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Obesity impacts the expression of Alzheimer's disease–related genes: The Framingham Heart Study

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Abstract

Introduction: We investigated associations of obesity with the expression of Alzheimer's disease (AD)–related genes in a large community-based cohort.

Methods: The sample consisted of 5619 participants from the Framingham Heart Study. Obesity metrics included body mass index (BMI) and waist-to-hip ratio (WHR). Gene expression was measured for a set of 74 AD-related genes, derived by integrating genome-wide association study results with functional genomics data.

Results: Obesity metrics were associated with the expression of 21 AD-related genes. The strongest associations were observed with *CLU, CD2AP, KLC3,* and *FCER1G.* Unique associations were noted with *TSPAN14, SLC24A4* for BMI, and *ZSCAN21, BCKDK* for WHR. After adjustment for cardiovascular risk factors, 13 associations remained significant for BMI and 8 for WHR. Dichotomous obesity metrics exhibited unique associations with *EPHX2* for BMI, and with *TSPAN14* for WHR.

Discussion: Obesity was associated with AD-related gene expression; these findings shed light on the molecular pathways linking obesity to AD.

KEYWORDS
Alzheimer's disease, dementia, functional genomics, gene expression, genome-wide association study, obesity

1 | INTRODUCTION

The number of people living with dementia is estimated to reach 131 million by 2050. This projection has momentous social and financial implications for patients, caregivers, and health-care systems.[1] Alzheimer's disease (AD) is the most common type of dementia.[2] Ongoing research efforts have expanded our comprehension of AD pathogenesis beyond the fundamental amyloid cascade and tau hyperphosphorylation hypotheses,[3] to include biological pathways associated with immunity,[4] inflammation,[5] and lipid metabolism.[6]

Similarly, obesity is a growing public health concern. In the United States, up to 85% of adults are projected to be overweight or obese by 2030.[7] Obesity has been linked to late-onset AD.[8,9] It has been hypothesized that obesity creates an environment of chronic low-grade systemic inflammation and increased oxidative stress that can contribute to neurodegeneration.[10] Furthermore, neurobiological changes related to vascular pathology often coexist with AD pathology and possibly have an additive or synergistic effect on cognitive decline.[11] Therefore, because obesity is a known risk factor for type 2 diabetes, heart disease, and stroke,[7] it might contribute to dementia risk by increasing the burden of vascular pathology in the brain. Nevertheless, the exact biological substrate of the relationship between obesity and AD has yet to be clarified.

One potential pathway linking obesity to AD may involve molecular changes in gene expression.[12] Recent investigations have identified several genetic variants associated with AD through genome-wide association studies (GWAS).[13] However, whether obesity is associated with differential expression of AD-related genes during the life course remains unknown.

The present study aimed to shed light on the molecular links between obesity and AD by investigating associations of obesity metrics with expression profiles of AD-related genes in a large community-based cohort. Moreover, considering prior evidence that obesity during mid-life is a stronger predictor of dementia,[8] and that the underlying biology linking obesity to dementia might be affected by sex-related differences,[14] potential effect modification by age and sex was explored.

2 | METHODS

2.1 | Participants

The Framingham Heart Study (FHS) is a community-based, longitudinal cohort study initiated in 1948 to investigate the risk factors associated with cardiovascular disease. Since its inception, the study has followed three generations of participants who are invited for follow-up examinations every 2 to 6 years.[15–17] The present cross-sectional analysis included 5619 non-Hispanic White participants with whole-blood gene expression profiling from the Offspring[16] and Third Generation[15] cohorts of the FHS,[17] who attended the eighth (2005 to 2008) and second examination cycles (2008 to 2011), respectively, and had available data on obesity metrics. All participants have pro-

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RESEARCH Of CONTEXT

1. **Systematic review**: The literature was reviewed using traditional (e.g., PubMed) sources. Current epidemiological evidence suggests that obesity, especially during mid-life, increases the risk of developing Alzheimer's disease (AD) later in life; however, the biological substrate of this relationship remains unclear.

2. **Interpretation**: Our findings suggest that obesity might be contributing to AD risk through modulating effects in the expression of genes regulating the underlying neurodegenerative process. There might also be differential susceptibility to these effects based on age and sex.

3. **Future directions**: The present work proposes a molecular link between obesity and AD, laying the groundwork for future research to confirm these findings and expand upon them, broadening our understanding of the underlying biology. Furthermore, identifying population groups that might be more prone to the detrimental effects of obesity might be important for prioritizing lifestyle interventions (e.g., weight loss) in the design of future healthy aging and dementia prevention strategies.

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vided written informed consent. Study protocols and consent forms have been approved by the institutional review board of the Boston University Medical Center.

2.2 | Selection of AD-related genes and expression profiling

To functionally characterize and prioritize individual AD-related genetic risk loci, we performed transcriptome-wide association studies (TWAS) using TWAS-FUSION.[18] The descriptive statistics from the AD single nucleotide polymorphism (SNP) main effects of the most recent large-scale meta-analyses of AD GWAS,[13] and precomputed SNP expression weights from 22 gene expression reference panels from blood tissue (Netherlands Twin Register [NTR]; Young Finns Study [YFS]; Genotype-Tissue Expression [GTEx]) were used (Suppl. Methods 1 in supporting information). To rule out that potential associations might reflect the random overlap between expression quantitative trait loci (eQTLs) and non-causal AD risk variants, colocalization analysis (COLOC) was performed at each significant locus,[19] to estimate the posterior probability of a shared causal variant (P ≥ 0.75) between gene expression and trait association, using a prior probability of 1.1×10^{-5} for the AD association. Functional validation of the eGenes was performed by testing for positional overlap of the best eQTLs from TWAS with enhancer (H3K4me1) and/or promoter (H3K4me3) regions across a broad category of relevant tissue types (blood, brain, heart) using HaploReg v4.1 software.[20]

Transcription-wide significant eGene–eQTL pairs with either high colocalization probability (≥0.75), or evidence of regulatory epigenome overlap in at least one tissue, were retained for studying their association with obesity metrics. Moreover, accounting for the pairwise correlation between gene expression features, we conducted the multiple degree-of-freedom omnibus analysis to test for shared effects of eGenes across the gene reference panels; a significance threshold of $P = 5.3*10^{-6}$ was set to identify eGenes functional across multiple reference panels, after Bonferroni correction for the total number of genes ($N = 9435$) tested. Then, the final set of AD-related genes was derived by combining eGenes satisfying TWAS selection criteria with eGenes reaching the significance threshold in omnibus analysis. Finally, the *APP*, *PSEN1*, *PSEN2*, and apolipoprotein E (*APOE*) genes (that were either not included in the GWAS summary statistics [*APOE*, *PSEN2*],[13] or not identified as high priority loci [*APP*, *PSEN1*]), were added in the final set, considering their association with AD risk.[21,22] The methodology followed for gene expression profiling has been described elsewhere.[23]

2.3 | Obesity metrics

The body mass index (BMI) was used as a measure of generalized obesity, and the waist-to-hip ratio (WHR) as a measure of abdominal obesity. Although BMI is the most commonly used indicator of obesity, we also included WHR, based on prior evidence suggesting it might be a better predictor of health-related outcomes, including the risk for cardiovascular disease, stroke, diabetes, and overall mortality.[24]

BMI was calculated by dividing the participant's weight by height squared (kg/m^2); generalized obesity was defined as BMI $\geq 30.0 \ kg/m^2$.[25] WHR was calculated by dividing the participant's waist circumference (cm) by hip circumference (cm); abdominal obesity was defined as a WHR > 0.90 for males and > 0.85 for females, based on World Health Organization (WHO) guidelines.[25]

2.4 | Cardiovascular risk factors

Blood pressure was calculated as the average of two measurements obtained 10 minutes apart with the participant resting in a sitting position. Laboratory values such as fasting blood glucose, total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, and triglycerides were measured using standard assays.

Cardiovascular risk factors (systolic blood pressure [SBP], smoking, diabetes mellitus, and history of cardiovascular disease [CVD]) were defined in accordance with the Framingham Stroke Risk Profile (Suppl. Methods 2 in supporting information).[26]

2.5 | Lifestyle factors

Adherence to potentially neuroprotective dietary habits was assessed by calculating the 2015 Dietary Guidelines Adherence Index (DGAI) score,[27] which reflects adherence to the dietary guidelines for Americans.[28] Physical activity was self-reported using the physical activity index.[29]

2.6 | Statistical analysis

Associations between different obesity metrics and AD-related gene expression were explored using linear mixed models with gene expression as the outcome. For each gene, two sets of models were constructed; one with BMI and the other with WHR as the main predictors. BMI and WHR were treated both as continuous as well as dichotomous measures (using the WHO-defined cutoffs for generalized obesity and abdominal obesity, respectively) to investigate for the potential presence of threshold effects. Familial relatedness was included as a random variance–covariance factor in the models. Confounder adjustment was conducted as follows: Model 1 was adjusted for age, sex, and differential cell counts; Model 2 was further adjusted for smoking, SBP, use of antihypertensive medications, diabetes, TC/HDL, and CVD.

Potential effect modification by age and sex was assessed by stratifying the participants into different groups based on age (< 55 years of age, ≥55 years of age) and sex (males, females), and repeating the aforementioned analyses in the respective subgroups.

Exploratory analyses were performed by further adjusting Model 2 for dietary habits and physical activity (Model 3), to account for potential confounding effects of lifestyle factors on the observed relationships.

The significance cutoff was set at 3.4×10^{-4} after Bonferroni correction for multiple hypotheses testing, accounting both for outcome multiplicity (the number of the different genes tested = 74) as well as the number of different obesity metrics studied (BMI, WHR).

Statistical analyses were performed using SAS Software, version 9.4 (SAS Institute). Graphics were created using R (R Core Team, 2022). Statistical tests were two-tailed.

3 | RESULTS

3.1 | Participant characteristics

Clinical and demographic characteristics of study participants, overall, as well as across different age and sex subgroups, are presented in Table 1. Mean age was 55 years and 54% of study participants were women. Thirty-one percent of participants met the definition of obesity based on BMI, and 78% had excess WHR. The cohort had a relatively low prevalence of cardiovascular risk factors and CVD (< 10%), whereas 33% of study participants reported use of antihypertensive medications. The vast majority of participants were cognitively healthy; only a small proportion had prevalent dementia ($n = 22, 0.4\%$).

3.2 | TWAS, COLOC, functional validation, and omnibus analysis

TWAS-FUSION identified 47 eGene–eQTL pairs with evidence for significant transcriptome-wide association with AD (Figure 1, Table S1

TABLE 1 Clinical and demographic characteristics of study participants

	Total sample (N = 5619)	<55 years (n = 2779)	≥55 years (n = 2840)	Women (n = 3041)	Men (n = 2578)
Age (years)	55 ± 13	44 ± 7	66 ± 8	55 ± 14	55 ± 13
Women	3041 (54)	1502 (54)	1539 (54)	3041 (100)	0 (0)
BMI (Kg/m2)	28.19 ± 5.68	27.88 ± 5.90	28.50 ± 5.44	27.46 ± 6.18	29.06 ± 4.89
Obese (BMI ≥ 30 Kg/m2)	1751 (31)	818 (29)	933 (33)	839 (28)	912 (35)
WHR	0.94 ± 0.09	0.91 ± 0.08	0.96 ± 0.09	0.90 ± 0.09	0.98 ± 0.07
Increased WHR	4359 (78)	1947 (70)	2412 (85)	2079 (68)	2280 (88)
Current smoker (n%)	530 (9)	306 (11)	224 (8)	279 (9)	251 (10)
Systolic blood pressure	122 ± 17	115 ± 14	128 ± 17	119 ± 18	124 ± 15
Anti-hypertensive medication	1859 (33)	357 (13)	1502 (53)	902 (30)	957 (37)
Diabetes	501 (9)	102 (4)	399 (14)	210 (7)	291 (11)
TC/HDL	3.43 ± 1.12	3.41 ± 1.16	3.44 ± 1.09	3.10 ± 1.00	3.81 ± 1.14
CVD	431 (8)	16 (0.6)	415 (15)	170 (6)	261 (10)

Note: Values are presented as means ± SD or counts (relative frequencies) for continuous and categorical variables, respectively.
Abbreviations: BMI, body mass index; CVD, cardiovascular disease; HDL, high-density lipoprotein; SD, standard deviation; TC, total cholesterol; WHR, waist-to-hip ratio.
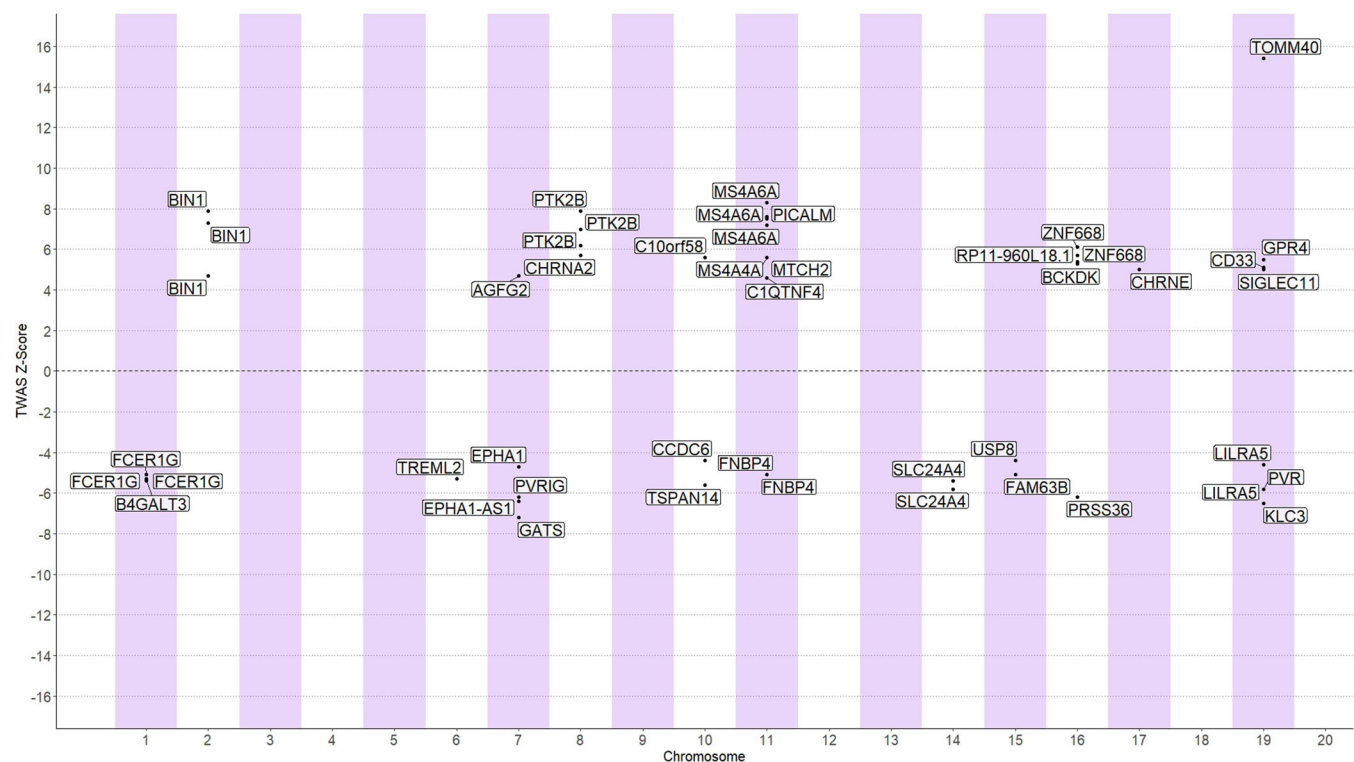

FIGURE 1 Summary of transcriptome-wide association study (TWAS) results. Each data point represents a gene grouped by chromosome (x-axis) and its respective z-score (y-axis), illustrating the direction of effect for expression of transcripts encoding Alzheimer's disease–related genes. Only significant transcriptome-wide associations are presented

in supporting information). Colocalization was observed for 25 TWAS-significant eQTLs (53%). In functional validation, positional overlap with enhancer and/or promoter elements was noted for the majority (83%) of TWAS signals, including eQTLs with weaker colocalization probability (PP4 < 75%). A total of 32 genes satisfied TWAS selection criteria. An additional 57 AD-related genes were identified by the omnibus analysis (Table S2 in supporting information). Overall, AD was associated with upregulated or downregulated expression of 89 genes (Table S3 in supporting information). After inclusion of the *APP*, *PSEN1*, *PSEN2*, and *APOE* genes, the final set consisted of 93 genes; expression profiles for 74 of those genes were available for analysis in the FHS.
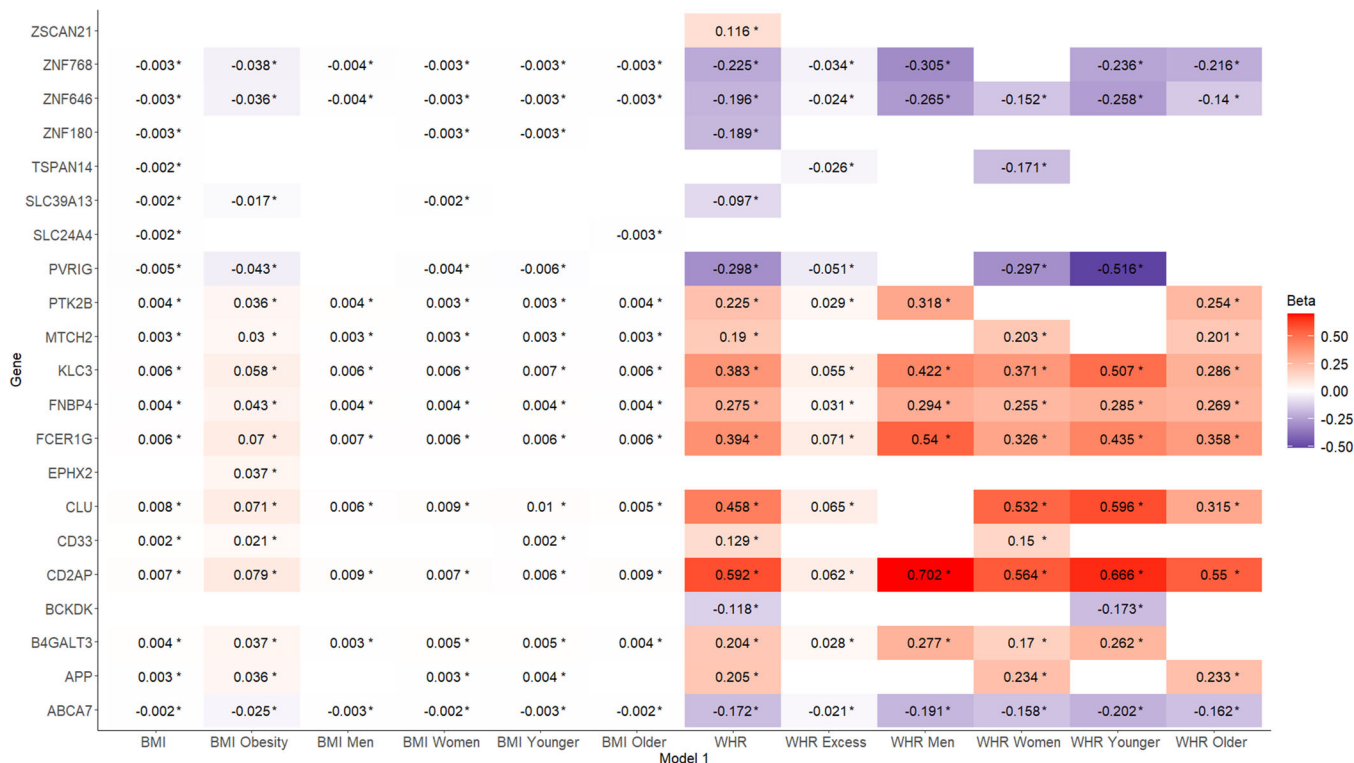
FIGURE 2 Associations of different obesity metrics (x-axis) with Alzheimer's disease–related gene expression (y-axis). Results from linear mixed models with gene expression as the outcome and the different obesity metrics as the main predictors. Models are adjusted for age, sex, and differential cell counts. BMI, body mass index; WHR, waist-to-hip ratio

3.3 | Obesity metrics and expression profiles of AD-related genes

Overall, obesity metrics were associated with the expression profiles of 21 AD-related genes (Figure 2; Tables S4, S7 in supporting information). After adjustment for cardiovascular risk factors, 14 of those associations remained significant (Figure 3; Tables S5, S8 in supporting information).

Linear mixed models with BMI and WHR as continuous measures revealed significant associations with the expression of 20 AD-related genes (Figure S1 in supporting information). Associations with *TSPAN14* and *SLC24A4* were unique to the BMI set, and associations with *ZSCAN21* and *BCKDK* were unique to the WHR set. Increased BMI and WHR were associated with increased expression of 10 and 11 genes, respectively, and with decreased expression of 8 and 7 genes, respectively. The strongest associations were observed with gene expression of *CLU, CD2AP, FCER1G,* and *KLC3.* No differences in the directionality of relationships between the BMI and WHR sets were observed.

Results from models with BMI and WHR as dichotomous measures (indicating generalized and abdominal obesity, respectively), are presented in Figure S2 in supporting information. Compared to models treating obesity as a continuous measure (Figure S3 in supporting information), one new association was identified for the BMI set (*EPHX2*), and one for the WHR set (*TSPAN14*). In contrast, three associations for the BMI set and seven for the WHR set that were significant

in the continuous models, became non-significant. Compared to models treating obesity as a continuous measure, the directionality of the overlapping relationships remained unchanged and the corresponding effect sizes were increased for the BMI set and decreased for the WHR set. Similar to the continuous models, the strongest associations were observed with gene expression of *CLU, CD2AP, FCER1G,* and *KLC3.*

After further adjustment for cardiovascular risk factors, 13 and 8 associations remained significant for the BMI and WHR sets, respectively (Figure S1). The directionality of the surviving relationships remained unchanged, with relatively small reductions in the corresponding effect estimates. Similar results were observed when obesity metrics were treated as dichotomous measures (Figure S2).

3.4 | Effect modification by sex

Stratified analyses by sex revealed that 4 out of the 18 identified associations between BMI and AD-related gene expression were only present in women (Figure S4A in supporting information). Furthermore, 3 associations, albeit present in the total sample, were not significant in the individual sex subgroups. In the models further adjusted for cardiovascular risk factors, 5 out of 13 associations were only present in women, and 4 associations were only present in the total sample but not in the individual sex subgroups; only 4 associations remained significant in men (Figure S4B). For the WHR set, 5 out of the 18 associations were only present in women, 2 were only present in men, 1 (*TSPAN 14*) was only present in women but not in the total
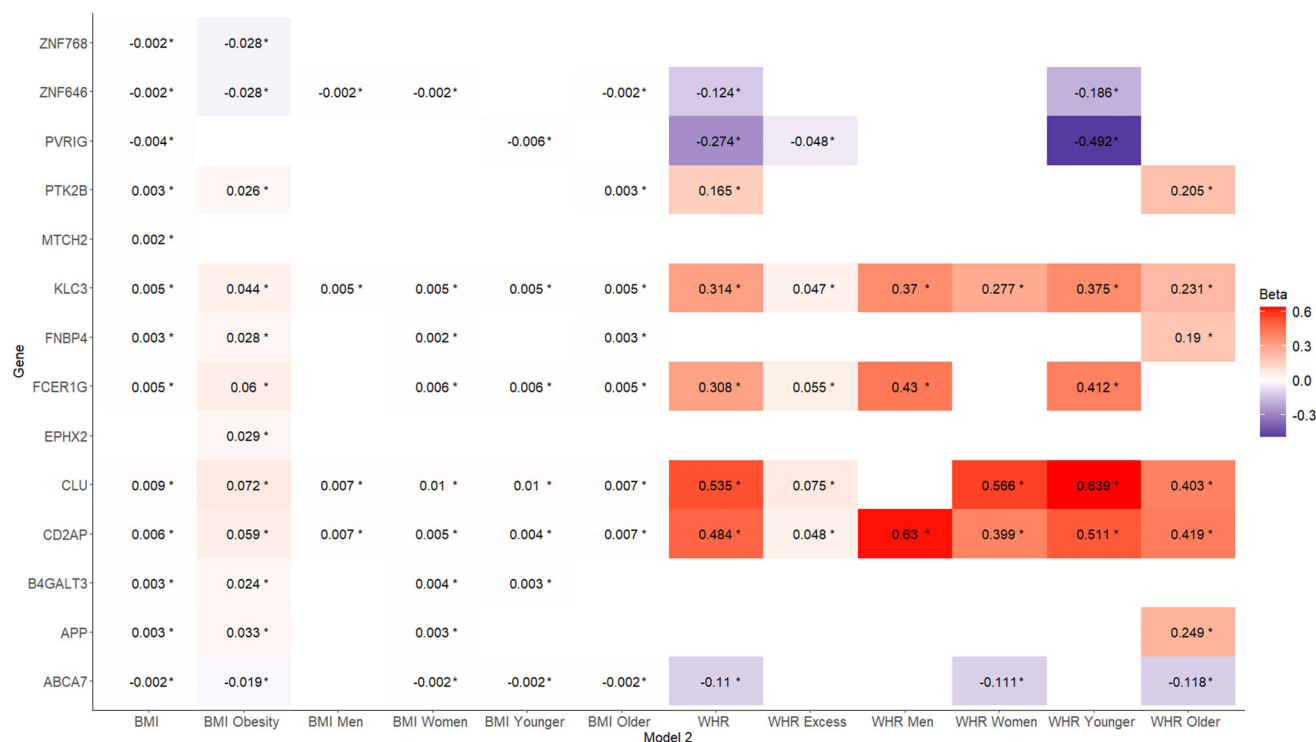
Gene	BMI	BMI Obesity	BMI Men	BMI Women	BMI Younger	BMI Older	WHR	WHR Excess	WHR Men	WHR Women	WHR Younger	WHR Older
ZNF768	-0.002*	-0.028*										
ZNF646	-0.002*	-0.028*	-0.002*	-0.002*		-0.002*	-0.124*				-0.186*	
PVRIG	-0.004*			-0.006*			-0.274*	-0.048*			-0.492*	
PTK2B	0.003*	0.026*				0.003*	0.165*					0.205*
MTCH2	0.002*											
KLC3	0.005*	0.044*	0.005*	0.005*	0.005*	0.005*	0.314*	0.047*	0.37*	0.277*	0.375*	0.231*
FNBP4	0.003*	0.028*		0.002*		0.003*						0.19*
FCER1G	0.005*	0.06*		0.006*	0.006*	0.005*	0.308*	0.055*	0.43*		0.412*	
EPHX2		0.029*										
CLU	0.009*	0.072*	0.007*	0.01*	0.01*	0.007*	0.535*	0.075*		0.566*	0.639*	0.403*
CD2AP	0.006*	0.059*	0.007*	0.005*	0.004*	0.007*	0.484*	0.048*	0.63*	0.399*	0.511*	0.419*
B4GALT3	0.003*	0.024*		0.004*	0.003*							
APP	0.003*	0.033*		0.003*								0.249*
ABCA7	-0.002*	-0.019*		-0.002*	-0.002*	-0.002*	-0.11*			-0.111*		-0.118*

Model 2

Beta
0.6
0.3
0.0
-0.3

FIGURE 3 Associations of different obesity metrics (x-axis) with Alzheimer's disease–related gene expression (y-axis). Results from linear mixed models with gene expression as the outcome and the different obesity metrics as the main predictors. Models are adjusted for age, sex, differential cell counts, and cardiovascular risk factors. BMI, body mass index; WHR, waist-to-hip ratio

sample or men, and 4 were only present in the total sample but not in the individual sex subgroups (Figure S5A in supporting information). Results from models further adjusted for cardiovascular risk factors are presented in Figure S5B.

3.5 | Effect modification by age

In stratified analyses by age, 4 out of the 18 associations for the BMI set were present only in the younger subgroup, 1 was present only in the older subgroup, and 2 were present in the total sample but not in the individual age subgroups (Figure S6A in supporting information). Correspondingly, for the WHR set, 3 out of the 18 associations were present only in the younger subgroup, 3 only in the older subgroup, and 4 were only present in the total sample but not in the individual age subgroups (Figure S7A in supporting information). Further adjustment for cardiovascular risk factors yielded similar findings, except the associations pertaining to the *FNBP4* and *APP* genes in the WHR set, which albeit not present in the total sample, were significant in the older subgroup (Figure S6B, S7B). Smaller effect sizes were noted in the older subgroup for the majority of overlapping relationships.

3.6 | Exploratory analyses

Further adjustment for lifestyle factors led to almost identical findings (Figure S8; Tables S6, S9 in supporting information).

4 | DISCUSSION

In the present study, obesity was associated with the expression of 21 AD-related genes. These relationships were studied in a large sample of > 5500 community-dwelling adults with a broad age range. After adjustment for cardiovascular risk factors, 14 associations remained significant, indicating that obesity might be modulating the expression of AD-related genes both directly and indirectly through vascular pathways. Further adjustment for lifestyle factors did not change the results. The fact that some associations became non-significant when obesity measures were treated as dichotomous could indicate the linear nature of underlying relationships, leading to increased power for continuous models to capture potential effects.

4.1 | Obesity and upregulated expression of AD-related genes

Obesity was associated with upregulated expression of 10 genes, after accounting for cardiovascular risk factors. The genes *CLU*, *CD2AP*, *FCER1G*, and *KLC3* exhibited the most robust associations in terms of effect size as well as significance, and were present across all obesity metrics. The largest body of evidence regarding a potential role in AD pathogenesis exists for the *CLU* gene. *CLU* is the third most associated risk gene for late-onset AD, explaining ≈9% of the attributable risk for the disease.[30,31] The encoded protein (clusterin) has been implicated in various molecular pathways of AD neurobiology, including amyloid aggregation and clearance, lipid metabolism,

neuroinflammation, as well as neuronal cell cycle control and apoptosis.[32] Clusterin levels have been found to be elevated in the brain and cerebrospinal fluid of individuals with AD,[32] and plasma levels have been associated with brain atrophy, baseline disease severity, and rapid clinical progression in patients with AD.[33,34] Furthermore, *CLU* genotypes were related to amyloid beta (Aβ) deposition on amyloid positron emission tomography imaging and hippocampal volumes on structural brain magnetic resonance imaging in the Alzheimer's Disease Neuroimaging Initiative (ADNI).[35] Plasma clusterin levels have also been positively correlated with BMI and WHR.[36] In fact, on array analysis, *CLU* was one of the top 15 extracellular matrix-related genes overexpressed in human obesity,[37] further highlighting its potential role as a molecular intermediary in the relationship between obesity and AD.[38] Interestingly, the *CD2AP* gene is also among the top 10 genetic predisposition factors for AD.[31] Accruing evidence suggests that its encoding protein, a scaffolding molecule regulating cell–cell interactions, cytoskeletal remodeling, and membrane trafficking, might also be implicated in AD pathogenesis, although the exact mechanisms remain unknown.[39] Similarly, *FCER1G* is a microglial-specific gene with higher expression in the adipose tissue of obese than that of non-obese individuals;[40] evidence for its potential role in AD pathogenesis comes from GWAS, DNA sequencing, and expression network analyses, supporting the involvement of microglial pathways in the development and progression of AD.[41]

4.2 | Obesity and downregulated expression of AD-related genes

After adjustment for cardiovascular risk factors, obesity was associated with downregulated expression of four genes (*PVRIG*, *ZNF646*, *ZNF768*, *ABCA7*). Strong genetic evidence suggests that *ABCA7* protects from AD pathogenesis.[42] The gene encodes a protein expressed in neurons, astrocytes, and microglia, responsible for mediating the assembly of lipids and exchangeable apolipoproteins, such as apoE, into HDL particles that are released into the extracellular space (lipid efflux). *ABCA7* deletion in mouse models has been associated with significant increases in insoluble Aβ deposition in the brain, either by increased production, or by reduced clearance.[43] Remarkably, prior studies in European ancestry populations have shown that AD-associated variants at the *ABCA7* locus raise the risk of late-onset AD by approximately 20%, whereas *ABCA7* loss-of-function mutations raise the risk of early-onset AD by 100% to 400%. Moreover, a loss-of-function mutation present in African ancestry populations increases AD risk by ≈80%.[42]

4.3 | Obesity, AD-related gene expression, and the role of cardiovascular risk factors

Obesity contributes directly to incident cardiovascular risk factors and leads to the development of CVD.[44] In turn, cardiovascular risk factors have been associated with the risk of AD;[45] therefore, they might act as mediators in the relationships between obesity and AD-

related gene expression. Indeed, a number of those relationships were no longer significant after adjustment for cardiovascular risk factors. The fact that this phenomenon was more prominent for WHR, which is more closely related to cardiovascular disease risk than BMI[24] (56% of the observed associations were affected vs. 28% for BMI), further supports this hypothesis. In the brain microenvironment, vascular pathology can drastically affect gene expression by a protein called hypoxia-inducible factor-1 (HIF-1), which controls the expression of > 700 target genes;[46] through this pathway, it might drive gene expression profiles that promote amyloidogenesis and initiation/propagation of AD pathobiology, especially in regions particularly vulnerable to hypoxia such as the hippocampus.[47] For instance, HIF-1 induction increases the expression of *BACE1* by binding to its promoter region.[48] *BACE1* is the major protease for β-cleavage of *APP* and upregulation of this enzyme leads to increased generation of Aβ40 and Aβ42.[48]

4.4 | Effect modification by sex

Two thirds of AD cases affect women, and notable sex differences in disease risk, presentation, and progression have been observed.[49] Those differences are likely multifactorial in etiology but might in part be related to differential susceptibility to disease risk factors such as obesity. Results from the English Longitudinal Study of Ageing (ELSA) revealed that women with abdominal obesity had a 39% increased risk for incident dementia compared to those with non-abdominal obesity; however, no such association was observed in men.[50] This differential susceptibility might originate from the molecular level. The present results support this hypothesis, as subgroup analysis by sex revealed that many associations between obesity and AD-related gene expression were observed only in female participants.

4.5 | Effect modification by age

Stratification of study participants by age revealed that the corresponding effect sizes for the majority of the associations between obesity and AD-related gene expression were smaller for older participants. Prior studies and meta-analyses have consistently demonstrated that midlife rather than late-life obesity correlates with incident dementia.[8,9] The present results support, and also provide a putative biological explanation for, prior epidemiological data, by suggesting that the regulatory effects of obesity on the expression of AD-related genes might weaken with age. Nevertheless, the definitive presence, and the exact nature of, molecular variations that might lead to differential responses of gene expression to obesity based on age and/or sex, remain for future mechanistic studies to determine.

5 | LIMITATIONS

GWAS findings are often difficult to interpret, because the causal variants driving the associations can be concealed by linkage

disequilibrium, and the causal genes mediating variant effects on the trait of interest are rarely ascertainable from GWAS results alone.[51] To overcome this issue, we used a series of cutting-edge integrative bioinformatics methods that combine GWAS results with functional genomics knowledge (TWAS, COLOC, and functional validation using histone regulatory mark information from blood, brain, and heart tissues) to functionally characterize and prioritize the individual AD genomic risk loci studied. Moreover, the observed associations of obesity with AD-related gene expression identified genes that have been implicated in AD pathobiology by prior research, further increasing the biological plausibility of our findings. Nevertheless, the possibility that some of the observed relationships might have been with genes that are not part of AD-related pathways cannot be excluded. Additionally, the cross-sectional design of the present analysis does not allow for conclusions about causation or derivation of temporal relationships, potentially raising the issue of reverse causality. This is particularly pertinent for studies exploring associations between risk factors and AD, given the long latency period between the onset of neuropathological changes in the brain and AD-related clinical symptoms.[52] However, the majority of study participants were cognitively healthy, and most of the observed associations were in fact stronger in younger participants (who are less likely to have accumulated AD-related pathology), decreasing the possibility that reverse causality accounts for the present findings. Finally, the study sample consisted of non-Hispanic White individuals; therefore, further studies are needed to evaluate the generalizability of the present findings to other racial/ethnic groups.

6 | CONCLUSION

The present study identified associations of obesity with the expression of 21 AD-related genes in a large sample of individuals living in the community. These associations, among others, implicated a number of genes known to be part of multiple pathways involved in AD neurobiology (e.g., *CLU*, *CD2AP*, *FCER1G*, *ABCA7*, *APP*, *CD33*, *PTK2B*). Therefore, one of the molecular mechanisms through which obesity contributes to AD risk might be its modulating effects on the expression profiles of genes regulating the underlying neurodegenerative cascade. The present findings provide important insights into the mechanistic intermediates of the pathway leading from obesity to dementia. Furthermore, they could assist public health and dementia prevention policies to target lifestyle interventions, such as weight loss, to population groups that might have the most benefit.

CONFLICTS OF INTEREST
The authors report no disclosures relevant to the manuscript. Author disclosures are available in the supporting information.

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