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# The expression of ferroptosis-related genes in osteoporosis based on GEO

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**Abstract. – OBJECTIVE: The aim of this study was to screen the differential genes related to ferroptosis in osteoporosis patients.**

**MATERIALS AND METHODS: GEO2R was used to screen the differential genes related to ferroptosis in osteoporosis patients by searching the relevant chips in the GEO database, and Spearman's correlation analysis was used to describe the correlation between quantitative variables without normal distribution. p-values lower than 0.05 were considered statistically significant. Another group of osteoporosis patients was selected in the GEO database to verify the significantly differentially expressed genes.**

**RESULTS: The results showed that 10 samples in chip GSE35956 were identified as research objects, and a total of 5 ferroptosis differential genes were screened out: *ATP5MC3, CDKN1A, MT1G, NCOA4, SLC1A5*, of which 3 up-regulated genes (*CDKN1A, MT1G, SLC1A5*), 2 down-regulated genes (*ATP5MC3, NCOA4*). The above differential genes were placed in 19 samples of chip GSE35959 for verification, and the same expression trend was obtained, but only the MT1G difference was statistically significant.**

**CONCLUSIONS: The gene correlation test found that *MT1G* and *ATP5MC3* had a strong negative correlation.**

*Key Words:*
Osteoporosis, Ferroptosis, GEO database, Differential gene, *MT1G*.

## Introduction

Osteoporosis is a prevalent etiological factor for fractures in individuals of middle and advanced age. Its primary pathological presentations involve a decline in bone mass and the deterioration of bone microarchitecture[1]. The equilibrium among osteoblasts, osteoclasts, and bone cells within the skeletal system serves as the principal determinant[2]. An excessive demise of bone cells and osteoblasts, coupled with the persistence of osteoclasts, can result in the disturbance of the metabolic equilibrium between bone formation and resorption, ultimately culminating in the development of osteoporosis. Recently, ferroptosis has received much attention as a newly discovered mode of programmed cell death characterized by depletion of glutathione leading to lipid peroxidation[3,4]. At the same time, many studies[5,6] have reported that ferroptosis plays an important role in the three types of cells above. For example, Gao et al[5] found that targeting ferroptosis can effectively save cell death in osteoporosis. Ling et al[6] found that melatonin significantly reduced the level of ferroptosis and had a bone-promoting effect by activating the Nrf2/HO-1 pathway. This suggests that inhibiting ferroptosis may be a potential therapeutic target for osteoporosis.

The Gene Expression Omni-Bus (GEO) database, established in 2000, is a comprehensive gene expression database developed and managed by the National Center for Biotechnology Information (NCBI) in the United States[7]. It encompasses high-throughput gene expression data, contributed by research institutions worldwide, ensuring unrestricted accessibility to researchers. The GEO database has garnered significant utilization in medical research endeavors within the past decade[8-10]. Consequently, this study employed the utilization of biological

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information data mining technology to examine the expression profile chip data of osteoporosis patients in the GEO database. The objective was to identify pivotal differentially expressed genes associated with ferroptosis and subsequently validate their correlation. This research establishes a foundation in the field of bioinformatics and offers novel avenues for future investigations into the molecular mechanisms underlying osteoporosis.

The objective of this study was to contribute novel insights into the diagnosis and treatment of osteoporosis, we conducted a study on the osteoporotic differential genes associated with ferroptosis, utilizing the GEO database.

## Materials and Methods

### Chip Data

In the GEO database (https://www.ncbi.nlm.nih.gov/geo/), a public database of gene expression profiles, the search term "osteoporosis" was used to search them, and then they were investigated one by one. Finally, the chip composite requirements numbered GSE35956 and GSE35959 were determined. The chip data contained 10 samples (5 cases in the control group and 5 cases in the experimental group) and 19 samples (5 cases in the control group, and 14 cases in the experimental group).

### Method

#### Differential gene screening

GEO2R (https://www.Ncbi.lm.nih.gov/geo/geo2r) was used to analyze the ferroptosis-related gene expression data of osteoporosis patients in the GSE35956 dataset in the GEO database and the screening thresholds were $*p < 0.05$, $**p < 0.01$.

#### Differential gene validation

The differentially expressed genes related to ferroptosis selected from the GSE35956 dataset in the GEO database were placed in the GSE35959 dataset for verification ($*p < 0.05$, $**p < 0.01$).

### Gene Correlation

We utilized the GEO database (https://www.ncbi.nlm.nih.gov/geo/) to acquire the dataset, downloading the data in MINiML format. We generated a boxplot using the downloaded data and created a PCA diagram using the ggord R software package. The two-gene correlation map is implemented by the R software package ggstatsplot, and the multi-gene correlation map is displayed by R software package pheatmap.

### Statistical Analysis

We used IBM SPSS Statistics 19.0 (IBM Corp., Armonk, NY, USA), Graph Pad Prism 8, and Excel 2010 to perform the statistical analysis of the data. The significance of two groups of samples was determined by the Wilcox test, and the significance of the three groups or more was determined by the Kruskal-Wallis test. Spearman correlation analysis was used to perform a two-gene correlation ($*p < 0.05$, $**p < 0.01$) between gene and gene expression.

## Results

### Mining of Differential Genes Related to Ferroptosis

Ten samples from the GSE35956 chip were selected as research objects, and GEO2R was used for preliminary analysis and screening. The screening criteria were $p < 0.05$ and $p < 0.01$. A total of 5 significantly different genes were obtained, among which 3 genes were up-regulated (*CDKN1A, MT1G, SLC1A5*). Two genes (*ATP5MC3 and NCOA4*) were down-regulated, and the distribution of differential genes is shown in Figure 1.

### Verification of Differential Genes

The 5 key differential genes selected were verified in 19 samples in the GSE35959 chip. The results showed that the osteoporosis group and the control group showed relatively evident expressions of iron-death-related genes. The expression levels of 3 key differential genes were up-regulated, while 2 genes were down-regulated in the samples of the osteoporosis group, as shown in Figure 2.

### The Correlation of Differential Genes

A correlation test was performed on 5 key genes. The correlation heat map and horizontal and vertical coordinates all represent genes, in which different colors represent correlation coefficients (red represents positive correlation, blue represents negative correlation). The darker the color, the stronger the correlation between the two; the results showed that *MT1G* and *ATP5MC3* had the most negative correlation, $*p < 0.05$. An asterisk stands for importance ($*p$) (Figure 3).

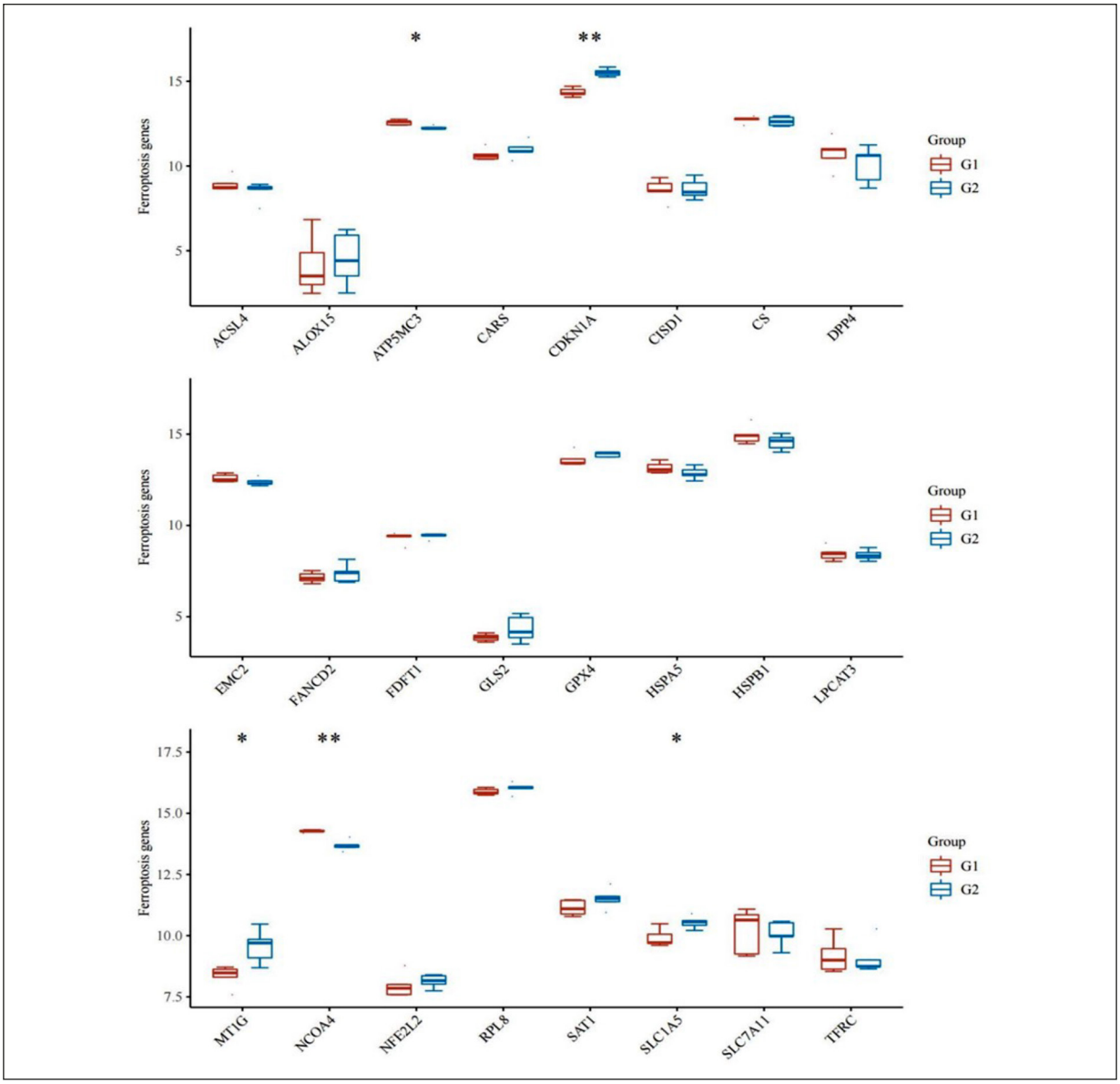


**Figure 1.** Mining of differential genes related to iron death. *$p < 0.05$, **$p < 0.01$.

## Discussion

Osteoporosis is a multifaceted pathological manifestation, governed by numerous transcription factors *in vivo*[11]. Research[12] has demonstrated a close association between bone metabolism and iron metabolism. Furthermore, ferroptosis is intricately linked to iron metabolism disorders and diverse bone diseases[13]. Consequently, it can be inferred that ferroptosis potentially exerts a direct impact on the advancement of osteoporosis. Given the escalating prevalence of osteoporosis patients in recent years, it is imperative to investigate the molecular mechanism underlying osteoporosis[14-16]. We employed bioinformatics techniques and tools to identify osteoporosis-specific genes associated with ferroptosis. Through the analysis of the GSE35956 chip data, it was determined that there were five differentially expressed genes related to ferroptosis in the context of osteoporosis (two down-regulated genes and three up-regulated genes). Utilizing the R software, *CDKN1A*, *MT1G*, and *SLC1A5* were identified as potential crucial genes involved in iron-induced cell death, all of which were up-regulated in the osteoporotic condi-

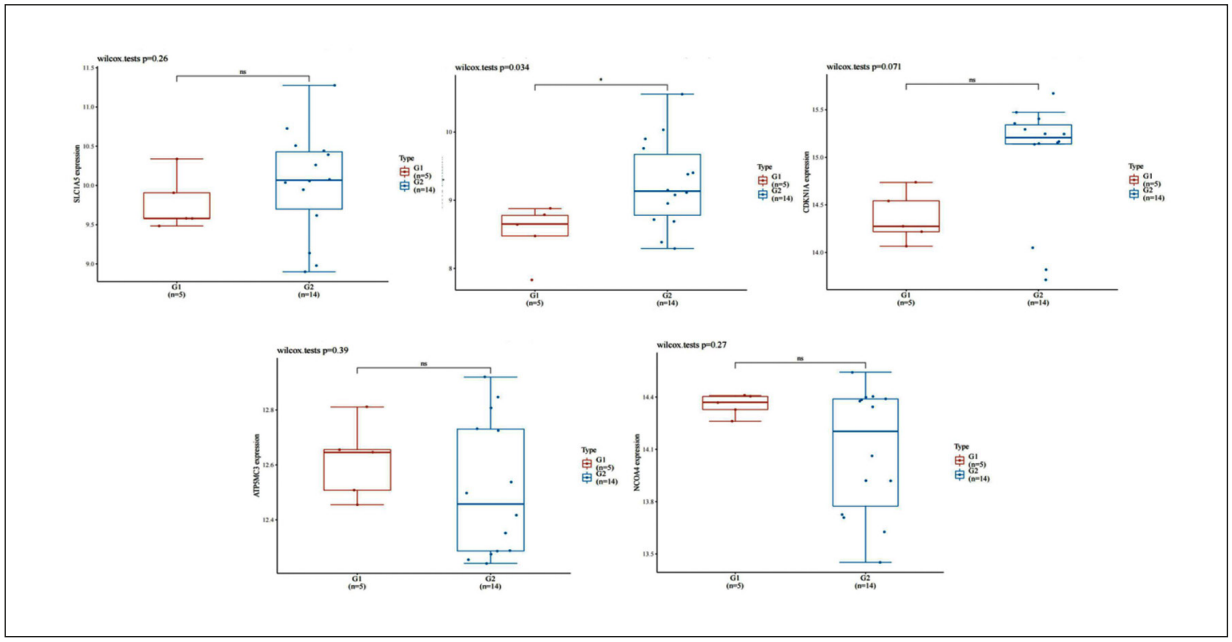


**Figure 2.** Verification of differential genes. *$p < 0.05$, **$p < 0.01$.

tion. This observation raises the possibility of a close association with osteoporosis. Studies[17] have shown the role of *CDKN1A* as an osteoporosis promoting factor, and knocking out *CDKN1A* can overcome the phenotypic and molecular changes in osteoporosis induced by dexmedetomidine treatment. Similarly, *CDKN1A* affects chondrocyte proliferation[18]. There have also been similar reports[19] of high expression of *CDKN1A* and cells entering G0 resting state, while low expression of *CDKN1A* and cell proliferation, indirectly demonstrating that *CDKN1A* inhibits cell metabolism. This study shows that *CDKN1A* plays an extremely important role in the growth and development of osteoporosis patients.

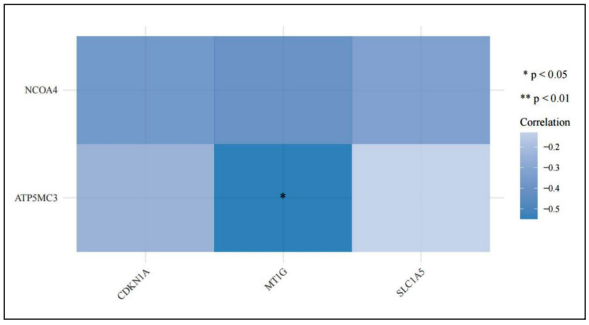


**Figure 3.** The correlation of differential genes. *$p < 0.05$, **$p < 0.01$.

Metallothionein 1G (*MT1G*), belonging to the metallothionein family (*MTs*), is a conserved protein with low molecular weight and high cysteine content. It interacts with the promoter region of DNA, thereby modulating the transcriptional expression of various genes, including stress-inducing genes[20,21]. Recent studies[22] have indicated that *MT1G* plays a role in sorafenib resistance by inhibiting ferroptosis, a novel mechanism of cell death regulation. The application of RNA interference to knock out *MT1G* leads to an elevation in glutathione depletion and lipid peroxidation, ultimately resulting in the induction of sorafenib-induced ferroptosis. In light of these findings, it can be concluded that this study has elucidated the underlying molecular mechanism responsible for sorafenib resistance, thereby proposing *MT1G* as a novel regulator of ferroptosis in hepatocellular carcinoma cells.

In this study, it is hypothesized that the overexpression of the *SLC1A5* gene may be induced in the presence of osteoporosis. Under normal physiological conditions, *SLC1A5* plays a crucial role in cell survival and growth. However, when exposed to Erastin and *RSL3*, *SLC1A5* promotes ferroptosis[23]. Transcription factors associated with iron-induced cell death have been identified in various tissues, including the brain[24], lung[25], skeletal muscle[26], testis[27], adipose tissue[28], large intestine, and kidney tissue[29]. Furthermore,

previous studies[30] have reported a correlation between *SLC1A5* and osteoclast formation, thus supporting the notion that *SLC1A5* is implicated in osteoporosis.

*ATP5MC3* has been implicated in iron-mediated cell death across various diseases, including clear cell carcinoma of the kidney. Additionally, a novel variant of *ATP5MC3* has been linked to dystonia and spastic paraplegia. In the present investigation, we observed a down-regulation of the *ATP5MC3* iron-death gene in osteoporosis patients, a finding that warrants further validation through basic experiments or animal models.

## Conclusions

In conclusion, the analysis of GSE35956 in the GEO database revealed the identification of five significant differential genes, namely *CDKN1A*, *MT1G*, *SLC1A5*, *ATP5MC3*, and *NCOA4*, under osteoporotic conditions. These genes potentially contribute to the advancement of osteoporosis by modulating ferroptosis through interaction. Consequently, these findings offer potential avenues for the discovery of novel preventive and therapeutic interventions for osteoporosis.

### Conflict of Interest
The authors declare that they have no conflict of interests.

### Informed Consent
Not applicable.

### Funding
None.

### Ethics Approval
Not applicable.

### Data Availability
The data used and analyzed during this research are available from the corresponding author upon reasonable request.

### Authors' Contribution
Concept: L.L.W., Y.X.S., H.H.C., Z.Z.L., Y.M. J., S.A.; Design: L.L.W., Y.X.S., H.H.C., S.A; Supervision: L.L.W., Y.X.S., H.H.C., S.A; Funding: L.L.W., Y.X.S., H.H.C., S.A; Materials: L.L.W., Y.X.S., H.H.C., Z.Z.L., Y.M. J., S.A; Data: L.L.W., Y.X.S., H.H.C., Z.Z.L., Y.M. J., S.A; Literature search: L.L.W., Y.X.S., H.H.C., Z.Z.L., Y.M. J., S.A; Writing: L.L.W., Y.X.S., H.H.C., Z.Z.L., Y.M. J., S.A; Critical revision: L.L.W., Y.X.S., H.H.C., S.A.

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