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Research article

To investigate the function of age-related genes in different subtypes of asthma based on bioinformatics analysis

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ABSTRACT

Asthma is a heterogeneous airway inflammatory disease that can be classified according to the inflammatory phenotype. The pathogenesis, clinical features, response to hormone therapy, and prognosis of different inflammatory phenotypes differ significantly. This condition also refers to age-related chronic ailments. Here, we intend to identify the function of aging-related genes in different inflammatory phenotypes of asthma using bioinformatic analyses. Initially, the research adopted the GSEA analysis to understand the fundamental mechanisms that govern different inflammatory phenotypes of asthma pathogenesis and use the CIBERSORT algorithm to assess the immune cell composition. The differentially expressed genes (DEGs) of eosinophilic asthma (EA), neutrophilic asthma (NA), and paucigranulocytic asthma (PGA) were identified through the limma R package. Aging-related genes, screened from multiple databases, were intersected with DEGs of asthma to obtain the asthma-aging-related DEGs. Then, the GO and KEGG pathway enrichment analyses showed that the NA- and EA-aging-related DEGs are involved in the various cytokine-mediated signaling pathways. PPI network and correlation analysis were performed to identify and evaluate the correlation of the hub genes. Further, the clinical characteristics of asthma-aging-related DEGs were explored through ROC analysis. 3 and 12 aging-related DEGs in EA and NA patients show high diagnostic accuracy, respectively (AUC >0.7). This study provided valuable insights into aging-related gene therapy for phenotype-specific asthma. Moreover, the study suggests that effective interventions against asthma may operate by disrupting the detrimental cycle of "aging induces metabolic diseases, which exacerbate aging".

1. Introduction

Bronchial asthma, also known as asthma, is a heterogeneous chronic airway inflammatory disease that affects approximately 358.3 million people worldwide, posing a severe public health concern [1,2]. It is characterized by variable and recurring clinical symptoms,

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and an individualized treatment strategy is essential to achieve disease remission [3]. Asthma is classified into various phenotypes based on clinical characteristics, including allergic asthma, non-allergic asthma, late-onset asthma, and obesity-related asthma. Furthermore, a classification based on the proportion of inflammatory cells in induced sputum has divided asthma inflammatory phenotypes into four distinct categories: eosinophilic asthma (EA), neutrophilic asthma (NA), paucigranulocytic asthma (PGA), and mixed granulocytic asthma (MGA) [4]. EA is characterized by type 2 inflammation, with eosinophil airway infiltration and the presence of inflammatory mediators such as cytokines IL-4, IL-5, and IL-13 [5]. In contrast, NA is characterized by non-type 2 inflammation. The inflammatory mediators involved in neutrophil airway infiltration are IL-6, IL-8, IL-17, IL-1β, TNF-α, and IFN-γ, which activate neutrophils and promote neutrophil inflammation [6,7]. NA patients usually present with persistent airflow restriction and severe or refractory asthma [8,9], and this phenotype may be related to respiratory infection, occupational exposure, or tobacco smoke exposure [10]. The cellular and molecular mechanisms of airway neutrophilic inflammation in NA patients have not been clarified, and there is still a lack of unified biomarkers and effective drug therapy for this phenotype [7]. PGA is characterized by the absence of airway neutrophil and eosinophilic infiltration, often indicating a "benign" asthma phenotype with a favorable response to asthma treatment. However, the expression mechanism in some patients with this phenotype remains unclear [11]. Previous studies have shown significant differences in pathological mechanisms, clinical features, and inflammatory mediators infiltration of asthma with different inflammatory phenotypes [12–14]. The in-depth understanding of the pathogenesis of asthma inflammatory phenotype has revolutionized the treatment strategies for asthma, guiding individualized and precise treatment approaches. However, there is still a lack of comprehensive understanding of asthma inflammatory phenotypes, particularly in the case of NA, which is insensitive to current asthma treatment drugs and lacks molecular therapeutic targets. Therefore, further study of the pathogenesis and explore the biomarkers and molecular therapeutic targets of the inflammatory phenotype of asthma is of utmost importance in advancing personalized and phenotypic-specific asthma treatment.

Aging is a significant risk factor for most chronic diseases, including cardiovascular disease, cancer, Alzheimer's disease, and other neurodegenerative diseases [15–17]. Atherosclerosis, characterized by the gradual accumulation of plaques composed of fats and proteins along arterial walls, underlies severe conditions such as heart disease, stroke, and ischemic conditions. Through an animal model, researchers observed a significant presence of aged macrophages in the artery wall where plaque initially forms. Over time, other aged cell types also appeared near these sites. These aged cells expressed many secreted factors that promote atherosclerosis compared to control cells. Removing these cells has shown the potential to hinder lesion growth and mitigate disease progression [18,19]. Likewise, in the context of osteoarthritis, researchers have identified substantial accumulations of senescent cells in the affected joints [20,21]. Eliminating these senescent cells has demonstrated pain relief, promoted the repair of damaged cartilage, and even prevented the onset of osteoarthritis in naturally aging mice [22]. In 2021, researchers from the University of Minnesota Medical School published a study in Nature confirming the adverse effect of senescent immune cells on aging mice [23]. Rather than being independent occurrences of unrelated diseases, multiple age-related conditions often manifest dysfunctions across various systems. Therefore, targeting conserved aging-related pathways should prevent or alleviate numerous clinical problems. This hypothesis has yet to be clinically validated, but several pieces of evidence support it. In addition, immune impairment (also known as immune senescence, which refers to age-related decline in immune function) is a significant cause of morbidity and mortality in patients.

Given that several cellular senescence-related changes have been associated with asthma, including oxidative stress, telomere shortening, autophagy/mitochondria, and inflammation, the researchers thus hypothesized that age-related respiratory changes may occur concurrently with asthma. The current prevalence of asthma in people over 65 is reported to be 4 %–13 %. Older individuals with asthma tend to exhibit a more severe disease phenotype than their younger counterparts. They are at increased risk of frequent

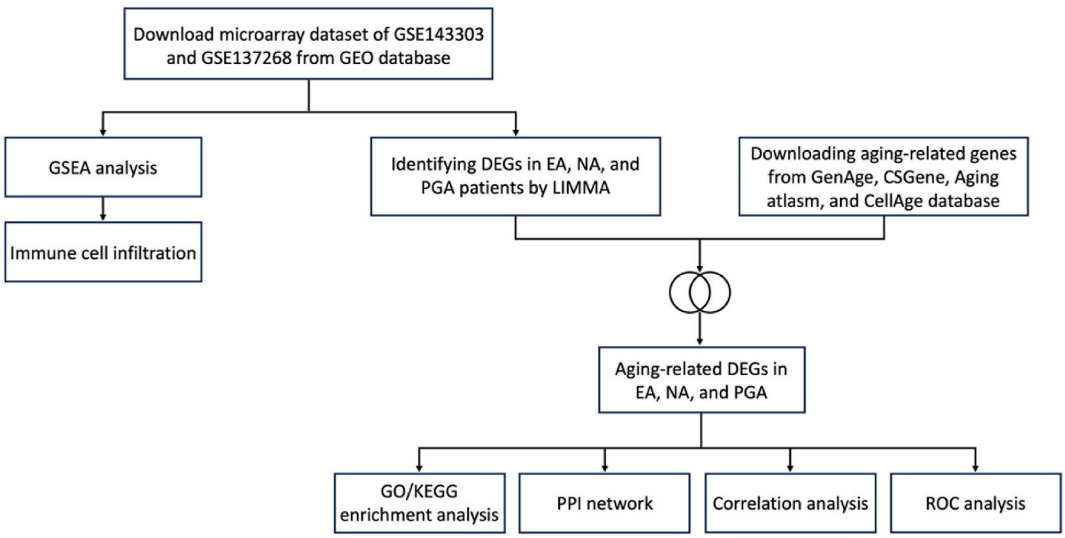

Fig. 1. The flow chart of this study.

hospitalization and are 5 times more likely to die from the disease [24,25]. Recent advances suggest that cellular senescence is a mechanism of airway inflammation. The airway smooth muscle cells of elderly asthmatics exhibit increased changes associated with aging. Cell surface protein Integrin b4 (ITGB4), which is responsible for maintaining airway integrity, is reduced in airway epithelial cells from elderly asthmatics. Furthermore, elderly asthmatics frequently demonstrate altered immune responses to immune senescence. To date, several potential therapeutic options for chronic lung disease have been proposed, with a focus on targeting cellular senescence [26,27].

Although it is generally believed that there is a link between aging and asthma, the specific contribution of aging in elderly asthmatic patients with different phenotypes remains to be established. This research will focus on the relationship between aging and bronchial asthma. Gene expression profiles of asthma patients were used to analyze the genetic signature related to aging and corresponding enrichment biological processes and provide new ideas for studying the mechanisms and treatments of the development of bronchial asthma. The flow chart of this study is depicted in Fig. 1.

2. Material and method

2.1. Data acquisition

To identify the aging-related genes in asthma patients with different inflammatory phenotypes, two microarray datasets of asthma (GSE143303 and GSE137268) were downloaded from the Gene Expression Omnibus datasets (GEO, https://www.ncbi.nlm.nih.gov/gds/). The keywords for searching were "asthma", "eosinophilic", and "homo sapiens". The details of the GSE datasets are summarized in Table 1. GSE143303, which contained 13 healthy samples, 22 EA samples, 9 NA samples, and 16 PGA samples, was acquired from the GPL10558 platform, and GSE137268, which included 15 healthy samples, 17 EA samples, 11 NA samples, and 16 PGA samples, was obtained from the GPL6104 platform. After the data standardization and normalization using the "limma" R package, the differently expressed genes (DEGs) between EA-healthy, NA-healthy, and PGA-healthy patients were analyzed by using the "limma" R package, respectively. Adjusted $p < 0.05$ and $|\mathrm{Log}_2$ fold change (FC)$| > 1$ were used as a threshold for significantly differential expression. The DEGs in different inflammatory phenotypes of asthma were visualized in heat maps and volcano plots using the R package ggplot2. A total of 1441 age-related genes were obtained from the CellAge database (https://genomics.senescence.info/cells/), GenAge database (https://genomics.senescence.info/genes/index.html), Aging Atlas database (https://ngdc.cncb.ac.cn/aging/index), and Cell senescence gene (CSGene) database (http://csgene.bioinfo-minzhao.org/). The DEGs associated with aging in different inflammatory phenotypes of asthma were intersected, and the overlapped genes were obtained.

2.2. Gene set enrichment analysis (GSEA)

GSEA analysis was performed to identify gene sets in different inflammatory phenotypes of asthma. The gene sets of "c2.cp.all.v2022.Hs.symbols.gmt" was used from the Molecular Signatures Database (MSigDB) (https://www.gsea-msigdb.org/gsea/msigdb/). The clusterProfiler R package was employed to conduct the GSEA analysis. The two indicators of adjusted $p < 0.05$ and false discovery rate (FDR) $q < 0.25$ were used to evaluate whether the pathways were statistically significant.

2.3. Analysis of the infiltration of immune cells

The immune infiltration in different inflammatory phenotypes of asthma was calculated by examining the signature of 22 kinds of immune cell expression spectrum matrix using the CIBERSORTx website (https://cibersortx.stanford.edu/). The relative percent, correlation index, and root-mean-squared prediction error (RMSE) of 22 kinds of immune cell infiltration in different inflammatory phenotypes of asthma were plotted.

2.4. Function enrichment analysis of aging-related DEGs

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) annotation analysis were standard methods for enrichment studies. The GO enrichment analysis was annotated to explore the biological significance of the aging-related DEGs. The biological process (BP), molecular function (MF), and cellular component (CC) categories of DEGs were performed using a David

Table 1
Characteristics of microarray datasets included.

Datasets	Platforms	Country	Year	Experiment type	Sample source	Description	Sample size
GSE143303	GPL10558	Australia	2021	Expression profiling by array	Endobronchial biopsies	Healthy	13
						EA	22
						NA	9
						PGA	16
GSE137268	GPL6104	Australia	2019	Expression profiling by array	Induced sputum	Healthy	15
						EA	17
						NA	11
						PGA	16

online tool (https://david.ncifcrf.gov/). KEGG pathway enrichment analysis of these aging-related DEGs was performed using the KEGG Orthology-Based Annotation System version 3.0 online analysis database (http://39.103.204.200). The two indicators of adjusted $p < 0.05$ and false discovery rate (FDR) $q < 0.25$ were used to evaluate whether the pathways were statistically significant.

2.5. Protein-protein interaction (PPI) network and potential hub gene analysis

To explore the potential interactions among the aging-related DEGs in different inflammatory phenotypes of asthma, the Search Tool for the Retrieval of Interacting Genes (STRING) database (https://cn.string-db.org/) was used, and a confidence score >0.4 was set as the cutoff criterion. The cytoHubba application was used to select significant modules from the PPI network in Cytoscape.

2.6. Correlation and diagnostic value analysis

The correlation analysis was used to show the relationship between the DEGs by the Spearman statistical method. Statistical analysis and visualization are performed in R version 4.2.1 involving R packages: igraph [1.3.4], ggraph [2.1.0], and the analysis results are visualized by a network diagram. The receiver operating characteristic (ROC) curve was utilized to evaluate the diagnostic performance of aging-related DEGs in different inflammatory phenotypes of asthma. The accuracy level of the area under the curve (AUC) can be classified as low (0.5–0.7), moderate (0.7–0.9), and high (above 0.9), with an AUC closer to 1, indicating a superior diagnostic efficacy. pROC [1.18.0] is used for ROC analysis and ROC verification. Statistical analysis and visualization were performed in R 4.2.1.

2.7. Statistical analysis

Graph production, data distribution, and statistical analyses were performed using QtiPlot. Analysis of variance (ANOVA) or t-tests was used to investigate significant differences between indicated groups. $p < 0.05$ was considered statistically significant.

3. Result

3.1. GSEA analysis in different inflammatory phenotypes asthma

To understand the basic mechanisms of different inflammatory phenotypes of asthma pathogenesis, the enriched gene set was

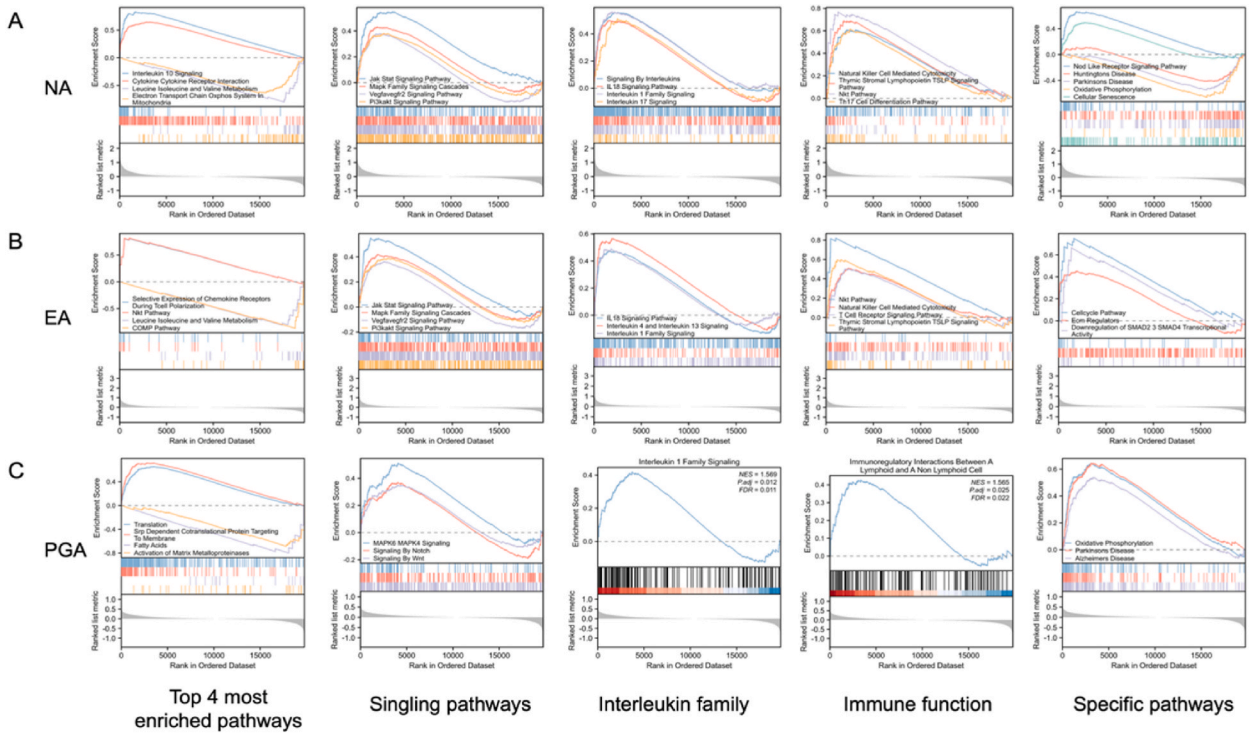

Fig. 2. GSEA analysis in different inflammatory phenotypes of asthma. Gene sets of the top 4 most enriched pathways, singling pathways, interleukin family, immune function, and specific pathways in NA (A), EA (B), and PGA (C) were plotted.

determined by GSEA. The top 4 most enriched gene sets, signaling pathways, interleukin family, immune function, and specific pathways enriched in different inflammatory phenotypes of asthma are shown in Fig. 2A–C. It is worth noting that the Th1/Th2 pathway gene set (Normalized enrichment score, NES = 1.55) was enriched in the EA group, and the Th17 cell differentiation pathway gene set (NES = 1.85) was enriched in the NA group. This result consists with previous research that shows that NA is primarily instigated by Th1 and, particularly, Th17 lymphocytes. Conversely, EA is typically orchestrated by inflammation associated with Th2 responses. The absence or suppression of Th2-mediated inflammatory pathways and an elevation in Th1 and/or Th17 inflammatory pathways are characteristic features of NA [3,28]. TSLP has previously been implicated in airway remodeling. It interacts with the initial OX40 of T cells, promoting the initiation of type 2 immune response. This, in turn, leads to elevated production of pro-inflammatory cytokines (such as IL-4, IL-5, and IL-13), further augmenting IgE levels, mast cell activation, mucus production, and heightened airway hyperresponsiveness [29,30]. In addition, TSLP and TLR3 ligands have been observed to facilitate the conversion of naïve T cells into Th17 cells. This process subsequently stimulates airway epithelial cells to attract neutrophils by releasing IL-8 and GM-CSF, initiating NA [8]. Moreover, TSLP expression was increased in the airways of asthmatics compared to healthy individuals [31]. In our research, a subset of the TSLP genes set was enriched in the NA (NES = 1.95) and EA (NES = 1.74) groups, respectively (Fig. 2). It is noteworthy that the gene sets associated with Huntington's disease, Parkinson's disease, and cellular senescence were found to be enriched in the NA group, and these gene sets were exclusively enriched in the NA group. This suggests that the development of NA diseases may be associated with senescence. These results are consistent with previous reports, supporting us in moving forward with the subsequent stages of our research.

3.2. Immune cell infiltration analysis in different inflammatory phenotypes of asthma

To understand the immune cell composition in different inflammatory phenotypes of asthma. 22 kinds of immune cell infiltration analysis on GEO transcriptome sequencing data were performed using the CIBERSORT algorithm. As shown in Fig. 3A–C, the relative percent of immune cells revealed that in NA, neutrophils, T cells CD4, macrophage M1, and monocytes with higher percentage; NK

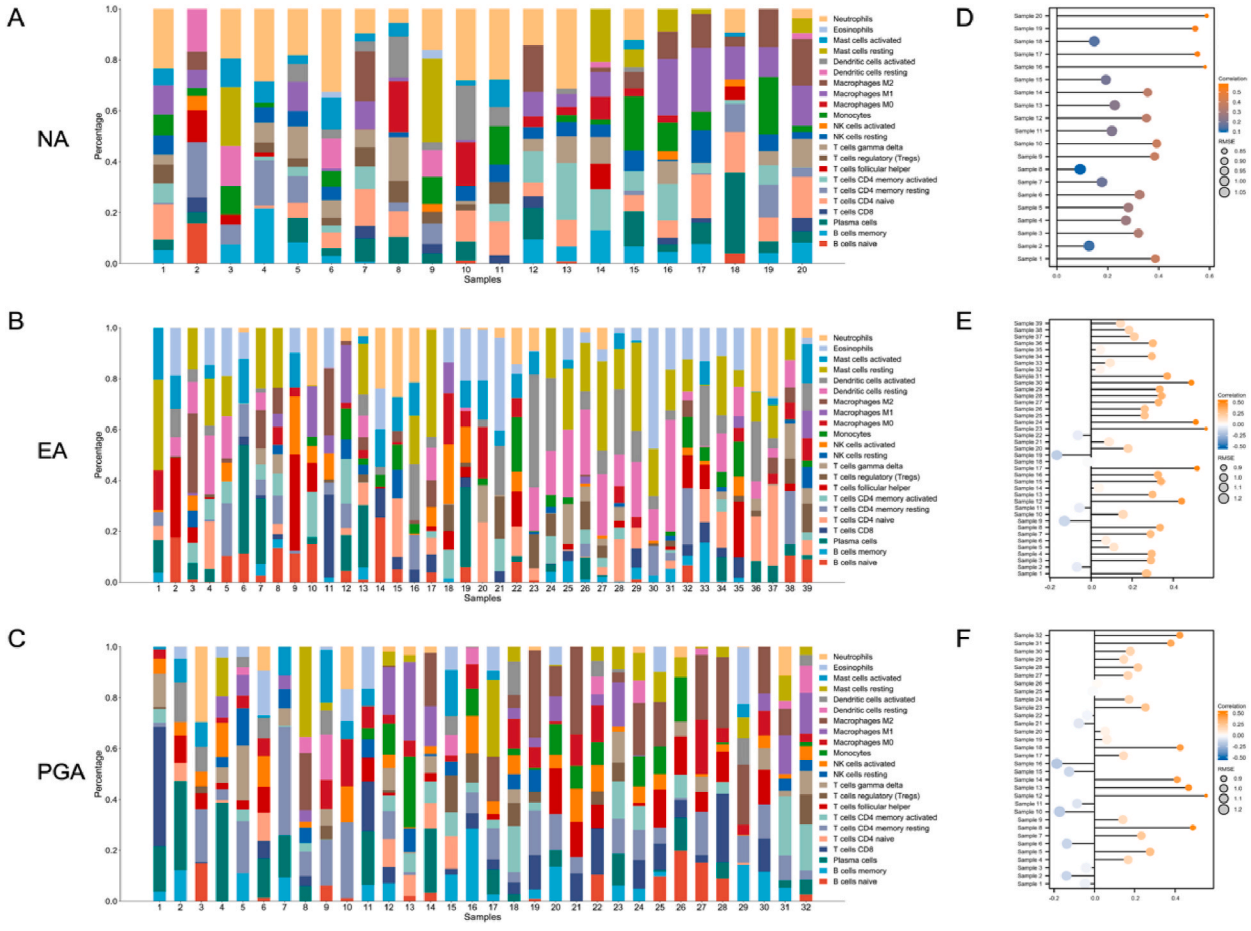

Fig. 3. Immune cell infiltration analysis in different inflammatory phenotypes of asthma. CIBERSORT algorithm for the relative percent of 22 kinds of immune cell infiltration analysis in NA (A), EA (B), and PGA (C) was plotted. The correlation and RMSE of 22 kinds of immune cell infiltration in NA (D), EA (E), and PGA (F) were visualized in lollipop plots.

cells, T cells CD8, and eosinophils with lower percentage. In EA, eosinophils, dendritic cells, and T cells CD4 with higher percentage; B cells memory, macrophage M1 with lower percentage. In PGA, macrophage M1, macrophage M2, macrophage M0, and T cells CD8 have a higher percentage; T cells CD4 have a lower percentage. The correlation and RMSE of 22 kinds of immune cell infiltration in NA, EA, and PGA were visualized in lollipop plots (Fig. 3D–F). The smaller the RMSE, the better the prediction.

3.3. Identifying the aging-related DEGs in different inflammatory phenotypes of asthma

To identify the aging-related DEGs in different inflammatory phenotypes of asthma, first, the GSE143303 and GSE137268 datasets containing 28 healthy samples, 39 EA samples, 20 NA samples, and 32 PGA samples were used to identify the DEGs between different inflammatory phenotypes of asthma and healthy control using adjusted $p < 0.05$ and $|\text{Log}_2 \text{ fold change (FC)}| > 1$ as a cutoff criterion. 64, 235, and 9 DEGs were identified in EA, NA, and PGA samples. The corresponding volcano plots, heatmaps, and PCA analysis were generated to visualize the results (Fig. 4A–C). The aging-related genes were searched in the CellAge database, GenAge database, Aging Atlas database, and CSGene database, and 1441 genes were identified (Fig. 5A). 12, 32, 0 aging-related DEGs in EA, NA, and PGA were included in both data were then intersected, respectively (Fig. 5B, Tables 2 and 3). Among 12 EA-aging DEGs, IL-7R were down-regulated ($\text{Log}_2\text{FC} = -1.16$) and 11 were up-regulated (HIST2H2AC, HIST2H2AA3, BIRC3, CCL1, MMP12, MMP10, CEACAM1, NLRP3, TNFSF14, CCL26, FSCN1). Data from asthma patients suggest that the severity of asthmatic airway inflammation is positively correlated with the thymic stromal lymphopoietin (TSLP) expression level. It binds to TSLPR and subsequently recruits the IL-7Rα to form the TSLP/TSLPR/IL-7Rα high-affinity triple complex, activating dendritic cells to induce Th2 cell polarization and drive Th2 cell development [32]. In addition, of 32 NA-aging DEGs, 2 were down-regulated (CKB, CYP26A1), which responds to nutrient metabolic process and cellular anion homeostasis, and 30 were up-regulated. The expression level of CCL20 was significantly increased in the NA group ($\text{Log}_2\text{FC} = 2.19$), which was previously reported to be related to glucocorticoid and chemotherapeutic resistance in various diseases [33–35]. The expression of EA-aging and NA-aging-related DEGs is shown in Fig. 5C.

3.4. Function enrichment analysis of aging-related DEGs in different inflammatory phenotypes of asthma

To further understand the potential biological functions of aging-related DEGs in different inflammatory phenotypes of asthma, GO enrichment analysis was performed by using the DAVID tool. The analysis showed that the EA-aging-related DEGs involved 262 biological processes, 1 cellular component, and 30 molecular functions. The NA-aging-related DEGs involved 302 biological processes, 4 cellular components, and 16 molecular functions. The NA-aging and EA-aging-related DEGs were involved in the cytokine-mediated signaling pathway, lymphocyte chemotaxis, and regulation of T cell activation. The NA-aging-related DEGs were mainly involved in the response to lipopolysaccharides, response to molecules of bacterial origin, and cellular response to biotic stimulus. While the biological processes of EA-aging-related DEGs were mainly concentrated on regulating T-cell-mediated immunity and cellular response to the virus. The 5 terms with the largest significant difference were selected and plotted (Fig. 6A, Tables 4 and 5). Subsequently, KEGG

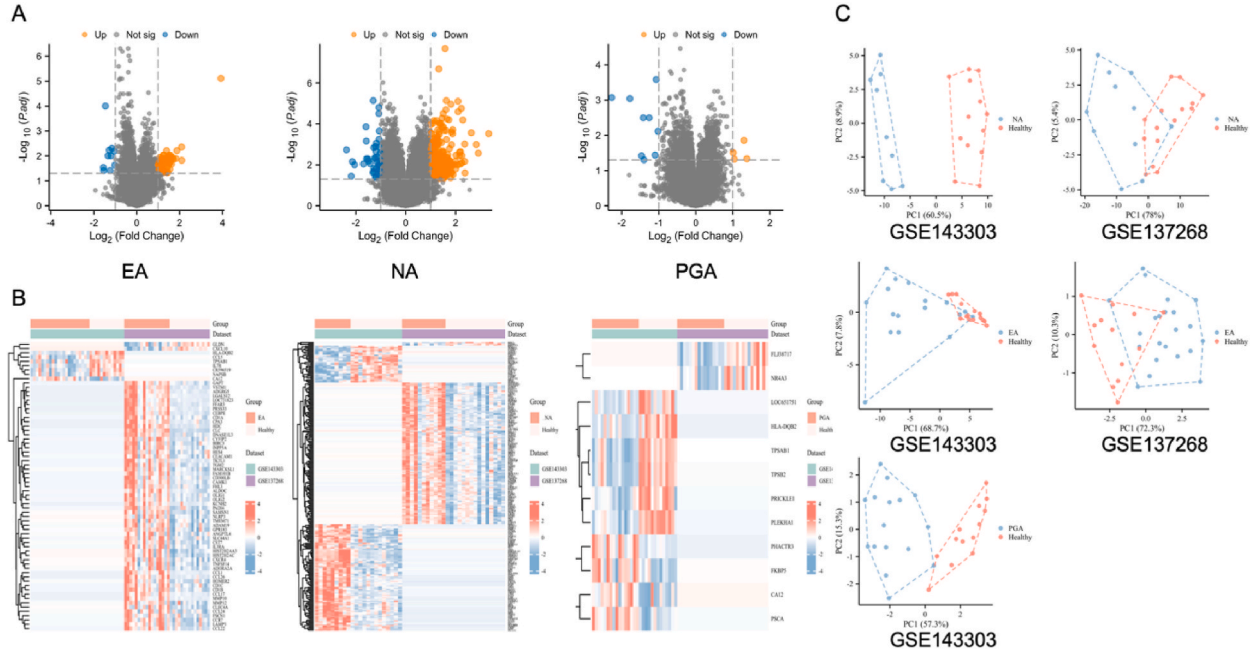

Fig. 4. Identifying the DEGs in different inflammatory phenotypes of asthma. DEGs in EA, NA, and PGA were visualized in volcano plots (A), heatmap (B), and PCA (C).

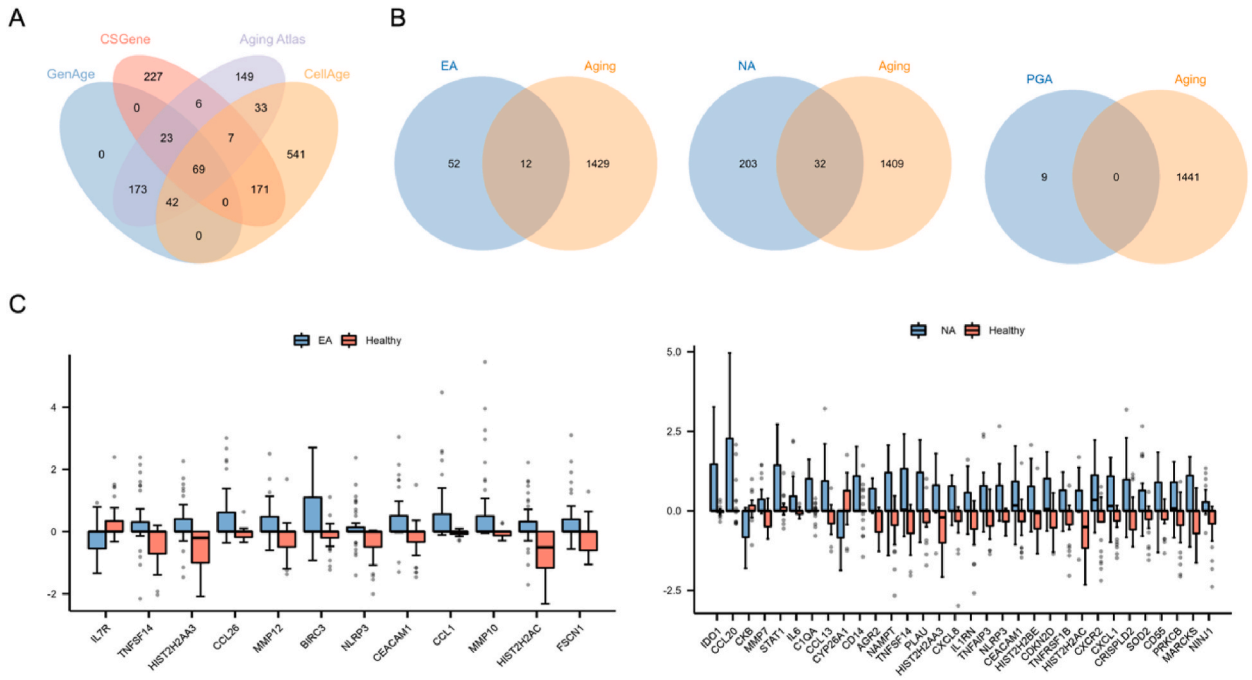

Fig. 5. Identifying the aging-related DEGs in different inflammatory phenotypes of asthma. (A) The aging-related genes searched in the CellAge database, GenAge database, Aging Atlas database, and CSGene database were visualized in a Venn diagram. (B) DEGs in EA, NA, and PGA intersected with aging-related genes in the Venn diagram, respectively. (C) The expression of aging-related DEGs in EA and NA was shown in boxplots, respectively.

Table 2
The analysis of aging-related DEGs in NA in GSE137268 and GSE143303 datasets.

Gene	Log_2FC	P. adjust	Type	Gene	Log_2FC	P. adjust	Type
CCL20	2.19	9.00E-03	Up	PLAU	1.27	0.028	Up
CXCL8	1.84	2.45E-04	Up	CRISPLD2	1.26	0.042	Up
TNFSF14	1.77	0.006	Up	AGR2	1.26	5.31E-04	Up
CCL13	1.76	1.61E-03	Up	STAT1	1.26	8.89E-03	Up
SOD2	1.73	6.30E-05	Up	TNFAIP3	1.23	0.021	Up
NAMPT	1.69	0.013	Up	CXCL1	1.18	3.67E-03	Up
HIST2H2AA3	1.67	0.005	Up	CD14	1.14	3.18E-02	Up
HIST2H2AC	1.64	0.005	Up	NLRP3	1.14	0.01	Up
IDO1	1.63	5.76E-04	Up	C1QA	1.11	1.05E-03	Up
CXCR2	1.63	0.015	Up	MMP7	1.09	2.28E-03	Up
IL1RN	1.35	0.0133	Up	NINJ1	1.08	0.026	Up
CEACAM1	1.34	0.02	Up	CD55	1.03	0.026	Up
PRKCB	1.33	0.02	Up	IL6	1.01	5.67E-03	Up
MARCKS	1.31	0.017	Up	TNFRSF1B	1	2.65E-02	Up
CDKN2D	1.31	0.008	Up	CKB	−1.01	1.02E-02	Down
HIST2H2BE	1.27	0.013	Up	CYP26A1	−1.58	5.11E-03	Down

pathway enrichment analysis was performed on the asthma-aging-related DEGs. The 4 metabolic pathways with the most significant number of genes were plotted in Fig. 6A. The results showed that the NA-aging and EA-aging-related DEGs were mainly enriched in 32 and 3 pathways, respectively. The NA-aging and EA-aging-related DEGs were both involved in the process of cytokine activity and cytokine receptor binding. Among them, the NA-aging-related DEGs were mainly involved in oxygen binding, lipopolysaccharide-binding, and tumor necrosis factor receptor binding. Meanwhile, the biological processes of EA-aging-related DEGs were mainly concentrated in the NF-kappa B signaling pathway. The molecules corresponding to the enriched terms are shown in Fig. 6B. GO/KEGG functional enrichment combined with Log_2FC values were demonstrated in Fig. 6C and Tables 6 and 7. Based on the provided logFC, the zscore was used to determine whether the corresponding term is positively or negatively regulated. The larger the absolute value of the zscore, the higher the degree of regulation to be.

Table 3
The analysis of aging-related DEGs in EA in GSE137268 and GSE143303 datasets.

Gene	Log_2FC	P. adjust	Type
IL7R	−1.16	4.97E-03	Down
HIST2H2AC	1.58	0.01	Up
HIST2H2AA3	1.53	0.011	Up
CCL26	1.21	0.014	Up
CCL1	1.27	0.025	Up
BIRC3	1.15	0.027	Up
MMP12	1.07	0.031	Up
MMP10	1.55	0.034	Up
CEACAM1	1.22	0.04	Up
NLRP3	1.03	0.042	Up
TNFSF14	1.26	0.047	Up
FSCN1	1.08	0.047	Up

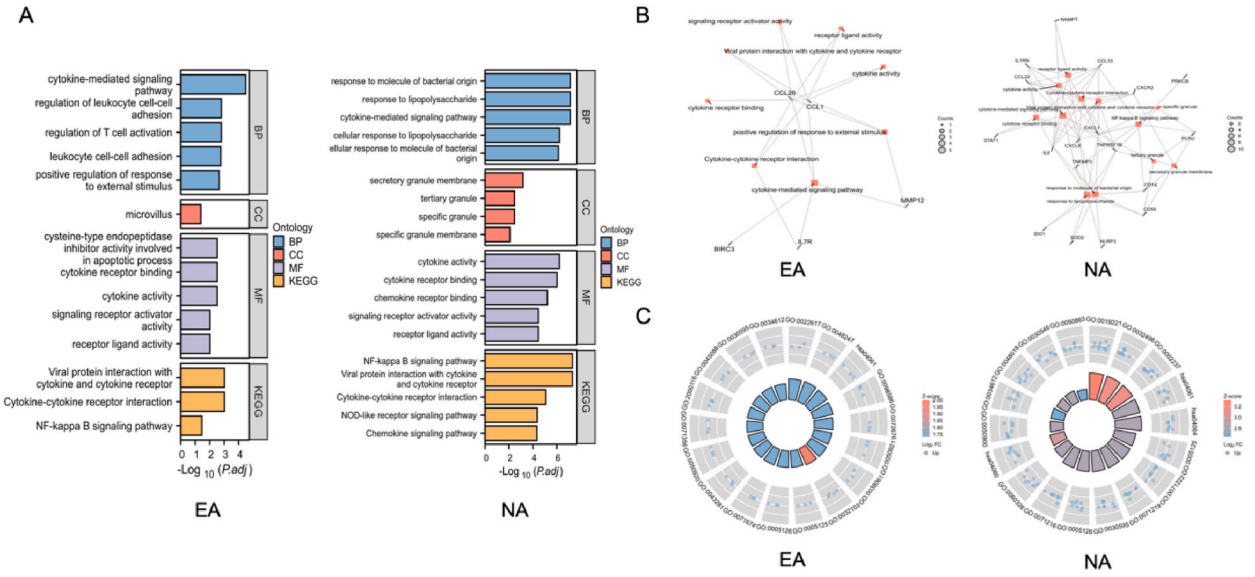

Fig. 6. Function enrichment analysis of aging-related DEGs in different inflammatory phenotypes of asthma. The 5 terms of GO (BP, CC, MF) and KEGG pathway with the largest significant difference enrichment analysis were shown in bubble diagram (A) and network diagram (B). GO/KEGG functional enrichment combined with Log_2FC values were demonstrated in the circle diagram (C).

Table 4
GO and KEGG pathway enrichment terms for EA-aging related DEGs.

Ontology	ID	Description	GeneRation	P. adjust	Gene ID
BP	GO:0019221	cytokine-mediated signaling pathway	6/10	3.27E-05	IL7R/BIRC3/CCL1/MMP12/CEACAM1/CCL26
BP	GO:0050863	regulation of T cell activation	4/10	0.002	IL7R/CEACAM1/NLRP3/TNFSF14
BP	GO:1903037	regulation of leukocyte cell-cell adhesion	4/10	0.002	IL7R/CEACAM1/NLRP3/TNFSF14
BP	GO:0007159	leukocyte cell-cell adhesion	4/10	0.002	IL7R/CEACAM1/NLRP3/TNFSF14
BP	GO:0032103	positive regulation of response to external stimulus	4/10	0.002	CCL1/MMP12/TNFSF14/CCL26
CC	GO:0005902	microvillus	2/10	0.04	CEACAM1/FSCN1
MF	GO:0005125	cytokine activity	3/10	0.003	CCL1/TNFSF14/CCL26
MF	GO:0005126	cytokine receptor binding	3/10	0.003	CCL1/TNFSF14/CCL26
MF	GO:0048018	receptor ligand activity	3/10	0.01	CCL1/TNFSF14/CCL26
MF	GO:0030546	signaling receptor activator activity	3/10	0.01	CCL1/TNFSF14/CCL26
MF	GO:0043027	cysteine-type endopeptidase inhibitor activity involved in apoptotic process	2/10	0.003	BIRC3/TNFSF14
KEGG	hsa04060	Cytokine-cytokine receptor interaction	4/7	0.001	IL7R/CCL1/TNFSF14/CCL26
KEGG	hsa04061	Viral protein interaction with cytokine and cytokine receptor	3/7	0.001	CCL1/TNFSF14/CCL26
KEGG	hsa04064	NF-kappa B signaling pathway	2/7	0.035	BIRC3/TNFSF14

Table 5
GO and KEGG pathway enrichment terms for NA-aging related DEGs.

Ontology	ID	Description	GeneRation	P. adjust	Gene ID
BP	GO:0019221	cytokine-mediated signaling pathway	11/31	7.51E-08	CXCL8/CCL13/IL6/STAT1/CCL20/CXCL1/IL1RN/CXCR2/CEACAM1/TNFRSF1B/TNFAIP3
BP	GO:0032496	response to lipopolysaccharide	10/31	7.51E-08	CXCL8/IDO1/IL6/CD14/CXCL1/NLRP3/TNFRSF1B/TNFAIP3/SOD2/CD55
BP	GO:0002237	response to molecule of bacterial origin	10/31	7.51E-08	CXCL8/IDO1/IL6/CD14/CXCL1/NLRP3/TNFRSF1B/TNFAIP3/SOD2/CD55
BP	GO:0071222	cellular response to lipopolysaccharide	8/31	5.99E-07	CXCL8/IL6/CD14/CXCL1/NLRP3/TNFRSF1B/TNFAIP3/CD55
BP	GO:0071219	cellular response to molecule of bacterial origin	8/31	7.33E-07	CXCL8/IL6/CD14/CXCL1/NLRP3/TNFRSF1B/TNFAIP3/CD55
CC	GO:0030667	secretory granule membrane	6/31	0.001	CD14/CXCR2/CEACAM1/TNFRSF1B/CD55/PLAU
CC	GO:0042581	specific granule	4/31	0.003	CXCL1/CEACAM1/TNFRSF1B/PLAU
CC	GO:0070820	tertiary granule	4/31	0.003	CXCL1/CEACAM1/CD55/PLAU
CC	GO:0035579	specific granule membrane	3/31	0.008	CEACAM1/TNFRSF1B/PLAU
MF	GO:0005125	cytokine activity	8/31	6.06E-07	CXCL8/CCL13/IL6/CCL20/TNFSF14/CXCL1/NAMPT/IL1RN
MF	GO:0005126	cytokine receptor binding	8/31	9.51E-07	CXCL8/CCL13/IL6/STAT1/CCL20/TNFSF14/CXCL1/IL1RN
MF	GO:0048018	receptor ligand activity	8/31	3.48E-05	CXCL8/CCL13/IL6/CCL20/TNFSF14/CXCL1/NAMPT/IL1RN
MF	GO:0030546	signaling receptor activator activity	8/31	3.48E-05	CXCL8/CCL13/IL6/CCL20/TNFSF14/CXCL1/NAMPT/IL1RN
MF	GO:0042379	chemokine receptor binding	5/31	6.12E-06	CXCL8/CCL13/STAT1/CCL20/CXCL1
KEGG	hsa04060	Cytokine-cytokine receptor interaction	9/26	8.17E-06	CXCL8/CCL13/IL6/CCL20/TNFSF14/CXCL1/IL1RN/CXCR2/TNFRSF1B
KEGG	hsa04061	Viral protein interaction with cytokine and cytokine receptor	8/26	4.95E-08	CXCL8/CCL13/IL6/CCL20/TNFSF14/CXCL1/CXCR2/TNFRSF1B
KEGG	hsa04064	NF-kappa B signaling pathway	8/26	4.95E-08	CXCL8/CCL13/CD14/TNFSF14/CXCL1/PRKCB/TNFAIP3/PLAU
KEGG	hsa04621	NOD-like receptor signaling pathway	7/26	4.37E-05	CXCL8/IL6/STAT1/CXCL1/NLRP3/NAMPT/TNFAIP3
KEGG	hsa04062	Chemokine signaling pathway	7/26	4.66E-05	CXCL8/CCL13/STAT1/CCL20/CXCL1/CXCR2/PRKCB

3.5. PPI network analysis of aging-related DEGs in different inflammatory phenotypes of asthma

To investigate the overall regulatory mechanism of aging-related DEGs in different inflammatory phenotypes of asthma, we constructed a PPI network by the STRING database, and statistical analysis and visualization were performed in R 4.2.1. The PPI network of NA-aging-related DEGs has 26 nodes and 71 edges (Fig. 7A, Table S1). The cytoHubba plugin was used to identify the 10 hub genes of the NA-aging-related DEGs PPI network, including IL-6, CXCL8, CXCL1, TNFAIP3, CCL20, IL-1RN, STAT1, CXCR2, NLRP3, CCL13 (Fig. 7B–Table 8). The GO and KEGG enrichment analysis suggests these hub genes were related to cytokine activity, cytokine-mediated signaling pathway, and leukocyte migration, showing that they may play a vital role in the occurrence or progression of NA (Fig. 7C). Among them, high expression of NLRP3 has been reported to cause asthma pathogenesis through activation of airway inflammatory response and cellular pyroptosis [36]. CXCL1 is expressed by macrophages, neutrophils, and epithelial cells and has neutrophil chemotactic activity. This chemokine is signaling via the CXCR2 receptor, initiating its effects [37]. Additionally, CXCR2 serves as a chemokine receptor for CXCL8 [38].

3.6. Correlation analysis of aging-related DEGs in different inflammatory phenotypes of asthma

We further conducted a two-by-two correlation analysis of NA-aging and EA-aging-related DEGs to demonstrate their interrelationships to the disease. Both statistical analysis and visualization were performed in R 4.2.1. Positive and negative signs indicate positive and negative correlations. $|r| > 0.95$: significant correlation; $|r| \geq 0.8$: highly correlated; $0.5 \leq |r| < 0.8$: moderately correlated; $0.3 \leq |r| < 0.5$: low correlation; $|r| < 0.3$: weak correlation (Fig. 8A Tables S2–3). To intuitively observe the expression difference of asthma-aging-related DEGs between asthma and health groups, the DEGs were grouped in asthma and health for correlation heat map grouping statistics (Fig. 8B). The results demonstrate that NA-aging-related DEGs have a strong correlation difference between the healthy and NA groups. In contrast, the EA-aging-related DEGs show a weak difference correlation compared to patients in the healthy group. Taken together, these results suggest that the aberrant functions of NA-aging-related DEGs may be closely associated with the development of NA.

Table 6
GO/KEGG functional enrichment combined with Log_2FC values for NA-aging related DEGs.

ID	Description	p.adjust	FDR	Gene ID	zscore
GO:0019221	cytokine-mediated signaling pathway	3.25E-08	1.62E-08	CCL20/CXCL8/CCL13/CXCR2/IL1RN/CEACAM1/STAT1/TNFAIP3/CXCL1/IL6/TNFRSF1B	3.32
GO:0032496	response to lipopolysaccharide	3.25E-08	1.62E-08	CXCL8/SOD2/IDO1/TNFAIP3/CXCL1/CD14/NLRP3/CD55/IL6/TNFRSF1B	3.16
GO:0002237	response to molecule of bacterial origin	3.25E-08	1.62E-08	CXCL8/SOD2/IDO1/TNFAIP3/CXCL1/CD14/NLRP3/CD55/IL6/TNFRSF1B	3.16
hsa04060	Cytokine-cytokine receptor interaction	5.48E-06	4E-06	CCL20/CXCL8/TNFSF14/CCL13/CXCR2/IL1RN/CXCL1/IL6/TNFRSF1B	3
hsa04061	Viral protein interaction with cytokine and cytokine receptor	3.44E-08	2.51E-08	CCL20/CXCL8/TNFSF14/CCL13/CXCR2/CXCL1/IL6/TNFRSF1B	2.83
hsa04064	NF-kappa B signaling pathway	3.44E-08	2.51E-08	CXCL8/TNFSF14/CCL13/PRKCB/PLAU/TNFAIP3/CXCL1/CD14	2.83
GO:0071222	cellular response to lipopolysaccharide	3.27E-07	1.63E-07	CXCL8/TNFAIP3/CXCL1/CD14/NLRP3/CD55/IL6/TNFRSF1B	2.83
GO:0071219	cellular response to molecule of bacterial origin	4E-07	2E-07	CXCL8/TNFAIP3/CXCL1/CD14/NLRP3/CD55/IL6/TNFRSF1B	2.83
GO:0030595	leukocyte chemotaxis	4.23E-07	2.1E-07	CCL20/CXCL8/TNFSF14/CCL13/CXCR2/CXCL1/NINJ1/IL6	2.83
GO:0005125	cytokine activity	3.11E-07	1.89E-07	CCL20/CXCL8/TNFSF14/CCL13/NAMPT/IL1RN/CXCL1/IL6	2.83
GO:0071216	cellular response to biotic stimulus	6.88E-07	3.42E-07	CXCL8/TNFAIP3/CXCL1/CD14/NLRP3/CD55/IL6/TNFRSF1B	2.83
GO:0005126	cytokine receptor binding	4.91E-07	2.97E-07	CCL20/CXCL8/TNFSF14/CCL13/IL1RN/STAT1/CXCL1/IL6	2.83
GO:0060326	cell chemotaxis	3.05E-06	1.52E-06	CCL20/CXCL8/TNFSF14/CCL13/CXCR2/CXCL1/NINJ1/IL6	2.83
GO:0050900	leukocyte migration	1.13E-05	5.61E-06	CCL20/CXCL8/TNFSF14/CCL13/CXCR2/CXCL1/NINJ1/IL6	2.83
GO:0048018	receptor ligand activity	2E-05	1.21E-05	CCL20/CXCL8/TNFSF14/CCL13/NAMPT/IL1RN/CXCL1/IL6	2.83
GO:0030546	signaling receptor activator activity	2E-05	1.21E-05	CCL20/CXCL8/TNFSF14/CCL13/NAMPT/IL1RN/CXCL1/IL6	2.83
GO:0034612	response to tumor necrosis factor	1.13E-05	5.61E-06	CCL20/CXCL8/CCL13/STAT1/TNFAIP3/CD14/TNFRSF1B	2.65
GO:0050863	regulation of T cell activation	4.29E-05	2.13E-05	TNFSF14/IDO1/CEACAM1/NLRP3/CD55/IL6/TNFRSF1B	2.65

Table 7
GO/KEGG functional enrichment combined with Log_2FC values for EA-aging related DEGs.

ID	Description	p. adjust	FDR	Gene ID	zscore
GO:0032103	positive regulation of response to external stimulus	0.002	0.001	CCL26/CCL1/MMP12/TNFSF14	2
GO:0022617	extracellular matrix disassembly	0.001	0.001	MMP12/MMP10/FSCN1	1.73
GO:0048247	lymphocyte chemotaxis	0.001	0.001	CCL26/CCL1/TNFSF14	1.73
GO:0098586	cellular response to virus	0.001	0.001	BIRC3/MMP12/NLRP3	1.73
GO:0072676	lymphocyte migration	0.002	0.001	CCL26/CCL1/TNFSF14	1.73
hsa04061	Viral protein interaction with cytokine and cytokine receptor	0.001	0.001	CCL26/CCL1/TNFSF14	1.73
GO:0050921	positive regulation of chemotaxis	0.002	0.001	CCL26/CCL1/TNFSF14	1.73
GO:0038061	NIK/NF-kappaB signaling	0.002	0.001	BIRC3/NLRP3/TNFSF14	1.73
GO:0071674	mononuclear cell migration	0.003	0.001	CCL26/CCL1/TNFSF14	1.73
GO:0043281	regulation of cysteine-type endopeptidase activity involved in apoptotic process	0.003	0.001	BIRC3/NLRP3/TNFSF14	1.73
GO:0005125	cytokine activity	0.003	0.001	CCL26/CCL1/TNFSF14	1.73
GO:0005126	cytokine receptor binding	0.003	0.001	CCL26/CCL1/TNFSF14	1.73
GO:0050920	regulation of chemotaxis	0.003	0.001	CCL26/CCL1/TNFSF14	1.73
GO:0071356	cellular response to tumor necrosis factor	0.003	0.001	CCL26/CCL1/BIRC3	1.73
GO:2000116	regulation of cysteine-type endopeptidase activity	0.003	0.001	BIRC3/NLRP3/TNFSF14	1.73
GO:0045088	regulation of innate immune response	0.003	0.001	BIRC3/MMP12/CEACAM1	1.73
GO:0030595	leukocyte chemotaxis	0.003	0.001	CCL26/CCL1/TNFSF14	1.73
GO:0034612	response to tumor necrosis factor	0.004	0.001	CCL26/CCL1/BIRC3	1.73

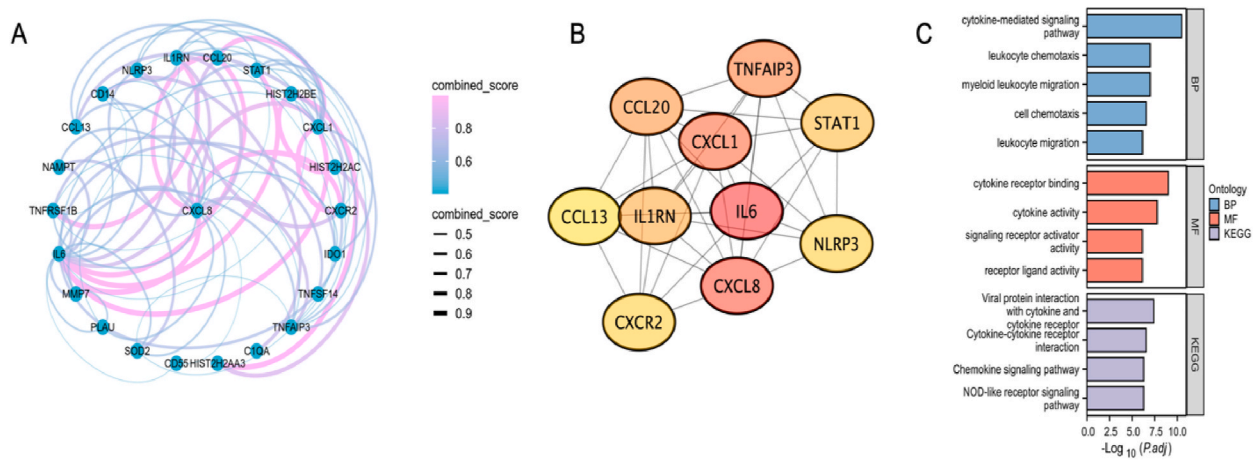

Fig. 7. PPI Network analysis of aging-related DEGs in NA. (A) PPI network of NA-aging-related DEGs. (B) Top 10 hub genes of the NA-aging-related DEGs. (C) The 5 terms of GO (BP, CC, MF) and KEGG pathway with the largest significant difference enrichment analysis of 10 hub genes were shown in the bubble diagram.

Table 8
Top 10 genes in NA-aging related DEGs network edges ranked by cytoHubba method.

Rank	Name	Score
1	IL6	855
2	CXCL8	854
3	CXCL1	768
4	TNFAIP3	552
5	CCL20	480
6	IL1RN	408
7	STAT1	270
8	CXCR2	240
8	NLRP3	240
10	CCL13	120

3.7. ROC analysis of aging-related DEGs in different inflammatory phenotypes of asthma

In order to distinguish blood-based biomarkers of different inflammatory phenotypes of asthma, ROC analysis was conducted to analyze the diagnostic value of asthma-aging-related DEGs. The AUC can be interpreted as the model's ability to accurately classify classes from 0.5 to 1. Generally, the closer the AUC is to 1, the better the diagnostic effect. The outcomes revealed that HIST2H2AC (AUC = 0.826), HIST2H2AA3 (AUC = 0.831), HIST2H2BE (AUC = 0.797), CDKN2D (AUC = 0.799), CKB (AUC = 0.729), CEACAM1 (AUC = 0.790), CYP26A1 (AUC = 0.774), CXCR2 (AUC = 0.750), CXCL1 (AUC = 0.821), PRKCB (AUC = 0.771), TNFSF14 (AUC = 0.795), and NLRP3 (AUC = 0.810), exhibited good predictive accuracy in NA patients (Fig. 9A). NLRP3 (AUC = 0.712), HIST2H2AA3 (AUC = 0.770), IL-7R (AUC = 0.743), and HIST2H2AC (AUC = 0.774) were able to predict clinical diagnosis in EA patients (Fig. 9B). We further used the GSE108417 dataset as a test set to validate the diagnostic value of asthma-aging-related DEGs. The AUC value was ranged from 0.556 to 1 and demonstrated a consistent outcome (Fig. 9C). Consequently, these results suggested that asthma-aging-related DEGs may be promising diagnostic biomarkers in the therapy of the two types of asthma.

4. Discussion

Asthma is a chronic inflammatory respiratory disease that results in the constriction of airways and restricted airflow within the lungs, which cause difficulty breathing and other symptoms. The conventional treatment of severe asthma is based on the use of high-dose inhaled corticosteroids (ICS) in combination with long-acting β2-adrenergic agonists (LABAs). Other medications, such as tiotropium and leukotriene modulators or oral corticosteroids, may also be necessary [39]. EA and NA are both specific types of asthma associated with an increase in different kinds of white blood cells (granulocytes) in the airway. Despite having similar clinical features, these two types of asthma have distinct pathophysiological mechanisms and respond differently to treatment. Bronchial biopsies from patients with NA revealed a key role of Th17 subpopulation in the $CD4^+$ T lymphocytes. This subpopulation secretes IL-17A and IL-17F, which induce bronchial epithelial cells and subepithelial airway fibroblasts to release neutrophil chemotactic agents (CXCL8 and CXCL1/GRO-α) [10]. Furthermore, specific receptors for IL-21 and IL-23 produced by Th17 cells act as autocrine amplifiers of the

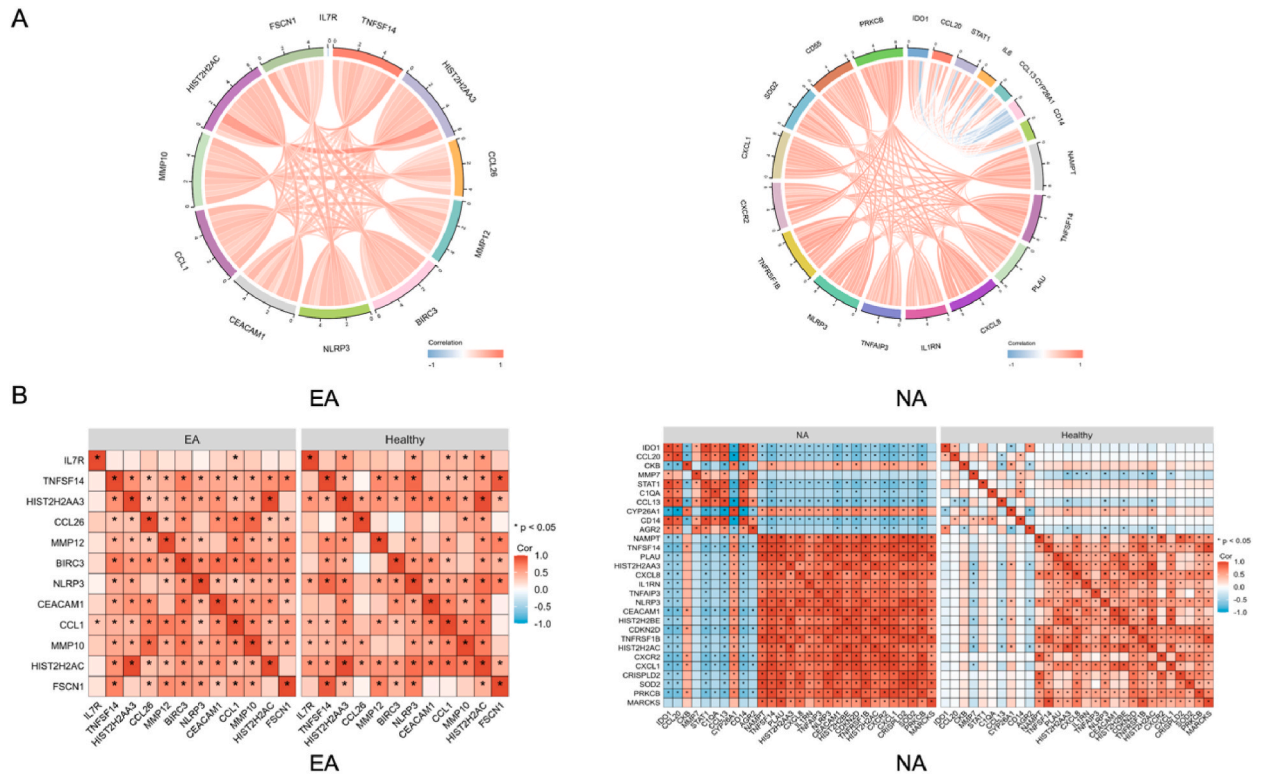

Fig. 8. Correlation analysis of aging-related DEGs in different inflammatory phenotypes of asthma. Correlation analysis of aging-related DEGs in EA and NA were shown in the chordal diagram (A) and heatmap (B).

Th17 response and bind to IL-23, stabilizing the Th17 phenotype and maintaining Th17 cells in a potent activation state. The differentiation of the immune phenotype of Th17 cells depends on the synergistic action of IL-1β, IL-6, and TGF-β. Activating caspase-1 by inflammatory vesicles converts IL-1β to an active form, thereby inducing the differentiation of Th17 cells [40]. Furthermore, neutrophil extracellular traps (NETs) promote the polarization of Th17 cells and neutrophil airway inflammation [41]. In addition to Th17 lymphocytes, IL-12-dependent Th1 cells are involved in the pathobiological process of severe neutrophilic asthma [42]. As for EA, activating, differentiating, and producing various cytokines (e.g., IL-4, IL-5, and IL-13) by specific Th2 cells can trigger allergic and eosinophilic non-allergic asthma. IL-4 is a cytokine that differentiates naïve T cells into Th2 cells. IL-5 is responsible for the maturation and release of eosinophils from the bone marrow. IL-13 induces the proliferation of IgE-producing B cells and the proliferation of endothelial cells. Group 2 innate lymphoid cells (ILC2s) play a pivotal role in eosinophilic non-allergic asthma. They can produce IL-5, which results in severe eosinophilic inflammation. In an Alternaria-induced mouse model of asthma, ILC2s, IL-33, IL-5, and IL-13, can drive eosinophilia and airway hyperresponsiveness (AHR) [43]. In addition, GM-CSF plays a pivotal role in eosinophil activation. The Th2 network containing the VCAM-1/CC chemokine/GM-CSF cascade may be a significant pathway for maintaining asthma eosinophil infiltration and activation, with or without IL-5 [44].

Currently, eosinophilic and neutrophil asthma are assessed clinically primarily by measuring cell concentrations in blood and/or sputum, and only a few relevant biomarkers are used clinically. The large-scale inhalation and systemic therapy may still not be able to control both types of asthma, and therefore requiring additional biological treatment. Molecular therapies targeting type 2 (high T2) eosinophilic asthma include IgE, IL-5 and its receptor, IL-4 receptor, and other upstream innate cytokines such as TSLP [45]. In contrast, molecularly targeted pharmacological treatments for low T2 (mainly neutrophilic severe asthma) are sorely lacking. C-X-C motif chemokine receptor 2 (CXCR2) antagonist, the antibody against anti-TNF-α, the antibody against anti-IL-17 receptor, anti-IL-1 receptor antagonist, anti-IL-6 antibody, IFN, KIT inhibitor, IL-13, and 5-lipoxygenase-activating protein (FLAP) inhibitor have been demonstrated to be effective adjunctive agents in the treatment of neutrophilic asthma. However, further clinical trials over a longer study period are necessary to confirm these findings [10]. Thus, discovering new clinical and quantifiable biomarkers for different inflammatory phenotypes of asthma could provide valuable insights into underlying disease mechanisms and help tailor asthma treatments to individual patients.

Population aging is a significant social challenge confronting our nation and the global community. The aging of the immune system contributes to the heightened vulnerability of older individuals to chronic inflammatory and autoimmune disease, leading to elevated susceptibility to infections and increased cancer vulnerability [46,47]. Elderly patients experiencing reduced physical and immune functionality often contend with a higher burden of respiratory illnesses. For instance, late-onset asthma emerges as a distinctive epidemiological disease marked by elevated morbidity and mortality rates as the global population continues to live longer

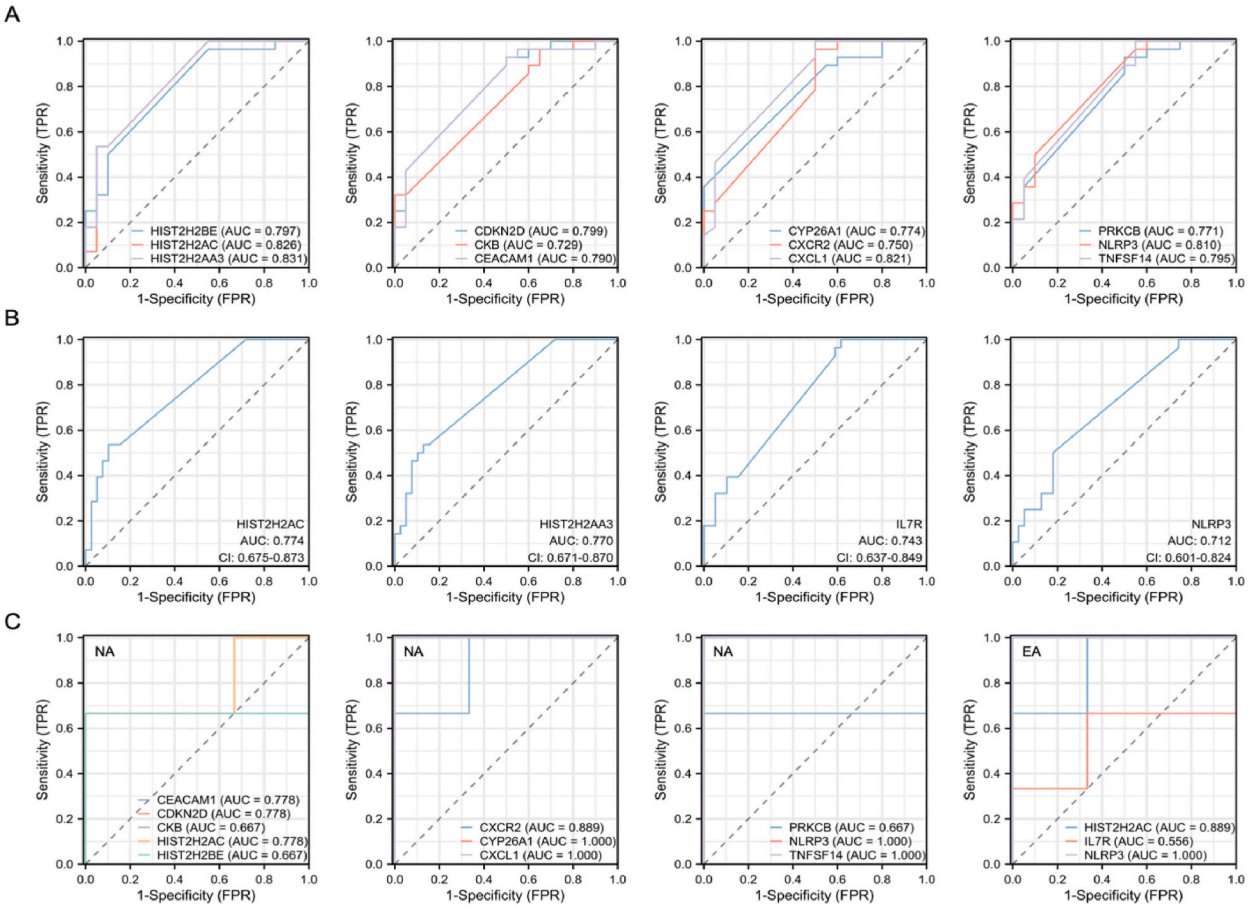

Fig. 9. ROC analysis of aging-related DEGs in NA (A), EA (B), and NA, EA in test set (C).

[48]. The prevalence of late-onset and severe asthma in patients with elderly asthmatics significantly surpasses that observed in non-elderly asthma cases. Comparatively, peripheral blood/sputum eosinophil counts and serum eosinophilic cationic protein levels are diminished in elderly individuals with asthma as opposed to their non-elderly counterparts. In contrast, sputum neutrophil counts tend to be higher in elderly asthmatics compared to non-elderly individuals [49]. Clinically, the manifestations of elderly asthma are characterized by a predominance of neutrophilic rather than eosinophilic inflammation. Notably, age-related asthma displays reduced atopic tendencies and is often accompanied by numerous comorbidities. The condition presents challenges in treatment response and prognosis [50], posing a substantial threat to life and health. In the clinical field, relief from this condition is primarily achieved through glucocorticoid medications. Herein, we discuss the existing understanding of molecular mechanisms and dysfunction regulating different aspects of aging-related asthma to formulate rational strategies for modulating the aging cell phenotype in the lung for therapeutic benefit. In our research, we observed a set of aging-related DEGs shared between both NA and EA. Specifically, the genes HIST2H2AC, HIST2H2AA3, CEACAM1, NLRP3, and TNFSF14 exhibited downregulation in both asthma subtypes (Tables 2 and 3). Among these genes, some have been previously implicated in age-dependent fibrosis [51], remodeling [52], and age-associated inflammation [53,54] and have been proposed as potential prognostic biomarkers for various diseases [55–57]. Most patients with asthma who respond well to inhaled glucocorticoid therapy are referred to as steroid-sensitive asthmatics, such as those with eosinophilic asthma [58]. However, in those patients who lack eosinophils in the airways and may even have neutrophilia, inhaled glucocorticosteroids and even oral glucocorticosteroids are ineffective in controlling their symptoms and are called steroid-resistant asthma [59,60]. This group of people with asthma often requires hospitalization, placing a heavy financial burden on the healthcare system. Whether the increase in neutrophils causes these patients to be insensitive to glucocorticoid therapy or whether it is a mechanism of neutrophil aggregation in the patient's airways that leads to the increase in neutrophils in their airways remains unclear. Many older asthmatics, mostly with NA, have persistent airway type 2 inflammation that continues to increase in severity despite glucocorticoid treatment. Therefore, we propose that aging may be essential in contributing to glucocorticoid resistance in asthmatics.

Due to the predominance of NA in elderly patients, we observed that aging-related DEGs in NA primarily affect the NOD-like receptor signaling pathway, type 2 immune response, T cell proliferation, cellular response to tumor necrosis factor, reactive oxygen species, lipopolysaccharide, and molecule of bacterial origin (Fig. 6), which have been previously reported in regulation asthma. For instance, the immune system plays a crucial role in skeletal aging. Although the role of neutrophils in bone is unclear, it has been

reported that neutrophils induce inflammatory bone loss by secreting CCL2 and CCL20 and summoning Th17 cells [61]. Moreover, CCL20 is a chemical attractant of helper T lymphocytes, neutrophils, and dendritic cells [62]. Persistent elevation of CCL20 is characteristic of severe asthma in human and mouse models. In the bronchial epithelium, glucocorticoids promote the expression of the pro-inflammatory cytokine CCL20, which may help explain the molecular basis of steroid-resistant asthma [63,64]. Lipopolysaccharides (LPS) are related to asthma severity and steroid resistance [65]. Bronchoscopy of asthma patients on and off hormonal therapy revealed significant changes in the composition of airway bacterial flora in lower airway samples from asthma patients compared to healthy controls. There was a correlation between airway microorganisms and asthma [66,67]. Mitochondria-derived reactive oxygen species may be a key driver of cellular senescence [68]. SOD2, the main antioxidant enzyme that scavenges ROS from the inner mitochondrial matrix, is the first line of defense against oxidative damage to mitochondria [69]. In mouse models, SOD2 deletion in mitochondria accelerates cell aging [70]. In addition, many studies have shown that the imbalance of oxidative stress is closely related to the pathological process of neutrophil asthma, and excessive ROS will reduce the efficacy of glucocorticoids in neutrophil asthma, leading to damaged airway remodeling and low lung function [71]. Research suggests that targeting neutrophil-associated mediators or neutrophil-mediated inflammatory responses is a novel strategy for asthma treatment.

As research advances, our understanding of asthma heterogeneity is essential for treatment strategies for the individual patient, leading to increasingly personalized and effective treatment approaches. Induced sputum cytology is the gold standard for determining the inflammatory phenotype of asthma and is commonly used to assess airway inflammation. With the rise of omics studies, breakthroughs have been made in using induced sputum to search for new biomarkers of severe asthma [72]. Age at the onset of asthma is now recognized as a key factor in distinguishing heterogeneous asthma phenotypes. Early-onset asthma is typically associated with significant family history and potential genetic factors, with a stronger correlation between age of onset and genetics. In older patients with asthma, the pathogenesis appears to differ from that observed in patients with early-onset asthma. The existing literature on the differences between severe early-onset and late-onset asthma, particularly with regard to their inflammatory markers, is limited. Further investigation is required to elucidate this aspect [73]. All aged cells eventually exhibit a complex, multicomponent senescence-related secretion phenotype (SASP). SASP involuntarily alters the behavior of neighboring cells and the tissue microenvironment. Chronic inflammation, a common feature of aging tissues, is a major risk factor for many age-related diseases (such as asthma), and the accumulation of chronic inflammation in aging tissues may be caused by SASP [74]. Experiments in human cells, tissues, and transgenic mouse models, as well as pharmacological interventions in cells and mice, strongly suggest that aged cells are involved in the pathology of asthma. Most of these adverse effects of aged cells may be attributed to the secretion of SASP containing many pro-inflammatory factors [75,76]. In this study, we further conducted ROC analysis to distinguish blood-based biomarkers of different inflammatory phenotypes of asthma with diagnostic value in asthma-aging-related DEGs. Such as HIST2H2AC, HIST2H2AA3, HIST2H2BE, CDKN2D, CKB, CEACAM1, CYP26A1, CXCR2, CXCL1, PRKCB, TNFSF14, and NLRP3 in NA patients. NLRP3, HIST2H2AA3, IL-7R, and HIST2H2AC in EA patients (Fig. 9). In conclusion, our research systematically screened and validated several blood-based biomarkers for asthma by various bioinformatics analyses and pointed out the age-related heterogeneous asthma targets may be a potential therapeutic target for patients with severe asthma.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Funding

Not applicable.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: Gene Expression Omnibus datasets (GEO, https://www.ncbi.nlm.nih.gov/gds/).

CRediT authorship contribution statement

Xinning Liu: Investigation, Formal analysis, Data curation. **Bing Li:** Data curation, Conceptualization. **Shuya Liu:** Software, Resources, Conceptualization. **Jinbao Zong:** Funding acquisition, Formal analysis. **Xin Zheng:** Investigation, Funding acquisition, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34766.

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