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Identification and validation of hub genes in children asthma and wheeze by bioinformatics methods.

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Abstract

Background: The pathogenic mechanisms of children asthma and the relationship between children asthma and children wheeze are complex and not fully understood. The purpose of this study was to identify the pathways and hub genes along with common Differentially Expressed Genes (DEGs) between children asthma and children wheeze, and to explore the specific insights for the clinical asthma and wheeze therapies in children.

Methods: The GSE123750 dataset was downloaded from the gene expression omnibus database. Differentially Expressed Genes (DEGs) between the children asthma and children wheeze groups were identified using the "Limma" package in the R-language. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses, along with Gene Set Enrichment Analysis (GSEA), were performed to explore potential signaling pathways associated with the DEGs. Weighted Gene Co-expression Network Analysis (WGCNA) was conducted to identify gene network modules associated with children asthma using microarray data. Functional enrichment analysis was performed on the co-expression genes within specific highlighted modules.

Results: A total of 334 DEGs were identified in children with asthma compared to those with wheezing. Furthermore, five KEGG pathways, namely ribosome, oocyte meiosis, p53 signaling pathway, B-cell receptor signaling pathway, and cellular senescence, exhibited significant enrichment. The Protein-Protein Interaction (PPI) network analysis in cytoscape highlighted four hub genes: *TOP2A, CDK1, CENPA,* and *KIF11*. GSEA results indicated a positive correlation between asthma and down-regulation of early-stage progenitor T-lymphocyte genes, up-regulation of plasma cell genes in bone marrow and blood, deadenylation-dependent mRNA decay, and down-regulation of genes in monocytes. The WGCNA analysis identified the pink module as being highly associated with asthma. Genes within this key module were primarily associated with organ or tissue-specific immune response, mucosal immune response, and the ribosome signaling pathway according to GO and KEGG pathway enrichment analyses. Furthermore, a Protein-Protein Interaction (PPI) network analysis using STRING and Cytoscape revealed one hub gene, *IMPA1*, among the key genes.

Conclusions: In conclusion, our study not only advances the current understanding of childhood asthma but also provides valuable insights for identifying potential biomarkers to improve early diagnosis of asthma in preschool children with wheezing. These findings can assist clinicians in making informed decisions regarding early treatment interventions.

Keywords: Asthma, Wheeze, Bioinformatics analysis, Differentially expressed genes, WGCNA.

Abbreviations: GEO: Gene Expression Omnibus; DEGs: Differentially Expressed Genes; WGCNA: Weighted Gene Co-Expression Network Analysis; PPI: Protein–Protein Interaction Network; TOM: Topological Overlap Matrix; GS: Gene Significance; MEs: Module Eigengenes; MM: Module Membership; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; GSEA: Gene Set Enrichment Analysis.

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Introduction

Asthma is a chronic heterogeneous, inflammatory airway disease that is characterized by airway hyper responsiveness, reversible airflow limitation, mucus overproduction and airway wall remodeling [1-3]. Among children, asthma is the most common chronic disease [4], with a rising prevalence worldwide [5]. In the United States, one out of every six children diagnosed with asthma still seeks treatment at an emergency department, and one out of every 20 children with asthma requires hospitalization [6].

Wheezing is not a disorder but rather a symptom characterized by a persistent whistling sound that occurs during breathing. This sound typically indicates a narrowing or obstruction in certain areas of the respiratory airways. Nearly 50% of young children experience wheezing by the time they reach 6 years of age. Preschool-aged children contribute to a disproportionately large share of healthcare and economic resources due to wheeze and asthma. However, the exact mechanisms underlying these episodes and their connection to the development of asthma in later stages of life remain largely unknown. Identifying which preschoolers with wheezing will eventually develop asthma poses a significant challenge [7-9].

In recent years, with the development of microarray technology [10,11], non-invasive biomarkers can be the key t personalized medicine in wheeze and asthma whereby patients receive specific treatments due to their identifiable molec signatures. Diverse biomarkers have been used in the diagn of children asthma [12,13]. However, none of these bio rkers has sufficient specificity and/or sensitivity in th clinical practice [14]. Furthermore, most previous studies on airway micro biota have relied on bronchoscopic bru or samples. Published prediction rules for id tify preschool children with asthma at school age lac a go st ard for diagnosis [15]. Consequently, reliable omar at can be used in routine medical setting re currently lacking. Therefore, there is an urgent for n iomarkers.

The aim of this study is to di a practical approach for determining which pr ch aged ildren with wheeze or asthma should receive tment and to identify some new biomarkers ildhoo thma. We investigated specific genes a enric ent pathways associated with wheeze and asthma. se constructed gene co-expression net orks a dentified highly specific hub genes for wheeze a hma. ub genes have the potential to serve as eutic targe and biomarkers for children with wheeze or a hma.

Materials and Methods

Retrieving data

The gene expression omnibus database is a public genome database (http://www.ncbi.nlm.nih.gov/geo). One microarray dataset (GSE123750) of comparing children asthma and the wheeze was extracted from the database. The gene expression data of GSE123750 was downloading from the platform Affymetrix HT HG-U133+PM array plate. The dataset was generated based on the samples of induced plasma from 104 children with asthma and 112 children with wheeze.

Data processing and identification of DEGs

First, the microarray data were preprocessed using t MA (robust multi-array average). Then the affy packag (https:// bioconductor.org/biocLite.R) in the R softw bio-c cto was used to read the data. The adjusted P-va djust P) and log Fold Change (log FC) were used t sele ficant DEGs. The cut-off criteria of |logFC| ≥ 0. d adjusted P-value<0.05 were consid d s tical significant. Additionally, DEGs were perfor ed by package in R software (version 4.1 Fi lly visualized the DEGs. Volcano plot and hea map w tted by ggplot2 and complex heat map package

GO Functional enrichment analysis

Functional en ich t of DEGs was analyzed in 3 categories of the Ge tology O) domains: Biological Process (BP), Cellular Com nt (CC), and Molecular Function (MF). GO fun tion nric ent analysis and KEGG pathways of the DE was p formed using the cluster profiler package in R.

PPI network and module analysis

STRING database (http://www.string-db.org/) was utilized to construct the PPI network of the DEGs-coded proteins. The avg local clustering coefficient was 0.423. To screen for the hub genes, cytoNCA plugin from cytoscape (version 3.9.1) software was used to visualize the most significant PPI network based on the betweenness. We identified top 4 hub genes, including *TOP2A, CDK1, CENPA, KIF11*.

Gene set enrichment analysis

GSEA is a computational method for calculating whether a set of genes defined by a priori show statistical significance between two disease type and biological processes. It was used to explore the differential gene sets between asthma and wheeze in dataset GSE123750. Compared with the GO and KEGG pathway enrichment analysis was only used to detect DEGs, GSEA was used to detect all genes in the dataset and judge its influence on phenotype. The annotated gene sets downloaded from the Molecular Signature Database (MSigDB), were considered as the reference gene sets. The number of permutations was set to 1,000, and the permutation type was set as "phenotype". P-values<0.05 and FDR<0.25 were significant in statistics.

Gene Set Enrichment Analysis (GSEA) plots of the most enriched gene sets in the asthma and wheeze group. The top 4 most (a-d) enriched pathways in the asthma group; (a): Genes down-regulated at early stages of progenitor T lymphocyte;

(b): Up-regulated of memory IgG IgA B cells vs. plasma cells; (c): Genes down-regulated in monocytes; (d): Deadenylation-dependent mRNA decay. The top 4 (e-h) most enriched pathways in the wheeze group; (e): Hepatic graft vs. host disease; (f): FGFR3 signaling in chondrocyte proliferation and terminal differentiation; (g): Genes up-regulated of effective memory CD4 T cells; (h): Glycosaminoglycan degradation.

WGCNA

Weighted Correlation Network Analysis (WGCNA) is a powerful algorithm to identify significant clusters and module Eigen gene. WGCNA is widely used to find the co-expressed gene modules with high biological significance and explore the relationship between gene networks and diseases. Therefore, we used the WGCNA to obtain the asthma and wheeze associated modules. More than 10000 genes were obtained by sequencing in the GEO dataset, and most of these genes did not have different expression between samples, we selected top 5,000 Median Absolute Deviation (MAD) genes to construct the representation matrix and the appropriate power parameter was decided by pickSoftThreshold function.

The "WGCNA" package in R.4.1.2 software were used to performed the WGCNA analysis. Before analysis, hierarchical clustering analysis was performed using the Hclust function in R language to exclude the outlier samples. Then the appropriate soft powers β (ranged from 1 to 20) was selected using the function of "pickSoftThreshold" in the WGCNA package according to the standard of scale-free network. Next, the soft power value β and gene correlations matrix among all gene pairs calculated by Pearson analysis were used to build adjacency matrix. Then the Topological Overlap Matrix (TOM) and the corresponding dissimilarity (1−TOM) were transformed from the adjacency matrix.

A hierarchical clustering dendrogram was further built and similar gene expressions were divided into different modules. Finally, the expression profiles of each module were summarized by the Module Eigengene (ME) and the correlation between the ME and the clinical feature was calculated. Therefore, modules with high correlation coefficient with clinical features were focused and the genes in these modules were selected for subsequent analyses. In this study, the soft threshold (power) was 7 in the WGCNA analysis. (The other parameters were the following: network type = "unsigned", min module size=30, merge cut height=0.25 and deep split=2).

Results

Identification of DEGs

334 DEGs were obtained from the GSE123750. It included 83 up-regulation genes and 251 down-regulation genes. The gene expression profile of DEGs containing 2 sets of sample data is shown in Figure 1.

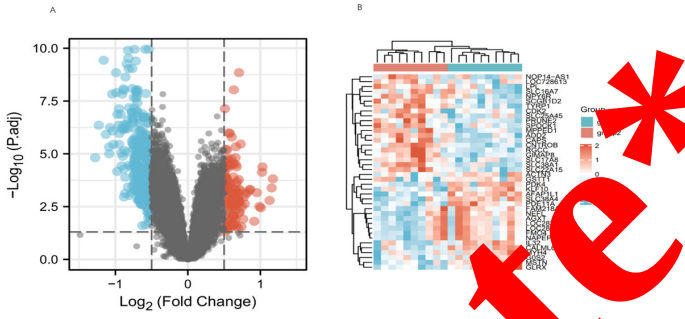

Figure 1. Identification of differentially expressed genes in the merged dataset. (A): Volcano plot of the genes, the blue dots represent the down-regulated genes and tangerine dots represent the up-regulated genes, while the gray dots showed genes with no significance. (B): Heat map plot of the differentially expressed genes.

Pathway analysis

In order to better understand the biological function of DEGs, we conducted GO function and KEGG enrichment analysis. GO results revealed that DEGs significantly enriched in cyclin-dependent protein serine/threonine kinase regulator activity, protein kinase regulator activity, kinase regulator activity of M phase, condensed nuclear chromosome kinetochore, cytosolic ribosome, immunoglobulin complex of CC, B cell activation, nuclear chromosome segregation, humoral immune response of BP. Moreover, KEGG analysis showed that the DEGs were enriched in p53 signaling pathway, Ribosome, Oocyte meiosis Figure 2.

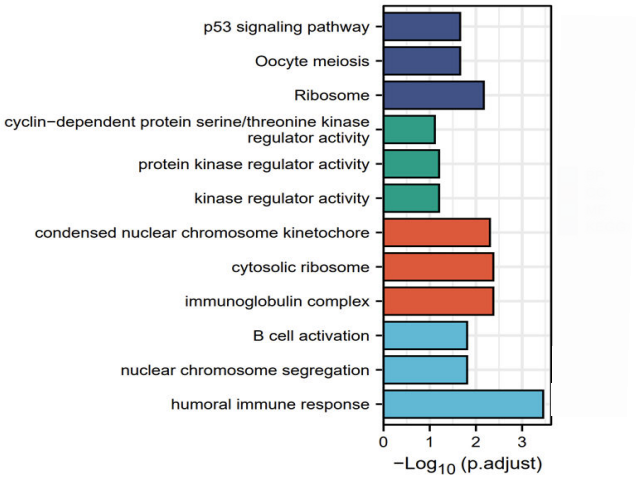

Figure 2. Gene ontology enrichment and KEGG enrichment analysis. ( ): BP; ( ): CC; ( ): MF; ( ): KEGG.

Analysis of the PPI network

STRING was used to construct DEGs PPI networks with confidence scores ≥ 0.4 and visualized using cytoscape software (version 3.9.1); we constructed a PPI network complex with 36 nodes and 302 edges. The top 4 genes, including *TOP2A, CDK1,*

CENPA, and *KIF11,* were taken to as hub genes based on the node degree score generated *via* cytoscape Figure 3.

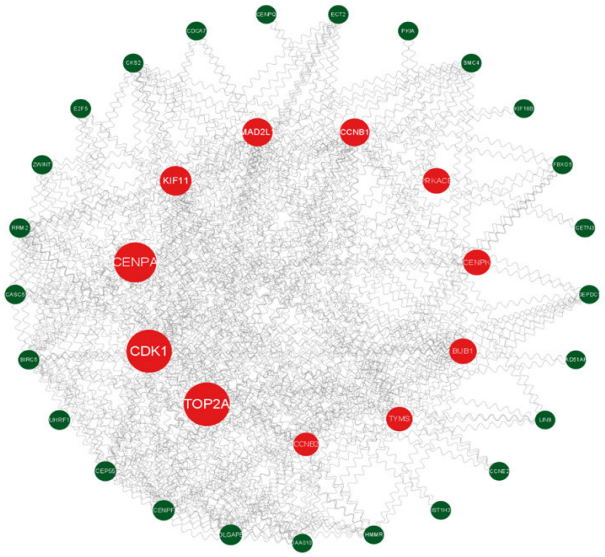

Figure 3. Four hub genes selected from PPI network. Hub genes screened by between centrality according to cytoNCA plugin.

Screening differential gene sets with GSEA

To further elucidate the different pathway involved in wheeze and asthma, GSEA was performed between the two groups in GSE123750. Pathways related to genes down-regulated at early stages of progenitor T lymphocyte, up-regulated of memory IgG IgA B cells *vs.* plasma cells, genes down-regulated in monocytes, and deadenylation-dependent mRNA decay were most enriched in asthma. Hepatic graft *vs.* host disease, FGFR3 signaling in chondrocyte proliferation and terminal differentiation, genes up-regulated of effective memory CD4 T cells, and glycosaminoglycan degradation were most significantly enriched in wheeze group Figure 4.

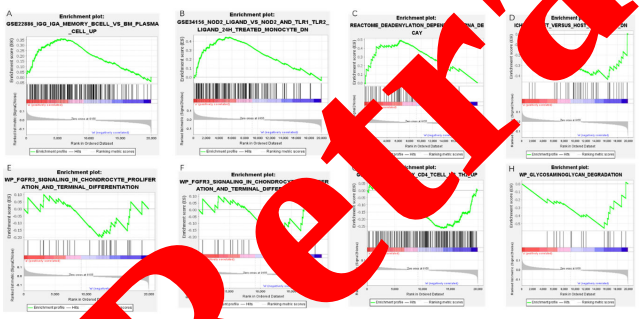

Figure 4. Gene Set Enrichment Analysis (GSEA) plots of the top enriched gene sets in the asthma and wheeze group. (A): Top 4 (a-d) most enriched pathways in the asthma group; (B): Up-regulated of memory IgG IgA B cells vs. plasma cells; (C): Genes down-regulated in monocytes; (D): Deadenylation-dependent mRNA decay. The top 4 (e-h) most enriched pathways in the wheeze group; (E): Hepatic graft vs. host disease; (F): FGFR3 signaling in chondrocyte proliferation and terminal differentiation; (G): Genes up-regulated of effective memory CD4 T cells; (H): Glycosaminoglycan degradation.

Enrichment analyses of module genes identified by weighted gene co-expression network analysis.

WGCNA was performed to get a deeper insight into the association between the key modules and asthma. Sample dendrogram is shown in Figure 5A. The scale independence and mean connectivity are shown in Figure 5B by setting the soft threshold to 7. Afterwards, a topological overlap matrix was generated; a series of modules were subsequently detected by hierarchical clustering, and we narrowed down the number of modules using dynamic tree cut algorithm, while key modules with high similarity were merged. We present the cluster dendrogram in Figure 5C. And find 12 modules were identified. The network heat map of DEGs is shown in Figure 5D. Finally, the module trait relationships are shown in Figure 5E. The pink module was significantly correlated with asthma and selected for further analysis.

The GO enrichment analysis of pink module genes were significantly enriched in processes related to organ or tissue specific immune response and mucosal immune response. The KEGG pathway enrichment analysis indicated that the pink module genes were significantly enriched ribosome pathways Figure 6A. The key genes in the pink module were analyzed with PPI network. There were 13 interactions among key genes in the pink module. One hub genes were found in PPI networks using cytohubba Figure 6B.

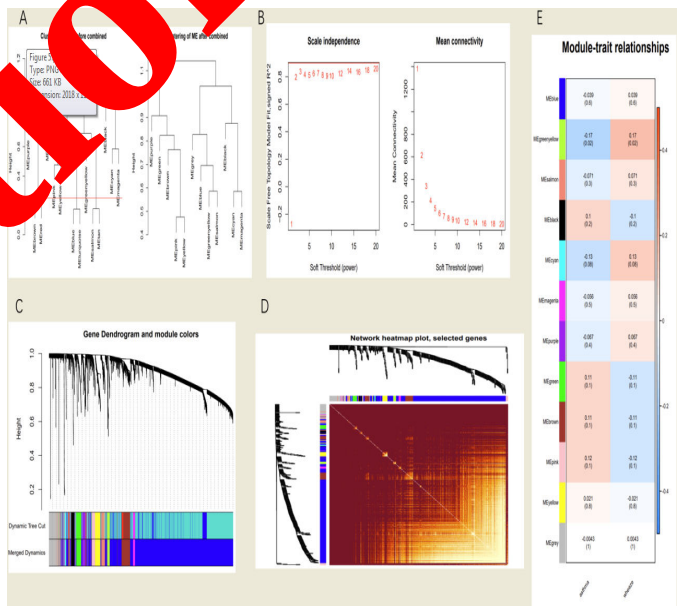

Figure 5. Identifcation of hub modules using the WGCNA analysis. (A): Sample dendrogram and trait heat map; (B): Analyses of network topology for various soft-thresholding powers and the scale-free topology were set as 0.9 roughly; (C): The dendrogram of the co-expression network was clustered based on the dissimilarity; (D): Network heat map of DEGs; (E): The module-trait heatmap showing the correlation between Module Eigengenes (ME) and traits.

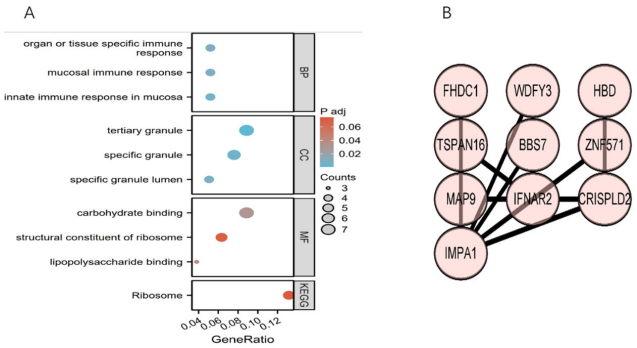

Figure 6. The gene functional enrichment analysis of pink module. (A): GO and KEGG enrichment in the pink module;(B): Protein–protein network of the hub genes in the pink module.

Discussion

The pathogenesis of asthma is multifactorial, with environmental and genetic factors interacting to produce some pathological features. In current investigation, 334 DEGs in total were identified, consisting of 83 up-regulated genes and 251 down-regulated genes. The results of GO functional classification indicated that the DEGs were mainly enriched in cyclin-dependent protein serine/threonine kinase regulator activity, protein kinase regulator activity, kinase regulator activity of MF, condensed nuclear chromosome kinetoch cytosolic ribosome, immunoglobulin complex of CC, B cell activation, nuclear chromosome segregation, humoral immune response of BP. KEGG analysis showed that the DEGs re enriched in p53 signaling pathway, ribosome, oocyte meiosis.

In the PPI network of DEGs, 4 (TOP2A, CDK1, C NPA, and KIF11) out of 334 DEGs. These 4 genes were all t regulated in pediatric patients with asthma. TOP2A code le enzyme topoisomerase II alpha, which plays a cial role in DNA replication and repair. Previous studies have own that TOP2A undergoes regulation thro ghout cell cycle, experiencing a notable surge be g from the midpoint of the S phase and persistin through itosis. This regulation encompasses three essent processes the activation of TOP2A through transcriptiona echanisms, the stabilization of TOP2A mRNA, a d acti on of the deubiquitylase enzyme USP [15]. coordinated processes ensure the proper fu g and sence of TOP2A during the various stages the ce cycle [16]. Studies have shown that TOP2A is ass ith ility in asthma patients [17,18].

C K1, als own as Cyclin-Dependent Kinase 1, is a protein plays a cal role in regulating the cell cycle. The gulation of the "CDK1/APC/C-Cdh1" signaling pathway is associated with the effective inhibition of Th17 differentiation and the exhibition of an anti-neutrophil effect in asthma [19]. Meanwhile, Cdk1 phosphorylates numerous proteins involved in regulating epigenetics, including writers and erasers of major histone marks. Consistent with these discoveries, inhibiting Cdk1 has an impact on the histone modification status of ES cells. Elevated levels of Cdk1 in ES cells phosphorylate and partially deactivate Dot1l, the H3K79 methyl transferase responsible for adding activating marks to gene bodies. Reduction of Cdk1 activity during ES cell differentiation relieves the repression of Dot1l, thereby enabling the coordinated expression of differentiation genes.

These analyses suggest that Cdk1 plays a crucial r in maintaining the epigenetic characteristics of ES cells [20]. GSEA data suggested that genes down-regulated at early st ge of progenitor T lymphocyte, up-regulated of memory IgG IgA B cells plasma cells, genes down-regulated in mon cyt and deadenylation-dependent mRNA decay enriched sthma patients. Several studies have implied th TH2 s e contribute to the development of chr inflammation commonly observed in asthma and o alle sorders [21-23]. Moreover, children diagno d w atopic diseases exhibit increased numbe of T me y B-cells in comparison to children without a y ato ations [24].

It has been reporte lay a significant role in the asthma severity or s bsequent recurrent wheezing [25]. IgG memory B cells are a spec l type of B cell that can survive in the body for a long time a emember previously infected pathogens. IgG memory B c ls can differentiate into plasma cells to prod ce tibodies so that they can respond quickly when inf d agai e generation of memory B and T cells, as well as l lived plasma cells, is a crucial feature of the ad mmu ystem. These specialized B cells play a vital r in viding protective immunity against recurring inf us a ents [26]. In the pathogenesis of atopic diseases, e ro of IgG Memory B cells as precursors for the p ation of pathogenic IgE plasma cells is of significant im ortance [27,28]. Hepatic graft *vs.* host disease, FGFR3 signaling in chondrocyte proliferation and terminal differentiation, genes up-regulated of effective memory $CD4^+$ T cells, and Glycosaminoglycan degradation were mainly enriched in wheeze group.

The subsequent WGCNA revealed one module that closely associated with asthma, namely, pink module that was positively associated with asthma. Function enrichment analyses showed that, in the category of CC, pink module genes were most significantly enriched in “tertiary granule”, “specific granule”, and “specific granule lumen”. Tertiary granule is a type of secretory granule that contains cathepsin and gelatinase and is readily exocytose upon cell activation; found primarily in mature neutrophil cells [29]. Airway neutrophils have been identified as a contributing factor to the severity of asthma and the occurrence of acute exacerbations. Recent research has shown that neutrophilic airway inflammation is associated with severe asthma in cases where there is non-type 2 inflammation.

This finding highlights the importance of considering non-type 2 inflammations in the diagnosis and treatment of severe asthma, as it may have a significant impact on the effectiveness of current therapies [30]. Elevated concentrations of extracellular DNA in sputum serve as a marker for a specific subset of asthma patients who have more severe symptoms. There was previous evidence indicating that the source of this extracellular DNA is neutrophils, supported by substantial

positive correlations between concentrations of eDNA, sputum neutrophil percentages, and MPO concentrations [31].

With regard to MF terms, pink module genes were predominantly involved in "carbohydrate binding", "structural constituent of ribosome", and "lipopolysaccharide binding". The relationship between ribosome and asthma may be related to Ribosomal Proteins (RPs). RPs is structural components of ribosome that participate in ribosome biogenesis and protein synthesis. Some studies suggest that abnormal expression or mutation of RPs may cause some human diseases [32]. Defects in the process of ribosome formation, translation, and the roles of specific Ribosomal Proteins (RPs), including mutations in RPs, have been associated with a diverse range of human congenital disorders collectively known as ribosomopathies [33].

These conditions are characterized by tissue-specific phenotypic abnormalities and an increased risk of developing cancer later in life [34]. Recent findings of somatic mutations in RPs across multiple types of tumors further highlight the links between ribosomal defects and cancer, emphasizing the potential significance of investigating the role of ribosomes in cancer development and progression [35]. Some previous studies have suggested that asthma and allergies can ind chronic and systemic immune system activation, which could potentially increase the risk of developing lung cancer [36]. However, the specific mechanisms are still not l understood. The relationship between lipopolysacchar binding and asthma. There is no definitive answer t this topic, but some studies suggest that Lipopolysacchari Binding Protein (LBP) may play an important role in reg ng the inflammatory response of asthma [37].

Some studies also found that lipopoly ccha de e sure can alleviate the symptoms of asthma i mice gulating the Th1/Th2 and Treg/Th17 balanc These results indicate that lipopolysaccharide bin g has a plex interaction with the occurrence and develop of asth and further research is needed to elucidate it As fo BP category, pink module genes were mainly clus ed i organ or tissue specific immune response", osal immune response", and "innate immune esp in m ". The results of pink module were furth confir g the roles of immune responses in the patho is Allergic asthma in childhood has been e tensive tudied, revealing the presence of eosinophilic i mmatio 39]. However, research has also shown that trophilic a mma displays a T-helper 17-shifted phenotype [40].

The susceptibility of newborns to respiratory infections may be linked to impaired neonatal neutrophil function regarding adherence, chemotaxis, and migration. Additionally, a deficiency of Polymorphonuclear Leukocytes (PMNs) during development may contribute to the development of childhood asthma. It is worth noting that a bias towards Th17-cell development may occur in human neonates, which may help regulate the initial colonization by microbes. Furthermore, it is important to highlight that childhood asthma phenotypes cannot be directly translated into adult phenotypes, which may include eosinophilic and neutrophilic asthma, among others. It is essential to prioritize the enhanced characterizati diverse childhood asthma phenotypes and their under immune mechanisms to devise more effective treat strategies that are tailored to individual patients [41].

The understanding that asthma involves more than st a rgic inflammation has led to a greater focus on the ro innat immunity in this disease. Recent advance ts in ea have identified new targets for preventing and ting asthma. These include identifying pathways f te s ation by environmental or endogenou Patho n-As ated Molecular Patterns (PAMPs) and Dange Asso d M ecular Patterns (DAMPs) that affect the activa on an vement of DCs, identifying innate s es, and discovering new subsets of T cells and lymphoid cells [42]. Previous studies have suggested that a we ened innate immune response may contribute to an increased susceptibility to asthma [43]. Furthermore, the NLRP3 infl mmasome is proposed to play a key role in i immunity by being involved in airway inflamma in ma or exacerbations induced by viral infections.

Th is i mol cular signaling complex controls proteolytic a ation f the highly inflammatory cytokines interleukin 18 an rle kin-1β and might regulate inflammatory processes, pecially in the neutrophilic asthma phenotype. Increased leukin-1β concentrations in serum, sputum, and Bronchoalveolar Lavage (BAL) of allergic asthmatics provide evidence for *NLRP3* activation [44]. Therefore, finding new biomarkers is essential. I*MPA1* was identified as a critical gene for asthma after further protein-protein interaction network research. *IMPA1* (Inositol Monophosphatase 1) is a protein-coding gene that encodes an enzyme responsible for the provision of inositol required for synthesis of phosphatidylinositol and polyphosphoinositides.

It has been implicated as the pharmacological target for lithium action in the brain [45]. Diseases associated with *IMPA1* include intellectual developmental disorder. Autosomal recessive 59 and autosomal recessive non-syndromic intellectual disability [46]. Among its related pathways are superpathway of D-myo-inositol (1,4,5) trisphosphate metabolism and Inositol phosphate metabolism [47]. Gene Ontology (GO) annotations related to this gene include protein homodimerization activity and magnesium ion binding [48]. A related study shows that the *IMPA1* mutation specifically affects neuronal progenitor cell survival and neuronal differentiation [49].

Not only that, *IMPA1* deficiency causes inositol decline, which elicits AMP-Activated Protein Kinase (AMPK) activation and mitochondrial fission without affecting ATP level. Mitochondrial fusion and fission are important for maintaining mitochondrial functions. Inositol is a metabolite that restricts AMPK-dependent mitochondrial fission. Inositol accumulation prevents AMPK-dependent mitochondrial fission. The AMP/inositol ratio is critical for AMPK activation. AMPK activation requires inositol decline to release AMPKγ for AMP binding [50].

Conclusion

In summary, our integrated bioinformatics study presented five hub genes *TOP2A, CDK1, CENPA, KIF11 and IMPA1*. These core genes establish relationship with children wheeze and asthma. These findings provide insights about the mechanisms underlying asthma progression, which is of great significance to the treatment of children asthma. Our study reveals the value of *IMPA1* in children asthma, where the role of *IMPA1* was previously unknown. Hence, AMPK is an inositol sensor that restricts mitochondrial fission when inactivated by inositol

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Availability of data and materials Microarray dataset (GSE123750) for this study is openly available in Gene Expression Omnibus database at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=gse123750.

Competing interests

The authors declare that they have no competing interest

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Authors' contributions

YF designed the study, analyzed the data and wrote the manuscript. JM contributed to data interpretation. YL and QL reviewed and revised the manuscript. YL and QL also supervised the study. All authors read and approved the final manuscript.

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