Bioinformatics Analysis in Different Expression Genes and Potential Pathways of CD4+ Cells in Childhood Allergic Asthma

Chenbei Li

First Affiliated Hospital of Nanchang University

Ao Kan

First Affiliated Hospital of Nanchang University

Xianjun Zeng (✉ xianjun-zeng@126.com)

First Affiliated Hospital of Nanchang University

Research Article

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Abstract

Background: Asthma is one of the most common chronic respiratory diseases in children. CD4+T cell plays a key role in the immune response which affects the pathogenesis of asthma. Genetic level of CD4+T cells on childhood allergic asthma remains unclear. In our study, we aimed to identify potential different expressed genes (DEGs) and pathways related to childhood allergic asthma by performing bioinformatics analysis of dataset.

Methods: GSE40887 dataset from Gene Expression Omnibus (GEO) was used for bioinformatics analysis. Limma package in R was used to detect DEGs. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were carried by Database for Annotation, Visualization and Integrated Discovery (DAVID). The construction of protein-protein interaction (PPI) network of the DEGs was performed by Search Tool for the Retrieval of Interacting Genes (STRING) and Cytoscape. Hub genes were identified through cytoHubba in Cytoscape.

Results: In all, 200 up-regulated genes and 69 down-regulated genes were identified. Up-regulated genes were mainly enriched in: protein self-association, cellular response to hydrogen peroxide, plasma membrane. As for down-regulated genes, enzyme binding and detection of chemical stimulus involved in sensory perception of smell, nucleolus were enriched. Only one KEGG pathway was enriched: olfactory transduction. In addition, top 10 hub genes were found including: PRPF19, CCAR1, CWC15, PRPF38A, PRCC, KRR1, RPS3, PDCD11, MKI67, and NUF2.

Conclusion: Several DEGs (SNORD46, RPS3, OR5E1P, OR56A5 and OR51B6, and PDCD11), one pathway (olfactory transduction) and one biological process (cellular response to hydrogen peroxide) were found might associate to childhood allergic asthma. Our results may provide inspiration for other researchers on DEGs and pathways in CD4+T cells of childhood allergic asthma.

Introduction

Asthma is not only one of the most common chronic respiratory diseases, but also a global health problem that has effects on approximately 300 million people around the world despite ages, countries, sex, and ethnic groups [1]. In children, it is the most frequently chronic disease due to poor asthma control [2]. Child asthma causes symptoms like airway inflammation, obstruction of the airway, bronchospasm and hyperresponsiveness of airway etc. [3, 4], these symptoms affect the subsequent daily lives and reduce the life quality among children [5, 6]. Factors that influence this complex disease include genetic and environmental factors [7, 8].

CD4 + T cells are T cells with the unique type of protein (CD4) on their surfaces, and they provide the assistance by triggering other cell's response during the immune response [9]. CD4 + T cells play a key role in the immune responses by helping B cells to make antibodies; these antibodies help macrophages to develop activity to kill microbes and recruit neutrophils, basophils and eosinophils at the site of infection [10]. T-helper cells type 1 (Th1), T-helper cells type 2 (Th2), T-helper cells type 17 (Th17) and

regulatory T cells are the subtypes of CD4 + T cells [11], and the imbalance of Th1 and Th2 also plays a vital role in asthma [12]. Considering the genetic factors, genes of CD4 + T cells regulating their function, up-regulated gene expression or down-regulated gene expression may affect the protein pathways that associate with asthma [13, 14].

The quantity and quality of genetic information are increasing due to the rapid development of high-throughput technology; this contributes to the identification of gene expression and its related protein; and it also helps investigators to discover the pathways of many complex diseases including childhood allergic asthma. Online databases such as Gene Expression Omnibus (GEO; <http://www.ncbi.nlm.nih.gov/geo>) provide available data of gene expression for bioinformatics analysis [15, 16], which helps investigators to discover the pathway and metabolism of diseases. Understanding of gene, protein and relevant pathway makes great contribution to developing new therapies against diseases. Up to now, no bioinformatics analysis based on identification of CD4 + T cells genes expressions and their related pathways in childhood allergic asthma have been performed.

Our study aimed to identify the different expression genes (DEGs) related to childhood asthma in CD4 + T cells by analyzing the gene expression data of childhood allergic asthma and healthy controls. Gene ontology (GO) enrichment analysis and pathway analysis were used to discover the potential function of DEGs. Hub genes involved in the childhood allergic asthma were also identified. Apart from that, PPI network was constructed to reveal the interaction of different proteins that correlated to DEGs. We hope that the DEGs and their functions, hub genes, proteins and pathways discovered in our study can enhance the understanding of the molecule metabolism of childhood allergic asthma and help scientists to have a better insight in genetic level of childhood allergic asthma.

Materials And Methods

Affymetrix microarray data of allergic childhood asthma

The data of allergic childhood asthma were obtained from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus database (GEO; <http://www.ncbi.nlm.nih.gov/geo>). The GEO accession of this data is GEO40887, gene expression of CD4 + cells samples was collected from peripheral blood of 3 children patients with allergic asthma and 5 healthy controls. Data of GEO40887 and the annotation files based on platform GPL6244 of Affymetrix Human Gene 1.0 ST Array were downloaded for analysis.

Different expression gene analysis

There are 19814 probes according to the annotation files, and these probes were converted into gene symbol in the expression data. The data was underwent background correction and normalization for further analysis. After that, Linear Models for Microarray Data (Limma) package was used in R to calculate the P-value and also identify different expression of genes between the allergic childhood asthma samples and healthy controls under condition that P-value < 0.05. Different expression genes

were classified into three categories: Up-regulated genes ($\log FC \geq 0.5$), down-regulated genes ($\log FC \leq -0.5$), and no-significant genes ($-0.5 < \log FC < 0.5$). Volcano Map was generated using the ggplot2 package of R, it can directly demonstrate the different expression genes and the distribution of genes. Besides volcano map, heat map was done using pheatmap function in the R pheatmap package.

Gene function analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID) is a powerful gene functional classification tool which helps researchers to discover and understand the function and biological information of number of genes [17]. Gene Ontology (GO) enrichment analysis and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were conducted on the data and using DAVID. Function annotation tools were used in the DAVID, and GOTERM_BP_Direct, GOTERM_CC_Direct, GOTERM_MF_Direct, KEGG_Pathway were selected. GO terms and KEGG pathway that P-value less than 0.05 were selected as they are significantly enriched. The selected GO terms and KEGG pathway were made into bubble maps together for reviewing the information ($-\log_{10}$ P-Value, false discovery rate (FDR), count). Bubble maps were divided into up-regulated DEGs and down-regulated DEGs. The maps were generated using ggplot function in R ggplot2 package.

Construction of PPI network

The Search Tool for the Retrieval of Interacting Genes (STRING; <http://string.embl.de/>) is a helpful tool for investigators to construct PPI network and analyze the interaction between proteins [18]. All up-regulated and down-regulated genes were analyzed using STRING to obtain the interaction of corresponding proteins (confidence score was set ≥ 0.4). After the interactions between corresponding proteins were found by STRING, Cytoscape software (version 3.6.1) cytoHubba was used to analyze and rank these genes by the MCC scores. Top 10 hubgene were used to construct the PPI network with Cytoscape software (version 3.6.1)

Results

Identification of different expression genes (DEG)

The dataset (GSE40887) was analyzed to identify genes that expressed differently in CD4 + T cells of childhood allergic asthma and healthy controls. In total, 8 CD4 + T cells from peripheral blood samples were obtained and analyzed. There were 269 DEGs in GSE40887, 200 of them are up-regulated genes and 69 of them are down-regulated genes. DEGs expressed between childhood allergic asthma and healthy controls are presented using volcano maps in Fig. 1. Table 1 shows Top 10 up-regulated genes and down-regulated genes in childhood allergic asthma. And all DEGs including non-significant, up-regulated and down-regulated genes were displayed using heat map in Fig. 2.

Table 1
Top 10 up-regulated and down-regulated DEGs in childhood allergic asthma.

Genes	logFC	P.Value	Group
LINC01561	2.176548756	0.000679943	up-regulated
SNORD46	2.043397504	0.001576536	up-regulated
RPS3	1.644047688	0.035559351	up-regulated
B4GAT1	1.583510857	0.002893113	up-regulated
FCRLB	1.491867533	0.034080293	up-regulated
FERMT3	1.428907627	0.012359682	up-regulated
SMCP	1.343828879	0.01068511	up-regulated
PHLDA3	1.307833081	0.017064122	up-regulated
FCRL2	1.301569798	0.007259615	up-regulated
DLSTP1	-1.376530143	0.013782394	down-regulated
MGAT4EP	-1.306714297	0.048682909	down-regulated
C11orf1	-1.265207413	0.001485408	down-regulated
SPATA42	-1.152625368	0.02870288	down-regulated
OR5E1P	-1.14898493	0.034142158	down-regulated
GPR3	-1.143712863	0.021928513	down-regulated
TUBGCP2	-1.132736597	0.044772999	down-regulated
OR56A5	-1.117357584	0.021737197	down-regulated
OR51B6	-1.083233805	0.032281767	down-regulated
C10orf113	-1.071010709	0.019692575	down-regulated
DEGs were ranked based on the logFC from high to low.			

The functional enrichment analysis of DEGs

The biological functions of total 269 DEGs were investigated through GO enrichment analysis and KEGG pathway analysis using DAVID. Bubble maps of functional enrichment analysis of up-regulated DEGs were displayed in Fig. 3 and down-regulated DEGs were displayed in Fig. 4. The 200 up-regulated genes were significantly enriched in 1 GO cellular component (CC), 9 GO molecule functions (MF), 7 GO biological processes (BP) and 1 KEGG pathways. The 69 down-regulated genes were significantly enriched in 4 MFs, 2 BPs, 1 CCs and 0 KEGG pathways. Only 1 KEGG pathway was enriched in DEGs: Olfactory transduction (P Value = 0.01746). Up-regulated genes were largely enriched in: GO MF protein

self-association (P Value = 1.01E-04), GO BP cellular response to hydrogen peroxide (P Value = 0.0026), GO CC plasma membrane (P Value = 0.021). As for down-regulated genes, GO MF enzyme binding (P Value = 0.017), GO BP detection of chemical stimulus involved in sensory perception of smell (P Value = 0.042), GO CC nucleolus (P Value = 0.047) were enriched.

Protein-Protein Interaction (PPI) network and Module analysis

All 269 DEGs were analyzed using STRING and this PPI analysis revealed that there are 159 nodes and 199 edges (Fig. 5). Protein-protein interactions were shown in edges and proteins were shown in nodes, labels filled with red color are down-regulated and those with yellow color are up-regulated genes in childhood allergic asthma. Blue color represents other proteins. CytoHubba method was used to rank the DEGs and MCC scores were used to rank the genes. As a result, top 10 hub genes are followed and their information was displayed: PRPF19 (score = 31), CCAR1 (score = 27), CWC15 (score = 26), PRPF38A (score = 25), PRCC (score = 24), KRR1 (score = 20), RPS3 (score = 20), PDCD11 (score = 18), MKI67 (score = 16), and NUF2 (score = 14). These genes were displayed in Table 2. The interaction between these top 10 hub genes were displayed in Fig. 6.

Red color nodes represent down-regulated DEGs; Yellow color nodes represent up-regulated DEGs; Blue color nodes represent other genes.

Table 2
Top 10 hub genes.

Rank	Name	MCC Score	Group
1	PRPF19	31	Up-Regulated
2	CCAR1	27	Up-Regulated
3	CWC15	26	Up-Regulated
4	PRPF38A	25	Up-Regulated
5	PRCC	24	Up-Regulated
6	KRR1	20	Down-Regulated
6	RPS3	20	Down-Regulated
8	PDCD11	18	Up-Regulated
9	MKI67	16	Down-Regulated
10	NUF2	14	Up-Regulated

The color from red to yellow represents MCC score from high to low. Blue color nodes represent other genes.

Discussion

In this study, an insight of gene expressions and their related proteins interactions in childhood allergic asthma was discovered based on the combination of genome array study and bioinformatics analysis. A total of 269 DEGs have been identified and 200 of them are up-regulated genes and the rest of them are down regulated genes. After functional analysis and PPI network construction, several pathways and hub genes related to childhood asthma were identified.

In the top 10 up-regulated and down-regulated DEGs and hub genes, we found several DEG that may be related to childhood allergic asthma.

Small nucleolar RNA, C/D box 46 (SNORD46) was one of the top 10 up-regulated DEGs in childhood allergic asthma with $\log FC = 2.04$. According to a recent study, SNORD46 functions as an oncogene in the lung cancer [19]. Other experiments identified that SNORD46 overexpressed in several types of cancer including lung cancer and regulated the cell proliferation, viability, impaired cell migration and invasion in lung cancer [20]. Airway narrowing is considered as the major symptom of asthma, which is caused by airway smooth muscle hyperplasia and hypertrophy (especially hyperplasia) [21]. SNORD46 was suspected of involvement in the airway smooth muscle hyperplasia of asthma. However, there are no researches focusing on SNORD46 and asthma. Further studies are needed to explore whether SNORD46 participates in the airway smooth muscle hyperplasia of asthma.

Ribosomal Protein S3 (RPS3) gene was not only top 10 up-regulated DEGs ($\log FC = 1.64$), but also one of the top 10 hub genes (score = 20). RPS3 is a ribosomal protein that plays a crucial role in the NF- κ B pathway, which binds to the p65 subunit so the NF- κ B complex's nuclear translocation will be promoted and the binding ability of p65 will be strengthened [22]. NF- κ B pathway mainly regulates the inflammation response and cellular activities like cell proliferation and cell death [23]. According to a study in 2012, the RPS3-p65 interaction in NF- κ B pathway is missing due to the knockout of RPS3 and it could significantly decrease the cytokine production in B cells, T cells proliferation and immunoglobulin κ light chain gene expression in B cells [24]. Also another research has confirmed that RPS3-p65 interaction influenced a lot in the immune response by regulating cytokine production and proliferation in T cells and immunoglobulin κ light chain gene expression in B cells [25]. High grade of immune responses such as high level of IgE, hypersecretion of mucus and high level of Th2 cytokines are characteristics of allergic asthma [26]. We hypothesized that the overexpression of RPS3 in NF- κ B pathway elevates immune response in individuals and results in allergic asthma. Moreover, it was found in a mouse study that the lung RPS3 protein level was significantly increased in mouse asthma and RPS3 silence RNA remarkably inhibited allergic airway inflammation [27], and this study provided evidence for our hypothesis but further research detecting relationship between human allergic asthma and RPS3 is required.

There are three down-regulated genes: OR5E1P ($\log FC = -1.14$), OR56A5 ($\log FC = -1.12$) and OR51B6 ($\log FC = -1.08$) coming from the olfactory receptor family, but their subfamilies are different. There are no current studies showing that these three genes are associated with asthma. However, a study in 2019 indicates that OR2AG2 from olfactory receptor family participates in the asthma pathogenesis and the suppression or genetic defects in OR2AG2 contribute to asthma pathogenesis [28]. According to the

study, olfactory receptors express in the airways which is similar to bitter taste receptor [28]. Besides, another study shows that OR2AG1 (paralog of OR2AG2) and OR1D2 are functionally expressed in the human airway smooth muscle cells (HSMCs) [29]. These two olfactory receptor genes are activated by different ligands [30, 31], which induce the brief increase of Ca^{2+} in HSMCs and both receptors can touch off cAMP-dependent signal transduction cascade [28]. cAMP possesses the ability to antagonize HASMC contraction and inhibits the cell proliferation, fights the airway narrowing [32]. In this way, cAMP can combat against asthma, and the activation of OR2AG1 and OR1D2 can trigger the inhibition of cell contraction by activating cAMP-dependent signaling pathway. Taken together, the deficiency or down-regulated of olfactory receptors: OR2AG2, OR2AG1 and OR1D2 play roles in asthma pathogenesis. Based on this, we hypothesize that the three down-regulated olfactory receptors in our study (OR5E1P, OR56A5 and OR51B6) have the similar characteristics like olfactory receptors (OR2AG2, OR2AG1 and OR1D2) and take part in the childhood allergic asthma pathogenesis. Since there are no current studies indicating that there exist relationships between these genes and asthma, further study is needed to confirm our hypothesis and investigate their effects in childhood allergic asthma pathogenesis.

MKI67 is a down-regulated gene ($\log FC = -0.55$) and one of the top 10 hub genes. It is also known as the Ki-67, a nuclear DNA binding protein that used as a marker for tumor grading [33]. Ki-67 is a marker of cellular cycle and proliferation [34], and it can be detected in G1, S, G2 and M phase of cell cycle [35]. Ki-67 is regulated by phosphorylation and only non-phosphorylation Ki-67 can form complex with DNA and functions in proliferation cells [36]. Besides this, Ki-67 can interact with RNA-binding protein during the mitosis [37]. According to one published study, CDK-1 phosphorylates Ki-67 during mitosis [38], and the transition from G1 to S is mediated by Ki-67. Taken together, Ki-67 plays an essential role in the cell cycle which regulates the cell proliferation. Since proliferation may cause the hyperplasia of airway which results in major symptom of asthma, we suspect that Ki-67 may participate in asthma pathogenesis. However, in our study, we identified Ki-67 as a down-regulated DEG in the childhood allergic asthma patients. Another research in 2010 detects 2 biomarkers including Ki-67 between severe asthma patients and healthy controls, and it is found that the proliferating cell nuclear antigen and Ki-67 were highly expressed [39]. The disagreement might be induced by different ages of patients or different subtypes of asthma. Further experiment is needed to clarify our results.

Besides two genes mentioned above (MKI67 and RPS3), PDCCD11 up-regulated gene ($\log FC = 0.73$) is also one of the top 10 hub genes (score = 18). According to studies, PDCCD11 induces the activation of NF- κ B pathway and results the apoptosis of cells [40]. PDCCD11 inducing the apoptosis by activating the transcription of the promoter [41]. Since NF- κ B pathway mainly regulates the inflammation response and cellular activities like cell proliferation and cell death [41], the up-regulated gene PDCCD11 may enhance the function of NF- κ B pathway. We suspected that the overexpression of PDCCD11 activates the NF- κ B pathway, and leads to high inflammation response and cellular activities. This may contribute to the pathogenesis of allergic asthma. However, there is no study indicating the relationship between PDCCD11 and childhood allergic asthma. Further research is required to detect the role of PDCCD11 in childhood allergic asthma.

In addition to genes including up or down regulating genes and hub genes, pathways were also identified and discussed.

According to the GO and KEGG enrichment analysis, only one KEGG pathway enriched in childhood allergic asthma: Olfactory transduction. Olfactory receptors play a critical role in the olfactory transduction and also have the ability to regulate important physiological effects and control the intracellular Ca^{2+} level [29]. Ca^{2+} level are critical for the airway smooth muscle cell contraction which is related to allergic asthma, since the HASMCs of asthmatics often show hypercontractility [42]. Olfactory receptor expresses differently in the human airway smooth muscle cells [43] and regulates the Ca^{2+} level, so we suspected that the pathway of olfactory transduction and its regulated DEGs (OR5E1P, OR56A5 and OR51B6 etc.) may participate in childhood allergic asthma.

In GO enrichment analysis, cellular response to hydrogen peroxide (H_2O_2) is an enriched biological process. A significant characteristic of airway inflammation in asthma patients is the activation of multiple inflammatory cells including eosinophils [44]. Airway inflammation of asthma is correlated to oxidative stress, which exists in the high oxidant activity [45]. H_2O_2 is a mediator of oxidative stress released by eosinophils to enlarge the process of airway inflammation [46]. Airway smooth muscle stimulated by H_2O_2 and contract, narrowing the airway which induces asthma [47]. According to study, H_2O_2 level is significantly higher in the asthma patients than normal controls [48]. Taken together, we concluded that cellular response is an important biological process in childhood patients. Since the cells like airway smooth muscle cells responding to H_2O_2 can induce the contraction which may narrow the airway and lead to asthma.

However, limitations are still occur in the study and need to be recognized. First, only one dataset from one database (GEO) was used during the study which reduces the credibility of the results. Second, the information of the samples including ages, regions and races of the patients remained unknown. Moreover, according to the results, there are 10 hub genes but not all of them have clear regulated mechanisms in asthma. Therefore, more evidence based on experiments are needed to investigate the mechanisms. Finally, we did not perform clinical researches to support our results.

In summary, we have discussed genes from $CD4^+$ T cells: SNORD46 (top 10 up-regulated genes), RPS3 (both top 10 up-regulated genes and top 10 hub genes), OR5E1P, OR56A5 and OR51B6 (three down-regulated genes from same family), MKI67 (down-regulated hub gene) and PDCD11 (up-regulated hub gene). Besides, olfactory transduction pathway and biological process cellular response to H_2O_2 have been discussed. Their importance and potential participation in childhood allergic asthma have been raised. Despite some of them need further research to confirm their roles or importance in childhood allergic asthma, our study provides inspiration for other researchers on DEGs and pathways in $CD4^+$ T cells of childhood allergic asthma.

Conclusion

SNORD46, RPS3, OR5E1P, OR56A5 and OR51B6, and PDCD11 are DEGs that expressions match the published articles' results and may involve in the pathogenesis of asthma. However, MKI67 is different and more studies are needed. Both olfactory transduction pathway and biological process cellular response to hydrogen peroxide are potential pathways in childhood allergic asthma. These provide inspiration and information for other researchers to study DEGs and pathways in CD4 + T cells of childhood allergic asthma. Development of therapies and treatment on molecular level might be enhanced.

Abbreviations

DEG: Different Expression Gene

GEO: Gene Expression Omnibus

GO: Gene Ontology

Limma: Linear Models for Microarray Data

KEGG: Kyoto Encyclopedia of Genes and Genomes

DAVID: Database for Annotation, Visualization and Integrated Discovery

PPI: Protein-protein Interaction

STRING: Search Tool for the Retrieval of Interacting Genes

Th1, Th2, Th17: T-helper cells type 1,2,17

SNORD46: Small nucleolar RNA, C/D box 46

RPS3: Ribosomal Protein S3

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets analyzed during the current study are available in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus database (GEO) repository, [<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE40887>]

Competing interests

Not applicable

Funding

Not applicable

Authors' contributions

All authors designed the study

CL analyzed the data of allergic childhood asthma patients and used R to graph the data, and was the major contributor in writing the manuscript.

AK discover the data from the database and analyzed the data of allergic childhood asthma patients.

XZ made revisions of the manuscript.

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Figures

Figure 1

Volcano map of DEGs between childhood asthma patients and healthy controls.

Figure 2

Heat map of DEGs between childhood asthma patients and healthy

Figure 3

Bauble maps of functional enrichment analysis of up-regulated DEGs.

Figure 4

Bauble maps of functional enrichment analysis of down-regulated DEGs.

Figure 5

Protein-protein interaction of DEGs.

Figure 6

Protein-protein interaction of top 10 hub genes.