ratio (Mann–Whitney U test); E, log2 fold change (L2FC) of differentially abundant taxa in the AD group (purple) and the control group (green), calculated with DESeq2 ($p < 0.05$, FDR) and plotted with LEfSe; F, principal coordinates analysis (PCoA) of Bray–Curtis dissimilarity (beta diversity) (ANOSIM R = 0.03, p = 0.047; PERMANOVA F = 2.01, p = 0.036); G, bacterial genera depleted in the AD group (Mann–Whitney U test); H, cladogram of differentially abundant taxa, with concentric circles running from kingdom at the center to species at the periphery; purple areas indicate taxa enriched in AD and green areas taxa enriched in controls (DESeq2, $p < 0.05$, FDR; LEfSe cladogram). Prefixes: p, phylum; c, class; o, order; f, family; g, genus; s, species

Figure 18 - Gut bacterial community composition in patients with Alzheimer's disease (AD) and controls

Note - Adapted from Kaiyrlykyzy et al. (2022) [146,p. 151]

In controls, the Firmicutes/Bacteroidetes (F/B) ratio ranged from 0.673 to 22.327 (median 2.803; IQR 1.29–5.20). In the AD group it ranged from 0.003 to 8.297 (median 1.176; IQR 0.506–4.74). The lower ratio in AD appeared to be driven mainly by a higher relative abundance of Bacteroidetes, which in this group ranged from 0.059 to 65.229 (median 0.399; IQR 0.158–1.187) (Mann–Whitney U test, p = 0.0073; Figure 18D, S1). The F/B values in our controls are close to those reported in our earlier pilot study of the Kazakh population (0.2 to 21), which is a range typical of healthy individuals [146,p. 151]. Lower F/B ratios, by contrast, have been described in a number of diseases.

Taxa associated with AD were identified by differential OTU abundance analysis using DESeq2. In the AD group, abundance was higher for Alphaproteobacteria (genus *Phyllobacterium*), Bacteroidia, Bacilli (notably *Lactobacillus salivarius*), Coriobacteriia, Erysipelotrichia, Gemmatimonadetes, Verrucomicrobiae, Synergistia (genus *Cloacibacillus*), and Clostridia. Abundance was lower for Rhodospirillales (Alphaproteobacteria), *Bacteroides* (Bacteroidia), *Bifidobacterium* (Actinobacteria), and *Monoglobus* (Clostridia). The most pronounced depletion in AD involved the genus *Bifidobacterium*, in particular *Bifidobacterium breve* (figure 18G). Several studies have suggested that this species may improve memory and cognitive function in older adults, possibly through the metabolites it produces [206,207]. These differences are summarized in the log2 fold-change plot and the cladogram (figure 18E, 18H).

Overall, the gut microbiome of patients with AD differed from that of controls in its composition rather than in within-sample diversity. Alpha diversity indices (Shannon, Simpson, and Chao1) did not differ between the groups, whereas beta diversity based on Bray–Curtis dissimilarity separated the samples by clinical status. Patients with AD also had a lower F/B ratio. Vogt et al. described a similar pattern in an observational study of 25 patients with AD, reporting reduced microbial diversity together with a relative increase in Bacteroidetes [95,p. 135]. The combination of greater Bacteroidetes abundance with lower diversity within this phylum suggests that this group of bacteria may be relevant to AD pathology [208].

Differential abundance analysis also pointed to dysbiosis in patients with AD, with depletion of *Bifidobacterium* and especially *B. breve*. This agrees with earlier work showing beneficial effects of this species on cognition in older adults and in animal models [209,210]. In transgenic mice, for example, six months of supplementation with *Bifidobacterium longum* 1714 reduced amyloid-beta deposition in the hippocampus and cerebral cortex [211]. In healthy people, the abundance of bifidobacteria has also been linked to levels of gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter [212]. Alongside the loss of *Bifidobacterium*, the AD group showed increased *Phyllobacterium*, a member of Pseudomonadota, as well as *Cloacibacillus porcorum* (Synergistetes), a taxon associated with inflammation, and members of the family Christensenellaceae. Taken together, fewer health-associated bacteria and more inflammation-associated bacteria point to gut dysbiosis in AD [146,p. 151].

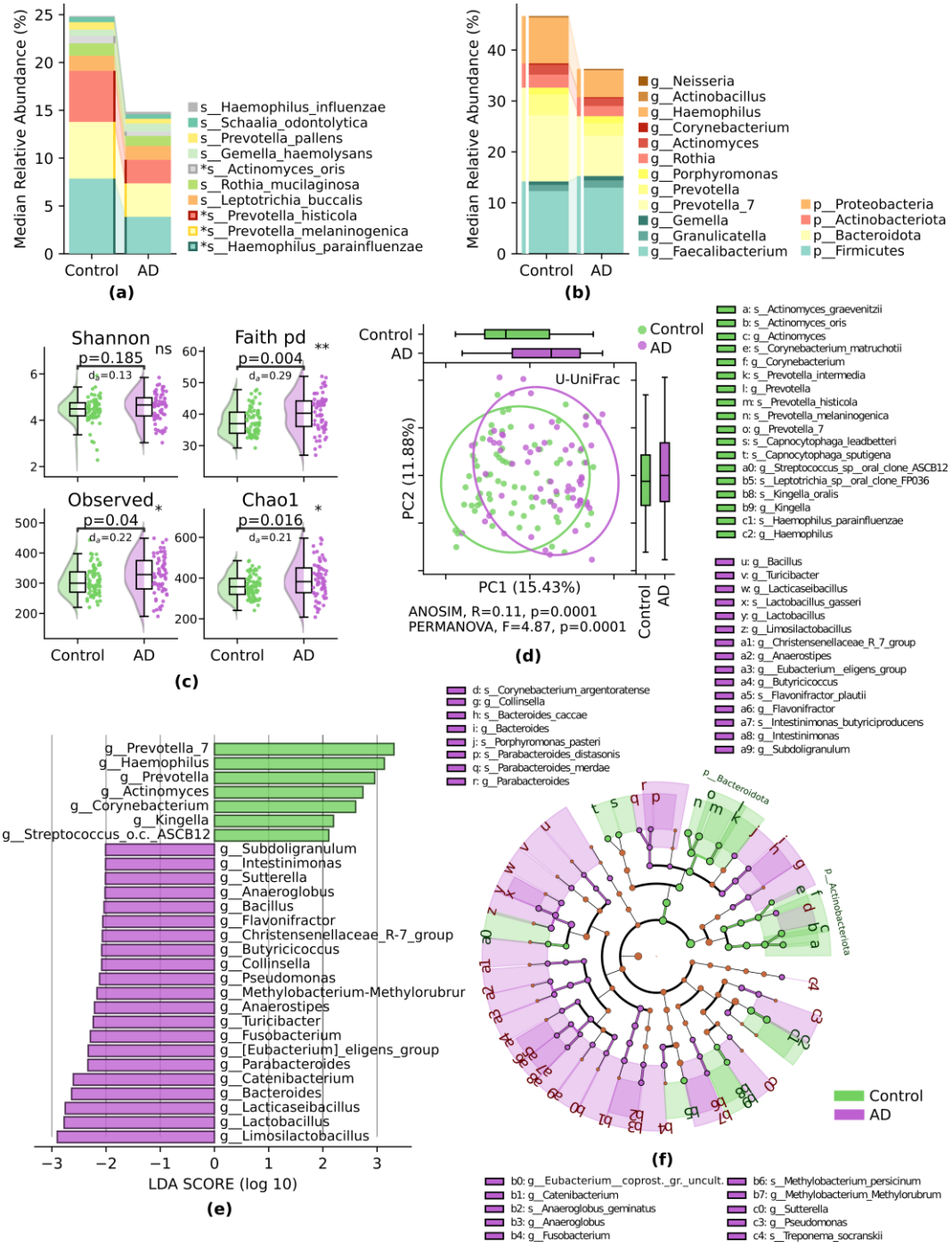
Diet is likely one of the main factors linking the gut microbiome to AD, although few studies in this field have measured it directly. A consistent finding across populations is the loss of fiber-fermenting bacteria that produce short-chain fatty acids (SCFAs) in AD and mild cognitive impairment [213-215]. In a Wisconsin cohort, lower fecal propionate and fewer propionate-producing bacteria were associated with amyloid positivity, and higher SCFA levels with slower cognitive decline [214,p. 708]. In a community-based Chinese cohort, the association between SCFA producers such as *Bifidobacterium pseudocatenulatum* and better cognition persisted after adjustment for dietary pattern [215,p. 106], suggesting that these taxa are more than markers of food intake. *Bacteroides*, on the other hand, is increased in American AD cohorts but decreased in Chinese ones, a difference attributed to regional diet and lifestyle [216], in our cohort, *Bacteroides* was also decreased. Intervention studies support a causal role for diet. A one-year Mediterranean diet in 612 older Europeans enriched taxa associated with better cognitive function and lower CRP and IL-17 levels [217], and a modified Mediterranean-ketogenic diet in adults with mild cognitive impairment increased *Akkermansia*, Christensenellaceae, and Erysipelotrichaceae and reduced *Bifidobacterium* [218]. Our Kazakhstani data fit this picture. Patients with AD had fewer *Bifidobacterium*, *Monoglobus*, and butyrate producers, a lower predicted capacity for SCFA production and polysaccharide degradation, and more *Akkermansia* and Christensenellaceae than controls [148,p. 974]. They also had lower BMI and triglyceride levels, and some were recruited from inpatient facilities where food choice is limited, a setting known to reduce microbial diversity in older people [219]. Part of this signature may therefore reflect reduced or altered food intake rather than the disease itself. Prospective studies that collect dietary data alongside microbiome profiles are needed in Central Asia, where traditional diets rich in meat and fermented milk have not yet been studied in relation to dementia.

3.7.2 Oral microbiome composition in Alzheimer's disease

The oral microbiome was analyzed in a subset of 135 participants (64 patients with AD and 71 controls). For regional comparisons, this subset was further divided into participants from Almaty (34 patients with AD and 45 controls) and from Astana (30 patients with AD and 26 controls).

The oral microbiome results have been published previously [147,p. 12]. At the phylum level, Firmicutes tended to be more abundant in the AD group, although the difference was not statistically significant, and Bacteroidota were less abundant at a trend level ($p = 0.06$) (figure 19a, b). At the species level, four species were significantly less abundant in AD than in controls: *Haemophilus parainfluenzae* ($p = 0.009$), *Prevotella melaninogenica* ($p = 0.02$), *Prevotella histicola* ($p = 0.01$), and *Actinomyces oris* ($p = 0.004$) (figure 19a). Alpha diversity was significantly higher in the AD group by Faith's phylogenetic diversity ($p = 0.004$), observed features ($p = 0.04$), and Chao1 ($p = 0.01$) (figure 19c). Beta diversity based on unweighted UniFrac distances also differed significantly between the groups (PERMANOVA $p = 0.0001$; ANOSIM $p = 0.0001$), with greater between-sample variability in the AD group (figure 19d). LEfSe analysis (LDA>2) identified taxa enriched in AD, including members of

the families Veillonellaceae, Porphyromonadaceae, Treponemataceae, Oscillospiraceae, Bacteroidaceae, Tannerellaceae, Methylobacteriaceae, Corynebacteriaceae, and Lactobacillaceae (figure 19e), shown in more detail in the cladogram (figure 19f).

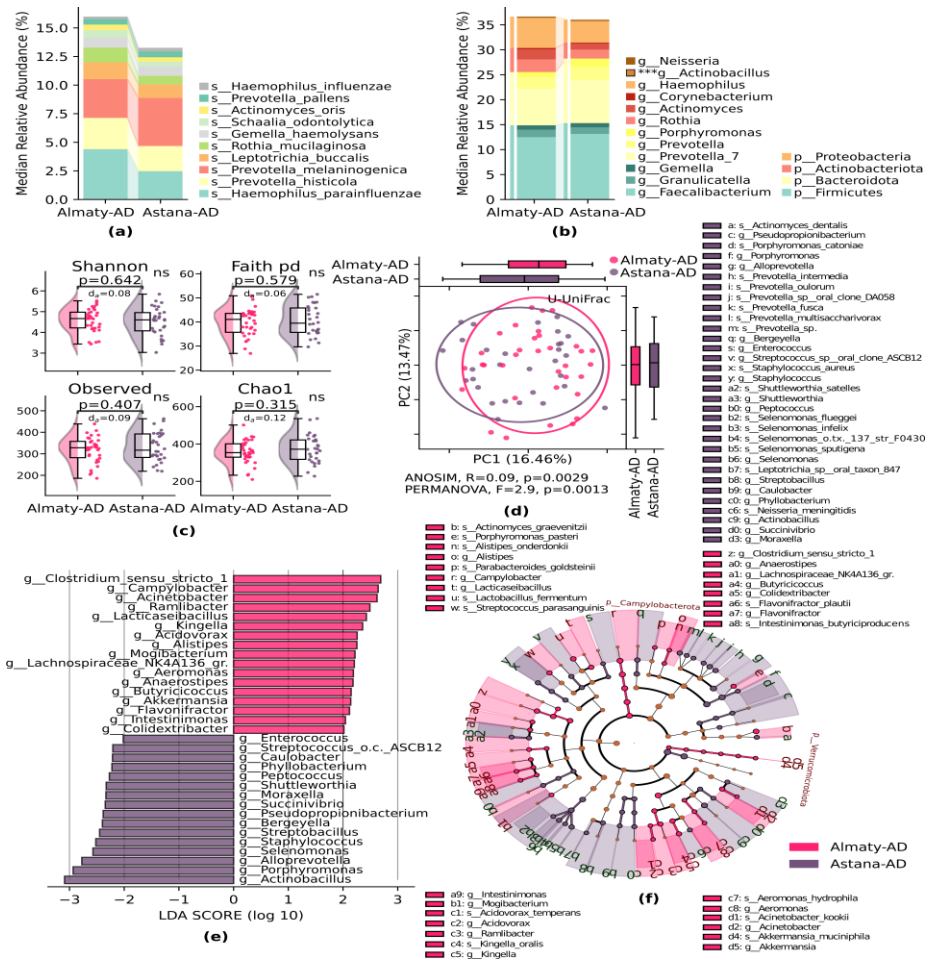


a) relative abundance at the species level; b) relative abundance at the genus level; c) alpha diversity; d) beta diversity; e) LEfSe analysis (LDA > 2); f) cladogram. $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$

Figure 19 - Oral microbiome in the AD and control groups

Note - Adapted from Issibayeva et al. (2024) [147,p. 12]

Comparison of patients with AD from Almaty and Astana [147,p. 12] showed a significant difference at the genus level for *Actinobacillus* ($p < 0.0001$) (figure 20b). Alpha diversity did not differ between the regional subgroups by any index ($p > 0.05$) (figure 20c), but beta diversity based on unweighted UniFrac distances differed significantly (PERMANOVA $p = 0.001$; ANOSIM $p = 0.002$), with greater variability in the Astana subgroup (figure 20d). LEfSe analysis identified region-specific taxa. The Astana AD subgroup was enriched in Akkermansiaceae, Butyricicoccaceae, Aeromonadaceae, Christensenellaceae, Comamonadaceae, and Clostridiaceae, whereas the Almaty AD subgroup was enriched in Porphyromonadaceae, Selenomonadaceae, Staphylococcaceae, Propionibacteriaceae, Weeksellaceae, Succinivibrionaceae, Microbacteriaceae, Peptococcaceae, Rhizobiaceae, P5D1-392, Caulobacteraceae, and Enterococcaceae (figure 20e), as shown in more detail in the cladogram (figure 20f).



(a) relative abundance at the species level; (b) relative abundance at the genus level; (c) alpha diversity; (d) beta diversity; (e) LEfSe analysis (LDA > 2); (f) cladogram. $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$

Figure 20 - Oral microbiome in the Almaty and Astana AD subgroups

Note - Adapted from Issibayeva et al. (2024) [147,p. 12]

Comparison with earlier studies gives a mixed picture. Our findings on bacterial richness, evenness, and community dissimilarity in AD agree with those of Holmer et al. [220]. Wu et al. [221] and Yang et al. [222], by contrast, reported lower oral microbial diversity in AD, unlike the higher diversity observed in our study, whereas Tadjoedin et al. and Fu et al. found no significant differences in alpha diversity or richness [223,224].

At the phylum level, the AD group had lower relative abundance of Proteobacteria, Actinobacteria, and Bacteroidota and higher abundance of Firmicutes, in line with Chen et al. [224,p. 3677]. The reduced abundance of *H. parainfluenzae*, *P. melaninogenica*, *P. histicola*, and *A. oris* in AD partly agrees with Liu et al. [225], who also linked lower *Actinomyces* abundance to the APOE ε4 genotype. Wu et al. likewise found low levels of this genus in patients with AD [221,p. 25], and Cristea et al. reported a lower abundance of the family Actinomycetaceae in a Canadian AD cohort [226]. *Actinomyces* is known as a pathogen involved in the development of periodontitis [227].

The lower abundance of *Prevotella* in AD is consistent with Yang et al. [222,p. 801]. *H. parainfluenzae* has recently been associated with good oral health in Moroccan adolescents with periodontitis [222,p. 801], which partly supports our observation. Some periodontitis-associated bacteria were therefore less abundant in our AD group, which is an unexpected finding. Our LEfSe results for *Lactobacillus* and *Lacticaseibacillus*, which were enriched in AD, differ from those of Wu et al., who found a lower abundance of these taxa [221,p. 25]. In an animal study, however, Liu et al. showed that an increase in *Lactobacillus* attenuated AD-related symptoms, which points to a protective role [228]. Notably, we did not observe higher levels of *Porphyromonas gingivalis* or *Aggregatibacter actinomycetemcomitans* in AD, although many studies have linked these species to the disease, as summarized in recent reviews by Weber et al. [228,p. 330] and Wan et al. [229]. In the regional analysis, however, *Porphyromonas* and *Actinobacillus* were more abundant in the Astana AD subgroup.

The regional differences we observed agree with evidence that environmental and geographical factors shape oral microbiome composition [230], even within a single ethnic group [157,p. 654]. At the same time, the regional AD subgroups differed little in predicted functional profiles or species richness, which suggests that AD may be associated with a distinct oral microbiome profile regardless of region. Two predicted metabolic pathways, HISTSYN-PWY (L-histidine biosynthesis) and PWY-2941 (L-lysine biosynthesis II), correlated negatively with total cholesterol and LDL-C and positively with MMSE score, whereas the bacterial classes *Bacteroidia* and *Erysipelotrichia* correlated with these pathways in the opposite direction. The histidine finding agrees with the metabolomic study by Nielsen et al., which showed downregulation of histidine metabolism in AD [231]. Because *Bacteroidia* correlated negatively with HISTSYN-PWY, and this pathway was associated with lower cholesterol, a recognized risk factor for AD [232-234], and with better cognition, these taxa and pathways may merit further study as potential treatment targets. The role of lysine biosynthesis is less clear: lysine has antioxidant properties and serves as an

energy source [235], but Yuan et al. have reported that it can promote tumor growth [236].

Several taxa showed the highest predictive value for AD in our analysis, and most have been linked to disease processes in earlier studies. In an animal study by Xia et al., *Bacteroides fragilis* activated microglia and promoted the development of AD [237]; *Methylobacterium–Methylorubrum* can cause infections in immunocompromised individuals, according to Kovaleva et al. Tran et al. linked *Anaerostipes* to the production of uremic toxins in chronic kidney disease [238], and *Shuttleworthia* has been associated with periodontitis [239-241]. *Lactobacillus* is known for its immunomodulatory properties [242], although its role in autoimmune and other chronic diseases appears to be twofold [242,p. 104].

Overall, our findings add to the evidence on oral microbial signatures and metabolic pathways as possible biomarkers and treatment targets in AD. Further study of the mechanisms linking the oral microbiota to AD may improve understanding of its pathogenesis and could inform prevention strategies based on oral health.

3.8 Logistic regression analyses

To identify independent predictors of AD beyond descriptive associations, three complementary logistic regression analyses were conducted across the major domains examined in this study. First, a hierarchical multivariable model was built to identify sociodemographic, psychosocial, and lifestyle predictors. Second, univariate and multivariable models were applied to biochemical and metabolic parameters. Third, logistic regression was used to identify independent dietary predictors from both the MIND diet framework and PCA-derived patterns. Together, these analyses provide a multi-domain picture of the independent risk architecture of AD in this population.

3.8.1 Sociodemographic and lifestyle predictors: multivariable models

Crude logistic regression analysis (unadjusted model)

To establish a baseline understanding of the risk landscape within the cohort, crude odds ratios (cORs) were calculated for each potential risk factor. These unadjusted analyses identify direct associations between specific variables and Alzheimer's disease status before controlling for confounding factors in multivariate analyses (table 17).

Table 17 - Crude odds ratios for Alzheimer's disease risk factors

Variable	Category	Crude OR	95% CI	p-value
1	2	3	4	5
Age		1.00	0.98–1.02	0.969
Gender	Female vs Male	1.25	0.82–1.91	0.298
APOE ε4	Carrier vs Non	2.08	1.34–3.25	0.001
Region	Astana vs Almaty	0.61	0.41–0.92	0.018
Race	Russian vs Kazakh	0.74	0.44–1.25	0.266
	Other vs Kazakh	1.30	0.63–2.71	0.480
Education	<12 vs ≥12 years	0.59	0.38–0.92	0.021

Continuation of table 17

1	2	3	4	5
Place of birth	Rural vs Urban	2.45	1.64–3.64	<0.001
Cannot read	Yes vs No	6.27	3.39–11.59	<0.001
Cannot write	Yes vs No	18.13	8.42–39.01	<0.001
Social category	Integrated vs Isolated	0.47	0.32–0.71	<0.001
Depression	Yes vs No	3.43	2.21–5.32	<0.001
Hypertension	Yes vs No	0.63	0.43–0.94	0.022

Multivariable analysis (adjusted models)

Four models were constructed to test the independence of these factors (table 18). The modeling followed a hierarchical approach to examine how various categories of risk factors, ranging from fixed demographics to modifiable lifestyle and social factors, impact the outcome.

Model 1 included basic non-modifiable factors: age, gender, race, and early-life factors like birthplace and literacy. Literacy and birthplace are crucial in the Kazakhstani context as proxies for early-life cognitive reserve and socio-economic status.

Model 2 has added biological markers (the APOE e4 allele) and clinical indicators, such as BMI, glucose levels, and blood pressure. This allowed for the assessment of metabolic and genetic contributions alongside demographic variables.

Model 3 included modifiable lifestyle behaviors, such as physical inactivity and smoking status. This tests whether lifestyle factors can explain the variance in the outcome beyond clinical and genetic risk factors.

Model 4 is the final model that incorporates social isolation and depression history. This final step evaluates whether social engagement and past depression episodes are independent predictors of the outcome.

The analysis reveals a steady improvement in model fit as more variables were added, with the Nagelkerke R^2 increasing from 0.18 in Model 1 to 0.46 in Model 4. Similarly, the AIC decreased from 469.5 to 290.9, indicating that Model 4 is the most informative (table 18).

Table 18 - Multivariable regression models for AD risk factors

Variable	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)	Model 4 OR (95% CI)
1	2	3	4	5
Age	0.99 (0.96–1.02)	0.99 (0.96–1.02)	0.98 (0.95–1.02)	0.97 (0.94–1.01)
Gender (Female)	1.21 (0.76–1.94)	1.36 (0.78–2.38)	1.23 (0.70–2.18)	1.03 (0.56–1.87)
Non-Kazakh	0.96 (0.58–1.59)	0.69 (0.37–1.28)	0.70 (0.37–1.31)	0.63 (0.32–1.22)
Birthplace (Rural)	2.41 (1.55–3.75)***	2.05 (1.23–3.42)**	1.86 (1.10–3.14)*	1.77 (1.02–3.07)*
Illiteracy	7.75 (4.46–13.45)***	5.05 (2.62–9.73)***	3.59 (1.79–7.22)***	3.48 (1.64–7.41)**

Continuation of table 18

1	2	3	4	5
APOE ε4 carrier	–	1.76 (1.02–3.04)*	1.77 (1.02–3.07)*	1.73 (0.97–3.11)
BMI	–	0.95 (0.90–1.00)*	0.95 (0.90–1.00)*	0.95 (0.90–1.00)
Fasting glucose	–	1.19 (1.00–1.42)*	1.20 (1.00–1.45)*	1.16 (0.95–1.41)
Physical inactivity	–	–	2.20 (1.26–3.86)**	1.75 (0.97–3.16)
Socially isolated	–	–	–	1.82 (1.03–3.22)*
Depression	–	–	–	3.52 (1.97–6.30)***
AIC	496.5	371.4	365.7	345.6
Nagelkerke R^2	0.248	0.243	0.270	0.351
Note - *p<0.05, **p<0.01, ***p<0.001				

Illiteracy was the strongest and most robust predictor across all models. In the fully adjusted Model 4, illiterate individuals had 3.48 times the odds of AD (OR 3.48; 95% CI 1.64–7.41; p=0.001). Rural birthplace remained significant across all four models (OR 1.77; 95% CI 1.02–3.07; p=0.042 in Model 4).

APOE ε4 carriage reached significance in Models 2–3 (OR 1.76–1.77; p=0.043) but was attenuated to borderline significance in Model 4 (OR 1.73; 95% CI 0.97–3.11; p = 0.065) after adjustment for social and psychosocial factors. BMI was protective in Models 2–3 (OR 0.95; p=0.034–0.041) but attenuated in Model 4. Fasting glucose showed a trend toward risk in Models 2–3 (OR 1.19–1.20; p=0.046) but became non-significant after adjustment for psychosocial factors.

Physical inactivity more than doubled the odds of AD in Model 3 (OR 2.20; 95% CI 1.26–3.86; p=0.006) but attenuated to borderline significance in Model 4 (OR 1.75; p = 0.065), suggesting shared variance with social isolation and depression. Social isolation was significantly and independently associated with AD in Model 4, with isolated individuals having 82% higher odds of AD (OR 1.82; 95% CI 1.03–3.22; p=0.039). Depression showed the strongest independent association with AD in the final multivariable model (OR 3.52; 95% CI 1.97–6.30; p<0.001).

The Nagelkerke R^2 increased from 0.248 in Model 1 to 0.351 in Model 4, and the AIC decreased from 496.5 to 345.6, confirming that the fully adjusted model provides the best fit. The substantial increase in explanatory power from Model 3 (R^2=0.270) to Model 4 (R^2=0.351) underscores the importance of psychosocial factors such as social isolation and depression in explaining AD risk in this population (figure 21).

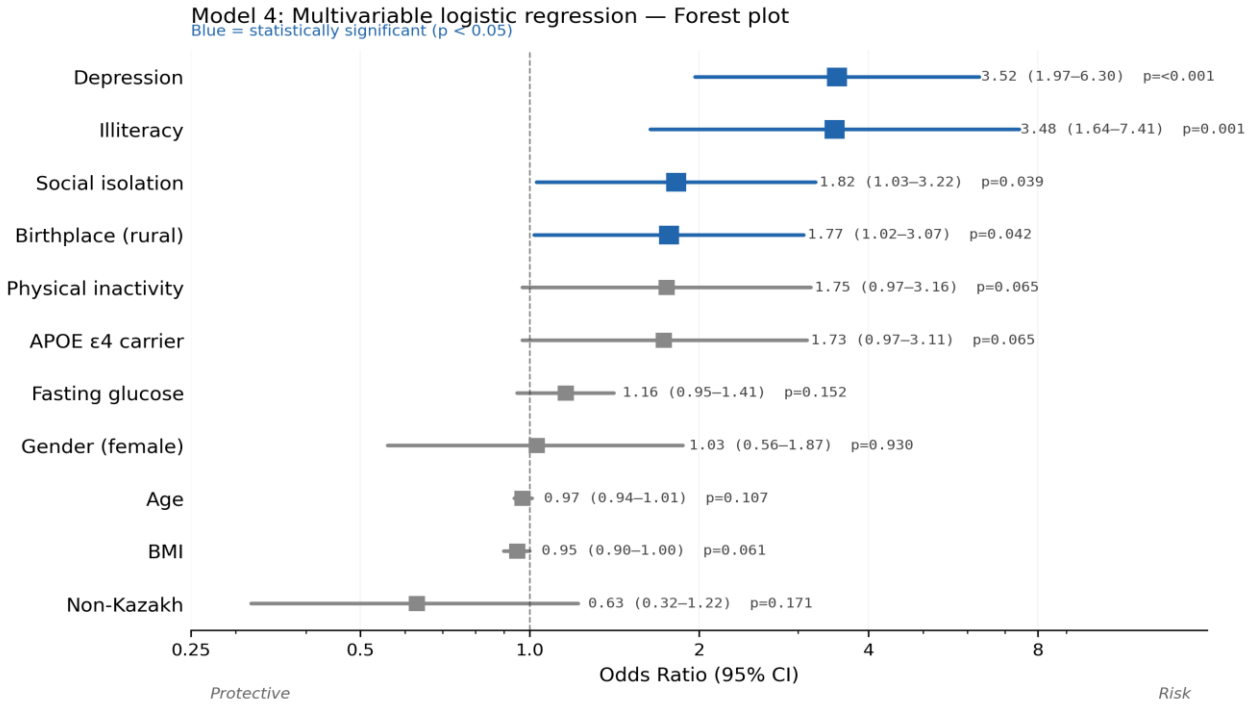


Figure 21 - Forest plot of the fully adjusted multivariable logistic regression model (Model 4) for Alzheimer's disease risk factors. Squares represent odds ratios (OR) and horizontal lines indicate 95% confidence intervals (CI). The vertical dashed line at OR=1.0 indicates the null effect. Blue markers denote statistically significant predictors ($p<0.05$)

These results support a multifactorial model of AD risk in Kazakhstan. Early-life factors, particularly illiteracy and rural birthplace, were the most persistent predictors, while APOE ε4 carriage, physical inactivity, social isolation, and depression provided additional independent contributions. The inverse association between formal education years and AD, combined with the strong positive association with illiteracy, suggests that functional cognitive capacity is a more meaningful marker of reserve than years of schooling. The cardiovascular paradox observed in descriptive analyses likely reflects selection and survival biases inherent in the cross-sectional design. Public health strategies in Kazakhstan should prioritize early-life literacy programs, promotion of physical activity, depression screening, and reduction of social isolation as pillars of dementia prevention.

3.8.2 Sociodemographic and lifestyle predictors: sensitivity analysis

To evaluate the robustness of the primary logistic regression findings reported above (Model 4), a sensitivity analysis was conducted on the final participant-level dataset (n=418; 177 AD cases, 241 controls). The final logistic regression model included functional illiteracy, rural birthplace, history of depression, social isolation, age, and sex as predictors of AD status. Sensitivity analyses were designed to address five methodological concerns arising from the study design: 1) reverse causation for functional illiteracy among severely demented participants; 2) confounding of illiteracy by APOE ε4 genetic status; 3) independence of the illiteracy finding from

formal education; 4) selection bias introduced by clinical control recruitment; and 5) geographic and demographic heterogeneity in the association structure. Results are presented in table 19.

Table 19 - Sensitivity analyses

Sensitivity model	n	Illiteracy OR (95% CI)	Depression OR (95% CI)	Rural birth OR (95% CI)	Social isolation OR (95% CI)
Primary model and reverse causation tests					
Main model (reference)	387	7.87 (4.31-14.35)***	3.55 (2.11-5.96)***	2.62 (1.61-4.25)***	1.69 (1.04-2.76)*
Excluding MMSE <15	327	3.28 (1.65-6.52)***	3.74 (2.19-6.40)***	2.47 (1.48-4.12)***	1.50 (0.90-2.50)
Excluding prior stroke	334	10.00 (4.97-20.12)***	3.21 (1.90-5.43)***	3.68 (2.08-6.50)***	1.23 (0.72-2.10)
Excluding stroke or TIA	311	10.51 (5.01-22.07)***	3.13 (1.80-5.42)***	3.91 (2.14-7.14)***	1.36 (0.79-2.36)
Confounder adjustment models					
APOE ε4 covariate	339	7.48 (3.87-14.47)***	3.55 (2.05-6.16)***	2.63 (1.58-4.40)***	1.65 (0.98-2.78)
Formal education covariate	383	10.19 (5.33-19.49)***	3.61 (2.11-6.17)***	3.05 (1.83-5.07)***	1.72 (1.03-2.85)*
Education replaces illiteracy	384	— (educ OR 0.60*)	3.14 (1.93-5.10)***	2.87 (1.80-4.57)***	2.45 (1.55-3.89)***
Excluding cardiovascular-comorbid controls	307	8.58 (4.01-18.36)***	4.20 (2.20-8.02)***	4.03 (2.27-7.15)***	2.23 (1.27-3.93)**
Note – All models adjusted for age and sex. Main model includes illiteracy, rural birthplace, depression, and Berkman-Syme SNI binary as additional predictors. ***p<0.001; **p<0.01; *p<0.05					

The main model included illiteracy, rural birthplace, depression, social isolation (Berkman-Syme binary), age, and sex as predictors in a logistic regression estimated on 387 participants with complete data. Functional illiteracy was the strongest predictor: OR 7.87 (95% CI 4.31–14.35, $p < 0.001$). Depression was independently associated with AD: OR 3.55 (95% CI 2.11–5.96, $p < 0.001$). Rural birthplace was significant: OR 2.62 (95% CI 1.61–4.25, $p < 0.001$). Social isolation, operationalized using the full Berkman–Syme SNI binary, was independently associated with AD: OR 1.69 (95% CI 1.04–2.76, $p = 0.036$). This model serves as the reference against which all subsequent analyses are compared.

Exclusion of participants with severe cognitive impairment (MMSE < 15) directly addresses the reverse causation hypothesis for functional illiteracy: participants with MMSE scores below 15 (severe dementia, n=55) may have lost literacy as a direct consequence of neurodegeneration rather than having had pre-morbid illiteracy. Excluding these participants leaves 327 participants (122 AD with MMSE≥15 and 178 controls) with complete covariate data. In this restricted sample, the illiteracy OR was 3.28 (95% CI 1.65–6.52, p<0.001). The attenuation from OR 7.87 to OR 3.28 is expected and mechanistically interpretable. Severely demented participants who can no longer read or write due to disease progression inflate the illiteracy association in the unrestricted sample. Critically, the association remains highly statistically significant after their exclusion, providing direct evidence that functional illiteracy is independently associated with AD status among participants with preserved residual cognitive function. Depression (OR 3.74, p<0.001) and rural birthplace (OR 2.47, p<0.001) were similarly unchanged.

Because controls in this cohort had a higher prevalence of cerebrovascular disease than AD, the final model was re-estimated after excluding participants with a documented history of stroke (n=53 excluded) and, separately, after excluding participants with stroke or TIA (n=76 excluded; table 19). In both restricted samples, illiteracy, rural birthplace, and depression remained robust independent predictors of AD, with odds ratios for illiteracy increasing to 10.00 and 10.51, respectively. Social isolation, by contrast, was attenuated to non-significance in both restricted models (OR 1.23, p=0.446 and OR 1.36, p=0.269), suggesting that part of the social isolation-AD association in the full sample may be confounded by post-stroke social withdrawal among controls with cerebrovascular disease. The persistence and strengthening of the illiteracy, rural birthplace, and depression associations after removing participants with cerebrovascular events provides further reassurance that these findings are not artifacts of differential cerebrovascular morbidity between cases and controls.

To test whether the illiteracy association reflects genetic susceptibility rather than cognitive reserve, APOE ε4 carrier status was added as a covariate (analysis restricted to 339 participants with available genotype data). The illiteracy OR was 7.48 (95% CI 3.87–14.47, p<0.001), essentially unchanged from the main model, indicating that the association is largely independent of APOE ε4. Depression (OR 3.55, p<0.001) and rural birthplace (OR 2.63, p < 0.001) were also unchanged. Illiteracy and APOE ε4 therefore capture distinct and additive components of AD vulnerability.

A critical question is whether functional illiteracy is merely a proxy for years of schooling. Sensitivity analysis adds the binary education variable (<12 years vs ≥12 years) as a covariate alongside illiteracy. The illiteracy OR increased to 10.19 (95% CI 5.33–19.49, p < 0.001). which is stronger than in the main model. This result confirms that functional literacy captures a dimension of early-life cognitive reserve that is independent of, and more sensitive than, formal years of schooling. The strengthening of the illiteracy effect when education is adjusted for reflects the paradoxical direction of the crude education–AD association in this cohort (clinical controls have more low-education participants), which partially suppresses the true illiteracy effect in unadjusted analyses.

In the next sensitivity analysis, we replaced illiteracy with the education binary as the primary educational predictor, with all other covariates unchanged. In this model, low formal education (<12 years) showed an OR of 0.60, a non-significant inverse association, reflecting the educational paradox observed in clinical control recruitment earlier. In contrast, social isolation became more strongly associated with AD (OR 2.45, 95% CI 1.55–3.89, p<0.001) in this specification. The substantively misleading direction of the education effect confirms that formal years of schooling is an inferior and less valid indicator of cognitive reserve compared with functional literacy in this study participants.

Controls recruited from internal medicine settings are more likely to have concurrent cardiovascular comorbidities, introducing selection bias. Sensitivity analysis excludes 80 controls with both hypertension and heart disease. All primary findings strengthened: illiteracy OR 8.58 (95% CI 4.01–18.36, p < 0.001), depression OR 4.20 (p < 0.001), rural birthplace OR 4.03 (p < 0.001), and social isolation OR 2.23 (p=0.006). This indicates that the primary associations are not materially inflated by clinical-control selection bias and are relatively robust to control-group composition.

3.8.3 Biochemical predictors of AD status

To identify individual biomarkers associated with AD status, unadjusted logistic regression models were fitted for each biochemical parameter separately. Odds ratios (OR) are reported per one standard deviation (SD) increase. Adiponectin showed the strongest univariate association with AD (OR=3.22, 95% CI: 1.97–5.27; AUC=0.776), followed by the De Ritis ratio (AST/ALT) (OR=1.44, 95% CI: 1.14–1.83; AUC=0.603). Among inversely associated biomarkers, ALT showed the strongest inverse association (OR=0.52, 95% CI: 0.38–0.71; AUC=0.621), followed by BMI (OR=0.61, 95% CI: 0.48–0.79; AUC=0.644), triglycerides (OR=0.68, 95% CI: 0.54–0.86; AUC=0.589), and AST (OR=0.65, 95% CI: 0.49–0.85; AUC=0.562). Glucose (OR=1.26, 95% CI: 1.00–1.59) and total bilirubin (OR=1.26, 95% CI: 1.03–1.55) were positively associated with AD. Total cholesterol, HDL, LDL, insulin, and CRP were not significant in univariate models (table 20).

Table 20 - Univariate logistic regression of individual biomarker associations with AD

Parameter	OR	95% CI	AUC	n
1	2	3	4	5
Adiponectin	3.22	1.97–5.27	0.776	245
AST/ALT ratio	1.44	1.14–1.83	0.603	389
Total Bilirubin	1.26	1.03–1.55	0.577	388
Glucose	1.26	1.00–1.59	0.608	389
HDL-C	1.13	0.92–1.39	0.530	389
LDL-C	1.03	0.84–1.26	0.500	389
Total Cholesterol	0.90	0.73–1.11	0.525	389
CRP	0.91	0.70–1.18	0.565	363
Insulin	0.89	0.69–1.14	0.551	327
Triglycerides	0.68	0.54–0.86	0.589	389
AST	0.65	0.49–0.85	0.562	389

Continuation of table 20

1	2	3	4	5
BMI	0.61	0.48–0.79	0.644	379
ALT	0.52	0.38–0.71	0.621	389
Note - ORs per 1 SD increase. Bold ORs indicate statistical significance (95% CI excludes 1.0)				

After adjustment for age and sex, the results remained essentially unchanged: adiponectin retained the strongest association (adjusted OR=3.15, AUC=0.772), followed by ALT (OR=0.52), BMI (OR=0.62), triglycerides (OR=0.68), AST/ALT ratio (OR=1.44), and bilirubin (OR=1.28). The stability of these estimates after demographic adjustment suggests that the biomarker associations are not confounded by age or sex.

Multivariable Model

A multivariable logistic regression model was constructed, including age, sex, BMI, and eight biochemical biomarkers (glucose, ALT, AST, total bilirubin, triglycerides, total cholesterol, HDL, and LDL). The model included 354 participants (126 AD, 228 controls) with complete data.

The model achieved an overall accuracy of 71.5%, with an AUC of 0.723 (table 21). The specificity was high (89.9%) while the sensitivity was lower (38.1%), indicating that the model was better at correctly identifying controls than AD cases. The most significant independent predictors of AD status were ALT (OR=0.56 per SD, inversely associated), glucose (OR=1.50, positively associated), BMI (OR=0.73, inversely associated), bilirubin (OR=1.32, positively associated), and triglycerides (OR=0.75, inversely associated). AST, total cholesterol, HDL, LDL, age, and sex were not independently significant in the multivariable model.

Incorporating APOE ε4 carrier status into the model (n=311: 120 AD, 191 controls) yielded an accuracy of 69.5% and an AUC of 0.731. APOE ε4 showed a modest independent association with AD (OR=1.32 per SD), while the biochemical predictors maintained their relative contributions: glucose (OR=1.52), ALT (OR=0.59), triglycerides (OR=0.65), bilirubin (OR=1.33), and BMI (OR=0.76). Notably, the metabolic predictors were not attenuated by the inclusion of APOE ε4, indicating that the metabolic associations are statistically independent of APOE ε4.

Table 21 - Multivariable logistic regression

Variable	Coefficient	OR (per SD)	Interpretation
1	2	3	4
Glucose	+0.408	1.50	Higher glucose is associated with higher odds of AD
Total bilirubin	+0.275	1.32	Higher total bilirubin is associated with higher odds of AD
HDL-C	+0.082	1.09	Not significant
LDL-C	−0.010	0.99	Not significant

Continuation of table 21

1	2	3	4
Total cholesterol	−0.151	0.86	Not significant
AST	−0.141	0.87	Not significant
Triglycerides	−0.290	0.75	Higher triglycerides are associated with lower odds of AD
BMI	−0.320	0.73	Higher BMI is associated with lower odds of AD
ALT	−0.576	0.56	Higher ALT is associated with lower odds of AD
Sex (male)	−0.167	0.85	Not significant
Age	−0.026	0.97	Not significant
Note - Model: accuracy=71.5%, AUC=0.723, sensitivity=38.1%, specificity=89.9%. ORs per 1 SD increase. Bold ORs indicate the strongest predictors			

Region-stratified logistic regression revealed notable differences in model performance and predictor structures between the two cities. In Astana, the model achieved 82.1% accuracy and an AUC of 0.804, with high specificity (95.8%) and moderate sensitivity (43.1%). The most important predictor was BMI (OR=0.38 per SD), indicating a strong inverse association: for each 1 SD decrease in BMI, the odds of AD roughly tripled. Triglycerides also showed a similarly strong inverse association (OR=0.44). Glucose was positively associated (OR=1.53), as were bilirubin (OR=1.34) and LDL (OR=1.44). AST was independently inversely associated (OR=0.72) in this subgroup. The excellent discriminatory performance in Astana aligns with the more severe disease phenotype, which may reflect more pronounced metabolic disturbance. In Almaty, the model achieved only 64.1% accuracy and an AUC of 0.687, with reversed diagnostic characteristics: higher sensitivity (72.1%) but lower specificity (55.6%). The predictor structure differed significantly from Astana. BMI was not a significant predictor (OR=1.02), nor were triglycerides (OR=1.01). Instead, total cholesterol (OR=0.53) and ALT (OR=0.60) showed the strongest links. Glucose still had a positive association (OR=1.40). The lower model performance and different predictor profile in Almaty reflect the milder AD phenotype, where metabolic disturbance is less evident and biochemical markers are less discriminative.

The gender-stratified female model showed an accuracy of 68.9% and an AUC of 0.696. The top predictors were ALT (OR=0.63), triglycerides (OR=0.73), glucose (OR=1.35), BMI (OR=0.77), and bilirubin (OR=1.21). HDL had a marginal positive association (OR=1.20). In males, the accuracy was 76.1%, and the AUC was 0.804, significantly higher than in the female model. Glucose was the dominant predictor (OR=2.18 per SD), making it more than twice as influential in males compared to females (OR=1.35). BMI showed a stronger inverse association (OR=0.58 vs. 0.77 in females). ALT (OR=0.55) and total cholesterol (OR=0.57) were also strong inverse predictors. Bilirubin exhibited a notably larger effect in males (OR=1.60) than in females (OR=1.21). The higher overall performance of the male model, despite the smaller sample size, suggests that the metabolic signature of AD is more distinct in

men, possibly because male AD patients display a sharper metabolic deviation from healthy controls, especially in glucose homeostasis.

Across all models, a consistent metabolic pattern was observed. Elevated glucose and bilirubin with reduced ALT, triglycerides, and BMI characterized the AD phenotype. Adiponectin was the strongest single discriminator when available (OR=2.81–3.22). The metabolic and genetic (APOE ε4) risk factors contributed independently. Region-specific models demonstrated that Astana showed strong metabolic discrimination (AUC=0.804) dominated by BMI and triglycerides, while Almaty showed weaker discrimination (AUC=0.687) with a different predictor structure centered on cholesterol. Gender-specific models revealed that males showed a stronger and more glucose-dominated metabolic signature (AUC=0.804) than females (AUC=0.696), consistent with the gender-specific findings observed throughout this study.

3.8.4 Dietary predictors of AD status

In unadjusted logistic regression, the total MIND score was not associated with AD (OR=0.957, 95% CI: 0.799–1.145, p=0.629). After adjustment for age, sex, and BMI, the result remained non-significant (OR=0.963, p=0.689). However, a multivariable model with individual MIND components as simultaneous predictors (adjusted for age and sex) identified two independently significant associations: leafy green vegetables were protective (OR=0.42, 95% CI: 0.22–0.80, p=0.009), while red meat consumption was a risk factor (OR=2.52, 95% CI: 1.18–5.37, p=0.017). These associations persisted after further adjustment for APOE ε4 carrier status (leafy greens: OR=0.47, p=0.015; red meat: OR=2.81, p=0.003), while APOE ε4 itself was not independently significant (OR=1.18, p=0.618).

3.9 Interpretation of findings

This dissertation set out to characterize the risk factor landscape of Alzheimer's disease in the Kazakhstani population by examining sociodemographic, lifestyle, genetic, metabolic, dietary, epigenetic, and microbiome-related determinants in a case-control framework. The convergent findings from multiple analytical approaches allow several overarching conclusions to be drawn and placed within the broader international context of dementia epidemiology.

The strongest and most consistent predictor of AD across all multivariable models was functional illiteracy, with illiterate individuals having 3.48-fold higher odds of AD in the fully adjusted model (OR 3.48; 95% CI 1.64–7.41). This finding substantially exceeds the population-attributable fractions typically reported for low education in high-income settings and aligns with the cognitive reserve hypothesis, which posits that early-life cognitive enrichment builds neural resilience against later neuropathological insult [82,p. 413]. Importantly, formal years of schooling showed a paradoxical inverse association with AD in crude analysis, suggesting that functional literacy, rather than mere educational attainment, is the more meaningful proxy for cognitive reserve in this context. This distinction has critical implications for how educational variables should be operationalized in dementia research across low- and

middle-income countries, where the quality and accessibility of education have varied considerably over time.

Rural birthplace emerged as the second most persistent predictor (OR 1.77; 95% CI 1.02–3.07 in Model 4), reflecting the cumulative life-course disadvantage experienced by individuals born in rural Kazakhstan. These individuals historically had reduced access to education, healthcare, and cognitively stimulating occupations. This finding is consistent with regional analyses in Eastern Europe and Central Asia that demonstrate that rural origins confer lasting vulnerabilities through reduced cognitive reserve and limited access to healthcare [167,p. 69].

Depression was the single strongest independent predictor in the final multivariable model (OR 3.52; 95% CI 1.97–6.30), a finding that resonates with the growing international consensus recognizing depression as both a prodromal feature and an independent risk factor for AD [93,p. 323]. Social isolation also contributed independently to AD risk (OR 1.82; 95% CI 1.03–3.22), supporting the hypothesis that social engagement confers neuroprotective benefits through cognitive stimulation and psychological well-being. The attenuation of physical inactivity to borderline significance in the final model (OR 1.75; p=0.065) suggests that its effect on AD risk is partly mediated through shared pathways with depression and social isolation, a pattern consistent with epidemiological evidence from the FINGER trial and related intervention studies [83,p. 2255].

The APOE ε4 allele was confirmed as a significant genetic risk factor in this Central Asian population, with an overall odds ratio of 2.08 (95% CI 1.34–3.25). However, the genetic architecture underlying this association proved highly heterogeneous. The ε4 AD association was strong in Astana (OR 3.05; p<0.001) but non-significant in Almaty (OR 1.20; p=0.757), and significant among women (OR 2.61; p<0.001) but absent among men (OR 1.08; p=1.000). The regional gradient is consistent with the well-documented latitudinal cline in ε4 frequency [168,p. 489], and the sex-specific finding aligns with evidence that post-menopausal hormonal changes may amplify ε4-mediated neuropathological mechanisms [243]. In the pilot GWAS, suggestive associations were identified in ADARB2, MAST4, ADAM23, and LINC01122, genes previously implicated in cognitive decline and synaptic function, providing some of the first genome-wide data from a Central Asian population [173,p. 253].

The dietary analyses revealed a population-level pattern of nutritional compromise. AD patients exhibited broadly lower intake of neuroprotective nutrients, including folate, selenium, vitamins C, D, and E, and beneficial fatty acids (MUFA, PUFA), compared with controls. The MIND diet assessment showed strikingly low adherence across the entire cohort (median score 4.0 out of 15), with no participant achieving high adherence. While the total MIND score did not discriminate between groups, component-level analysis identified leafy green vegetables as protective (OR 0.42; 95% CI 0.22–0.80) and red meat consumption as a risk factor (OR 2.52; 95% CI 1.18–5.37). PCA-derived dietary patterns proved more discriminative than the Western-derived MIND framework, with the Vegetable-rich pattern showing the largest case-control difference (Cohen's d=−0.87; OR 0.70). These findings underscore

the need for culturally adapted dietary assessment tools in dementia research outside Western settings.

The biochemical profiling revealed a distinctive metabolic signature in AD, characterized by elevated fasting glucose and bilirubin alongside reduced ALT, triglycerides, and BMI. Adiponectin emerged as the most powerful biochemical discriminator (OR 3.22; 95% CI 1.97–5.27; AUC=0.776), with AD patients showing approximately three times higher serum levels compared to controls. The finding of increased ADIPOQ promoter methylation at the CpG site at −74 nt in AD patients (20.97% vs 5.77%; p=0.006), along with elevated AdipoR1 expression in PBMCs (p=0.04), indicates a complex dysregulation of the adiponectin signaling pathway. The positive correlation between adiponectin and MMSE scores within the AD group suggests a compensatory neuroprotective response, although whether elevated adiponectin is causally protective or simply a reactive marker of ongoing neurodegeneration remains unresolved.

The microbiome analyses revealed significant compositional alterations in both the gut and oral compartments. In the gut, AD patients displayed a reduced Firmicutes-to-Bacteroidetes ratio and depletion of beneficial taxa, particularly *Bifidobacterium breve*, alongside enrichment of pro-inflammatory organisms. In the oral cavity, AD patients showed increased alpha diversity with depletion of commensal species (*Haemophilus parainfluenzae, Prevotella, Actinomyces*). Regional differences in oral microbiome composition between Almaty and Astana-based AD patients were notable, reflecting the influence of geography, diet, and environment on microbial ecology even within the same ethnic group [244]. The identification of metabolic pathways, such as L-histidine biosynthesis and L-lysine biosynthesis, that correlated with both microbial taxa and clinical parameters provides preliminary evidence for mechanistic links between microbial dysbiosis and cognitive impairment [231,p. 100].

Taken together, these findings support a multifactorial model of AD risk in Kazakhstan in which early-life socio-educational disadvantage (illiteracy, rural birth), psychosocial factors (depression, social isolation), genetic susceptibility (APOE ε4), metabolic dysregulation (glucose, adiponectin, hepatic markers), dietary patterns (low vegetable intake, high red meat consumption), and microbiome dysbiosis converge to shape disease vulnerability. The pronounced regional and sex-specific heterogeneity observed across nearly all domains underscores the importance of context-sensitive approaches to dementia research and prevention in Central Asia.

3.9.1 Implications for public health

The findings of this dissertation carry substantial implications for public health policy and dementia prevention strategy in Kazakhstan and the broader Central Asian region. Several priority areas emerge from the data.

Literacy and educational interventions across the life course

The prominent role of illiteracy as a predictor of AD risk underscores the long-term cognitive consequences of inadequate early-life education. For Kazakhstan, where older birth cohorts, particularly women and individuals of rural origin, experienced limited educational access during the Soviet and early post-Soviet periods,

these findings suggest that the current burden of dementia is partly rooted in historical educational disparities. Strengthening lifelong learning programs, adult literacy initiatives, and cognitively stimulating activities for older adults should be integrated into national dementia prevention frameworks, consistent with the 2024 Lancet Commission recommendations [65,p. 572].

Screening for depression and social isolation in older adults

Depression and social isolation were among the strongest modifiable predictors of AD in this cohort. Given that depression is often underdiagnosed and undertreated among older adults in Kazakhstan, incorporating routine depression screening into primary care and geriatric services could facilitate early identification of individuals at elevated dementia risk. Community-based social engagement programs, particularly for widowed and isolated older women, could simultaneously address both social isolation and depression, thereby potentially reducing downstream dementia burden [96,p. 7].

Promoting physical activity and addressing sedentary lifestyles

Physical inactivity was markedly more prevalent among AD cases and more than doubled AD odds in the intermediate regression model (OR 2.20). Public health campaigns that encourage regular physical activity among older Kazakhstanis, especially in urban settings where sedentary lifestyles are increasingly common, should be considered a cost-effective component of dementia prevention. Evidence from the FINGER trial and similar multidomain interventions supports the integration of physical activity promotion with cognitive and cardiovascular risk management [87,p. 4].

Culturally adapted dietary guidelines for brain health

The strikingly low adherence to the MIND diet across the entire cohort highlights a fundamental dietary mismatch between Western-derived brain health recommendations and the traditional Central Asian diet. Both AD patients and controls consumed excessive meat and refined grains while falling short of recommendations for fish, fruit, vegetables, and nuts. The identification of leafy green vegetables as protective and red meat consumption as a risk factor provides an evidence base for culturally specific dietary messaging. Public health nutrition programs in Kazakhstan should promote increased consumption of locally available plant-based foods, cruciferous and leafy vegetables, berries, and legumes, while encouraging moderation in red and processed meat intake. These dietary recommendations can be framed within the context of traditional Kazakh cuisine rather than requiring wholesale adoption of Mediterranean or Western dietary patterns.

Integration of metabolic and genetic risk assessment

The finding that adiponectin, fasting glucose, ALT, triglycerides, and bilirubin collectively discriminate AD cases from controls suggests that routine metabolic panels could contribute to risk stratification. Adiponectin profiling, if validated in larger longitudinal cohorts, could complement existing biomarker strategies for early AD risk identification. The pronounced regional heterogeneity in the APOE ε4–AD association (strong in Astana, absent in Almaty) highlights the need for population-specific genetic

risk assessment rather than the uncritical application of risk estimates derived from European or East Asian cohorts.

Microbiome health as a modifiable risk factor

The identification of gut and oral microbiome dysbiosis in AD patients, including depletion of *Bifidobacterium breve* and alterations in inflammatory microbial signatures, opens new avenues for prevention. Interventions that promote microbiome health, including fiber-rich diets, probiotic supplementation, and improved oral hygiene, could be integrated into broader dementia prevention strategies. These approaches align with existing WHO recommendations for integrated management of non-communicable diseases and could generate multi-system health benefits [3,p. 2].

Regional and sex-sensitive approaches to dementia prevention

The consistent finding of regional (Astana vs. Almaty) and sex-specific differences across genetic, metabolic, dietary, and microbiome domains indicates that a one-size-fits-all approach to dementia prevention would be inadequate for Kazakhstan. Women, who bore a disproportionate burden of low education, social isolation, depression, and APOE ε4 carriage in this cohort, should be specifically targeted by prevention programs. Similarly, the distinct risk profiles observed in Astana (dominated by illiteracy, social isolation, and metabolic derangement) and Almaty (characterized by depression, physical inactivity, and lipid abnormalities) call for regionally tailored public health strategies.

Alignment with global dementia prevention frameworks

These findings are directly relevant to the implementation of the WHO's Global Action Plan on the Public Health Response to Dementia 2017–2025 and the 2024 Lancet Commission's expanded list of modifiable risk factors. The data from this dissertation provide population-specific evidence that can inform Kazakhstan's national dementia strategy and contribute to the still-limited evidence base on AD risk factors in Central Asia and the post-Soviet region.

3.9.2 Limitations of the study

Several limitations should be considered when interpreting the findings of this dissertation.

First, the cross-sectional, case-control design prevents establishing causality. The observed links between risk factors and AD may be due to reverse causation, especially for variables like BMI, dietary habits, physical activity, and depression, which can be affected by the neurodegenerative process itself. For instance, the lower BMI and reduced caloric intake seen in AD patients might be consequences of disease-related anorexia and weight loss rather than pre-existing risk factors. Similarly, the higher prevalence of depression among AD cases could reflect prodromal neuropsychiatric symptoms instead of an independent cause.

Second, the control group was recruited predominantly from clinical settings (internal medicine and primary care), which may have introduced selection bias. Controls who actively attend healthcare facilities may have higher rates of diagnosed chronic conditions such as hypertension, heart disease, and diabetes, potentially explaining the cardiovascular paradox observed in this study. This recruitment strategy

may also have contributed to the paradoxical finding of higher formal education among AD cases, as controls from clinical settings in Kazakhstan may be more representative of lower socioeconomic strata. While controls were matched to cases by age group, gender, and ethnicity, education and socioeconomic status were not used as matching criteria and should be considered potential residual confounders in the interpretation of sociodemographic findings. Similarly, the observed higher prevalence of functional illiteracy among AD cases may partly reflect reverse causation, whereby early neurodegeneration leads to progressive decline in reading and writing engagement rather than representing solely a pre-existing risk factor. A population-based control group would have reduced this source of bias.

Third, the dietary assessment relied on a retrospective Food Frequency Questionnaire, which is subject to recall bias, a limitation that is particularly salient in cognitively impaired individuals. Although proxy respondents assisted AD patients in completing the FFQ, the accuracy of dietary recall in dementia patients remains a recognized methodological challenge. The use of the FETA nutrient analysis tool, which was developed for UK populations, may not fully capture the nutritional composition of traditional Kazakh foods, potentially introducing measurement error.

Fourth, the pilot GWAS was conducted with a relatively small sample size (n=180 after quality control), which limits the statistical power to detect genome-wide significant associations. The suggestive signals identified in ADARB2, MAST4, ADAM23, and LINC01122 require replication in larger, independent cohorts before they can be considered validated risk loci.

Fifth, the microbiome analyses (both gut and oral) were based on 16S rRNA gene sequencing, which provides taxonomic resolution primarily at the genus level and cannot fully characterize functional metabolic pathways. Shotgun metagenomic sequencing would offer greater resolution for both taxonomic classification and functional annotation. The sample sizes for microbiome analyses were also modest, and findings should be considered exploratory.

Sixth, the adiponectin and epigenetic analyses were performed in a subset of participants primarily from the Astana region, limiting generalizability to the broader Kazakhstani population. The absence of midlife BMI data prevents a definitive assessment of the temporal relationship between adiposity, adiponectin levels, and subsequent AD risk.

Seventh, several potentially important confounders were not systematically assessed, including midlife blood pressure trajectories, hearing impairment, air pollution exposure, occupational complexity, and detailed medication histories. Furthermore, although diagnoses were established by neurologists using standardized clinical criteria, the possibility of mixed Alzheimer's/vascular dementia cases cannot be entirely excluded, as definitive differentiation requires neuroimaging and CSF biomarker confirmation that was not available in this study. The absence of neuroimaging and cerebrospinal fluid biomarker data means that the AD diagnosis relied on clinical criteria, which, while standard for epidemiological studies in resource-limited settings, may introduce diagnostic misclassification.

Finally, the study was conducted in two major cities (Astana and Almaty), and the findings may not be generalizable to rural populations or other regions of Kazakhstan where risk factor profiles, healthcare access, and dietary patterns differ substantially.

3.9.3 Suggestions for future research

The findings and limitations of this dissertation point toward several priority areas for future investigation.

Longitudinal cohort studies are critically needed in Kazakhstan and the wider Central Asian region. Prospective designs that follow participants from midlife onward would allow the examination of temporal relationships between risk factors and subsequent cognitive decline, addressing the main issue of reverse causation found in cross-sectional studies. These cohorts should include repeated cognitive assessments, biomarker measurements, and detailed life-course exposure histories, such as midlife cardiovascular data, occupational complexity, and educational pathways.

Larger GWAS and whole-genome sequencing studies in Central Asian populations are essential to fill the current gap in the global genetic architecture of AD. The suggestive loci identified in this pilot GWAS (ADARB2, MAST4, ADAM23, LINC01122) should be prioritized for targeted replication. Multi-ethnic GWAS meta-analyses that include Central Asian samples alongside East Asian, European, and other populations would help disentangle shared from population-specific genetic risk factors and improve the transferability of polygenic risk scores.

The development and validation of culturally adapted dietary assessment tools is a key research goal. The poor performance of the MIND diet score in this population emphasizes the need for brain health dietary indices that mirror the traditional Central Asian food environment. Future research should create and validate region-specific dietary scores that include locally relevant foods (kumys, beshbarmak, kurt, and other traditional dishes) and examine their links with cognitive outcomes in prospective studies.

The adiponectin findings warrant further investigation through longitudinal and interventional studies. Prospective studies should determine whether elevated adiponectin precedes or follows cognitive decline, and whether it serves a compensatory neuroprotective function or reflects a pathological process. Intervention studies that modulate adiponectin levels through exercise, dietary modification, or pharmacological agents could help clarify the causal direction of this association. The novel finding of increased ADIPOQ promoter methylation in AD should be replicated in independent cohorts and explored through multi-tissue epigenetic analyses to understand the relationship between peripheral blood and brain-specific methylation patterns.

The microbiome findings call for shotgun metagenomic sequencing and metabolomic profiling to move beyond taxonomic descriptions toward functional mechanistic understanding. Longitudinal microbiome studies that track changes in gut and oral microbial composition alongside cognitive decline trajectories would help establish whether dysbiosis precedes or accompanies neurodegeneration. Intervention studies testing probiotics (particularly Bifidobacterium breve), prebiotics, and dietary

modifications on both microbiome composition and cognitive outcomes in Kazakhstani older adults are warranted.

The pronounced sex-specific heterogeneity observed across genetic, metabolic, and psychosocial domains calls for studies specifically designed to investigate the mechanisms underlying women's disproportionate vulnerability to AD in this population. Hormonal profiling, menopause history, and reproductive factors should be incorporated into future study designs. Similarly, the striking regional differences between Astana and Almaty warrant investigation through expanded multi-regional studies that include rural areas and other cities across Kazakhstan.

Finally, multidomain intervention trials based on the FINGER study design should be tested in the Kazakhstani context. These trials should include culturally tailored dietary counseling, physical activity encouragement, depression screening and treatment, social engagement initiatives, and cognitive training. Recognizing illiteracy, depression, social isolation, physical inactivity, and dietary habits as key modifiable risk factors in this population offers a solid evidence base for planning such interventions.

CONCLUSIONS

This dissertation provides one of the first comprehensive, multi-domain characterizations of Alzheimer's disease risk factors in the Kazakhstani population to our knowledge. The study enrolled 177 individuals with clinically diagnosed AD and 241 age-matched cognitively healthy controls from the cities of Astana and Almaty and examined sociodemographic, lifestyle, genetic, metabolic, dietary, epigenetic, and microbiome-related determinants using complementary analytical approaches. The principal conclusions are as follows:

1. Hierarchical multivariable logistic regression identified four independent predictors of AD in the fully adjusted model. Functional illiteracy was the most robust predictor, exceeding effect sizes typically reported for low formal education in Western cohorts. Depression and social isolation were the most important psychosocial risk factors, and rural birthplace was the second most persistent sociodemographic predictor. Physical inactivity was strongly associated with AD in unadjusted and intermediate models, with its effect attenuating in the fully adjusted model, suggesting partial mediation through shared pathways with depression and social isolation. Formal educational attainment showed a paradoxical crude association with AD that reversed on adjustment, indicating that functional literacy, rather than years of schooling, is the more meaningful proxy for cognitive reserve in this population. Each successive block of variables produced a stepwise improvement in model fit, underscoring the multifactorial nature of AD risk in Kazakhstan and the important contribution of modifiable socio-educational and psychosocial exposures.

2. The APOE ε4 allele was confirmed as a significant genetic risk factor for AD, with a pronounced gene-dose effect in ε4/ε4 homozygotes. The association exhibited regional heterogeneity (strong in Astana but non-significant in Almaty) and was sex-specific, significant among women but absent among men. A pilot genome-wide association study identified suggestive associations in ADARB2, MAST4, ADAM23, and LINC01122, providing some of the first genome-wide data from a Central Asian population.

3. A distinctive metabolic signature of AD was identified, characterized by elevated fasting glucose and bilirubin alongside reduced ALT, triglycerides, and BMI, indicating hepatic and lipid metabolic dysregulation that partly diverges from the cardiovascular risk profiles typical of Western AD cohorts. Biochemical markers were independent of APOE ε4 genotype, indicating that metabolic alterations represent a pathophysiological axis separate from genetic susceptibility in this population.

4. Serum adiponectin was the strongest single biochemical discriminator of AD status, with AD patients showing approximately threefold higher levels than controls. Increased ADIPOQ promoter methylation and elevated AdipoR1 expression in PBMCs indicate complex epigenetic and transcriptional dysregulation of the adiponectin signaling pathway. The positive correlation between serum adiponectin and MMSE scores within the AD group is consistent with a compensatory neuroprotective response, though the causal direction of this association remains unresolved.

5. Multiplex cytokine profiling of 41 plasma analytes revealed a predominantly pro-inflammatory systemic immune signature in AD. After FDR correction, two cytokines retained significance: IL-1α was elevated approximately 2.1-fold and IL-17A was elevated 1.6-fold in AD patients compared to controls. Fourteen of 17 nominally significant analytes were elevated in AD, with three reduced (IL-8, EGF, FGF-2). The concurrent upregulation of an innate immune mediator (IL-1α) and an adaptive immune effector (IL-17A), together with nominally elevated MCP-1, IP-10, and TNF-β, supports chronic low-grade systemic inflammation as a feature of AD in this population. These findings are mechanistically coherent with the adiponectin dysregulation and are consistent with systemic inflammation being a feature of AD in this cohort.

6. Dietary analysis using complementary approaches demonstrated that AD patients had broadly lower intake of neuroprotective nutrients, including folate, selenium, and vitamins C, D, and E, as well as beneficial fatty acids. Low MIND diet adherence was observed across the entire cohort, with no participant achieving high adherence. The total MIND score did not differentiate between study groups, whereas PCA-derived patterns outperformed the MIND framework: the vegetable-rich pattern showed the largest case-control difference, with leafy green vegetables inversely associated and red meat consumption positively associated with AD, even after adjustment for APOE ε4 status.

7. Both gut and oral microbiome compartments showed significant compositional alterations in AD patients. In the gut, a reduced Firmicutes-to-Bacteroidetes ratio and depletion of *Bifidobacterium breve* were observed alongside enrichment of pro-inflammatory taxa. In the oral cavity, increased alpha diversity and depletion of commensal species (*Haemophilus parainfluenzae, Prevotella, Actinomyces*) were identified. The identification of L-histidine and L-lysine biosynthesis pathways correlated with both microbial taxa and clinical parameters provides preliminary evidence for mechanistic links between microbial dysbiosis and cognitive impairment. Regional differences in microbiome composition between Astana and Almaty AD patients further highlight the influence of geography and environment on the microbiome–neurodegeneration axis.

In summary, pronounced regional (Astana vs. Almaty) and sex-specific heterogeneity was observed across nearly all domains examined. In Astana, Alzheimer's disease was primarily associated with functional illiteracy, social isolation, APOE ε4 carriage, and metabolic disturbances, whereas in Almaty, depression, physical inactivity, and lipid abnormalities were more prominent. Among women, the clustering of low educational attainment, social isolation, depression, and APOE ε4 suggests a syndemic pattern in which gender-related social inequalities and genetic susceptibility jointly amplify vulnerability to dementia.

Overall, this dissertation indicates that multiple domains of early-life socio-educational disadvantage, psychosocial factors, genetic predisposition, metabolic alterations, dietary patterns, inflammatory dysregulation, and microbiome dysbiosis are associated with Alzheimer's disease in Kazakhstan and consistent with a multifactorial model. These findings provide a population-specific evidence base for

the development of targeted, culturally adapted strategies for dementia prevention in Kazakhstan and contribute to the limited body of knowledge on AD risk factors in Central Asia.

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