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Integrative Genomic Analysis of m6a-SNPs Identifies Potential Functional Variants Associated with Alzheimer's Disease

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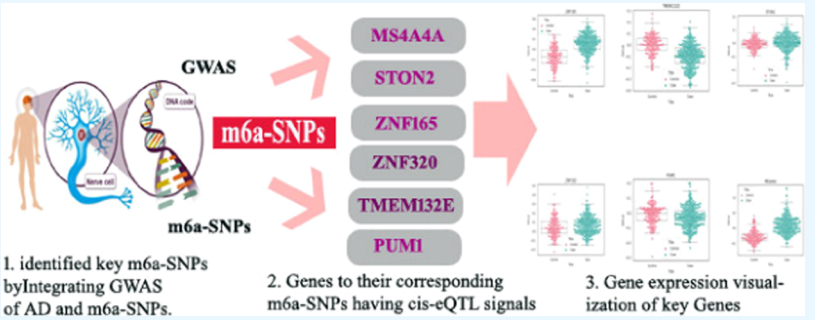

ABSTRACT: Alzheimer's disease (AD) is a neurodegenerative disorder that affects 35 million people worldwide. However, no potential therapeutics currently are available for AD because of the multiple factors involved in it, such as regulatory factors with their candidate genes, factors associated with the expression levels of its corresponding genes, and many others. To date, 29 novel loci from GWAS have been reported for AD by the Psychiatric Genomics Consortium (PGC2). Nevertheless, the main challenge of the post-GWAS era, namely to detect significant variants of the target disease, has not been conducted for AD. N6-methyladenosine (m6a) is reported as the most prevalent mRNA modification that exists in eukaryotes and that influences mRNA nuclear export, translation, splicing, and the stability of mRNA. Furthermore, studies have also reported m6a's association with neurogenesis and brain development. We carried out an integrative genomic analysis of AD variants from GWAS and m6a-SNPs from m6AVAR to identify the effects of m6a-SNPs on AD and identified the significant variants using the statistically significance value (p-value <0.05). The cis-regularity variants with their corresponding genes and their influence on gene expression in the gene expression profiles of AD patients were determined, and showed 1458 potential m6a-SNPs (based on p-value <0.05) associated with AD. eQTL analysis showed that 258 m6a-SNPs had cis-eQTL signals that overlapped with six significant differentially expressed genes based on p-value <0.05 in two datasets of AD gene expression profiles. A follow-up study to elucidate the impact of our identified m6a-SNPs in the experimental study would validate our findings for AD, which would contribute to the etiology of AD.

1. INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia.[1] It is characterized by a gradual decrease in cognitive functions, which usually begins with memory loss. Individuals with this condition typically rely on caregivers before death.[2] AD is the most frequent neurodegenerative disease, with around 35 million persons afflicted with this morbidity.[3] AD is highly heritable, with estimates ranging between 60 and 80%.[4]

As of now, there is no treatment available to prevent AD progression or slow it down due to the lack of AD basal processes. However, AD is said to be a multifactorial disorder that is associated with factors such as age, environment, and genetics. Moreover, there are presently no disease-modifying medications to reverse the pathological alterations causing this disorder. However, minimal exposure to common risk factors might prevent or slow down the onset of dementia in some people, while in some people, an early diagnosis of the disease or risk of the disease is still important because it gives time to the patients and their caregivers for readiness and planning their future accordingly; also, access to medication in early stages may help manage the symptoms. Additionally, health-care providers can identify patients at risk having mild changes in their routine and lifestyle that can halt or reduce the progression of disease by providing prompt diagnoses. The best course of action is early intervention because the patient's

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level of function is preserved for a longer period of time. Previously, the e4 allele of the apolipoprotein E (*APOE*) gene was found to have increased chances of developing AD in 1993. With the notable exception of some African populations, this link has since been found in practically all ethnic groups.[5,6] Our understanding of the genetics of AD was anticipated to advance quickly by the identification of *APOE* and the significance of the genetic component linked with AD, but other genetic risk factors contributing to AD were not identified until the late 2000s and later on due to the development of high-throughput genomic approaches. Specifically, this made the researchers capable of analyzing tens of thousands of people and millions of genetic variations through genome-wide association studies (GWASs); more and more GWAS studies have identified several risk loci associated with AD.

GWASs in genetics research are used to link specific genetic variations with diseases.

PGC2 recently published a large-scale GWAS that found 29 risk loci for AD.[7] One major problem with the GWAS is that many reported AD-associated variants are found in the intronic region of the genome, where they have no evident biological importance.[8,9] As GWAS-identified variants lie in noncoding regions, which are known as regulatory elements of the genome, the identification of functional variants and pathways in which their corresponding genes may be implicated was done by integrating multiple factors, for example, expression of quantitative trait loci, active enhancers, and/or linking variants, with their corresponding gene expression in various cell types and diseased tissues. A recent research has claimed that the functional variations in many genes cause GWAS signals to regulate the expression of their corresponding genes in myeloid cells (and thus potentially in microglia).[10]

N6-methyladenosine (m6A) is the most abundant modification of RNA; the M6a in mRNA is a reversible alteration that dynamically mediates its function.[11] m6a affects RNA in multiple ways such as degradation of mRNA, alteration in translation and splicing, and nuclear RNA export.[10−15] In addition to RNA degradation, m6A plays a role in translation and nuclear export. Methyltransferases (writers) activate m6A, while demethylases (erasers) deactivate it and m6A binding proteins recognize it (readers). Wilms tumor 1-associating protein (WTP1) and methyltransferase-like protein 3 are two of the most well-known writers today,[16] whereas erasers include *AlkB* homolog 5[17] and obesity-associated protein FTO,[18] and readers include *YTHDF1*,[19] *YTHDF2*,[20] *YTHDF3*,[21] *YTHDC1*,[22] *YTHDC2*,[23] *hnRNP* A2/B1,[24] *hnRNPC*,[25] and *hnRNPG*.[26]

Research shows that m6A is the most common RNA modification inside the brain. It has been associated with neurogenesis,[27] learning and memory,[28] brain development,[29] and axon regeneration.[30] m6A's rigorous regulation of brain activities is required for normal brain development, regulated by neuronal functions (e.g., *hnRNP A2/B1, METTL14, YTHDF* 1,2,3), which have been identified as key regulators of m6a pathways.[31] Additionally, multiple m6A players have been identified as mutant or dysregulated in epilepsy, intellectual impairment, depression, schizophrenia, and neurodevelopmental disorders.[32] Alzheimer's disease (AD) is a progressive neurodegenerative disease linked to changes in synapses, wherein m6A is considered to operate as a regulator. Maintaining appropriate mRNA levels through degradation and stabilization is also critical for maintaining healthy brain function in reaction to variations in neuronal function and activity. Interference with RNA metabolism, more specifically mRNA splicing, has been reported to be associated with age-related diseases: for example, FTD,[33] Parkinson's disease,[34] and Alzheimer's disease.[35] Indeed, epitranscriptomic regulation is another control of epigenetic as well as transcriptional control that might contribute as a third layer of regulation. Studies found the dynamic regulation of m6A affecting the brain of humans and mice during development and ageing, which demonstrate the role of m6A in Alzheimer's disease in more detail. Overall, RNA m6A methylation regulates the mRNA expression levels of transcripts that result in aging affected by the 3′ untranslated region (UTR), as well as the protein levels of AD-associated genes.[36] It is known that m6a methylation has effects on neurodevelopment and Alzheimer's disease; as studies have shown their impacts in early and later neurodevelopment specifically, genes with tissue-specific mRNA expression have been linked with m6a methylation. m6A is preferentially found in alternate UTR regions throughout aging and is correlated with a significant decrease in mRNA transcription. Additionally, the first genetic evidence that the absence of m6A writers and readers may influence the molecular etiology of Alzheimer's disease and may identify a novel function for m6A in controlling the protein levels of Alzheimer's disease-associated RNAs. Finally, m6A functions as a critical posttranscriptional modulator in the regulation of neurodevelopment, aging, and neurodegeneration.[36]

Given that m6A plays a key role in Alzheimer's disease,[36] and the effect of m6A -SNPs in Alzheimer's disease is unknown, identifying m6A-SNPs linked to Alzheimer's disease is critical and might give a new annotation for the pathogenic mechanism of Alzheimer's disease risk loci revealed by GWAS. Therefore, the goal of this work was to find m6A-SNPs linked to Alzheimer's disease using a public GWAS and the m6AVar database, as well as to illustrate their potential functions.

2. MATERIALS AND METHODS

2.1. GWAS Summary Statistics Data Retrieval. We retrieved the data from a large-scale publicly available GWAS on AD (https://ctg.cncr.nl/software/summary_statistics), which is composed of 12.24 million SNPs; the data are taken from 71,880 cases and 383,378 controls.[7]

2.2. Retrieving m6a-SNPs from m6aVAR. m6as are important in regulating the transcription process, but their exact mechanism is yet not explored. Evidence provides insights that single-nucleotide variants (SNVs) have a role in gene regulation, and that the m6a-associated SNVs would possibly be helpful to understand their noncoding influence on m6a. Previously, Zuo et al. developed a catalog for the m6a-SNPs' repository; we retrieved m6a-SNPs from the m6Avar database, which can be accessed at http://m6avar.renlab.org. This catalog is basically taken from three different sources: high, medium, and low confidence. Each category has been annotated with its confidence rank: the high-confidence category contains miCLIP/PA-m6-seq data; the medium-confidence m6a-SNPs have been taken from MeRIP-seq data; and the low-confidence m6a-SNPs have been taken from transcriptome-wide prediction.[37] The m6Avar database is also helpful in providing functional annotation information of the corresponding SNVs, which ultimately are useful for examining the role of m6a-SNPs in transcriptional regulation.

2.3. Integrating AD-GWAS and m6a Variants. We integrated both GWAS and m6a-SNP data utilizing the

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ACS Omega 2023, 8, 13332−13341

m6AVAR database to identify the common variants associated with AD. We set the threshold of *P*-value <0.05 to be considered statistically significant. The significant variants based on *P*-value were further used for functional and regulatory effects on the corresponding genes.

2.4. eQTL Analysis. Although a GWAS provides enough information about diseases, it gives less information to help in specific identification of the causal variant. This makes it difficult to predict the important variants that have an association with the expression of genes that are either located near the gene or at the distal end. In this regard, the expression quantitative trait loci (eQTL), on the other hand, pinpoint the causal variants in the pool of noncoding variants.[38,39] Furthermore, epigenomics particularly facilitates the noncoding influence on genes. HaploReg (http://compbio.mit.edu/HaploReg) is one of the tools that is used to explore the role of the noncoding genome. This tool links the noncoding genomes to their corresponding genes by utilizing the information of the 1000 Genomes project.[40] SNVs located within 1 mega base pair area are called cis-acting eQTLs, and those that are located distant from it are called trans-eQTLs. Cis-acting eQTL analysis is important in that it maximizes the probability of such variants with their corresponding genes, while the trans-eQTL gives information on the global influence of their corresponding genes.[40]

The HaploR we used for this analysis is an R-package and was used to import annotations like the eQTL signal and their corresponding genes. This package is available for common use at https://github.com/izhbannikov/haploR.

2.5. Functional Annotations with RegulomeDB. The functional annotation enhances the probability to provide information on the effects of individual m6a-SNP on posttranscriptional regulation. For this purpose, we used the RegulomeDB database (http://www.regulomedb.org/). This database screens a large pool of variants that gives scores to potential elements.[41] Based on the scoring, we can then easily predict the binding, gene expression, and even the possible magnitudes of binding. More details about this are given in Table 1.

Table 1. Ranking for Functional Annotation

category	description
	Likely to Affect Binding and Related to Gene Expression
1a	eQTL + TF binding + matched TF motif + matched DNase footprint + DNase peak
1b	eQTL + TF binding + any motif + DNase footprint + DNase peak
1c	eQTL + TF binding + matched TF motif + DNase peak
1d	eQTL + TF binding + any motif + DNase peak
1e	eQTL + TF binding + matched TF motif
1f	eQTL + TF binding/DNase peak
	Expected to Affect the Binding
2a	TF binding + matched TF motif + matched DNase footprint + DNase peak
2b	TF binding + any motif + DNase footprint + DNase peak
2c	TF binding + matched TF motif + DNase peak
	Less Likely to Affect the Binding
3a	TF binding + any motif + DNase peak
3b	TF binding + matched TF motif
	Minimal Binding Evidence
4	TF binding + DNase peak
5	TF binding or DNase peak
6	motif hit

2.6. Gene Ontology (GO) Analysis via Enrichr. To know and characterize the list of m6a genes in terms of biological functions, we performed enrichment analysis via Enrichr.[42−44] Enrichr is a powerful tool that provides the information on a particular gene such as which molecule is associated with the gene, what biological function it performs, and where it is in the cell. Further, the analysis is ranked based on the overall score from the *p*-value and *z*-score, and the results can be visualized in multiple formats.

2.7. Protein−Protein Interaction (PPI) Analysis Using STRING. Normally proteins interact with one or multiple partner proteins and perform physiological functions; therefore, understanding protein−protein interactions is important. The normal functioning can be influenced by a partner protein. A partner protein may also lead to abnormal functioning if there is a disruption caused by an alteration in protein expression level or three-dimensional (3D) structure or any kind of other changes. We therefore used the STRING database accessible at https://string-db.org/ to identify the interactions of query proteins with other partner proteins.[45]

2.8. Differential Gene Expression Analysis. Differential gene expression analysis is a useful technique because the product of a gene is a protein, and the expression level of a protein is crucial to its normal biological activity.[46,47] To know the expression level of our list of genes, for which cis-eQTL signals were shown, we aligned our m6a-SNPs identified genes with the gene expression profiles of microarray and miRNA datasets of AD cases and controls. We downloaded a microarray dataset from Gene expression omnibus (GEO), an online data repository of gene expression data of so many diseases, and most of them could be accessed. We accessed the database and downloaded the AD dataset (https://www.ncbi.nlm.nih.gov/geo/); the accession number GSE33000 contained 310 AD cases and 110 controls with matching genotype and clinical data from postmortem prefrontal brain tissues. Besides that, we also used an mRNA-seq dataset of 143 samples from two BAs analyzed in the mRNA-Seq experiment.

The GEO database also has an in-built online tool GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) that can be used for differential gene expression analysis within the database. Further, we downloaded the desired list of genes having *P*-value <0.05 to overlap with the genes (cis-eQTL signals).

The expression data of the overlapped genes were visualized with Python seaborn and matplotlib packages.

3. RESULTS

3.1. Identification of m6a-SNPs Associated with AD. The finding of common variants in both datasets from different cohorts would reveal mechanistic insights into the ways in which these SNPs from the GWAS cohort study impact specific pathways resulting in a pathology. Here, we identified a total of 22,148 unique m6a-SNPs after integrating the AD-GWAS dataset, which contained 12.24 million SNPs and 1.2 million m6a-SNPs from the m6aVar database. Further, we detected 1458 m6a-SNPs by setting the *p*-value <0.05 genome-wide suggestive threshold, which were considered significant variants associated with AD.

3.2. eQTL Analysis. We investigated to find the nearest genes to their corresponding m6a-SNPs that were associated with AD by using the HaploR package that patched annotations from HaploReg Browser. In total, we found 258 potential m6a-SNPs (*p*-value <0.05) that were having cis-eQTL signals.

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Table 2. Top Significant m6a-SNPs with Their Corresponding Differentially Expressed Genes

m6a-SNP_id	gene name	gene title	GENCODE_distance	differentially expressed genes	cis-eQTL signal	regulomeDB rank
rs1026256	*MS4A4A*	membrane-spanning 4-domains, subfamily A, member 4	18083	yes	yes	6
rs12895215	*STON2*	stonin 2	1102	yes	yes	5
rs73396550	*ZNF165*	zinc finger protein 165	6775	yes	yes	6
rs8100166	*ZNF320*	zinc finger protein 320	2892	yes	yes	3a
rs4341789	*TMEM132E*	transmembrane protein 132E	9966	yes	yes	5
rs6425690	*PUM1*	pumilio homolog 1 (Drosophila)	6010	yes	yes	7

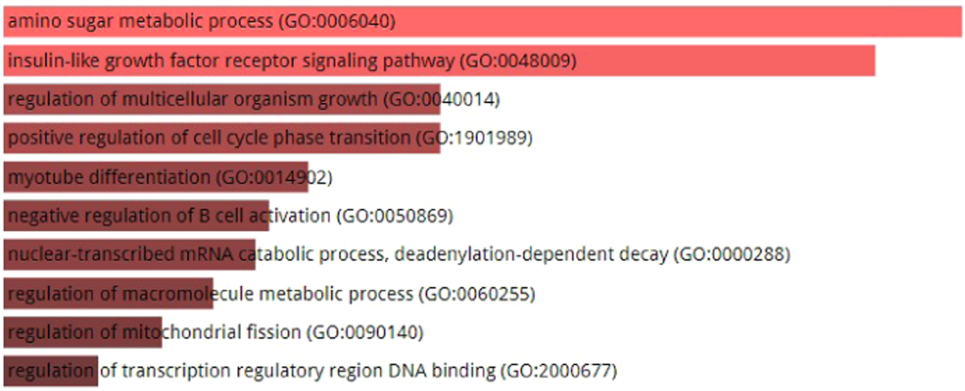

Figure 1. Biological functions of m6a genes with GO terms.

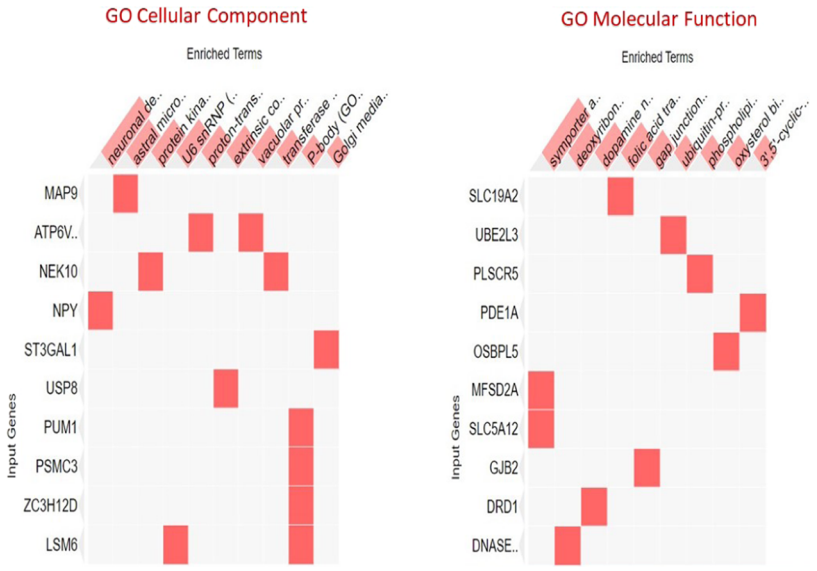

Figure 2. GO molecular function and cellular components for m6a genes.

3.3. Functional Annotation via RegulomeDB. We looked for potential functionality for the m6a-SNPs that had shown cis-eQTL signals by using RegulomeDB, which prioritized variants based on transcription regulations such as binding with transcription factors, protein binding, and DNase peak binding. In total, we found four m6a-SNPs that were likely to affect gene expression: 11 2a and 2b m6a-SNPs were expected to affect binding, and 12 3a m6a-SNPs were expected to affect the binding of the transcription factor and DNase peak binding. Some m6a-SNPs were found that had minimal evidence of binding with transcription factors, motif hits, and DNase peaks.

3.4. Pathway Enrichment Analysis. *3.4.1. Biological Process.* Genes corresponding to cis-eQTL m6a-SNPs have been enriched in many biological processes such as amino sugar metabolic processes, the insulin signaling pathway, protein sialylation, axon point recognition, negative regulation of interleukin-13 and interleukin-5 production, negative regulation of myelination, smooth muscle cell apoptotic process, and synapse organization; see Table 2 for details.

3.4.2. GO Molecular Function. In molecular function, these genes were enriched in multiple activities such as symporter activity, deoxyribonuclease 1, the dopamine neurotransmitter receptor, and gap junction channel activity involved in cell communication by electrical coupling, 3′,5′-cyclic-GMP phosphodiesterase, and the ubiquitin-protein transferase activator (Figure 1).

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ACS Omega 2023, 8, 13332−13341

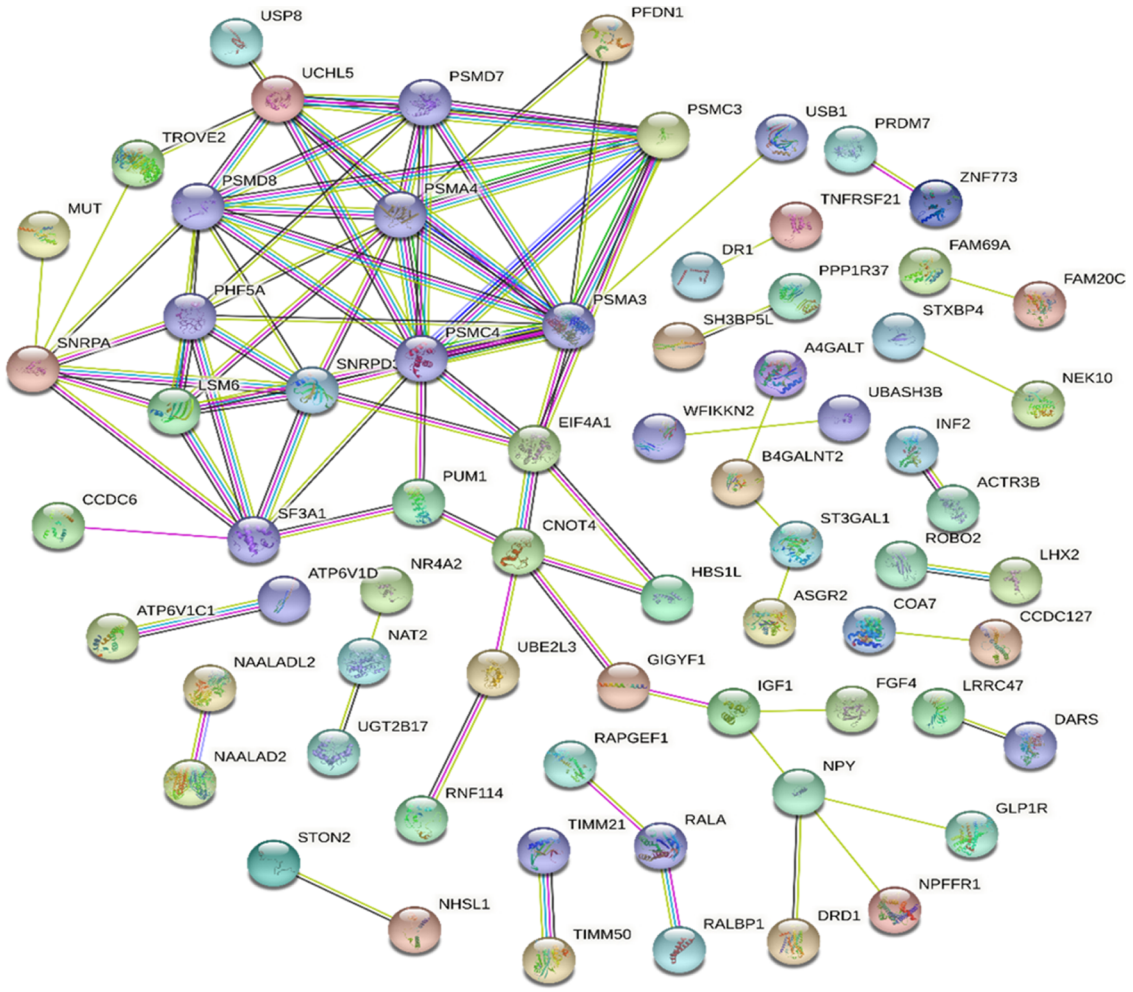

Figure 3. Protein−protein interactions.

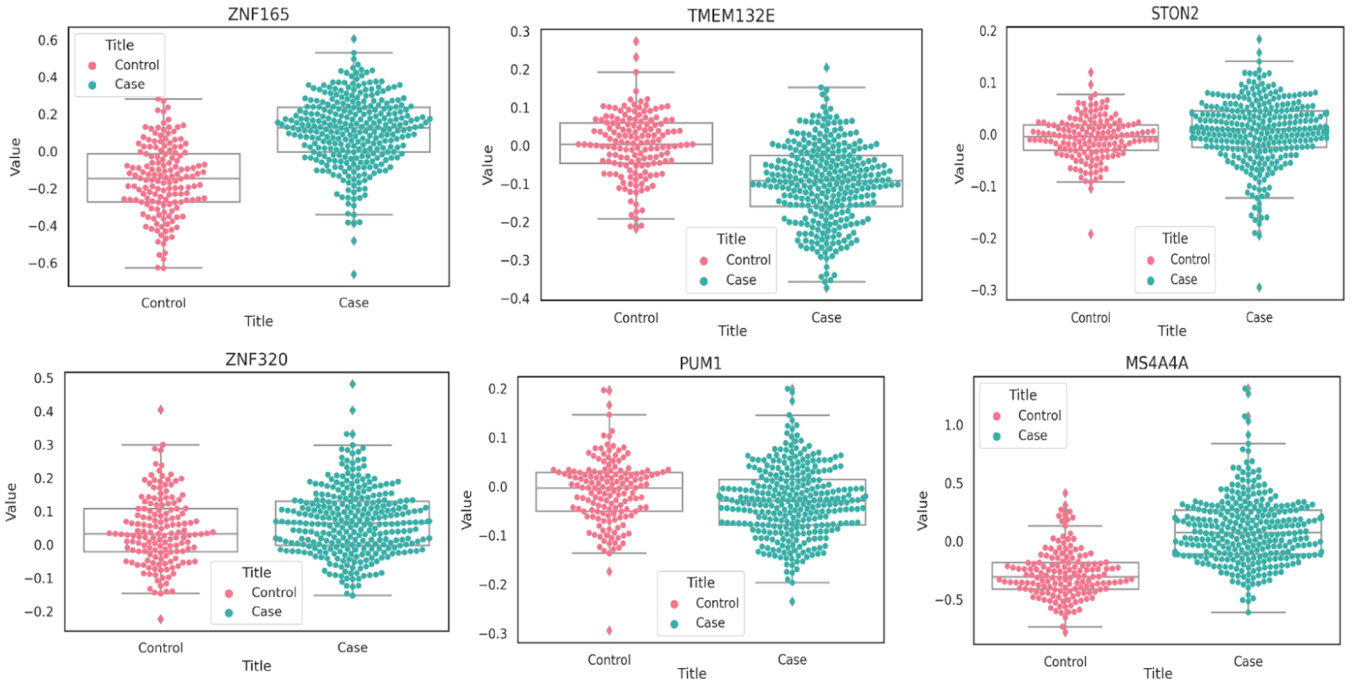

Figure 4. Differentially expressed genes in GSE33000.

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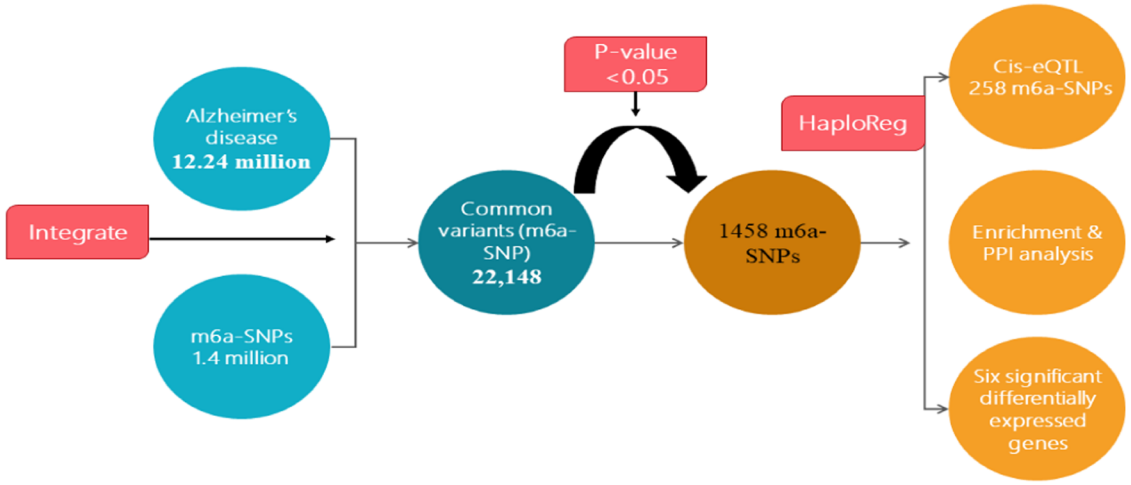

Figure 5. Workflow and design of the study.

3.4.3. GO Cellular Component. In cellular components, the m6a genes were involved in neuronal dense core vesicles, astral microtubules, the protein kinase complex, U6 snRNP, proton-transporting V-type ATPase, the extrinsic component of the endosome membrane, vacuolar proton transportation, transferase complex, and Golgi medial cisterna (Figure 2).

3.5. Protein−Protein Interaction Using the STRING Database. In our list of query proteins, these proteins showed a strong protein−protein interaction network, which includes PSMD8, PSMD7, PSMA4, UNCH5, PHF5, SNRPD, PSMC4, LSM6, SNRPA, TROVE2, SF3A1, PUM1, and EIF4A1, as shown in Figure 3. These were either experimentally determined or showed co-occurrence and co-expression. We also checked the functions of these proteins in our network. In biological processing, proteins in the network were found to be involved in the regulation of the cellular amine metabolic process and NIK/NF-kappaB signaling. According to cellular components, we found their associations with proteasome regulatory particles, the proteasome complex, and the spliceosomal snRNP complex. According to the local network cluster, they have a relationship with proteasome regulatory particles, proteasome α-type mixed, the proteasome complex, and ubiquitin conjugation.

3.6. Gene Differential Expression Analysis. We overlapped our list of genes having cis-eQTL signals (p-value <0.05) with publicly available gene expression datasets to find the expression level of genes in AD cases that might be regulated by m6a-SNPs from the AD cohort. Concisely, for the identified 258 m6a-SNPs in which cis-eQTL signals were shown, we analyzed the mRNA expression level of the local genes in a microarray dataset and one mRNA-seq dataset. In the GSE33000 microarray dataset, 89 differentially expressed genes had p-value <0.05, for which cis-eQTL signals were displayed, while 6 were found to be unique genes that were differentially expressed in the microarray dataset and also in the mRNA-seq dataset. These six genes were cis-eQTL signaled and found to be differentially expressed in AD; the details of the top six m6a-SNPs and their corresponding differentially expressed genes are shown in Table 2 and their expression values are illustrated in Figure 4.

4. DISCUSSION

Our findings showed that m6a-SNPs affect their local genes by altering the expression of the corresponding genes between AD cases and controls. Further, we explored the m6a-SNPs associated with AD, which showed their role in methylation, transcription factor binding, and DNase and protein binding. Our findings of numerous m6a-SNPs that affect the expression of AD-associated genes and their variants were previously reported in an AD-GWAS (Figure 5).

m6a modification involves RNA modification and has a drastic impact on homeostasis, nuclear export, and splicing. This modification of RNA is said to be the most abundant modification of RNA in eukaryotes. A recent study has found that m6a controls the protein expression level of certain genes that are associated with AD. The distinct AD-associated pathways also play a pivotal role in neurodegenerative diseases.

However, the influence of genetic variants has not been explored for RNA modification that might be associated with AD due to their small role in RNA degradation in brain regions such as the cerebral cortex and hippocampus. Due to these concerns over RNA modifications and their associations with the brain, the identification of m6a-SNPs that affect the level of expression of genes responsible for AD and the overlapping genes with differential expression analysis datasets of the AD case−control study is yet to be accomplished. To date, there is no such study conducted on the identification of m6a-SNPs associated with AD, and how m6a-SNPs are associated with AD is yet needed to be explicated; therefore, we identified m6a-SNPs associated with AD by altering the RNA and expression of RNA, which might play a potential role in AD.

Further, we analyzed the identified genes to see whether they have a role in AD, which had been validated in previous studies; we found that the *MS4A4A* gene deserves greater concern due to a recent study conducted to measure the gene expression levels of AD patients in the cerebellum and the temporal cortex brain region. In another study of loaded cis-SNPs, their SNPs were different, while our identified m6a-SNP has shown the cis-eQTL signal for the *MS4A4A* (rs1026256) gene, which was previously associated with AD risk.[48] Moreover, AD is a neurodegenerative disorder and *PUM1* is an RNA binding protein, although its decreased protein level is associated with neurodegeneration.[49] Our identified m6a-SNP (rs6425690) showed a cis-eQTL signal and was very similar to

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ACS Omega 2023, 8, 13332−13341

the *PUM1* gene, which was downregulated among the AD cases of the microarray dataset and miRNA. Anxiety and depression are twice likely to contribute to develop AD; *TMEM* genes are candidate genes associated with panic disorder, and anxiety and depression are more likely associated with panic disorder.[50] *TMEM132E* to cis-m6a-SNP (rs4341789) was found to be a differentially expressed gene in the microglia and cerebellum brain region of AD cases, so m6a-SNP (rs4341789) might be a potential variant and *TMEM132E* gene might play a role in the etiology of AD.

ZNF family proteins have been found in association with Parkinson's disease. While Parkinson's and AD are both brain disorders, the former leads to dementia, while dementia is always associated with AD.[51] *ZNF165* (rs73396550) and *ZNF320* (rs8100166) to cis-m6a-SNPs were differentially expressed in AD patients and these genes might be susceptible to AD. The *STON2* (rs12895215) gene functions in neural endocytosis and evidence shows that endocytosis plays a pivotal role in an amyloid-β peptide, which is the main component of AD.[52,53]

During the past ten years, our understanding of the genomics of AD has improved significantly. The genetic information has already altered how the AD research community views the progress, concisely by bringing attention to the role and significance of microglia in AD. This knowledge about AD genetics is still by no means a complete understanding of AD, and with similar multifactorial aging-related disorders, significant efforts are still required to fully understand the underlying genetics linked with AD. Various new and complementary approaches will probably be necessary in this situation. Postgenomic research is needed to interpret the results of the unknown genetic risk factors of AD. This poses the biggest obstacle in the research on the pathophysiological mechanisms underlying AD. One major temptation is to attribute novel identified susceptible genes that were previously considered to be crucial in AD. Even if this is quite likely the case, this will advance a hypothesis-free approach and make that approach a hypothesis-driven one.

One major issue that we face is to create read-outs using new models that are more or less independent of our current understanding, which can be solved by integrating Omics, an all-encompassing strategy that has already been established ("omics" is a database made for brain-relevant cell types). These initiatives are unquestionably needed for the most comprehensive and fruitful results. No matter what, the combination or integration of these techniques can either individually reveal novel, unexpected processes or confirm the established mechanisms underlying AD.

Prioritization of tissues and genes linked with complex disorders has been identified by several enrichment analyses and colocalization or eQTL analyses; still, the lack of comprehensive and functional information restricts current approaches. For instance, gene expression profiling derived from bulk tissues is commonly integrated by these approaches. However, only the most prevalent cell types can provide gene expression datasets from those bulk tissues, which is not enough information on the cell composition and density of these cell types.[54] Aside from this, these colocalization approaches do not pinpoint the causality; for example, a gene with a trait affected by a variant is identified by an independent approach, which cannot be differentiated from a single causal version of colocalization.

Therefore, further experimental validation of candidate genes is required to pinpoint the causality, which can be achieved by combining GWAS variants with single-cell assays or by testing the identified genes using advance gene-editing techniques. The available methods for colocalization can become more useful by utilizing single-cell techniques, because it is possible to profile single-cell transcriptomes in large cohorts due to these assays, which can provide a fine mapping of single-cell eQTLs by the unprecedentedly high-throughput growth. In 2018, such a study was conducted, which profiled 45 thousand single cells derived from more than 40 healthy people[55] and found contrast eQTL results from gene expression profiling in various cell types: the rs4804315 signaled for ZNF414 was associated with overexpression in NK cells but reduced expression in T cells. This study suggests that single-cell eQTL relationships could be made available for post-GWAS integrative analysis in the near future.

Recently, the availability and effectiveness of gene-editing technologies have rapidly increased. Specifically, CRISPR/Cas9 is capable of and highly accurate in deletion of specific genomic regions.[56] Genes have been systematically modified by using a CRISPR technique known as CRISPR screening.[57] CRISPR screening has a wide range of uses; it can determine which genes are crucial for the development of complex disorders like cancer and neurodegenerative diseases, which makes this a useful platform for the identification of novel therapeutic targets.[58]

This technology can also be used for studying the noncoding genome or can also be studied via CRISPR-interference (CRISPRi), which can prevent regulatory elements from contacting their corresponding genes with the help of guide RNAs and also the Cas9 enzyme in the nonactive state.[59] Aside from this, CRISPR activation (CRISPR-a) can boost the transcription by fusing an activator of the Cas9 enzyme;[60] by utilizing these tools, we can map the functional information for the regulatory variants linked to disorders. These gene-editing techniques, if used in cell types that are identified by the SNPs from particular diseases, can ideally confirm the risk factors lying in the noncoding region of the genome and can help in devising quick and effective therapeutics for complex disorders, but currently, these techniques are mostly used only for cell lines. There are several reasons behind this; for example, the large quantity of cells and maintaining these cells in culture for a longer time make it difficult to apply mutagenesis to these primary cells.

Some of these limitations will probably be overcome by advanced systems like improved Cas9 delivery systems.[61,62] Still, more technological advancement is required and expected in the very near future before gene editing may be regularly used as a GWAS follow-up strategy. We are expecting that future developments can convert the GWAS results of AD and other complex diseases into therapeutically useful gene sets, including the incorporation of single-cell data in GWASs, validation of candidate genes through gene editing, and cellular phenotyping.

5. CONCLUSIONS

Here in this study, we identified m6a-SNPs associated with AD that were confirmed in a previous GWAS cohort study of AD; further, we inspected the potential functionality of our findings, which helped in validating them. The identified six genes (*MS4A4A*, *STON2*, *ZNF165*, *ZNF320*, *TMEM132E*, and *PUM1*) were differentially expressed in AD cases, and they

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ACS Omega 2023, 8, 13332−13341

also have a role in other neurodegenerative diseases. A follow-up study to check these genes in vivo to validate our findings would be required to confirm our findings and may contribute to the etiology of Alzheimer's disease.

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Notes

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