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Identification of differentially expressed genes in childhood asthma

Nian-Zhen Zhang, BS[a], Xiu-Juan Chen, BS[b], Yu-Hua Mu, BS[c], Hewen Wang, BS[a,*]

Abstract

Asthma has been the most common chronic disease in children that places a major burden for affected people and their families.

An integrated analysis of microarrays studies was performed to identify differentially expressed genes (DEGs) in childhood asthma compared with normal control. We also obtained the differentially methylated genes (DMGs) in childhood asthma according to GEO. The genes that were both differentially expressed and differentially methylated were identified. Functional annotation and protein–protein interaction network construction were performed to interpret biological functions of DEGs. We performed q-RT-PCR to verify the expression of selected DEGs.

One DNA methylation and 3 gene expression datasets were obtained. Four hundred forty-one DEGs and 1209 DMGs in childhood asthma were identified. Among which, 16 genes were both differentially expressed and differentially methylated in childhood asthma. Natural killer cell mediated cytotoxicity pathway, Jak-STAT signaling pathway, and Wnt signaling pathway were 3 significantly enriched pathways in childhood asthma according to our KEGG enrichment analysis. The PPI network of top 20 up- and downregulated DEGs consisted of 822 nodes and 904 edges and 2 hub proteins (UBQLN4 and MID2) were identified. The expression of 8 DEGs (GZMB, FGFBP2, CLC, TBX21, ALOX15, IL12RB2, UBQLN4) was verified by qRT-PCR and only the expression of GZMB and FGFBP2 was inconsistent with our integrated analysis.

Our finding was helpful to elucidate the underlying mechanism of childhood asthma and develop new potential diagnostic biomarker and provide clues for drug design.

Abbreviations: 15[S]-HETE = 15 (S)-hydroxyeicosatetraenoic acid, 15-LO = 15-lipoxygenase, AHR = airway hyperresponsiveness, BioGRID = Biological General Repository for Interaction Datasets, CLC = Charcot–Leyden crystal galectin, CTL = cytotoxic T lymphocytes, DEGs = differentially expressed genes, DMGs = differentially methylated genes, FDR = false discovery rate, GEO = Gene Expression Omnibus, GO = Gene Ontology, IFNG = interferon gamma, KEGG = Genes and Genomes, NC = normal control, NK = natural killer, PPI = protein–protein interaction, Th1 = T-helper cells type 1, Th2 = T-helper cells type 2, TSS = Transcription Start Site.

Keywords: childhood asthma, differentially expressed genes, integrated analysis, methylation, PPI network

1. Introduction

Asthma is a common allergic and chronic respiratory disease, which is characterized by airway inflammation and obstruction and airway hyperresponsiveness (AHR).[1] With rapidly increased prevalence, asthma has been the most common chronic disease in children[2] despite treatment of multiple drugs, which place a major burden for affected people and their families.[3] Asthma is a complex disease that is affected by genetics, environmental exposure and sensitization, and their integration.[4] Although the exact pathogenesis of asthma remains elusive, inflammation was the most principal pathogenesis. [3] T cells and IgE-mediated response are crucial for allergic response.[5] As subtypes of T cells, T-helper cells type 1 (Th1) play a role in antagonism of allergic response and virus defense, T-helper cells type 2 (Th2) can produce pro-inflammatory cytokines.[6] The production of IgE and imbalance between Th1 and Th2 cytokines were reported to play pivotal roles in asthma.[6–8] The eosinophils are also associated with the pathogenesis of asthma because of the function in host defense.[9,10] In addition, atopy was reported to be closely correlated with asthma as well.[6] Besides asthma-associated gene variants,[11] DNA methylation was reported to be related to childhood asthma in peripheral blood cells.[12,13]

With advanced high throughput technology, the amount of available genetic information increased rapidly. Considerable efforts have taken place to identify genes and pathways that are associated with complex diseases such as childhood asthma. The information on differentially expressed genes (DEGs), differentially methylated genes (DMGs), and pathways involved with the pathogenesis of childhood asthma is obtained primarily from studies employing microarrays in peripheral blood, bronchial and epithelial biopsy specimens,[14–17] and nasal lavage samples.[18] Because of the difference in sample size, protocols, platforms, and analytical methods in multiple microarray studies, integrated analysis of microarray studies from multiple

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sources is more accurate and meaningful than individual microarray study.

In this study, we identified the DEGs associated with asthma in children by integrated analysis of multiple gene-expression profiles in childhood asthma compared with normal control (NC). Function annotation and protein–protein interaction (PPI) network construction were performed to interpret the biological function of DEGs and identify the pathways enriched in childhood asthma. We also obtained the DMGs in childhood asthma according to GEO and we performed a comprehensive analysis by using a bioinformatics approach to discover the regulatory roles of DEGs and DMGs in the development of childhood asthma. Hopefully, the DEGs, DMGs, and pathways found in this study may advance the knowledge about the cellular and molecular events occurring in childhood asthma and raise new strategies of treatment for childhood asthma.

2. Methods

2.1. Eligible gene-expression and DNA methylation profiles of childhood asthma

On the basis of the Gene Expression Omnibus database (GEO, http://www.ncbi.nlm.nih.gov/geo), the microarray datasets of blood samples in childhood asthma and NC groups were selected. The gene expression and DNA methylation data for childhood asthma were downloaded from GEO. All the selected datasets were standardized or raw datasets.

2.2. Differential gene expression analysis

After downloading the selected datasets, background correction and normalization of datasets were performed. After the normalization processing using the Linear Models for Microarray Data (Limma) package in R, 2-tailed Student *t* test was used to obtain the *P* value and identify the DEGs between childhood asthma and NC with $P < .01$. Heat maps were generated using the heatmap.2 function in the R gplots package. The gene expression boxplots overlapped with beeswarm of selected DEGs were generated by beeswarm package in R.

2.3. Differential methylation analysis

DMGs were defined as genes involved with differentially methylated sites that can regulate the expression of their downstream genes. The differentially methylated sites in children with asthma compared with NC were identified by Wilcoxon rank-sum test. $P < .01$ indicated the significant difference. TSS200 orTSS1500 indicated the region between position −200 bp or −1500 bp from the Transcription Start Site (TSS), respectively.[19] The promoter-related DMGs were identified by using 450K Illumina arrays with TSS200 or TSS1500.

2.4. Correlation analysis between DMGs and DEGs

The genes that were not only differentially expressed but also differentially methylated were identified to analyze the association between gene expression and DNA methylation. As DNA methylation makes negative influences on gene expression, the DEGs whose expression and DNA methylation play opposite regulation in children with asthma were identified.

2.5. Functional annotation of DEGs

To further research the biological function of DEGs, we performed Gene Ontology (GO)[20] and Kyoto Encyclopedia of Genes and Genomes (KEGG)[21] pathway enrichment analysis using the online-based software GeneCodis3.[22] False discovery rate (FDR) <0.05 was defined as the selected criterion of statistically significant difference.

2.6. PPI network construction

Identification of the crucial protein involved with childhood asthma is a meaningful research work. As an approach to analyze the function of protein, PPI network is superior to the functional analysis of single protein. In this study, top 20 DEGs were used to construct the PPI network through Biological General Repository for Interaction Datasets (BioGRID, http://thebiogrid.org/) and Cytoscape.

2.7. QRT-PCR confirmation

Three children with asthma and 3 NCs were recruited from Rizhao People's Hospital from June 2016 to August 2016. The 6 blood samples were obtained from 3 children with asthma and 3 NC children. The patient characteristics are displayed in Supplementary Table 1, http://links.lww.com/MD/C251. All individuals provided written informed consent for use of their samples in this study. This study was approved by the Ethics Committee of Rizhao People's Hospital.

With Trizol reagent (Invitrogen, China), total RNA was isolated and cDNA was obtained from isolated RNA with SuperScript III Reverse Transcriptase (Life Technologies, Carlsbad, CA). In an ABI 7500 real-time PCR system (Applied Biosystems, Carlsbad, CA), quantitative PCR was performed with Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). We analyzed the relative gene expression by using $2^{-\Delta\Delta Ct}$ method. The human 18srRNAs were served as endogenous controls for mRNA expression in analysis.

3. Results

3.1. Identification of DEGs in childhood asthma

Three datasets (GSE40732, GSE27011 and GSE40888) were obtained (Table 1). In total, 441 DEGs (251 upregulated

Table 1

Gene expression and methylation datasets used in this study.

Data type	GEO ID	Sample (Case:Normal)	Platform	Year
mRNA	GSE40732	97: 97	GPL16025NimbleGen Homo sapiens Expression Array [100718_HG18_opt_expr]	2015
mRNA	GSE27011	36: 18	GPL6244[HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array [transcript (gene) version]	2011
mRNA	GSE40888	65: 40	GPL6244 [HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array [transcript (gene) version]	2014
DNA methylation	GSE40732	97: 96	GPL16025 NimbleGen Homo sapiens Expression Array [100718_HG18_opt_expr]	2015

Table 2

The top 10 upregulated and downregulated DEGs in childhood asthma.

Gene ID	Gene symbol	P	Gene ID	Gene symbol	P
Upregulated DEGs			Downregulated DEGs		
59352	*LGR6*	7.03E-07	54556	*ING3*	2.64E-06
7011	*TEP1*	8.34E-07	51193	*ZNF639*	1.17E-05
3002	*GZMB*	1.08E-06	1983	*EIF5*	1.66E-05
10417	*SPON2*	9.60E-06	28971	*C11orf67*	5.83E-05
83888	*KSP37*	9.93E-06	29101	*SSU72*	5.89E-05
55020	*FLJ20699*	1.33E-05	11043	*MID2*	1.31E-04
1178	*CLC*	1.54E-05	6157	*RPL27A*	1.89E-04
53637	*EDG8*	2.18E-05	9643	*MORF4L2*	2.42E-04
30009	*TBX21*	3.61E-05	54557	*SGTB*	2.44E-04
246	*ALOX15*	4.15E-05	65056	*GPBP1*	2.60E-04

DEGs and 190 downregulated DEGs) between childhood asthma and NC were identified (Supplementary Table 2, http://links.lww.com/MD/C251). The top 10 upregulated and downregulated DEGs are displayed in Table 2. Figure 1 is the heat-map of top 100 DEGs in childhood asthma compared with NC. The gene expression boxplots overlapped, with beeswarm of selected DEGs (GZMB, FGFBP2, CLC, TBX21, ALOX15, IL12RB2, UBQLN4, and MID2) displayed in Fig. 2. MID2 was downregulated, while the other 7 DEGs were upregulated in childhood asthma compared with NC.

3.2. Correlation of DNA methylation and mRNA expression

A total of 5195 differentially methylated sites including 541 hypermethylated sites and 4654 hypomethylated sites were identified with $P < .01$. By using 450K Illumina arrays with TSS200 or TSS1500, we identified 1209 DMGs including 280 DMGs involved with 231 hypermethylated sites and 929 DMGs involved with 938 hypomethylated sites (Supplementary Table 3, http://links.lww.com/MD/C251). Among which, 2 DEGs were both downregulated and hypermethylated in childhood asthma and 14 DEGs were both upregulated and hypomethylated in childhood asthma compared with NC (Table 3).

3.3. Functional annotation of DEGs

The most significantly enriched GO terms in childhood asthma compared with NC were viral reproduction (FDR = 1.32E-05), signal transduction (FDR = 4.49E-05), protein binding (FDR = 8.82E-16), DNA binding (FDR = 2.39E-04), nucleus (FDR = 9.95E-20), and cytoplasm (FDR = 1.38E-17). The top 15 most significantly enriched GO terms, including "biological process," "molecular function," and "cellular component" are displayed in Fig. 3. According to the KEGG enrichment analysis (Table 4 and Fig. 3), natural killer (NK) cell mediated cytotoxicity (FDR = 0.000529783), Jak-STAT signaling pathway (FDR = 0.0199405), and Wnt signaling pathway (FDR = 0.0417312) were 3 significantly enriched pathways in childhood asthma. Seven upregulated DEGs (SH2D1B, PRF1, KLRD1, NCR1, ITGAL, GZMB, and CD244) and 2 downregulated DEGs (NRAS and KIR2DL1) were enriched in pathway of NK cell mediated cytotoxicity. Moreover, both SH2D1B and KLRD1 were hypomethylated in childhood asthma compared with NC. Five upregulated DEGs (IL12RB1, IL12RB2, CCND3, IL2RB, and CBL) and 2 downregulated DEGs (SPRY2 and IL6ST) were enriched in Jak-STAT signaling pathway. Five upregulated DEGs (WNT16, PLCB2, CTBP1, DAAM2, and CCND3) and 1 downregulated DEG (MAPK8) were enriched in Wnt signaling pathway. Among them, CCND3 was hypomethylated in childhood asthma compared with NC.

3.4. PPI network construction

The PPI network of top 20 upregulated and downregulated DEGs consisted of 822 nodes and 904 edges (Fig. 4). Edges were used to represent protein–protein interactions. Nodes were used to represent the proteins, among which, the red and green ellipses represented the proteins encoded by upregulated and downregulated DEGs between childhood asthma and NC, respectively. The blue ellipses represented other proteins. Two hub proteins including UBQLN4 (degree = 164) and MID2 (degree = 131) were identified according to the PPI network.

3.5. QRT-PCR confirmation

We selected 8 DEGs (GZMB, FGFBP2, CLC, TBX21, ALOX15, IL12RB2, UBQLN4, and MID2) to verify the integrated analysis, among which, 5 genes (*CLC*, *TBX21*, *ALOX15*, *IL12RB2*, and *UBQLN4*) were upregulated and the other 3 were downregulated. Only the expression of GZMB and FGFBP2 was inconsistent with our integrated analysis (Fig. 5).

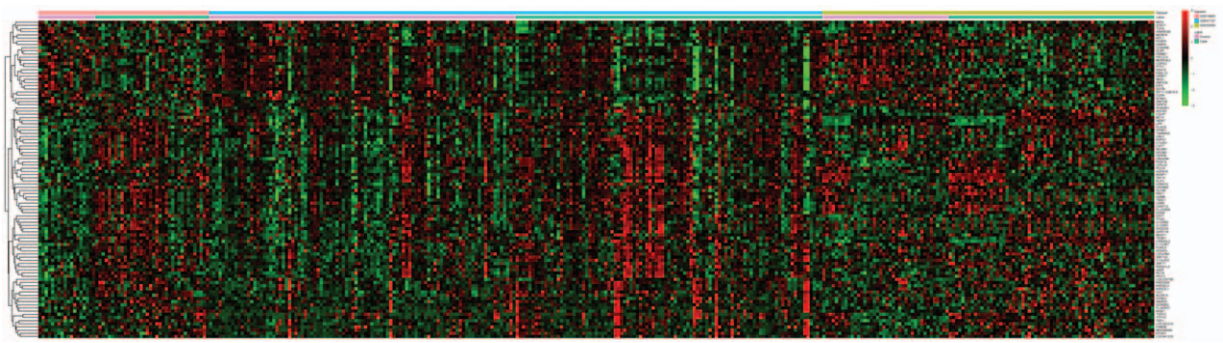

Figure 1. The heat-map of top 100 upregulated and downregulated DEGs in childhood asthma. Row and column were used to represent DEGs and samples. The color scale indicated the expression levels of DEGs. Red and green color indicated the upregulation and downregulation in childhood asthma, respectively.

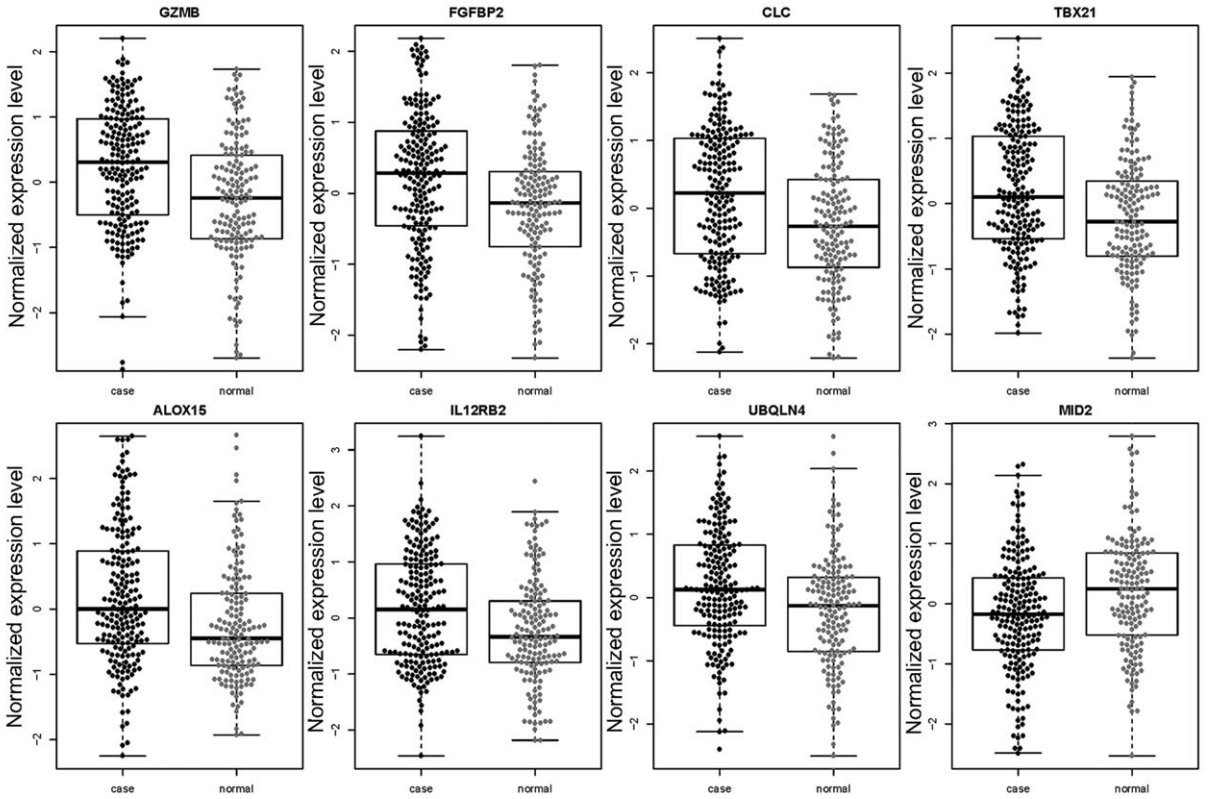

Figure 2. The gene expression boxplots overlapped with beeswarm of selected DEGs in childhood asthma. (A) GZMB; (B) FGFBP2 (C) CLC; (D) TBX21; (E) ALOX15; (F) UBQLN4; (G) MID2. The x-axis shows case and normal groups and y-axis shows normalized expression level. Case and normal group represented the blood samples of children with asthma and normal control. The black plots indicated the expression level of each case and the grey plots indicated the expression level of each normal samples.

4. Discussion

On the basis of the integrated analysis of microarray studies and bioinformatics analysis, our study identified the DEGs and DMGs in children with asthma compared with NC group associated with childhood asthma. The genes that were both differentially expressed and differentially methylated in childhood asthma were identified as well. After functional annotation, PPI network construction, and

Table 3

The genes both differentially expressed and differentially methylated in childhood asthma.

Gene ID	Gene symbol	P^*	Expression	Methylated site	$P^\dagger$	Methylation
8644	AKR1C3	.0021893	Down	cg07665352	.004471977	Hypomethylation
246	ALOX15	.0000415	Up	cg07057744	.006125529	Hypomethylation
896	CCND3	.009901588	Up	cg07891064	.005706163	Hypomethylation
1524	CX3CR1	.006405838	Up	cg22917487	.007074546	Hypomethylation
1524	CX3CR1	.006405838	Up	cg22917487	.007074546	Hypomethylation
79368	FCRL2	.001831486	Up	cg04559323	.006494941	Hypomethylation
124602	KIF19	.009867946	Up	cg01360413	.00141283	Hypomethylation
124602	KIF19	.009867946	Up	cg20467929	.002576607	Hypomethylation
3824	KLRD1	.004685502	Up	cg09261289	.003153825	Hypomethylation
3824	KLRD1	.004685502	Up	cg13828440	.008730343	Hypomethylation
23499	MACF1	.000941204	Up	cg09790512	.008697439	Hypomethylation
23209	MLC1	.001345852	Up	cg20194973	.004905963	Hypomethylation
260429	PRSS33	.001303107	Up	cg01569052	.00520898	Hypomethylation
260429	PRSS33	.001303107	Up	cg26934402	.009663874	Hypomethylation
117157	SH2D1B	.003714097	Up	cg12274567	.003441373	Hypomethylation
8470	SORBS2	.000612513	Up	cg21797147	.001169765	Hypomethylation
23224	SYNE2	.000329229	Up	cg16503753	.008830177	Hypomethylation
7504	XK	.008996166	Up	cg08902818	.004123384	Hypomethylation
84766	EFCAB4B	.0075864	Down	cg03397307	.006571632	Hypermethylation
133418	EMB	.006290756	Down	cg19459207	.004140322	Hypermethylation

* *P*. The *P* value of expression.

† *P*. The *P* value of methylation.

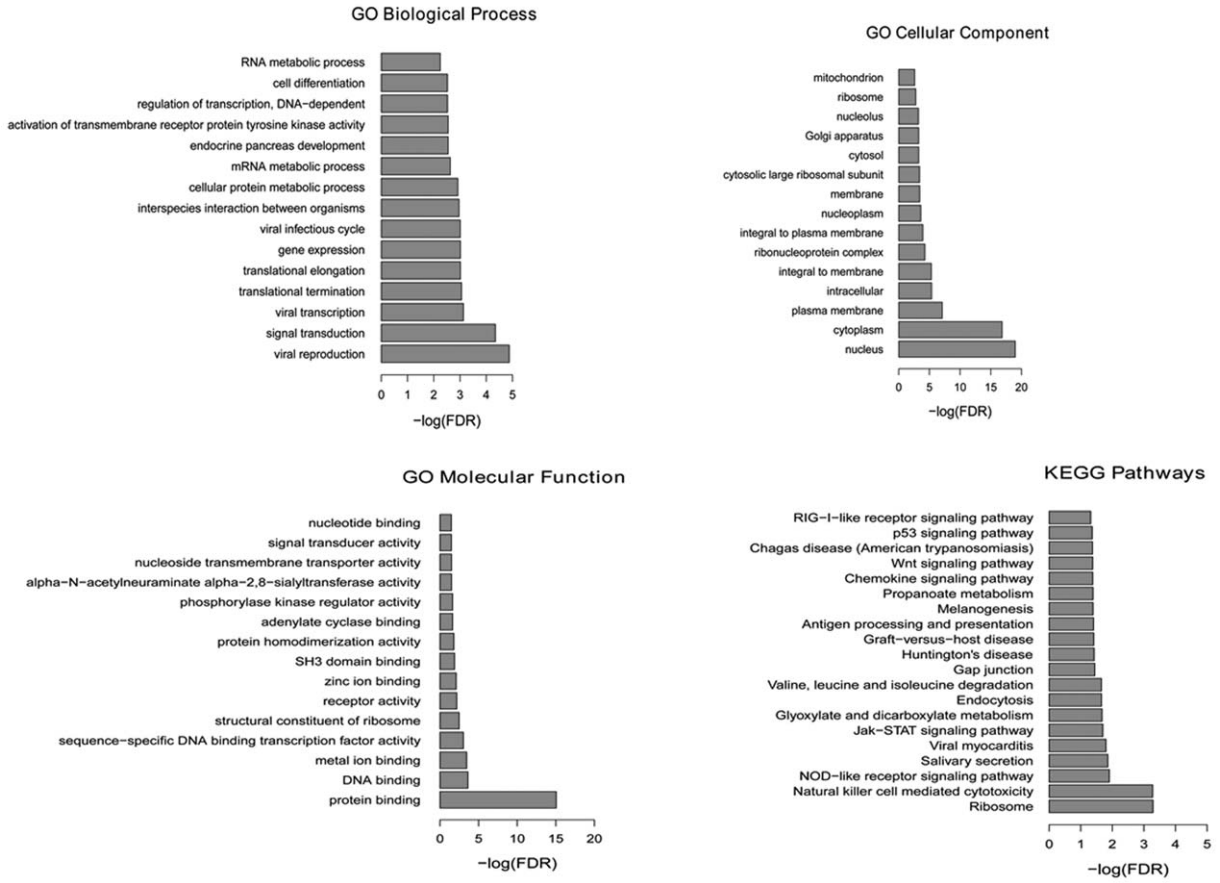

Figure 3. The top 15 most significantly enriched GO terms and enriched KEGG pathways of DEGs in childhood asthma.

qRT-PCR confirmation, we identified several genes associated with childhood asthma.

Among the top 10 upregulated and downregulated DEGs, we identified several DEGs including ALOX15, which may be associated with childhood asthma.

15-Lipoxygenase (15-LO) pathway was reported to be associated with the pathogenesis of asthma by reducing the pericellular concentration of 15(S)-hydroxyeicosatetraenoic acid (15[S]-HETE),[23,24] and preventing AHR and asthmatic inflammation.[25,26] As a main metabolite of the 15-LO

Table 4

The enriched KEGG pathways in childhood asthma.

KEGG ID	KEGG term	No. of genes	FDR	Gene list
Kegg:03010	Ribosome	8	0.000515567	*RPL4,RPS25,RPL37A,RPL5,RPS21,RPL7,RPL32,RPL27A*
Kegg:04650	Natural killer cell mediated cytotoxicity	9	0.000529783	*SH2D1B,PRF1,NRAS,NCR1,KLRD1,ITGAL,GZMB,KIR2DL1,CD244*
Kegg:04621	NOD-like receptor signaling pathway	5	0.0124332	*CARD8,CASP8,BIRC3,MAPK8,NFKB1*
Kegg:04970	Salivary secretion	6	0.0138448	*ADRB2,ATP2B4,CAMP,PLCB2,ADCY7,SLC4A2*
Kegg:05416	Viral myocarditis	5	0.0158712	*PRF1,MYH13,CASP8,CAV1,ITGAL*
Kegg:04630	Jak-STAT signaling pathway	7	0.0199405	*IL12RB1,IL12RB2,IL6ST,CCND3,IL2RB,SPRY2,CBL*
Kegg:00630	Glyoxylate and dicarboxylate metabolism	3	0.0212109	*ACO1,GLYCTK,PCCA*
Kegg:04144	Endocytosis	8	0.0219837	*ADRB2,SH3GLB1,TSG101,CAV1,ZFYVE20,IL2RB,CBL,SMURF1*
Kegg:00280	Valine, leucine and isoleucine degradation	4	0.0220365	*ACADM,BCKDHB,ABAT,PCCA*
Kegg:04540	Gap junction	5	0.0358327	*PDGFD,NRAS,PLCB2,ADCY7,TUBB6*
Kegg:05016	Huntington's disease	7	0.0376254	*CREB3L2,CASP8,PLCB2,NDUFB3,NDUFA3,IFT57,TBPL1*
Kegg:05332	Graft-versus-host disease	3	0.0385031	*PRF1,KLRD1,GZMB*
Kegg:04612	Antigen processing and presentation	4	0.0393373	*NFYA,KLRD1,KIR3DL3,KIR2DL1*
Kegg:04916	Melanogenesis	5	0.0408008	*CREB3L2,WNT16,NRAS,PLCB2,ADCY7*
Kegg:00640	Propanoate metabolism	3	0.040875	*ACADM,ABAT,PCCA*
Kegg:04062	Chemokine signaling pathway	7	0.0416242	*CCL23,NRAS,CX3CR1,PLCB2,ADCY7,NFKB1,DOCK2*
Kegg:04310	Wnt signaling pathway	6	0.0417312	*WNT16,PLCB2,MAPK8,CTBP1,DAAM2,CCND3*
Kegg:05142	Chagas disease (American trypanosomiasis)	5	0.0423453	*GNAL,CASP8,PLCB2,MAPK8,NFKB1*
Kegg:04115	p53 signaling pathway	4	0.0434829	*CASP8,SESN1,GADD45G,CCND3*
Kegg:04622	RIG-I-like receptor signaling pathway	4	0.0482344	*CASP8,MAPK8,NFKB1,IKBKE*

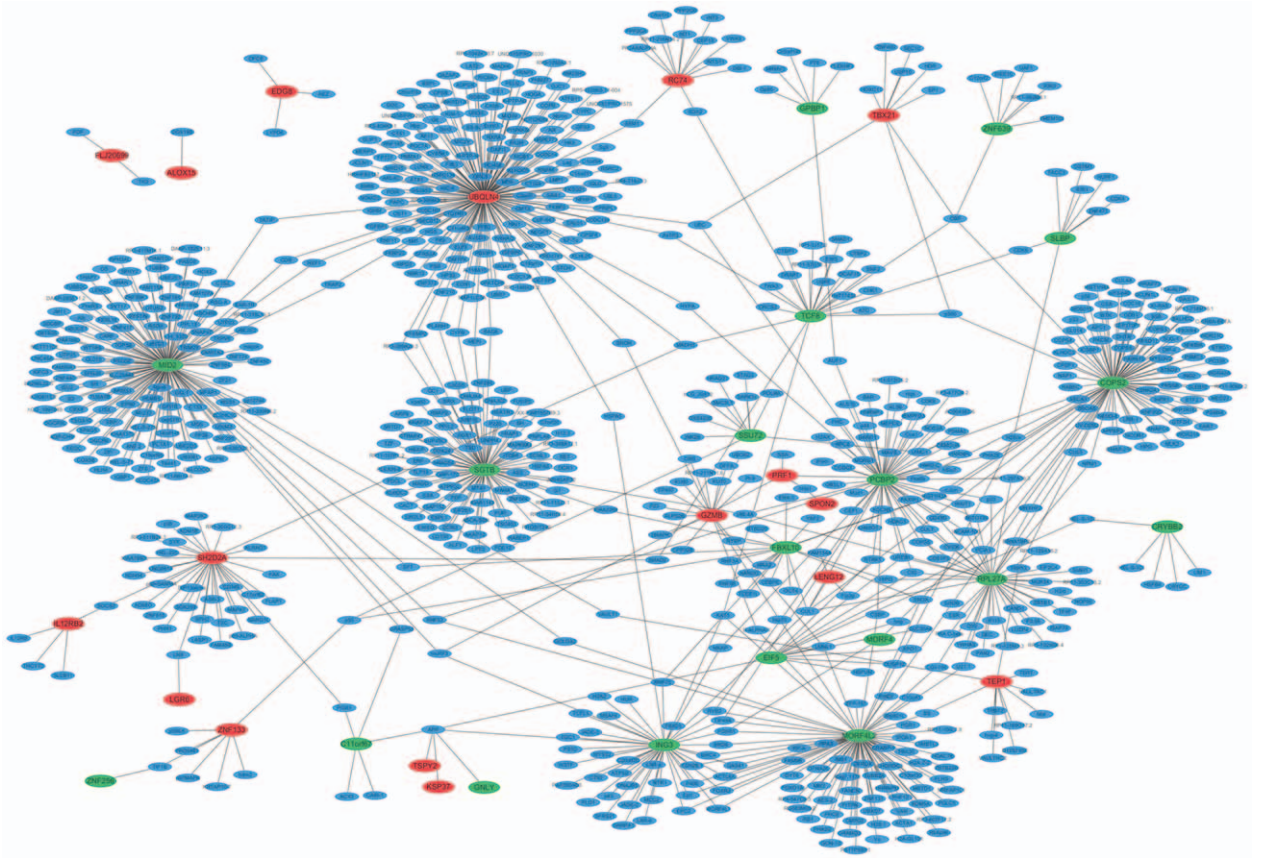

Figure 4. The PPI network of the top 20 upregulated and downregulated DEGs in childhood asthma. The PPI network consisted of 822 nodes and 904 edges; the red and green ellipses represented the proteins encoded by upregulated and downregulated DEGs, respectively. The blue ellipses were represented the other proteins. Edges represented protein–protein interactions.

pathway, 15[S]-HETE is a pro-inflammatory mediator that is catalyzed by ALOX15 [27] and it was significantly upregulated in children with asthma.[27] ALOX15, arachidonate 15-lipoxygenase, is an enzyme that is expressed in multiple lung cells such as airway epithelial cells[23] and neutrophils[28] and a positive association between ALOX15 and the AHR phenotype was also detected.[29] In our study, ALOX15 was both upregulated and hypomethylated in childhood asthma compared with NC. The upregulation of ALOX15 may induced by the hypomethylation. Our finding provided evidences for previous studies and we hypothesized that ALOX15 may play a crucial role in childhood asthma by regulating the 15-LO pathway.

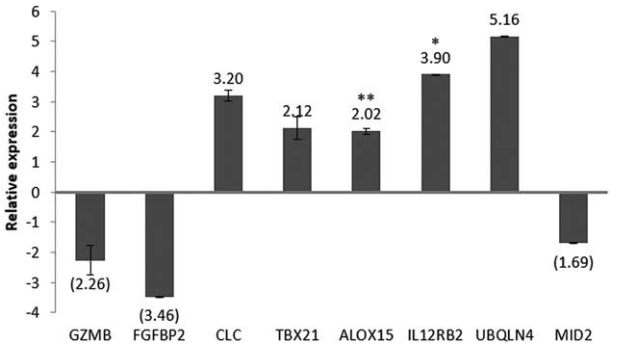

Figure 5. q-RT-PCR results of selected DEGs in childhood asthma. "*" was represented $P < .05$ and "**" was represented $P < .01$.

TBX21 belonged to the T-box gene family and encodes a Th1-specific T-box transcription factor, which is expressed in T cells.[30] The polymorphisms of TBX21 were associated with the risk of asthma phenotypes.[31] The lineage commitment in CD4 T helper cells can be regulated by TBX21.[32] TBX21 induce helper T cells to differentiate into Th1 cells and induce the production of interferon gamma (IFNG) by Th1 cells.[32] In addition, IFNG can also produce Th1 cells. As an imbalance between Th1 and Th2 was reported to elevate the risk for asthma in children,[33,34]*TBX21* was suspected to be a key gene in childhood asthma because of the regulation of Th1 andTh2 cells. TBX21 has been reported to be significantly decreased in the lung tissue of patients with asthma,[35] while the expression of TBX21 was upregulated in blood of children with asthma in our study that needs further research.

FGFBP2, fibroblast growth factor binding protein 2 (also called killer-specific secretory protein of 37 kDa, Ksp37), is a Th1/Tc1-specific secretory protein that was mainly expressed in cytotoxic T lymphocytes (CTLs) and NK cells. FGFBP2 have been demonstrated to be involved with the pathogenesis of atopic asthma because of the function in CTL-mediated immunity.[36] The number of FGFBP2-positive $CD8^+$ T cells in the peripheral blood with mild atopic asthma was marked increased, and upregulated FGFBP2 was detected in the peripheral blood of patients with asthma,[36] which was consistent with our integrated analysis. However, our qRT-PCR results show that

FGFBP2 was downregulated in children with asthma compared with NC. This discrepancy might be induced by small sample size of qRT-PCR. Further research with large sample size was needed to clarify our finding.

CLC, charcot-Leyden crystal galectin (also called galectin-10), was expressed in eosinophils, basophils, and $CD4^+$, $CD25^+$ regulatory T cells. CLC was reported to be associated with eosinophilic inflammation.[37] The expression of CLC was upregulated in the peripheral blood of patients with aspirin-induced asthma,[38] which plays the same pattern with our study.

GZMB is a member of granzyme family of serine proteases. Recently, de novo expression of GZMB has been demonstrated to induce a qualitative change of the composition of granules of mature blood basophils.[39] As several major mediators of allergy and asthma, such as histamine, leukotriene C4, IL-4, and IL-13, are formed by basophils,[40–44] GZMB may play a role in asthma by regulating the make-up of basophils. Deficiency of GZMB can elevate the production of Th2 cytokines and break the balance between Th1 and Th2 cytokines, so that the susceptibility to allergen-induced asthma was increased.[45] In addition, children with downregulated GZMB may induce the inability of regulating immune response to infection.[46] No previous study reported the association between GZMB and childhood asthma. In our study, GZMB was also downregulated in blood of children with asthma based on qRT-PCR results. We speculated that GZMB might be a mediator of allergic inflammation including asthma. However, our integrated analysis indicated that GZMB was upregulated in children with asthma compared with NC. Further research and biological test were needed to explore the precise role of GZMB in children with asthma.

According to the KEGG enrichment analysis, NK cell mediated cytotoxicity is a significantly enriched pathway in childhood asthma. As a type of immune cells, NK cells were reported to play a role in allergic inflammation in patients with asthma.[47,48] Previous study indicated that conventional NK cells play a pro-inflammatory role in the process of allergic disease, and makes a contribution on regulating the development of airway eosinophilia and Th2 cytokine production.[49] We speculated that the pathway of NK cell mediated cytotoxicity and its regulated DEGs may play roles in childhood asthma. Among these DEGs, SH2D1B and KLRD1 were both upregulated and hypomethylated in childhood asthma compared with NC, which suggested that methylation may contribute to the expression of these 2 DEGs.

Jak-STAT signaling pathway was also a significantly enriched pathway in childhood asthma. As the expression of IgE is closely associated with the prevalence of atopic asthma, Jak-STAT signaling pathway was suspected to play a role in the development and progression of asthma by regulating the level of IgE and the balance between Th1 and Th2. [50] Hence, we concluded that the Jak-STAT signaling pathway related DEGs may be associated with childhood asthma.

Among which, IL12RB2 is a specific IL-12 receptor that is expressed in activated T and NK cells. IL12RB2 was implicated to be a biologic candidate in asthma pathogenesis.[51] The expression of IL12RB2 can be detected in Th1 cells but not in Th2 cells,[52] and increased IL12RB2 make a contribution to converting the production of cytokine from Th2 to Th1 or Th0 type in allergic asthma.[53] In our study, the increased IL12RB2 was detected in the blood of children with asthma. Hence, the level of IL12RB2 was served as a potential marker to reflect the balance between Th1 and Th2.

Wnt signaling pathway is another significantly enriched pathway in children with asthma in the present study. Wnt signaling pathway has been demonstrated to be associated with lung function in children with asthma[54] and it can regulate the development of airway remodeling in patients with asthma as well.[55] Wnt signaling pathway and its regulated DEGs were concluded to be involved with physiological and pathological processes of childhood asthma. Moreover, CCND3 was a common DEG both in Jak-STAT signaling pathway and Wnt signaling pathway that was upregulated and hypomethylated in childhood asthma. CCND3 may play roles in these 2 pathways by regulating its methylation level.

In the present work, UBQLIN4 was upregulated and MID2 was downregulated in children with asthma. Moreover, UBQLIN4 and MID2 were 2 hub proteins in the PPI network that emphasized their importance on childhood asthma.

5. Conclusion

Several DEGs and DMGs as well as 3 pathways, including NK cell mediated cytotoxicity, Jak-STAT signaling pathway, and Wnt signaling pathway, were found to be associated with childhood asthma, which was helpful to elucidate the underlying mechanism with molecular level, develop new potential diagnostic biomarker, and provide clues for drug design. However, the sample size for qRT-PCR was small, which is a limitation of this study. Further research with a larger sample size was needed.

Author contributions

Conceptualization: Nian-Zhen Zhang, hewen wang.
Formal analysis: Xiujuan Chen.
Methodology: Nian-Zhen Zhang, Xiujuan Chen, Yuhua Mu.
Project administration: Nian-Zhen Zhang.
Resources: Nian-Zhen Zhang, Yuhua Mu.
Supervision: hewen wang.
Validation: Xiujuan Chen, Yuhua Mu, hewen wang.
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