REVIEW
of the official reviewer for dissertation work
Kaiyrlykyzy Aiym
on the theme **«Risk factors associated with Alzheimer's disease in Kazakhstan»**
presented for the degree of Doctor of Philosophy (PhD) in the specialty **«8D10101 – Public Health»**.

№	Criteria	Eligibility (one of the options must be checked)	Justification of the position of the official reviewer
1.	The topic of the thesis (as of the date of its approval) corresponds to the directions of development of science and/or state programs	1.1 Compliance with priority areas of science development or government programs:	The topic corresponds to the priority direction of science development «Life and Health Sciences» and to the objectives of state health policy on non-communicable diseases and healthy ageing.
		1) The thesis was completed within the framework of a project or target program financed from the state budget (indicate the name and number of the project or program) 2) The thesis was completed within the framework of another state program (indicate the name of the program) 3) The dissertation corresponds to the priority direction of the development of science, approved by the Higher Scientific and Technical Commission under the Government of the Republic of Kazakhstan (indicate the direction)	The dissertation was completed within the framework of grant funding of the Ministry of Science and Higher Education of the Republic of Kazakhstan (projects AP14871338 and AP09259730) and the Nazarbayev University Collaborative Research Program grant No. 091019CRP2112. The work also corresponds to the priority direction «Life and Health Sciences» approved by the Higher Scientific and Technical Commission and is in line with the WHO Global Action Plan on the Public Health Response to Dementia.
2.	Importance for science	The work **makes**/does not make a significant contribution to science, and its importance is **well disclosed**/not disclosed	The work makes a significant contribution to science. Central Asian populations are practically absent from global studies of dementia risk, and for Kazakhstan there were no systematic data on the determinants of Alzheimer's disease. The dissertation provides the first multidomain characterization of AD risk in a Kazakhstani case-control cohort and combines sociodemographic, psychosocial, genetic,biochemical, epigenetic (ADIPOQ), immunological, dietary and gut and oral microbiome data in one sample. From the standpoint of population genetics,

			the APOE allele frequencies and the genotype data from Kazakh participants are a valuable reference for the region. The importance of the work is well disclosed in the Introduction and in the Discussion.
3.	The principle of independence	Self-reliance level: **1) High;** 2) Medium; 3) Low; 4) No independence	The level of independence is high. The doctoral student took part in participant recruitment and data collection in Astana and Almaty, ran the laboratory work for the adiponectin/ADIPOQ block, built the analytical database, carried out the statistical analyses and interpreted the results. She is the first author of the key publications on the adiponectin system and the gut microbiome. Her contribution to the work done jointly with co-authors is clearly identified in the text.
4.	The principle of inner unity	4.1 Justification of the relevance of the thesis: **1) Justified;** 2) Partially justified; 3) Not justified.	The relevance is justified convincingly. It rests on the growing burden of dementia, modifiable risk factors, the under-representation of Central Asian populations in this evidence base, and the absence of national epidemiological data on AD in Kazakhstan.
		4.2 The content of the thesis reflects the topic of the thesis: **1) Reflects;** 2) Partially reflects; 3) Does not reflect	The content of the thesis reflects the topic of the thesis. All seven objectives deal with risk factors and biomarkers of AD in the Kazakhstani population, and each objective is addressed in a dedicated subsection of Chapter 3.
		4.3. The purpose and objectives correspond to the topic of the thesis: **1) correspond;** 2) partially correspond; 3) do not correspond	The purpose (to investigate risk factors and emerging biomarkers of AD in the Kazakhstani population through a multidomain case-control approach) and the seven objectives correspond to the topic and are consistent with the conclusions.
		4.4 All sections and provisions of the thesis are logically interconnected: **1) completely interconnected;** 2) the interconnection is partial; 3) there is no interconnection	The sections are logically connected: from literature review, methods to domain-specific results and interpretation, limitations and future research. The links between domains (adiponectin and inflammation, diet and microbiome, APOE adjustment of metabolic and dietary findings) are discussed explicitly. The literature review section on microbiome could be shortened in favor of

			genetic epidemiology, which is closer to the results presented.
		4.5 The new solutions (principles, methods) proposed by the author are reasoned and evaluated in comparison with the known solutions: **1) there is a critical analysis;** 2) partial analysis; 3) the analysis does not represent one's own opinions, but quotes from other authors	The author compares her results critically with international data: APOE ε4 frequencies and effect sizes in European and East Asian populations, the inapplicability of the Western MIND diet score, and the agreement and disagreement of oral microbiome findings with other cohorts findings. The comparison of the array-based genetic findings with known AD loci (e.g., large GWAS meta-analyses) is less developed.
5.	Scientific novelty principle	5.1 Are the scientific results and provisions new? **1) completely new;** 2) partially new (25-75% are new); 3) not new (less than 25% are new)	The results are new for Kazakhstan and Central Asia: functional illiteracy as the strongest independent predictor of AD; regional (Astana vs Almaty) and sex differences in the APOE ε4-AD association; candidate loci from the first array-based association analysis of AD in Kazakh participants; elevated serum adiponectin and ADIPOQ promoter methylation in AD; IL-1α and IL-17A; gut and oral microbiome signatures of AD in a Central Asian population.
		5.2 Are the dissertation findings new? **1) completely new;** 2) partially new (25-75% are new); 3) not new (less than 25% are new)	The conclusions are new and are formulated on the basis of the author's own data. Conclusions 1, 4, 6 and 7 are the most original. Conclusion 2 contains the expected confirmation of APOE ε4 as a risk factor, which is new for this population, together with original data on its heterogeneity.
		5.3 Technical, technological, economic or management decisions are new and reasonable: 1) completely new; **2) partially new (25-75% are new);** 3) not new (less than 25% are new)	The management proposals are partially new: multidomain prevention programs adapted to Kazakhstan, development of culturally adapted dietary indices, and the use of literacy rather than years of schooling in risk assessment. They are well- reasoned. However, multidomain prevention itself is an established international approach.

6.	The validity of the main findings	All main conclusions **<u>are</u>**/are not based on scientifically significant evidence or well-grounded (for qualitative research and areas of training in the arts and humanities)	The main conclusions are based on scientifically significant evidence: an adequate sample size for the case-control comparison, multivariable models with adjustment for age, sex, region and ethnicity and sensitivity analyses . The author separates confirmatory from exploratory findings correctly. The findings for the array-based association analysis and for the regional and sex heterogeneity of APOE ε4 are, by design, hypothesis-generating and should be worded as such (see item 11).
7.	The main provisions for the defense	It is necessary to answer the following questions for each provision separately: 7.1 Is the provision proven? 1) proven; 2) rather proven; 3) rather not proven; 4) not proven 7.2 Is it trivial? 1) yes; 2) no 7.3 Is it new? 1) yes; 2) no 7.4 Application level: 1) narrow; 2) medium; 3) wide 7.5 Is it proven in the article? 1) yes; 2) no	Provision 1 (illiteracy, depression, social isolation, rural birthplace): The provision is proven. It is not trivial, and it is new. Its level of application is wide. It has been partially proven in publications: it has been presented in conference abstracts. The association remains significant after participants with severe dementia are excluded, although reverse causation cannot be fully ruled out. Provision 2 (APOE ε4 and pilot GWAS): The provision is rather proven. It is not trivial, and it is new. Its level of application is medium. It has been partially proven in publications: it has been presented in conference abstracts, and the genotype manuscript is in preparation. The overall effect of the ε4 allele is proven. The regional and sex differences require a formal test of interaction, and the signals from the array-based analysis are suggestive only. Provision 3 (metabolic profile independent of APOE ε4): The provision is rather proven. It is not trivial, and it is new. Its level of application is medium. It has been partially proven in publications. Provision 4 (adiponectin, ADIPOQ methylation, AdipoR1): The provision is proven. It is not trivial, and it is new. Its level of application is medium. It has been proven in an article published in BMC Medical Genomics (2022).

			Provision 5 (cytokine profile, IL-1α and IL-17A): The provision is proven. It is not trivial, and it is new. Its level of application is medium. It has been proven in an article published in the International Journal of Alzheimer's Disease (2024). Provision 6 (dietary patterns, MIND vs PCA): The provision is rather proven. It is not trivial, and it is new. Its level of application is wide. It has been partially proven in publications, as it has so far been presented in conference abstracts. Provision 7 (gut and oral microbiome): The provision is rather proven. It is not trivial, and it is new. Its level of application is wide. It has been proven in articles published in Scientific Reports (2022), Pathogens (2024) and the International Journal of Alzheimer's Disease (2024).
8.	The principle of reliability Reliability of sources and information provided	8.1 Choice of methodology - is justified or the methodology is described in sufficient detail **1) yes;** 2) no	The choice of methodology is justified and described in sufficient detail for most blocks. The description of genotype quality control, imputation and microbiome bioinformatics would benefit if completed.
		8.2 The results of the thesis were obtained using modern methods of scientific research and methods of processing and interpreting data using computer technologies: **1) yes;** 2) no	Modern methods were used: real-time PCR genotyping, Illumina Infinium ImmunoArray-24 BeadChip with processing in Array Analysis CLI, BCFtools and PLINK 1.9/2.0, methylation-specific PCR, multiplex immunoassay on FLEXMAP 3D, 16S rRNA sequencing (V3–V4) with phyloseq, DESeq2 and LEfSe, and statistical analysis in R.
		8.3 Theoretical conclusions, models, identified relationships and patterns have been proven and confirmed by experimental research (for areas of training in pedagogical sciences, the results have been proven on the basis of a pedagogical experiment): **1) yes;**	The identified relationships are confirmed by the author's own laboratory and field data. Hypotheses that go beyond the data (compensatory role of adiponectin, gradient for APOE ε4, mechanistic links to microbial pathways) are presented correctly as hypotheses requiring prospective confirmation.

		2) no	
		8.4 Important statements are **confirmed** / partially confirmed / not confirmed by references to current and reliable scientific literature	Important statements are confirmed by references to current and reliable literature (Lancet Commission 2024, GBD 2021, Alzheimer's Association 2025, meta-analyses on APOE).
		8.5 Used literature sources are **sufficient**/not sufficient for a literature review	The reference list contains 244 sources, about half of them published in 2021–2026, including leading journals in the field. This is sufficient for a literature review.
9	Practical value principle	9.1 The thesis has theoretical value: **1) yes;** 2) no	Yes. The work refines the cognitive reserve concept (functional literacy rather than years of schooling) and adds Central Asian data on gene–environment heterogeneity of APOE ε4, the adiponectin pathway, and the gut–brain axis.
		9.2 The thesis is of practical importance and there is a high probability of applying the results obtained in practice: **1) yes;** 2) no	Yes. The results provide a population-specific evidence base for national dementia prevention programs, screening of high-risk groups (low literacy, depression, social isolation, women carrying APOE ε4), and the design of multidomain intervention studies. The APOE and genotype data can be used for population genetic reference and for future pharmacogenetic and nutrigenetic studies in Kazakhstan.
		9.3 Are the practice suggestions new? 1) completely new; **2) partially new (25-75% are new);** 3) not new (less than 25% are new)	Partially new: the recommendations on literacy-based risk assessment and culturally adapted dietary indices are new for the region. The general multidomain prevention framework follows international practice.
10.	The quality of writing and design	Academic writing quality: **1) high;** 2) average; 3) below average; 4) low.	The dissertation is written in clear academic English and is well structured; tables and figures are informative. It complies with requirements for dissertations in general.
11.	Notes on a thesis		While evaluating the work positively overall, I would like to make the following comments, mainly on the genetic part, which is closest to my area of expertise:

			1. Terminology of the genetic study. The Infinium ImmunoArray-24 is an immune-focused array with dense coverage of immune-related loci and only sparse coverage of the rest of the genome. The analysis should therefore be called an «exploratory array-based association study» rather than a GWAS, and the claim of «the first GWAS in Central Asia» should be softened. **2. Epigenetic analysis.** MSP of a single CpG site gives qualitative data. Quantitative bisulfite pyrosequencing or targeted bisulfite sequencing of the promoter region is recommended for future work. **3. Gene-diet interaction.** Since APOE genotype and dietary patterns were measured in the same participants, an analysis of APOE ε4- dietary pattern interaction would strengthen the nutritional findings and is recommended as a direction for future work. These comments are recommendations and do not reduce the overall positive evaluation of the dissertation.
12.	Scientific level of the doctoral student's articles on the topic of research (in case of defense of the dissertation in the form of a series of articles, the official reviewers comment on the scientific level of each article of the doctoral student on the topic of research)		The dissertation is defended as a monograph. The main results have been published in 16 works, including 5 articles in international peer-reviewed journals indexed in Scopus and Web of Science with an impact factor, 1 article in a journal recommended by the Committee for Quality Assurance in the Sphere of Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan, and 10 abstracts in international conference proceedings The articles are of a high scientific level. Among them are Kaiyrlykyzy A. et al., BMC Medical Genomics (2022), on adiponectin, its receptors and ADIPOQ promoter methylation; Kaiyrlykyzy A. et al., Scientific Reports (2022), on gut microbiota in AD; Issilbayeva A. et al., Pathogens (2024), on the oral microbiome; and Kozhakhmetov S. et al., International Journal of Alzheimer's Disease (2024), on inflammatory markers and gut dysbiosis. The publications reflect the main provisions

	the official reviewers comment on the scientific level of each article of the doctoral student on the topic of research)		in international conference proceedings The articles are of a high scientific level. Among them are Kaiyrlykyzy A. et al., BMC Medical Genomics (2022), on adiponectin, its receptors and ADIPOQ promoter methylation; Kaiyrlykyzy A. et al., Scientific Reports (2022), on gut microbiota in AD; Issilbayeva A. et al., Pathogens (2024), on the oral microbiome; and Kozhakhmetov S. et al., International Journal of Alzheimer's Disease (2024), on inflammatory markers and gut dysbiosis. The publications reflect the main provisions of the dissertation and meet the requirements for publication of the results.
13.	Decision of the official reviewer (pursuant to paragraph 28 of the present Model Regulations)		The dissertation of Kaiyrlykyzy Aiym «Risk factors associated with Alzheimer's disease in Kazakhstan» is a completed, independent research work that solves a scientific problem of importance for public health in Kazakhstan. It meets the requirements of the Model Regulations on the Dissertation Council for PhD dissertations. **I believe that Kaiyrlykyzy Aiym deserves to be awarded the degree of Doctor of Philosophy (PhD) in the specialty 8D10101 – Public Health.**

In reviews, official reviewers indicate one of the following solutions:

1) **to award the degree of Doctor of Philosophy (PhD) or Doctor of Specialization;**

2) send the thesis for revision (except for cases of thesis defense in the form of a series of articles);

3) refuse to award the degree of Doctor of Philosophy (PhD) or Doctor of Specialization.

Copies of the reviews of the official reviewers are handed over to the doctoral student no later than 5 (five) working days before the defense of the thesis.

Official Reviewer

Candidate of Biological Sciences, Professor, Head of the National Scientific Shared Laboratory of Biotechnology, National Center for Biotechnology

Национальный центр биотехнологии ОТДЕЛ ОРГАНИЗАЦИОННО-КАДРОВОЙ РАБОТЫ

_____ (signature) Elena V. Zholdybayeva

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