Identification of mitophagy-related subgroups and biomarker in AIS osteopenia based on bioinformatics and machine learning approaches

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Article

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Abstract

Background: Adolescent idiopathic scoliosis (AIS) is a complicated spinal deformity with an unknown origin. The progression of AIS and the severity of spinal curvature are both substantially linked to osteopenia. Mitophagy is critical for the balance of osteogenic and osteoclastic development in BMSCs, but its significance in AIS osteopenia is unknown. The goal of this work was to look into the mechanism of mitophagy in AIS osteopeniapatients' BMSCs and give a new diagnostic and therapeutic target for AIS osteopenia.

Methods: The gene expression profiles of BMSCs from AIS patients are available in the Gene Expression Omnibus (GEO) collection. Consensus cluster analysis of mitophagy-related genes was used to identify molecular isoforms.Using machine learning, identify mitophagy-related diagnostic indicators of osteopenia in AIS. The biological function and immunological features of diagnostic biomarkers were then assessed using GSEA and ssGSEA.

Results: Immune cell infiltration was found to differ between mitophagy-related subtypes, implying that the development of AIS osteopenia may be associated with immune cell infiltration. UBA52 was identified as the best mitophagy-related diagnostic biomarker for osteopenia in AIS by machine learning methods, and GSEA revealed that UBA52 mostly affected osteopenia in AIS through oxidative phosphorylation. In addition, UBA52 regulates immune cell infiltration and may contribute to osteopenia in AIS patients.

Conclusion: According to our findings, AIS patients can be split into two mitophagy subgroups. Furthermore, we used machine learning to identify UBA52, a mitophagy-related diagnostic marker, and discovered that UBA52 played a significant role in increasing osteopenia in AIS.

1. Introduction

Adolescent idiopathic scoliosis (AIS) is a complicated spinal deformity characterized by three-dimensional curvature, the etiology of which remains unidentified. This condition primarily manifests in individuals between the ages of 10 and 18 years[1–3].AIS can raise the risk of respiratory failure and cardiovascular disease, resulting in a poor prognosis and considerably increased mortality[4]. The primary treatment for AIS currently is corrective surgery, which can have a negative impact on patients' quality of life and significantly increase their financial burden[5, 6]. Hence, comprehending the biomolecular mechanism underlying the pathogenesis of AIS and devising genetic-level interventions and treatments are crucial areas of investigation. These endeavors hold the potential to enhance the effectiveness and timeliness of AIS treatment.

The etiology of AIS remains unclear at present. Various theories regarding the occurrence of AIS adamantly asserts that AIS is a complicated illness impacted by a number of variables, including bone metabolism, the central nervous system, genetics, biomechanics, and other relevant aspects[7–10]. Since the initial publication by the Burner team in 1982, there has been a growing scholarly interest in the matter of osteopenia in AIS[11, 12]. Osteopenia refers to a condition characterized by a Z-score of

femoral neck areal bone mineral density (aBMD) that falls below − 1[13].The prevalence of AIS osteopenia ranges from 20–38%, which is much greater compared to the occurrence observed in normal teenagers[14]. High blood bone turnover markers in AIS patients are associated with a high likelihood of AIS curve progression, according to temporal study of local bone production and resorption[15]. An increasing number of experts subscribe to the notion that the evolution of AIS and the severity of the spinal curvature may be attributed, at least in part, to bone loss[16–18]. Nevertheless, the exact cause of osteopenia associated with AIS has not been completely understood. The primary objective of this investigation was to examine the molecular mechanisms behind osteopenia associated with AIS and identify prospective targets for the diagnosis and therapy of AIS-related osteopenia.

Bone marrow stem cells (BMSCs) serve as precursor cells for the development of bone tissue and adipose tissue throughout the process of bone marrow cavity creation. The maintenance of bone homeostasis relies on the delicate equilibrium between the development of BMSCs into osteogenic and adipogenic lineages[19]. Furthermore, a growing body of research in recent years indicates that the dysregulation of mitophagy levels has a detrimental impact on the equilibrium of bone metabolism and serves as a pivotal factor in the development of bone-related disorders[20]. Mitochondrial autophagy pertains to the process by which cells selectively degrade and eliminate old or damaged mitochondria by autophagy. This mechanism is crucial for maintaining mitochondrial homeostasis and preserving the integrity of the intracellular environment[21]. Several studies have documented the role of SIRT3 in promoting osteoclast development and production, primarily through its stimulation of mitophagy[22]. The involvement of PINK-Parkin signaling has been observed to have significant implications in the context of mitophagy-related damage in osteoblasts and osteoclasts associated with osteoporosis[23]. Nevertheless, there is currently a lack of research on the investigation of mitophagy in osteopenia associated with AIS. Our study aims to identify diagnostic biomarkers related to mitophagy in the genetic profile of BMSCs in AIS patients using machine learning algorithms. Additionally, we seek to explore a novel molecular mechanism perspective of AIS-related osteopenia.

2. Methods and materials

2.1. Study design

To enhance comprehension of the research methodology, we have depicted the sequential progression of our investigation in Fig. 1.

2.2. Data preparation

The keyword "adolescent idiopathic scoliosis" was used to search the GEO database, resulting in the retrieval of the dataset GSE110359, which contains the expression profile of BMSCs. The study consisted of a sample size of 12 persons who were diagnosed with AIS, along with an additional 5 healthy controls. In the R programming language, the data underwent background correction and normalization using the

"limma" package. Furthermore, a comprehensive set of 29 genes associated with mitophagy was acquired from the GeneCards database (https://www.genecards. org) .

2.3. Clustering of Mitophagy-driven subgroups in AIS

Following the extraction of mitophagy-related gene expression profiles from GSE110359, the "ConsensusClusterPlus" R software was used to classify mitophagy-related molecular subtypes based on the expression of these genes. Principal component analysis(PCA), was used to show how the subtypes differed from one another.

2.4. Evaluation and comparison of immune-infiltrating cells

The analysis of immune cell infiltration in AIS patients, the investigation of immune cell infiltration in distinct subgroups, and the monitoring of changes in the relationship between immune infiltrating cells and biomarkers were all conducted using single-sample gene set enrichment analysis (ssGSEA). "Limma", "reshape2 ", "ggplot2" and "tidyverse" applications were utilized to evaluate and present the results.

2.5. Machine learning algorithms for detecting diagnostic biomarkers

Three machine learning algorithms were utilized to screen mitophagy-related diagnostic indicators of osteopenia in AIS: random forest (RF), support vector machine recursive feature elimination (SVM-RFE), and least absolute shrinkage and selection operator (LASSO). "RandomForest", "e1071", and "glmnet" were respectively used to conduct LASSO regression, SVM, and RF. Using venn diagrams, identify the best diagnostic biomarkers related with mitophagy in AIS osteopenia.

2.6. Differential expression and ROC curve analysis of diagnostic biomarker

A t-test was employed, with a statistical significance level set at $p < 0.05$, to evaluate the disparities in diagnostic biomarkers between the cohorts with the disease and the control cohorts. Following that, the diagnostic effectiveness of diagnosis biomarker for disease diagnosis was evaluated by the utilization of receiver operating characteristic (ROC) analysis, employing the "pROC" package.

2.7.GSEA of the diagnostic biomarker

Gene set enrichment analysis (GSEA) was utilized to find functional categories and pathways enriched for diagnostic biomarkers in order to conduct additional research into the potential role of diagnostic biomarkers in osteopenia in AIS. The R package "clusterprofiler" was utilized to perform GSEA.The reference gene set was h.all.v6.2.sytmbols.gmt from the Molecular Signatures Database (MSigDB), and the cut-off criteria was p adjusted value 0.05.

2.8. Statistical analysis

The analytical approach involved the utilization of R program version 4.2.3. This version facilitated the application of t-tests, as well as non-parametric tests, to examine variations in data distribution properties among different groups.In order to establish statistical significance, it is necessary for the p-values to be below the threshold of 0.05, indicating a significant level of significance.

3. Results

3.1. Identification of Mitophagy molecular subtypes

To give a comprehensive and simple depiction of the distinctive methodology employed in this work, the analytical process is clearly illustrated in Fig. 1. Through the utilization of consensus cluster analysis, we were able to identify two distinct subtypes of mitophagy in individuals with osteopenia associated with AIS. Cluster A consisted of nine patients, while cluster B consisted of three patients (Fig. 2A). The correlation analysis revealed a significant association between mitophagy-related genes and immune cell infiltration in AIS (Fig. 2B). The application of PCA unveiled statistically significant variations in the observed distribution patterns between two clearly defined clusters (Fig. 2C). Furthermore, notable disparities in the majority of mitophagy genes were seen when comparing the two categories (Fig. 2D).

3.2. Detailed examination of DEGs linked to Mitophagy subtypes

In order to provide more insight into the functional importance of the previously indicated mitophagy pattern, 57 differently expressed genes (DEGs) were discovered between the two mitophagy isoforms (Fig. 3A). Based on these 57 DEGs, we next used a consensus clustering technique to split AIS patients into two DEGs subtypes (Fig. 3B-C). The PCA results show that the distributions of the two gene clusters differ(Fig. 3D) . Furthermore, as demonstrated by the ssGSEA results, we discovered that there were notable disparities in infiltration between the two genetic subtypes of plasmacytoid dendritic cells,Type 1 T helper cell and Type 17 T helper cell (Fig. 3E). These results confirm the efficacy of the mitophagy cluster.

3.3. Identification mitophagy related diagnostic markers

The LASSO regression analysis successfully discovered a set of nine genes that possess unique and discernible characteristics(Fig. 4A). Following that, a total of five diagnostic biomarkers were effectively identified by the utilization of SVM-RFE algorithm (Fig. 4B,C). Furthermore, RF was employed to pick the top five genes with the highest signature weights for further investigation(Fig. 4D,E). The present study utilized a Venn analysis approach to establish that UBA52 is the optimal mitophagy related diagnostic biomarker for osteopenia in AIS(Fig. 4F).

3.4. Efficacy verification of UBA52

Through the process of differential analysis, it was determined that the expression of UBA52 was dramatically elevated in AIS-BMSC (Fig. 5A). Furthermore, the study of the ROC curve demonstrated that UBA52 had favorable diagnostic efficacy, as evidenced by an AUC value of 0.825 (Fig. 5B).

3.5. GSEA of UBA52 in AIS osteopenia

We discovered that UBA52 was primarily localized in the inner mitochondrial membrane and the mitochondrial matrix in AIS BMSCs, based on GO of GSEA (Fig. 6A). Furthermore, the GSEA-based KEGG effective pathway enrichment study indicates that oxidative phosphorylation is the primary enrichment of the UBA52 effective route(Fig. 6B).

3.6. Analysis of immune cell infiltration

In the study conducted on dataset GSE110359, the application of single-sample gene set enrichment analysis (ssGSEA) revealed notable disparities in the immune cell infiltration patterns between the disease and control cohorts. Specifically, the analysis indicated that CD56dim natural killer cells and Mast cells had decreased numbers in AIS when compared to the corresponding normal samples (Fig. 7A,B). Furthermore, UBA52 exerts a positive correlation modulation on MDSC, gamma delta T cells, and activated CD8 T cells in AIS (Fig. 7C).

Discussion

The investigation of pathogenic mechanisms behind bone abnormalities has emerged as a prominent research focus in the quest to comprehend the pathophysiology of AIS[3]. Moreover, a growing body of evidence indicates that the dysregulation of mitophagy is a significant factor in the process of stem cell differentiation and the development of bone metabolism diseases[24, 25]. Hence, it was hypothesized that genes associated with mitophagy may play a role in the pathogenesis and advancement of osteopenia in AIS. To investigate the underlying biological mechanism of mitophagy in relation to osteopenia in AIS, an extensive analysis of AIS-BMSC gene expression profiles was conducted utilizing bioinformatics analysis and machine learning algorithms.

Based on mitophagy gene classification, we conducted a consistent cluster analysis of AIS patients in this study. The findings indicated that two distinct subtypes of AIS could be distinguished by mitophagy-related genes. We further conducted consistent cluster analysis of AIS patients based on the differential genes of the subtypes and generated two subtypes in order to further explore the role of the aforementioned two mitophagy subtypes. Prior research has demonstrated that regulatory T cells, also known as Treg cells, suppress osteoclast development and production, lower osteoclast activity, and negatively control osteoclasts in bone metabolism[26, 27]. In order to determine whether mitophagy-related genes may regulate Treg cells and impact bone metabolism in AIS patients, we performed a differential analysis of immune cell infiltration by ssGSEA in DEGs subtypes. The results demonstrated that there were significant differences in type 1 T helper cell and type 17 T helper cell infiltration between the two subtypes.

Machine learning has been extensively used in biomedicine to enhance diagnosis and therapy[28].In recent years, the utilization of advanced machine learning algorithms for the detection of medical biomarkers has been increasingly prevalent[29]. Finally, using three machine learning methods, we were able to identify UBA52 as a mitophagy-related diagnostic biomarker for osteopenia in AIS. The Ubiquitin-52 amino acid fusion protein (UbA52) has been extracted from the library of the human genome and is comprised of five exons that are dispersed throughout a span of 3,400 base pairs[30]. UBA52 can be broken down into RPL40 and ubiquitin by cleaving, with ubiquitin located at the amino terminus and RPL40 at the carboxyl terminus[31]. Ubiquitination is a common posttranslational modification that causes substrate breakdown[32]. Additionally, the gene UBA52 plays a crucial role in the early stages of embryonic development prior to implantation, and it has been found to have the ability to facilitate the formation of tumors in non-small cell lung cancer and colorectal cancer[33, 34]. UBA52 has not previously been described in AIS-related osteopenia, and this work discovered that UBA52 was considerably overexpressed in BMSCs from AIS patients, and that GSEA showed that UBA52 is mainly involved in oxidative phosphorylation (OXPHOS) regulation. One of the most essential metabolic functions of mitochondria is OXPHOS[35]. OXPHOS is involved in energy balance and is thought to be a potential target for a number of disorders[36, 37]. Previous research has shown that mitochondrial malfunction and decreased OXPHOS activity always result in osteogenic differentiation of BMSCs[38]. Thus, UBA52 may affect osteogenic differentiation of BMSCsd in AIS patients and promote osteopenia in AIS by attenuating mitochondrial function and activity of OXPHOS. In addition, the immune system affects skeletal development because it has a shared developmental niche with bone. Innate and adaptive immune cells can contribute to the pathophysiology of osteopenia by generating pro-inflammatory mediators[39]. For instance, Th17 cells play a major role in beginning and promoting osteoclast production, or bone resorption[40]. According to our research, UBA52 may encourage the infiltration of immune cells such as MDSC, gamma-delta T cells, and activated CD8 T cells. This infiltration may raise inflammatory factors and have an impact on the osteogenic differentiation of BMSCs.

Although the survey's findings were positive, it was important to realize that there were certain limitations.It is prospective investigations are necessary to establish the existence of identified predictive markers. In order to determine a connection between osteopenia and mitophagy phenotypes in AIS, mechanistic studies are also necessary. Therefore, it is crucial to carry out more practical experimental studies in the future study.

Conclusion

We identified various mitophagy-related subtypes and signature genes in AIS osteopenia based on cluster analysis and machine learning of mitophagy gene expression in AIS patients. This helps to provide new ideas for mitophagy-related pathogenesis of AIS osteopenia and provide new directions for future research.

Declarations

Author Contributions

Xigao Cheng designed the study. Jiahao Liu searched the data from the database. Jiahao Liu and Hui Wu performed the analysis of the data. Xinxin Miao wrote the original draft of the manuscript. Tianlong Wu supervised this work revised the manuscript. All authors had read and approved the final manuscript.

Availability of data and materials

The data in its entirety were accessible through the GEO database (https://www.ncbi. nlm.nih.gov/geo/). The data analyzed and presented in this manuscript are available upon reasonable request from the corresponding author.

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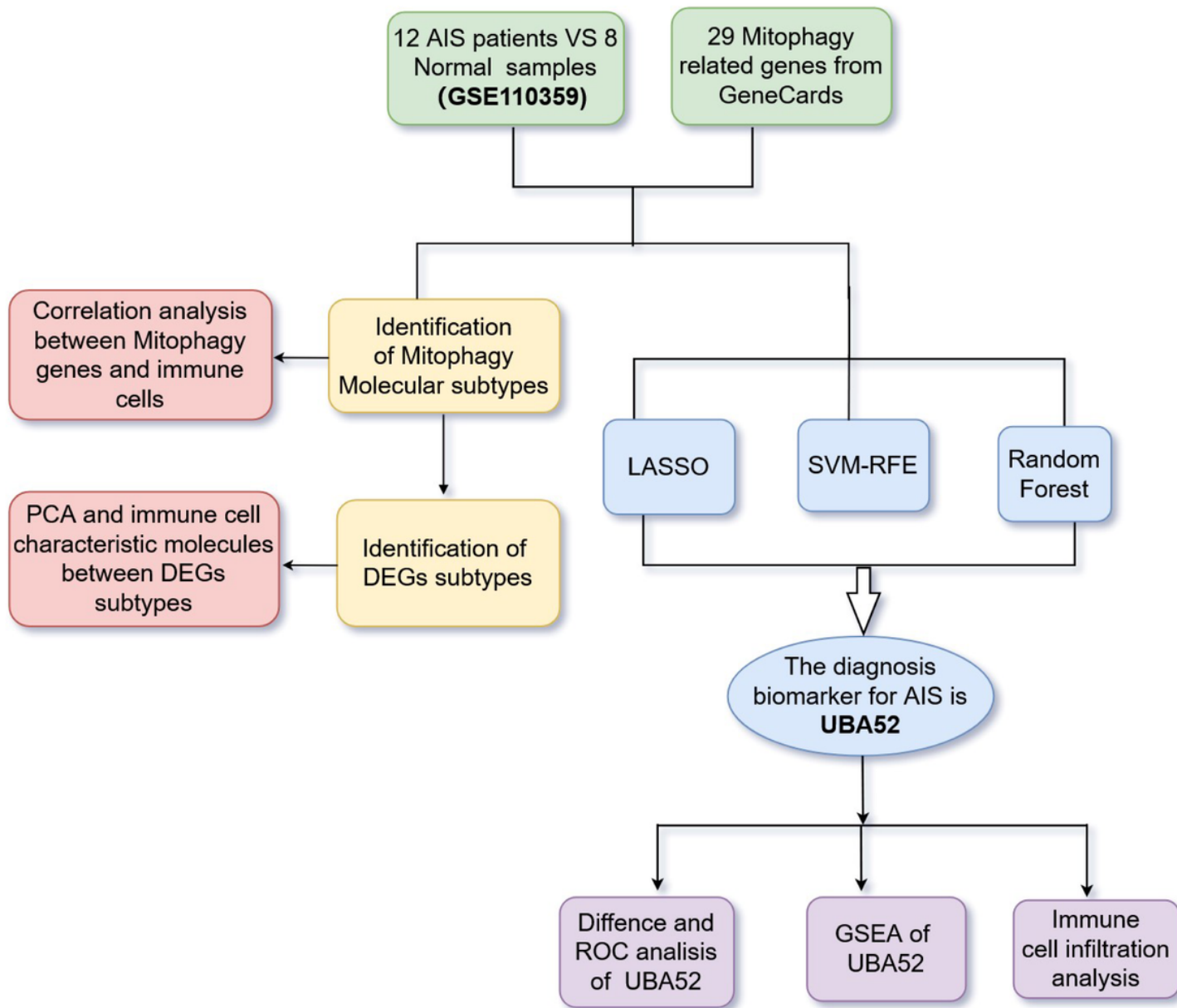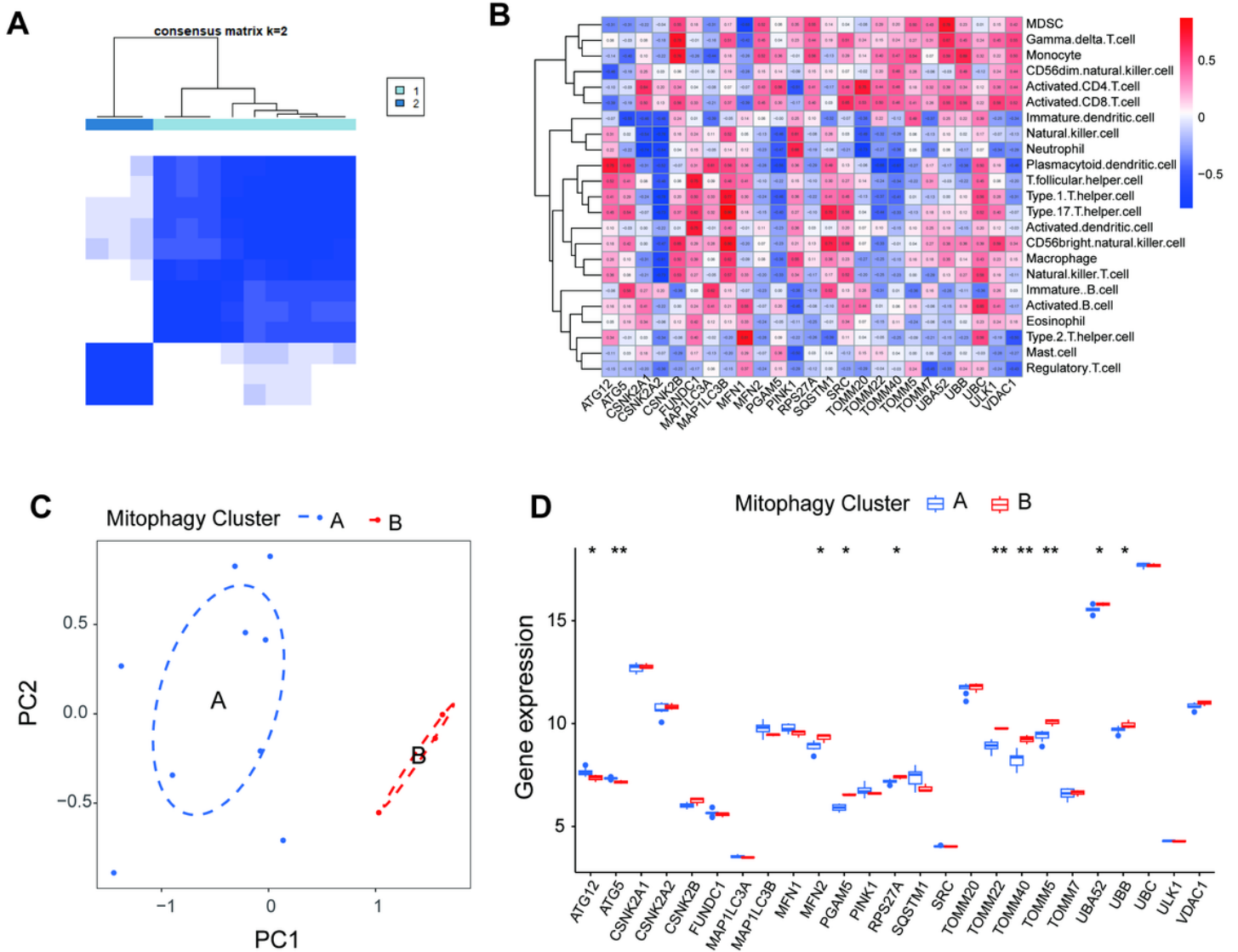
Figures

Figure 1

Analysis flow chart

Figure 2

Identification of Mitophagy Molecular subtypes

(A) The consensus matrix of 12 samples when *k* = 2. (B) Analysis of the correlation between mitochondrial autophagy genes and immune cells. (C) PCA plot of the two subtypes.(D) Analysis of differences in mitochondrial autophagy genes between subtypes, *P<0.05, **P<0.01 and ***P<0.001.
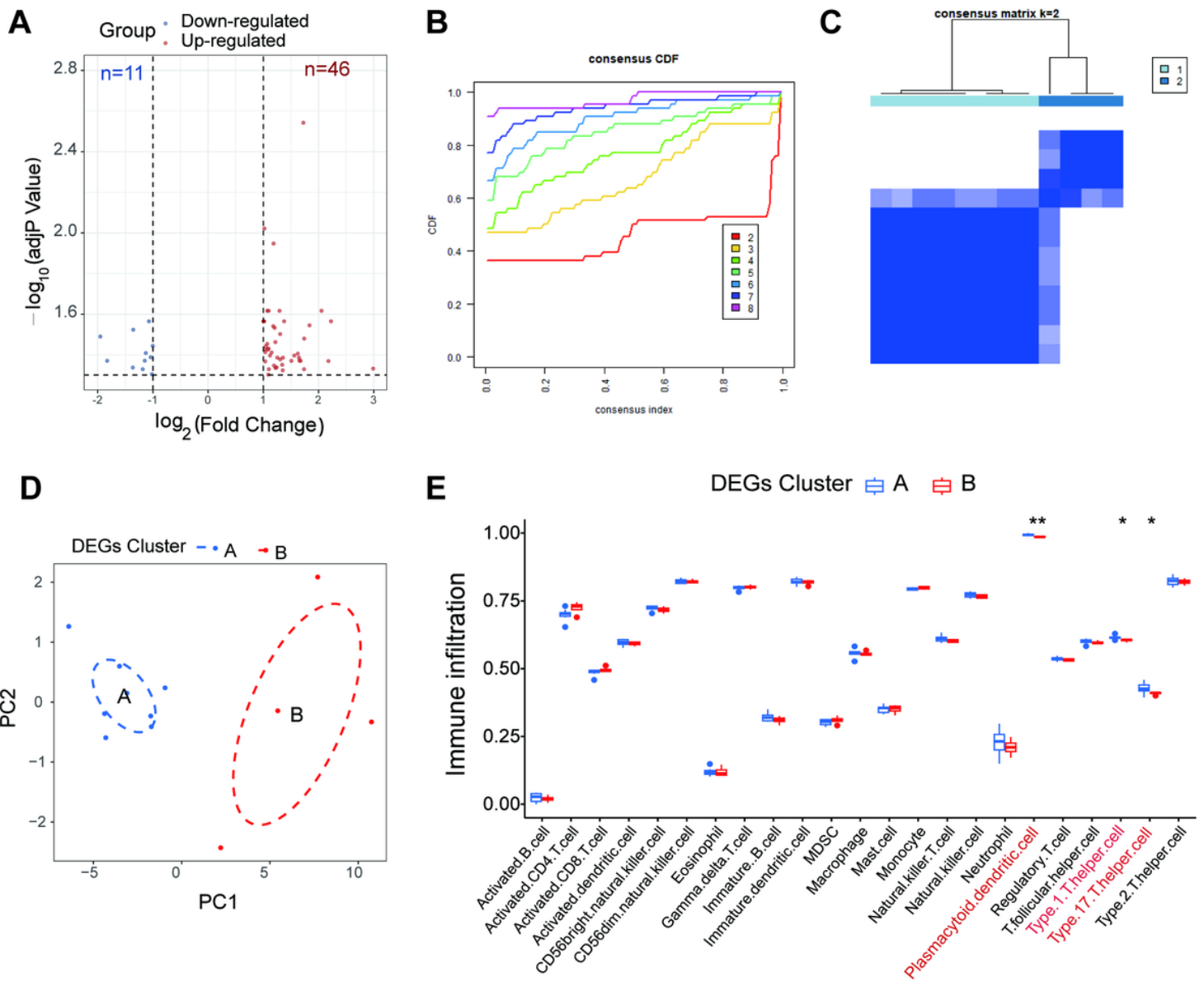
Figure 3

Comprehensive analysis of DEGs associated with Mitophagy subtypes

(A) The volcano plot of DEGs. (B) The CDF curve for *k* = 2-8. (C) The consensus matrix when *k* = 2. (D) PCA plot of the two DEGs subtypes. (E) The immune cell infiltration between the two subtypes was analyzed by the ssGSEA.*P<0.05, **P<0.01 and ***P<0.001.

Figure 4

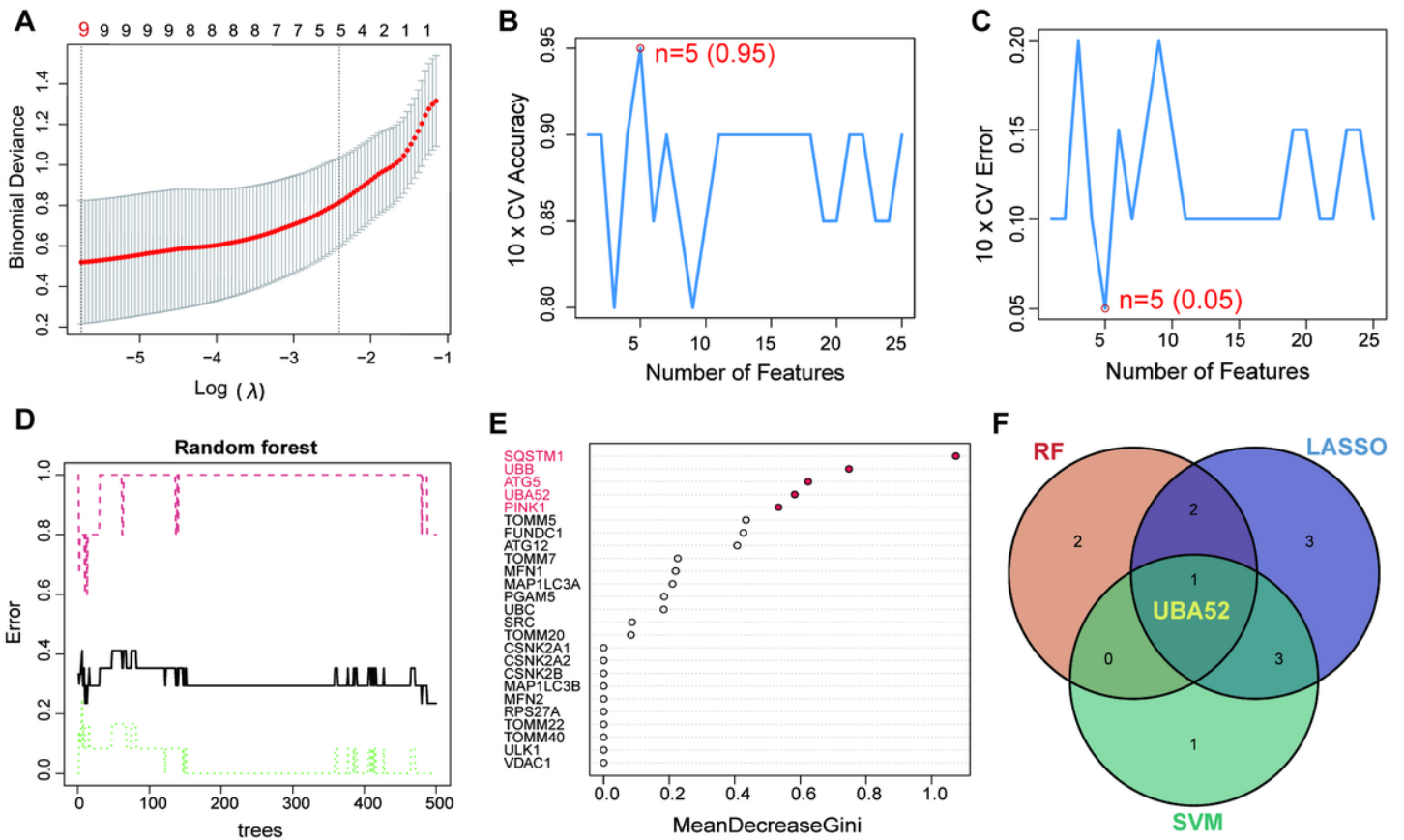**The screening of the diagnostic biomarkers in AIS osteopenia**

(A) Identification diagnostic biomarkers via LASSO. (B,C)Screening diagnostic biomarkers by SVM. (D,E) RF to screen mitophagy related diagnostic biomarkers in AIS osteopenia. (F) Veen plot showing best diagnostic biomarker as UBA52.
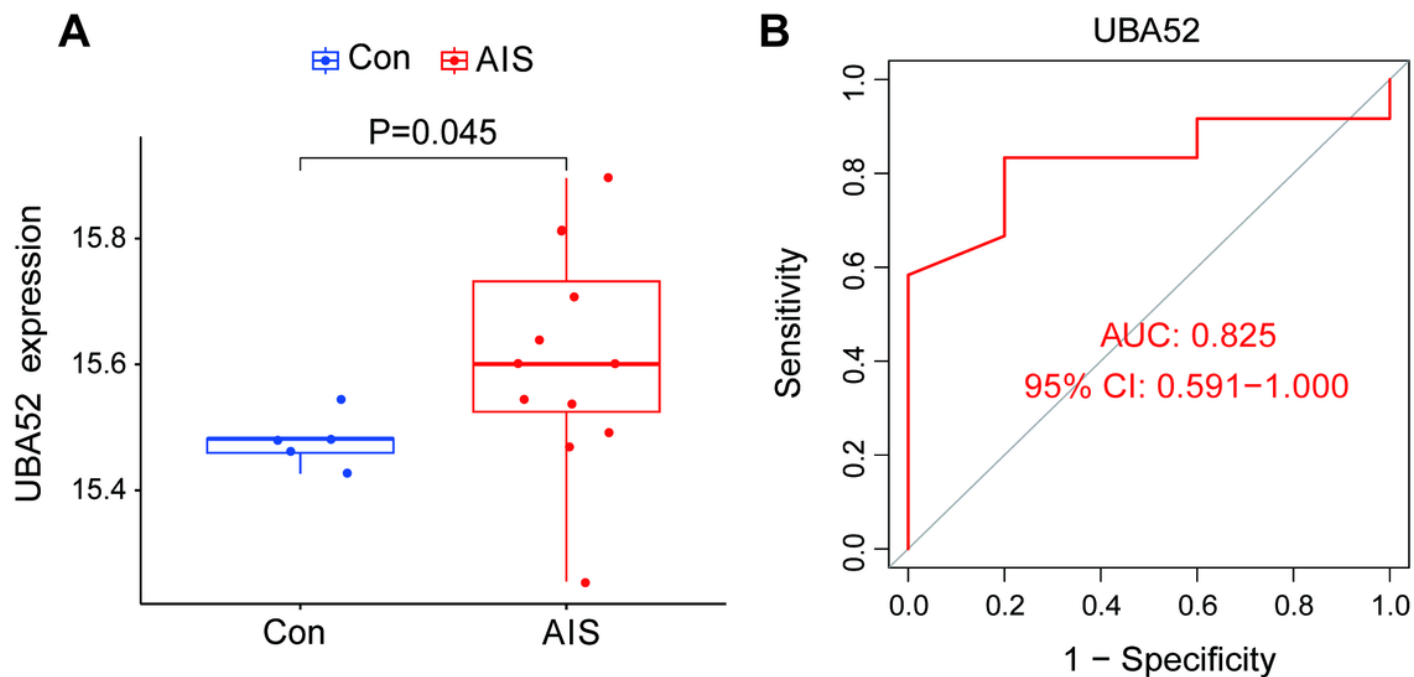
Figure 5

The validation of the diagnostic biomarker

(A) Differential expression of UBA52 in GSE110359. (B) ROC curve evaluating the UBA52 diagnostic efficacy in GSE110359.
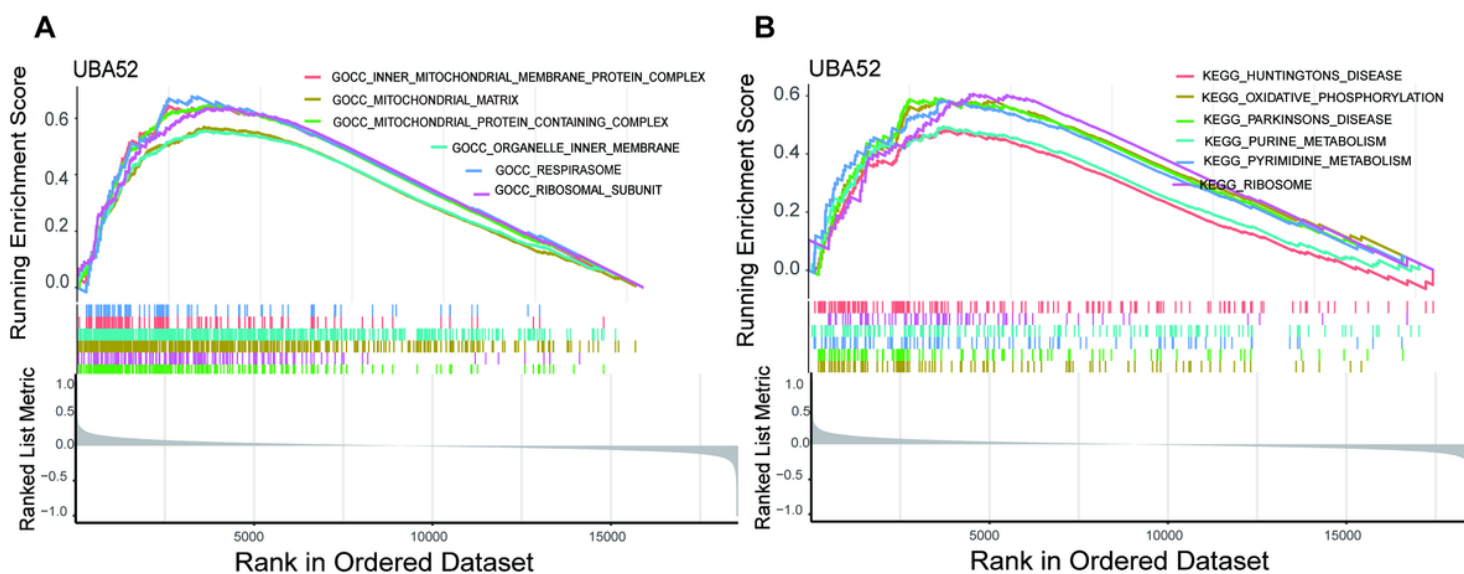

Figure 6

GSEA and GSVA of UBA52 in AIS osteopenia

(A) GO analysis based on GSEA of UBA52 in AIS osteopenia. (B) KEGG analysis based on GSEA of UBA52 in AIS osteopenia.