The Emerging Role of Ferroptosis-Autophagy-Mitophagy in Alzheimer's Disease Pathophysiology and Therapy

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ABSTRACT

Alzheimer's disease (AD) is emerging as a pathological state characterized by dysregulated proteostasis and metabolic homeostasis, in which oxidative stress and mitochondrial dysfunction converge to accelerate neurodegeneration. Recent evidence suggests ferroptosis, an iron-dependent form of regulated cell death, as a critical inflammatory trigger, causing oxidative stress and neuronal loss in AD, which is explicitly mediated by dysregulated autophagy and mitophagy mechanisms. More specifically, aberrant ferritinophagy and impaired PINK1/Parkin-mediated mitophagy may promote labile iron accumulation, mitochondrial ROS generation, and lipid peroxidation, thereby reducing the ferroptosis threshold of susceptible neurons. To disentangle this dynamic, we advance a unified multi-omics approach combining transcriptomics, proteomics and metabolomics studies produced from extensive cohorts of AD with computational inference of causality via networks along with single-cell resolution. This approach allows identification of concordant and discordant ferroptosis-autophagy signatures that span multiple stages of the pathway and prioritization of causal hubs such as GPX4, NCOA4 and PINK1 and evaluate their relationships with cognitive decline. Connectivity mapping and structural modelling also facilitate finding and proving candidate therapeutics that help restore redox balance and mitophagy flux. Finally, complex systems-level Ordinary Differential Equation modeling of iron-ROS-lipid peroxide dynamics is a predictive scaffold for intervention testing. Combined, this multi-level approach identifies the mechanisms that drive the crosstalk between ferroptosis and autophagy pathways in AD and presents a systems-level matrix to find avenues for therapeutic intervention.

1. INTRODUCTION

High levels of iron entering the brain and its abnormal handling within cells have become critical early events in the progression of Alzheimer's disease. In healthy neurons iron uptake is controlled to maintain metabolic processes such as mitochondrial respiration and neurotransmitter synthesis. In human cells, iron is stored in ferritin or used within iron-sulfur clusters for maintaining bioenergetic reactions, which is often disrupted in Alzheimer's disease. The enhanced iron burden often changes the intracellular environment, specially in the hippocampus and cortex, a sensitive region for Alzheimer's patients. Ferrous iron is found to play a crucial role in redox cycling reactions to produce highly reactive hydroxyl radicals which in turn brings about oxidative damage to membrane lipids, structural proteins and nucleic acids. This iron-induced oxidative load sets the stage for extensive metabolic instability.

In Alzheimer's disease affected neurons often show reduced electron transport chain efficiency, decreased ATP production, and increased reactive oxygen species (ROS) leakage. Excess iron and mitochondrial ROS coexist, resulting in lipid membranes vulnerable to peroxidative damage. The accumulation of lipid hydroperoxides not only compromises membrane integrity but also activates regulated cell death pathways. Among these, ferroptosis-a distinct iron-dependent form of cell death characterized by uncontrolled lipid peroxidation-has garnered significant attention. Impaired antioxidant defenses, particularly in glutathione-dependent lipid repair systems, lower the threshold for ferroptotic damage in susceptible neuronal populations [1] [2].

Recent studies show the autophagy-mediated regulation of intracellular iron and mitochondrial quality control are closely interconnected. Ferritinophagy, the targeted degradation of ferritin via autophagic pathways, can increase cytosolic iron levels under pathological conditions, thereby exacerbating oxidative stress. Whereas, impaired mitophagy obstructs the removal of damaged mitochondria, leading to sustained ROS production. In Alzheimer's disease, where autophagic flux is often disrupted, both iron release and mitochondrial dysfunction become self-perpetuating processes. This interplay creates a vicious cycle in which iron accumulation, oxidative stress, and defective organelle turnover collectively heighten neuronal susceptibility to ferroptosis [3].

Thus the interconnected impact of ferroptosis-mitophagy-autophagy impairment have been implicated in Alzheimer's disease pathology, and their combined impact has been addressed in the study and this gap has been studied via a system-level approach that captures molecular interactions. Integrating transcriptomic, proteomic, and metabolomic data from large AD cohorts can reveal coordinated patterns in ferroptosis-autophagy signaling. Coupled with network-based causal inference, single-cell resolution analyses, and computational modeling of iron, ROS, and lipid peroxide dynamics, this approach can identify key regulatory hubs and critical vulnerability thresholds in the disease process [4].

2. Integrated Mechanistic Framework of the Ferroptosis-Mitophagy-Autophagy (FMA) Network

Recent work shows Alzheimer’s disease not only accounts for protein aggregation, but also iron metabolism disruption, mitochondrial quality control and autophagic flux that collectively impact neuronal susceptibility. In this work we represent this interaction in terms of the Ferroptosis-Mitophagy-Autophagy (FMA) axis-an integrated regulatory network under which iron handling, redox homeostasis, and organelle turnover dynamically influence one another. In this perspective, ferroptosis is the final result while ferritinophagy and mitophagy are upstream regulators of intracellular iron availability and mitochondrial ROS burden. The FMA axis can thus be a mechanistic scaffold explaining how metabolic imbalance evolves to irreversible neuronal loss [5].

2.1 Ferroptosis as an Iron-Dependent Neurodegenerative Trigger

Ferroptosis is a controlled type of cell death spurred by iron-catalyzed lipid peroxidation and inadequate antioxidant protection. Unlike apoptosis or necroptosis, ferroptosis is characterized by accumulation of phospholipid hydroperoxides in cellular membranes and is tightly linked to glutathione-dependent detoxification systems. In neurons, the high metabolic demands, availability of abundant polyunsaturated fatty acids, and dependence on mitochondrial respiration favor a permissive environment for ferroptotic injury when redox buffering capacity declines.

Increased intracellular iron levels and long-term oxidative stress in Alzheimer's disease lead to the peroxidation of membrane lipids. This effect is also enhanced by reduction of glutathione availability or impaired function of lipid peroxide-reducing enzymes, when built up beyond a repairable threshold, results in compromised membrane integrity, thus resulting in bioenergetic collapse and cell death [6].

2.2 Ferritinophagy and Labile Iron Pool Expansion

Under physiological conditions, the ferritinophagy pathway which is responsible for autophagic degeneration of ferritin mediated by NCOA4 - which mobilizes stored iron for metabolic needs, maintains iron homeostasis without increasing labile iron pool. Whereas, in Alzheimer’s disease due to impaired autophagic flux, ferritin turnover is enhanced, resulting in enhanced ferritinophagy or defective lysosomal processing which increases ferrous iron availability. This process accelerates reactive oxygen species generation. In AD neurons, where antioxidant capacity is already compromised, even modest increases in free iron can substantially lower the threshold for ferroptosis. Thus the aberrant ferritinophagy represents a mechanistic bridge between autophagy dysfunction and iron-driven oxidative damage[3].

2.3 PINK1/Parkin-Mediated Mitophagy Failure and ROS Amplification

Mitochondrial integrity is found to be critical for neuron survival, a specialized form of autophagy, known as mitophagy, focuses on removing damaged mitochondria, to maintain bioenergetic efficiency and limit oxidative stress. The PINK1/Parkin pathway which is initiated by PINK1, is responsible for the recognition and selective degradation of depolarized mitochondria. Recent research shows in Alzheimer's disease, PINK1/Parkin signaling impairment causes disruption of mitochondria and thus resulting in accumulation of dysfunction and damaged mitochondria, which further disrupt electron transport chain function and increased superoxide and other reactive species leakage. The continuous mitochondrial ROS production results in lipid peroxidation and destabilizes iron-sulfur cluster-containing enzymes, causing metabolic decline [7].

2.4 Convergence of Iron, ROS, and Lipid Peroxidation in AD Neurons

The interconnected cross-talk of the Ferroptosis-Mitophagy-Autophagy (FMA) axis shows a prominent pathological role in iron accumulation, mitochondrial ROS production, and lipid peroxidation. The radicals formed are catalyzed by iron, whereas mitochondria serves as the main source of ROS and lipids provide the substrates for peroxidative chain reactions. Iron elevation occurs due to the ferritinophagy pathway, resulting in mitophagy as ROS generation fails to be controlled by mitochondria, thus resulting in synergizing and amplifying cellular damage.

In AD neurons, this convergence generates a feed-forward loop. Elevated iron enhances ROS formation; ROS oxidises membrane lipids and mitochondrial components; lipid peroxidation further impairs membrane-bound enzymes and ion channels, exacerbating bioenergetic dysfunction. Over time, this self-amplifying cycle erodes cellular resilience and primes neurons for ferroptosis. The FMA axis therefore represents a coordinated network rather than isolated pathways, with each component reinforcing the others [3].

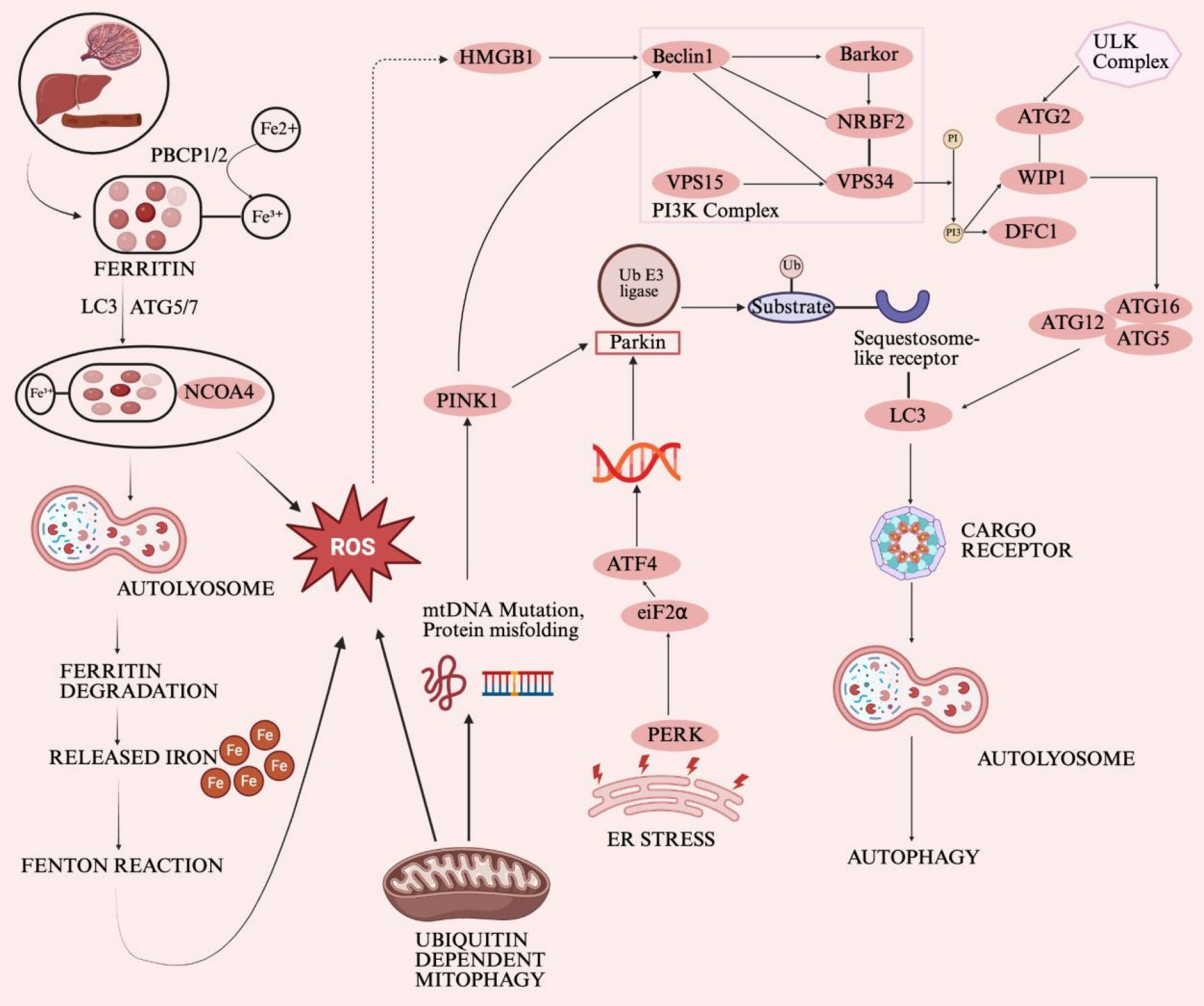

Fig:1 Interlinked ferritinophagy-autophagy-mitophagy network supporting neuronal resilience in Alzheimer's disease.

3. Methods

3.1. Data source

Hippocampus,temporal lobe, and fusiform gyrus gene expression array data of control and AD subjects and corresponding clinical features were obtained from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) in the National Center of Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/). Our study comprised data from 165 subjects derived from GEO Datasets GSE153873 GSE95587, and GSE173955.

3.2. Differential Expression Analysis

Differential gene expression analysis was performed separately for each dataset using the R package **DESeq2**. Raw counts were normalized using the median-of-ratios method implemented in DESeq2. Genes with low counts across samples were filtered prior to analysis to reduce noise. Differentially expressed genes (DEGs) were identified using the Wald test. Statistical significance thresholds were set at |log2 fold change (FC)| > 1 and adjusted P value < 0.05.

Volcano plots and boxplots of DEGs were generated using the R package **ggplot2**, while heatmaps were constructed using the **p-heatmap** package.

3.3. Pathway-Based Gene Shortlisting

To identify biologically relevant candidates, DEGs from each dataset were cross-compared with curated gene sets representing apoptosis, mitophagy, and autophagy pathways. The genes that were part of the target pathways FMA axis have been obtained using KEGG and Reactome databases.

3.4. Visualization of Overlapping Gene Signatures

For the visualization of shared pathway-associated genes, Venn diagrams were generated using the R package which facilitated the identification of conserved molecular signatures across independent cohorts among apoptosis-, mitophagy-, and autophagy-related DEGs.

3.5. Functional Enrichment Analysis

Gene Ontology (GO) and KEGG pathway enrichment analysis were accomplished through clusterProfiler package in R. Results of enrichment with P < 0.05 were considered statistically significant. Plotting functional clustering of the shortlisted genes was visually executed with dot plots and bar graphs for enrichment outputs.

3.6. Protein-Protein Interaction Network Construction

To explore functional connectivity of the shortlisted genes analysis of protein-protein interaction (PPI) was performed using the STRING database (https://string-db.org/) [8]. A confidence test interaction score of > 0.4 was regarded as significant. Interaction networks were imported into Cytoscape (version 3.9.1) for visualization and topological interpretation. Hub genes were identified using the cytoHubba plugin with the Maximum Clique Centrality (MCC) algorithm. The MCODE plugin was applied to detect highly interconnected subnetworks.

4. Result and Discussion

The present study utilized three publicly available RNA-sequencing datasets-GSE153873, GSE95587, and GSE173955-comprising post-mortem brain tissues from individuals with Alzheimer's disease and non-demented controls. Samples were derived from disease-relevant regions including the hippocampus and fusiform gyrus, with representation of both male and female subjects. GSE153873 further included age-stratified control groups, while GSE95587 and GSE173955 provided clinically annotated AD and control samples. Each dataset was analyzed separately to maintain cohort-specific biological integrity as represented in table.1 below. There were a total 534 downregulated genes and 494 upregulated genes. The DEGs are shown in the volcano plot (Fig. 2)

Table:1 Demographic information between patients and controls

GEO Accession	**Brain Region / Tissue**	**Total Samples**	**AD Cases**	**Non-AD Controls**
GSE153873	Lateral temporal lobe / hippocampus	30	*Not directly reported in summary* (mixed groups: AD / Old / Young)	*Not directly reported*
GSE95587	Fusiform gyrus (post-mortem)	117	Approx. ~80+ AD	Approx. 30+ Controls
GSE173955	Hippocampus (post-mortem)	18	8	10

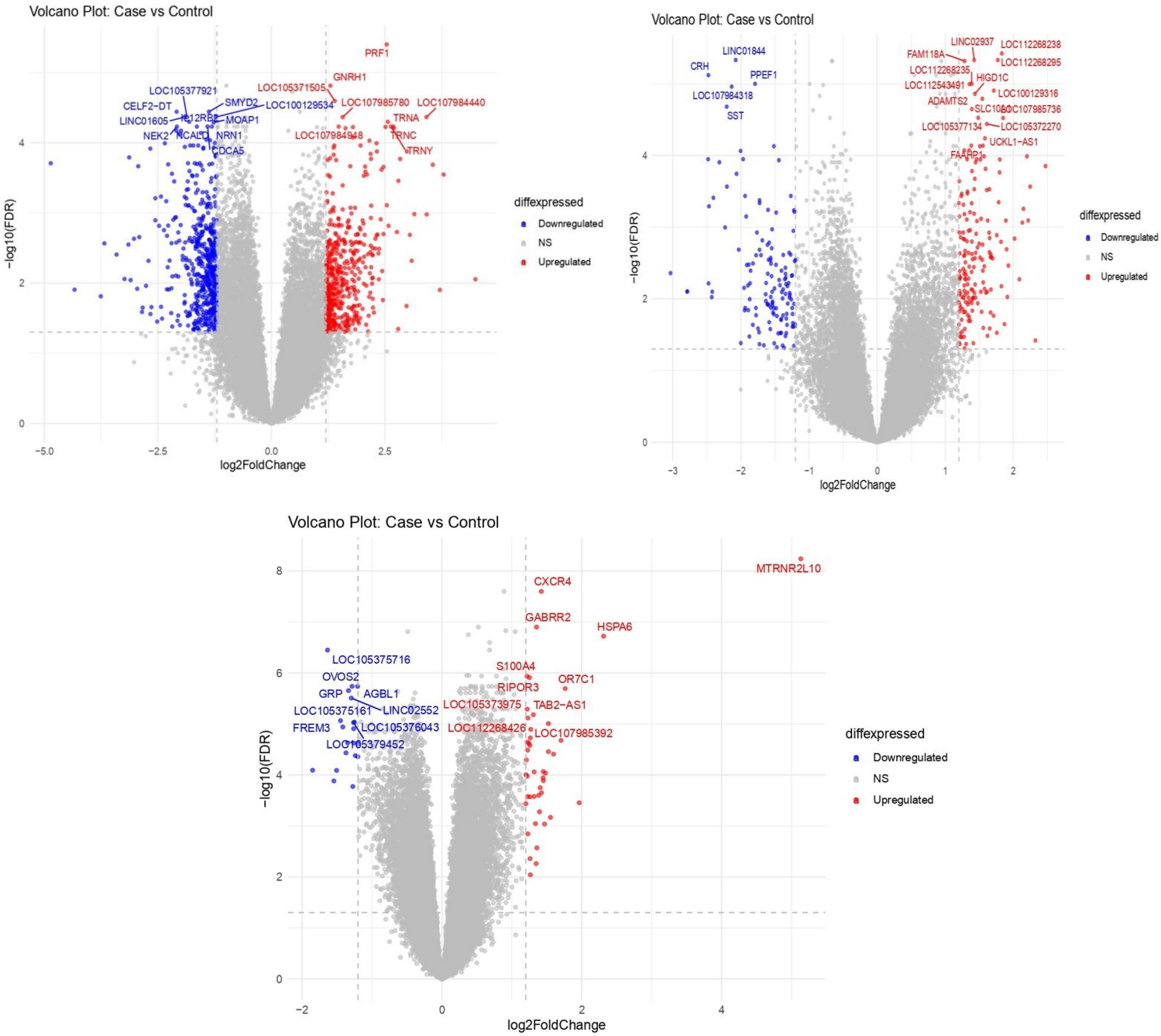

Fig:2 DEGs screening in AD IN DATASETS: GSE153873 GSE95587, and GSE173955.

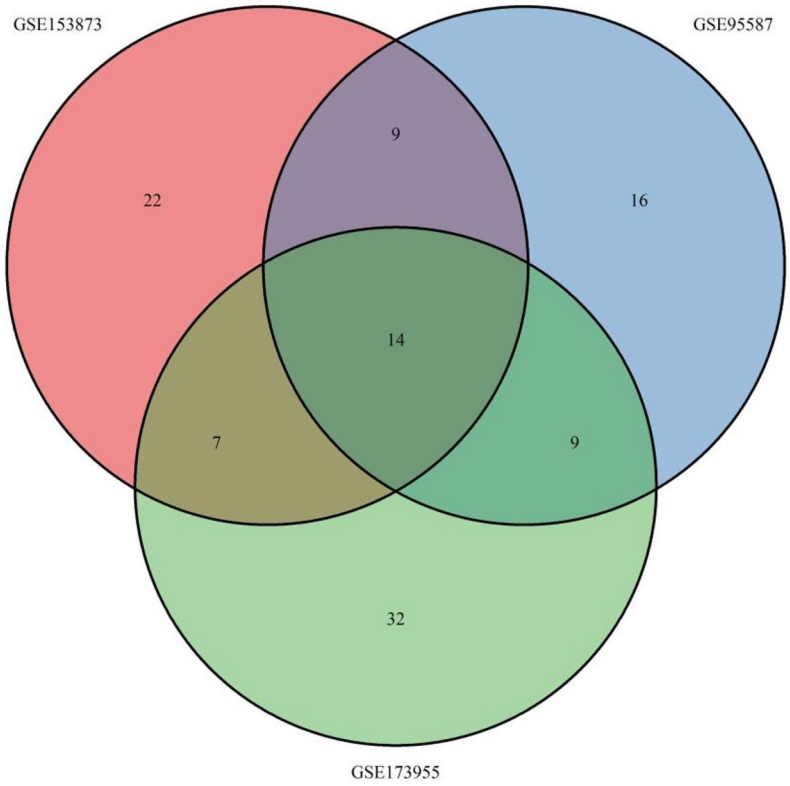

Fig: 3 Venn diagram showing overlapping genes of ferroptosis-autophagy-mitophagy related genes and DEG.

4.1. Expression of ferroptosis- autophagy- mitophagy related genes in AD

Functional link between the Ferroptosis-Mitophagy-Autophagy (FMA) axis and neurodegenerative disorders were established, further, suggesting that coordinated dysregulation of iron-dependent cell death and impaired mitochondrial and autophagic quality control mechanisms contributes significantly to the progression of Alzheimer's disease pathology. The Venn diagram represents the integrative analysis of ferroptosis-, autophagy-, and mitophagy-related differentially expressed genes (DEGs) identified independently from the three transcriptomic datasets (GSE153873, GSE95587, and GSE173955). Following differential expression analysis and pathway-based shortlisting, overlapping genes were systematically compared to determine conserved and dataset-specific signatures.

Through data analysis we identified 14 genes consistently dysregulated across all three cohorts, indicating a shared molecular core potentially underlying coordinated disruption of iron metabolism and mitochondrial quality control mechanisms in Alzheimer's disease. Pairwise comparisons revealed 9 shared genes between GSE153873 and GSE95587, 7 between GSE153873 and GSE173955, and 9 between GSE95587 and GSE173955. Whereas, 22, 16, and 32 genes were distinctively detected in GSE153873, GSE95587, and GSE173955, respectively, reflecting cohort-specific variations.

4.2. PPI Network construction of ferroptosis-mitophagy-autophagy related genes

Through a protein-protein network constructed, a significant crosstalk between iron metabolism, mitochondrial quality control and autophagic regulation was established. The nodes depicted as hub genes, potentially coordinating the regulation of redox balance, lipid peroxidation, and organelle turnover. Whereas , the overlapping

connections among these three biological processes support the idea of a unified Ferroptosis-Mitophagy-Autophagy (FMA) axis, rather than separate, isolated signaling pathways.

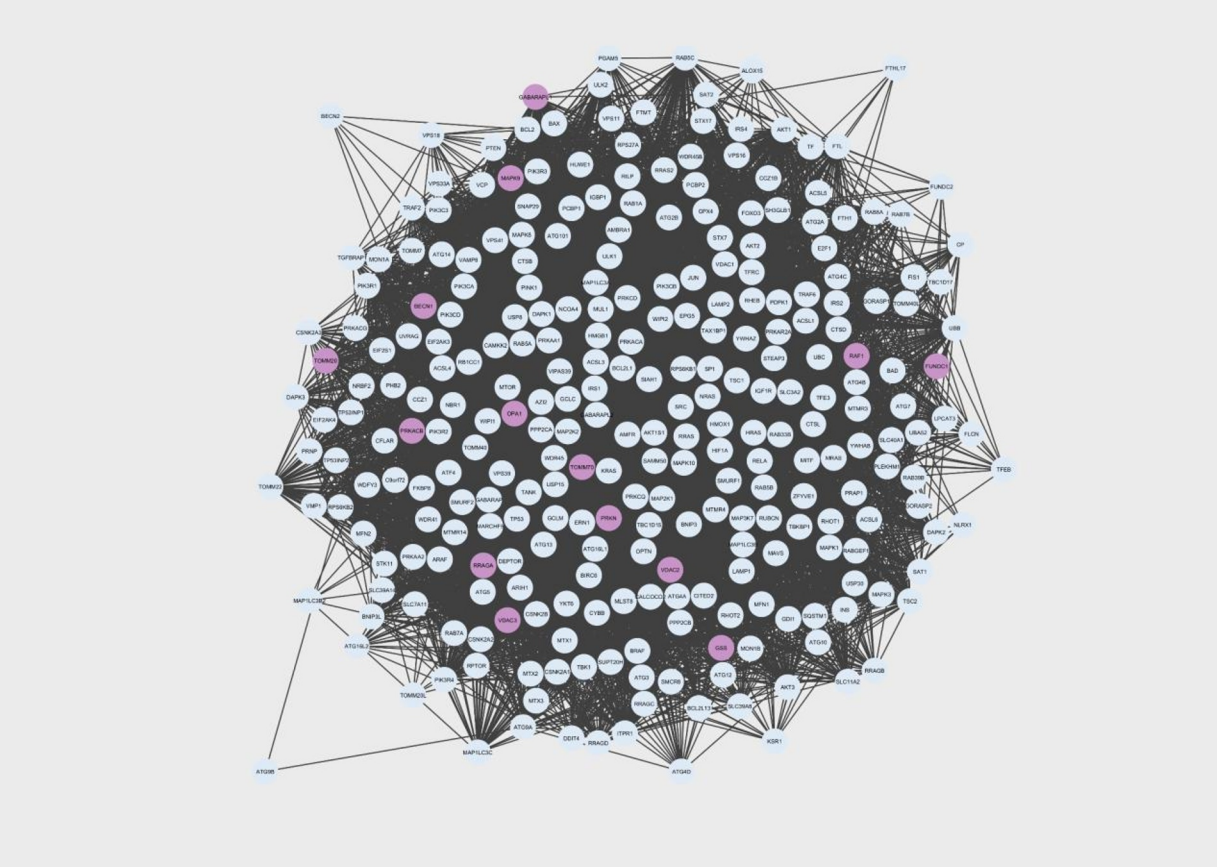

Fig: 4 Ferroptosis-Autophagy-Mitophagy-related gene interactions.

5. Conclusion

Acting as a potential catalyst for oxidative stress and as a cofactor in metabolic and repair processes, iron plays a dual role in neuronal physiology. Ferritinophagy, autophagy, and mitophagy works in concert under tightly regulated conditions, to maintain intracellular iron balance, remove damaged proteins and organelles, and sustain mitochondrial integrity. These integrated quality-control systems can prevent excessive labile iron accumulation, lipid peroxidation, and support efficient energy production. Thus maintaining these pathways, help to reduce amyloid-β burden, modulate tau pathology, and protect synaptic function, resulting in increased neuronal resilience. Whereas, the imbalance of this balance shifts iron metabolism toward both redox destabilisation and susceptibility to ferroptosis, leading to oxidative injury and neurodegeneration thus, balanced activity in the related Ferroptosis-Mitophagy-Autophagy axis would likely move iron from being a cause of toxicity to becoming a target for therapeutic interventions, offering potential biomarkers and intervention strategies for slowing the progression of Alzheimer's disease.

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