

Review

Glycosylation in aging and neurodegenerative diseases

Weilong Zhang, Tian Chen, Huijuan Zhao, and Shifang Ren*

NHC Key Laboratory of Glycoconjugates Research, Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Fudan University, Shanghai 200032, China

*Correspondence address. Tel: +86-21-54237701; E-mail: renshifang@fudan.edu.cn

Received 29 April 2024 Accepted 23 June 2024

Abstract

Aging, a complex biological process, involves the progressive decline of physiological functions across various systems, leading to increased susceptibility to neurodegenerative diseases. In society, demographic aging imposes significant economic and social burdens due to these conditions. This review specifically examines the association of protein glycosylation with aging and neurodegenerative diseases. Glycosylation, a critical post-translational modification, influences numerous aspects of protein function that are pivotal in aging and the pathophysiology of diseases such as Alzheimer's disease, Parkinson's disease, and other neurodegenerative conditions. We highlight the alterations in glycosylation patterns observed during aging, their implications in the onset and progression of neurodegenerative diseases, and the potential of glycosylation profiles as biomarkers for early detection, prognosis, and monitoring of these age-associated conditions, and delve into the mechanisms of glycosylation. Furthermore, this review explores their role in regulating protein function and mediating critical biological interactions in these diseases. By examining the changes in glycosylation profiles associated with each part, this review underscores the potential of glycosylation research as a tool to enhance our understanding of aging and its related diseases.

Key words aging, glycosylation, neurodegenerative diseases

Introduction

Aging manifests as a progressive decrease in biological functions across an organism and is influenced by myriad factors at both the cellular and molecular levels [1–4]. Despite considerable investigative efforts, the molecular bases driving functional decreases during aging remain only partially elucidated, presenting challenges in the development of effective interventions and the precision of experimental studies in aging research [5,6]. The discovery and analysis of aging biomarkers have enhanced our understanding of this complex process, shedding light on critical aspects such as genomic instability, telomere attrition, epigenetic changes, loss of proteostasis, dysregulated autophagy, impaired nutrient sensing, and mitochondrial dysfunction. While these biomarkers are indicative of distinct aging dimensions, they collectively emphasize the importance of an integrative approach to understanding the aging process [7].

Glycosylation, a post-translational modification, is fundamentally linked to a variety of biological processes and critically influences protein functionalities such as folding, solubility, stability, and enzyme activity. This modification plays an essential role in

protecting proteins from proteolytic degradation, ensuring appropriate subcellular localization, and maintaining cellular homeostasis [8–11]. Notably, glycosylation has a profound impact on the aging process, as it is closely associated with various age-related physiological changes, and alterations in glycosylation patterns have been shown to correlate with aging and affect organismal health and longevity [12–14]. Emerging evidence from recent studies underscores the connection between glycosylation dynamics and the aging process, suggesting a pivotal role of glycosylation in modulating lifespan and age-related disorders.

Understanding the interplay between glycosylation and aging is crucial, particularly in the context of age-related pathologies such as neurodegenerative disorders, cancer, type 2 diabetes, metabolic syndrome and chronic inflammatory conditions. These disorders share mechanistic pathways with the natural aging process, indicating that alterations in glycosylation may play a role in disease pathogenesis and progression [15]. Among these diseases, neurodegenerative diseases have garnered extensive attention in recent research due to their significant impact on health span and quality of life in aging populations. Accumulating evidence suggests

that glycosylation modifications are closely linked to the pathophysiology of these disorders, possibly through mechanisms involving protein misfolding and aggregation, which are hallmark features of neurodegenerative diseases.

The relationship between glycosylation and aging or neurodegenerative diseases is particularly compelling as it offers insights into the molecular underpinnings that bridge chronic disease and aging. Research into the molecular basis of aging and neurodegenerative diseases can significantly enhance our understanding of how these processes are interlinked, potentially leading to novel diagnostic and therapeutic strategies. These strategies could focus on modifying glycosylation pathways to mitigate the adverse effects of aging and disease progression.

Given the established connections between glycosylation and aging or age-related diseases, this review focuses primarily on the characteristics of glycosylation modifications in the context of aging and selected neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. By delving into recent research advancements, we aim to highlight how glycosylation impacts these diseases within the broader spectrum of aging, thereby offering new avenues for intervention that could improve diagnosis, treatment, and ultimately patient outcomes. This focused approach allows for a detailed exploration of potential therapeutic targets within glycosylation pathways that could influence both aging and the pathogenesis of neurodegenerative diseases in the future.

N-linked Glycosylation

Protein glycosylation, a fundamental biochemical process, is predominantly categorized into N-linked and O-linked types, occurring predominantly within the endoplasmic reticulum (ER) and the Golgi apparatus. In this process, glycosyl donors covalently bind to proteins through a complex enzymatic process facilitated by

a repertoire of approximately 200 types of glycosyltransferases [16]. N-linked glycosylation in eukaryotic cells involves two conserved steps: the initial assembly of oligosaccharides on a lipid carrier—dolichol phosphate, and the subsequent transfer of oligosaccharides to selected asparagine residues on nascent polypeptides translocated into the ER lumen [17]. The process begins with the stepwise addition of monosaccharides by glycosyltransferases, which are activated via nucleotides, forming glycan chains. The formed lipid-linked oligosaccharides (LLOs) are then translocated from the cytoplasmic side to the lumen side of the ER membrane or to the cell membrane in prokaryotes, where they serve as glycosylation donors. In eukaryotes, these oligosaccharide chains may undergo further elongation within the ER lumen. Proteins that possess a glycosylation consensus sequence (N-X-S/T, where X is any amino acid except proline) and are transported into the ER lumen or extracellular space in prokaryotes act as receptors. Oligosaccharyl-transferase (OST) subsequently catalyzes the attachment of the entire oligosaccharide chain to the asparagine residue of the target polypeptide. Following this initial glycosylation event, N-glycans can be extensively modified in eukaryotes, a process coupled with the secretory pathway, leading to the formation of complex, species- or cell type-specific N-linked glycan structures [12,18,19] (Figure 1).

O-linked Glycosylation

Another principal type of protein glycosylation is O-linked glycosylation. Unlike N-linked glycosylation, most O-linked glycosylation occurs in the Golgi apparatus. The primary process involves various glycosyltransferases attaching single monosaccharide residues to the hydroxyl groups of serine or threonine, followed by the sequential addition of diverse monosaccharide residues. O-glycosylation can be categorized into mucin-type and non-mucin-type based on the monosaccharide residues attached to serine or threonine. In mucin-type glycosylation, the initial

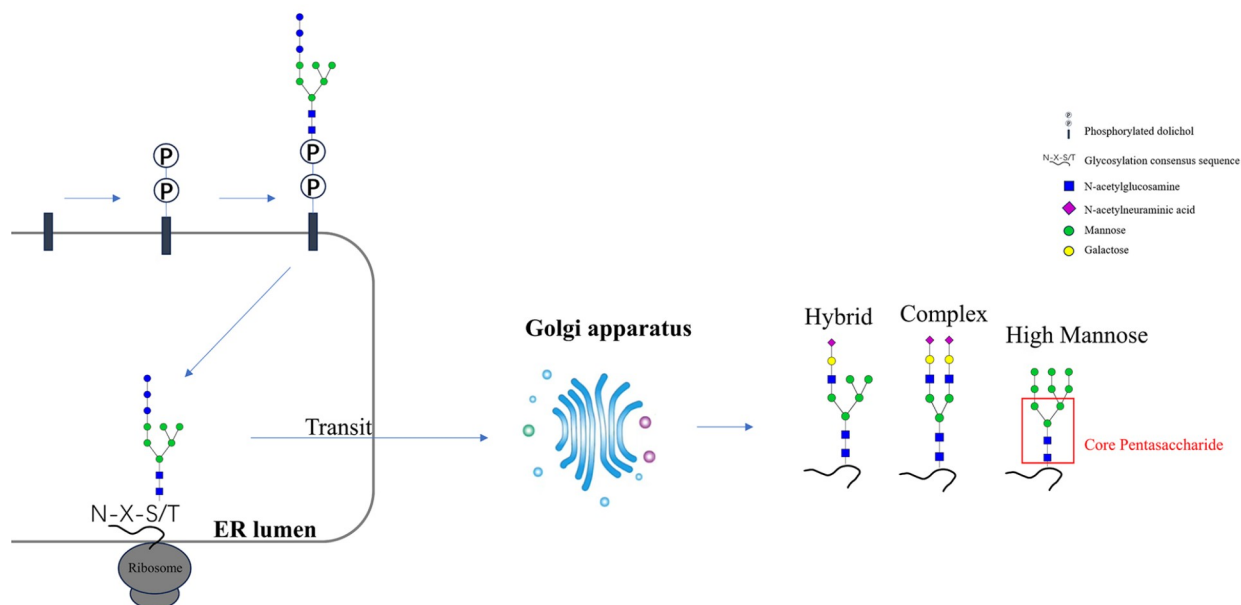


Figure 1. Simplified diagram of the N-linked glycosylation process Proteins synthesized and folded in the ER are glycosylated at the consensus sequence N-X-S/T. These glycoproteins then transit to the Golgi apparatus, where they undergo further modifications to acquire complex, hybrid, or high-mannose N-glycan chains.

monosaccharide attached is N-acetylgalactosamine, whereas in non-mucin-type glycosylation, the initial residues can include N-acetylglucosamine, galactose, fucose, and others. Compared with N-linked glycosylation, O-glycosylation is more diverse and complex, driven by the heterogeneity of monosaccharide residues, which contributes to the varied and complex O-glycosylation mechanisms in mammals [20–22]. Additionally, a pivotal post-translational modification, O-GlcNAc glycosylation, also known as O-GlcNAcylation, is a specific type of O-glycosylation. O-GlcNAc glycosylation involves the attachment of a single N-acetylglucosamine (GlcNAc) molecule to serine or threonine residues on proteins through an O-glycosidic bond. This modification differs from other forms of glycosylation, such as N-linked glycosylation [23–25]. This glycosylation is a highly dynamic process in eukaryotes and is regulated by two highly conserved enzymes: O-GlcNAc transferase (OGT) which catalyzes the addition of β -O-GlcNAc, and O-GlcNAcase (OGA) which mediates its removal [26–28]. In human biological processes, O-GlcNAc glycosylation plays a significant role and is closely linked to aging and chronic age-associated diseases, including neurodegenerative disorders [29–31]. Figure 2 illustrates a simplified diagram depicting the O-linked glycosylation process.

Glycosylation in Chronological Aging

This part aims to systematically explore the latest advancements in research on the role of glycosylation, specifically within the context of chronological aging, independent of age-associated diseases. This focused approach is crucial, as it allows for a clearer delineation of the effects of natural aging processes, separated from pathological states, thereby providing a purer lens through which to view the intrinsic aging process. Effective aging biomarkers can help identify patients at higher risk of age-related diseases at an earlier stage so that targeted anti-aging treatments can be administered. Aging is a complex and long-term evolutionary process influenced by both genetic and environmental factors. As research has advanced, glycosylation has emerged as a critical regulator in the aging

process, and the degree of protein glycosylation serves as a real-time indicator of intracellular and intercellular processes, thereby making it a potential biomarker for aging and age-related diseases.

Research into aging dates back to 1939, when caloric restriction in mice and rats was found to prolong lifespan [32]. For decades, scientists have continuously searched for biomarkers that can identify aging and age-related diseases. This ongoing quest has led to the exploration of various physiological markers, among which glycosylation has recently been highlighted as a potential indicator of aging. Notably, galactosylation and sialylation have been reported to decrease significantly with aging, and similar trends have also been observed in some age-related diseases. These findings have been summarized in previous reviews [15,33–35]. In this context, our review is specifically dedicated to consolidating recent research developments over the past several years concerning glycosylation modifications in aging. We aim to delineate the latest advancements that extend beyond the scope of previous reviews.

Following the overview of the role of glycosylation in aging, it is critical to delve into the molecular intricacies and genetic underpinnings that facilitate these changes. The process of human protein glycosylation is complex, and the regulatory mechanisms *in vivo* are poorly understood. To explore the underlying mechanisms, genome-wide association studies (GWAS) have been employed to identify genetic variants associated with glycosylation processes. This approach facilitates the discovery of genetic loci that potentially influence the synthesis of plasma protein N-glycans, providing insights into the molecular basis of glycosylation and its implications in aging and related diseases. Notably, three prior GWAS studies have identified sixteen loci linked to the synthesis of plasma protein N-glycans, with twelve of these loci being replicated in independent samples [36–38]. To mitigate the risk of false positives, further research was conducted to validate the discovery of the 16 loci. This study involved a large sample size of 4,802 individuals from 4 cohorts, and 15 of the original 16 loci were successfully replicated. These findings confirmed the robustness of

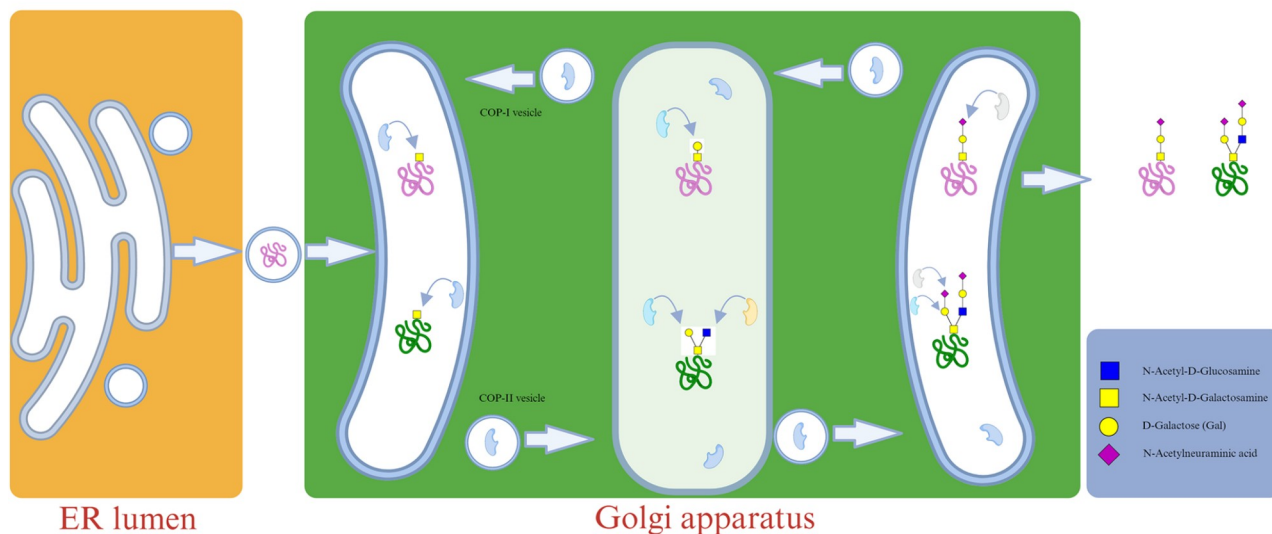


Figure 2. Simplified diagram of the O-linked glycosylation process After being synthesized and folded in the ER, proteins in the secretory pathway enter the Golgi apparatus. As they progress through the Golgi stacks, they acquire complex O-glycan chains based on the distribution of glycosyltransferases.

GWAS in identifying genetic regulators of glycosylation. This study suggests that further functional investigations can be conducted on these genes, particularly those encoding glycosyltransferases such as MGAT5, B3GAT1, and others, to explore their roles in protein glycosylation processes more deeply [39]. In addition to these genetic studies, prompted by extensive prior research indicating a link between IgG galactosylation and aging, researchers have conducted further GWAS analyses specifically targeting the characteristics of IgG galactosylation. GWAS analysis identified 37 candidate genes, and the subsequent use of the CRISPR/dCas9 system confirmed the upregulation of 3 genes, namely, *EEF1A1*, *MANBA* and *TNFRSF13B*, which changed the glycan composition of IgG, confirming that these 3 genes participate in galactosylation of IgG in the *in vitro* expression system [40]. Moreover, some researchers also used Mendelian randomization (MR) to analyze the causal relationship between IgG N-glycosylation and aging and reported the potential causal effect of lower GP23 levels on late aging [41]. The findings from GWAS and Mendelian randomization studies provide some evidence that glycosylation modifications, particularly IgG N-glycosylation, may play a significant role in the aging process. These studies not only identify genetic loci associated with glycosylation traits but also suggest a causal link between specific glycosylation patterns and aging. Together, these studies provide some possible insights into how glycosylation serves as a crucial biological mechanism in aging. In addition to plasma whole proteins and individual proteins such as immunoglobulins, the role of glycosylation in aging extends to the tissue-specific level. Integration of the genomic and transcriptomic data of both healthy and diseased tissues revealed that specific glycosylation patterns correlate with aging. This research elucidated the genome-wide alterations in glycosylation associated with aging, and further demonstrated the potential of glycosylation as a prognostic biomarker [42].

Environmental factors, including lifestyle choices, also play a significant role in the aging process, influencing glycosylation patterns in various ways. Researchers have developed a new

biomarker of biological aging, namely, the glycan age clock, which suggests the interplay between genetic and environmental factors in determining biological age [43]. This complex interaction determines the speed of the glycan clock, emphasizing the potential of glycosylation markers in prognostic applications.

These studies highlight the significant role of glycosylation in aging. GWAS has identified and validated critical genetic loci involved in protein glycosylation, suggesting potential targets for further functional investigations. Studies focusing on IgG galactosylation have demonstrated the intricate genetic regulation of glycosylation and its association with aging. Additionally, the introduction of the glycan age clock as a biomarker emphasizes the interplay between genetic and environmental factors in determining biological aging, reinforcing the potential of glycosylation markers in prognostic applications.

With the accumulation of recent research results on the factors influencing age-related glycosylation modifications, new opportunities to intervene in the aging process have emerged. For instance, Greto *et al.* [44] investigated whether weight loss interventions affect inflammation and aging-related IgG glycosylation changes in a longitudinal cohort of bariatric surgery patients. A low-calorie diet resulted in a significant reduction in bisecting GlcNAc levels in IgG N-glycans, which are often associated with aging and inflammation. During the one-year follow-up through weight loss, significant increases in delactosylated and sialylated glycans were observed, along with significant reductions in agalactosylated and core fucosylated IgG N-glycans. These findings demonstrate that weight loss significantly affects IgG N-glycosylation, resulting in decreased biological age. Similarly, another study by Šimunić-Briški *et al.* [45] revealed that regular moderate physical exercise reduced the glycan age index and the inflammatory potential of immunoglobulin G. Additionally, in our previous study, calorie restriction was found to reduce the overall serum glycan level. The reduction in galactosylated glycans and sialylated glycans may be associated with the benefits of calorie restriction in antiaging, providing a possible molecular basis for the beneficial effects of calorie restriction from

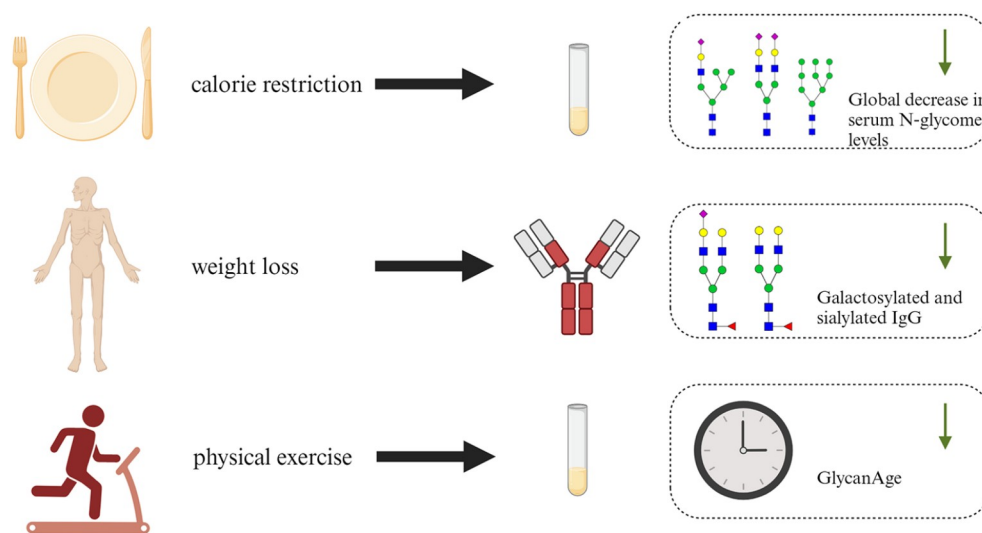


Figure 3. Opportunities to intervene in the aging process Aging interventions (left) affect biomolecules (middle), leading to measurable changes (right).

the perspective of glycosylation changes [46]. Figure 3 shows various opportunities for intervention in the aging process.

In addition to the relationship between N-glycosylation and aging described above, some studies have also focused on the relationship between O-glycosylation or related proteins and aging. In the aging process, the protective functionality of the mucus barrier significantly deteriorates. Muc2 mucin, an essential element of the colonic mucus layer, is significantly altered. Recent assessments of mucus layers in mice of different age groups have shown that, as age progresses, there are alterations in the expression of glycosyltransferases, which directly influence the glycan structures of intestinal mucin Muc2. For instance, elevated levels of Core 1 β -1,3-galactosyltransferase (C1GalT1) can lead to the addition of more galactose residues to mucins, whereas decreased expressions of Core 2 β 1,6-N-acetylglucosaminyltransferase (C2GnT) and Core 3 β 1,3-N-acetylglucosaminyltransferase (C3GnT) may reduce the addition of N-acetylneuraminic acid or other sugars. These changes can affect the normal properties of the mucus layer, compromising its structure and increasing its susceptibility to microbial penetration, thereby destabilizing the intestinal environment [47,48]. Additionally, studies on mucins in the submandibular glands of mice during aging have revealed distinct glycosylation patterns across different age groups, identifying three types of mucins: age-, youth-, and life-long-specific mucins. These studies revealed a trend toward a decreased proportion of fucosylated glycans and an increased proportion of sialylated glycans with aging [49]. Moreover, cellular protein O-GlcNAcylation, a dynamic mechanism widely linked to age-associated diseases, results in elevated O-GlcNAc levels across different tissues in elderly mice. Carbamoyl phosphate synthetase 1 (CPS1), which undergoes significant O-GlcNAcylation in the aged liver, has been thoroughly examined. The O-GlcNAcylation of CPS1 is recognized as a crucial nutrient-responsive regulatory step in the urea cycle during aging and dietary limitations [50]. These findings highlight the importance of both N- and O-glycosylation in the aging process, offering new perspectives for understanding and potentially mitigating age-related physiological changes.

Stem cells play pivotal roles in tissue development, homeostasis, and regeneration. Decades of research have highlighted the decline in stem cells during tissue and organismal aging, with recent insights into the role of glycosylation in stem cell aging [51]. Studies on CD90-mediated glycosylation-dependent glucose influx mechanisms illustrate how glycosylation can mitigate aging in adipose-derived stem cells (ASCs). CD90, an extensively glycosylated membrane protein, regulates glucose entry into ASCs. Reduced expression of CD90 interferes with the glucose metabolism-driven redox balance, thus promoting aging in ASCs. Fibroblast growth factor 21 (FGF21) enhances the glycosylation of CD90 on the surface of adipose stem cells, increasing its level, promoting glucose influx, and improving oxidative stress conditions. In a high-fat diet-induced obese mouse model, long-term low-dose FGF21 treatment was found to improve adipose tissue function and overall metabolic dysregulation, increasing insulin sensitivity without significantly affecting body weight or food intake [52]. In other studies, long-term culture studies on human umbilical cord mesenchymal stem cells (hUMSCs) demonstrated that the N-glycan patterns of cellular glycoproteins are associated with mesenchymal stem cell aging. For instance, after 7 days of culture, N-glycan peaks predominantly represent high mannose-type glycans, whereas after 30 days, they

mainly consist of the complex N-glycan of Fuc1Hex5HexNAc4 [53]. These findings on stem cell glycosylation add another layer of understanding to the complex mechanisms of aging.

In addition to studying the relationship between glycosylation modifications and aging from the perspectives of glycosylation products and glycosylation-related genes involved in the synthesis process, some studies have focused on the relationship between glycosylation donors and aging. This has helped us gain a more comprehensive understanding of the connection between glycosylation modifications and aging. For example, one study investigating the impact of aging on nucleotide sugar composition across various tissues was performed by employing an innovative LC-MS/MS technique. One study revealed alterations associated with aging in nucleotide sugar concentrations, including a marked reduction in UDP-glucuronic acid in the kidneys and decreased amounts of UDP-N-acetylgalactosamine in the brains of older mice [54].

Building on the findings mentioned above, ongoing research continues to explore the relationship between aging and glycosylation. For example, studies on the porcine nucleus pulposus (NP), associated with intervertebral disc degeneration which is a condition related to aging, have revealed significant changes in outer arm fucosylation, the abundance of certain oligomannose-type N-glycans, and the content of α (2,3)-linked sialic acids across different age groups. These findings highlight the impact of glycosylation patterns on the health and degeneration of the intervertebral disc [55]. Furthermore, McDonald *et al.* reported that age-related decreases in ovarian steroids might regulate the expression of N-glycosyltransferases in mouse gonadotropins, explaining previously reported age-related N-glycosylation alterations in the FSH β subunit in human pituitary glands [56].

These studies demonstrate that the development of various technologies, such as advanced glycomics, glycoproteomics, and CRISPR/dCas9, has deepened the research into glycosylation modifications and aging. Moreover, the cross-disciplinary integration of other techniques, such as single-cell RNA sequencing (scRNA-seq), mass spectrometry imaging, and spatial transcriptomics, is poised to further promote research on glycosylation and aging. From the initial identification of age-related disease-specific glycan profiles and the construction of biomarkers for predicting biological age, elucidating the roles of abnormal glycosylation modifications associated with aging and age-related diseases and various possible influencing factors, as well as exploring changing mechanisms and corresponding molecular regulatory networks, there has also been an increased focus on the impacts of various interventions. We organize the research articles by year, accompanied by a short description of the results in Table 1.

Glycosylation in Neurodegenerative Diseases

Neurodegenerative disorders (NDs) represent a diverse collection of complex diseases marked by the gradual deterioration and decline of neurons across different areas of the nervous system, resulting in deficits in movement and/or cognitive ability [57–59]. These conditions, including Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS), are incurable and pose significant challenges because of their increasing prevalence [60]. Despite extensive research, effective treatments to slow, halt, or prevent the progression of NDs remain elusive, with the

Table 1. Glycosylation and chronological aging

Sample type	Results	Publication date	Ref
Human plasma	Replication of 15 sites related to N-glycosylation of human plasma proteins across 4,802 samples from four different cohorts.	2021	[39]
Plasma	A calorie-restricted diet significantly reduced the quantities of IgG N-glycans containing bisecting GlcNAc, whereas bariatric surgery notably elevated levels of digalactosylated and sialylated glycans, and markedly reduced the presence of agalactosylated and core fucosylated IgG N-glycans.	2021	[44]
Submandibular gland	The submandibular glands in mice produce three varieties of mucins, each characterized by unique glycan compositions. The expression of these mucins changes as the mice age.	2021	[49]
N/A	Integrating genomic and transcriptomic data from healthy and pathological tissues to uncover new understanding of the intricate function of glycosylation in humans.	2022	[42]
Mouse tissues and cells	CPS1 O-GlcNAcylation is a crucial nutrient-detecting regulatory phase in the urea cycle amid aging and dietary limitations.	2022	[50]
Serum/IgG	Environmental factors have a strong impact on the result measured with the glycan clock.	2022	[43]
Human umbilical cord mesenchymal stem cells (hUMSCs)	The N-glycan patterns of cellular glycoproteins are associated with mesenchymal stem cell aging.	2022	[53]
IgG Glycan Information	The upregulation of TNFRSF13B, EEF1A1, and MANBA, changed the glucose composition of IgG, confirming that these three genes participated in galactosylation of IgG in vitro expression system .	2023	[40]
Cell	Administration of fibroblast growth factor 21 (FGF21) effectively diminished senescent characteristics by increasing the glycosylation of the CD90 protein.	2023	[52]
IgG	Consistent, lifelong moderate physical activity exerts an anti-inflammatory influence on both the female and male populations.	2023	[45]
Serum	Mannose varieties consistently displayed reduced concentrations. Meanwhile, O-acetylated sialoglycans demonstrated an increasing pattern mainly observed in H5N4Ge2Ac1and H5N4Ge2Ac2.	2023	[46]
Colon tissues	Changes in mRNA expression levels of the enzymes C1GalT1, C1GalT2, and C2gnts involved in O-glycan biosynthesis with age in the mouse colon.	2023	[48]
Nucleus pulposus tissue	Antennary structures, galactosylation, fucosylation, and sialylation in the nucleus pulposus change from young to mature stages, with an increase in outer arm fucosylation and a decrease in $\alpha(2,3)$ -linked sialylation as aging progresses.	2023	[55]
Mice	Find that age-related declines in ovarian steroids might regulate the expression of N-glycosyltransferases in mouse gonadotropins, explaining previously observed age-related N-glycosylation alterations in the FSH β subunit in human pituitary glands.	2023	[56]
IgG glycan information	Find the effect of lower GP23 levels on late aging.	2024	[41]
Mouse tissue	Alterations in nucleotide sugar concentrations associated with aging, such as a notable reduction in UDP-glucuronic acid in the kidneys and decreased amounts of UDP-N-acetylgalactosamine in the brains of older mice.	2024	[54]

mechanisms underlying neuronal dysfunction and death still largely unexplored [61]. Aging is the principal risk factor for neurodegenerative diseases which are predominantly observed in older individuals. This progressive, irreversible pathophysiological process is characterized by a deterioration in tissue and cellular function and an increased risk of several age-related diseases, including neurodegenerative conditions [62]. Alzheimer’s disease and related dementia (ADRD) exemplify conditions in which aging plays a significant role in their development. Cognitive deterioration and neurodegenerative alterations become apparent as individuals age, even in elderly adults who do not manifest dementia, highlighting

the link between aging and neurodegenerative conditions [63,64]. Glycosylation plays a crucial role in the central nervous system (CNS), influencing receptor function and cell adhesion [65]. This process is essential for normal neural function, as proteins within the CNS are extensively glycosylated. Recent studies have indicated that in neurodegenerative diseases, various glycosylation patterns change, suggesting the potential significance of glycosylation in the pathology of these diseases [66]. Moreover, glycosylation has been identified to play a significant role in the development of biomarkers for neurodegenerative diseases, highlighting the relationship between glycosylation and neurodegeneration.

ALS

First described in 1800 by the French neuroscientist Jean-Martin Charcot, ALS is a fatal disorder of somatic muscle dysfunction caused by degeneration of upper and lower motor neurons. The mechanism of ALS development is poorly understood, and there are currently no effective treatments [67–68]. The earliest widely studied relationship between neurodegenerative diseases and glycosylation was ALS. Although the exploration of their relationship is still in its early stages, promising results have been achieved, indicating potential diagnostic and pathological insights.

A study of ALS patient serum glycosylation revealed that ALS patient serum-derived N-glycans presented high levels of sialylated glycans and low levels of core fucosylated glycans. As IgG is a major serum glycoprotein affected by sialylation or core fucosylation, analysis of IgG glycans alone revealed a distinct glycan, A2BG2, in the serum IgG of ALS patients. Subsequent experiments revealed that the presence of A2BG2 increased the affinity of IgG for CD16 on effector cells, thereby increasing antibody-dependent cytotoxicity (ADCC). This study suggested that IgG glycans can be used as ALS biomarkers and may also be involved in the process of neuronal damage [69]. In another study examining protein N-glycosylation in the cerebrospinal fluid (CSF) of ALS patients, glycomic analysis via liquid chromatography (LC) and mass spectrometry (MS) was used to analyze glycosylation patterns in the cerebrospinal fluid (CSF) of 29 ALS patients and matched controls. The glycans A2G2S and FA2G2S are significantly increased in ALS patients [70]. Furthermore, studies focusing on the glycans of IgG in CSF by combining HPLC and MALDI-TOF-MS analysis revealed that ALS patients have increased levels of IgG galactosylation, which has comparable diagnostic predictive value to phosphorous neurofilament heavy chains in ALS diagnosis [71]. There is growing evidence of glycosylation disorders in central nervous system diseases, including neurodegenerative diseases. Recognizing the importance of glycosylation in neurological disorders and the need for a centralized repository of glycan structural data, the GlyConnect glycoprotein database (<https://glyconnect.expasy.org/brain>) was developed. This database consolidates detailed structural data on brain and cerebrospinal fluid glycans obtained through advanced glycomics and glycoproteomics techniques. This database may aid further research into glycobiology, particularly in the context of neurodegenerative diseases [65].

In addition to glycan characteristics, some studies have explored the relationships between glycoproteins and ALS. For example, the glycoprotein nonmetastatic melanoma protein B (GPNMB) has been identified as an ALS-associated factor via mouse DNA microarray analysis. SOD1 (G93A) inhibited GPNMB glycosylation and increased motor neuron vulnerability [72].

Furthermore, O-GlcNAc modification also plays an important role in ALS. An ALS transgenic mouse model overexpressing mutant superoxide dismutase (mSOD) was evaluated. The level of O-GlcNAc immunoreactivity in the spinal cord of mSOD mice was lower than that in the spinal cord of control mice. A series of experiments revealed that the neurodegeneration observed in mSOD-treated mice is related to a decrease in O-GlcNAc levels in neurons (including motor neurons) [73].

The exploration of the glycosylation profiles of patients with ALS has provided significant insights, suggesting a complex interplay between glycoprotein modifications and disease pathology. Notably, variations in glycan structures, such as increased sialylation

and altered fucosylation in serum IgG and distinctive glycosylation patterns in cerebrospinal fluid, offer promising biomarkers for ALS diagnosis and indicate their potential role in neuronal damage mechanisms. Resources such as the GlyConnect glycoprotein database support research on glycomics for understanding neurodegenerative diseases, facilitating further research in this critical area.

From a new perspective, exploring how glycosylation interacts with other cellular processes implicated in ALS, such as mitochondrial dysfunction or oxidative stress, might be fruitful. Investigating these intersections could reveal novel therapeutic targets or diagnostic tools that leverage the systemic nature of glycosylation across various biochemical pathways. This approach could not only enhance our understanding of ALS but also improve our insight into other neurodegenerative diseases where similar pathophysiological mechanisms may be at play.

PD

PD is a common neurological disease. It is a progressive degenerative disease characterized by motor symptoms (resting tremor, motor retardation, postural instability and stiffness) and non-motor symptoms (cognitive decline, depression, anxiety, autonomic nervous dysfunction and sleep disorders). Earlier studies have identified male sex and age as independent risk factors for PD. The growing elderly population contributes to an increase in Parkinson's disease (PD) patients [74].

Early studies identified IgG glycan as a potential PD marker [75]. By 2019, researchers began focusing on serum N-glycosylation. Through the development of a capillary electrophoresis (CE) method in combination with label-free quantitation and supporting vector machine-based feature selection, lower sialylation and increased fucosylation were found in Parkinson's disease patients, particularly on tri-antennary glycans with 2 and 3 terminal sialic acids [76].

Pathologically, PD is the result of selective degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc), and its pathological hallmark is depigmentation of the substantia nigra and locus coeruleus, with neuronal loss in the SNpc. Changes in tissue glycosylation are also important for understanding neurodegenerative diseases. In an analysis of O-glycosylation in the human striatum and substantia nigra tissue in PD patients and healthy controls, Wilkinson *et al.* [77] reported a significant increase in sialylation in the substantia nigra of PD patients. Rebelo *et al.* [78] characterized human Parkinson's disease nigrostriatal N-glycan as well as related transcriptome/proteome and reported that oligomannosylated N-glycans increased by 2% in the substantia nigra. Additionally, Hu *et al.* employed semisynthesis to produce homogenous α -synuclein monomers with specific site modifications (pS87 and gS87). Cryo-EM analysis revealed that O-GlcNAcylation at S87 interacts with K80 and E61, resulting in a unique iron-like fold [79]. These findings indicate that glycosylation may play an important role in PD pathology, highlighting the importance of modifications such as sialylation and oligomannosylation in the substantia nigra, as well as the impact of posttranslational modifications on the α -synuclein fibril structure.

Blood and urine are both promising sources for discovering noninvasive biomarkers, indicating promise for early and sensitive diagnosis. In serum samples, one study detected an increased abundance of glycans containing core fucose, sialic acid, and

bisecting N-acetyl glucosamine in PD patients by employing glycoproteomics methods. This alteration was observed at both the overall glycan level and specific glycosites of glycopeptides. Additionally, five Parkinson's disease-associated proteins exhibiting such N-glycosylation changes were identified. The authors suggested that these site-specific N-glycosylation changes in serum hold promise as potential biomarkers for Parkinson's disease diagnosis [80]. Similarly, in urine samples, the same group revealed a reduced abundance of N-glycans, particularly biantennary galactosylated N-glycans, in PD patients through mass spectrometry-based glycomics and glycoproteomics approaches. The downregulation of sialylation may be attributed to the reduction in ST3GAL2. Moreover, site-specific N-glycosylation analysis revealed PD-specific N-glycosylation sites in proteins such as AMBP, UMOD, and RNase 1. These findings suggest that N-glycosylation changes in urine could potentially serve as disease-specific glycosylation biomarkers in PD [81].

To evaluate the etiological implications of glycosylation variation in PD, some studies have focused more on the abnormal glycosylation modifications of specific proteins in PD. The adenine nucleotide translocator (ANT), located in the inner mitochondrial membrane, is a transporter responsible for the conversion of ADP/ATP between the mitochondrial matrix and the cytoplasm. The major functions of ANT1 include mediating mitochondria, ATP/ADP transport, uncoupling and proton pumping. As a member of the mitochondrial transporter family, ANT1 can mediate the transfer of ATP from the mitochondria to the cytoplasm and ADP from the cytoplasm to the mitochondria. This ATP/ADP exchange is an important guarantee for ATP synthesis. Previous studies have shown that downregulated ANT1 is associated with PD through the formation of protein aggregates with α -synuclein [82]. Zhang *et al.* [83] reported that changes in ANT1 glycosylation are associated with PD. This investigation revealed the role of protein glycosylation in PD pathogenesis. An increasing number of associations between glycosylation changes in specific proteins and PD have been discovered. A study reported that high glycosylation of MAP6 is involved in the pathogenesis of PD [84].

The exploration of glycosylation in PD provides significant

insights into its potential role in both diagnosis and pathogenesis. The study of altered glycosylation patterns in proteins such as IgG and the identification of specific glycosylation changes in biological fluids such as blood and urine suggest promising avenues for biomarker development. Additionally, the observed alterations in the glycosylation of specific proteins, such as ANT1 and MAP6, indicate the potential impact of post-translational modifications on PD molecular mechanisms.

Future research should focus on integrating glycosylation studies with genetic and environmental risk factors for PD to develop a more comprehensive understanding of disease mechanisms. This approach could help elucidate how glycosylation interplays with other molecular pathways influenced by these risk factors, potentially leading to targeted therapies that address the underlying causes of glycosylation changes. This may help identify targeted therapeutic strategies for Parkinson's disease and may offer personalized and effective interventions on the basis of an individual's specific glycosylation profile and genetic makeup. Figure 4 summarizes the glycosylation changes reported in ALS and PD.

AD

AD is the primary cause of dementia worldwide and is characterized by the accumulation of beta-amyloid plaques and hyperphosphorylated tau protein neurofibrillary tangles. The disease initiates with alterations in amyloid-beta ($A\beta$), leading to the propagation of tau pathology. This neurodegenerative disorder results in cognitive and functional decline linked to aging and specific neuropathological features. Early manifestations include defects in memory encoding and storage, followed by broader cognitive and behavioral changes. Despite its increasing incidence due to an aging population, no disease-modifying treatments are currently available [85–88].

As summarized in the Chronological Aging section, glycosylation plays a significant role in aging, a predominant factor in AD progression, suggesting the need to focus on the role of glycosylation in neurodegenerative diseases [63]. The accumulation of beta-amyloid peptide ($A\beta$) in the brain is a hallmark of Alzheimer's disease [89]. Amyloid precursor protein (APP) is a type I

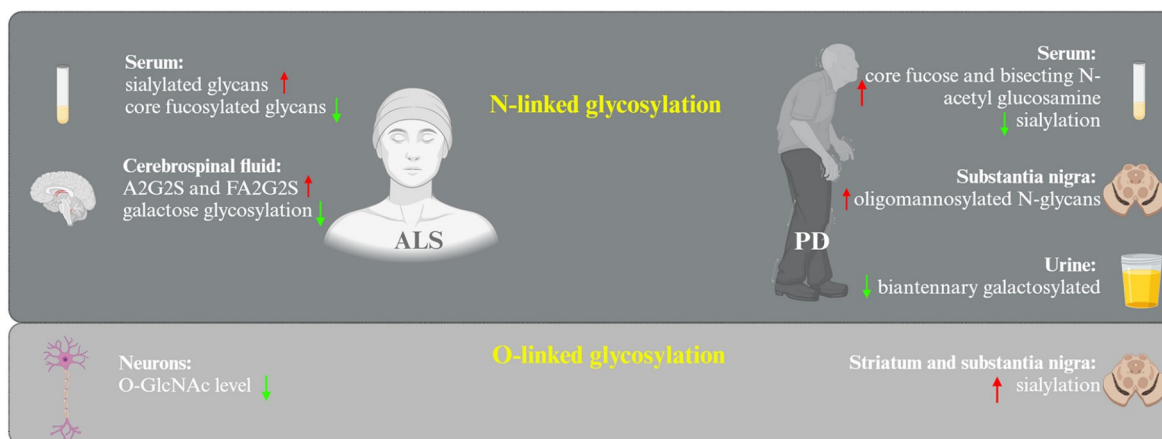


Figure 4. Glycosylation changes in ALS and PD patients The diagram illustrates glycosylation changes in ALS and PD patients. The upper section shows N-linked glycosylation changes, whereas the lower section shows O-linked glycosylation changes. The arrows indicate the direction of changes in glycosylation levels in various tissues and fluids.

transmembrane protein that is a precursor to amyloid-beta (Aβ) peptides. Studies using wild-type APP and N-glycosylation site mutant APP-SNAP plasmids have demonstrated that N-glycosylation significantly affects the transport and processing of proteins, specifically altering the cellular localization of APP and modulating the level of Aβ42 [90]. With respect to O-glycosylation, Tachida *et al.* [91] reported that APP exhibiting minimal O-glycosylation is transported to the surface of endothelial cells and subsequently returns to the Golgi apparatus for additional O-glycan alterations. Furthermore, the suppression of genes responsible for initiating APP O-glycosylation markedly decreases the synthesis of Aβ. Additionally, another investigation explored the role of glycosylation in naturally occurring autoantibodies (nAbs) against the amyloid-beta (Aβ42) isoform, revealing that N-glycosylation is crucial for the beneficial effects of autoreactive Aβ antibodies on Aβ42 aggregation, toxicity, and phagocytosis. Notable differences in glycan structures between AD patients and controls were observed, underscoring the potential of N-glycosylation as a target for monoclonal Aβ antibody development and novel diagnostic methods [92].

Hyperphosphorylation of the tau protein and related processes are other characteristics of AD [89]. Tau is a microtubule-associated protein, and its abnormal hyperphosphorylation leads to the deposition of pathological tau proteins in the brain [93–95]. Previous studies have shown that reduced brain glucose metabolism and O-GlcNAcylation lead to excessive phosphorylation of the tau protein, diminishing its ability to bind to microtubules and impairing its function. Additionally, changes in glycosylation patterns can further alter the levels of tau protein with abnormal phosphorylation, directly impacting the pathology of Alzheimer’s disease [96–98]. O-GlcNAc glycosylation modulates Tau phosphorylation and aggregation, with O-GlcNAcylation at key sites such as S400, S412, and S413, reducing phosphorylation and slowing fibrillar assembly in Alzheimer’s disease, whereas phosphorylation at sites such as pS396 and pS404 by GSK3β promotes aggregation [99]. In a brain-specific SIRT1-deficient mouse model (SIRT1 flox/Cre+), increased site-specific phosphorylation of Tau is coupled with enhanced O-GlcNAcylation triggered by reduced O-GlcNAcase (OGA) and increased O-GlcNAc transferase (OGT) protein levels [100]. An *in vivo* study using the OGA inhibitor Thiamet G (TMG) revealed that increased brain O-GlcNAc levels reduced hyperphosphorylated Tau and significantly decreased mouse hyperactivity [101]. Park *et al.* also validated O-GlcNAcylation-based therapy as a potential intervention for AD [102,103]. However, the side effects of inhibitors such as TMG remain a concern [104]. Further research led to the discovery of a novel inhibitor, compound 39, which

demonstrated excellent OGA inhibition, no cytotoxicity, and favorable pharmacokinetic properties in mice [105]. Overall, glycosylation plays a crucial role in understanding the involvement of the tau protein in Alzheimer’s disease, and glycosylation-related interventions also show promise for Alzheimer’s disease treatment.

In biomarker research, a comprehensive glycomic analysis of AD-related blood and brain tissues identified N-glycosylation as a mechanistic biomarker for the early diagnosis of AD/ADRD [106]. Further studies by Tena *et al.* [107] on serum glycoproteins from 195 participants (including AD patients and controls) revealed alterations in serum glycoprotein glycosylation and identified fucosylation as a potential marker of AD. Significant reductions in the fucosylation of immunoglobulin G1 (IgG1) and IgG2 have been identified as diagnostic indicators. This study also distinguished AD patients from healthy controls through unique glycopeptide profiles of complement proteins and apolipoprotein B. A large-scale N-glycoproteomic analysis of brain tissues from AD patients revealed the predominant presence of highly mobile and complex fucosylated and bisected N-glycans, with Man5 N-glycan emerging as the most frequent one. This analysis also highlighted a notable characteristic of the brain’s N-glycoproteome: sialylation, which is relatively rare and occurs in less than 9% of glycoforms. Furthermore, the research revealed variations in glycosylation at individual sites, including differences in antenna number, fucosylation frequency, and bisection, which are correlated with disease progression. These glycosylation differences extend to subcellular compartments, indicating alterations across the stages of AD [108]. Recent studies have identified glycopeptide forms associated with cognitive resilience and revealed that the glycopeptides NPTX2a, NPTX2b, NECTIN1, NPTX2c, HSPB1, PLTP, NAG, and VAT are linked to cognitive decline, suggesting that targeting these glycopeptides could be beneficial for cognitive restoration [109]. Alzheimer’s disease is largely influenced by genetic factors, with the APOE allele showing the strongest correlation with the disease [85]. Additionally, because molecular and cellular pathological changes precede clinical onset by several years, pre-symptomatic biomarker research is particularly crucial [61]. A pivotal study involving 233 individuals demonstrated the predictive value of bisecting N-acetylglucosamine in conjunction with total tau levels in blood for AD. This correlation, along with an intermediate tau/bisecting N-acetylglucosamine ratio, is linked to an increased risk of developing AD. A comprehensive model that includes this ratio, apolipoprotein E (APOE) ε4 status, and cognitive scores showed high predictive accuracy for AD, underscoring the biomarker’s potential in early diagnosis [110]. Table 2 lists possible glycosylation-related biomarkers associated with neurodegenerative diseases.

Table 2. Possible glycosylation-related biomarkers in neurodegenerative diseases

Results	Diseases	Sample type	Ref
Reductions in fucosylation of Immunoglobulin G1 (IgG1) and IgG2, glycopeptide profiles of complement proteins and apolipoprotein B.	AD	Serum	[106]
Variations in the number of antennae, frequency of fucosylation, and degree of bisection correlate with the progression of the disease.	AD	Brain tissues	[108]
Identified glycopeptidforms associated with cognitive resilience.	AD	Dorsal lateral prefrontal cortex	[109]
Bisecting N-acetylglucosamine in conjunction with total tau levels.	AD	Serum	[110]
Site-specific N-glycosylation changes.	PD	Serum	[80]
Site-specific N-glycosylation in proteins such as AMBP, UMOD, and RNase1.	PD	Urine	[81]

Table 3. Functional differences of different glycosylation in neurodegenerative diseases

Diseases	N-glycosylation	Function	O-glycosylation	Function
AD	Decreased fucosylated and oligomannose N-glycans (Brain)	Neuronal excitotoxicity	O-GlcNAc levels (Frontal cortex)	Induced cell death
AD	N-glycans (APP)	APP transport and secretion	OGA inhibition (APP)	Prevents A β -induced Tau hyperphosphorylation and hippocampal damage
AD	High bisecting GlcNAc levels (BACE1)	BACE1 biological processes	Suitable level of O-GlcNAc (Tau)	Regulation of Phosphorylation, blocking Tau aggregates
AD	(Tau)	Maintenance of PHF structure		
PD	Sialylated and fucosylated N-glycans (TREM2)	Affects protein stability	O-GlcNAc (α -synuclein)	Affects α -synuclein aggregation, phosphorylation

With respect to other aspects of the relationship between Alzheimer's disease and glycosylation, an analysis of protein glycosylation-related genes (PGRGs) in AD revealed their involvement in disease pathogenesis. This research differentiated AD into two subtypes with distinct biological processes and neuroinflammation levels based on protein glycosylation patterns. This study identified potential therapeutic agents and highlighted the importance of immune cell infiltration differences between the subtypes. Specifically, SERPINA3 was identified as a key diagnostic marker influenced by a complex network of competing endogenous RNAs and closely linked to immune cell dynamics [111]. Furthermore, Haukedal and colleagues discovered that genetic variations in the Sortilin-related receptor 1 linked to Alzheimer's disease intensified fragmentation of the Golgi apparatus and subsequent alterations in glycosylation [112]. Hawkinson and colleagues utilized MALDI mass spectrometry imaging to perform a comprehensive analysis of spatial N-glycans, finding strong region-specific N-glycan variations associated with AD in mice and humans [113]. These findings highlight the complexity of glycosylation in AD and its potential implications in diagnosis and therapy. Table 3 outlines the functional differences observed in various glycosylation patterns across neurodegenerative diseases.

According to the studies discussed above, our understanding of glycosylation in neurodegenerative diseases is becoming increasingly comprehensive. This encompasses a spectrum from serum glycomics to detailed investigations of individual protein glycosylation and from studies on N-glycosylation to those focusing on O-glycosylation. Additionally, the exploration of glycosylation biomarkers for neurodegenerative diseases continues vigorously. Research into pre-symptomatic biomarkers, in particular, may represent a crucial area for deeper investigation.

Perspectives

Understanding the complex roles of glycosylation in aging and neurodegenerative diseases opens new avenues for biomedical research. Glycosylation, a post-translational modification, affects protein folding, stability, and cellular signaling. Changes in glycosylation patterns in aging and neurodegenerative diseases indicate their crucial roles in these processes. This review highlights changes in glycans in disease processes, their potential as biomarkers, their value as therapeutic targets, and the latest relevant research involved. However, the complexity of glycosylation patterns and their regulation present significant challenges. To fully harness their diagnostic and therapeutic potential, future

research should involve comprehensive glycan profiling of various tissues and fluids across different aging stages and neurodegenerative diseases. While the complexity of glycosylation poses research difficulties, understanding the precise mechanisms by which glycosylation changes lead to cellular dysfunction and disease requires further investigation beyond merely observing glycosylation alterations as endpoints.

Acknowledgement

The authors acknowledge that Figures 2–4 and the graphical abstract were created with BioRender.com.

Funding

This work was supported by the grant from the National Key R&D Program of China (2022YFC3400803).

Conflict of Interest

The authors declare that they have no conflict of interest.

References

- Ukrainitseva S, Arbeev K, Duan M, Akushevich I, Kulminski A, Stallard E, Yashin A. Decline in biological resilience as key manifestation of aging: potential mechanisms and role in health and longevity. *Mech Ageing Dev* 2021, 194: 111418
- Cindrić A, Krištić J, Martinić Kavur M, Pezer M. Glycosylation and aging. *Adv Exp Med Biol* 2021: 341–373
- Wagner K-H, Cameron-Smith D, Wessner B, Franzke B. Biomarkers of aging: from function to molecular biology. *Nutrients* 2016, 8: 338
- Lipsky MS, King M. Biological theories of aging. *Dis Mon* 2015, 61: 460–466
- Schmeier C, Kretz A, Wengerodt D, Stojiljkovic M, Witte OW. Dissecting aging and senescence-current concepts and open lessons. *Cells* 2019, 8: 1446
- Galkin F, Zhang B, Dmitriev SE, Gladyshev VN. Reversibility of irreversible aging. *Ageing Res Rev* 2019, 49: 104–114
- López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: an expanding universe. *Cell* 2023, 186: 243–278
- Eichler J. Protein glycosylation. *Curr Biol* 2019, 29: R229–R231
- Pearce OM. Cancer glycan epitopes: biosynthesis, structure and function. *Glycobiology* 2018, 28: 670–696
- Varki A. Biological roles of glycans. *Glycobiology* 2017, 27: 3–49
- Olden K, Bernard BA, Humphries MJ, Yeo T-K, Yeo K-T, White SL, Newton SA, et al. Function of glycoprotein glycans. *Trends Biochem Sci* 1985, 10: 78–82
- Esmail S, Manolson MF. Advances in understanding N-glycosylation

- structure, function, and regulation in health and disease. *Eur J Cell Biol* 2021, 100: 151186
13. Gudelj I, Lauc G, Pezer M. Immunoglobulin G glycosylation in aging and diseases. *Cell Immunol* 2018, 333: 65–79
 14. Knežević A, Gornik O, Polašek O, Pučić M, Redžić I, Novokmet M, Rudd PM, *et al.* Effects of aging, body mass index, plasma lipid profiles, and smoking on human plasma N-glycans. *Glycobiology* 2010, 20: 959–969
 15. Paton B, Suarez M, Herrero P, Canela N. Glycosylation biomarkers associated with age-related diseases and current methods for glycan analysis. *Int J Mol Sci* 2021, 22: 5788
 16. Chen B, Liu W, Li Y, Ma B, Shang S, Tan Z. Impact of N-linked glycosylation on therapeutic proteins. *Molecules* 2022, 27: 8859
 17. Aebi M. N-linked protein glycosylation in the ER. *Biochim Biophys Acta* 2013, 1833: 2430–2437
 18. Cherepanova N, Shrimal S, Gilmore R. N-linked glycosylation and homeostasis of the endoplasmic reticulum. *Curr Opin Cell Biol* 2016, 41: 57–65
 19. Schwarz F, Aebi M. Mechanisms and principles of N-linked protein glycosylation. *Curr Opin Struct Biol* 2011, 21: 576–582
 20. Zhu Q, Wang H, Chai S, Xu L, Lin B, Yi W, Wu L. O-GlcNAcylation promotes tumor immune evasion by inhibiting PD-L1 lysosomal degradation. *Proc Natl Acad Sci USA* 2023, 120: e2216796120
 21. Thompson N, Wakarchuk W. O-glycosylation and its role in therapeutic proteins. *Biosci Rep* 2022, 42: BSR20220094
 22. Mulagapati S, Koppolu V, Raju TS. Decoding of O-linked glycosylation by mass spectrometry. *Biochemistry* 2017, 56: 1218–1226
 23. Estevez A, Zhu D, Blankenship C, Jiang J. Molecular interrogation to crack the case of O-GlcNAc. *Chemistry* 2020, 26: 12086–12100
 24. Issad T, Masson E, Pagesy P. O-GlcNAc modification, insulin signaling and diabetic complications. *Diabetes Metab* 2010, 36: 423–435
 25. Rexach JE, Clark PM, Hsieh-Wilson LC. Chemical approaches to understanding O-GlcNAc glycosylation in the brain. *Nat Chem Biol* 2008, 4: 97–106
 26. Lubas WA, Frank DW, Krause M, Hanover JA. O-linked GlcNAc transferase is a conserved nucleocytoplasmic protein containing tetratricopeptide repeats. *J Biol Chem* 1997, 272: 9316–9324
 27. Kreppel LK, Blomberg MA, Hart GW. Dynamic glycosylation of nuclear and cytosolic proteins. Cloning and characterization of a unique O-GlcNAc transferase with multiple tetratricopeptide repeats. *J Biol Chem* 1997, 272: 9308–9315
 28. Dong DL, Hart GW. Purification and characterization of an O-GlcNAc selective N-acetyl-beta-D-glucosaminidase from rat spleen cytosol. *J Biol Chem* 1994, 269: 19321–19330
 29. Lee BE, Suh PG, Kim JI. O-GlcNAcylation in health and neurodegenerative diseases. *Exp Mol Med* 2021, 53: 1674–1682
 30. Nie H, Yi W. O-GlcNAcylation, a sweet link to the pathology of diseases. *J Zhejiang Univ Sci B* 2019, 20: 437–448
 31. Saha A, Bello D, Fernández-Tejada A. Advances in chemical probing of protein O-GlcNAc glycosylation: structural role and molecular mechanisms. *Chem Soc Rev* 2021, 50: 10451–10485
 32. McCay CM, Maynard LA, Sperling G, Barnes LL. The Journal of Nutrition. Volume 18 July–December, 1939. Pages 1–13. Retarded growth, life span, ultimate body size and age changes in the albino rat after feeding diets restricted in calories. *Nutr Rev* 1975, 33: 241–243
 33. Ohtsubo K, Marth JD. Glycosylation in cellular mechanisms of health and disease. *Cell* 2006, 126: 855–867
 34. Urata Y, Takeuchi H. Effects of notch glycosylation on health and diseases. *Dev Growth Differ* 2020, 62: 35–48
 35. Loaeza-Reyes KJ, Zenteno E, Moreno-Rodríguez A, Torres-Rosas R, Argueta-Figueroa L, Salinas-Marín R, Castillo-Real LM, *et al.* An overview of glycosylation and its impact on cardiovascular health and disease. *Front Mol Biosci* 2021, 8: 751637
 36. Lauc G, Essafi A, Huffman JE, Hayward C, Knežević A, Kattla JJ, Polašek O, *et al.* Genomics meets glycomics-the first GWAS study of human N-glycome identifies HNF1 α as a master regulator of plasma protein fucosylation. *PLoS Genet* 2010, 6: e1001256
 37. Huffman JE, Knežević A, Vitart V, Kattla J, Adamczyk B, Novokmet M, Igl W, *et al.* Polymorphisms in B3GAT1, SLC9A9 and MGAT5 are associated with variation within the human plasma N-glycome of 3533 European adults. *Hum Mol Genet* 2011, 20: 5000–5011
 38. Sharapov SZ, Tsepilov YA, Klaric L, Mangino M, Thareja G, Shadrina AS, Simurina M, *et al.* Defining the genetic control of human blood plasma N-glycome using genome-wide association study. *Hum Mol Genet* 2019, 28: 2062–2077
 39. Sharapov SZ, Shadrina AS, Tsepilov YA, Elgaeva EE, Tiys ES, Feoktistova SG, Zaytseva OO, *et al.* Replication of 15 loci involved in human plasma protein N-glycosylation in 4802 samples from four cohorts. *Glycobiology* 2021, 31: 82–88
 40. Frkatović-Hodžić A, Mijakovac A, Miškec K, Nostaeva A, Sharapov SZ, Landini A, Haller T, *et al.* Mapping of the gene network that regulates glycan clock of ageing. *Aging* 2023, 15: 14509–14552
 41. Sun W, Jian X, Zhang J, Meng X, Wang H, Zheng D, Wu L, *et al.* The causality between human immunoglobulin G (IgG) N-glycosylation and aging: a mendelian randomization study. *Molecules* 2024, 29: 1281
 42. Sobral D, Francisco R, Duro L, Videira PA, Grosso AR. Concerted regulation of glycosylation factors sustains tissue identity and function. *Biomedicines* 2022, 10: 1805
 43. Mijakovac A, Frkatović A, Hanić M, Ivok J, Martinić Kavur M, Pučić-Baković M, Spector T, *et al.* Heritability of the glycan clock of biological age. *Front Cell Dev Biol* 2022, 10: 982609
 44. Greto VL, Cvetko A, Štambuk T, Dempster NJ, Kifer D, Deriš H, Cindrić A, *et al.* Extensive weight loss reduces glycan age by altering IgG N-glycosylation. *Int J Obes* 2021, 45: 1521–1531
 45. Šimunić-Briški N, Dukarić V, Očić M, Madžar T, Vinicki M, Frkatović-Hodžić A, Knjaz D, *et al.* Regular moderate physical exercise decreases glycan age index of biological age and reduces inflammatory potential of Immunoglobulin G. *Glycoconj J* 2024, 41: 67–76
 46. Fan J, Sha J, Chang S, Zhao H, Niu X, Gu Y, Gu J, *et al.* Generalized low levels of serum N-glycans associate with better health status. *Aging Cell* 2023, 22: e13855
 47. Burger-van Paassen N, van der Sluis M, Bouma J, Korteland-van Male AM, Lu P, Van Seuningen I, Boehm G, *et al.* Colitis development during the suckling-weaning transition in mucin Muc2-deficient mice. *Am J Physiol Gastrointest Liver Physiol* 2011, 301: G667–G678
 48. Sang X, Wang Q, Ning Y, Wang H, Zhang R, Li Y, Fang B, *et al.* Age-related mucus barrier dysfunction in mice is related to the changes in muc2 mucin in the colon. *Nutrients* 2023, 15: 1830
 49. Kameyama A, Thet Tin WW, Nishijima R, Yamakoshi K. Alteration of mucins in the submandibular gland during aging in mice. *Arch Oral Biol* 2021, 121: 104967
 50. Wu J, Liu J, Lapenta K, Desrouleaux R, Li MD, Yang X, Liu F. Regulation of the urea cycle by CPS1 O-GlcNAcylation in response to dietary restriction and aging. *J Mol Cell Biol* 2022, 14: mjac016
 51. Liu B, Qu J, Zhang W, Izipisua Belmonte JC, Liu GH. A stem cell aging framework, from mechanisms to interventions. *Cell Rep* 2022, 41: 111451
 52. Zhou Z, Zhang H, Tao Y, Zang J, Zhao J, Li H, Wang Y, *et al.* FGF21 alleviates adipose stem cell senescence via CD90 glycosylation-dependent glucose influx in remodeling healthy white adipose tissue. *Redox*

- Biol* 2023, 67: 102877
53. Wang J, Zhao K, Chen L, Zhou J, Sun Q, Chen J, Su R, *et al.* Proteomics and post-translational modifications analysis of umbilical mesenchymal stem cells aging. *Anal Biochem* 2022, 652: 114770
 54. Imae R, Manya H, Tsumoto H, Umezawa K, Miura Y, Endo T. Changes in the amount of nucleotide sugars in aged mouse tissues. *Glycobiology* 2024, 34: cwae032
 55. Günay B, Matthews E, Morgan J, Tryfonidou MA, Saldova R, Pandit A. An insight on the N-glycome of notochordal cell-rich porcine nucleus pulposus during maturation. *FASEB BioAdv* 2023, 5: 321–335
 56. McDonald R, Larsen M, Liu Z, Southeekal S, Eudy J, Guda C, Kumar TR. RNA-seq analysis identifies age-dependent changes in expression of mRNAs encoding N-glycosylation pathway enzymes in mouse gonadotropes. *Mol Cell Endocrinol* 2023, 574: 111971
 57. Agnello L, Gambino CM, Lo Sasso B, Bivona G, Milano S, Ciaccio AM, Piccoli T, *et al.* Neurogranin as a novel biomarker in Alzheimer's disease. *Lab Med* 2021, 52: 188–196
 58. Cheng YJ, Lin CH, Lane HY. From menopause to neurodegeneration—molecular basis and potential therapy. *Int J Mol Sci* 2021, 22: 8654
 59. Cummings J. Disease modification and neuroprotection in neurodegenerative disorders. *Transl Neurodegener* 2017, 6: 25
 60. Gitler AD, Dhillon P, Shorter J. Neurodegenerative disease: models, mechanisms, and a new hope. *Dis Model Mech* 2017, 10: 499–502
 61. Katsumo M, Sahashi K, Iguchi Y, Hashizume A. Preclinical progression of neurodegenerative diseases. *Nagoya J Med Sci* 2018, 80: 289–298
 62. Guo J, Huang X, Dou L, Yan M, Shen T, Tang W, Li J. Aging and aging-related diseases: from molecular mechanisms to interventions and treatments. *Signal Transduct Target Ther* 2022, 7: 391
 63. Gonzales MM, Garbarino VR, Pollet E, Palavicini JP, Kellogg Jr. DL, Kraig E, Orr ME. Biological aging processes underlying cognitive decline and neurodegenerative disease. *J Clin Invest* 2022, 132: e158453
 64. Johnson IP. Age-related neurodegenerative disease research needs aging models. *Front Aging Neurosci* 2015, 7: 168
 65. Costa J, Hayes C, Lisacek F. Protein glycosylation and glycoinformatics for novel biomarker discovery in neurodegenerative diseases. *Ageing Res Rev* 2023, 89: 101991
 66. Yang J, Li H, Zhao Y. Dessert or poison? The roles of glycosylation in Alzheimer's, Parkinson's, Huntington's disease, and amyotrophic lateral sclerosis. *ChemBioChem* 2023, 24: e202300017
 67. Grad LI, Rouleau GA, Ravits J, Cashman NR. Clinical spectrum of amyotrophic lateral sclerosis (ALS). *Cold Spring Harb Perspect Med* 2017, 7: a024117
 68. Hardiman O, Al-Chalabi A, Chio A, Corr EM, Logroscino G, Robberecht W, Shaw PJ, *et al.* Amyotrophic lateral sclerosis. *Nat Rev Dis Primers* 2017, 3: 1–9
 69. Edri-Brami M, Rosental B, Hayoun D, Welt M, Rosen H, Wirguin I, Nefussy B, *et al.* Glycans in sera of amyotrophic lateral sclerosis patients and their role in killing neuronal cells. *PLoS One* 2012, 7: e35772
 70. Goncalves M, Tillack L, de Carvalho M, Pinto S, Conradt HS, Costa J. Phosphoneurofilament heavy chain and N-glycomics from the cerebrospinal fluid in amyotrophic lateral sclerosis. *Clinica Chim Acta* 2015, 438: 342–349
 71. Costa J, Streich L, Pinto S, Pronto-Laborinho A, Nimtz M, Conradt HS, de Carvalho M. Exploring cerebrospinal fluid IgG N-glycosylation as potential biomarker for amyotrophic lateral sclerosis. *Mol Neurobiol* 2019, 56: 5729–5739
 72. Tanaka H, Shimazawa M, Kimura M, Takata M, Tsuruma K, Yamada M, Takahashi H, *et al.* The potential of GPNMB as novel neuroprotective factor in amyotrophic lateral sclerosis. *Sci Rep* 2012, 2: 573
 73. Shan X, Vocadlo DJ, Krieger C. Reduced protein O-glycosylation in the nervous system of the mutant SOD1 transgenic mouse model of amyotrophic lateral sclerosis. *Neurosci Lett* 2012, 516: 296–301
 74. Hayes MT. Parkinson's disease and parkinsonism. *Am J Med* 2019, 132: 802–807
 75. Russell AC, Šimurina M, Garcia MT, Novokmet M, Wang Y, Rudan I, Campbell H, *et al.* The N-glycosylation of immunoglobulin G as a novel biomarker of Parkinson's disease. *Glycobiology* 2017, 27: 501–510
 76. Váradi C, Nehéz K, Hornyák O, Viskolcz B, Bones J. Serum N-glycosylation in parkinson's disease: a novel approach for potential alterations. *Molecules* 2019, 24: 2220
 77. Wilkinson H, Thomsson KA, Rebelo AL, Hilliard M, Pandit A, Rudd PM, Karlsson NG, *et al.* The O-glycome of human nigrostriatal tissue and its alteration in parkinson's disease. *J Proteome Res* 2021, 20: 3913–3924
 78. Rebelo AL, Drake RR, Marchetti-Deschmann M, Saldova R, Pandit A. Changes in tissue protein N-glycosylation and associated molecular signature occur in the human Parkinsonian brain in a region-specific manner. *PNAS Nexus* 2024, 3: pgad439
 79. Hu J, Xia W, Zeng S, Lim YJ, Tao Y, Sun Y, Zhao L, *et al.* Phosphorylation and O-glcNAcylation at the same α -synuclein site generate distinct fibril structures. *Nat Commun* 2024, 15: 2677
 80. Xu M, Jin H, Wu Z, Han Y, Chen J, Mao C, Hao P, *et al.* Mass spectrometry-based analysis of serum N-glycosylation changes in patients with parkinson's disease. *ACS Chem Neurosci* 2022, 13: 1719–1726
 81. Xu M, Jin H, Ge W, Zhao L, Liu Z, Guo Z, Wu Z, *et al.* Mass spectrometric analysis of urinary N-glycosylation changes in patients with parkinson's disease. *ACS Chem Neurosci* 2023, 14: 3507–3517
 82. Winter J, Hammer E, Heger J, Schultheiss HP, Rauch U, Landmesser U, Dörner A. Adenine nucleotide translocase 1 expression is coupled to the HSP27-mediated TLR4 signaling in cardiomyocytes. *Cells* 2019, 8: 1588
 83. Zhang W, Liu J, Chen Q, Ding W, Li S, Ma L. Identification of ADP/ATP translocase 1 as a novel glycoprotein and its association with parkinson's disease. *Neurochem Res* 2022, 47: 3355–3368
 84. Ma L, Song J, Sun X, Ding W, Fan K, Qi M, Xu Y, *et al.* Role of microtubule-associated protein 6 glycosylated with Gal-(β -1,3)-GalNAc in Parkinson's disease. *Aging* 2019, 11: 4597–4610
 85. Scheltens P, De Strooper B, Kivipelto M, Holstege H, Chételat G, Teunissen CE, Cummings J, *et al.* Alzheimer's disease. *Lancet* 2021, 397: 1577–1590
 86. Knapskog AB, Engedal K, Selbæk G, Øksengård AR. Alzheimers sykdom—diagnostikk og behandling. *Tidsskr Nor Laegeforen* 2021, 141
 87. Soria Lopez JA, González HM, Léger GC. Alzheimer's disease. *Handb Clin Neurol* 2019, 167: 231–255
 88. Weller J, Budson A. Current understanding of Alzheimer's disease diagnosis and treatment. *F1000Res* 2018, 7: F1000
 89. O'Brien RJ, Wong PC. Amyloid precursor protein processing and Alzheimer's disease. *Annu Rev Neurosci* 2011, 34: 185–204
 90. Lin T, van Husen LS, Yu Y, Tjernberg LO, Schedin-Weiss S. Lack of N-glycosylation increases amyloidogenic processing of the amyloid precursor protein. *Glycobiology* 2022, 32: 506–517
 91. Tachida Y, Iijima J, Takahashi K, Suzuki H, Kizuka Y, Yamaguchi Y, Tanaka K, *et al.* O-GalNAc glycosylation determines intracellular trafficking of APP and A β production. *J Biol Chem* 2023, 299: 104905
 92. Kronimus Y, Albus A, Hasenberg M, Walkenfort B, Seifert M, Budeus B, Gronewold J, *et al.* Fc N-glycosylation of autoreactive A β antibodies as a blood-based biomarker for Alzheimer's disease. *Alzheimers Dement* 2023, 19: 5563–5572
 93. Avila J, Lucas JJ, Pérez M, Hernández F. Role of tau protein in both physiological and pathological conditions. *Physiol Rev* 2004, 84: 361–384

94. Pîrșcoveanu DFV, Pirici I, Tudorică V, Bălșeanu TA, Albu VC, Bondari S, Bumbea AM, *et al.* Tau protein in neurodegenerative diseases a review. *Rom J Morphol Embryol* 2017, 58: 1141–1150
95. Kovacs GG. Tauopathies. *Handb Clin Neurol* 2017, 145: 355–368
96. Robertson LA, Moya KL, Breen KC. The potential role of tau protein O-glycosylation in Alzheimer's disease. *J Alzheimer Dis* 2004, 6: 489–495
97. Zhao J, Lang M. New insight into protein glycosylation in the development of Alzheimer's disease. *Cell Death Discov* 2023, 9: 314
98. Haukedal H, Freude KK. Implications of glycosylation in Alzheimer's disease. *Front Neurosci* 2020, 14: 625348
99. Cantrelle FX, Loyens A, Trivelli X, Reimann O, Despres C, Gandhi NS, Hackenberger CPR, *et al.* Phosphorylation and O-GlcNAcylation of the PHF-1 epitope of tau protein induce local conformational changes of the C-terminus and modulate tau self-assembly into fibrillar aggregates. *Front Mol Neurosci* 2021, 14: 661368
100. Yin X, Li Y, Fan X, Huang F, Qiu Y, Zhao C, Zhou Z, *et al.* SIRT1 deficiency increases O-GlcNAcylation of tau, mediating synaptic tauopathy. *Mol Psychiatry* 2022, 27: 4323–4334
101. Rostgaard N, Jul PH, Garmer M, Volbracht C. Increasing O-GlcNAcylation attenuates tau hyperphosphorylation and behavioral impairment in rTg4510 tauopathy mice. *J Integr Neurosci* 2023, 22: 135
102. Park J, Ha HJ, Chung ES, Baek SH, Cho Y, Kim HK, Han J, *et al.* O-GlcNAcylation ameliorates the pathological manifestations of Alzheimer's disease by inhibiting necroptosis. *Sci Adv* 2021, 7: eabd3207
103. Pan D, Gu JH, Zhang J, Hu Y, Liu F, Iqbal K, Cecic N, *et al.* Thiamme2-G, a novel O-GlcNAcase inhibitor, reduces tau hyperphosphorylation and rescues cognitive impairment in mice. *J Alzheimer Dis* 2021, 81: 273–286
104. Huynh VN, Wang S, Ouyang X, Wani WY, Johnson MS, Chacko BK, Jegga AG, *et al.* Defining the dynamic regulation of O-GlcNAc proteome in the mouse cortex—the O-GlcNAcylation of synaptic and trafficking proteins related to neurodegenerative diseases. *Front Aging* 2021, 2: 757801
105. Li X, Han J, Bujaranipalli S, He J, Kim EY, Kim H, Im JH, *et al.* Structure-based discovery and development of novel O-GlcNAcase inhibitors for the treatment of Alzheimer's disease. *Eur J Med Chem* 2022, 238: 114444
106. Yu L, Huo Z, Yang J, Palma-Gudiel H, Boyle PA, Schneider JA, Bennett DA, *et al.* Human brain and blood N-glycome profiling in Alzheimer's disease and Alzheimer's disease-related dementias. *Front Aging Neurosci* 2021, 13: 765259
107. Tena J, Tang X, Zhou Q, Harvey D, Barajas-Mendoza M, Jin LW, Maezawa I, *et al.* Glycosylation alterations in serum of Alzheimer's disease patients show widespread changes in N-glycosylation of proteins related to immune function, inflammation, and lipoprotein metabolism. *Alzheimers Dement (Amst)* 2022, 14: e12309
108. Suttapitugsakul S, Stavenhagen K, Donskaya S, Bennett DA, Mealer RG, Seyfried NT, Cummings RD. Glycoproteomics landscape of asymptomatic and symptomatic human Alzheimer's disease brain. *Mol Cell Proteomics* 2022, 21: 100433
109. Buchman AS, Yu L, Klein HU, Zammit AR, Oveisgharan S, Nag S, Tickotsky N, *et al.* Glycoproteome-wide discovery of cortical glycoproteins that may provide cognitive resilience in older adults. *Neurology* 2024, 102: e209223
110. Zhou RZ, Vetrano DL, Grande G, Duell F, Jönsson L, Laukka EJ, Fredolini C, *et al.* A glycan epitope correlates with tau in serum and predicts progression to Alzheimer's disease in combination with APOE4 allele status. *Alzheimers Dement* 2023, 19: 3244–3249
111. Ma Z, Yang F, Fan J, Li X, Liu Y, Chen W, Sun H, *et al.* Identification and immune characteristics of molecular subtypes related to protein glycosylation in Alzheimer's disease. *Front Aging Neurosci* 2022, 14: 968190
112. Haukedal H, Corsi GI, Gadekar VP, Doncheva NT, Kedia S, de Haan N, Chandrasekaran A, *et al.* Golgi fragmentation—one of the earliest organelle phenotypes in Alzheimer's disease neurons. *Front Neurosci* 2023, 17: 1120086
113. Hawkinson TR, Clarke HA, Young LEA, Conroy LR, Markussen KH, Kerch KM, Johnson LA, *et al.* In situ spatial glycomic imaging of mouse and human Alzheimer's disease brains. *Alzheimers Dement* 2022, 18: 1721–1735