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Research Report

Genome-Wide Mendelian Randomization Identifies Ferroptosis-Related Drug Targets for Alzheimer's Disease

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Abstract.
Background: Alzheimer's disease (AD) currently lacks effective disease-modifying treatments. Recent research suggests that ferroptosis could be a potential therapeutic target. Mendelian randomization (MR) is a widely used method for identifying novel therapeutic targets.
Objective: Employ genetic information to evaluate the causal impact of ferroptosis-related genes on the risk of AD.
Methods: 564 ferroptosis-related genes were obtained from FerrDb. We derived genetic instrumental variables for these genes using four brain quantitative trait loci (QTL) and two blood QTL datasets. Summary-data-based Mendelian randomization (SMR) and two-sample MR methods were applied to estimate the causal effects of ferroptosis-related genes on AD. Using extern transcriptomic datasets and triple-transgenic mouse model of AD (3xTg-AD) to further validate the gene targets identified by the MR analysis.
Results: We identified 17 potential AD risk gene targets from GTEx, 13 from PsychENCODE, and 22 from BrainMeta (SMR $p < 0.05$ and HEIDI test $p > 0.05$). Six overlapping ferroptosis-related genes associated with AD were identified, which could serve as potential therapeutic targets *(PEX10, CDC25A, EGFR, DLD, LIG3,* and *TRIB3*). Additionally, we further pinpointed risk genes or proteins at the blood tissue and pQTL levels. Notably, *EGFR* demonstrated significant dysregulation in the extern transcriptomic datasets and 3xTg-AD models.
Conclusions: This study provides genetic evidence supporting the potential therapeutic benefits of targeting the six druggable genes for AD treatment, especially for *EGFR* (validated by transcriptome and 3xTg-AD), which could be useful for prioritizing AD drug development in the field of ferroptosis.

Keywords: Alzheimer's disease, ferroptosis, Mendelian randomization, 3xTg-AD

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INTRODUCTION

Over the past three decades, there has been a noticeable increase in the global prevalence and societal and economic impact of Alzheimer's disease (AD), underscoring the imperative for extensive research into its pathogenesis and potential treatments.[1] The pathogenesis of AD is complex, involving multiple factors such as amyloid-β (Aβ) toxicity, tau protein, genetic mutations, synaptic damage, and neuroinflammation.[2,3] Ferroptosis, an iron-dependent form of regulated cell death, has offered fresh insights into comprehending the pathogenesis of AD.[4]

Recent studies suggest that ferroptosis might be involved in the pathological process of AD.[5,6] There are several similarities between the characteristics of AD pathogenesis and those of ferroptosis, such as iron accumulation and lipid peroxides.[7,8] As a result, ferroptosis is gradually being acknowledged as a unique cell death mechanism that contributes to the pathogenesis of AD. Interestingly, we also observed a close correlation between neuronal death and ferroptosis in AD patients. Elevated levels of iron and markers of lipid peroxidation have been detected in the brains of AD patients.[4] Dysregulation of iron metabolism, specifically the accumulation of iron within neurons, has been implicated in the initiation of ferroptosis. This aberrant iron accumulation leads to increased reactive oxygen species (ROS) production, which in turn triggers lipid peroxidation and the subsequent death of neurons through ferroptosis.[4,9]

Substantial efforts have been invested in the search for treatments that can modify AD, but significant progress remains elusive due to the unknown pathophysiology of the disease.[10] Given the presence of iron deposits in the brains of AD patients, ferroptosis inhibitors offer promising prospects for AD treatment.[11] Excess iron can worsen oxidative damage, and targeting ferroptosis may help alleviate cognitive deficits and oxidative stress in AD.[12,13] However, the research on ferroptosis inhibitors and AD is still in its early stages. Large-scale human genetic studies provide a unique avenue for innovative drug development for complex diseases. This is because drug targets backed by genetic evidence have a higher likelihood of success in drug discovery pipelines.[14]

MR analysis has been widely utilized to adapt licensed drugs and uncover novel therapeutic targets by amalgamating summary data from disease genome-wide association study (GWAS) and quantitative trait loci (QTL) studies.[15] The levels of gene expression can be viewed as a form of long-term exposure, and QTLs situated in the genomic region of "druggable" genes are always regarded as proxies.[16] Additionally, previous studies have used MR methods to assess the causal effects of disease exposure or biomarkers on various neurodegenerative diseases, further validating the reliability of our research approach and enriching our background knowledge for this study.[17–19]

This MR study employing brain-specific and blood-specific expression QTLs (eQTLs) and protein QTLs (pQTLs) of ferroptosis-related genes as instrumental variables, with ferroptosis-related genes as the exposure factor and AD GWAS summary-level data as the outcome factor, enables us to identify potential drug targets associated with ferroptosis in AD. This strategy employs genetic information to evaluate the causal impact of ferroptosis-related genes on the risk of AD and can offer valuable insights for the development of targeted therapies for AD.

MATERIAL AND METHODS

Ferroptosis-related genes list

The principle and flow diagram of MR study are depicted in Fig. 1. FerrDb, a central database dedicated to the study of ferroptosis regulators and their disease associations, is accessible via http://www.zhounan.org/ferrdb/. Genes related to ferroptosis were extracted from this database for subsequent analysis.

QTL datasets used for genetic instrumental variables selection

We leveraged four QTL datasets of brain to derive genetic instrumental variables for ferroptosis-related genes. Moreover, we utilized eQTL and pQTL data from available blood tissue to exhaustively search for ferroptosis-related targets related to AD. The GTEx eQTL datasets were acquired from the SMR website (https://yanglab.westlake.edu.cn/software/smr/#eQTLsummarydata).

The PsychENCODE eQTL datasets were generated using brain tissues (specifically, the prefrontal cortex) from 1,378 human individuals.[20] We accessed the LD reference panel of the European (EUR) population from the 1000 Genomes project provided by OpenGWAS API.[21] The BrainMeta v2 cis-eQTL summary data, downloaded from the SMR web-

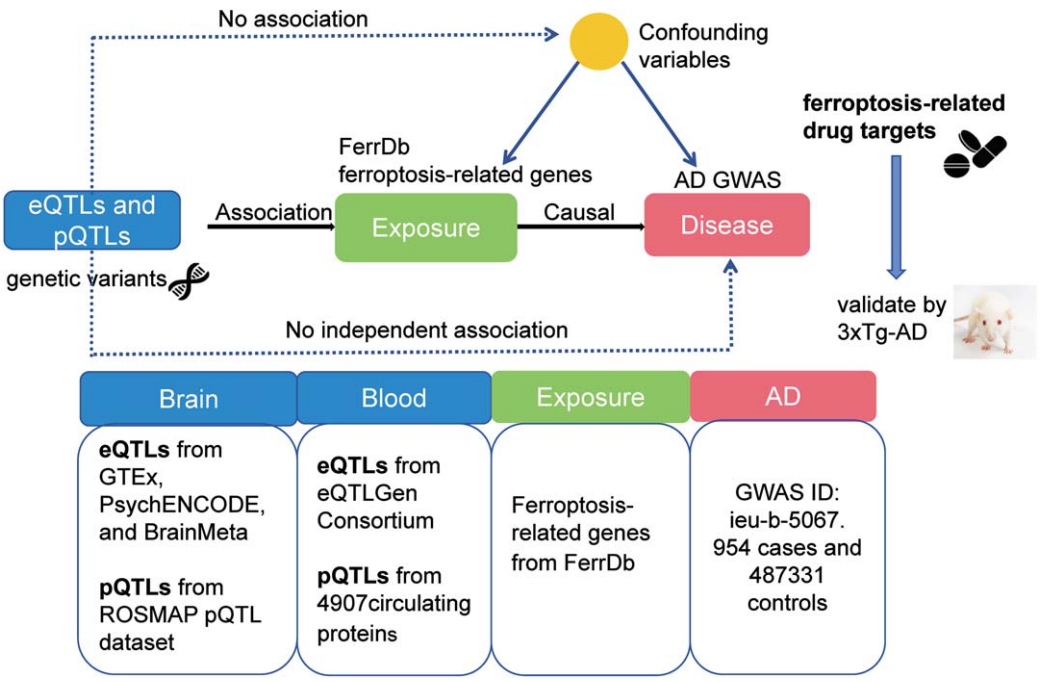

Fig. 1. The principle and flowchart of MR analysis.

site, underwent eQTL mapping using RNA-seq data from 2,865 brain cortex samples, collected from 2,443 unrelated individuals of European ancestry with genome-wide single nucleotide polymorphism (SNP) data. The pQTL data of brain originated from a recent study, which can be downloaded from Synapse (https://doi.org/10.7303/syn23627957).[22]

The summary-level eQTL data of blood tissues were procured from the eQTLGen Consortium, comprising a total of 31,684 individuals (https://www.eqtlgen.org/). The pQTL summary study is grounded in a large-scale GWAS on the blood proteome (https://www.decode.com/summarydata/). We extracted summary-level statistics of genetic associations with levels of 4,907 circulating proteins from a large-scale pQTL study conducted in 35,559 Icelanders.

GWAS summary statistics of AD

The summary statistics for AD from GWAS were retrieved from the MRC IEU Open GWAS Project (https://gwas.mrcieu.ac.uk/). The GWAS results, identified by the GWAS ID ieu-b-5067, served as the outcome data in the MR analysis. This analysis incorporated data from 954 cases and 487,331 controls.

Mendelian randomization

SMR method

In our study, we utilized the SMR method to explore the relationship between gene expression levels and outcomes of interest, leveraging summary-level data from both GWAS and eQTL studies, specifically focusing on eQTLs as instrumental variables.[23] To isolate brain-specific eQTLs, we analyzed all GTEx results and pinpointed cis-SNPs linked to gene expression. Following this, we delved deeper to ascertain if genes whose expression was influenced by these SNPs were implicated in ferroptosis-related gene targets. We then narrowed down to genetic variants associated with the expression of ferroptosis-related genes, setting a stringent threshold of eQTL significance at $p < 5 \times 10^{-08}$ for further analysis.

To ensure the validity of our findings, we performed the HEIDI test within the SMR software to discern whether the observed associations between gene expression and outcomes could be attributed to linkage scenarios.[23] A p-value less than 0.05 from the HEIDI test suggests a likelihood of linkage influencing the observed association.[24] Our selection criteria focused on genes exhibiting an SMR p-value below 0.05, coupled with a HEIDI p-value greater than 0.05,

highlighting them as promising candidates linked to AD.

One SNP could be associated with the expression of multiple genes, a phenomenon known as horizontal pleiotropy. To assess the likelihood of horizontal pleiotropy, we identified nearby genes (within a 1 Mb window) whose expression was significantly associated with the genetic instrumental variant. We then performed SMR analysis to ascertain if the expression of these genes was related to AD outcomes.

Two-sample MR method

"TwoSampleMR" R package was used to conduct the two-sample MR analysis.[25] In this study, we employed cis-pQTL data as genetic instruments (exposure) and GWASs of AD as the outcome trait data. The two-sample MR method evaluates the relationship between protein expression and AD. The Wald ratio was applied when only one pQTL was available for a given protein. In instances where two or more genetic instruments were available, we implemented the inverse variance weighted MR (MR-IVW) method followed by a heterogeneity analysis.

PPI analysis and expression of the identified gene targets in different cell types of the human brain

The online tool, Search Tools for the Retrieval of Interacting Genes (STRING), was used to scrutinize protein interactions. We filtered the Protein-Protein Interaction (PPI) pairs based on a confidence score exceeding 0.40 and visualized the PPI network using Cytoscape V3.8.2 software.[26] We leveraged the Cortical Development Expression Viewer (CoDEx) data portal to investigate the expression patterns of genes significant in the SMR analysis at the single-cell level.[27]

Triple-transgenic mouse model of AD

3xTg-AD is an important preclinical animal model used for studying the pathogenesis and potential treatments for AD. This mouse model was created by incorporating three transgenes (APP, PS1, and Tau mutation) into the mouse genome to mimic key features of AD pathology observed in human patients.[28] By combining these three mutations in a single mouse model, the 3xTg-AD model recapitulates various aspects of AD pathology, including Aβ plaque deposition, neurofibrillary tangle formation, synaptic dysfunction, and cognitive decline.

Males of the 3xTg-AD strain, aged between 6-7 weeks, were obtained from the Changzhou Cavens Laboratory Animal Co., Ltd. (Wuhan, China; license number: SCXK (Su) 2016-0010). These mice were maintained in a pathogen-free laboratory at Wuhan Myhalic Biotechnology Co., Ltd. (Wuhan, China), under precise conditions: a temperature range of 20–26°C, 50% ± 10% humidity, and a 12-h day-night light cycle. Standard rodent chow and unrestricted access to water were provided until they reached 9 months of age. Males of the C57BL/6J strain, aged 7 months, were purchased from Wuhan Youdu Biotechnology Co., Ltd. (Wuhan, China; license number: SCXK (E) 2021-0025). These mice were housed under identical conditions to those of the 3xTg-AD mice, including comparable dietary regimens up to 9 months of age. All animal research was conducted with the approval of the Animal Ethics Committee of Wuhan Myhalic Biotechnology Co., Ltd. (ethical approval number: HLK-20230518-001). The experimental procedures complied with the ARRIVE guidelines and the American Veterinary Medical Association Guidelines for the Euthanasia of Animals (2020). No interventional experimental procedures were employed on the mice. Following anesthesia with isoflurane, the mice were decapitated, and their entire brains were rapidly harvested for western blot analyses.

Validation of the gene targets by transcriptome and western blotting

Validation of external transcriptomic datasets

To provide additional validation for the gene targets identified via SMR analysis, we employed transcriptomic datasets pertaining to AD from the Gene Expression Omnibus (GEO) database. Specifically, dataset GSE122063, sourced from the GPL16699 platform, encompassed transcriptomic data from 56 individuals diagnosed with AD and 44 control samples.[29] Gene expression profiling was conducted on the frontal and temporal lobes of AD patients and non-demented controls (referred to as Controls) sourced from the University of Michigan Brain Bank.

Western blotting

We used a lysis buffer for the acquisition of overall protein from the complete brain of 3xTg-AD. After assessing with a BCA kit, we segregated the protein samples on a gel comprising of sodium dodecyl sulfate-polyacrylamide and shifted them

onto membranes composed of polyvinylidene difluoride (PVDF). Following an hour-long incubation with 5% skim milk, both primary antibodies and GAPDH were employed overnight at a temperature of 4°C. Subsequently, a secondary antibody connected with horseradish peroxidase was administered to the membrane and allowed to incubate for an hour at ambient temperature. To render the proteins visible, we employed a chemiluminescence kit.

The following primary antibodies were used: CDC25A (55031-1-AP, proteintech, Wuhan, China), LIG3 (26583-1-AP, proteintech, Wuhan, China), TRIB3 (13300-1-AP, proteintech, Wuhan, China), DLD (16431-1-AP, proteintech, Wuhan, China), EGFR (A11351, Abclonal, Wuhan, China), PEX10 (A16949, Abclonal, Wuhan, China), and β-Tublin (10094-1-AP, proteintech, Wuhan, China).

RESULTS

Ferroptosis-related genes list

A total of 369 genes were identified as drivers, 348 as suppressors, 11 as markers, and 116 were left unclassified. Some genes may duplicate across different categories. Consequently, we have gathered 564 genes related to ferroptosis for further analysis (Supplementary Table 1).

SMR to prioritize candidate gene targets in brain tissue

SMR analysis can be used to ascertain causal relationships and investigate whether alterations in gene or protein expression contribute to disease onset. Consequently, the significant genes or proteins identified by SMR can serve as potential therapeutic targets. By using cis-eQTL SNPs from GTEx (spanning 13 brain regions) as genetic instruments, we pinpointed 17 potential gene targets for AD (SMR $p < 0.05$ and HEIDI test $p > 0.05$) (Fig. 2A). We also found 13 significant correlations from PsychENCODE, and 22 from BrainMeta (Fig. 2B, C). Additional details can be found in the Supplementary Table 1.

When we combined the three gene lists, we were able to identify 30 potential genes. When we found the common genes among the three lists, we discovered six overlapping candidate genes, including *PEX10, CDC25A, EGFR, DLD, LIG3,* and *TRIB3* (SMR $p < 0.05$ and HEIDI test $p > 0.05$) (Fig. 3A, Tables 1 and 2). These genes, including *PEX10, EGFR, DLD, and LIG3,* function as inducers of ferroptosis, promoting its occurrence. Conversely, *CDC25A* and *TRIB3* serve as an inhibitor of ferroptosis, preventing its onset. The detailed SMR locus plot for the six genes can be found in Supplementary Figure 1.

In addition to the gene expression-level candidate targets, we also conducted a two-sample MR analysis by incorporating the pQTL of all proteins from brain tissues. In total, we selected 65 LD-independent pQTL instruments from 65 proteins. We then intersected these 65 potential proteins with 564 ferroptosis-related targets, and finally obtained 5 candidate proteins associated with AD, including CFL1, MGST1, GLS2, DLD, and EIF2AK4 ($p < 0.05$) (Fig. 2D). When overlapping the results from eQTLs and pQTLs derived from brain tissue, we identified two common genes, DLD and EIF2AK4 (Fig. 3B).

We also conducted similar explorations in blood tissues. Using cis-eQTL SNPs from the eQTLGen Consortium as genetic instruments, we identified 20 potential AD targets (SMR $p < 0.05$ and HEIDI test $p > 0.05$) (Fig. 2E). We observed that the candidate genes identified from brain and blood tissue screenings have a high degree of overlap. The overlapping genes between the two tissues include *PEX10, CDC25A, PARP14, PARP11, CDH1, ACSF2, EGLN2, TRIB3,* and *ATF4* (Fig. 3C). We also identified 2 potential ferroptosis-related proteins using cis-pQTL SNPs from circulating 4,907 proteins levels, including BCAT2 and ECH1 ($p < 0.05$) (Fig. 2F). When comparing the results of eQTLs and pQTLs obtained from blood tissue, we found no overlapping genes (Fig. 3D). More details can be found in Supplementary Table 1.

PPI analysis and expression of the identified genes in different cell types of the human brain

We carried out a PPI analysis of the 30 genes obtained from the brain eQTLs results, using the STRING database (https://string-db.org/). The PPI network exhibited significantly more connections than anticipated ($p < 0.001$). The top 10 genes (*SLC7A11*, *SLC40A1*, *SLC3A2*, *RPTOR*, *GABARAPL2*, *EGFR*, *ATM*, *ATF4*, *TXN*, and *TRIB3*) were identified as potential hub genes, based on their Degree with CytoHubba (Supplementary Figure 2). We examined the expression patterns of these prioritized genes using single cell RNA sequencing data. Among the genes associated with AD, *PEX10, CDC25A, EGFR, DLD, LIG3*, and *TRIB3*,

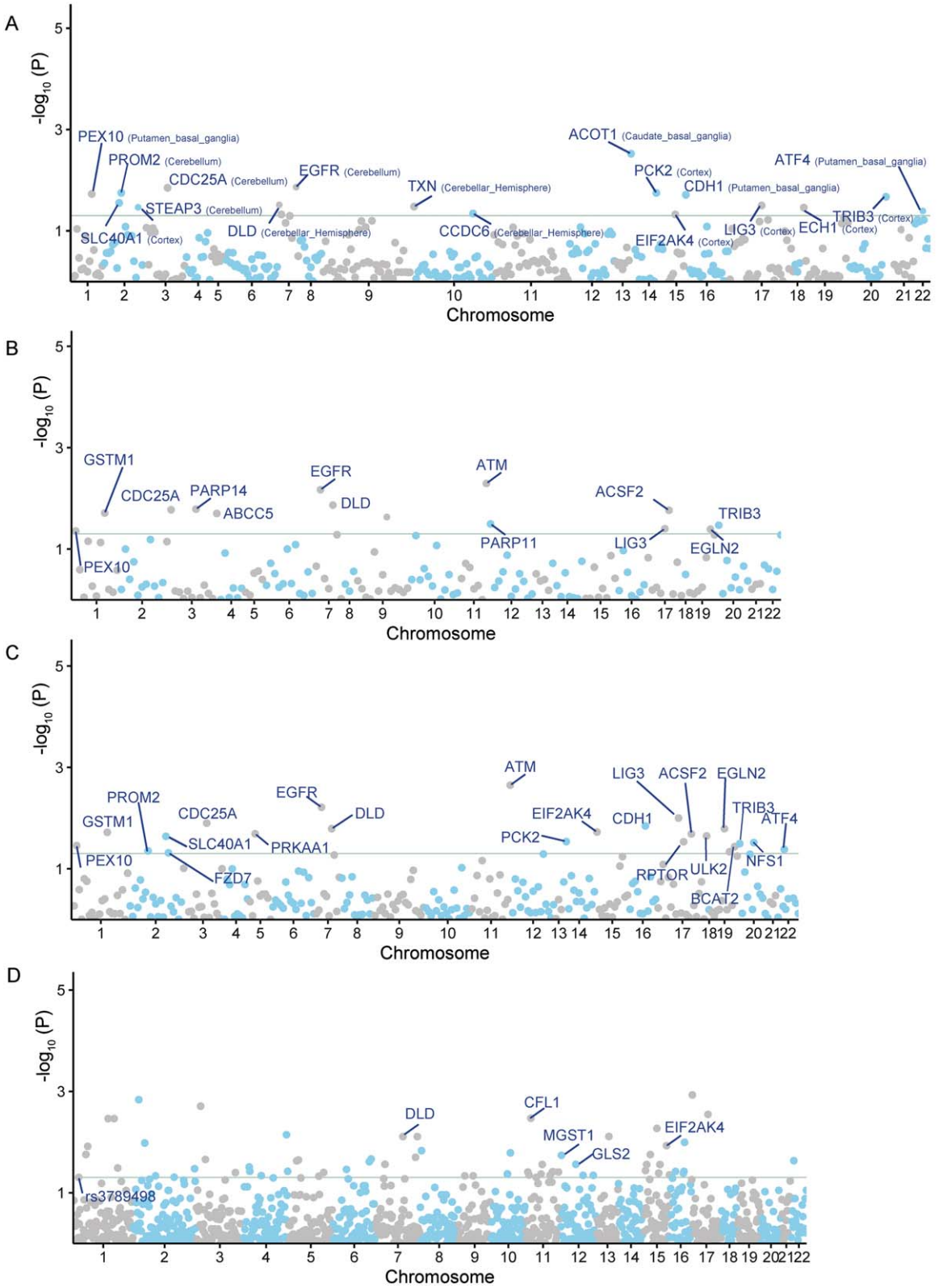

Fig. 2. (*Continued*)

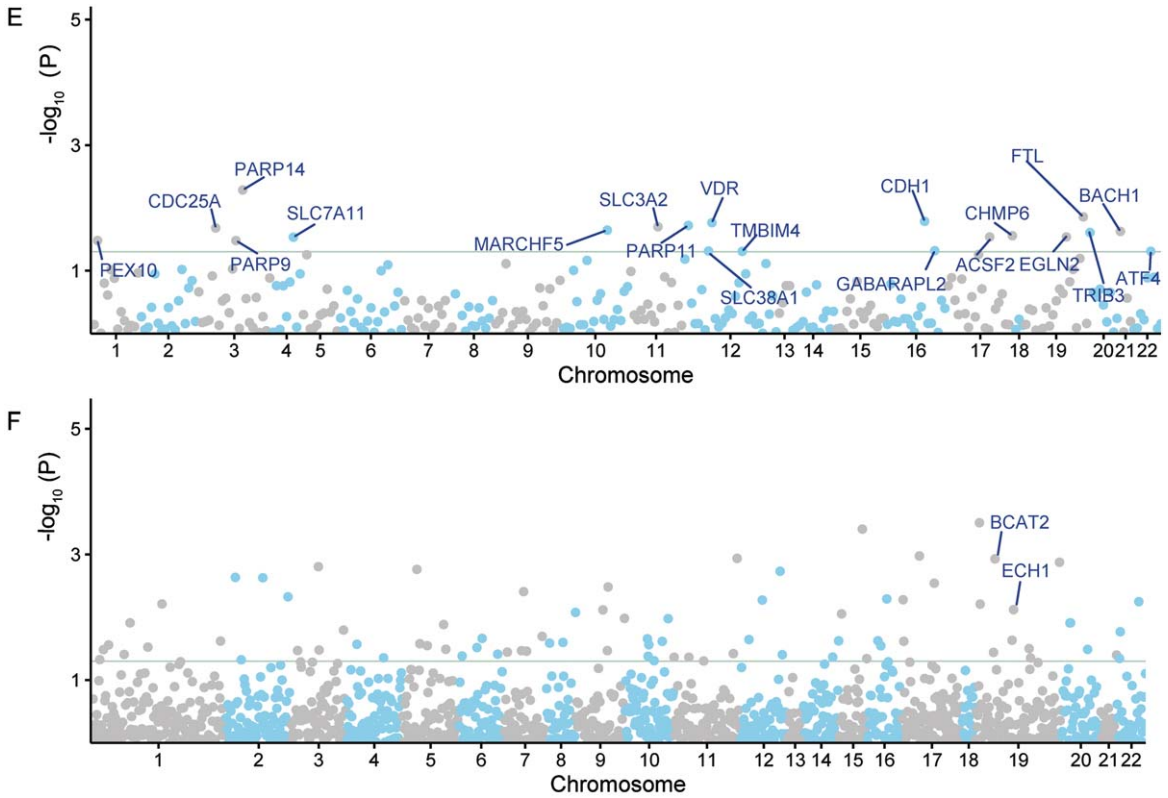

Fig. 2. The Manhattan plots of MR analysis results using QTLs and AD GWAS summary statistics. The gray horizontal line is the Bonferroni corrected significant level. A) The MR result using GTEx brain eQTL as instruments; B) The MR result using PsychENCODE eQTL as instruments; C) The MR result using BrainMeta eQTL as instruments; D) The MR result using ROSMAP pQTL as instruments; E) The MR result using cis-eQTL SNPs from eQTLGen Consortium as genetic instruments; F) The MR result using cis-pQTL SNPs from circulating 4,907 proteins levels as genetic instruments.

were observed to be widely expressed across various brain cell types at relatively high levels (Supplementary Figure 3).

Validation of gene targets by transcriptome and western blotting

Using cis-eQTL SNPs from GTEx, PsychENCODE, and BrainMeta as genetic instruments, we identified six putative gene targets associated with AD. The expression levels of these genes were further validated by analyzing in external transcriptomic datasets. We found that *EGFR, DLD, PEX10*, and *LIG3* were significantly expressed compared to the control group ($p < 0.05$), while *TRIB3* and *CDC25A* did not appear to show statistical significance (Fig. 4). To enhance validation at the protein level, brain tissue was procured from 3xTg-AD mice, followed by western blotting experiment.

In the 3xTg-AD, western blotting demonstrated that both *EGFR* ($p < 0.05$) and *TRIB3* ($p < 0.05$) were downregulated in the whole brain tissues compared with the control group (Fig. 5). Transcriptome and Western blotting analyses both revealed significant dysregulation of EGFR, underscoring its potential as a therapeutic target for AD.

DISCUSSION

Using the MR method, we have identified six ferroptosis-related gene targets associated with AD. Notably, *EGFR* showed significant dysregulation in external transcriptomic datasets and 3xTg-AD models, suggesting its potential role in regulating ferroptosis in AD. These findings suggest *EGFR* could serve as a promising target for therapeutic interventions aimed at modulating ferroptosis in AD treatment strategies.

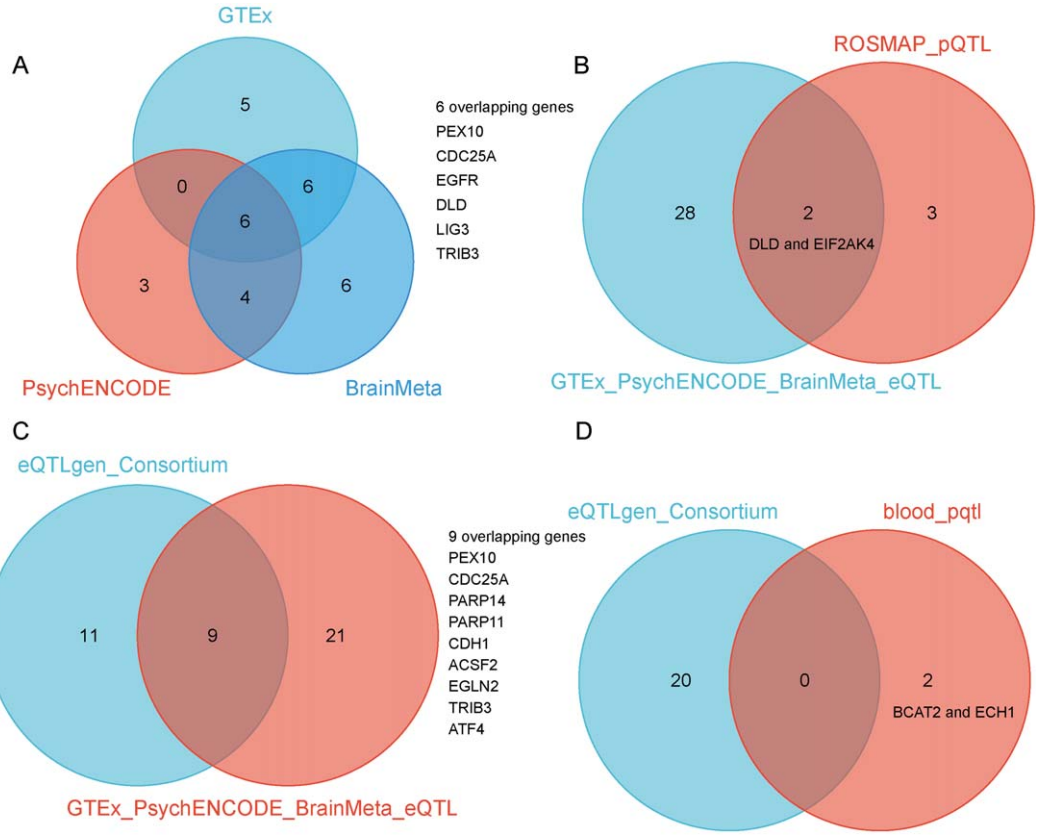

Fig. 3. A) SMR method identified six overlapping ferroptosis-related gene targets associated with AD when using GTEx, PsychENCODE, and BrainMeta brain eQTL as genetic instruments, including *PEX10, CDC25A, EGFR, DLD, LIG3,* and *TRIB3*. B) Venn diagram showed the intersection of AD risk genes identified by GTEx, PsychENCODE, and BrainMeta brain eQTL and ROSMAP brain pQTL as genetic instruments. C)Venn diagram showed the intersection of AD risk genes identified by GTEx, PsychENCODE, and BrainMeta brain eQTL and eQTLGen blood eQTL as genetic instruments. D) Venn diagram showed the intersection of AD risk genes identified by eQTLGen blood eQTL and blood pQTL as genetic instruments.

The ferroptosis-related gene targets associated with AD

Existing research supports the involvement of the ferroptosis-related gene targets that we identified in the pathological process of AD. *EGFR* signaling has been linked to various cellular processes, such as neurodevelopment and neurodegenerative process.[30] Recent research has demonstrated that *EGFR* signaling can influence ferroptosis by adjusting the expression of key genes involved in ferroptosis, such as *GPX4* and *SLC7A11*.[31] Some studies have indicated the potential involvement of TRIB3 in neurodegenerative disorders through the modulation of ferroptosis.[32,33]

The *PEX10* encodes a peroxisomal membrane protein involved in peroxisome formation.[34] Peroxisomal dysfunction is linked to neurodegenerative disorders such as AD, which shows impaired peroxisomal function and lipid metabolism.[34,35] *CDC25A* regulates cell cycle and various cellular processes, which are interconnected with ferroptosis.[36] *LIG3*, an enzyme crucial for DNA repair, has been linked to impaired DNA repair mechanisms in the process of ferroptosis in AD.[37,38] Mitochondrial dysfunction has been associated with both ferroptosis and AD, indicating that *DLD* may indirectly contribute to the dysregulation observed in both conditions.[39,40]

The association of EGFR with ferroptosis

EGFR activation leads to downstream signaling through pathways such as the Ras-*MAPK* pathway, *PI3K-AKT* pathway, and *STAT3* pathway. These pathways regulate various cellular processes, including proliferation, survival, and differentiation.[41] However, they also influence the expression of genes involved in iron metabolism and oxidative stress,

Table 1
SMR results from GTEx brain eQTLs

GTEx_brain	Gene	topSNP	b_SMR	se_SMR	*p*_SMR	*p*_HEIDI
Amygdala	TRIB3	rs62191434	–0.0004	0.0002	0.0401	0.9869
Anterior_cingulate	DLD	rs10271464	–0.0007	0.0003	0.0306	0.7389
	TRIB3	rs62191434	–0.0004	0.0002	0.0441	0.9696
Caudate	DLD	rs4518	–0.0006	0.0003	0.0474	0.7126
	TRIB3	rs62191434	–0.0003	0.0001	0.0349	0.9785
Cerebellar	CDC25A	rs13097450	0.0003	0.0001	0.0141	0.9997
	DLD	rs2237686	–0.0011	0.0005	0.0224	0.7021
Cerebellum	CDC25A	rs3731550	0.0003	0.0001	0.0182	0.9988
	EGFR	rs76928645	0.0014	0.0005	0.0109	0.8670
	TRIB3	rs62191434	–0.0005	0.0002	0.0431	0.9742
Cortex	DLD	rs4518	–0.0006	0.0003	0.0485	0.9015
	LIG3	rs1634800	–0.0003	0.0002	0.0316	0.9639
	TRIB3	rs11698987	–0.0003	0.0001	0.0212	1.0000
Frontal_Cortex	DLD	rs4266570	–0.0007	0.0003	0.0328	0.9751
	TRIB3	rs62191440	–0.0004	0.0002	0.0415	0.8672
Hippocampus	TRIB3	rs6051554	–0.0005	0.0002	0.0363	0.9685
Hypothalamus	LIG3	rs1088450	–0.0004	0.0002	0.0306	0.6199
	TRIB3	rs62191440	–0.0006	0.0003	0.0431	0.8981
Nucleus_accumbens	LIG3	rs1634800	–0.0004	0.0002	0.0332	0.7828
	TRIB3	rs62191440	–0.0004	0.0002	0.0361	0.9917
Putamen	PEX10	rs10910063	–0.0009	0.0004	0.0187	0.5846
	LIG3	rs3135967	–0.0003	0.0002	0.0348	0.8546
	TRIB3	rs62191440	–0.0003	0.0002	0.0369	0.9939
Substantia	TRIB3	rs6051544	–0.0003	0.0002	0.0374	0.9950

Table 2
SMR results from PsychENCODE and BrainMeta brain eQTLs

	Gene	topSNP	b_SMR	se_SMR	*p*_SMR	*p*_HEIDI
PsychENCODE	PEX10	rs12085089	–0.0021	0.0011	0.0441	0.2550
	CDC25A	rs3731497	0.0013	0.0005	0.0168	0.8560
	EGFR	rs74504435	0.0012	0.0004	0.0068	0.1970
	DLD	rs35765154	–0.0018	0.0007	0.0136	0.7330
	LIG3	rs2339122	–0.0019	0.0009	0.0397	0.5720
	TRIB3	rs62191434	–0.0011	0.0005	0.0339	0.7850
BrainMeta	PEX10	rs12092052	–0.0007	0.0003	0.0348	0.1240
	CDC25A	rs17647717	0.0005	0.0002	0.0134	0.9618
	EGFR	rs74504435	0.0004	0.0002	0.0061	0.2023
	DLD	rs8440	–0.0003	0.0001	0.0162	0.9991
	LIG3	rs3135967	–0.0003	0.0001	0.0293	0.9971
	TRIB3	rs62191440	–0.0004	0.0002	0.0319	0.8966

thereby indirectly affecting ferroptosis.[42,43] *EGFR* signaling can modulate iron uptake and utilization within cells. For instance, *EGFR* activation can increase the expression of divalent metal transporter 1 (DMT1), which facilitates iron uptake.[44] Additionally, *EGFR* signaling can affect the activity of enzymes involved in iron processing, such as ferritin and hephaestin, influencing intracellular iron levels.[43]

The accumulation of ROS due to increased iron availability can lead to lipid peroxidation, a hallmark of ferroptosis. *EGFR* signaling can enhance ROS production through various mechanisms, including upregulation of NADPH oxidases (NOXs).[45,46] This increased oxidative stress can damage cellular membranes and organelles, promoting ferroptotic cell death.

Interestingly, therapeutic agents targeting *EGFR*, such as tyrosine kinase inhibitors (TKIs), can also modulate ferroptosis by altering iron metabolism and oxidative stress.[47,48] For example, erlotinib, an *EGFR* TKI, has been shown to induce ferroptosis by disrupting iron uptake and enhancing lipid peroxidation. The interaction between EGFR and ferroptosis represents a promising area of research with potential clinical applications.[49] Further elucidation of the

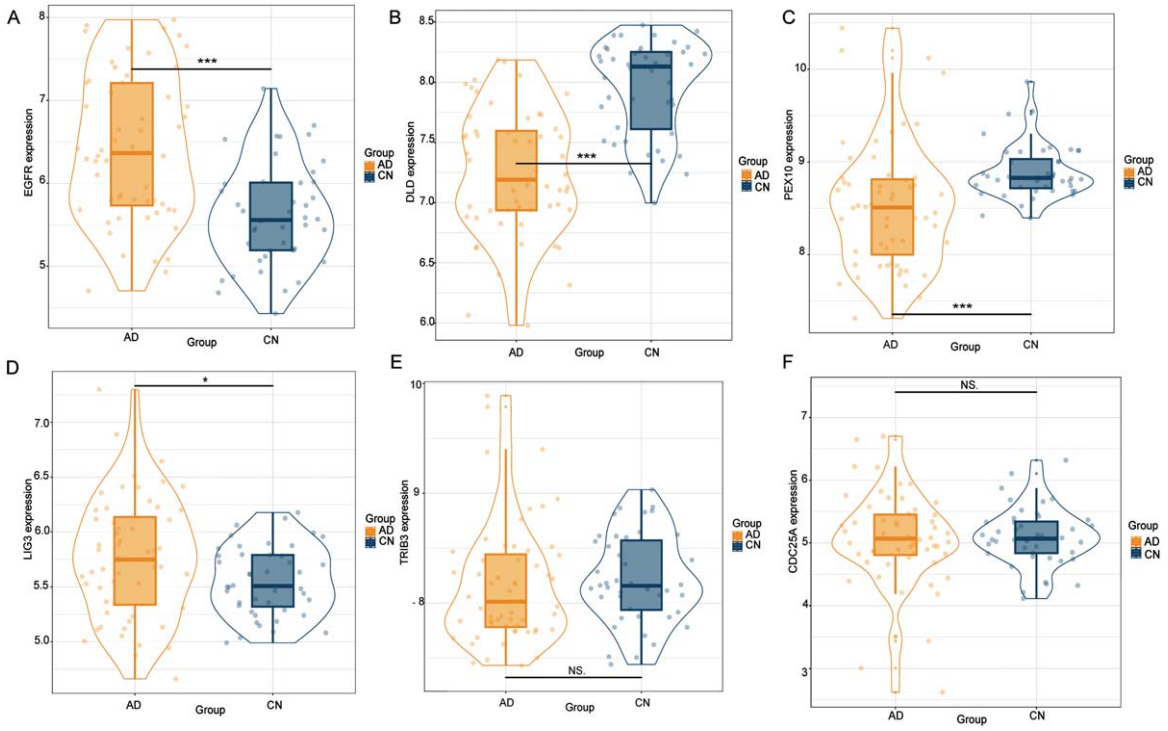

Fig. 4. The external transcriptome dataset corroborated the identification of six gene targets through SMR analysis. Notably, *EGFR, DLD, PEX10,* and *LIG3* demonstrated significant expression differences relative to the control group ($p < 0.05$). Conversely, *TRIB3* and *CDC25A* failed to reach statistical significance.

underlying molecular mechanisms will facilitate the development of targeted therapeutic strategies aimed at leveraging ferroptosis for AD treatment.

EGFR serving as a therapeutic target for AD

In recent times, there's been growing interest in repurposing existing anti-cancer drugs that target the *EGFR* for AD research, given the link between *EGFR* overactivity and several neurodegenerative conditions, including AD.[50,51] Research has highlighted that blocking *EGFR* can reduce the activity of reactive astrocytes, lessen Aβ toxicity and inflammation, and encourage the regrowth of axons.[52] Furthermore, other investigations have noted positive changes in behavior and cognitive functions, alongside decreased amyloid formation and enhanced autophagy, as a result of *EGFR* blockade in different animal models of AD.[53]

Mansour and his colleagues conducted a detailed review of past research, pinpointing overlooked neuroprotective routes and underscoring the importance of exploring *EGFR* inhibitors as possible treatments for AD.[53] They found that Aβ peptides intensify oxidative stress mediated by NADPH oxidase (NOX).[53,54] Activating *EGFR* leads to an increase in ROS via the PI3K pathway, which then sets off the Ras-Rac1 sequence, culminating in NOX activation.[55] Interestingly, NOX appears to be closely associated with AD, suggesting it could serve as a valuable indicator for developing new medications.

Additionally, another *EGFR*-driven process that contributes to oxidative stress and axon loss involves the ZNRF1–AKT–GSK3β–CRMP2 pathway.[56] ZNRF1, a ubiquitin ligase, is prevalent in neurons throughout the central nervous system (CNS). When neurons are damaged, ZNRF1 breaks down AKT, blocks GSK-3β, and disrupts microtubule stability, leading to axonal damage in the Wallerian model.[56] More research is needed to understand the relationship between NOX-mediated oxidative stress, *EGFR*, and ZNRF1 in live models of AD.

Limitations

Our study acknowledges several limitations. Initially, despite a notable correlation between eQTLs in blood and brain, these observations are confined

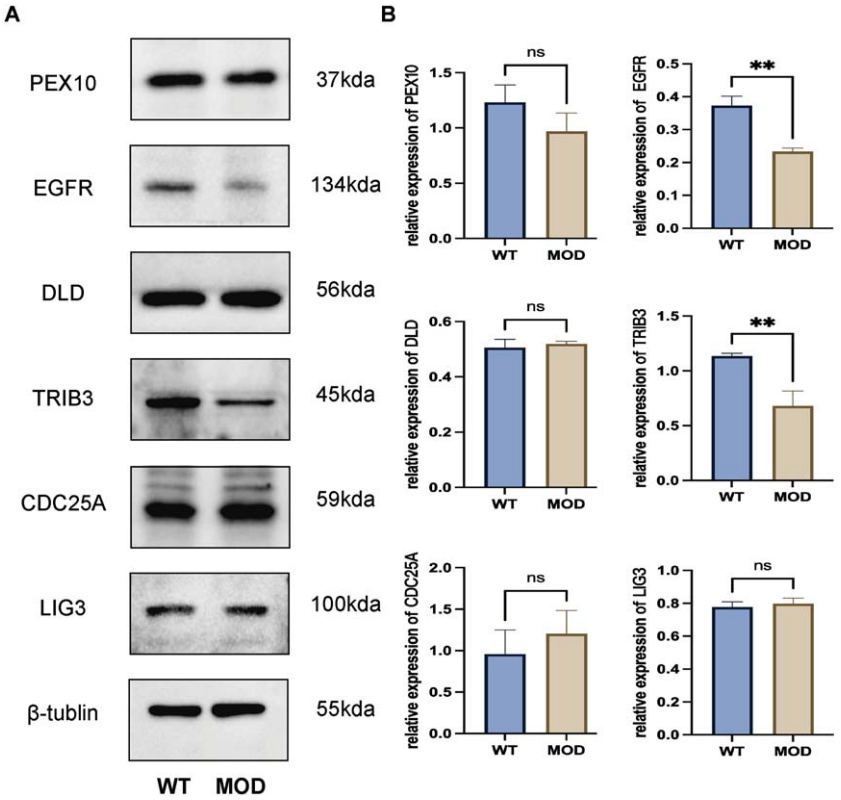

Fig. 5. Validation of the gene targets by western blotting using 3xTg-AD mice. Both *EGFR* ($p < 0.05$) and *TRIB3* ($p < 0.05$) were downregulated in the whole brain tissues compared with the control group. WT, wild type; MOD, model of 3xTg-AD mice.

to genes expressed in both tissues and possessing eQTLs in them. While these studies can be beneficial for estimating the long-term effects of exposure beyond the scope of randomized controlled trials, they fail to directly reveal the impact of short-term drug treatments on disease risk. Secondly, the complete removal of confounding bias and/or horizontal pleiotropy remains a formidable challenge. Lastly, the discovery of additional candidate genes in the 3xTg-AD mice does not negate their association with AD. Additional laboratory and clinical data are necessary to substantiate these findings.

Conclusions

This study provides genetic evidence supporting the potential therapeutic benefits of targeting the six druggable genes for AD treatment, especially for EGFR (validated by transcriptome and 3xTg-AD), which could be useful for prioritizing AD drug development in the field of ferroptosis.

AUTHOR CONTRIBUTIONS

Ying Wang (Conceptualization; Data curation; Formal analysis; Methodology; Software; Visualization; Writing – original draft; Writing – review & editing); Xinhua Song (Methodology; Validation); Rui Wang (Conceptualization; Methodology); Xinzi Xu (Writing – review & editing); Yaming Du (Writing – review & editing); Guohua Chen (Funding acquisition; Project administration; Supervision); Junhua Mei (Funding acquisition; Project administration; Supervision).

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available in the FerrDb repository (http://www.zhounan.org/ferrdb/), MRC IEU Open GWAS Project (https://gwas.mrcieu.ac.uk/), eQTLGen Consortium (https://www.eqtlgen.org/), and SMR website (https://yanglab.westlake.edu.cn/software/smr/#eQTLsummarydata).

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/ADR-240062.

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