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REVIEW ARTICLE

Risk of Alzheimer's disease in Down syndrome: Insights gained by multi-omics

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Abstract

Individuals with Down syndrome (DS) are highly susceptible to Alzheimer's disease (AD). The integration of genomics, transcriptomics, epigenomics, proteomics, and metabolomics enables unprecedented understanding of DS-AD, offering a detailed picture of this complex issue. The vast -omics data also present challenges that reflect the complexity of genetic information flow. These studies nonetheless reveal critical mechanisms behind AD risk, including unique observations in DS that differ from those seen in the general population and familial dominant AD. In addition, the correlations between the AD polygenic risk score and proteins related to female infertility and autoimmune thyroiditis corroborate clinical observations. Metabolomic data reveal disrupted metabolic networks, offering prospects for a dynamic score to create specialized nutritional interventions. By adopting a multidimensional perspective with integrated reductionism, the evolving landscape presents an opportunity to identify promising directions for developing precision strategies to mitigate the impact of AD in the DS population.

KEYWORDS
Alzheimer's disease, Down syndrome, epigenomics, genomics, metabolomics, -omics, polygenic risk score, proteomics, transcriptomics

Highlights

- Individuals with Down syndrome (DS) are highly susceptible to Alzheimer's disease (AD).
- DS-AD is characterized by its polygenic nature, extending beyond chromosome 21 with significant contributions from various chromosomes.
- DS-AD also presents unique features that differ from those observed in the general population and familial dominant AD.
- Our review consolidates key findings from genomics, transcriptomics, epigenomics, proteomics, and metabolomics, providing a comprehensive view of the molecular mechanisms underlying DS-AD.

- We highlight promising research directions to further elucidate the pathogenesis of DS-AD.

1 | DOWN SYNDROME AND ALZHEIMER'S DISEASE

Down syndrome (DS) or trisomy 21 (T21), first described by Dr. John Langdon Down in 1866,[1] is a genetic anomaly characterized by the presence of an additional copy of chromosome 21 (Chr21),[2] occurring at a rate of 13.65 per 10,000 live births in the United States.[3] Complete T21 accounts for ≈ 95% of DS cases, frequently resulting from maternal meiotic non-disjunction of the Chr21 pair, with an increased risk associated with the mother's age.[4] In 4% of cases, a fragment of Chr21 is attached (Robertsonian translocation) to another chromosome, such as Robertsonian t(14;21) or t(21;21), with approximately one third being inherited, more commonly from the mother.[4] An infrequent form is mosaic DS, in which varying proportions of cells in the body have an extra copy of Chr21, often resulting in milder symptoms.[5] This additional genetic material affects the development of the body and brain, leading to characteristic physical features, including almond-shaped eyes, a small nose, epicanthic folds, macroglossia, short neck, and hypotonia, alongside mild to moderate intellectual disabilities.[6] To date, individuals with DS have a lifespan reaching into their 60s.[7] Individuals with DS are at a notably higher risk than the general population of developing Alzheimer's disease (AD). After reaching the age of 30 years, nearly all adults with DS develop the characteristic neuropathological changes of AD, that is, amyloid beta (Aβ) plaques and tau tangles.[8] Approximately 50% to 70% of DS patients may experience dementia by the time they reach 60 to 70 years of age[9], which is 5 to 6 times higher than individuals aged ≥ 65 in the general population.

DS-AD is receiving extensive research attention.[10] AD is characterized by irreversible neuronal degeneration, driven by multiple interrelated mechanisms.[11] The extracellular plaques formed by Aβ (particularly Aβ42 and Aβ40) and the neurofibrillary tangles (NFTs) composed of abnormal clumps of hyperphosphorylated tau proteins (p-tau181 and p-tau217) in the brains of individuals with AD serve as central mechanisms in the pathogenesis of AD.[12,13] As a result of T21, DS-AD involves the overexpression of the amyloid precursor protein (*APP*) gene, which is located on Chr21q21.3. Analyses of human AD brains show that tau pathology begins approximately a decade before the formation of Aβ plaques,[14,15] and tau protein dysfunction is more closely correlated with dementia than amyloid.[16] Targeting tau protein may be more effective in improving cognitive function.[14,17] Tau positron emission tomography (PET) scans in 312 individuals across the AD spectrum demonstrated that tau spreads through neuronal communication pathways during normal aging, with this process being accelerated by the presence of brain Aβ.[18] In DS, tau deposition plays a significant role in early cognitive decline, with findings indicating that tau PET is a more sensitive biomarker than Aβ PET for predicting early memory problems in adults with DS.[19]

The cross-sectional study by Flores-Aguilar et al. extensively characterizes the evolving neuroinflammatory profile across the lifespan of individuals with DS, revealing early alterations in microglial morphology and inflammatory cytokine expression.[20] Although neuroinflammation is not formally part of the amyloid, tau, neurodegeneration (ATN) framework[21] of DS-AD prediction, Aβ accumulation, tau hyperphosphorylation, and chronic neuroinflammation form a vicious cycle in which each process reinforces the others, driving the progression of neurodegeneration. Proteins such as glial fibrillary acidic protein (GFAP; a marker of astrocyte activation[22]) and neurofilament light chain (NfL; a marker of axonal damage[23]) are increasingly recognized as indicators of neuroinflammation and neuronal injury in AD.[24] In addition, mitochondrial dysfunction,[25] oxidative stress, synaptic dysfunction, and excitotoxicity may be involved in AD pathogenesis, with these mechanisms being interrelated and capable of amplifying each other.[26–28]

Basal forebrain cholinergic neurons (BFCNs) play a crucial role in cognitive function by providing cholinergic innervation to critical brain regions such as the cortex and hippocampus. In addition to impaired neuronal development in DS, the degeneration of BFCNs in DS-AD occurs through the mechanisms described above, making them a therapeutic target for both DS and AD. Cholinergic enhancement through acetylcholinesterase inhibitors has the potential to slow cognitive decline in DS-AD.[29] With no cure or proven preventive measures for AD to date, understanding the specific pathological mechanisms underlying the development of DS-AD is crucial for the development of strategies to reduce the risk of developing the disease or slow its progression.

The recent review by Martini et al.[30] offers an insightful analysis of the link between DS and AD, particularly focusing on the genetic predispositions linked to T21. Their work highlights how genes on Chr21 contribute to metabolic imbalances, immune dysregulation, cerebrovascular issues, and oxidative stress in DS.[30] In a broader context, the genetic contributors to AD in this population extend beyond Chr21, incorporating a broad array of genes that play pivotal roles in the disease's complex pathology. Notably, the apolipoprotein E (*APOE*) gene on chromosome 19, especially its ε4 variant, is recognized for its substantial risk factor in sporadic AD,[31,32] potentially aggravating DS-AD by influencing lipid metabolism and the clearance of Aβ. Rare variants in the presenilin 1 (*PSEN1*) gene at Chr14 and the presenilin 2 (*PSEN2*) gene at Chr1 are associated with familial dominant forms of AD,[33–35] whereas their expression is downregulated in developmental and adult DS brains.[36,37] The complex genomic landscape of DS-AD may involve synergistic effects between the overexpressed genes on T21 and genes on other chromosomes, which adds to the complexity of the AD phenotype in DS. This intricate genomic interplay underscores the need for a more holistic approach. Multi-omics research

emerges as a pivotal breakthrough, weaving together genomics, transcriptomics, epigenomics, proteomics, and metabolomics to illuminate the molecular complexities of DS-AD. This integrated approach transcends traditional research methods, offering personalized insights that promise to revolutionize disease management and treatment. By identifying specific biomarkers and therapeutic targets, multi-omics paves the way for early detection, preventive strategies, and targeted therapies tailored to the unique molecular profile of DS-AD. This advancement not only deepens our understanding but also heralds a hopeful future, in which the challenges of DS and AD are effectively managed, significantly improving the quality of life and health outcomes for affected individuals.

2 | GENOMICS INSIGHTS

2.1 | Genetics on Chr21

In the general population, *APP* and *ADAMTS1* on Chr21 have been associated with an increased genetic risk of AD (see the supporting information). The additional copy of *APP* at Chr21 in individuals with DS leads to an augmented production of Aβ peptides, consequently culminating in the formation of Aβ plaques. Compelling evidence for the role of *APP* gene dosage in AD risk come from individuals with partial T21 and *APP*-related familial dominant AD. *APP* is not located in the DS critical region (DSCR). DS patients with partial T21 lacking the *APP* gene triplication do not progress to AD dementia, even into their sixth decade of life, suggesting a direct link between *APP* dosage and AD risk in DS.[38] Furthermore, mosaicism, in which T21 is present in only a subset of an individual's cells, introduces an additional layer of complexity.[39] Inherited mutations affecting chromosome segregation can elevate the risk for both DS and AD, highlighting the importance of mitotic errors in AD development.[40] Mosaicism may contribute to both familial and sporadic AD.[40] Even low-degree T21 mosaicism promotes early-onset AD (EOAD).[41] These T21 cells increase AD risk by elevating the dosage of the *APP* gene and promoting Aβ production, similar to the mechanism seen in DS. However, AD tends to develop later in individuals with mosaicism compared to those with full T21, as the majority of normal diploid cells modulate this effect.[40]

Individuals with DS begin to accumulate Aβ at a very young age, starting in childhood, which is significantly earlier than in the general population. This accumulation continues to increase throughout their lifespan.[42] Interestingly, the sensitivity to gene dosage manifests as significant overexpression of APP in the adult DS brain rather than during the fetal stage.[43] This finding challenges the notion of a straightforward gene dosage effect throughout life, yet it does not explain the early-life accumulation of Aβ. Other factors beyond *APP* gene dosage alone may contribute to the early onset of Aβ accumulation in individuals with DS, which remains to be addressed by further study.

In addition to T21, mutations within the *APP* gene can cause familial dominant AD by disrupting normal APP processing. Specifically, mutations near the β- and γ-secretase cleavage sites shift APP processing toward the amyloidogenic pathway, increasing the production of Aβ42, a highly aggregation-prone peptide.[44,45] The recognition of *APP* as a significant genetic contributor to DS-AD opens avenues for therapeutic interventions. Targeted drug development focusing on modulating *APP* expression or its processing pathways could offer promising strategies to mitigate Aβ plaque formation.[46] The study by Hung et al. demonstrates the effectiveness of antisense oligonucleotides (ASOs) targeting *APP* and reducing its protein levels, rescuing endolysosome and autophagy dysfunction, and significantly decreasing intracellular and extracellular Aβ aggregates in human-induced pluripotent stem cell (hiPSC)–derived cortical neurons with *APP* duplication.[47] ASOs being developed for *APP* offer promising potential for DS-AD.[48] Additionally, gene editing techniques, such as clustered regularly interspaced palindromic repeats (CRISPR) CRISPR-associated protein 9 (Cas9), present a futuristic but potentially viable approach to correct or modify the dosage sensitivity of *APP* directly in the genome,[49] offering a tailored approach to prevent AD in individuals with DS.

Beyond *APP*, other critical genes located on Chr21, such as dual specificity tyrosine phosphorylation regulated kinase 1A (*DYRK1A*) and DNA methyltransferase 3 like (*DNMT3L*), play crucial roles in DS brain development. *DYRK1A* at Chr21q22.13[50] has been identified as a key gene within DSCR, and its dosage is critical in the central nervous system during development and aging[51] and correlated with developmental cognitive deficits in DS.[52] The kinase encoded by *DYRK1A* directly phosphorylates the tau protein at multiple sites,[53] contributing to its hyperphosphorylation. The amyloid burden caused by *APP* overexpression due to T21 and tau hyperphosphorylation driven by *DYRK1A* are synergistically linked. Subtle differences in the spatial distribution, timing, and magnitude of tau burden have been observed between individuals with DS and those with *APP*-related autosomal-dominant AD,[54] potentially linked to the DSCR genes. *DYRK1A* also affects gene expression through histone modification and is implicated in various cellular processes.[55,56] *DNMT3L* at Chr21q22.3 encodes a nuclear protein with similarity to DNA methyltransferases (DNMTs).[57] While *DNMT3L* does not possess DNA methyltransferase activity, it stimulates the DNA methylation functions of *DNMT3A* and *DNMT3B* through direct interaction in a dose-dependent manner.[58] Overexpression of *DNMT3L* during neurodevelopment replicates a facet of the genome-wide DNA methylation pattern characteristic of DS.[59] Understanding the dose-dependent roles of *DYRK1A* and *DNMT3L* in the epigenetic landscape of the DS brain offers valuable insights for potential therapeutic approaches, such as silencing these genes through CRISPR-Cas9.[60]

2.2 | Genetic insights from other chromosomes in the general population

APOE is a major susceptibility locus for AD in the general population[61] (supporting information). In DS individuals, the presence of the *APOE* ε4 allele compounds the AD risk, leading to an even earlier onset of AD symptoms.[31,32] A cross-sectional study of 80 young children and 240 adults with DS showed varied association between *APOE* ε4 and

attention across the lifespan. While ε4 carriers displayed an attentional advantage in young children, possession of an ε4 allele was linked to a disadvantage among older adults.[62] A cohort study of 464 DS adults revealed that carriers of the *APOE* ε4 allele exhibited earlier clinical symptoms and biomarker changes (e.g., Aβ, tau, and neurodegeneration) associated with AD.[63] Additionally, carriers also showed reduced metabolism in subcortical and parieto-occipital regions, along with diminished medial temporal volume.[63]

Among the AD genome-wide association study (GWAS) loci in the general population[64,65] (Table S1 in supporting information), the phosphatidylinositol binding clathrin assembly protein gene (*PICALM*) at Chr11q14.2 has been shown to specifically affect the age at onset of DS-AD.[66] *PICALM* is known for its role in clathrin-mediated endocytosis,[67] a process critical for synaptic functioning and the regulation of Aβ levels. In the context of DS-AD, its impact may involve distinct mechanisms driven by the unique molecular features of DS, such as *APP* triplication. These distinct features likely modify *PICALM*'s role in regulating *APP* internalization, trafficking, and Aβ clearance, linking cellular trafficking to amyloid pathology.[68]

Presenilins encoded by *PSEN1* and *PSEN2* serve as the catalytic core of the γ-secretase complex, which plays a crucial role in *APP* processing and Aβ generation. Rare variants in *PSEN1* and *PSEN2* are associated with familial dominant forms of AD[33–35] (supporting information). γ-secretase inhibitors and modulators have been developed as potential treatments for AD in the general population. However, their effectiveness in DS-AD may differ due to the altered expression levels of *PSEN1* and *PSEN2* and the overproduction of *APP*. These treatments could be beneficial in DS patients with *PSEN1* and *PSEN2* rare mutations. However, their broader utility depends on the specific levels and activity of γ-secretase in the DS brain. Transcriptome data from developmental and adult DS brains indicate lower expression levels of *PSEN1* and *PSEN2*,[36,37] highlighting the unique molecular context of DS-AD.

2.3 | Polygenic nature of AD and complex contributions from DS: integrating blood proteomics

Investigations into the AD polygenic risk score (PRS) based on the AD GWAS loci have provided additional valuable information on the genetic susceptibility of individuals with DS to AD[69,70] (supporting information). In particular, the AD PRS affects memory in DS people, regardless of their *APOE* status, suggesting a commonality in the genes and pathways that contribute to AD and those affecting dementia and memory deterioration in the DS population.[70]

Our study indicates that the AD PRS is correlated with blood levels of proteins related to female infertility (in line with the observed inverse correlation between the parity of children and AD risk in women[71]), autoimmune thyroiditis, and fetal growth retardation.[72] This association is noteworthy given the unique reproductive patterns observed in women with DS, who generally have fewer children.[73] This reduction in parity, previously linked to an increased AD risk in the general female population, may similarly elevate AD risk in the DS population, adding a sociobiological layer to the genetic risk factors for AD. The correlation between AD PRS and proteins linked to female infertility underscores a complex interplay between reproductive health and neurodegenerative processes, particularly through the neuroprotective roles of estrogen.[74] The involvement of CCAAT enhancer binding protein beta (*CEBPB*) on Chr 20q13, follicle-stimulating hormone subunit beta (*FSHB*) on Chr11p14, leptin (*LEP*) on Chr7q32, luteinizing hormone beta polypeptide (*LHB*) on Chr19q13, and LIF, interleukin 6 family cytokine (*LIF*) on Chr22q12, offers a molecular bridge between these domains.[72] *CEBPB* is a transcription factor, pivotal in regulating inflammatory and immune responses.[75] Its interaction with estrogen signaling can modulate neuroinflammatory responses, involved in AD's pathogenesis.[76] *FSHB* and *LHB* are integral to gonadal function regulation, impacting estrogen production.[77] Dysregulation of these hormones can lead to altered estrogen levels, affecting its ability to modulate Aβ clearance and provide neuroprotection. It is worth noting, however, that the relationship between nulliparity and estrogen levels is complex. While estrogen is known to exert neuroprotective effects, estrogen levels across the lifespan are indeed higher in women who do not bear children, as established in the context of breast cancer research.[78] The intricacies of estrogen's role in neuroprotection warrant further investigation, particularly in the context of nulliparity and AD risk. While estrogen's neuroprotective effects are well documented, its interplay with reproductive factors such as nulliparity may introduce additional complexities that necessitate a more nuanced understanding of its mechanisms in neurodegenerative diseases. *LEP*, involved in energy homeostasis and appetite regulation, also plays a role in neuroprotective processes.[79] Leptin can influence neuroinflammatory pathways and modulate hippocampal synaptic function, crucial for learning and memory.[80]

Autoimmune thyroiditis, particularly Hashimoto's thyroiditis, is more prevalent in individuals with DS compared to the general population,[81] suggesting a complex link between immune system dysregulation and the increased AD risk in DS. This link is further supported by the correlation between AD PRS and proteins encoded by genes located on specific chromosomes: CD3 gamma chain (*CD3G*) on chr11q23, glycoprotein hormones, alpha polypeptide (*CGA*) on Chr6q14, C-X-C motif chemokine ligand 9 (CXCL9) on Chr4q21, Fas cell surface death receptor (*FAS*) on Chr10q23, and leukocyte immunoglobulin-like receptor B1 (*LILRB1*) on Chr19q13.[72] *CD3G* is integral to T-cell receptor (TCR) signaling and T-cell activation.[82] Alterations in *CD3G* expression could impact immune surveillance mechanisms in the brain, potentially influencing the inflammatory milieu associated with AD pathology in DS. *CGA* forms a part of the thyroid-stimulating hormone (TSH).[83] *CXCL9* is involved in immune cell recruitment to inflammation sites. *FAS* mediates apoptosis. *LILRB1* modulates immune responses, including T-cell–mediated reactions.

Low birth weight is common in neonates with DS.[84] This association adds a layer of complexity to our understanding of genetic factors influencing not only cognitive health but also prenatal development in individuals with DS. The intricate relationships between AD risk and fetal growth are mediated by the correlation with proteins encoded by insulin-like growth factor 1 (*IGF1*) on Chr12q23, insulin-like growth factor binding protein 3 (*IGFBP3*) on Chr7p12, *LEP*, selectin

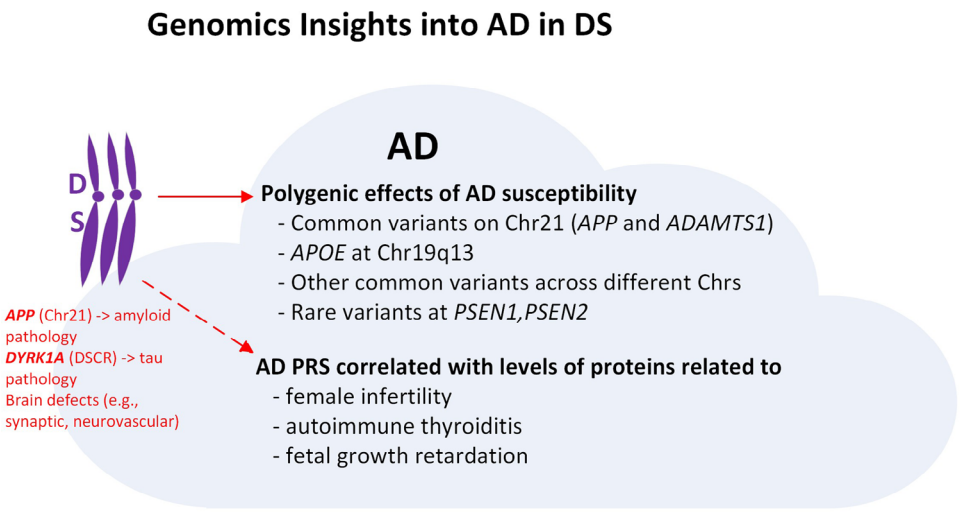

FIGURE 1 Genomic insights into the polygenic nature of DS-AD involving effects from various chromosomes. Interesting genetics and clinical connections are highlighted. Multiple AD loci from both Chr21 and other chromosomes have been identified. The PRS for AD is associated with proteins related to conditions like female infertility, autoimmune thyroiditis, and fetal growth retardation. As one significant factor among many, T21 in DS leads to the overexpression of *APP*. The DS critical region (DSCR) contains *DYRK1A*, a key gene implicated in tau hyperphosphorylation. Brain defects in DS (e.g., synaptic, neurovascular) increase the risk of AD. In addition to these genetic mechanisms, DS may contribute to AD through mechanisms related to female infertility, autoimmune thyroiditis, and fetal growth retardation. AD, Alzheimer's disease; Chr21, chromosome 21; DS, Down syndrome; PRS, polygenic risk score; T21, trisomy 21.

P (*SELP*) on Chr1q24, and transmembrane protein 70 (*TMEM70*) on Chr8q21.[72] *IGF1* plays a critical role in both prenatal growth and postnatal brain development. *IGFBP3* regulates the availability of *IGF1* by binding to it. *TMEM70* is involved in mitochondrial function and energy metabolism.[85]

2.4 | Perspective and limitations of genetics

Unraveling the intricate relationships among AD risk, thyroid function, reproductive history, and fetal growth provides a comprehensive perspective on the multifaceted nature of health considerations in this population. These findings also highlight the complex interplay between susceptibility to AD and various health factors in DS. Looking ahead, the integration of more sophisticated PRS models, particularly those delineating specific biological processes like neuroinflammation and microglial activation, endocytosis, and fibril regulation in AD[86,87] hold immense potential. While a global PRS may underestimate the disease heterogeneity, these specific models provide a data-driven assessment of individualized risk components, elucidating genetic variants specifically linked to each person's unique pathogenic mechanisms. These pathway-specific PRSs demand extensive analysis across diverse traits or diseases that share common pathways. Fortunately, the availability of large, well-characterized GWAS datasets to the public makes such targeted PRSs increasingly feasible. Meanwhile, managing multiple PRSs for the same disease demands a sophisticated clinical approach and decision-making process. This personalized strategy will not only deepen our understanding of the genetic and disease interactions in DS-AD but also pave the way for customized interventions and precision medicine in neurodegenerative disorders.

Despite these promising developments, it is important to acknowledge limitations inherent to PRS approaches, especially in the context of sampling. The utility of PRS models depends significantly on the population being studied, whether it is individuals with DS, those with familial AD, or the general population of a specific ethnicity. Sampling biases can influence PRS predictive power, underscoring the need for caution when interpreting results.[88] Furthermore, etiologic heterogeneity, such as differences between the younger and older patients, is inevitable in AD.[89] This underscores the critical importance of evaluating PRSs with careful consideration of confounding factors, such as aging, environmental influences, comorbid conditions, and genetic background.[90]

Insights gained from genetic studies in the general population underscore effects from various chromosomes and have significantly advanced our understanding of AD, providing clues about how genetic risk factors influence pathways relevant to DS-AD (Figure 1). For example, analysis of AD GWAS loci highlights biological processes such as protein processing and immune response regulation. Altered protein processing and immune responses in DS may exacerbate neuroinflammation and neurodegeneration, accelerating the onset and progression of DS-AD.[91] However, it is crucial to differentiate those findings generated from the general population (supporting information) from those specific to DS due to distinct physiological differences. While the PRS has shown great promise in precision medicine for AD in the general population, its application to DS is more complex. In DS, the overexpression of *APP* from an early age leads to profound amyloid pathology, potentially altering how PRS interacts with other AD-associated loci. Factors such as T21-related gene dosage and distinct immune and neuroinflammatory responses may affect the predictability of AD risk through PRS in DS, requiring refinement of

these tools to address DS-specific biological contexts. Further studying the interplay between AD-predisposing variants and T21 could lead to exciting and potentially groundbreaking discoveries. However, it is crucial to approach this research with respect and empathy for the individuals and communities affected. Discoveries in the genomic interplay of AD-predisposing variants and T21 should be aimed at developing better treatments, care strategies, and support systems for individuals with DS who are at risk of developing AD. The ultimate goal should be to enhance their well-being and quality of life, rather than simply advancing scientific knowledge for its own sake.

3 | TRANSCRIPTOMICS FINDINGS IN DS BRAINS

Transcriptomics, which involves the study of the complete set of RNA transcripts, provides a dynamic and real-time snapshot of gene expression patterns. This gene expression profiling perspective is pivotal in deciphering the downstream effects of genetic variations, offering a comprehensive understanding of how genes are actively translated into functional proteins, including influencing the intricate biological processes associated with DS-AD pathogenesis.

3.1 | Key gene expression changes in developmental DS brain

Olmos-Serrano et al. conducted a multi-region transcriptome analysis of brains from individuals with DS and euploid controls, encompassing brain tissues from mid-fetal development to adulthood (GEO GSE59630).[36] The study uncovered genome-wide alterations in gene expression, impacting a substantial number of genes across multiple brain regions with temporal and spatial specificity.[36]

One of the most critical findings from this analysis was the identification of key synaptic genes involved in neuronal communication and development, which were significantly downregulated in developmental DS brains. Synaptic dysfunction, characterized by impaired communication between neurons, is a central feature of both DS and AD. Specifically, the synaptic genes neuronal pentraxin 2 (*NPTX2*), rabphilin 3A (*RPH3A*), and brain-derived neurotrophic factor (*BDNF*), which are positively associated with global cognitive function across multiple excitatory neuron subtypes in a large-sample single-cell RNA sequencing (scRNA-seq) study of the human prefrontal cortex by Mathys et al.,[92] have been observed in downregulation in DS. These downregulated genes may contribute to the development of dysfunctional neuronal circuits, a hallmark that both DS and AD share. *RPH3A* encodes an effector of the small GTPase Rab3A, facilitating the docking and fusion of synaptic vesicles with the presynaptic membrane.[93] *BDNF* encodes a member of the neurotrophin family, promoting the survival, differentiation, and growth of neurons.[94] *NPTX2* encodes a member of the neuronal pentraxins family, interacting with AMPA receptors to modulate synaptic formation and strength.[95]

However, it is important to recognize that while neurons are directly impacted, it is becoming increasingly clear that non-neuronal factors also play a significant role in DS-AD progression. Vascular dysfunction, including the degeneration of blood–brain barrier (BBB) integrity and reduced cerebral blood flow, has been implicated in both DS and AD.[96] Platelet and endothelial cell adhesion molecule 1 (*PECAM1*), expressed on the surface of endothelial cells, is crucial for maintaining vascular integrity.[97] Upregulation of *PECAM1* is observed in developmental DS brains, which could be a compensatory response to maintain vascular health or might contribute to pathogenesis if it leads to dysregulated angiogenesis or inflammation.

3.2 | Key gene expression changes in the adult DS brain

Compared to the developmental DS brain, the adult DS brain exhibits additional dysregulation in pathways specific to neurodegenerative processes, including cytoskeletal regulation, vesicle trafficking, and heightened immune and oxidative stress responses, which are closely associated with the progression of AD pathology.[37] These changes, as represented by the overrepresentation of gene sets related to antigen presentation, integrin-mediated signaling, actin cytoskeleton organization, and vesicle-mediated transport, mark a transition from developmental dysfunction to neurodegenerative processes. Immune activation and oxidative stress responses contribute significantly to the inflammatory milieu characteristic of AD, while dysregulation of cytoskeletal dynamics and vesicle transport mechanisms further disrupt synaptic integrity and neuronal signaling, exacerbating cognitive deficits. Additionally, persistent activation of growth and developmental pathways in the adult DS brain may drive maladaptive cellular responses, compounding neurodegeneration.[37]

3.3 | The role of non-neuronal cells in DS-AD pathogenesis

The use of cutting-edge technology such as scRNA-seq has played a pivotal role in revolutionizing our comprehension of cellular heterogeneity and the dynamics of gene expression at an unprecedented resolution.[98] Notably, single-nucleus RNA sequencing (snRNA-seq) has emerged as a powerful technique for studying complex tissues, particularly those challenging to dissociate into single cells, such as brain tissue.[99] The brain's complexity, with its myriad closely linked cell types, poses challenges for scRNA-seq due to the difficulty of cell dissociation, which can lead to cell damage and altered gene expressions. snRNA-seq offers a solution by avoiding cell dissociation, allowing for the detailed analysis of the brain's cellular diversity. This technique facilitates the identification of distinct neuronal and non-neuronal cells, enabling research into cell-specific gene expression and the discovery of molecular markers for various brain regions and cell types.[100] Specifically, the DS study highlighted abnormalities in the expression of genes linked to the development of oligodendrocytes (ODC) and the myelination process. Among these genes, several have also been identified to be of trajectory differential expression (t-DEX) in ODC, microglia (MG), and astrocytes (ASC) in AD brains by Morabito et al.'s snRNA-seq study on AD brains[101] (Figure 2A[102]).

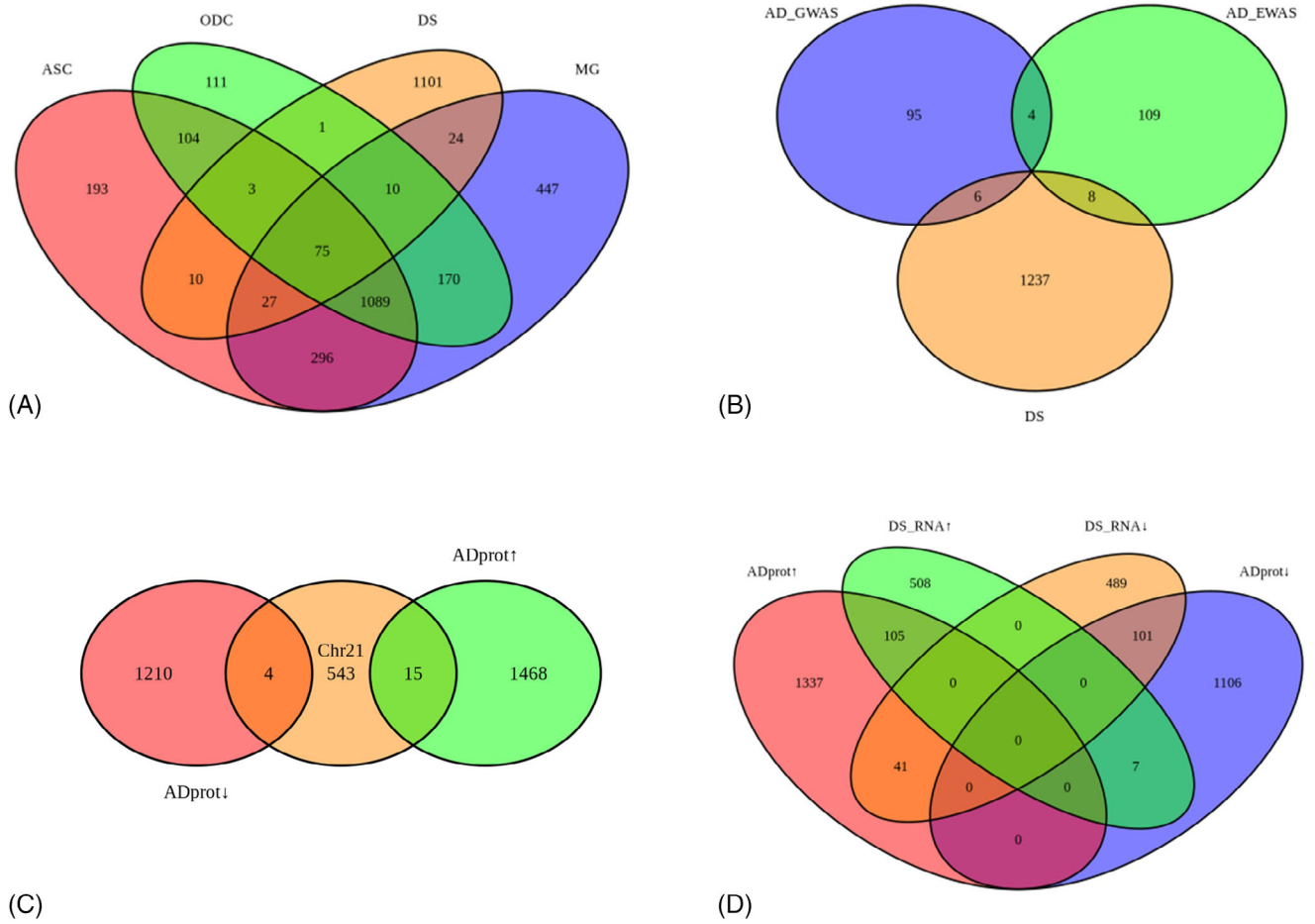

FIGURE 2 Venn diagrams of omics data integration. A, Genes with DEX in developmental DS brains and single-nucleus transcriptomics in AD brains. B, Genes with DEX in developmental DS brains and genes identified in AD GWAS and AD EWAS. C, Genes with DEX in AD proteome and genes encoded by Chr21. D, Genes with DEX in AD proteome and genes with DEX in developmental DS brains. Venn diagrams were generated using the VennDiagram package.[102] AD, Alzheimer's disease; ASC, astrocytes; Chr21, chromosome 21; DEX, differential expression; DS, Down syndrome; EWAS, epigenome-wide association studies; GWAS, genome-wide association studies; MG, microglia; ODCs, oligodendrocytes.

In addition to the roles of neurons and vascular dysfunction, it is essential to recognize the crucial contribution of non-neuronal cells to the pathogenesis of DS-AD, as they play key roles in amyloid clearance, neuroinflammation, and maintaining overall brain homeostasis. The findings from the transcriptomics studies on AD brains, which reveal genome-wide alterations in gene expression in non-neuronal cells ODC, MG, and ASC, open important avenues for further exploration into their role in DS-AD pathogenesis. ODC, MG, and ASC collectively contribute to the intricate cellular environment of the brain and are implicated in different aspects of AD. Dysfunction in these cell types can contribute to the pathogenesis of AD, affecting myelination, immune responses, Aβ clearance, and overall brain homeostasis.[103–105] Remarkably, *GFAP* was upregulated in all three cell types. A subset of genes ($n = 75$) exhibiting differential expression in developmental DS brains was found of t-DEX in all three cell types—ODC, MG, and ASC—in AD brains. Over-representation analysis highlighted five Gene Ontology biological process (GO-BP) gene sets, including cell junction organization, regulation of membrane potential, cell-substrate adhesion, synapse organization, and axon development (Table 1). All these BPs are closely linked to AD pathology. For instance, disturbances in the organization of cell junctions, particularly the impairment of BBB function, has been recognized as a significant contributor to AD pathology.[106] Minor changes in membrane potential may alter the firing characteristics of neurons, resulting in substantial dysfunction in the brain.[107] Although these changes have been identified, the crucial task remains to ascertain whether they are a primary driver of the disease or a consequence of its progression. This distinction is pivotal for a deeper understanding of the disease mechanism and could be instrumental in guiding future research efforts aimed at developing targeted therapies for DS-AD.

3.4 | Integrating DS transcriptomics and AD genetics

Integrating genetic findings with transcriptomic data provides deeper insight into the molecular mechanisms underlying DS-AD. In dorsal frontal cortex (DFC, related to long-term memory encoding[109]), β-

TABLE 1 Overrepresented Gene Ontology biological processes with DEX in developmental and adult DS brains and t-DEX in oligodendrocytes (ODC), microglia (MG), and astrocytes (ASC) in AD brains.[a]

Gene set	Description	Size	Expect	Ratio	*p*	FDR	Genes
GO:0034330	Cell junction organization	285	1.33	6.78	6.59E-06	0.0056	*APOD*, *BCL2*, *CDH12*, *GJA1*, *NFASC*, *PARD3*, *PTPRJ*, *TESK2*, *TGFB2*
GO:0042391	Regulation of membrane potential	414	1.93	4.67	0.000123	0.0423	*AKAP6*, *BCL2*, *GJA1*, *GRIN1*, *GRIN2A*, *GRM1*, *KCNE4*, *KCNH8*, *KCNQ3*
GO:0031589	Cell-substrate adhesion	332	1.55	5.17	0.000149	0.0423	*APOD*, *BCL2*, *CD44*, *EPHB1*, *MERTK*, *PTPRJ*, *SEMA3E*, *TESK2*
GO:0050808	Synapse organization	385	1.79	4.46	0.000407	0.0729	*CNTN2*, *COL4A1*, *EPHB1*, *ERC2*, *GRIN1*, *NFASC*, *SEMA3E*, *SPARCL1*
GO:0061564	Axon development	490	2.28	3.94	0.000429	0.0729	*APOD*, *BCL2*, *CNTN2*, *EPHB1*, *GRIN1*, *NCAM2*, *NFASC*, *PARD3*, *SEMA3E*,

Abbreviations: AD, Alzheimer's disease; DEX, differential expression; DS, Down syndrome; FDR, false discovery rate; t-DEX, trajectory differential expression.

[a]Seventy-five genes with DEX in the DS brain and t-DEX in the three cell types in the AD brain: *ACSL1*, *ACSS3*, *AGBL4*, *AKAP6*, *ALDH1L1*, *AOAH*, *APOD*, *ARHGAP10*, *ASPH*, *BCL2*, *CABLES1*, *CADPS*, *CD44*, *CDH12*, *CEBPD*, *CNTN2*, *COL4A1*, *CSMD1*, *DLGAP2*, *ELL2*, *EPB41*, *EPHB1*, *EPN2*, *ERC2*, *EYA2*, *FAM49B*, *FRAS1*, *GALNT2*, *GJA1*, *GRIN1*, *GRIN2A*, *GRM1*, *HECW1*, *HIF3A*, *HS3ST5*, *KCNE4*, *KCNH8*, *KCNQ3*, *LDLRAD3*, *LIMK2*, *MED12L*, *MERTK*, *NCAM2*, *NFASC*, *NKAIN3*, *NR4A1*, *NT5DC1*, *OSMR*, *PARD3*, *PARP8*, *PELI1*, *PKNOX2*, *PLSCR4*, *POU6F2*, *PRAM1*, *PTPRJ*, *PYGL*, *RAB31*, *RFX4*, *RNF220*, *RYR3*, *SEMA3E*, *SLC14A1*, *SLC25A13*, *SNAP91*, *SPARCL1*, *SRGAP1*, *STK33*, *STON2*, *TESK2*, *TGFB2*, *THSD4*, *TMTC2*, *TNFAIP8*, *YAP1*. Over-representation analysis by the WebGestalt (WEB-based Gene SeT AnaLysis Toolkit) web tool.[108]

secretase 1 (*BACE1*), and *PSEN1* (its rare variants correlated with familial AD by affecting Aβ precursor processing) showed lower expression in developmental DS brains.[36] In addition, downregulation of *PSEN2* expression has been observed in the adult DS brain by Lockstone et al.[37] β-secretase is responsible for the initial cleavage for APP,[110] while γ-secretase encoded by *PSEN1* and *PSEN2* cleaves the remaining membrane-bound C-terminal fragment of APP following the action of β-secretase. Inhibitors of *BACE1* have been a major focus in the development of treatments to reduce plaque formation.[111] These paradox changes of *BACE1* and *PSEN1* expression in developmental DS brains, where one might expect higher activity to correlate with AD progression, suggest that β- and γ-secretase inhibitors may not be the focus in DS-AD treatment. In cerebellar cortex (CBC, contributing to both the deterioration of motor functions and cognitive impairments[112]), *APOE* had higher expression in developmental DS brains. In addition, a number of AD-associated genes identified by GWASs were also identified of DEX in the DS brain (Figure 2B). In DFC, AD-associated genes heparan sulfate-glucosamine 3-sulfotransferase 5 (*HS3ST5*, chr6), granulin precursor (*GRN*, chr17), and pleckstrin homology domain containing A1 (*PLEKHA1*, chr10) were downregulated in DS patients; signal peptide peptidase like 2A (*SPPL2A*, chr15), and FERM domain containing kindlin 2 (*FERMT2*, chr14), were upregulated in DS patients. In CBC, AD-associated gene *PLEKHA1* was downregulated in DS patients; protein kinase D3 (*PRKD3*, chr2), *SPPL2A*, and *FERMT2*, were upregulated in DS patients (Table 2). This observation underscores their altered expression levels in DS-AD. The inference that common AD-associated genetic variants may exacerbate the risk of DS-AD adds a layer of complexity to our understanding. Importantly, the genes with altered expression levels in DS and influenced by AD-associated variants may serve as potential therapeutic targets.

3.5 | Perspective and limitations of transcriptomics

Taken together, transcriptomics studies offer insights into the intricate molecular mechanisms of AD risk in individuals with DS, providing valuable information on potential therapeutic targets and intervention pathways. For example, interventions aimed at normalizing the expression of genes involved in ODC development or myelination processes could be beneficial.[134] Lifestyle interventions aimed at enhancing brain plasticity and resilience may also be effective in counteracting the transcriptomic alterations associated with DS-AD.[135] The identification of gene expression alterations in specific cell types and brain regions unveils the complex interactions contributing to AD pathogenesis in the context of DS. Building on these findings, the observed transcriptomic changes in genes harboring AD-associated genetic variants highlight their significance in identifying potential therapeutic approaches. However, it is crucial to note that, while these genes possess AD-associated variants, the direct influence of these variants on the transcriptomic alterations in DS, and any potential interactions with T21, including but not limited to epistatic effects, gene dosage impacts, epigenetic modifications, and alterations in signaling pathways, remains to be fully elucidated. Future research should therefore prioritize clarifying the relationships between these AD-associated genetic variants and the unique transcriptomic profiles observed in individuals with DS.

TABLE 2 AD-associated genes with DEX in DS brains.

Gene	Functional role	Mechanistic insights	Relevance to DS-AD
Downregulated in DS brains			
HS3ST5	Encodes a sulfotransferase, adding sulfate groups to heparan sulfate, enabling normal heparan sulfate function.[113]	In AD brains, heparan sulfate binds and accelerates Aβ aggregation, mediates Aβ internalization, and induces cytotoxicity.[114] It also binds tau, influencing secretion, internalization, and aggregation.[115] Downregulation disrupts heparan sulfate sulfation patterns, potentially exacerbating Aβ aggregation and tau pathology.	Aberrant heparan sulfate sulfation may amplify Aβ-mediated cytotoxicity and tau interactions, worsening DS-AD pathology.
GRN	Encodes granulin precursor, cleaved into active peptides (granulins/epithelins) that function as growth factors.[116,117]	*GRN* mutations are implicated in neurodegenerative diseases such as frontotemporal lobar degeneration[118,119] and Lewy body dementia.[120] Downregulation *GRN* may compromise neuronal maintenance and repair mechanisms.[121]	Reduced granulin levels may compromise neuronal resilience and exacerbate neurodegeneration in DS-AD.
PLEKHA1	Encodes a plasma membrane protein that binds phosphatidylinositol 3,4-bisphosphate, playing a role in signaling complex formation.[122]	In neurons, *PLEKHA1* is involved in synaptic signaling and *APP* processing.[123] Downregulation may disrupt membrane functions, leading to impaired *APP* metabolism and Aβ accumulation. Variants are also linked to hypothyroidism,[124] autoimmune thyroid disease,[125] and other autoimmune diseases.[126]	Downregulation may exacerbate synaptic dysfunction and Aβ pathology, contributing to DS-AD progression.
Upregulated in DS brains			
SPPL2A	Encodes an intramembrane protease that degrades CD74, the invariant chain of MHC class II, impacting antigen presentation and immune response.[127]	Upregulation in microglia may alter immune responses by degrading CD74, enhancing neuroinflammation. Elevated *SPPL2A* levels during early Aβ-mediated processes may create a feedback loop, intensifying Aβ production and pathology.[128]	Upregulated *SPPL2A* may drive neuroinflammation and exacerbate Aβ pathology in DS-AD.
FERMT2	Encodes a protein integral to extracellular matrix structures,[129] modulating Aβ and tau proteostasis in neuronal cells.[130]	Genome-wide siRNA screening identifies *FERMT2* as a key modulator of *APP* metabolism.[131] Underexpression of *FERMT2* is correlated with increased Aβ peptide production, attributed to elevated levels of mature *APP* at the cell surface and an enhanced recycling.[131] In contrast, depletion reduces phosphorylated tau and extracellular Aβ.[130]	*FERMT2* is a regulator of proteostasis, and excessive activity could disrupt the fine-tuned balance of *APP* metabolism and tau regulation.
PRKD3	Encodes a kinase involved in vesicle trafficking and signaling pathways,[132] with emerging links to synaptic function and neuroinflammation.[133]	Overexpression of *PRKD3* may disrupt neuronal signaling and contribute to inflammation. It is a potential therapeutic target for AD, with midostaurin, an FDA-approved multi-targeted protein kinase inhibitor for the treatment of both solid and non-solid tumors, being explored to modulate PRKD3 activity.[133]	Dysregulated *PRKD3* activity may aggravate neuroinflammation and synaptic deficits in DS-AD.

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; DEX, differential expression; DS, Down syndrome; FDA, US Food and Drug Administration.

Nevertheless, while transcriptomics is a powerful and informative tool in DS and AD research, it is important to maintain caution. The potential for bias exists due to the more informative nature of transcriptomic studies compared to other -omic approaches, which might skew the perception of intervention opportunities. Therefore, corroborative evidence from additional -omic technologies is crucial to validate and support these potential interventions. For example, transcriptomic profiling identifies dysregulated genes involved in ODC development and myelination processes. Proteomics can reveal protein levels, post-translational modifications (PTMs), protein isoforms, and interactions crucial for these processes. Meanwhile, metabolomics provides insights into metabolic disturbances affecting ODC function. Integrating other -omics data may further enrich our understanding, allowing for a holistic exploration of the multifaceted molecular signatures associated with DS-AD. These comprehensive data facilitate the identification of promising targets for interventions. Furthermore, predictive models using multi-omics data can determine the optimal timing for interventions. In the following sections, we will explore how other -omics approaches further elucidate the molecular landscape of DS-AD, complement transcriptomic insights, and provide a more holistic understanding of the multifaceted mechanisms underlying this condition.

4 | EPIGENOMICS INSIGHTS INTO DS-AD

4.1 | Integrating AD epigenomics with DS brain transcriptomics

Epigenomics is important for its unique focus on the regulation of gene expression through heritable and reversible modifications. Its dynamic nature, involvement in development and disease, and potential for therapeutic interventions make epigenomics a critical component in the broader landscape of -omics sciences. Epigenomics has proven to be a valuable tool in unraveling the complexities of DS-AD, laying the groundwork for future therapeutic strategies and interventions.[136,137] Notably, the epigenomic analyses of the fetal DS cortex have revealed significant alterations in DNA methylation patterns, showing an increase in both hypermethylated and hypomethylated CpG sites throughout the genome.[138] Accelerated epigenetic aging in the blood of individuals with DS has been observed prenatally.[139] Furthermore, the discovery of accelerated epigenetic aging in adult DS, as measured by the epigenetic clock,[140] elucidates that DS significantly increases the biological age of blood and brain tissue by an average of 6.6 years based on DNA methylation levels.[141]

Epigenetic alterations, including changes in histone acetylation and DNA methylation, affect multiple genes involved in AD pathology in vulnerable brain regions, with potential implications for therapeutic approaches like epigenomic engineering (supporting information). Among the AD epigenome-wide association study (EWAS) genes,[142] eight genes were identified of DEX in developmental DS brain, including seven genes upregulated in developmental DS brain (in DFC: *PCNT*, *C10orf99*, *RHBDF2*; in CBC: *SLC15A2*, *CPEB4*, *GJB6*, *HOXB1*), and one gene *RNF112* downregulated in developmental DS CBC.[36] In the adult DS brain, two genes were upregulated, *ABLIM1* (GO:0009887, organogenesis) and *RHOB* (GO:0007049, cell cycle). Conversely, two genes were downregulated, *ANK1* (GO:0007010, cytoskeleton organization and biogenesis) and *SPN* (GO:0006968, cellular defense response).[37]

4.2 | Functional insights from key epigenetically altered genes

The upregulated genes in developmental DS brains participate in diverse cellular processes. The pericentrin gene (*PCNT*) at Chr21q22.3 is involved in organizing cellular structures, particularly the centrosome.[143] The centrosome is a cellular organelle that serves as the main microtubule-organizing center in animal cells. It plays a pivotal role in various cellular processes, including cell division, intracellular transport, and the maintenance of cell structure.[144] Overexpression of *PCNT* in DS is related to the presence of an extra copy of Chr21. The identification of *PCNT* as a locus in AD EWAS implies that this gene is sensitive to changes in gene dosage. Other genes are located on different autochromosomes. *C10orf99* (encoding G protein-coupled receptor 15 ligand, *GPR15LG*) at Chr10q23.1 encodes a cytokine,[145] potentially contributing to neuroinflammation. The rhomboid 5 homolog 2 gene (*RHBDF2*) at Chr17q25.1 regulates epidermal growth factor signaling and controls the activation and substrate selectivity of ADAM metallopeptidase domain 17 (ADAM17)-dependent shedding events, for example, the ectodomain shedding of tumor necrosis factor α.[146] The solute carrier family 15 member 2 gene (*SLC15A2*) at Chr3q13.33 encodes a proton-coupled peptide transporter, possibly maintaining the integrity and selective permeability of the BBB.[147] The cytoplasmic polyadenylation element binding protein gene 4 (*CPEB4*) at Chr5q35.2, containing unstructured low complexity domains (LCDs), can form nucleolar aggregates and may mediate AD pathology.[148] The gap junction protein beta 6 gene (*GJB6*) at Chr13q12.11 encodes connexin 30, integral to gap junctions.[149] Connexin 43, encoded by the gap junction protein alpha 1 gene (*GJA1*), is a significant player in AD pathogenesis. Sustained *GJA1* upregulation can cause neuronal strain, leading to eventual cellular degeneration and death.[150] The homeobox B1 gene (*HOXB1*) at Chr17q21.32 encodes a transcription factor with an essential role in morphogenesis. A subpopulation of noradrenergic neurons with developmental *HOXB1* expression plays a unique role in modulating stress-related behavior.[151]

In contrast to the above upregulated genes, the ring finger protein 112 gene (*RNF112*) at Chr17p11.2 is downregulated in developmental DS brains. *RNF112*, also known as neurolastin, belongs to the RING finger protein family of transcription factors and is a brain-specific dynamin family GTPase with functional GTPase and RING domains. It plays a crucial role in dendritic spine formation, synaptic function, and synaptic plasticity during development.[152] Mice lacking the *Rnf112* gene (*Rnf112*−/−) had smaller brains compared to wild-type littermates, with impairments in brain functions, specifically affecting motor balance, spatial learning, and memory.[153]

In the adult DS brain, the genes exhibit a shift toward processes associated with neurodegeneration, such as *ABLIM1* and *RHOB*, which are upregulated and linked to cell cycle dysregulation and cytoskeletal dynamics, and impaired cellular defense and cytoskeletal organization, reflected in the downregulation of *ANK1* and *SPN*. These changes signify the onset of neurodegenerative mechanisms. This transition reflects an age-related evolution of the molecular profile in DS brains, moving from developmental dysregulation in early life to mechanisms driving neurodegeneration in adulthood. These genes, identified in AD EWASs, highlight an epigenetic regulatory influence in AD and a transcriptomic shift in DS, suggesting intersecting pathways that converge on DS and neurodegenerative processes. The converged pathways originate in the developmental stage and transition in adulthood and may serve as potential therapeutic targets for gene-specific epigenetic editing.[154]

4.3 | Perspective and limitations of epigenomics

The integration of epigenomics with DS-AD research opens avenues for targeted therapeutic strategies, including gene-specific epigenetic editing. When integrated with transcriptomics, it offers a synergistic and comprehensive understanding of the molecular mechanisms

underpinning DS-AD. The recognition of epigenetic alterations in AD-susceptible brain regions highlights the importance of these modifications in the disease process, offering possibilities for intervention. Specifically, understanding the roles of *DYRK1A*, *DNMT3L*, and other identified genes in DS epigenetics is essential for advancing the potential of epigenomic interventions in the treatment and understanding of both DS and AD. Drugs that specifically target epigenetic modifications, such as inhibitors of DNA methylation or histone deacetylases (HDACs),[155] could be tailored to normalize the epigenetic marks associated with AD pathology. The potential for using epigenome editing tools, like CRISPR-dCas9 systems equipped with epigenetic modifiers, offers a futuristic but highly promising approach to directly correct the aberrant epigenetic landscape in DS-AD.[156] Furthermore, lifestyle and environmental interventions that can positively influence the epigenome, such as diet, physical exercise, and cognitive engagement, present non-invasive strategies to mitigate AD risk in DS.[157]

Despite these promising avenues, there remain significant challenges in fully understanding and using epigenomics in DS-AD research. The heterogeneity of epigenetic modifications across different brain regions and cell types[138] necessitates more refined, cell-specific epigenomic mapping techniques. Additionally, while tools like CRISPR-dCas9 provide immense therapeutic potential, their safety, precision, and long-term effects require extensive preclinical and clinical validation. Future studies must also address the dynamic nature of epigenetic changes over time, particularly in response to aging and environmental factors, to better understand their impact on DS-AD progression. Integrating other -omics approaches with epigenomics will help overcome current limitations and provide a holistic understanding of the molecular networks driving DS-AD pathogenesis.

5 | PROTEOMIC FINDINGS

5.1 | Integration of AD brain proteomics with DS brain transcriptomics

The correlations between plasma protein biomarkers of Aβ40 and Aβ42, total tau, and NfL with cognitive and functional impairments in DS adults have been demonstrated by a large cross-sectional cohort study.[158] The investigation into brain proteomics further accelerates advancements in AD and DS research. Pires et al. identified secernin-1 (SCRN1) as a novel p-tau binding protein, predominantly accumulating in AD, DS-AD, and primary age-related tauopathy (PART).[159]

The proteomic findings of AD in the general population are discussed in Table S2 in supporting information. Among the proteomic findings, key AD-related proteins encoded by *APP*, *MAPT* (encoding tau), *GFAP*, and *NEFL* (encoding NfL) are all upregulated in AD brains.[160] Discrepancies in proteomics findings for AD may arise due to technical differences between studies, such as variations in assay sensitivity, platform type, or sample handling, as well as small sample sizes that limit statistical power and contribute to inconsistent results.[161,162] In this regard, comparing AD brain proteomics with DS genetics and brain transcriptomics helps corroborate proteomic findings. Notably, two layers of associations between AD and DS are suggested by AD brain proteomics with 1483 proteins upregulated and 1214 proteins downregulated.[160] While 15 out of 1483 upregulated proteins are encoded by Chr21, only 4 of 1214 downregulated proteins are encoded by Chr21 ($p = 0.035$), implying a link between increased Chr21 dosage in DS and AD pathogenesis (Figure 2C). Furthermore, consistent changes were observed in the developmental DS brain transcriptome:[36] 105 upregulated proteins have been upregulated in the developmental DS brain transcriptome, and 41 upregulated proteins have been downregulated in the developmental DS brain transcriptome (Figure 2D, 105/620 vs. 41/631, $p = 8.98E-09$); 101 downregulated proteins have been downregulated in the developmental DS brain transcriptome, and 7 downregulated proteins have been upregulated in the developmental DS brain transcriptome (101/631 vs. 7/620, $p = 7.42E-21$). These consistent changes of AD brain proteome and developmental DS brain transcriptome further underscore the associations between DS and AD risk. Several biologically significant processes (BPs) have been identified with high statistical significance (Table 3), offering critical insights into how DS contributes to AD pathology (Table 4).

While increased expression of Chr21 genes occurs due to T21, certain proteins encoded by Chr21 show reduced levels in AD proteomics. Among the seven downregulated proteins with upregulated RNAs in the DS brain, the intersectin 1 gene (*ITSN1*) at Chr21q22.11 is a notable example. *ITSN1* is involved in regulating clathrin-coated vesicle formation and synaptic vesicle recycling.[177] Interestingly, while *ITSN1* RNA is upregulated in DS brains due to T21, there is an observed reduction in the brain levels of *ITSN1* compared to younger counterparts.[178]

Further insights are gained from changes observed in AD brain proteomics and the adult DS brain transcriptome. A total of 44 upregulated proteins align with upregulation in the adult DS brain transcriptome, while 2 upregulated proteins show downregulation in adult DS brain transcriptome (44/138 vs. 2/55, $p = 5.53E-04$). Several BPs have been highlighted by these genes with statistical significance (Table 3). Similarly, 10 downregulated proteins align with downregulation in the adult DS brain transcriptome, while 3 downregulated proteins show upregulation in the adult DS brain transcriptome (10/55 vs. 3/138, $p = 8.74E-04$). These consistent changes between the AD brain proteome and the adult DS brain transcriptome further underscore the associations between DS and AD risk. While the downregulated genes are fewer in number and lack significant enrichment for specific BP gene sets, they are involved in critical pathways such as synaptic transmission, potassium ion transport, protein amino acid dephosphorylation, small GTPase-mediated signal transduction, and cytoskeleton organization and biogenesis.[37] Compared to the developmental DS brain, the focus in adult DS brain shifts toward cellular signaling and physiological responses that may reflect compensatory or degenerative mechanisms. The upregulation of small GTPase-mediated signaling and cell morphogenesis regulation underscores ongoing cellular adap-

TABLE 3 overrepresented Gene Ontology biological process with DEX in the AD brain proteome and developmental and the adult DS brain transcriptome.[a]

Gene set	Description	Size	Expect	Ratio	*P*	FDR
a. Upregulated AD proteins and upregulated RNAs in developmental DS brain						
GO:0007015	Actin filament organization	388	2.65	5.29	3.69E-07	0.0002
GO:0034330	Cell junction organization	285	1.94	6.17	5.32E-07	0.0002
GO:0002446	Neutrophil mediated immunity	496	3.38	4.14	6.61E-06	0.0015
GO:0036230	Granulocyte activation	500	3.41	4.10	7.25E-06	0.0015
GO:1902903	Regulation of supramolecular fiber organization	329	2.24	4.90	1.48E-05	0.0023
GO:0031589	Cell-substrate adhesion	332	2.26	4.86	1.61E-05	0.0023
GO:0071559	Response to transforming growth factor beta	238	1.62	5.54	3.56E-05	0.0037
GO:0032970	Regulation of actin filament-based process	362	2.47	4.45	3.56E-05	0.0037
GO:1990778	Protein localization to cell periphery	301	2.05	4.87	3.90E-05	0.0037
GO:0007229	Integrin-mediated signaling pathway	101	0.69	8.71	6.51E-05	0.0055
b. Downregulated AD proteins and downregulated RNAs in developmental DS brain						
GO:0050905	Neuromuscular process	107	0.63	9.54	3.89E-05	0.0330
GO:0014075	Response to amine	44	0.26	15.47	1.26E-04	0.0461
GO:0099177	Regulation of trans-synaptic signaling	417	2.45	4.08	1.63E-04	0.0461
GO:0097366	Response to bronchodilator	51	0.30	13.35	2.25E-04	0.0478
GO:0051648	Vesicle localization	305	1.79	4.46	4.22E-04	0.0717
GO:0001505	Regulation of neurotransmitter levels	335	1.97	4.06	7.81E-04	0.0935
GO:0031644	Regulation of neurological system process	128	0.75	6.65	9.36E-04	0.0935
GO:0099504	Synaptic vesicle cycle	193	1.13	5.29	9.55E-04	0.0935
GO:0006836	Neurotransmitter transport	268	1.57	4.45	1.00E-03	0.0935
GO:0099003	Vesicle-mediated transport in synapse	203	1.19	5.03	1.24E-03	0.0935
c. Upregulated AD proteins and upregulated RNAs in adult DS brain						
GO:0007264	Small GTPase mediated signal transduction	493	1.32	6.80	4.81E-06	0.0040
GO:0022604	Regulation of cell morphogenesis	243	0.65	9.20	4.25E-05	0.0135
GO:1902903	Regulation of supramolecular fiber organization	375	1.01	6.96	5.48E-05	0.0135
GO:0055094	Response to lipoprotein particle	31	0.08	36.07	7.63E-05	0.0135
GO:0014812	Muscle cell migration	86	0.23	17.34	8.05E-05	0.0135
GO:0071402	Cellular response to lipoprotein particle stimulus	34	0.09	32.89	1.01E-04	0.0141
GO:0097242	Amyloid beta clearance	37	0.10	30.22	1.30E-04	0.0156
GO:0044703	Multi-organism reproductive process	199	0.53	9.37	1.81E-04	0.0190
GO:0044706	Multi-multicellular organism process	207	0.56	9.00	2.18E-04	0.0194
GO:0007229	Integrin-mediated signaling pathway	113	0.30	13.20	2.32E-04	0.0194

Abbreviations: AD, Alzheimer's disease; DEX, differential expression; DS, Down syndrome; FDR, false discovery rate; ORA, over-representation analysis.

[a]ORA by the WebGestalt (WEB-based Gene SeT AnaLysis Toolkit) web tool.[108] a. 105 AD upregulated proteins and upregulated in DS brain transcriptome: *SEPT2*, *ACSM5*, *ACSS3*, *ADD3*, *ALDH1L1*, *ANP32B*, *APAF1*, *APOE*, *APPL2*, *ARPC1B*, *ASPH*, *BBOX1*, *BBS2*, *BCL2*, *C5AR1*, *CD44*, *COL4A1*, *COMT*, *CRLF3*, *CSK*, *CTNNA1*, *CYBRD1*, *DARS*, *DBF4B*, *DKK3*, *DPY19L3*, *EPB41*, *EPS15*, *F11R*, *FERMT2*, *FKBP5*, *FLT1*, *GALM*, *GDF10*, *GJA1*, *GLUD1*, *GPC4*, *GRAMD3*, *H1F0*, *HADHA*, *HCLS1*, *HEATR5A*, *HMGN1*, *HSDL2*, *HVCN1*, *IFI30*, *IFT122*, *INPPL1*, *ITGAL*, *ITGB4*, *ITGB8*, *ITM2C*, *KIF5B*, *LAIR1*, *LDHB*, *LIX1*, *METTL7A*, *MGST1*, *MT1A*, *NEK6*, *NOTCH2*, *NPL*, *NUBP2*, *PARD3*, *PECAM1*, *PHKA2*, *PLCD1*, *PLP2*, *PLSCR4*, *PRAM1*, *PRCP*, *PRMT2*, *PSTPIP1*, *PTTG1IP*, *PXN*, *PYGL*, *RAB31*, *RASA4*, *RDX*, *RELL1*, *RNF114*, *S100A10*, *S100B*, *SDC4*, *SEC14L2*, *SLC15A2*, *SLC6A11*, *SON*, *SRGAP1*, *SSPN*, *STK33*, *STON2*, *SYTL4*, *TAX1BP3*, *TGFB2*, *TMOD3*, *TP53BP2*, *TRIP10*, *TWF1*, *UNC119B*, *UNC13D*, *VASN*, *VSIG4*, *WASF2*, *YAP1*; b. 101 AD downregulated proteins and downregulated in DS brain transcriptome: *ABLIM2*, *ACHE*, *ACTR3B*, *ADAM11*, *ADAP1*, *AGBL4*, *AP3B2*, *ARFGAP1*, *ARFGAP2*, *ARHGEF2*, *ASAP2*, *ASTN1*, *ATP2B3*, *ATP5H*, *BAI1*, *BCL2L2*, *BDNF*, *BRSK2*, *C2CD2L*, *CA4*, *CADPS*, *CBLN4*, *CDH12*, *CDK5R2*, *CECR6*, *COX5B*, *CSMD1*, *DIRAS1*, *DLAT*, *DLGAP2*, *DLK2*, *DPYSL4*, *DUSP3*, *DYNC1LI1*, *EFNA3*, *EFR3B*, *EPHB6*, *ERC2*, *EXOC6*, *FAM131B*, *FAM134A*, *FAM49B*, *GRIN1*, *GRIN2A*, *GTPBP10*, *HECW1*, *HPRT1*, *HSPA12A*, *HYOU1*, *IGSF8*, *KCNH1*, *KCNQ3*, *LARP1B*, *LIMK1*, *MET*, *MGST3*, *MRPL2*, *NDUFS2*, *NFASC*, *NGEF*, *NLGN2*, *NPTX2*, *NRBF2*, *NUMBL*, *PCSK1*, *PDCD6IP*, *PGBD5*, *PIK3R4*, *PIP5K1C*, *PLEKHA1*, *PLXNA1*, *PNKD*, *PORCN*, *RDH14*, *RGS4*, *ROGDI*, *RPH3A*, *SERAC1*, *SH3RF1*, *SLC25A44*, *SLC27A4*, *SLC7A8*, *SNX4*, *SYNGAP1*, *SYNGR3*, *SYT12*, *TBC1D10B*, *TFRC*, *TMEM163*, *TMEM177*, *TMEM38A*, *TPPP*, *TRIM3*, *TSC22D2*, *TTC39C*, *TTC9B*, *UBXN6*, *UQCRC1*, *VPS36*, *WDR7*, *ZDHHC5*. 41 upregulated proteins and downregulated in DS brain transcriptome: *ACSL1*, *BACE1*, *C9orf142*, *CLCN7*, *CTSD*, *DAGLB*, *DHX16*, *FARP2*, *FBL*, *FRZB*, *GNA11*, *GRN*, *GTPBP2*, *H2AFY*, *HMGA1*, *LACTB2*, *LIPA*, *MAP3K2*, *NFKB1*, *NUP210*, *OSTM1*, *PCIF1*, *PDYN*, *PES1*, *PGAM2*, *PLXNB1*, *PRKCI*, *PTBP1*, *QSOX1*, *RCC2*, *RHPN2*, *RING1*, *RNPEP*, *RRAGA*, *SLC39A14*, *SLC6A9*, *SMYD5*, *STIM2*, *TRAM1*, *UNC5B*, *WDR46*; 7 downregulated proteins and upregulated in DS brain transcriptome: *FAM49A*, *GJB6*, *ITSN1*, *MKLN1*, *PURG*, *SCO1*, *SNAP91*; c. 44 upregulated proteins with upregulation in the adult DS brain: *ARFGAP3*, *PDE4DIP*, *NIPBL*, *EEF1A1*, *CRK*, *RECK*, *METAP2*, *RPS6KA5*, *SMARCA1*, *CAPG*, *STXBP3*, *CLU*, *NECAP2*, *SON*, *RAB9A*, *WASF2*, *PEA15*, *FABP7*, *RBPJ*, *SMC3*, *APOE*, *ITGB4*, *EPS8*, *SNX2*, *NOTCH2*, *ITGB1*, *SPARC*, *DDR1*, *SEPT10*, *PLXNB1*, *SEPT2*, *CPNE3*, *PLTP*, *IGFBP5*, *FAM107A*, *SMC1A*, *HLA-DRB1*, *OSBPL11*, *ITGB2*, *RDX*, *HLA-DRA*, *CA2*, *SPP1*, *ADAMTS1*.

TABLE 4 Insights from overrepresented Gene Ontology biological process in AD brain proteomics and DS brain transcriptomes.

Gene set	Functional role	Mechanistic insights	Relevance to DS-AD
a. Upregulated AD proteins and upregulated RNAs in the developmental DS brain			
GO:0007015 actin filament organization	Maintains cellular structure and function.	Disruptions in actin filament organization alter neuronal structure, affecting synaptic integrity and plasticity.[163,164]	Upregulation of actin filament organization in DS-AD may initially compensate for cytoskeletal instability. However, persistent abnormalities in actin dynamics can disrupt synaptic function.
GO:0034330 cell junction organization	Regulates the integrity of the BBB.	Alterations in cell junctions increase BBB permeability, allowing harmful substances (e.g., neurotoxic agents) to enter the brain.[165,166]	Upregulated cell junction organization genes may reflect a response to BBB dysfunction in DS-AD.
GO:0002446 neutrophil mediated immunity	Contributes to immune defense and inflammation.	Systemic inflammation have been underlined in AD pathogenesis.[167] Hyperactivation of neutrophils promotes neuroinflammation and oxidative stress, releasing pro-inflammatory mediators.[168] Neutrophil hyperactivation have been suggested of association with the rate of cognitive decline in AD.[169]	Upregulated neutrophil-mediated immunity in DS-AD may drive chronic inflammation, accelerating neurodegeneration and cognitive decline.
GO:0071559 response to transforming growth factor beta	Regulates neuronal development and synaptic plasticity.	Dysfunction in TGF-β signaling increases Aβ deposition and NFT formation.[170]	The perturbation of TGF-β signaling underscores the intricate relationship between trophic factor responses, synaptic plasticity, and the neurodegenerative processes in DS-AD.
b. Downregulated AD proteins and downregulated RNAs in developmental DS brain			
GO:0014075 response to amine	Supports neurotransmission and synaptic signaling.	Changes in response to dopamine, serotonin, and norepinephrine impact neuronal survival and synaptic function.[171]	Disrupted neurotransmitter signaling is highlighted in DS-AD.
GO:0051648 vesicle localization	Ensures proper neurotransmitter release.[172]	Disruptions in vesicle transport and localization impair synaptic neurotransmitter release.[173]	Downregulated vesicle localization genes in DS-AD leads to synaptic dysfunction and communication deficits.
c. Upregulated AD proteins and upregulated RNAs in adult DS brain			
GO:0007264 Small GTPase mediated signal transduction	Regulates intracellular signaling, cytoskeletal dynamics, and vesicle trafficking.	Dysregulation affects synaptic plasticity and neuronal communication.[174]	Upregulation may reflect attempts to compensate for impaired signaling but could contribute to disrupted neuronal connectivity.
GO:1902903 Regulation of supramolecular fiber organization	Maintains cytoskeletal integrity and extracellular matrix structure.	Impaired fiber organization leads to cytoskeletal instability and extracellular matrix remodeling.[175]	May compensate for cellular stress but eventually contribute to structural brain deterioration.
GO:0071402 Cellular response to lipoprotein particle stimulus	Drives cellular adaptations to lipid-related signals, including inflammatory responses.	Links to amyloid processing and neuroinflammation through altered lipid metabolism.[176]	May exacerbate inflammatory cascades, promoting AD pathology in DS.
GO:0097242 Aβ clearance	Removes Aβ peptides to prevent plaque accumulation.	Impaired clearance exacerbates amyloid aggregation and neuronal toxicity.	Reflects efforts to mitigate amyloid burden.

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; BBB, blood–brain barrier; DS, Down syndrome; NFT, neurofibrillary tangle.

tations, while pathways like Aβ clearance and responses to lipoproteins suggest a shift toward addressing AD pathology in the adult DS brain.

5.2 | Amyloid plaque analysis and PTMs in AD and DS-AD *post mortem* older adult brains

A recent proteome study revealed that the amyloid plaques found in sporadic EOAD and DS-AD *post mortem* older adult brains share common proteins, yet there are variations in the levels of certain proteins between the two.[179] While the fundamental mechanisms of amyloid plaque formation may be shared, the differing levels of specific proteins could contribute to the distinct pathological trajectories observed in EOAD and DS-AD. For example, proteins such as SMOC1 (SPARC-related modular calcium binding 1) and phosphorylated Aβ were significantly enriched in DS-AD plaques, while higher levels of oligomeric Aβ were observed in EOAD plaques. SMOC1 is a matricellular protein involved in cellular signaling and the regulation of extracellular matrix components.[180] Its protein levels are known to be elevated in the AD brain.[181] The enrichment of SMOC1 in DS-AD plaques indicates a potential mechanistic role for extracellular matrix dysregulation in driving the accelerated and early-onset amyloid deposition observed in DS-AD.

AD proteomics reveals potential therapeutic targets through identified proteins, pathways, and the intricate interplay between genetic factors and molecular processes, emphasizing the need for targeted interventions to mitigate neurodegenerative processes. Importantly, oxidative stress has been implicated in contributing to neurodegeneration in the brains of individuals with DS,[182] as well as in AD in the general population.[183] The integration of DS transcriptomic data enhances our understanding of how specific molecular mechanisms influence the proteomic landscape in DS-AD. Additionally, the discovered associations between altered protein profiles and disease progression suggest the potential development of biomarkers, reflective of the specific risk present in individuals with DS, for early detection and disease monitoring in DS-AD. The complex landscape of PTMs revealed in AD pathogenesis (supporting information), particularly the role of oxidative stress, offers unique opportunities for designing interventions that target specific modification pathways. Addressing oxidative stress in the context of DS-AD becomes particularly important, given its significant role in the pathogenesis of both DS and AD.

5.3 | Perspective and limitations of proteomics

The foundational knowledge of specific protein alterations linked to DS-AD pathogenesis opens avenues for developing targeted therapies. Specifically, using small molecule inhibitors to target proteins involved in actin filament or cell junction organization could help restore cellular and synaptic integrity. Additionally, antioxidant treatments that mitigate oxidative stress–induced protein modifications could unveil new therapeutic directions. However, insights from AD proteomics integrated with AD genetics also call for caution. The triggering receptor expressed on myeloid cells 2 (*TREM2*) is associated with the genetic risk for AD, with loss-of-function mutations impairing microglial functions essential for managing AD pathology.[184] Agonistic anti-*TREM2* antibodies, which bind directly to *TREM2* and activate its signaling pathways, enhance microglial function, improving clearance of amyloid plaques and cellular debris.[184] However, the immunotherapy approach targeting *TREM2* is controversial. *TREM2* overexpression is observed in AD brain proteomics, though it is likely a compensatory response to ongoing neurodegeneration. Increased *TREM2* levels contribute to chronic inflammation by stimulating the ongoing release of inflammatory cytokines.[185] Therefore, the therapeutic focus may need to prioritize restoring the impaired function of specific *TREM2* variants rather than simply enhancing *TREM2* activity.

While proteomic studies have yielded valuable insights into DS-AD pathogenesis, discrepancies in proteomic datasets often arise due to variability in methodologies, sample handling, and data analysis. Addressing these challenges requires the refinement of proteomic methodologies and the standardization of experimental protocols to improve reproducibility. Delving deeper into disease proteomics entails advanced strategies such as subcellular localization profiling using techniques like subcellular fractionation coupled with mass spectrometry to uncover the spatial distribution of proteins within cellular compartments.[186] Dynamic quantitative proteomics approaches enable the exploration of temporal changes in protein expression and turnover rates, offering insights into disease progression dynamics.[187] Single-cell proteomics technologies dissect cellular heterogeneity and identify rare cell populations driving disease progression,[188] while functional proteomics screens systematically interrogate protein function and drug–target interactions.[189] Furthermore, integrating proteomic data with other -omics approaches, such as transcriptomics and metabolomics, remains essential for achieving a more comprehensive understanding of molecular pathways and regulatory networks driving DS-AD.

6 | METABOLOMICS

6.1 | Metabolic alterations in AD and DS-AD

Metabolomics, a rapidly evolving field within systems biology, has become a potent tool for unraveling the complex molecular landscape associated with AD.[190,191] Lipidomic analyses of the AD brain reveal an enrichment of lysobisphosphatidic acid (LBPA), sphingomyelin, ganglioside GM3, and cholesterol esters, highlighting disruptions in neuronal membrane stability, signaling pathways, and lysosomal function.[192] Blood metabolomics in AD sheds light on intricate metabolic networks, particularly in lipid and amino acid metabolism, as well as pathways related to glucose and energy substrate use.[161,190] However, discrepancies exist among findings. Some studies have reported altered levels of glutamic acid, tyrosine, and other metabolites in cerebrospinal fluid (CSF) or plasma, while others found no significant changes. Similarly, sphingolipids and phosphatidylcholines often show differences

TABLE 5 Omics insights into the complex landscape of DS-AD.

Field	Interconnections	Key findings
Genetics	- Epigenomics - proteomics	- T21 drives AD • Common genetic variants at the *APP* and *ADAMTS1* loci on Chr21q21.3 are associated with increased susceptibility to AD. • Rare variants in *APP* are associated with familial dominant form of AD. • DS critical region: *DYRK1A* is associated with tau hyperphosphorylation; *DYRK1A* and *DNMT3L* contribute to epigenetic regulation. - Genetic studies have identified common variants at multiple loci associated with AD across various chromosomes. - Rare variants in *PSEN1* and *PSEN2* are associated with familial dominant forms of AD. - The AD PRS is correlated with the blood levels of proteins implicated in conditions such as female infertility, autoimmune thyroiditis, and fetal growth retardation.
Transcriptomics	- Genetics - Genetics	- Genome-wide transcriptomic changes impact oligodendrocyte development and myelination in developmental and adult DS brains. - Genes in developmental and adult DS brains exhibit DEX, similarly found in oligodendrocytes, microglia, and astrocytes of AD brains. - Familial AD genes *PSEN1* and *PSEN2* show reduced expression in adult DS brains, arguing against β- and γ-secretase inhibitors for DS-AD treatment. - AD-associated genes identified by GWASs demonstrate differential expression in developmental and adult DS brains, highlighting potential therapeutic targets.
Epigenomics	- Transcriptomics	- Significant alterations in DNA methylation in the fetal DS cortex indicate accelerated epigenetic aging. - Critical epigenetic genes located on Chr21, such as *DYRK1A* and *DNMT3L*, play roles in DS brain development and offer potential as therapeutic targets for gene-specific epigenetic editing. - Among the AD EWAS genes, 8 were identified with DEX in developmental and adult DS brain transcriptome.
Proteomics	- Transcriptomics - Transcriptomics - Transcriptomics	- Proteome studies identify 2697 proteins differentially expressed in AD brains, associated with various neuropathologies and *TREM2* overexpression implicating chronic inflammation. - 105 proteins upregulated in AD brains are also upregulated in developmental and adult DS brain transcriptome. - 101 proteins downregulated in AD brains are also downregulated in developmental and adult DS brain transcriptome.
Metabolomics		- Altered blood metabolic profiles, along with disrupted energy homeostasis and metabolites in human *post mortem* brain tissue and CSF, are prominent in AD. - Metabolic disorders, including mitochondrial defects, oxidative stress, and impaired glucose and lipid metabolism, are key contributors to the DS phenotype. - A dynamic metabolomic score could be instrumental in shaping metabolic interventions for DS, such as ketogenic diets or supplements targeting mitochondrial function, supporting brain health and potentially delaying AD progression.

Abbreviations: AD, Alzheimer's disease; CSF, cerebrospinal fluid; DEX, differential expression; DS, Down syndrome; EWAS, epigenome-wide association study; GWAS, genome-wide association study; T21, trisomy 21.

between AD and control groups in plasma, yet other lipid markers demonstrate mixed results depending on the biofluid analyzed, along with variations in cohort characteristics and analytical methods used.[161] Nonetheless, this emerging field offers valuable insights into potential disease mechanisms and identifies promising therapeutic targets/opportunities. Moreover, the application of metabolomics holds significant promise in bridging the gap between preclinical research conducted in animal models of AD and its translation to human studies. Analysis of human *post mortem* brain tissue and CSF has revealed significantly altered energy homeostasis and metabolites in AD[193,194] (supporting information). Conversely, metabolic disorders, including mitochondrial defects, increased oxidative stress levels, and impaired glucose and lipid metabolism, ultimately result in reduced energy production and cellular dysfunctions, contributing significantly to the DS phenotype, including DS-AD.[195,196]

6.2 | Metabolomics insights into DS-AD

Metabolomics provides valuable insights into the metabolic disruptions underlying DS-AD, where T21 drives widespread alterations in lipid metabolism, amino acid pathways, and mitochondrial function.[195,196] These metabolic abnormalities are fundamental to both the DS phenotype and the increased risk of EOAD. Significant plasma metabolic disruptions in DS involve one-carbon metabolism and the tricarboxylic acid (TCA) cycle, both critical for energy production and neurotransmitter synthesis.[197] Plasma and urinary metabolomic profiles also consistently reflect mitochondrial dysfunction, linking DS to altered cellular energy homeostasis.[198] Dysregulation of metabolic pathways also involves amino acids like leucine, phenylalanine, tyrosine, and alanine.[199] Leucine contributes to neurotransmitter pathways via the glutamine/glycine system,

while phenylalanine impacts dopamine synthesis, directly influencing cognitive processes.

At the core of these metabolic disruptions are molecular mechanisms driven by T21, which exacerbate oxidative stress and mitochondrial dysfunction. Overexpression of Chr21-encoded genes such as superoxide dismutase 1 (*SOD1*) and regulator of calcineurin 1 (*RCAN1*) leads to elevated reactive oxygen species (ROS) and lipid peroxidation, impairing mitochondrial integrity and functionality and exacerbating energy deficits.[200] These widespread physiological alterations in DS disrupt neuronal membranes, impair synaptic function, and contribute to Aβ aggregation. Dierssen et al. characterize DS as a metabolic disorder, emphasizing the central role of dysregulated insulin signaling in linking systemic metabolic alterations.[195]

6.3 | Perspective and limitations of metabolomics

Some metabolomics studies have developed promising diagnostic models for the early detection and intervention of AD using panels of blood-based metabolomic biomarkers.[161] Further study will help to confirm and reveal more specific changes of metabolism in DS-AD, potentially leading to the development of a dynamic metabolomic score for assessing DS-AD risk. Such a score could prove instrumental in clinical management by providing insights into the unique metabolic profile of individuals with DS and guiding potential metabolic interventions. Nutritional therapies aimed at correcting metabolic dysregulations, such as ketogenic diets or supplements targeting mitochondrial function, could offer new ways to support brain health and delay AD progression in DS. Furthermore, the identification of specific metabolic markers can lead to the development of metabolic therapies tailored to the unique needs of individuals with DS, potentially improving their quality of life and cognitive function.

Despite its potential, variability in findings across metabolomics studies, driven by differences in cohort demographics, sample types, and analytical platforms, can hinder the reproducibility and generalizability of results. Moreover, the dynamic nature of the metabolome, influenced by factors such as age, diet, and comorbid conditions, complicates the interpretation of metabolic changes. Future research should prioritize longitudinal studies to capture temporal shifts in metabolism while using standardized protocols to reduce methodological variability. Integrating metabolomics with other -omics approaches, including transcriptomics and proteomics, will be essential for uncovering the complex interplay of metabolic and molecular networks in DS-AD.

7 | CONCLUDING REMARKS AND PERSPECTIVES

DS and AD share overlapping molecular and metabolic pathways, encompassing alterations at the genetic, epigenetic, protein, and metabolite levels. Emerging strategies, such as immunotherapy targeting core AD pathology,[201] can be enhanced by integrating -omics approaches for precision medicine. In parallel, therapeutic strategies targeting specific DS-associated genes and dysfunctions (e.g., *APP* overexpression due to T21, *DYRK1A* in the DSCR) could potentially delay or mitigate DS-AD progression. The complexity of DS-AD necessitates diverse research strategies and tailored interventions, with comprehensive insights from -omics studies (Table 5). For example, paradoxical alterations in *BACE1* and *PSEN1* expression within the DS brain challenge the anticipated correlation between increased enzyme activity and the progression of AD. Additionally, correlations between the AD PRS and proteins related to female infertility and autoimmune thyroiditis suggest potential links among reduced parity, autoimmune conditions, and heightened DS-AD risk, pointing to novel intervention opportunities. However, further investigation of the mechanisms is warranted, considering alternative hypotheses such as hormonal dysregulation or immune system dysfunction, as well as the complexity of DS comorbidities. Metabolomics in AD provides insights into intricate metabolic networks and metabolic disorders that significantly contribute to the DS phenotype. In essence, these collective omics-driven revelations furnish a comprehensive understanding, laying the foundation for targeted therapeutic interventions and precision clinical management in the challenging realm of neurodegenerative disorders.

Developing innovative diagnostic tools and therapeutic strategies is essential to address the unique progression patterns of DS-AD. In particular, cerebrovascular changes provide important insights into disease progression. In DS adults, cortical microinfarcts are common with age and in those more affected by AD.[202] Meanwhile, cerebral microbleeds have been detected in a subset of DS children as early as age 5 years, and these individuals may be at the highest risk of developing DS-AD earlier and progress more rapidly.[203] Meanwhile, the advent of emerging technologies such as artificial intelligence (AI)–driven analysis for the comprehensive integration of multi-omics data,[204] advanced imaging techniques for the early detection of AD pathology, and CRISPR-Cas9 for precise gene editing, heralds a new era in understanding and managing DS-AD. These advancements promise to catalyze breakthroughs in early diagnosis, targeted therapy development, and personalized treatment approaches.

To effectively identify specific therapeutic targets for DS-AD, the strategies involve gathering comprehensive multi-omics data from large and diverse patient cohorts, integrating clinical data such as patient history and treatment response, and using improved AI models to manage data imbalance and noise while enhancing interpretability through algorithm development and feature importance scoring. Guidelines should be formulated by emphasizing cross-disciplinary collaboration among geneticists, bioinformaticians, and clinicians, establishing standardized protocols for generating and sharing multi-omics data, and prioritizing experimental validation using in vitro and in vivo models. In vitro models, such as cell cultures and organoids derived from patient-derived iPSCs,[205] offer a controlled environment for studying molecular pathways and screening potential therapeutic interventions. These models can recapitulate key aspects of DS-AD pathology, allowing for the investigation of molecular mechanisms and the evaluation of candidate targets in a controlled setting. In vivo models, including transgenic mouse models and non-human primates, provide valuable insights into disease pathogenesis and therapeutic

efficacy in a more complex biological context.[206,207] These models enable researchers to study disease progression, test the effects of candidate interventions, and assess safety and efficacy parameters. Building on these models, we may leverage single-cell multi-omics technologies and spatial profiling techniques, such as using scRNAseq to capture dynamic molecular changes across disease progression stages and tissue microenvironments, using long-read sequencing for higher resolution gene isoform analysis,[208] and integrating multi-omics data for patient stratification and precision medicine applications.

In our quest for scientific advancement, it is paramount to center our efforts on improving the quality of life for individuals with DS. While obtaining a comprehensive understanding of DS-AD may take time, a focused exploration of specific mechanisms may offer a targeted approach that could yield therapeutic effects. In this regard, the utility of specific omics-derived biomarkers that would identify DS patients who are at greatest risk of developing early and more severe dementia would be of high value. It is highly likely that children who present with microbleeds on magnetic resonance imaging in early childhood are the primary risk group for this who may then warrant preventive therapy with safe and effective drugs that block the aggregation and precipitation of amyloid and tau proteins in the brain.[209] Looking ahead, longitudinal studies will play a critical role in tracking AD progression in DS individuals from an early age into adulthood, helping to identify pivotal intervention points that could significantly influence disease trajectory and improve patient outcomes.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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