

# Bioinformatics Analysis of Oxidative Stress-Related Genes and Immune Infiltration Patterns in Vitiligo

Mingmei Yang<sup>1,2</sup>, Huiying Wang<sup>1</sup>, Ruzhi Zhang<sup>1</sup>

<sup>1</sup>Department of Dermatology, Third Affiliated Hospital of Soochow University, Changzhou, Jiangsu Province, 213003, People's Republic of China;

<sup>2</sup>Department of Dermatology, Affiliated Changzhou Children's Hospital of Nantong University, Changzhou, Jiangsu Province, 213003, People's Republic of China

Correspondence: Ruzhi Zhang, Email zhangruzhi628@163.com

**Background:** Vitiligo is an autoimmune disorder characterized by pigment loss, and current treatment options remain inadequate.

**Objective:** This study aims to identify oxidative stress-related biomarkers and hub genes associated with vitiligo diagnosis through genomic analysis and to examine the role of immune cell infiltration in the pathogenesis of vitiligo.

**Methods:** The mRNA expression profile dataset GSE75819 was retrieved from the GEO database. Differential expression of oxidative stress-related genes in vitiligo was analyzed using R software. Protein-protein interaction (PPI) analysis, gene ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted on the differentially expressed genes (DEGs). Immune cell infiltration between vitiligo and normal control groups was assessed using the CIBERSORT algorithm. Additionally, two machine learning algorithms were employed to identify hub genes, perform enrichment analyses, and evaluate their correlation with immune infiltration.

**Results:** A total of 415 Oxidative Stress-DEGs were identified in vitiligo, including 317 up-regulated and 98 down-regulated genes. PPI analysis highlighted the significance of certain ribosomal protein genes. KEGG enrichment analysis suggested an association between vitiligo and various neurodegenerative conditions, particularly through pathways such as oxidative phosphorylation and ribosome biogenesis. GO enrichment analysis indicated that the hub genes were significantly enriched in mitochondrial-related activities. Significant differences in immune infiltration patterns were observed between vitiligo patients and normal controls. Machine learning algorithms identified oxidative stress-related key genes associated with vitiligo, notably the DCT gene, whose expression was strongly linked to the activity of specific immune cell subsets and melanin biosynthetic pathways.

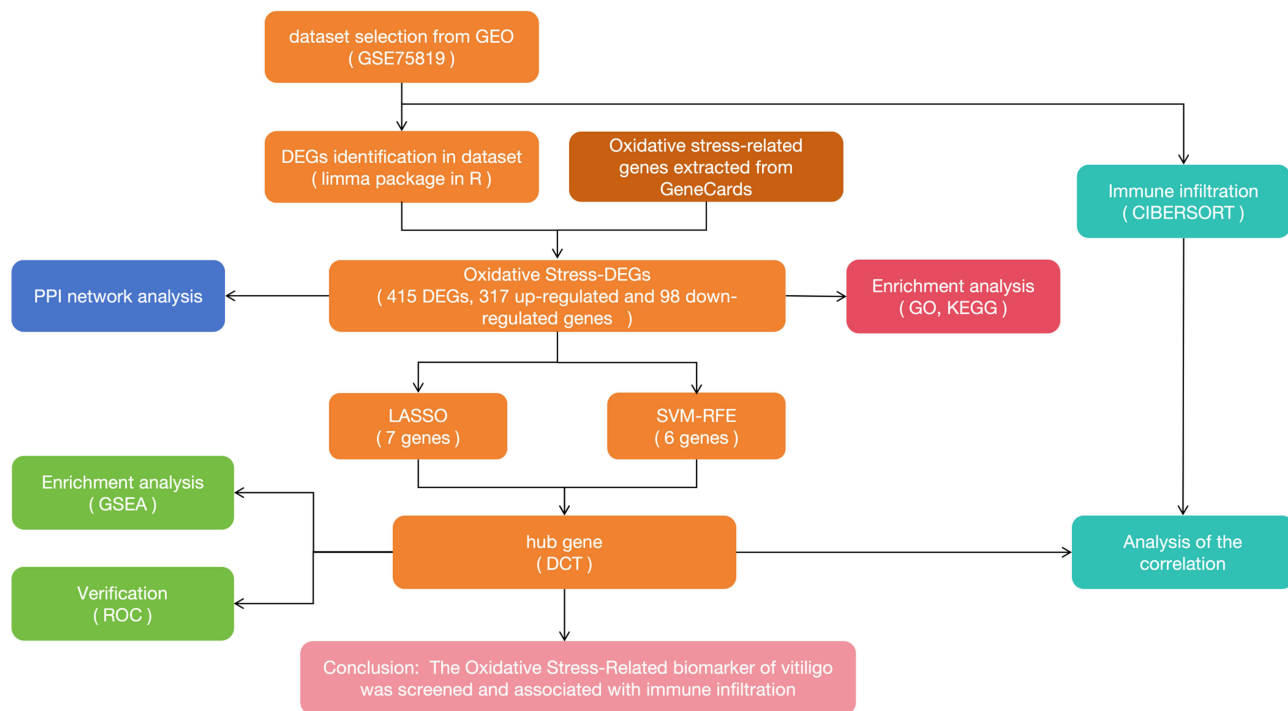
**Conclusion:** Oxidative stress-related DEGs, ribosomal proteins, immune infiltration, and hub genes related to melanin biosynthesis, particularly DCT, are closely associated with the pathogenesis of vitiligo. These findings enhance our understanding of vitiligo and may aid in identifying therapeutic targets for the disease.

**Keywords:** vitiligo, oxidative stress, DEGs, hub gene, DCT, immune infiltration

## Introduction

Vitiligo is a common autoimmune dermatological condition with an incidence of approximately 0.5% to 1%, characterized by the selective loss of melanocytes.<sup>1</sup> However, depending on geographical region, the prevalence of vitiligo varies, ranging from less than 0.1% to more than 8% worldwide.<sup>2</sup> The countries with the highest reported prevalence are India (8.8%), Mexico (2.6–4%) and Japan ( $\geq 1.68\%$ ).<sup>3</sup> Several meta-analyses have shown that individuals with vitiligo experience a reduced quality of life and are more likely to suffer from psychological issues such as depression, anxiety, and shame, leading to low self-esteem and social isolation.<sup>4,6</sup> Vitiligo that develops in childhood can have long-term effects on self-esteem and is associated with significant psychological trauma, with up to 95% of adolescents (aged 15–17 years) being affected.<sup>7</sup> Current treatments for vitiligo are suboptimal; they are not effective for all patients and only offer short-term relief, with a recurrence rate of 40% within a year of stopping treatment.<sup>8</sup> Consequently, there is a pressing

## Graphical Abstract



need to identify the underlying causes of vitiligo and develop more effective treatments. However, the etiology and pathogenesis of the disease remain poorly understood.

The pathophysiology of this complex disorder is influenced by a dynamic interplay between immunological and non-immunological factors. Besides melanocytes, various cell types, including keratinocytes, fibroblasts, natural killer cells, and innate lymphoid cells, are involved in the disease process.<sup>9</sup> The recurrence of vitiligo in the same locations suggests a potential link with memory T cells. CD8 T cells play a crucial role in melanocyte destruction through damage-associated molecular patterns (DAMPs) and adaptive immune responses.<sup>10,11</sup> Increasing evidence indicates that the pathogenesis of vitiligo involves multiple biological processes, such as genetic predisposition, oxidative stress, mitochondrial dysfunction, autoimmunity, autoinflammation, neural factors, apoptosis, molecular adhesion disorders, and other multifactorial mechanisms. Among these, oxidative stress is a key factor in the onset and progression of vitiligo. It is believed that the immune response triggered by oxidative stress is a primary cause of the disease, with mitochondrial damage resulting from oxidative stress being a significant contributor to melanocyte death. However, the oxidative stress-related genes associated with vitiligo remain largely unexplored and require further investigation. Identifying and elucidating these genes will provide valuable biomarkers for the treatment of this condition.

Bioinformatics has become an indispensable tool in aetiology research, enabling the elucidation of genetic variations, gene expression patterns, protein interaction networks, and metabolic dysregulations associated with disease. The application of genomics, transcriptomics, proteomics, metabolomics, and other high-throughput methods has significantly deepened our understanding of disease-related molecular features and signaling pathways. In this study, bioinformatics tools were utilized to analyze data from vitiligo patients and healthy controls obtained from the Gene Expression Omnibus (GEO). The vitiligo-related dataset GSE75819 includes samples from 15 vitiligo patients and 15 healthy controls, allowing for the identification of differentially expressed genes (DEGs) between the two groups. Additionally, oxidative stress-related genes were extracted from the GeneCards database. The DEGs identified from the GSE75819 dataset were then intersected with the oxidative stress-related genes from GeneCards to obtain oxidative stress-related

DEGs (Oxidative Stress-DEGs). To uncover potential functions and enriched pathways, we analyzed protein-protein interaction (PPI) networks, Gene Ontology (GO) terms, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for genes differentially expressed in association with oxidative stress. Machine learning methods were then employed to identify key genes implicated in vitiligo. Additionally, we examined the relationship between these key genes and immune infiltration to gain a deeper understanding of the immune processes involved in vitiligo and to identify potential diagnostic and therapeutic targets.

## Methods

### Collection of Vitiligo Transcriptome Data

Transcriptome data for vitiligo were sourced from the National Center for Biotechnology Information Gene Expression Omnibus (NCBI-GEO). We specifically utilized the GSE75819 dataset, which is a publicly available high-quality dataset with balanced sample groups, providing standardized, log<sub>2</sub>-transformed mRNA expression profiles from 15 vitiligo patient samples and 15 control samples. This dataset was chosen due to its detailed annotation, standardized processing, and relevance to the study's objectives. The gene expression data were aligned with gene names, and the microarray platform used for this dataset was GPL6884.

### Differential Analysis

The expression matrix from the GSE75819 dataset was normalized, and probes were annotated using the dataset's annotation file. Differentially expressed genes (DEGs) associated with vitiligo were identified using the “limma” package in R software. Genes were considered differentially expressed if the adjusted p-value was < 0.05 and the absolute fold change was > 1. A total of 7905 oxidative stress-related genes were extracted from GeneCards (<https://www.genecards.org/>) based on the keyword “oxidative stress” and a relevance score (>10). The DEGs identified from the GSE75819 dataset were intersected with the oxidative stress-related genes from GeneCards to obtain oxidative stress-related DEGs (Oxidative Stress-DEGs) that were both vitiligo-related and oxidative stress-related. Heatmaps and volcano plots were generated using the “heatmap” and “ggplot2” packages in R software to visualize the expression patterns of the identified Oxidative Stress-DEGs.

### Protein-Protein Interaction (PPI) Network Analysis

The identified Oxidative Stress-DEGs were further analyzed using PPI network analysis via the STRING database (<http://www.string-db.org>).<sup>12</sup> A minimum interaction score threshold of 0.700 was set to ensure robust biological relevance. The resulting network visualization excluded unrelated nodes, with each node representing a distinct protein derived from a protein-coding gene locus. This analysis enabled the identification of potential functional clusters and key regulatory genes involved in vitiligo pathology.

### Functional Enrichment Analysis

To elucidate the biological roles and functions of the identified target genes, we conducted a functional enrichment analysis. We used Gene Ontology (GO) categorization, a standard method for gene functional annotation, to classify genes according to their molecular function (MF), biological process (BP), and cellular component (CC). Concurrently, we applied Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis to explore genomic functional information and identify pathways potentially implicated in vitiligo.

Using the ClusterProfiler R package, we systematically performed GO enrichment analysis on the shortlisted mRNAs to highlight their roles in cellular metabolism and biological regulation. Additionally, KEGG pathway enrichment analysis was conducted to pinpoint the molecular pathways significantly associated with the Oxidative Stress-DEGs. This integrative approach aimed to provide a comprehensive understanding of the molecular mechanisms underlying vitiligo pathogenesis and to identify pathways that could be targeted for therapeutic intervention.

## Immune Infiltration Analysis

We employed the CIBERSORT algorithm to estimate the cellular composition of mixed tissue samples. This algorithm infers the relative proportions of 22 immune cell types from gene expression data, evaluating the accuracy of these estimates using correlation and root mean square error (RMSE) metrics.<sup>13</sup> To ensure accuracy, methods such as support vector regression (SVR), permutation testing, standardization, and quantile normalization were applied. For each sample, we determined cell proportions and assessed the reliability of the results. To ensure the accuracy of the immune cell proportion estimates, we set a permutation threshold (perm > 1000) and a p-value threshold ( $p < 0.05$ ). To compare the levels of immune infiltration between vitiligo and normal controls, we used the vioplot package in R. CIBERSORT results may vary due to differences in gene expression quality and sample preparation.

## Machine Learning Identification and Analysis of Key Genes

We combined the least absolute shrinkage and selection operator (LASSO) method with support vector machine recursive feature elimination (SVM-RFE) to identify key vitiligo-related genes.<sup>14</sup> The binomial family was chosen to address binary classification challenges, and the optimal regularization parameter was determined through cross-validation. This integrated machine learning approach enabled the identification of genes with significant relevance to the disease pathology. We assessed the diagnostic value and specificity of these key genes using ROC curve analysis with the “pROC” package. AUC values greater than 0.6 were considered statistically significant. Additionally, we used the CIBERSORT algorithm to analyze correlations between key genes and infiltrating immune cells. To explore the biological pathways associated with these genes, we conducted Gene Set Enrichment Analysis (GSEA).

## Statistical Analysis

All statistical analyses were conducted using R Statistical Software (version 4.3.2). The significance of fold changes in the microarray data was assessed using *t*-tests. The Pearson correlation coefficient was employed to examine the relationship between immune cells and genes. Unless otherwise specified, results with a p-value < 0.05 were considered statistically significant.

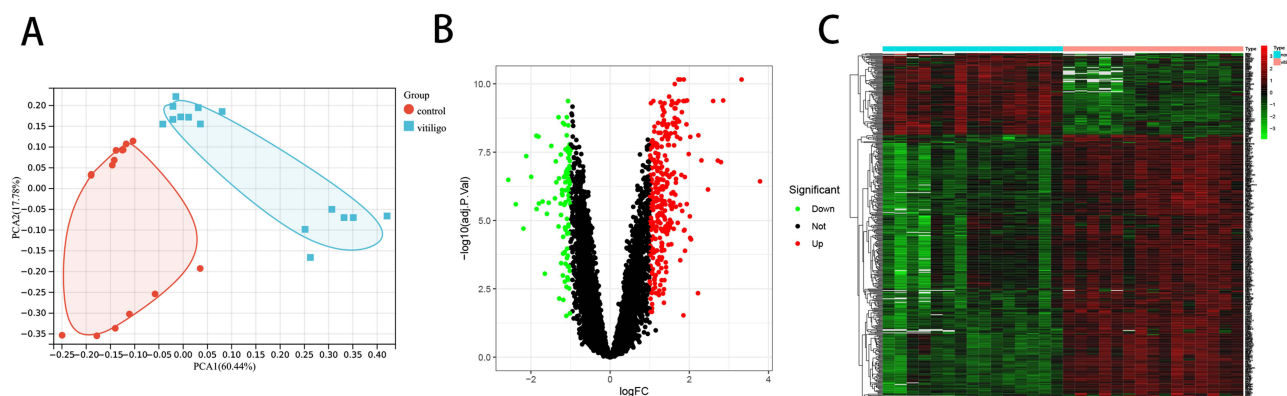
## Results

### Differential Analysis

To elucidate the genetic basis of oxidative stress in vitiligo, we used GEO's GSE75819 dataset to analyze the original mRNA expression profiles of vitiligo patients and healthy controls to obtain differential genes. We extracted 7905 oxidative stress-related genes from GeneCards, intersecting with DEGs from the GSE75819 dataset. Principal component analysis (PCA) was used to visually compare gene expression variances between the vitiligo and control groups, revealing distinct clustering patterns for each cohort (Figure 1A). This analysis identified 415 differentially expressed oxidative stress-related genes, with 317 genes upregulated ( $\log_2$  fold change > 1) and 98 genes downregulated ( $\log_2$  fold change < -1). Differential expression patterns were illustrated using a volcano plot, which highlighted the overall differences in oxidative stress-related gene expression (Figure 1B). A heat map displayed the expression profiles of these differentially expressed genes (DEGs) across samples (Figure 1C). The top 5 upregulated genes were CXCL10, ENY2, MCEE, PDCD5, and TBCA, while the top 5 downregulated genes were HPS6, VAMP2, NRTN, CTSD, and ARHGDI1A.

### Protein-Protein Interaction (PPI) Network Analysis

To explore the interactions between differentially expressed oxidative stress-related genes, we conducted a PPI network analysis on the top 200 DEGs using data from the STRING database. The analysis revealed that these DEGs interact with each other (Figure 2A). To identify hub genes, we performed a node connectivity analysis, as shown in the bar graph in Figure 2B, which presents the correlation scores for each gene. The top ten genes with the highest correlation scores, indicating their potential importance, are RPL9, RPL31, RPS20, RPL7, RPL24, RPL35A, RPL17, RPL21, RPL26, and



**Figure 1** Differentially expressed oxidative stress-related genes in vitiligo and healthy samples. **(A)** PCA compared normal controls and vitiligo samples. **(B)** Volcano plot illustrating DEGs between the two groups, with red representing upregulated genes and green indicating downregulated genes. **(C)** Cluster heat map showing the expression profiles of DEGs between the two groups.

RPL34. Most of these genes encode ribosomal proteins, which are crucial components of both the large and small ribosomal subunits.

## Functional Enrichment Analysis

To investigate the potential biological functions of the hub genes in vitiligo, we conducted a functional enrichment analysis using R software. The KEGG pathway analysis revealed associations between vitiligo and several neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis, and prion diseases (Figure 3A). Notably, genes such as COX6C, NDUFA4, and UQCRH were significantly involved in pathways like oxidative phosphorylation and ribosome biogenesis.

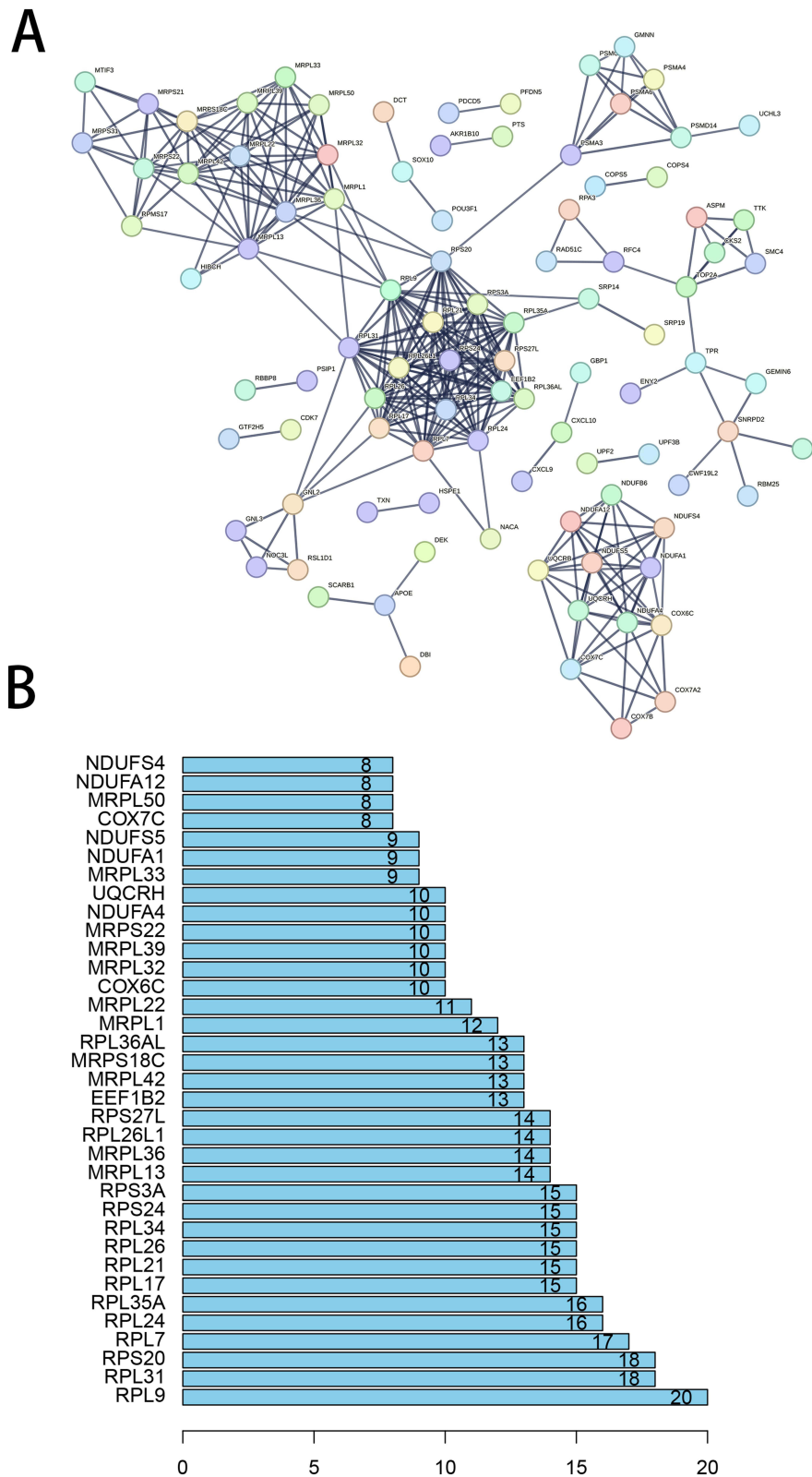
GO analysis showed that the most enriched terms were related to mitochondrial activities. These included mitochondrial translation, mitochondrial gene expression, ATP synthesis coupled with electron transport, and mitochondrial ATP synthesis coupled with electron transport in the biological process (BP) category. In the cellular component (CC) category, terms such as ribosomal subunit, ribosome, mitochondrial inner membrane, mitochondrial protein-containing complexes, and respiratory chain complexes were highly represented. For molecular function (MF), key terms included the structural constituent of ribosome, oxidoreduction-driven active transmembrane transporter activity, NADH dehydrogenase (ubiquinone) activity, and NADH dehydrogenase (quinone) activity (Figure 3B). Overall, these findings suggest a potential link between vitiligo and mitochondrial dysfunction.

## Analysis of Immune Infiltration

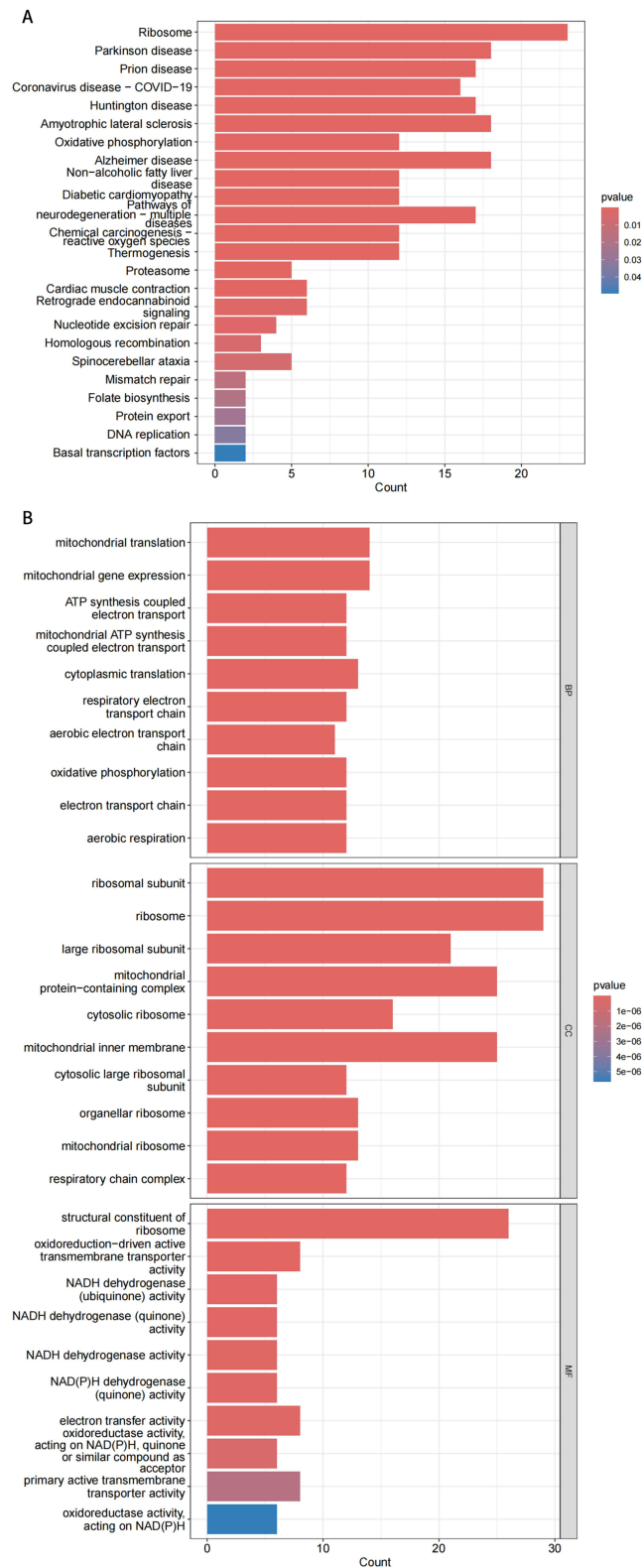
To investigate immune cell infiltration in vitiligo, we quantified differences in immune infiltration between vitiligo and normal groups across 22 immune cell subtypes using the CIBERSORT algorithm. The heatmap displays the expression profiles for each sample, with rows representing immune cell types and columns representing individual samples (Figure 4A). The violin plot shows the distribution and median levels of immune cell infiltration, revealing significant differences in CD4 naive T cells ( $p < 0.001$ ), regulatory T cells (Tregs) ( $p < 0.001$ ), monocytes ( $p < 0.001$ ), and neutrophils ( $p < 0.001$ ) between the groups (Figure 4B). These results suggest complex regulation of specific immune cell subsets within the vitiligo microenvironment, offering insights for further investigation into their roles in disease pathogenesis.

## Screening of Hub Genes

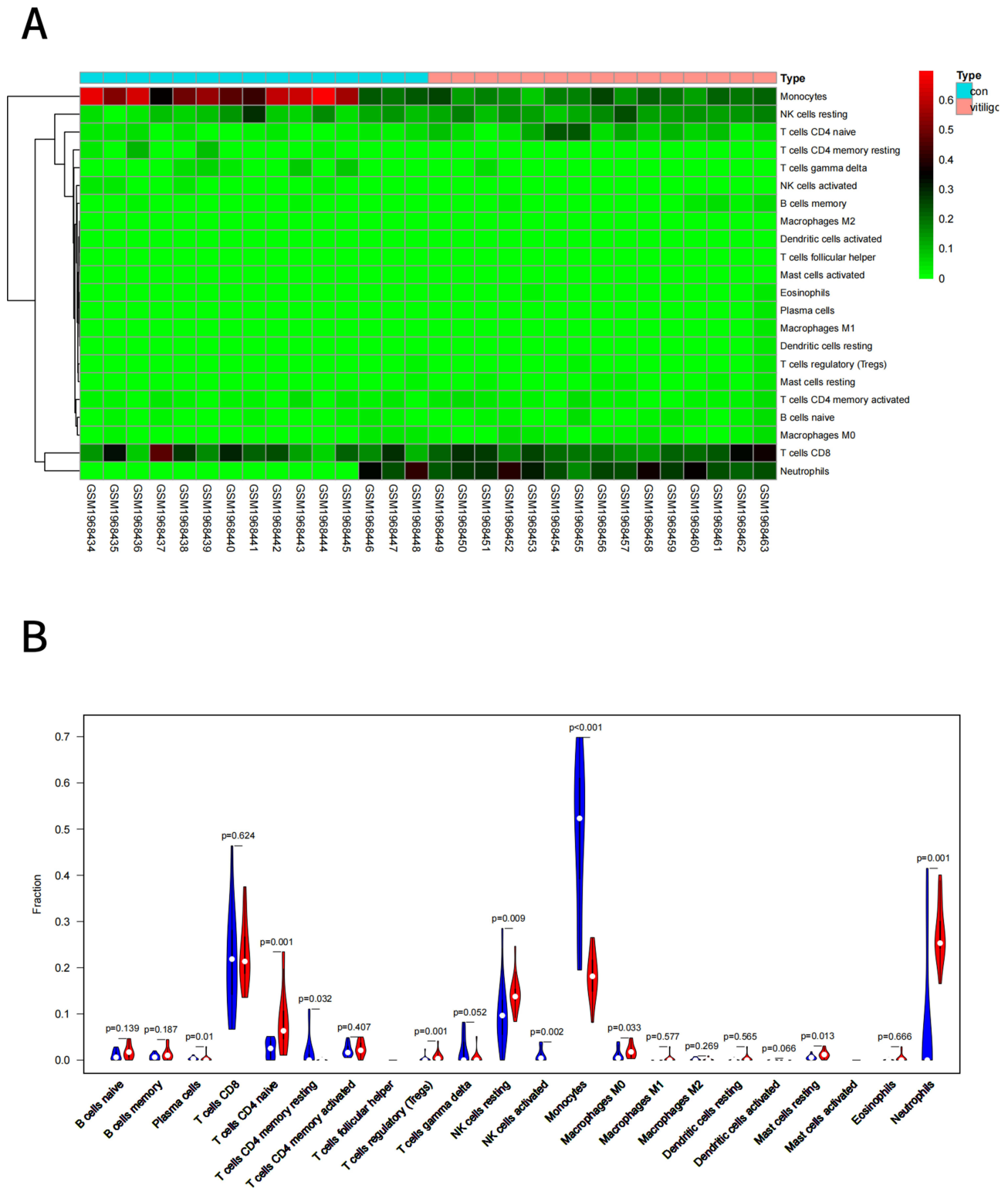
The hub genes associated with oxidative stress in vitiligo were identified using LASSO regression and the support vector machine-recursive feature elimination (SVM-RFE) algorithm. The SVM-RFE algorithm identified six biological markers: MTIF3, MRPL1, DCT, TTK, CXCL10, and MRPL42 (Figure 5A). The LASSO regression algorithm identified



**Figure 2** PPI analysis of the top 200 differentially expressed oxidative stress-related genes. **(A)** PPI network: Nodes represent proteins, with node size indicating the relative importance of each protein. Edges between nodes represent interactions, with different colors denoting various interaction types or functional classifications. **(B)** Bar chart of the PPI network: The X-axis indicates the number of interacting proteins for each target protein, while the Y-axis lists the target proteins.



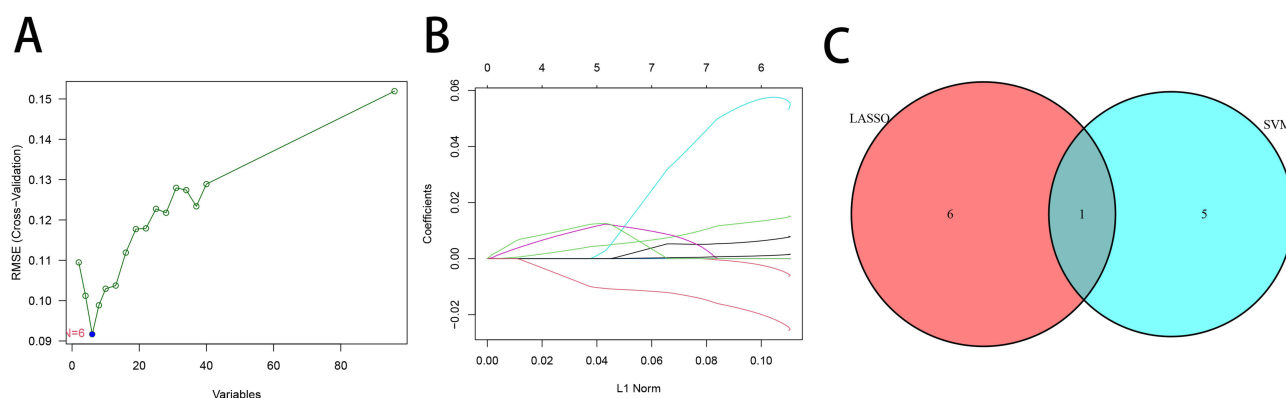
**Figure 3** Functional enrichment analysis of hub DEGs. **(A)** Bar plot showing the results of KEGG pathway analysis. **(B)** Bar plot depicting the results of GO analysis.



**Figure 4** Landscape of immune infiltration in vitiligo compared to normal controls. **(A)** Heatmap showing the percentage distribution of 22 immune cell subtypes. **(B)** Violin plot illustrating the differences in immune cell infiltration between normal subjects (blue) and vitiligo patients (red).

seven predictors: ASPM, DCT, GNL3, POU3F1, PSIP1, RPL31, and SCARB1 (Figure 5B). By comparing results from both methods, DCT was identified as a key gene, common to both algorithms (Figure 5C).

Model performance was evaluated using the root mean square error (RMSE), assessed through cross-validation (Figure 5A). The RMSE initially decreased as the number of variables increased, reaching a minimum point that



**Figure 5** (A) Plot of biomarker selection by the SVM-RFE algorithm, displaying the RMSE as a function of the number of variables in a model predicting vitiligo. (B) Graph showing coefficient paths versus the L1 norm in a LASSO model analyzing vitiligo-related data, with each line representing a different coefficient. (C) Venn diagram illustrating the overlap between variables selected by the LASSO and SVM-RFE methods in the study of vitiligo.

indicated the model's optimal complexity and generalizability. Beyond this point, the RMSE increased, suggesting overfitting. The LASSO coefficient path plot showed how coefficients changed with increasing L1 regularization (Figure 5B). Each line represented a variable, with intersections on the horizontal axis indicating exclusion from the model.

## Analysis of the Hub Gene DCT

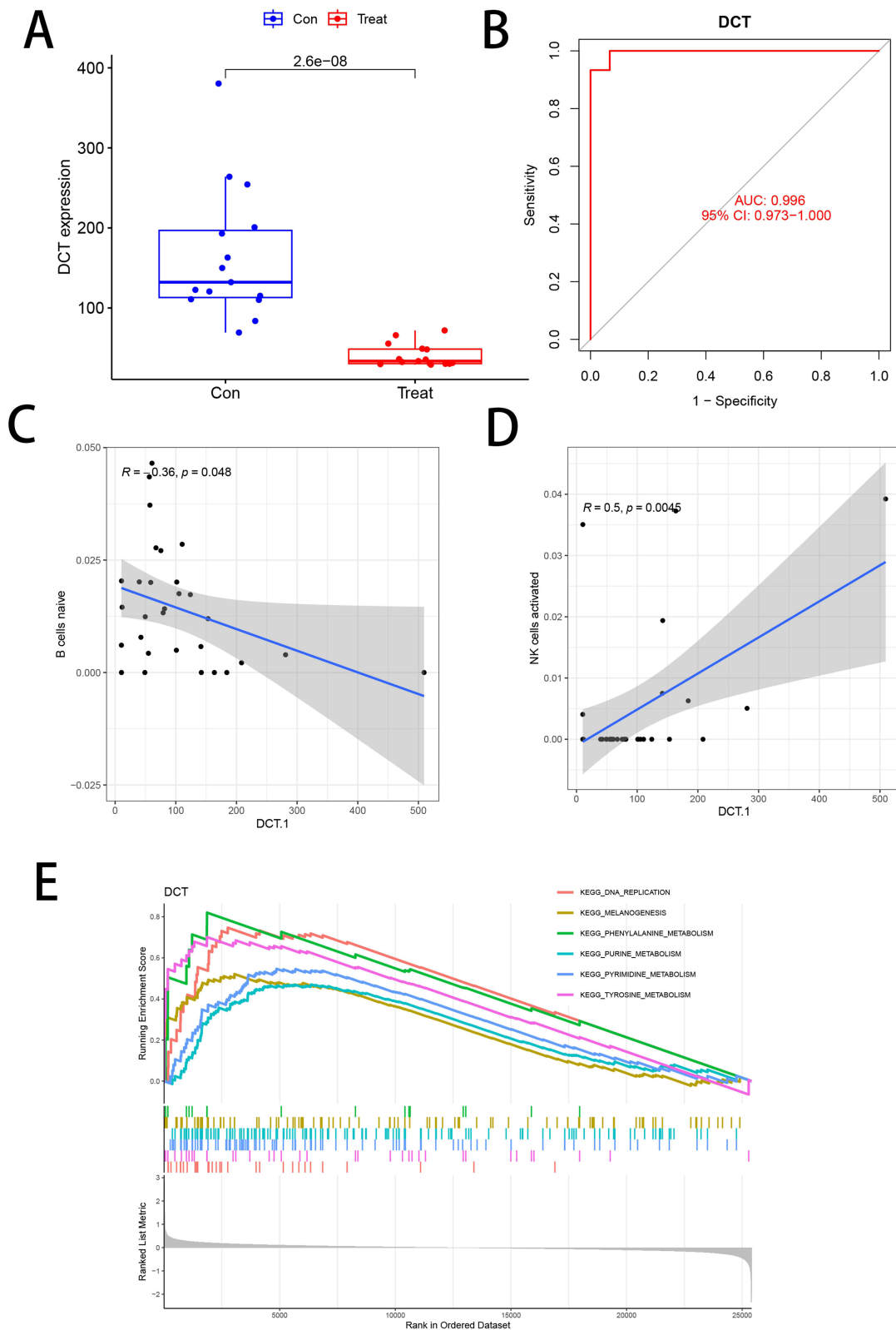
To evaluate the potential predictive role of the DCT gene in vitiligo, we conducted a series of analyses, including receiver operating characteristic (ROC) curve analysis, correlation with immune-infiltrating cells, and single-gene GSEA enrichment analysis. A box plot analysis showed significant downregulation of the DCT gene in the vitiligo treatment group (treat) compared to the healthy control group (control), with a p-value of 2.6e-08 (Figure 6A). The ROC curve for DCT indicated an area under the curve (AUC) of 0.996, demonstrating its high sensitivity and specificity as a diagnostic marker for vitiligo (Figure 6B).

Correlation analysis revealed a negative correlation between DCT expression and the proportion of naive B cells ( $R = -0.36$ ). In contrast, there was a strong positive correlation with activated NK cells ( $R = 0.5$ ), suggesting that increased DCT expression is associated with higher levels of activated NK cells (Figure 6C and D). KEGG pathway analysis using single-gene GSEA implicated the DCT gene in several pathways relevant to vitiligo, including DNA replication, phenylalanine metabolism, tyrosine metabolism, pyrimidine metabolism, purine metabolism, and notably, melanin biosynthesis (Figure 6E). The association with melanin biosynthesis is particularly noteworthy due to its central role in pigmentation disorders like vitiligo. Additionally, the gene's potential impact on phenylalanine, tyrosine, and tryptophan metabolism, which are crucial for pigmentation processes, suggests a complex role in the pathophysiology of the disease. Overall, these results suggest that the DCT gene may influence immunological characteristics in vitiligo.

## Discussion

Vitiligo is characterized by the presence of depigmented patches on the skin. Although the exact cause of vitiligo is not fully understood, research suggests that it may result from a combination of genetic predisposition, autoimmune reactions, environmental factors, stress, and skin trauma.<sup>15</sup> In individuals with vitiligo, the immune system mistakenly attacks and destroys melanocytes, the cells responsible for skin pigmentation, leading to the loss of skin color.<sup>16</sup> Current treatments for vitiligo include medication, laser therapy, phototherapy, and surgery. Recent advances in understanding the genetic and immune regulatory mechanisms involved in vitiligo have provided promising insights into the development of more effective therapeutic strategies.<sup>17</sup>

Oxidative stress is considered a critical initial event in the degeneration of melanocytes in vitiligo. It is believed that in vitiligo, melanocytes are damaged by an accumulation of reactive oxygen species (ROS), which disrupts the structural and functional integrity of DNA, lipids, and proteins.<sup>18</sup> Furthermore, oxidative stress exacerbates the disease by



**Figure 6** Analysis of the hub gene DCT. **(A)** Boxplot comparing DCT expression in the vitiligo treatment group and healthy control group. **(B)** ROC curve for DCT. **(C)** Correlation between DCT expression levels and the proportion of naive B cells. **(D)** Correlation between DCT expression levels and the proportion of activated NK cells. **(E)** KEGG pathway analysis of the DCT gene.

activating the immune system. At high oxidative stress levels, damage-associated molecular patterns (DAMPs) are released from keratinocytes or melanocytes in the skin and induce downstream immune responses during vitiligo.<sup>19</sup> Studies analyzing skin samples have shown that oxidative stress significantly upregulates pro-inflammatory chemokines such as CXCL9 and CXCL10, as well as cytokines like IFN- $\gamma$  and TNF- $\alpha$ . These molecules recruit immune cells, particularly activated CD8<sup>+</sup> T cells, which mediate the destruction of melanocytes, ultimately leading to melanocyte apoptosis.<sup>20,21</sup>

In this study, we conducted an in-depth analysis of oxidative stress-related gene expression differences between vitiligo patients and healthy individuals using mRNA expression profile data from the GEO public database (GSE75819). PCA revealed a clear distinction between vitiligo and control tissues at the gene expression level, indicating significant molecular differences between the two groups. Further analysis identified 415 Oxidative Stress-DEGs, with 317 up-regulated and 98 down-regulated. The top five upregulated genes identified were CXCL10, ENY2, MCEE, PDCD5, and TBCA, while the top five downregulated genes were HPS6, VAMP2, NRTN, CTSD, and ARHGDI1A.

Previous studies have shown that CXCL10 levels are significantly higher in both active and stable vitiligo compared to controls ( $p < 0.05$ ) and are even higher in active vitiligo than in stable vitiligo ( $p < 0.05$ ). This increase is attributed to the oxidative damage-induced rise in the pro-inflammatory factor IL-15, which promotes the expression of the IFN- $\gamma$ -induced chemokine CXCL10.<sup>22</sup> Additionally, the upregulation of MCEE has been found to inhibit enhanced glycolysis, promote fatty acid oxidation, and restore mitochondrial oxidative phosphorylation, thereby alleviating oxidative stress and correcting energy metabolism disorders.<sup>23</sup> PDCD5 is recognized as a novel molecule that promotes programmed cell death.<sup>24</sup> Recent studies have shown that PDCD5 translocates to the nucleus in response to DNA damage and interacts with p53 to promote apoptosis.<sup>25</sup> PDCD5 may facilitate melanocyte apoptosis or cause their detachment and migration from the basal layer. TBCA is an activator of NADPH oxidase, which is crucial in oxidative stress and apoptosis, particularly in the induction of ROS production.<sup>26</sup> A significant decrease in VAMP2 has been linked to increased apoptotic markers, including reduced NADPH production rates.<sup>27</sup> The lysosomal proteolytic enzyme CTSD is the only aspartic-type protease expressed ubiquitously in all human cells, with particularly high expression in the brain. The absence of CTSD can lead to neurodegenerative diseases, as it is responsible for degrading several neuronal proteins that accumulate in these conditions. Proteins such as the amyloid precursor,  $\alpha$ -synuclein, and huntingtin are physiological substrates of CTSD, and without efficient degradation by this enzyme, they would accumulate abnormally, ultimately contributing to neurodegeneration.<sup>28,29</sup>

PPI network analysis using the STRING database highlighted the significance of certain ribosomal protein genes that may play a critical role in the pathogenesis of vitiligo. KEGG pathway enrichment analysis further associated vitiligo with several neurodegenerative diseases and identified key genes related to oxidative phosphorylation and ribosomes, such as COX6C, NDUFA4, and UQCRH. Oxidative stress induced by ROS causes cellular damage and contributes to the pathogenesis of neurodegenerative diseases. Additionally, oxidative stress from neurotransmitters and environmental neurotoxins further threatens protein folding and the integrity of organelle membranes in neurons. Failure to degrade these altered materials impairs neuronal function and ultimately leads to neurodegeneration.<sup>28</sup> GO analysis highlighted several key BPs, CCs, and MFs, particularly those related to mitochondrial function.

Immune infiltration is a hallmark of vitiligo pathology, and the infiltration of specific immune cells into the affected skin can lead to the destruction of melanocytes. Oxidative stress and immune infiltration are interrelated processes that synergistically accelerate the progression of vitiligo, especially in early lesions, where immune cell aggregation may be triggered by antigen release induced by oxidative stress and subsequent immune activation. CD8<sup>+</sup>T cells are key mediators of melanocyte destruction, releasing IFN - $\gamma$  and TNF - $\alpha$  to induce melanocyte apoptosis.<sup>10</sup> Skin biopsies have revealed CD8<sup>+</sup> and CD4<sup>+</sup> T-cell infiltration in the margins of active lesions, with an increased CD8<sup>+</sup> to CD4<sup>+</sup> ratio.<sup>30</sup> Active vitiligo lesions demonstrate infiltration of several types of innate immune cells, including dendritic cells, Langerhans cells, macrophages, and natural killer (NK) cells.<sup>31</sup> These findings further support the central role of immune cells in the pathogenesis of vitiligo. Immune infiltration analysis in our study revealed significant differences in the expression patterns of specific immune cell subsets between vitiligo patients and healthy individuals, with notable increases in T cells CD4 naive, T cells regulatory (Tregs), NK cells resting, neutrophils infiltration and decrease in monocytes infiltration. Another study on immune infiltration analysis has shown that the abundance of activated CD4<sup>+</sup>T

cells, CD8+T cells, immature dendritic cells and B cells, myeloid derived suppressor cells (MDSCs), gamma delta T cells, mast cells, regulatory T cells (Tregs), and T helper 2 (Th2) cells is higher in vitiligo lesions. However, the abundance of CD56 bright natural killer (NK) cells, monocytes, and NK cells is relatively low.<sup>32</sup> These findings provide important insights into the immunopathology of vitiligo.<sup>33</sup>

Hub genes were identified using machine learning algorithms, with both LASSO and SVM-RFE methods confirming the relevance of the DCT gene in vitiligo. Further analyses, including box plots, ROC curve, and correlation analysis, supported the potential of the DCT gene as a biomarker for vitiligo. KEGG pathway analysis also suggested the involvement of the DCT gene in several biological pathways, particularly in melanin biosynthesis, underscoring its importance in the depigmentation seen in vitiligo. Pathological studies revealed significant correlations between DCT gene expression and immune cell activity in vitiligo.<sup>34</sup> Specifically, DCT expression levels were negatively correlated with the proportion of B cells and positively correlated with the proportion of activated NK cells.<sup>35</sup> This suggests altered activity in these immune cell populations in vitiligo patients. The decrease in B cells may indicate impaired immune surveillance, while the increase in activated NK cells may reflect a response to persistent inflammation or autoimmune reactions.<sup>36–38</sup> KEGG pathway analysis further highlighted the significance of pathways such as tyrosine metabolism, melanin biosynthesis, and tryptophan metabolism. Abnormalities in tyrosine metabolism can directly impact melanin synthesis, which is a primary cause of depigmentation in vitiligo.<sup>39,40</sup> Disruption of melanin biosynthesis pathways can lead to melanocyte dysfunction, contributing to the development of vitiligo.<sup>41–43</sup> Additionally, alterations in tryptophan metabolism may influence immune system balance, potentially playing a role in the autoimmune pathology of vitiligo.<sup>44–46</sup>

The DCT gene (dopachrome tautomerase), also known as tyrosinase-related protein-2 (TRP-2), is an important enzyme in the melanin biosynthesis pathway. DCT plays a protective role in melanocytes by reducing oxidative stress. It facilitates the conversion of dopachrome into 5,6-dihydroxyindole-2-carboxylic acid (DHICA), which generates less reactive oxygen species (ROS) compared to alternative pathways in melanin synthesis.<sup>47</sup> By mitigating ROS accumulation, DCT supports the survival and functionality of melanocytes under oxidative stress conditions, which are a hallmark of vitiligo pathogenesis. DCT expression influences immune cell activity within vitiligo lesions. Our research indicates that DCT expression levels are negatively correlated with B cell proportions and positively correlated with activated NK (natural killer) cell levels. This suggests that alterations in DCT expression may modulate local immune responses, potentially contributing to the autoimmune destruction of melanocytes. Dysregulated redox balance due to reduced DCT expression exacerbates cellular damage, contributing to melanocyte apoptosis and depigmentation. As a melanocyte-specific antigen, DCT can trigger autoimmune responses in vitiligo. CD8+ T cells may target DCT-expressing melanocytes, leading to their destruction.<sup>48</sup> Increased immune infiltration, particularly by cytotoxic T cells, amplifies local inflammation and melanocyte damage, perpetuating the disease cycle. Salinas-Santander et al found that the DCT gene was overexpressed in the skin of Mexican vitiligo patients.<sup>49</sup> DCT has been identified as a biomarker associated with vitiligo in several studies.<sup>48,50,51</sup> The dual role of DCT in regulating oxidative stress and immune responses makes it a promising biomarker and therapeutic target for vitiligo.

Our current study focused on a single dataset (GSE75819), and the findings are exploratory and need to be validated in independent datasets and patient-sourced samples to confirm their relevance and practical application. This study has several limitations. First, the database used provides a limited number of individuals for investigation. Future studies should include meta-analyses of multiple data sets to enhance the robustness and generalizability of the findings. Meta-analysis allows for the integration of different datasets, which can reduce bias associated with a single dataset and provide a more complete understanding of the underlying molecular mechanisms of vitiligo. Second, the limitations of our study highlight the need for experimental validation. The RNA expression levels of DEGs in vitiligo patients and healthy controls were not validated by qRT-PCR or immunohistochemical analysis in skin or blood samples. Third, we did not conduct experiments in cell cultures or mouse models to confirm the expression and underlying mechanisms of key genes. Finally, our analysis of the association between hub genes and immune infiltration, as well as their role in regulating immune response and inflammation in vitiligo, was based solely on bioinformatics analysis and requires further validation.

## Conclusion

This study provides new insights into the molecular mechanisms of vitiligo and identifies potential targets for the development of specific biomarkers. Immune infiltration studies revealed dynamic changes in specific immune cell subsets in vitiligo. Additionally, the machine learning-based selection of hub genes, particularly the DCT gene, underscores its potential role in the pathogenesis of vitiligo, likely through melanin biosynthesis pathways. Our comprehensive analysis suggests that vitiligo is a complex disease involving multiple genes and pathways, offering valuable clues for a deeper understanding of its pathophysiology, and providing a foundation and possibility for the development of targeted therapies for oxidative stress and immune dysfunction in vitiligo.

## Data Sharing Statement

The datasets are available in the NCBI-GEO repository (<https://www.ncbi.nlm.nih.gov/geo/>). All data generated or analyzed during this study are included in this published article. Data supporting the findings of this study may be obtained from the corresponding author upon reasonable request.

## Ethical Statement

This study was approved by the Ethics Committee of Changzhou Children's Hospital (approval number: [2023]CL096).

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors declare that they have no competing interests in this work.

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