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HMOX1 as a potential drug target for upper and lower airway diseases: insights from multi-omics analysis

Enhao Wang[1†], Shazhou Li[1†], Yang Li[2*] and Tao Zhou[1*]

Abstract

Background Oxidative stress is key in inflammatory airway diseases. Heme oxygenase 1 (HMOX1) regulates oxidative stress, but its role in airway diseases needs exploration.

Methods Differentially expressed genes (DEGs) between healthy nasal mucosa and chronic rhinosinusitis with nasal polyps (CRSwNP) were identified from Gene Expression Omnibus (GEO). Candidate genes were further screened using Gene Set Enrichment Analysis (GSEA) and Random Forest (RF) algorithms. Causal inference between candidate genes and upper and lower airway diseases (CRSwNP, allergic rhinitis (AR), and asthma (AS)) was conducted using bidirectional two-sample Mendelian randomization (TwoSampleMR) analysis. Single-cell RNA sequencing (scRNA-seq) data were used to determine the cellular localization and intercellular interactions of candidate genes. Molecular docking was used to identify potential therapeutic agents.

Results HMOX1 expression was significantly elevated in CRSwNP. TwoSampleMR analysis indicated a negative causal relationship between HMOX1 exposure and the occurrence of upper and lower airway diseases (CRSwNP [(odds ratio (OR)/95% confidence interval (CI): 0.945/(0.893–0.999), $P=0.044$], AR [OR/95% CI: 0.997/(0.994–0.999), $P=0.007$], and AS [OR/95% CI: 0.935/(0.895–0.977), $P=0.003$]). scRNA-seq data revealed HMOX1 localization in M2 macrophages. Molecular docking identified 15 antioxidants, including Acetylcysteine and Quercetin, that can upregulate HMOX1 expression.

Conclusion HMOX1 may have a protective role in the pathogenesis of upper and lower airway diseases (CRSwNP, AR, and AS) by modulating oxidative stress. Antioxidants that increase HMOX1 expression could offer new therapeutic avenues for these diseases.

Clinical trial Not applicable.

Keywords HMOX1, Chronic rhinosinusitis with nasal polyps, Allergic rhinitis, Asthma, Upper and lower airway diseases

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Introduction

Chronic rhinosinusitis with nasal polyps (CRSwNP), allergic rhinitis (AR), and asthma (AS) are prevalent upper and lower respiratory tract diseases that significantly impact global health, quality of life, and socioeconomic status. CRSwNP affects approximately 14% of adults in the United States, about 10.9% in Europe, and around 8% in China [1]. Patients with CRSwNP frequently experience nasal congestion, loss of smell, and facial pain, leading to decreased quality of life and increased medical costs due to recurrent infections and surgeries [2]. AR affects up to 30% of adults and 40% of children worldwide, presenting with symptoms such as sneezing, itching, nasal congestion, and rhinorrhea. These symptoms disrupt daily activities and sleep, creating a significant economic burden due to medical expenses and productivity losses [3, 4]. AS affects over 300 million people globally, characterized by episodic wheezing, shortness of breath, chest tightness, and coughing. This disease not only causes severe morbidity and mortality but also incurs significant economic costs due to frequent hospitalizations and healthcare utilization [5, 6]. The concept of "united airway disease" or "one airway, one disease" emphasizes the interconnectedness of the upper and lower airways. This concept indicates that diseases affecting the upper respiratory tract, such as CRSwNP and AR, are often associated with lower respiratory tract diseases like AS. These diseases share common inflammatory pathways and mediators, suggesting that managing one can positively affect the others [7–9].

Oxidative stress plays a critical role in the pathogenesis of CRSwNP, AR, and AS. It arises from an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify these reactive intermediates or repair the resulting damage. In CRSwNP, oxidative stress contributes to chronic inflammation and tissue remodeling [10]. Similarly, in AR, oxidative stress exacerbates inflammation and the hyper-responsiveness of nasal mucosa to allergens [11]. In AS, oxidative stress is associated with airway inflammation, hyper-reactivity, and remodeling, contributing to disease severity and progression [12]. Heme oxygenase 1 (HMOX1) is an essential antioxidant enzyme that regulates oxidative stress by degrading heme into biliverdin, free iron, and carbon monoxide. HMOX1 exerts anti-inflammatory and cytoprotective effects by restoring redox balance and plays a significant role in various inflammatory diseases [13]. In cardiovascular diseases, neurodegenerative disorders, and metabolic syndrome, HMOX1 mitigates oxidative damage and inflammation, highlighting its therapeutic potential [14, 15]. Although HMOX1 has been shown to protect against oxidative stress-induced damage and inflammation in some airway diseases, its role in CRSwNP, AR, and AS remains underexplored [16, 17].

M2 macrophages are a subtype of macrophages that play an indispensable role in resolving inflammation and promoting tissue repair. Unlike M1 macrophages, which have pro-inflammatory functions and are involved in the initial immune response, M2 macrophages produce anti-inflammatory cytokines and growth factors that facilitate tissue healing and remodeling [18]. However, the role of M2 macrophages in airway inflammatory diseases remains controversial, with many studies suggesting that M2 macrophages may have a detrimental role in these conditions [19].

Mendelian randomization (MR), a study design that leverages genetic variants assigned at conception, is becoming more popular for investigating causal relationships because it is less susceptible to confounding compared to traditional observational studies [20]. Additionally, it often produces results that are more consistent with those of randomized controlled trials.

This study utilizes bulk sequencing data analysis, single-cell sequencing data analysis, bidirectional TwoSampleMR analysis, immunohistochemistry, and molecular docking to preliminarily elucidate the role of HMOX1 in upper and lower respiratory tract inflammatory diseases.

Methods

The flow chart of this study is shown in Fig. 1.

Bulk RNA data analysis

We downloaded the bulk RNA data from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/gds/, accessed 1 May 2023) (Additional file 1: Table S1) [21–25]. These datasets offer reliable expression profiles of CRSwNP, exclusively derived from human samples. We used the ggplot2 and ggalt R packages to generate principal component analysis (PCA) plots of the transcriptomic data for all samples. The limma R package was employed to identify Differentially Expressed Genes (DEGs) between the CRSwNP and healthy control groups, the criteria for identifying DEGs were set to |logfc|>1and adjust $P<0.05$. Gene Set Enrichment Analysis (GSEA) (BP: subset of GO (7608)) was conducted using https://www.bioinformatics.com.cn/, accessed 20 May 2023, 1 February 2024. The intersection genes between the REACTOME_INTERLEUKIN_4_AND_INTERLEUKIN_13_SIGNALING pathway and DEGs were obtained from http://www.ehbio.com/test/venn/#/. The heatmap of the intersection genes was also generated using https://www.bioinformatics.com.cn/. The randomForest R package was used to obtain all DEGs with high importance scores. The expression data from GSE179265, GSE72713, GSE36830, and GSE23552 were utilized to validate the differential expression of HMOX1 between the control and CRSwNP groups.

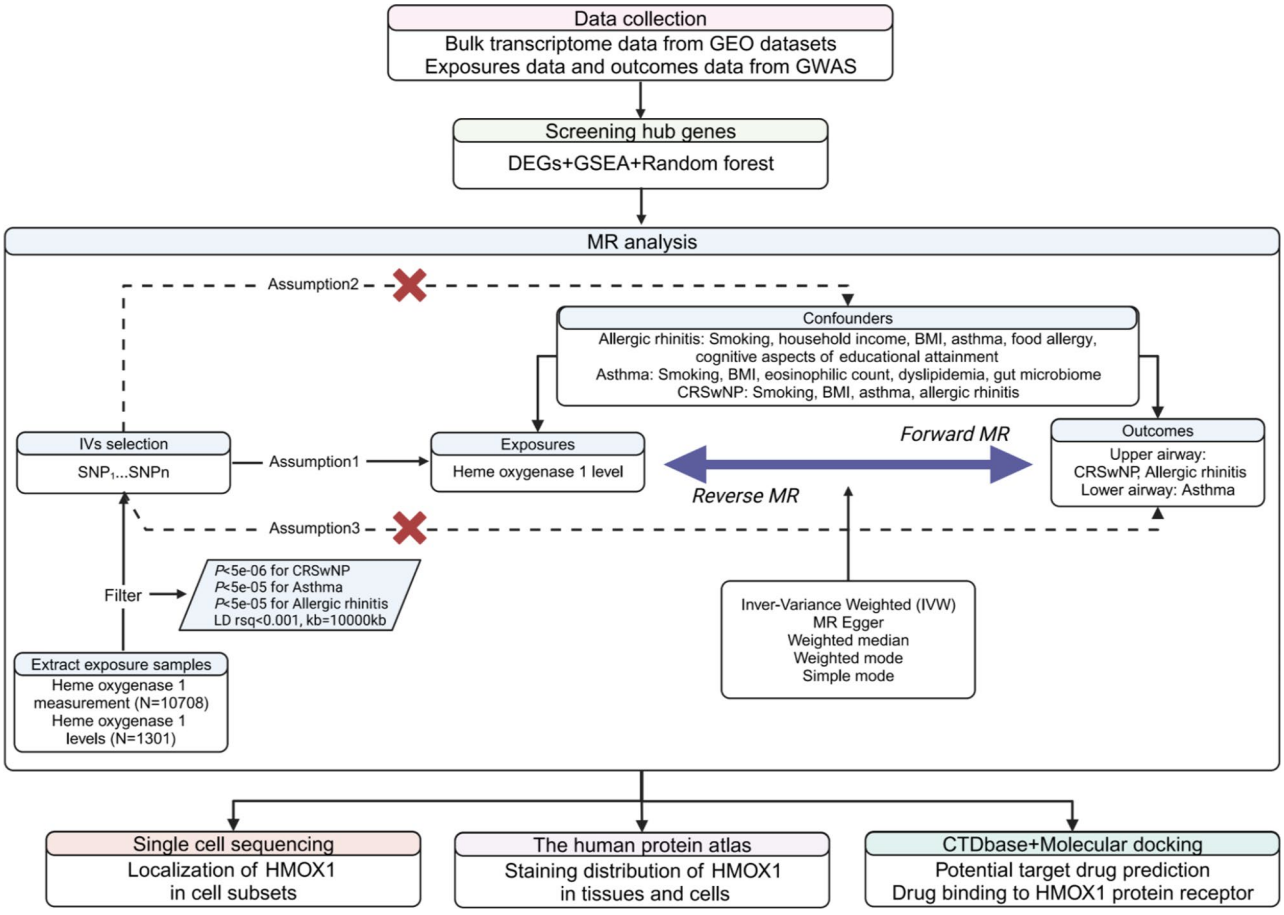

Fig. 1 Flow chart of this study

Study design of MR analysis

We conducted a bidirectional two-sample MR analysis to investigate the effect of HMOX1 exposure on CRSwNP or AR or AS, while adhering to the three fundamental assumptions of MR: (1) the genetic instrumental variables (IVs) are strongly associated with the HMOX1 exposure; (2) the IVs are independent of confounders (confounders of CRSwNP: smoking, BMI, AS, AR [26–28]; confounders of AR: smoking, household income, BMI, AS, food allergy, cognitive aspects of educational attainment [29–31]; confounders of AS: smoking, BMI, eosinophilic count, dyslipidemia, gut microbiome [32–34]); (3) the outcome (CRSwNP or AR or AS) is influenced solely by the exposure through the IVs. Our analysis utilized publicly available Genome-Wide Association Studies (GWAS) summary statistics from the MR base app (http://app.mrbase.org/, accessed 20 April 2024, App version: 1.4.3 8a77eb). No new data were collected, and no additional ethical approval was required. Finally, a reverse MR analysis was conducted to mitigate the potential impact of CRSwNP or AR or AS on HMOX1 expression levels.

Data sources and selection of instrumental variants

The genetic data for HMOX1 used in this study were obtained from the MR Base database (http://app.mrbase.org/, accessed 20 April 2024, App version: 1.4.3 8a77eb) and the ieu open gwas project website (https://gwas.mrcieu.ac.uk/datasets/, accessed 21 April 2024), which contain a collection of summary statistics from numerous GWAS. This resource allows for the manual identification of instruments, enabling the use of these traits as exposures by identifying independent GWAS significant hits from these summary associations. We limited the analyses to unrelated individuals who were no more closely related than the third degree and identified as White of European ancestry based on self-report and genetic profiling. The exposure and outcome data were obtained from the European Bioinformatics Institute (EBI), UK Biobank (UKB), and FinnGen (Round 10) (FINN) (Additional file 1: Table S2).

The selection of instrumental variables for bidirectional Mendelian randomization followed specific criteria. With HMOX1 expression level as the exposure factor and nasal polyp or AR or AS as the outcome. Consider the insufficient number of IVs, we established a threshold

of $P < 5 \times 10^{-6}$ for nasal poly, $P < 5 \times 10^{-5}$ for AR, and $P < 5 \times 10^{-5}$ for AS, then performed linkage disequilibrium (LD) clumping (r2 = 0.001 and kb = 10,000) to obtain independent single nucleotide polymorphisms (SNPs). The strength of each IV was evaluated by computing the F-statistic, which incorporated the total sample size, the number of SNPs used, and the proportion of variance explained. SNPs with F-statistics > 10 were considered suitable as potential instruments. Next, the LDtrait tool (https://ldlink.nih.gov/) was utilized to eliminate any SNPs linked to potential confounders [35]. Ultimately, the exposure and outcome SNPs were harmonized to possess identical effect alleles, and SNPs that were palindromic or ambiguous were omitted.

MR analysis

We utilized the inverse variance-weighted (IVW) method as the primary approach for our MR analysis to determine the causal effects of HMOX1 on nasal polyps, allergic rhinitis, or asthma. To ensure robust estimates, additional MR methods including MR Egger, weighted median, simple mode, and weighted mode were employed. The Wald ratio method estimated the effects of each SNP, while Cochrane's Q test assessed heterogeneity among SNP instruments. In cases of significant heterogeneity ($P < 0.05$), the random-effects IVW test was applied for more conservative and reliable estimates. The weighted median test provided consistent estimates when at least 50% of the weights originated from valid IVs, whereas the MR-Egger regression allowed for pleiotropy in more than half of the IVs. Sensitivity analyses included the MR-Egger intercept test, the global test for outliers (MR-PRESSO), and leave-one-out analysis. The MR-Egger intercept and MR-PRESSO global test were used to detect pleiotropy, while the leave-one-out analysis checked if individual SNPs drove significant results [36, 37]. All statistical analyses were performed using the MR BASE app (accessed 25 April 2024) configured with R (version 4.1.3). The IVW, weighted median, simple mode, weighted mode, and MR Egger regression methods were implemented via the "TwoSampleMR" package (version 0.5.5), and the MR-PRESSO test was conducted using the "MRPRESSO" package.

Single-cell RNA sequencing (scRNA-seq) data analysis

We obtained scRNA-seq data of CRSwNP patients (GSE156285) from the GEO database [38]. This included human nasal mucosa tissue samples from one healthy control and one CRSwNP patient (Additional file 1: Table S3). The publicly available datasets used in this study had obtained the necessary ethical approvals. The "Seurat" R package was utilized for the analysis of scRNA-seq data. To annotate the cell clusters, we used the "SingleR" R package with the Human Primary Cell Atlas as the reference dataset. We used the "CellChat" R package for cell communication analysis.

Characteristics of the infiltration of immune cells in the samples

The CIBERSORT algorithm was applied to assess immune cell infiltration in tissues from patients with CRSwNP and healthy controls. This method converted the normalized gene expression data into the proportions of various infiltrating immune cells.

Protein-protein interaction (PPI) network

We examined the interactions between DEGs and HMOX1. All PPI analyses were performed using version 12.0 of the STRING database (https://cn.string-db.org/, accessed 1 May 2024).

The human protein atlas (THPA) public data acquisition

Obtain public data from THPA website (https://www.proteinatlas.org/, accessed 1 June 2024). Specifically, download histological images, protein expression profiles and single cell tissues overview data for the tissues and proteins of interest.

Potential therapeutic drugs prediction

We searched various chemicals in the CTDbase database (accessed 15 June 2024) to obtain information on drug interactions related to HMOX1. Subsequently, we analyzed small molecular ligands that might act on these genes. Considering the protective effect of HMOX1 on upper and lower airway diseases, we selected drugs that enhance HMOX1 expression levels.

Molecular docking

We acquired the two-dimensional structures of each small molecule ligand drug from the PubChem database (https://www.rcsb.org/. accessed 20 June 2024). These structures were imported into Chem3D software (version 14.0.0.117) to calculate the minimum free energy and convert them into three-dimensional (3D) structures. The 3D structures of the target proteins, the receptors, were sourced from the RCSB Protein Data Bank (PDB, https://www.rcsb.org/). We used PyMOL (version 3.1.0a0) to import these structures and remove any water molecules and ligands. The AutoDock Tool (version 1.5.6) was employed to prepare the receptors and ligands by converting them into PDBQT formats and creating a 3D grid box for the receptor for subsequent molecular docking simulations. Molecular docking analysis was conducted with AutoDock Vina (version 1.1.2). Finally, the optimal predicted binding site was visualized using PyMOL (https://www.pymol.org/, accessed 30 June 2024).

Subjects and clinical specimens

This study was approved by the Ethics Committee of Wuhan Union Hospital (Wuhan, China) (UHCT-IEC-SOP-016-03-01). Written informed consent was obtained from all patients before enrollment. Six patients with CRSwNP and six control subjects who underwent septoplasty due to anatomical deviation were recruited. The control group had no allergic symptoms and no chronic inflammation in the sinuses. Nasal polyp (NP) tissue samples from CRSwNP patients and inferior turbinate (IT) tissue samples from healthy subjects were collected during surgery.

Animals and murine CRS model

The C57BL/6 mice were housed in a specific pathogen-free animal facility with standard temperature and light control conditions. Six-week-old female mice were used for our experiments. A mixture of 2 Unit AP (Sigma-Aldrich, St. Louis, MO) and 75 μg OVA (Sigma-Aldrich) was diluted with sterile saline to 20 μL and administered intranasally to each mouse three times per week for 12 consecutive weeks ($n = 5$ per group). Control mice received 20 μL of saline intranasally at the same intervals ($n = 5$ per group). After intranasal administration, mice were maintained in a head-down position to prevent the reagents from flowing into the lungs. Twenty-four hours after the final intranasal administration, sinus tissues were collected for RNA sequencing. All animal experiments were approved by the Animal Ethics Committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (Wuhan Union Hospital).

Immunofluorescence staining

Cell components were stained with DAPI, CD163 antibody (Proteintech, 16646-1-AP), and HMOX1 antibody (Proteintech, 10701-1-AP). The differences in protein staining among groups were observed using an Olympus confocal microscope.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism 8.0 (GraphPad Software, La Jolla, CA). For comparisons between two groups, an unpaired Student's t-test was applied for normally distributed data, while a two-tailed Mann-Whitney U test was used for non-normally distributed data. Statistical significance is denoted as follows: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Results

Screening key DEGs

First, the study collected five cohorts (GSE136825, GSE179265, GSE72713, GSE36830, GSE23552) from the GEO database (Fig. 2A), with GSE136825 serving as the training set and the other cohorts as test sets. PCA analysis revealed significant heterogeneity between CRSwNP and healthy control samples (Fig. 2B). Bulk RNASeq analysis showed that compared with control group, there were 1514 increased DEGs and 1303 decreased DEGs in CRSwNP group. The volcano plot displayed the DEGs, highlighting the top five upregulated (CP, HMOX1, NR2E1, ARRB2, BGLN3) and downregulated genes (NRXN1, SCGN, ACTC1, SYNM, SLC48A1) (Fig. 2C). All 21,733 genes were used for GSEA analysis. GSEA enrichment analysis indicated that genes in CRSwNP were enriched in the REACTOME_SIGNALING_BY_INTERLEUKINS and REACTOME_INTERLEUKIN_4_AND_INTERLEUKIN_13_SIGNALING pathways (Fig. 2D-E). There were 28 intersecting genes between the DEGs and the IL4-IL13 pathway (Fig. 2F-G). The RF algorithm showed these genes exhibited high stability and fewer sample residuals, with HMOX1 receiving the highest importance score (Fig. 2H-I). Finally, HMOX1 was validated in the other four cohorts to ensure its ubiquity (all $P < 0.05$) (Fig. 2J-M). HMOX1, an important antioxidant enzyme, is significantly expressed in CRSwNP, one of the upper and lower airway diseases. This suggests the necessity for further research into the relationship between HMOX1 and upper and lower airway diseases.

HMOX1 exposure levels are negatively causally associated with the incidence of upper and lower airway diseases (CRSwNP, AR, AS)

In this study, we included up to 9 SNPs for CRSwNP, 54 SNPs for AR and 69 SNPs for AS (Additional file 1: Table S4-6). TwoSampleMR analysis showed that using the IVW method, HMOX1 exposure is negatively causally associated with the incidence of NP [odds ratio (OR)/95% confidence interval (CI): 0.945/(0.893,0.999), $P = 0.044$], AR [OR/95% CI: 0.997/(0.994,0.999), $P = 0.007$], and AS [OR/95% CI: 0.935/(0.895,0.977), $P = 0.003$] (Fig. 3A). The scatter plots displayed the effect sizes of each MR method, showing a gradual decrease in the incidence of CRSwNP, AR, and AS with increasing HMOX1 expression (Fig. 3B-D). Leave-one-out analysis was conducted to assess the influence of individual SNPs on the final MR results. The findings indicated that, upon sequentially omitting individual SNPs, the bidirectional residual causal effects of HMOX1 on CRSwNP, AR, and AS remained consistent with the main MR study results, suggesting that no single SNP had a significant impact on the final outcome (Fig. 3E-G). This supports the conclusion that HMOX1 is a protective factor for these three airway diseases. Forest plots showed that IVW indicated a causal negative association between HMOX1 and the three airway diseases (Additional file 1: Fig. S1A, S3A, S5A). Funnel plots demonstrated a symmetrical distribution of genetic variations in HMOX1, indicating a low

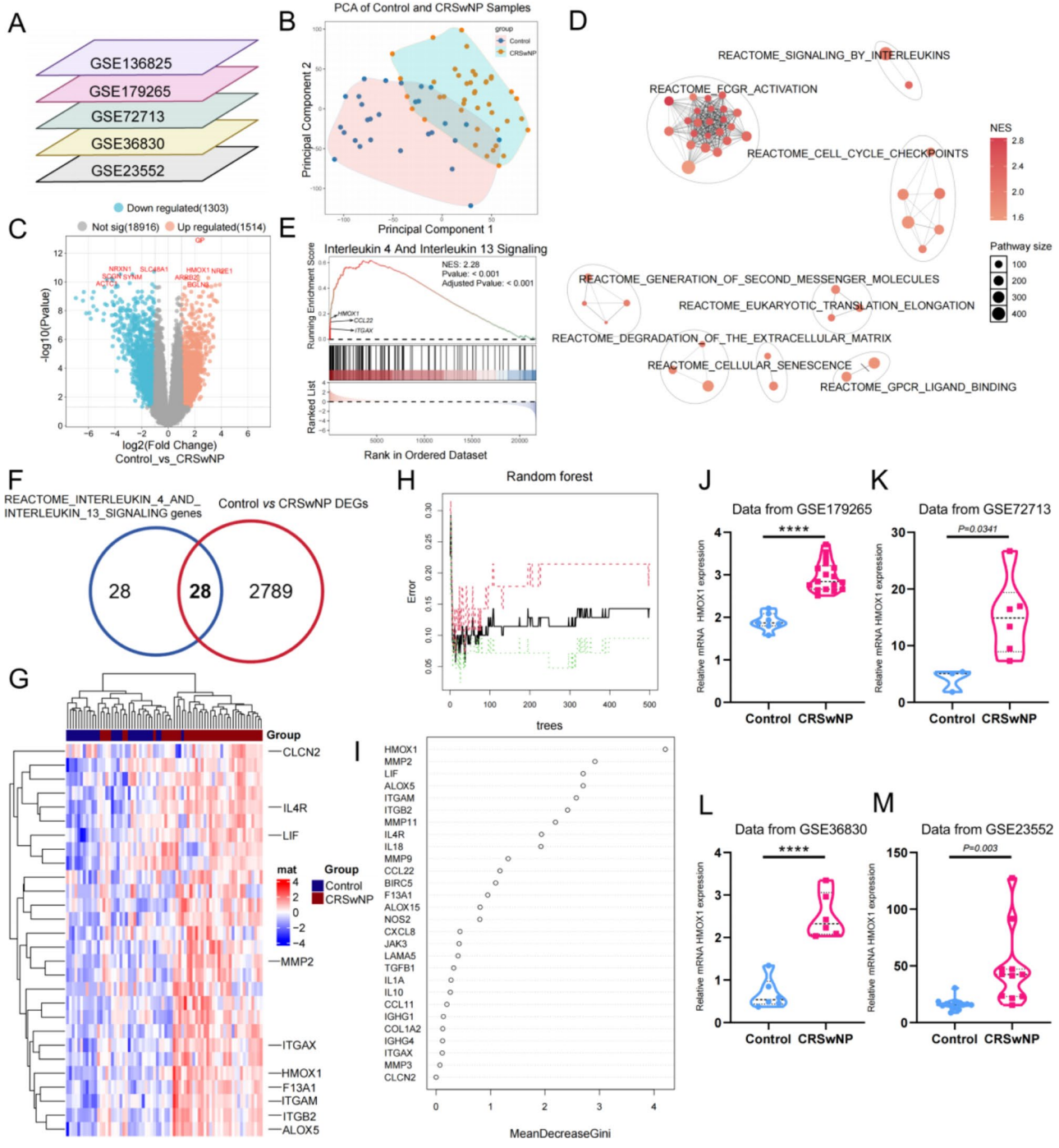

Fig. 2 Identification of merge genes from DEGs and GSEA Analyses. (**A**) Schematic of the included Bulk transcriptome sequencing datasets. (**B**) PCA diagram of all samples of GSE136825. (**C**) Volcano map of DEGs between healthy control samples and CRSwNP samples. (**D**) Top 50 enrichment pathways in GSEA analysis. (**E**) IL4-IL13 pathway in GSEA analysis. (**F**) Venn plot of merge genes of DEGs and GSEA. (**G**) Expression heatmap of merge genes. (**H**) Error decision tree: the green curve represents the control error, the red curve indicates the error in diagnosing CRSwNP, and the black curve denotes the overall error across all samples. (**I**) Relative importance of explanatory variables as ranked by the RF algorithm. (**J-M**) Differences between groups of HMOX1 expression levels in four test datasets. * for $P < 0.05$, ** for $P < 0.01$, *** for $P < 0.001$, **** for $P < 0.0001$

likelihood of significant heterogeneity (Additional file 1: Fig. S1B, S3B, S5B). Reverse MR studies were then conducted to test the causal effects of the three airway diseases on HMOX1 outcomes, showing no evidence of reverse causality (Additional file 1: Fig. S2,S4,S6). No evidence of pleiotropy or heterogeneity was observed in these results (Additional file 1: Table S7-12). Both the leave-one-out analysis and the forest plots confirmed the

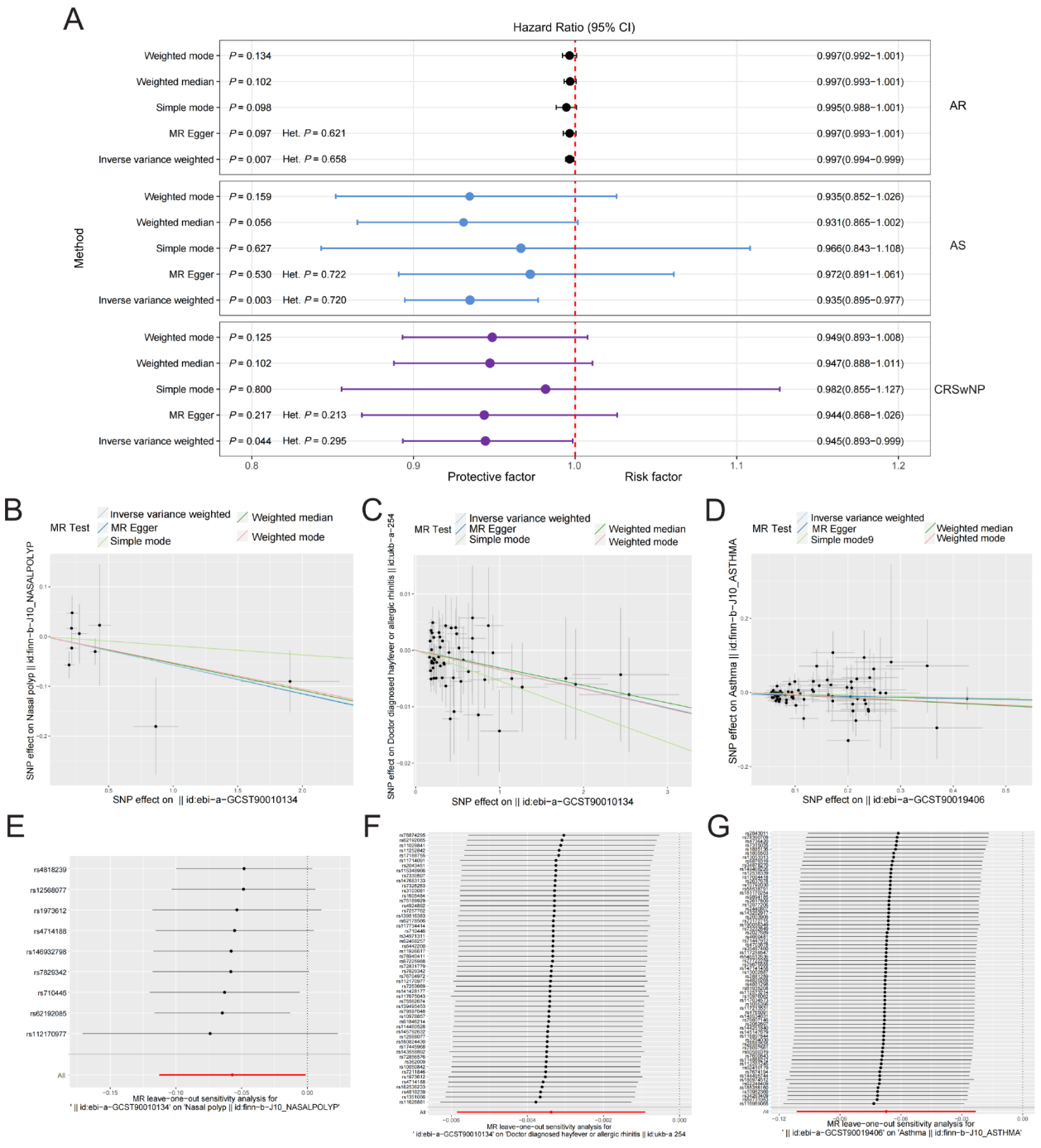

Fig. 3 Two-sampleMR analysis of HMOX1 exposure level and CRSwNP/AR/AS outcomes. (**A**) Summary forest map of five methods of Mendelian randomization. (**B-D**) Scatter plot of the causal association between HMOX1 exposure levels and CRSwNP/AR/AS outcomes. (**E-G**) Leave-one-out plot of causal association between HMOX1 exposure levels and CRSwNP/AR/AS outcomes

robustness of the MR analysis. Based on these findings, we can infer that HMOX1 is a protective factor against CRSwNP, AR, and AS.

HMOX1 is closely associated with airway and lung macrophages

Bulk-seq results indicate differential immune cell infiltration between the Control and CRSwNP groups. Plasma cells ($P<0.05$), resting memory CD4 T cells ($P<0.01$), and activated NK cells ($P<0.01$) showed reduced infiltration in the CRSwNP group, whereas M2 macrophages ($P<0.001$), activated dendritic cells ($P<0.05$), and Neutrophils ($P<0.01$) showed increased infiltration in the CRSwNP group (Fig. 4A-B). Correlation analysis revealed a strong positive correlation between HMOX1 and M2 macrophage infiltration (Fig. 4C). scRNA-seq results demonstrated that cells in nasal mucosa samples can be divided into 9 cell subsets (Fig. 5A, B,C, E). The proportions of Epithelial cells, NK cells, Monocytes, Fibroblasts, and Endothelial cells decreased in CRSwNP, while the proportions of T cells, Mast cells, B cells, and M2 macrophages increased in CRSwNP (Fig. 5D). The expression levels of inflammatory genes (IL4, IL5, IL13, IL33, IFNG, GATA3) are elevated in CRSwNP (Fig. 5F). Despite differences in methodology, both Bulk-seq and scRNA-seq revealed disparities in the immune microenvironment between Control and CRSwNP. Additionally, scRNA-seq showed that HMOX1 is distributed in M2 macrophages and Monocytes (Fig. 5G). Cellular communication exists between different cell subsets (Fig. 5H-I). The bubble plot displays receptor-ligand pairs involved in communication between M2 macrophages and both immune and non-immune cells (Fig. 5J; Additional file 1: Fig. S7). Furthermore, using the THPA database, we found that HMOX1 is highly expressed in the spleen, lymph node, lung, and liver (Fig. 6A), with the highest immunohistochemical staining scores in the lung, bone marrow, and other organs (Fig. 6B). THPA database immunohistochemistry images showed medium to high intensity staining of HMOX1 in macrophages in normal lung tissue (Fig. 6C-F), consistent with lung scRNA data from the THPA database (Additional file 1: Fig. S8). Lung macrophages, as the primary source of airway macrophages, exhibit high HMOX1 expression, further suggesting the important role of HMOX1 and macrophages in airway diseases.

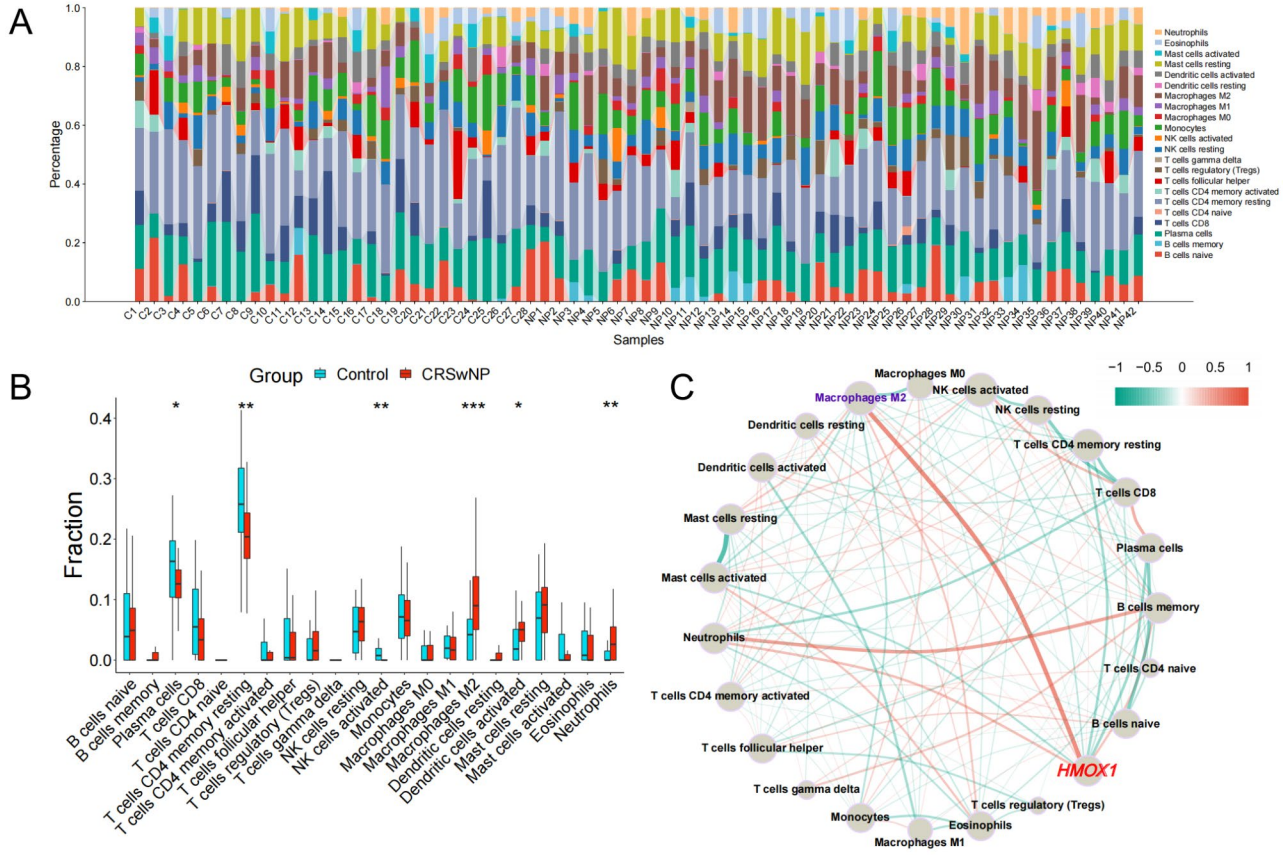

Fig. 4 The analysis of immune cell infiltration in the control and CRSwNP groups, and the relationship between HMOX1 expression and immune cell infiltration. (**A**) Proportions of twenty-two immune cell types in each sample. (**B**) Comparison of immune cell infiltration levels between control and CRSwNP samples. (**C**) Correlation between HMOX1 expression and the infiltration of the twenty-two immune cell types. * for $P<0.05$, ** for $P<0.01$, *** for $P<0.001$

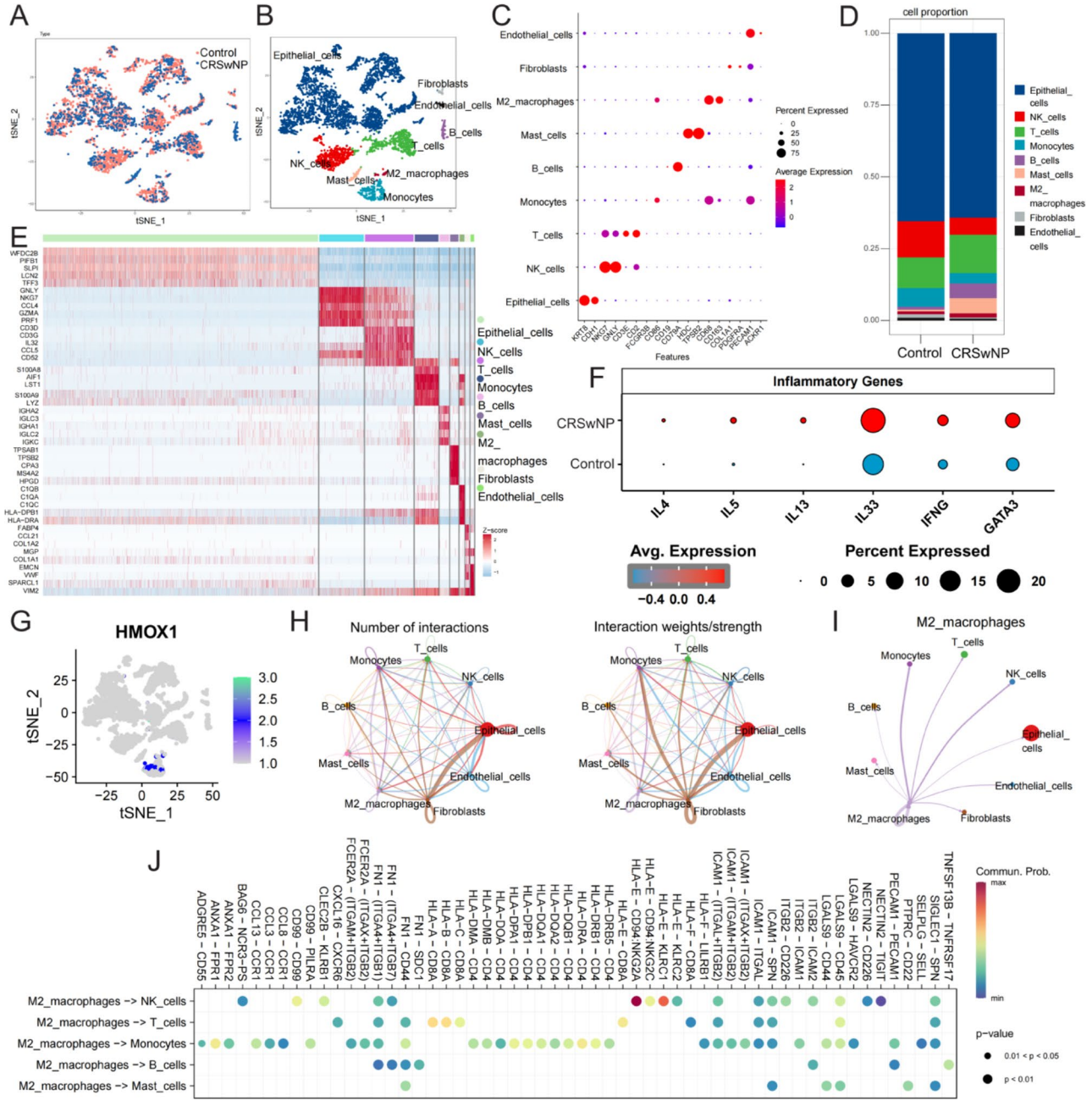

Fig. 5 scRNA landscape of human nasal mucosa samples. (**A-B**) tSNE plots displaying samples from two individuals (one control and one CRSwNP sample), with cells categorized into 9 major cell subsets. (**C**) Bubble plot of annotation genes for each cell subset. (**D**) Proportions of all cell subsets in the samples. (**E**) Heatmap of the top 5 expressed genes in each cell subset. (**F**) Differential expression of inflammatory genes between groups. (**G**) T-SNE plot showing the distribution of HMOX1 across all cell subsets. (**H**) Inter-cellular communication among all cell subsets. (**I**) Communication between M2 macrophages and other cell subsets. (**J**) Receptor-ligand pairs in communication between M2 macrophages and immune cells

HMOX1 has potential interactions with antioxidant genes, and antioxidants can enhance the expression of HMOX1

Considering that HMOX1 is a protective factor in upper and lower airway diseases, we used PPI analysis to identify its interactions with other genes that regulate oxidative stress. The results showed that HMOX1 has direct and indirect interactions with antioxidant genes such as SOD3, GPX2, and GPX3 (Additional file 1: Fig. S9). Additionally, we screened small molecule compounds that could potentially enhance the expression of HMOX1 and performed molecular docking simulations. Fifteen antioxidants, including Acetylcysteine and Quercetin, were found to increase the expression of HMOX1 (Fig. 7) (Additional file 1: Table S13). This suggests the potential for incorporating these compounds into existing

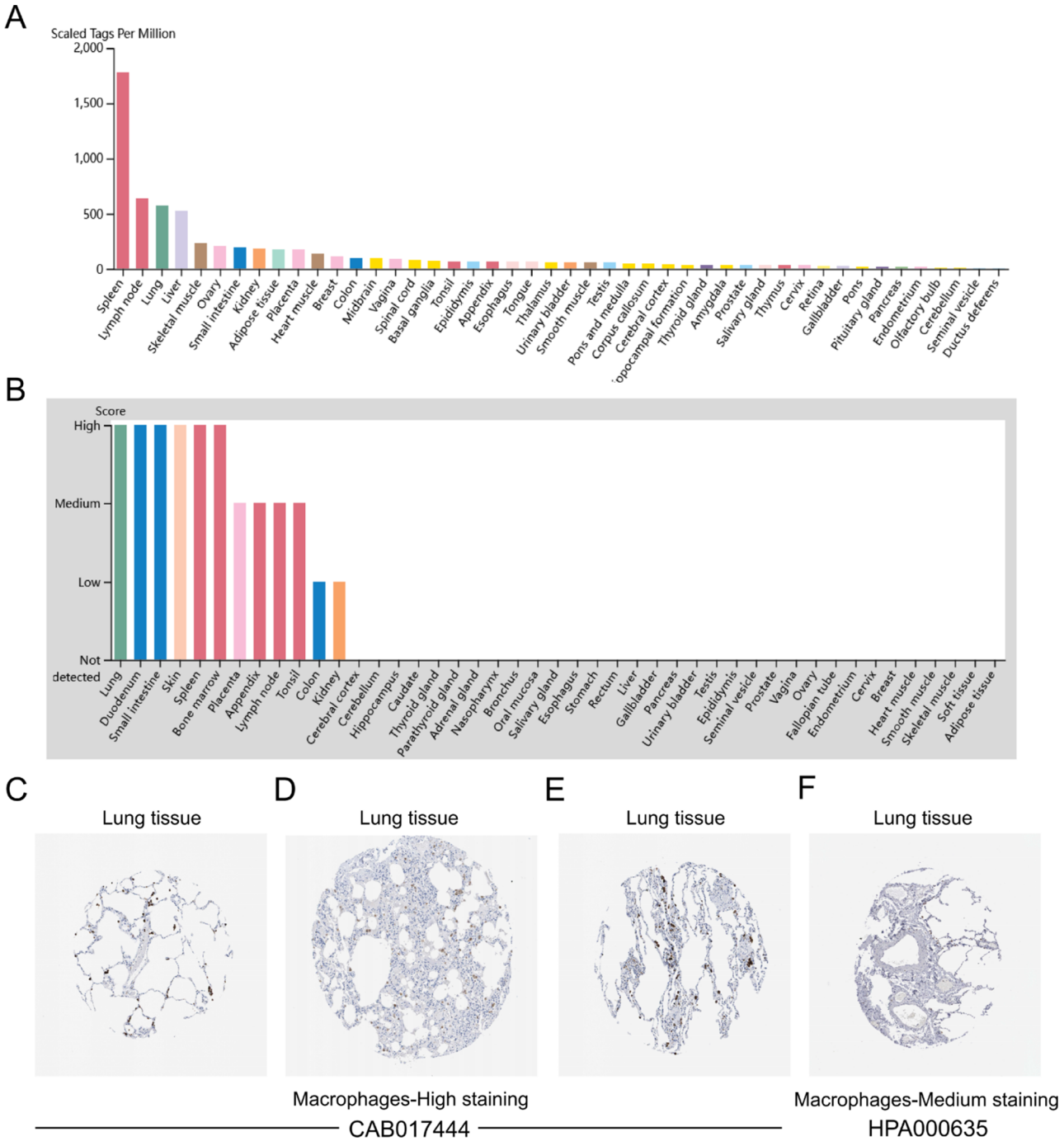

Fig. 6 HMOX1 expression information in the THPA Dataset. (**A**) HMOX1 RNA expression in human normal tissue plotted as rates per kilobase million. Data were obtained from HPA Dataset available from proteinatlas.org. (**B**) Expression intensity of HMOX1 protein in different tissues. (**C-F**) Immunohistochemistry analysis data for HMOX1 expression in macrophages of normal lung tissue from the HPA database. Bar value, 200 μm. Antibody number: CAB017444, HPA000635

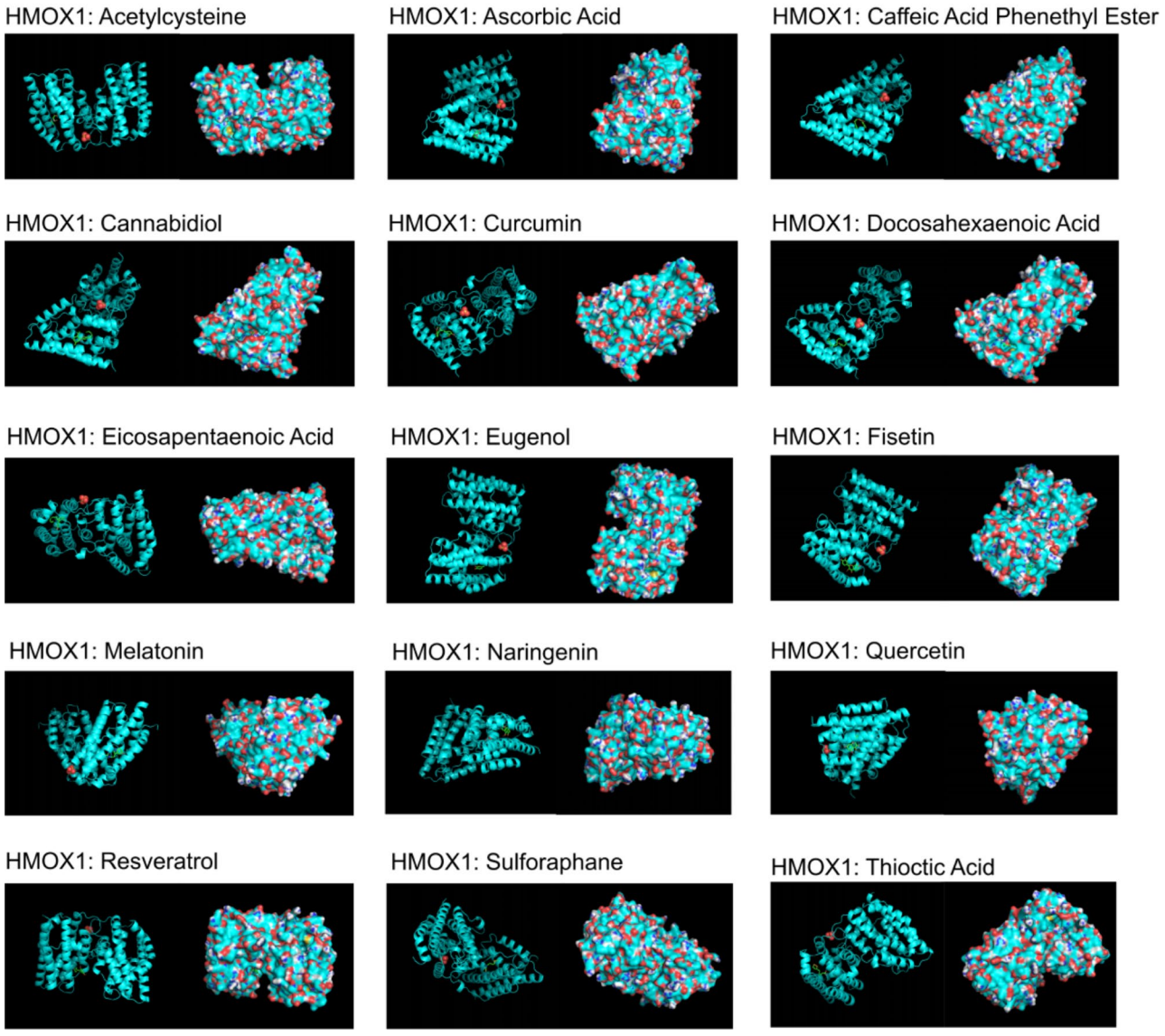

Fig. 7 Molecular docking diagram of HMOX1 and potential therapeutic drugs

treatment strategies for upper and lower airway diseases in the future.

HMOX1 is closely associated with CD163 + M2 macrophages, and multiple antioxidant genes are upregulated in the nasal tissue of CRS mice

Immunofluorescence staining revealed that the number of HMOX1 + CD163 + double-positive M2 macrophages was significantly higher in CRSwNP compared to healthy controls (Fig. 8A-B). Furthermore, we previously established a murine CRS model and performed transcriptome sequencing of mouse nasal mucosa, which demonstrated that HMOX1 and antioxidant genes such as SOD3, GPX2, and GPX3 expression levels in the nasal tissue of CRS mice were significantly higher than those in control mice (Fig. 8C-D).

Discussion

Our MR analysis revealed a significant negative causal relationship between HMOX1 expression and the development of CRSwNP, AR, and AS. This finding indicates HMOX1's protective role, where increased HMOX1 exposure corresponds to lower disease risk. This protective function likely stems from HMOX1's established anti-inflammatory and antioxidant properties, which play crucial roles in modulating airway inflammation [39]. Indeed, we found that HMOX1 expression was elevated in CRSwNP in the transcriptomic data. This phenomenon can be understood as a stress-induced increase in HMOX1 antagonizing inflammation, which is consistent with some studies [40]. However, this endogenous HMOX1 increase appears insufficient to fully suppress inflammation. Similar stress-induced HMOX1 elevations

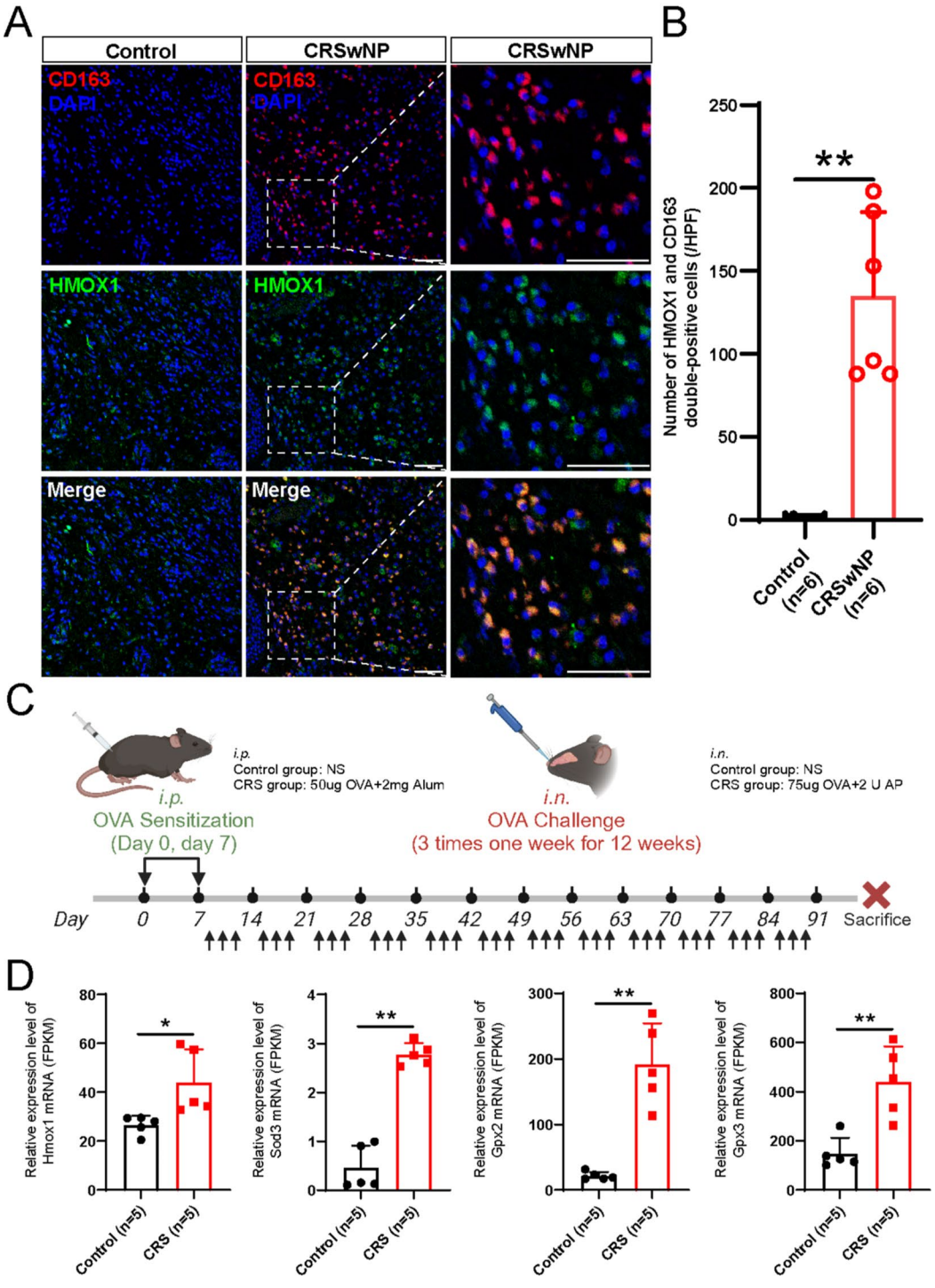

Fig. 8 Clinical specimens experiments and murine CRS model. (**A**) Double immunostaining was performed on nasal tissues from control subjects and CRSwNP patients using antibodies against CD163 (red) and HMOX1 (green). Nuclei in the lamina propria were counterstained with DAPI (blue). The right panels show magnified views of the boxed areas. Scale bar = 50 μm. (**B**) Statistical analysis of the number of HMOX1 and CD163 double-positive cells under High Power Field (HPF). (**C**) Establishment protocol for the CRSwNP mouse model. Mice were administered intranasal AP + OVA three times per week for 12 weeks to monitor CRSwNP development. Control mice received saline at the same intervals. *N* = 5 per group. (**D**) Differential expression of HMOX1 and multiple antioxidant genes in the nasal mucosa between the two groups of mice

have been documented in AR and AS studies under inflammatory conditions, demonstrating a common pattern across upper and lower airway diseases [41–43]. This further indicates the potential value of supplementing exogenous HMOX1.

The anti-inflammatory effects of HMOX1 are mediated through its by-products, particularly CO, which exerts anti-inflammatory effects by inhibiting pro-inflammatory cytokine production and promoting anti-inflammatory cytokines [44]. Oxidative stress and inflammation are key drivers of airway diseases, including CRSwNP, AR, and AS. The role of HMOX1 in these pathways is crucial, as it helps to maintain redox homeostasis and modulate inflammatory responses.

The 'one airway, one disease' paradigm highlights the intricate interconnection between upper and lower airway diseases [7]. Common pathophysiological mechanisms, particularly chronic inflammation and oxidative stress, underscore the importance of adopting an integrated approach to airway disease management. The demonstrated protective effects of HMOX1 across both upper and lower airway diseases strengthen this unified concept, suggesting that HMOX1-targeted therapeutic strategies may offer comprehensive benefits across the spectrum of airway disorders.

Sc-RNA sequencing data indicate that HMOX1 is predominantly expressed in M2 macrophages, which possess anti-inflammatory and tissue repair functions. This association suggests that HMOX1 may exert its protective effects by regulating macrophage polarization. This phenomenon has also been observed in some studies where myeloid HMOX1 expression regulates macrophage polarization and protects against hepatic ischemia-reperfusion injury at least partially by supporting the M2 phenotype [45]. However, there is significant controversy regarding the role of M2 macrophages in upper and lower airway diseases, so the relationship between HMOX1 and M2 macrophages in CRSwNP, AR, and AS requires further investigation.

The potential to modulate HMOX1 expression opens new avenues for the treatment of airway diseases. Small molecules such as acetylcysteine and resveratrol have been shown to possibly upregulate HMOX1 expression in our study. Acetylcysteine, a precursor to glutathione, enhances cellular antioxidant capacity and has been used in respiratory diseases for its mucolytic and antioxidant properties [46]. Resveratrol, a polyphenolic compound, has demonstrated anti-inflammatory and antioxidant effects in various models of airway disease [47]. These agents could serve as potential therapeutic options to enhance HMOX1 expression, thereby leveraging its protective effects against airway inflammation and oxidative stress.

Understanding the precise mechanisms by which HMOX1 exerts its protective effects is crucial for developing targeted therapies. Current evidence suggests that HMOX1 modulates various signaling pathways involved in inflammation and oxidative stress. For example, HMOX1 can inhibit the NF-κB signaling pathway, which is a key regulator of inflammatory responses [48]. This prompts us to activate by inhibiting NF-κB, HMOX1 may reduce the production of pro-inflammatory cytokines and chemokines, thereby mitigating inflammation in the airways. Moreover, HMOX1 has been shown to activate the Nrf2 signaling pathway, which enhances the expression of various antioxidant and cytoprotective genes. Activation of Nrf2 leads to increased production of glutathione, superoxide dismutase, and other antioxidants that help to neutralize ROS and protect airway tissues from oxidative damage [49, 50]. The dual role of HMOX1 in modulating both NF-κB and Nrf2 pathways underscores its potential as a therapeutic target for airway diseases.

The clinical implications of enhancing HMOX1 expression are significant, as it offers a novel approach to managing airway diseases. Current treatments for CRSwNP, AR, and AS primarily focus on symptom relief and controlling inflammation. However, these treatments often have limited efficacy and can be associated with adverse effects. By targeting HMOX1, it may be possible to develop therapies that not only alleviate symptoms but also address the underlying pathophysiological mechanisms of these diseases. Future clinical trials should aim to evaluate the efficacy and safety of HMOX1 modulators in patients with airway diseases. Additionally, follow-up studies could explore the potential benefits of combining HMOX1 modulators with existing therapies to enhance their overall effectiveness.

The study inevitably has certain limitations, primarily in four aspects: First, the inverse variance weighted results in the MR analysis are relatively weak; Second, two-sample MR cannot directly model the interaction between exposure and outcome; Third, the exclusion of confounding factors did not account for all potential confounders, and new confounders, especially non-genetic factors related to economic status, education level, and smoking, may emerge in the future; Fourth, the conclusions still need to be validated through future fundamental experiments; Fifth, the data were derived from high-income white countries and may not be applicable to other ethnicities or low- and middle-income countries.

Conclusion

HMOX1 may have a protective role in the pathogenesis of upper and lower airway diseases (CRSwNP, AR, and AS) by modulating oxidative stress. Antioxidants that increase HMOX1 expression could offer new therapeutic avenues for these diseases.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12931-025-03124-w.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

Supplementary Material 9

Supplementary Material 10

Supplementary Material 11

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Author contributions

Conceptualization, WEH, LY and ZT; methodology, WEH and LSZ; software, WEH and LSZ; data curation, WEH, LY and LSZ; writing—original draft preparation, WEH and LSZ; writing—review and editing, WEH, LSZ, LY and ZT; project administration, WEH, LSZ and ZT. All authors have read and agreed to the published version of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

All experimental protocols were approved by the Ethics Committee of Union Hospital (wuhan union hospital), Tongji Medical College, Huazhong University of Science and Technology (UHCT-IEC-SOP-016-03-01).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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