Editorial

DOI: 10.5582/bst.2024.01365

Serum proteomics reveals early biomarkers of Alzheimer's disease: The dual role of *APOE-ε4*

Ya-nan Ma[1], Ying Xia[1], Kenji Karako[2], Peipei Song[3,*], Wei Tang[2,3], Xiqi Hu[1,*]

[1] Department of Neurosurgery, Haikou Affiliated Hospital of Central South University Xiangya School of Medicine, Haikou, China;
[2] Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan;
[3] National Center for Global Health and Medicine, Tokyo, Japan.

SUMMARY: Alzheimer's disease (AD), the leading cause of dementia, significantly impacts global public health, with cases expected to exceed 150 million by 2050. Late-onset Alzheimer's disease (LOAD), predominantly influenced by the *APOE-ε4* allele, exhibits complex pathogenesis involving amyloid-β (Aβ) plaques, neurofibrillary tangles (NFTs), neuroinflammation, and blood-brain barrier (BBB) disruption. Proteomics has emerged as a pivotal technology in uncovering molecular mechanisms and identifying biomarkers for early diagnosis and intervention in AD. This paper reviews the genetic and molecular roles of *APOE-ε4* in the pathology of AD, including its effects on Aβ aggregation, tau phosphorylation, neuroinflammation, and BBB integrity. Additionally, it highlights recent advances in serum proteomics, revealing *APOE-ε4*-dependent and independent protein signatures with potential as early biomarkers for AD. Despite technological progress, challenges such as population diversity, standardization, and distinguishing AD-specific biomarkers remain. Directions for future research emphasize multicenter longitudinal studies, multi-omics integration, and the clinical translation of proteomic findings to enable early detection of AD and personalized treatment strategies. Proteomics advances in AD research hold the promise of improving patient outcomes and reducing the global disease burden.

Keywords: Alzheimer's disease (AD), *APOE-ε4*, proteomics, neuroinflammation, blood-brain barrier (BBB), multi-omics

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia, accounting for approximately 80% of all dementia cases worldwide (*1*). This neurodegenerative disorder is primarily characterized by a gradual decline in cognitive function, accompanied by memory impairment, reduced language skills, and spatial disorientation. Currently, around 55 million people globally are affected by dementia, and this number is expected to exceed 150 million by 2050, placing a significant burden on society and healthcare systems (*2*). Late-onset Alzheimer's disease (LOAD) is the most prevalent form of AD and its incidence rises sharply in individuals over the age of 65, posing a major public health challenge globally (*3*).

The exact cause of AD is not yet fully understood, but research has indicated that its pathogenesis involves a range of complex biological processes, including the deposition of amyloid-β (Aβ) plaques, the formation of neurofibrillary tangles (NFTs), neuroinflammation, and neuronal loss (*4*). Current Aβ-targeted therapeutic strategies have made some progress in slowing disease progression, but their efficacy remains limited (*5*). Therefore, identifying new potential therapeutic targets and biomarkers is crucial to the early diagnosis of and intervention in AD.

In recent years, the rapid development of proteomics technology has enabled researchers to reveal the biological characteristics that precede the onset of LOAD by analyzing circulating proteins in individual body fluids (*6,7*). Proteomics studies specifically focus on identifying early warning signals of the disease by analyzing biomarkers in blood and cerebrospinal fluid (CSF), even allowing for interventions before clinical symptoms manifest (*8*). However, most existing proteomic studies are limited to small-scale cross-sectional research and lack validation in large prospective cohorts. Notably, the genetic factors for AD, and especially the influence of the apolipoprotein E (*APOE*) gene, have been widely confirmed to be closely associated with LOAD onset (*9*).

Among the three main *APOE* gene alleles (ε2, ε3, ε4), the ε4 allele is the strongest risk factor for LOAD (*10*). Studies have shown that about 25% of the general

population carries the *APOE-ε4* allele, whereas over 50% of AD patients are *APOE-ε4* carriers (*11*). The risk of developing LOAD for *APOE-ε4* carriers is three times higher than that in the general population, and for homozygotes, the risk can be as high as 12 times (*4*). The relationship between *APOE-ε4* and LOAD has been extensively studied, but how *APOE-ε4* specifically regulates the protein networks related to AD remains a key focus of current research. Identifying *APOE-ε4*-dependent and independent molecular characteristics is crucial for a deeper understanding of the mechanisms underlying AD. Moreover, proteomic analyses hold promise for providing new potential biomarkers for early diagnosis and personalized treatment of AD while offering insights into the role of *APOE-ε4* in disease progression.

2. Background and challenges of AD

2.1. Pathogenesis of AD

The pathological mechanisms of AD are diverse and complex, primarily involving the formation of Aβ plaques, abnormal phosphorylation of tau protein, synaptic dysfunction, mitochondrial damage, and neuronal death (*4*). The two hallmark pathological changes are the formation of Aβ plaques in the brain and the aggregation of hyperphosphorylated tau protein, which forms NFTs. These pathological phenomena lead to damage and death of neurons, ultimately disrupting neural networks and resulting in cognitive and functional decline.

In addition to Aβ and tau protein pathology, chronic inflammation and oxidative stress are also considered key factors in the progression of AD. In particular, neuroinflammation has emerged in recent years as a significant mechanism influencing AD onset and progression. Microglia, the immune cells of the central nervous system, become activated in response to Aβ and tau pathology, triggering inflammatory responses that further exacerbate neuronal damage (*12*).

These pathological mechanisms play critical roles in the progression of AD, but not all patients follow the same disease patterns. Recent research has revealed that AD exhibits significant genetic heterogeneity and phenotypic diversity, with genetic factors playing a crucial role in its onset and progression (*13*). Among these, the ε4 allele of the *APOE* gene has been recognized as one of the strongest genetic risk factors associated with AD.

2.2. Genetic association of APOE with AD

The *APOE* gene, located on chromosome 19, encodes APoE, which plays a key role in lipid metabolism and transport. There are three major alleles of *APOE*: ε2, ε3, and ε4. The ε4 allele significantly increases the risk of LOAD. Individuals carrying one copy of the ε4 allele have a threefold increased risk of developing AD compared to the general population, while individuals with two copies (homozygotes) have up to a twelvefold increased risk (14). In contrast, the *APOE-ε2* allele has a protective effect, reducing the risk of AD.

The mechanism of APOE's influence is primarily through its effects on the deposition and clearance of Aβ (*15*). Research has suggested that the *APOE-ε4* allele may promote the aggregation and deposition of Aβ in the brain, accelerating neurodegenerative changes (*16*). Moreover, *APOE-ε4* carriers often have impaired blood-brain barrier (BBB) function, exacerbating the accumulation of Aβ in the brain. In addition, *APOE-ε4* may interfere with synaptic function and neuronal plasticity by affecting cholesterol and lipid metabolism, exacerbating the pathology of AD.

Although the risk associated with *APOE-ε4* has been extensively studied, its precise impact on protein regulation processes and its specific role in LOAD remains incompletely understood. Notably, the effects of *APOE-ε4* vary significantly among individuals, suggesting complex interactions between genetic and environmental factors. Thus, further understanding of the *APOE-ε4*-dependent and independent protein characteristics will provide new insights into the pathogenesis of LOAD and inform the development of personalized therapeutic strategies.

2.3. Proteomics in AD

Proteomics is a technology that enables large-scale analysis of protein expression, modification, and interactions, revealing key biological processes. In recent years, proteomics has been widely used in AD research, particularly in identifying proteins in blood and CSF that may be associated with the risk of AD. By uncovering disease-related molecular features, proteomic studies provide important clues for understanding the mechanisms of AD pathogenesis.

Several studies have found that circulating protein levels in serum are highly correlated with the pathological processes of AD. Certain proteins in the blood can reflect neuronal damage or inflammatory states in the brain, making them potential biomarkers for the disease. However, most existing studies are primarily cross-sectional, with small sample sizes and lacking longitudinal follow-up data. Therefore, identifying protein characteristics that change over the long term before the onset of AD, and particularly those dependent or independent of *APOE-ε4*, holds significant scientific value.

3. Mechanistic association of *APOE-ε4* with AD

The *APOE* gene is one of the most extensively studied genetic risk factors in AD research. Located on

chromosome 19, it encodes APoE, which plays a crucial role in lipid metabolism, primarily by regulating the transport and distribution of cholesterol and lipids to maintain brain function. Among *APOE* alleles, the ε3 allele is the most common neutral genotype, ε2 is thought to have some protective effects, and ε4 significantly increases the risk of LOAD.

3.1. Function of *APOE-ε4* in AD

ApoE is a lipoprotein that functions mainly in the brain and peripheral tissues. Its primary role is to transport, metabolize, and store lipids by binding to lipids, cholesterol, and their receptors (*17*). Lipid metabolism in the brain is critical to maintaining neuronal function, synaptic plasticity, and cell membrane integrity. During neuronal regeneration and repair, ApoE facilitates the transport of cholesterol and phospholipids, assisting in membrane repair and neuronal regeneration (*17*) (Figure 1). ApoE's function is essential to maintaining brain health and neuronal plasticity.

The impact of different *APOE* alleles on ApoE function varies. Compared to *APOE-ε3*, ApoE encoded by the *APOE-ε4* allele is less efficient in lipid transport and metabolism, particularly in the brain, where its ability to clear Aβ is significantly reduced (*18*). The accumulation and deposition of Aβ is a hallmark of the pathology of AD, and the presence of *APOE-ε4* contributes to increased Aβ accumulation, exacerbating the progression of AD.

3.2. *APOE-ε4* and Aβ metabolism

The influence of *APOE-ε4* on Aβ metabolism is one of the primary mechanisms by which it increases the risk of AD. The production and clearance of Aβ are crucial processes for maintaining the dynamic balance of Aβ levels in the brain, and different *APOE* alleles play an important regulatory role in this process. Studies have shown that the clearance rate of Aβ is significantly lower in *APOE-ε4* carriers compared to those with *APOE-ε3* or *ε2* alleles (*19*).

ApoE encoded by *APOE-ε4* plays a key role in promoting Aβ deposition (Figure 1). ApoE binds directly to Aβ, forming insoluble complexes that promote the aggregation of Aβ in the brain. Compared to *APOE-ε3* and *APOE-ε2*, *APOE-ε4* more readily accelerates the deposition of Aβ around blood vessels and neurons, forming characteristic Aβ plaques (*18*). Significant interactions between ApoE4 and Aβ have been observed in primary immortalized astrocytes, making protein aggregation complexes more likely (*20*).

APOE-ε4 not only promotes Aβ deposition but also significantly reduces its clearance efficiency. The clearance of Aβ primarily relies on phagocytosis, lipoprotein-mediated transport, and translocation across the BBB (*21*). Soluble oligomeric forms of Aβ42

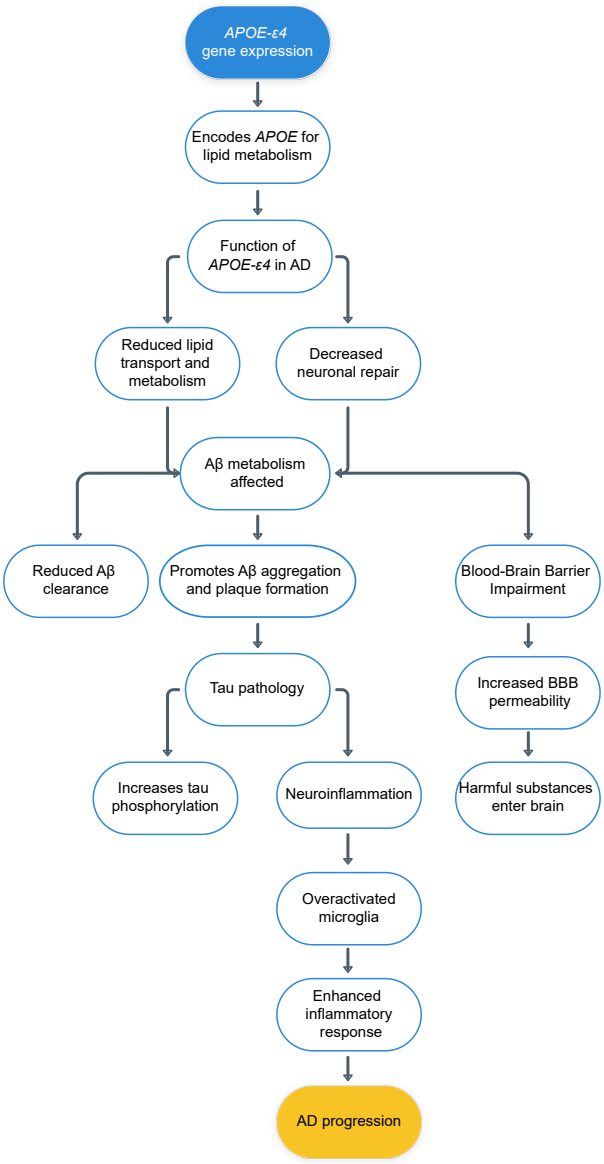

Figure 1. The mechanistic impact of *APOE-ε4* on Alzheimer's disease development. *Abbreviations*: AD, Alzheimer's disease; BBB, blood-brain barrier.

(including dimers, trimers, and small oligomers) are typically cleared through systemic circulation. Compared to *APOE ε3/ε3* carriers, *APOE ε4/ε4* carriers exhibit higher levels of Aβ oligomers. Prolonged exposure to Aβ42 dimers and trimers can lead to progressive dendritic spine loss and hippocampal synapse reduction (*22,23*). Low-density lipoprotein receptor-related protein 1 (LRP1) plays an essential role in Aβ clearance, and ApoE4 enhances Aβ production by accelerating LRP-mediated amyloid precursor protein (APP) endocytosis (*24,25*). Moreover, *APOE-ε4* carriers have a weaker BBB, with Aβ aggregates being cleared by pericytes through LRP1/ApoE interactions, a process that ApoE4 disrupts, resulting in impaired translocation of Aβ from the brain to peripheral circulation, further exacerbating Aβ accumulation in the brain (*26,27*). Additionally, *APOE-ε4* inhibits the phagocytic and degradative

abilities of microglia to clear Aβ, further contributing to Aβ accumulation. Collectively, these factors lead to earlier Aβ deposition in *APOE-ε4* carriers.

3.3. *APOE-ε4* and tau pathology

In addition to its role in Aβ metabolism, *APOE-ε4* also significantly impacts the phosphorylation and aggregation of tau protein, another key pathological hallmark of AD (*28*). Tau protein is a component of neuronal microtubules and plays a crucial role in maintaining cytoskeletal stability. However, abnormally phosphorylated tau protein forms NFTs, another hallmark of the pathology of AD.

Studies have shown that tau protein phosphorylation and aggregation are more severe in the brains of *APOE-ε4* carriers (*28*). This may be due to the indirect effects of *APOE-ε4* on lipid metabolism and synaptic function, which promote pathological changes in tau protein (*29*) (Figure 1). Moreover, neuroinflammatory responses in the brains of *APOE-ε4* carriers exacerbate the progression of tau pathology (*30*). Particularly in the hippocampus, tau pathology is significantly more pronounced in *APOE-ε4* carriers, which is closely associated with accelerated cognitive decline (*31*).

3.4. *APOE-ε4* and the BBB

APOE-ε4 affects not only Aβ and tau metabolism but also has a significant impact on the integrity of the BBB (*26*). The BBB is essential for maintaining central nervous system homeostasis by preventing harmful substances from entering the brain. *APOE-ε4* carriers tend to have diminished BBB function, characterized by a reduced capillary basement membrane area and increased thrombinogen concentrations in the microvascular walls and perivascular nerve membranes that accelerate breakdown of the BBB (*32-34*). This breakdown allows peripheral toxins and inflammatory factors to more easily enter the brain, exacerbating the pathology of AD.

Studies have also found that impaired BBB function in *APOE-ε4* carriers further decreases the efficiency of Aβ clearance, creating a vicious cycle that accelerates the progression of AD (*35*) (Figure 1). Therefore, BBB disruption is considered a key mechanism by which *APOE-ε4* promotes the development of AD *via* multiple pathways.

3.5. *APOE-ε4* and neuroinflammation

Neuroinflammation is a critical pathological mechanism in the progression of AD. ApoE protein is typically synthesized by microglia and astrocytes. *APOE-ε4* promotes excessive activation of microglia and astrocytes, intensifying inflammation in the brain. Microglia, as the immune cells of the central nervous system, play a key role in responding to Aβ pathology. There is a higher risk of Aβ deposition in *APOE-ε4* carriers, and the toxicity of Aβ plaques is also significantly enhanced. Aβ aggregation triggers neuroinflammatory responses, leading to neuronal death (*36*). In *APOE-ε4* carriers, however, microglial function shifts from Aβ clearance to pro-inflammatory responses, further damaging neurons (*37*). ApoE4 induces neuroinflammation by activating the pro-inflammatory prostaglandin E2 (PGE2) pathway or inhibiting the triggering receptor expressed on myeloid cells 2 (TREM2) pathway (*38*).

In addition, *APOE-ε4* regulates the release of inflammatory factors, promoting the infiltration of more immune cells into the brain and further exacerbating neuroinflammation (Figure 1). Compared to ApoE3 mice, ApoE4 mice exhibit significantly increased levels of TNF-α and IL-6 in the brain (*30*). ApoE4 increases the expression of inflammatory factors in human astrocytes (*30*). This excessive inflammatory response accelerates Aβ and tau pathology progression. ApoE4 can also activate the cyclosporin A-matrix metalloproteinase-9 (CypA-MMP9) pathway, leading to neuronal loss and synaptic disruption (*39*). Additionally, ApoE4 induces the activation of Ca2+-dependent phospholipase A2 (cPLA2), affecting the arachidonic acid (AA) signaling cascade that is typically associated with chronic brain inflammation (*40*). Neuroinflammation plays a particularly important role in the early stages of AD in *APOE-ε4* carriers.

3.6. Individual variability in *APOE-ε4*

APOE-ε4 is widely considered to be a key risk factor for AD, but not all *APOE-ε4* carriers develop AD. In fact, approximately 24% of *APOE-ε4* carriers do not develop AD during their lifetime (*41*). This suggests that the effects of *APOE-ε4* may depend on other genetic, environmental, and lifestyle factors (*42*) (as shown in Figure 2). Studies have shown that lifestyle interventions such as a healthy diet, regular physical activity, maintaining cognitive engagement, and managing cardiovascular health may help reduce the risk of AD in *APOE-ε4* carriers (*43-47*). Additionally, the influence of other genes, individual immune status, and sex differences also affect AD risk in *APOE-ε4* carriers (*48*). Compared to male ApoE4 mice, primary microglia from female ApoE4 mice have higher levels of IL1b, TNF-α, IL6, and NOS2 (*49*). A study has suggested that female have a higher incidence of AD than males (*50*). The *APOE4* allele similarly increases the risk of amyloid abnormalities in both male and female, but its impact on tau is more significant in female (*50*). However, another study has suggested that sex differences in dementia risk may partially depend on age and/or geographic region (*51*). A recent meta-analysis found no significant differences in the relationship between *APOE-ε4* and AD between males and females, but between the ages of 55

Figure 2. Risk factors for Alzheimer's disease (AD)

and 70, females had a higher risk of AD associated with *APOE-ε4* than males (*52*). Thus, *APOE-ε4* is thought to have its most potent effects during this age range. Whether the impact of *APOE-ε4* is dependent on sex hormones such as estrogen remains unclear (*53*).

4. Serum proteomic characteristics of AD and future directions

The rapid development of proteomics has opened new avenues for studying complex neurodegenerative diseases such as AD. Serum proteomics, in particular, offers unique advantages in discovering new biomarkers and understanding disease mechanisms. Given that blood is relatively easy to obtain and reflects dynamic changes in systemic diseases, serum proteomic characteristics hold great potential for early diagnosis and research into the pathology of AD.

Recent studies have shown that proteins in the serum may reflect pathological changes in the brain and CSF, even before clinical symptoms appear. Therefore, identifying serum proteomic features associated with AD, and especially those dependent or independent of the *APOE-ε4* genotype, provides new perspectives for early diagnosis, understanding the molecular mechanisms of the disease, and developing personalized treatment strategies.

4.1. Serum proteins as early biomarkers

The early stages of AD, and particularly the asymptomatic phase or mild cognitive impairment (MCI) stage, represent a critical window for therapeutic intervention. Early intervention has the potential to slow or reverse disease progression, but there is a lack of biomarkers that are able to accurately diagnose AD in this stage. Serum proteomics research, through large-scale screening, can identify proteins that exhibit abnormal levels years or even decades before AD clinical symptoms manifest.

In different stages of the pathology of AD, levels of specific proteins in the serum change significantly. For example, proteins associated with neuronal damage, inflammatory responses, and metabolic dysregulation have been found to be abnormally expressed even in the preclinical stages of AD. These proteins could not only serve as early biomarkers for AD but also reflect the core pathological processes of neurodegenerative diseases.

A recent longitudinal analysis of serum proteomes in 5,294 participants identified 329 proteins associated with AD, some of which were linked to *APOE-ε4*-dependent pathways while others were linked to independent pathways (*54*). Notably, some *APOE-ε4*-independent proteins, such as glycoprotein non-metastatic protein B (GPNMB), netrin 1 (NTN1), SPARC-related modular calcium binding 1 (SMOC1), and spondin 1 (SPON1), displayed a high degree of consistency with AD-related proteins in the brain and CSF. This suggests that these proteins may reflect early neuronal pathway changes and could be used as early biomarkers for predicting and diagnosing AD.

4.2. The significance of *APOE-ε4*-dependent and independent protein characteristics

Research has found that the serum proteomic characteristics of AD patients can be divided into two categories based on *APOE-ε4* carrier status: dependent and independent. *APOE-ε4*-dependent protein characteristics primarily reflect pathological processes related to lipid metabolism, Aβ metabolism, and neuroinflammation regulated by *APOE-ε4*. For example, *APOE-ε4*-dependent proteins such as ARL2, S100A13, and TBCA are closely related to AD, and levels of their expression are significantly influenced by the *APOE-ε4* genotype (*54*). AD-related changes in these proteins are more pronounced in *APOE-ε4* carriers, suggesting that they may serve as biomarkers specific to *APOE-ε4* carriers and help to identify early cases of AD in this high-risk group.

In contrast, *APOE-ε4*-independent proteins reflect broader neuronal dysfunction and metabolic abnormalities and are potentially indicative of a wider range of AD patients. Proteins such as GPNMB, NTN1, SMOC1, and SPON1 are closely associated with neuronal survival, synaptic function, and extracellular matrix changes. These proteins exhibit similar pathological patterns in the serum, CSF, and brain tissue (*54*). This consistency suggests that these proteins could become universal biomarkers across different APOE genotypes, allowing for broader AD detection and monitoring.

Moreover, the discovery of *APOE-ε4*-independent protein characteristics offers new insights for treating AD in non-*APOE-ε4* carriers. The specificity and broad applicability of these protein markers suggest that they have significant potential for personalized treatments in the future. By combining different sets of protein characteristics, scientists could better identify individual risk and tailor personalized treatment plans.

4.3. Limitations and challenges of proteomics

Proteomics research has brought new hope to AD diagnosis and treatment, but several limitations and challenges remain. First, most current proteomics studies focus on specific populations, and particularly Nordic populations. Whether these findings can be generalized to other populations, and especially those with different racial, geographic, and lifestyle backgrounds, needs to be validated further. Differences in population genetics and environmental exposure may significantly affect protein expression. Therefore, future studies should include more diverse populations to ensure the generalizability of findings.

Second, proteomics technology itself presents certain technical challenges. Different proteomic platforms vary in sensitivity, specificity, and methods of data processing, leading to potential inconsistencies between studies. For example, protein levels measured using different platforms may display different patterns of association across cohorts (*55,56*). Therefore, to improve the reproducibility and consistency of studies, standardizing experimental protocols and methods of data analysis in proteomics research is essential.

Moreover, proteomics studies have revealed many potential biomarkers associated with AD, but further research is needed to determine whether these biomarkers can accurately differentiate AD from other types of dementia. Biomarkers of AD may overlap with other neurodegenerative diseases, such as Parkinson's disease and Lewy body dementia. Therefore, identifying proteins that can specifically distinguish AD is crucial.

4.4. Directions for future research

To overcome the current limitations and further advance the early diagnosis and personalized treatment of AD, further studies are needed.

4.4.1. Large-scale, multicenter prospective cohort studies

To enhance the generalizability of their findings, future studies should involve multicenter collaborations that include populations of different races, geographic backgrounds, and age groups in prospective cohort studies. This approach will help identify proteomic biomarkers that are common across various ethnic and cultural groups and reveal the impact of environmental and lifestyle factors on protein characteristics.

4.4.2. Longitudinal studies and dynamic monitoring

As a slowly progressing disease, AD's pathological process may evolve over several decades. Therefore, longitudinal studies are essential for revealing the temporal dynamics of proteomic characteristics. Long-term follow-up studies will enable researchers to better understand changes in protein levels and identify proteins that are abnormal before symptoms appear, improving the accuracy of early diagnosis.

4.4.3. Integration of proteomics with multi-omics

Single -omics studies often cannot fully capture the complexity of diseases. Future research should integrate proteomics with other -omics, such as genomics, metabolomics, and transcriptomics, to create more comprehensive molecular network models. This multi-omics approach can reveal interactions between different biomolecules, providing deeper insights into the pathogenesis of AD and new avenues for personalized treatment.

4.4.4. Personalized treatment strategies

Future research should focus on developing personalized treatment strategies based on proteomic characteristics. By identifying specific proteomic profiles in patients, and particularly *APOE-ε4*-dependent and independent proteins, researchers can design precise interventions for different risk groups. For instance, therapies targeting Aβ and neuroinflammation may be developed for *APOE-ε4* carriers, while treatments focused on neuronal protection and metabolic regulation could be tailored for non-*APOE-ε4* carriers.

4.4.5. Clinical translation of novel biomarkers

Although many protein markers have been discovered to be associated with the risk of AD, extensive work is still required to use these biomarkers clinically. Future studies should focus on validating these markers in clinical settings and developing simple, accurate, and cost-effective detection tools for widespread use in routine medical practice.

5. Conclusion

In conclusion, the use of serum proteomics in AD research holds great promise, not only offering new solutions for early diagnosis and risk prediction but also laying the foundation for the development of personalized treatments. As technological advances continue and research goes further in depth, the clinical translation of proteomic biomarkers will help to improve

the quality of life for patients and alleviate the significant public health burden of AD worldwide.

Study of AD is reaching a new milestone, where the integration of proteomics, genetics, and other multi-omics technologies will enable scientists to better understand the complexity of the disease and provide a solid scientific basis for the development of new therapeutic approaches. Future research should focus on further validating these biomarkers in clinical settings and exploring how to integrate them into existing healthcare systems with the ultimate goals of early detection of, precise intervention in, and personalized treatment for AD.

Funding: This work was supported by a grant from the National Natural Science Foundation of China (No. 82460268) and the Hainan Province Clinical Medical Research Center (No. LCYX202410, LCYX202309) and Grants-in-Aid from the Ministry of Education, Science, Sports, and Culture of Japan (24K14216).

Conflict of Interest: The authors have no conflicts of interest to disclose.

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Received November 17, 2024; Revised January 14, 2025; Accepted January 19, 2025.

**Address correspondence to:*
Xiqi Hu, Department of Neurosurgery, Haikou Affiliated Hospital of Central South University Xiangya School of Medicine, Haikou, China 570208.
E-mail: 218302048@csu.edu.cn

Peipei Song, National Center for Global Health and Medicine, Tokyo, Japan 162-8655.
E-mail: psong@it.ncgm.go.jp

Released online in J-STAGE as advance publication January 23, 2025.