European Review for Medical and Pharmacological Sciences 2012; 16: 1961-1966

Screening of differentially expressed genes and small molecule drugs of pediatric allergic asthma with DNA microarray

X.-Q. WANG[1], X.-M. WANG[2], T.-F. ZHOU[1], L.-Q. DONG[3]

[1]Department of Pediatric Cardiology, West China Second University Hospital, Sichuan University, No. 20, Section 3, Renmin Nan Lu, Chengdu, Sichuan, People's Republic of China
[2]Department of Pediatric Cardiology, Chengdu Women's and Children's Central Hospital, No. 1617, Riyue Road, Chengdu, Sichuan, People's Republic of China
[3]Department of General Pediatrics, West China Second University Hospital, Sichuan University, No. 20, Section 3, Renmin Nan Lu, Chengdu, Sichuan, People's Republic of China

Abstract. – BACKGROUND: Asthma is a disease resulting from a complex interaction of multiple genetic and environmental factors. More than 200 asthma candidate genes have been identified in the past decades by using genetic association studies, positional cloning and knockout mouse approaches.

AIM: This study was to identify differentially expressed genes and provide direction for medicine design related to pediatric allergic asthma with DNA microarray.

MATERIALS AND METHODS: The gene expression profile of pediatric allergic asthma GSE18965 was downloaded from Gene Expression Omnibus database which includes 16 samples, 7 normal and 9 pediatric allergic asthma samples. The differentially expressed genes between normal and disease samples were identified by using R language. The co-expression coefficient was calculated among the differentially expressed genes to construct co-expression networks with String Software. Software DAVID and FuncAssociate were used to analyze the functions of genes in the co-expression networks.

RESULTS: A total of 133 genes were identified as differentially expressed genes between normal and disease samples, and 8 related small medicine molecules were also obtained (penbutolol, felbinac, iodixanol, josamycin, oxolamine, 3-nitropropionic acid, scriptaid, and sanguinarine) from database CMAP. The differentially expressed genes were enriched in several biological processes, in which viral transcription and lysosome were the most significant GO term of up- or down-regulated genes.

CONCLUSIONS: Our present findings shed new light on the molecular mechanism of allergic asthma and provide three small molecular medicines (3-nitropropionic acid, scriptaid, and sanguinarine) which have the potential to use in clinic for treatment of allergic asthma in future.

Key Words:
Co-expression network, Differentially expressed gene, Functional annotation, Pediatric allergic asthma.

Introduction

As a chronic respiratory disease, asthma is resulting from a complex interaction of multiple genetic and environmental factors. More than 200 asthma candidate genes have been identified in the past decades by using genetic association studies, positional cloning and knockout mouse approaches[1], but only in the recent years it has been possible to perform whole-genome investigations largely due to the Genome-Wide Association Studies (GWAS)[2], microarray and high throughput sequencing, that have soon shown to be powerful tools to identify novel loci and susceptibility variants for common diseases.

Genome-wide microarray studies of pooled DNA samples are shown to be a valuable tool to identify candidate differentially expressed genes associated to a phenotype in a fast, scalable and economical way[3]. These methods have been applied to different microarray platforms and different diseases, particularly those related to complex traits as intellectual and psychological abilities, multiple sclerosis or Alzheimer disease[4]. Many tools and analysis pipelines have also been developed to improve the ability to identify true associations among thousands of potential candidates[5].

In this study, DNA microarrays were used to identify genes differentially expressed in pediatric allergic asthma between airway epithelial cells from healthy and atopic children. Significance of differential expression was tested by limma and adjusted for multiple testing with the Benjamini and Hochberg (BH) procedure (adjusted $p \leq 0.05$). In addition, significantly associated small molecules drugs were also selected and synergic network was constructed in allergic asthma.

Corresponding Author: Li-Qun Dong, MD; e-mail: Christinexiao5@gmail.com

Materials and Methods

Affymetrix Chip Data

One transcription profile of GSE18965 was downloaded from a public functional genomics data repository Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) database, which is based on the GPL570 platform data (Affymetrix Human Genomic U133 2.0 chip). This chip contains 16 samples, of which 7 and 9 airway epithelial cells samples were from healthy and atopic children, respectively[6]. The annotation information of chip probes comes from Affymetrix Inc.

Data processing and Differentially Expressed Genes Analysis

The raw data were processed by Affy package in R language[7]. Significance of differential expression was tested by R package-limma[8] and adjusted for multiple testing with the Benjamini and Hochberg (BH)[9]. Only the genes with False Discovery Rate (FDR) < 0.05 and |logFC| > 1 were selected as differentially expressed genes.

Related Small Medicine Molecule Selection

The selected genes were divided into up- and down-regulated groups and put into the Connectivity map (cmap). Related small medicine molecules were screened out through comparing our differentially expressed genes with expression patterns of genes collected in Comparative Mapping (CMAP) database. In this study, the |score| > 0.9 was considered as the high relationship threshold.

Construction of Small Molecular Medicine Regulated Co-Expression Network

String software[8] was used to calculate co-expression coefficient within differentially expressed genes. In this study, interactions with co-expression coefficient > 0.5 were selected to construct co-expression network under small molecular medicine regulation.

Gene Function Annotation

The up- and down-regulated differentially expressed genes were put into the Database for Annotation, Viasualization and Integrated Discovery (DAVID)[9] and FuncAssociate[10] softwares for Gene Ontology (GO) term enrichment analysis. $p < 0.05$ was set as the threshold for the analysis using the hypergeometric distribution.

Results

Screening Differentially Expressed Genes in Pediatric Allergic Asthma

Based on normalized expression data after data processing, a total of 133 genes were selected as differentially expressed genes with a FDR < 0.05 and |logFC| >1.

Related Small Molecular Medicine Screening

Totally 8 related small medicine molecules were obtained (Table I), of which, 5 negatively related (penbutolol, felbinac, iodixanol, josamycin, and oxolamine) and 3 (3-nitropropionic acid, scriptaid, and sanguinarine) positively related. These results indicate treatment with penbutolol, felbinac, iodixanol, josamycin, and oxolamine can approach to the same disease statue of the atopic children samples in this study. But the application of 3-nitropropionic acid, scriptaid, and sanguinarine can treat allergic asthma.

Construction of Small Molecular Medicine Regulated Co-Expression Network

Table II listed the 29 selected co-expression pairs. Together with the 8 related small medicine molecules, we constructed small medicine molecules regulation network. In the network, there were individually 14 up- and 9 down-regulated genes (Figure 1).

Gene Function Annotation

GO enrichment analysis was performed using the hypergeometrical distribution to find the significant biological process of up- and down-regulated genes. The results are shown in Table II and Table III. Among them, the most significant GO term of up-regulated genes was viral transcription, which involved 9 genes, Ribosomal Protein L388 (RPL3), RPS10, RPL27, RPS11, RPL27A,

Table I. Small molecular medicine from CMAP database.

CMAP name	Enrichment
Penbutolol	–0.996
Felbinac	–0.971
Iodixanol	–0.934
Josamycin	–0.908
Oxolamine	–0.902
3-nitropropionic acid	0.903
Scriptaid	0.908
Sanguinarine	0.947

Table II. Gene function annotation list of down-regulated genes.

GO ID	*p*-value	GO name
GO:0005764	1.29E-06	Lysosome
GO:0019318	1.58E-06	Hexose metabolic process
GO:0005773	2.06E-06	Vacuole
GO:0006007	2.83E-06	Glucose catabolic process
GO:0005996	3.67E-06	Monosaccharide metabolic process
GO:0046364	4.21E-06	Monosaccharide biosynthetic process
GO:0046165	1.09E-05	Alcohol biosynthetic process
GO:0034637	1.37E-05	Cellular carbohydrate biosynthetic process
GO:0070013	2.32E-05	Intracellular organelle lumen
GO:0043233	3.69E-05	Organelle lumen
GO:0006006	4.51E-05	Glucose metabolic process
GO:0044262	4.53E-05	Cellular carbohydrate metabolic process
GO:0006066	5.40E-05	Alcohol metabolic process
GO:0031974	5.40E-05	Membrane-enclosed lumen
GO:0016051	5.40E-05	Carbohydrate biosynthetic process
GO:0044437	6.06E-05	Vacuolar part

RPL37A, eukaryotic translation initiation factor 5A (EIF5A), EIF5B, and eukaryotic translation elongation factor 1 delta (EEF1D). The most significant GO term of down-regulated genes was lysosome, which involved 6 genes, aminolevulinate, delta-, synthase 1 (ALAS1), aconitase 1, soluble (ACO1), glutathione peroxidase 3, plasma (GPX3), Phosphogluconate dehydrogenase (PGD), vitamin K epoxide reductase complex, subunit 1 (VKORC1), and dicarbonyl/L-xylulose reductase (DCXR).

Discussion

Most of allergic asthma has been attributable to infection with respiratory viruses, including rhinovirus, influenza, virus and respiratory syncytial virus[11]. Viral transcription and translation in airway epithelial cells will lead to more viral proteins accumulation and eventually progeny virus particles are released, which further exacerbate bronchial asthma[12]. As expected, viral transcription related proteins were identified up-regulated in this study (RPL38, RPS10, RPL27, RPS11, RPL27A, EIF5A, EIF5B, and EEF1D), which is in accordance with previous studies that indicate the acid ribosomal protein P1 and P2 have been tested in several clinical studies involving patients suffering from asthma[13].

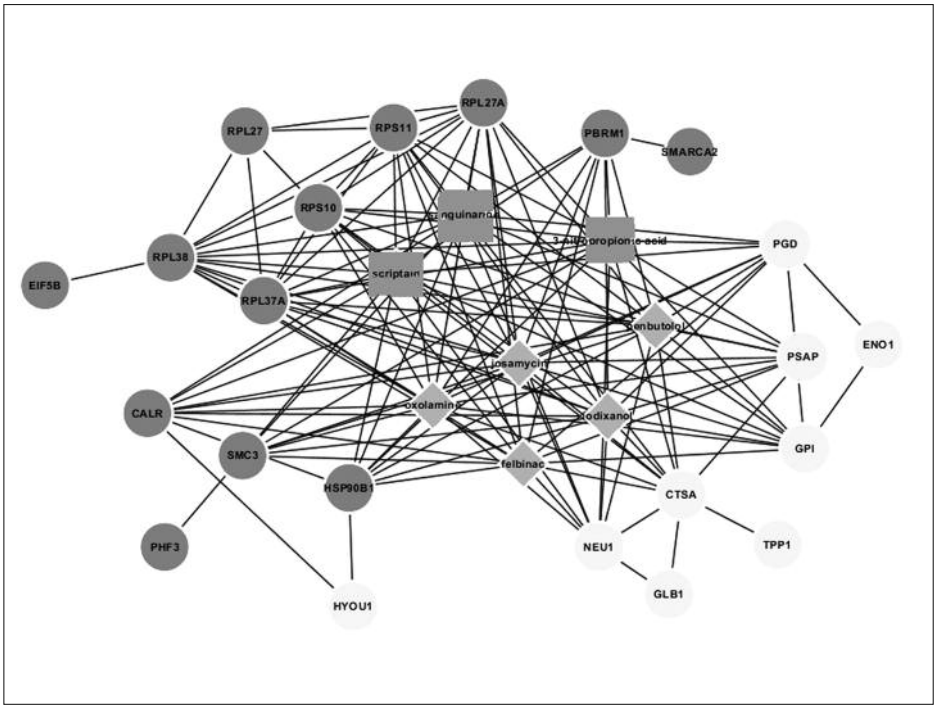

Figure 1. The co-expression network associated with small molecular medicines the white and squares represent individually the small molecular medicines negatively and positively associated, and the black and gray circles represent down- and up-regulated genes.

Table III. Gene function annotation list of up-regulated genes.

GO ID	*p*-value	GO name
GO:0019083	1.99e-11	Viral transcription
GO:0006415	2.46e-11	Translational termination
GO:0019058	3.23e-11	Viral infectious cycle
GO:0043624	4.76e-11	Cellular protein complex disassembly
GO:0006414	5.06e-11	Translational elongation
GO:0043241	5.06e-11	Protein complex disassembly
GO:0034623	1.56e-10	Cellular macromolecular complex disassembly
GO:0006412	1.60e-10	Translation
GO:0034645	9.02e-07	Cellular macromolecule biosynthetic process
GO:0043933	1.03e-06	Macromolecular complex subunit organization
GO:0009059	1.08e-06	Macromolecule biosynthetic process
GO:0051208	1.57e-06	Sequestering of calcium ion
GO:0022414	1.85e-06	Reproductive process
GO:0016071	2.04e-06	Mrna metabolic process
GO:0032774	2.07e-06	Rna biosynthetic process
GO:0044267	2.42e-06	Cellular protein metabolic process
GO:0005829	2.70e-06	Cytosol
GO:0043228	3.43e-06	Non-membrane-bounded organelle
GO:0043232	3.43e-06	Intracellular non-membrane-bounded organelle
GO:0044260	5.57e-06	Cellular macromolecule metabolic process
GO:0003723	1.29e-05	Rna binding
GO:0051238	1.46e-05	Sequestering of metal ion
GO:0019538	1.81e-05	Protein metabolic process
GO:0043170	2.13e-05	Macromolecule metabolic process
GO:0044249	3.71e-05	Cellular biosynthetic process
GO:0090304	4.25e-05	Nucleic acid metabolic process
GO:0005198	4.89e-05	Structural molecule activity
GO:0009058	5.41e-05	Biosynthetic process

Virus can induce airway hyper-responsiveness via stimulating T lymphocytes and chemotaxis of acidophilic leukocytes[3], which can further produce various pro-inflammatory cytokines and mediators to induce inflammatory reaction[14]. In addition, reactive oxygen species, derived from high concentrations of inflammatory cells such as polymorphonuclear neutrophils, are often increased, but the antioxidant enzymes (such as GPx-3) are decreased in patients with asthma, which finally contributes to oxidative stress[15]. GPx-3 is a selenocysteine-containing protein with antioxidant properties. GSH-Px activity has been demonstrated to be lower in whole blood, plasma, and platelets of asthmatic than in non-asthmatic subjects[16, 17]. In mammalian tissues, ROS destabilizes cell organelles, including lysosomes, and releases hydrolases, such as cathepsins to decompose viral protein[18]. The lysosomal membranes are metabolized directly by glutathione peroxidase (GSH-Px)[19]. Therefore, the low-expression of GPX3 leads the inhibition of lysosome function, and hence gives rise to the development of pediatric allergic asthma.

In addition, allergic asthma has already been shown to be influenced by several environmental factors, including diet[20], air pollution[21], and drug[22], which are known asthma risk factors. In this study, we also identified five small molecular medicines (penbutolol, felbinac, iodixanol, josamycin, and oxolamine) that could induce the development of allergic asthma. Penbutolol is a new non-selective β-adrenoceptor blocker that should not be used in asthmatics due to risk of bronchospasm[23]. Josamycin is a 16-membered macrolide antibiotic that is particularly indicated for the treatment of infections of the skin, ear, nose and throat. However, macrolide therapy would result in deterioration of asthma in a patient with diffuse panbronchiolitis[24].

Although there are some useful medicines in asthma treatment, such as B adrenaline receptor

agonist[25] and phosphodiesterase inhibitor[26], these medicines are far from enough for asthma treatment. Recent studies indicate that resveratrol attenuates experimental allergic asthma in mice by restoring inositol polyphosphate 4 phosphatase (a crucial molecular checkpoint to in controlling PI3K-Akt signaling pathway in allergic airway inflammation)[27]. In this study, we screened three small molecular medicines (3-nitropropionic acid, scriptaid, and sanguinarine) which may be effective for allergic asthma treatment. For example, sanguinarine, derived from the root of Sanguinaria canadendid, has been shown to possess anti-inflammatory properties and used to treat various inflammatory diseases[28]. Recently, sanguinarine has been demonstrated to promote reduced expression of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, two surface molecules crucial for the pathogenesis of inflammatory diseases such as allergic asthma[29].

In conclusion, the present findings shed new light on the molecular mechanism of allergic asthma and have implications for future research. The changes in the viral transcription (RPL3, RPS10, RPL27, RPS11, RPL27A, RPL37A, EIF5A, EIF5B, and EEF1D) and lysosome function (ALAS1, ACO1, GPX3, PGD, VKORC1, and DCXR may be associated with the exacerbation of allergic asthma. This study also provides five small molecular medicines (penbutolol, felbinac, iodixanol, josamycin, and oxolamine) which can mimic the same disease statue and is used to further explore the molecular mechanism of the allergic asthma, and three small molecular medicines (3-nitropropionic acid, scriptaid, and sanguinarine) which have the potential to use in clinic for treatment of allergic asthma in future.

References

1) Vercelli D. Discovering susceptibility genes for asthma and allergy. Nat Rev Immunol 2008; 8: 169-182.
2) Wu H, Romieu I, Shi M, Hancock DB, Li H, Sienra-Monge JJ, Chiu GY, Xu H, del Rio-Navarro BE, London SJ. Evaluation of candidate genes in a genome-wide association study of childhood asthma in mexicans. J Allergy Clin Immunol 2010; 125: 321-327.e313.
3) Docherty SJ, Butcher LM, Schalkwyk LC, Plomin R. Applicability of DNA pools on 500 K SNP microarrays for cost-effective initial screens in genomewide association studies. BMC Genomics 2007; 8: 214.
4) Viding E, Hanscombe KB, Curtis CJ, Davis OS, Meaburn EL, Plomin R. In search of genes associated with risk for psychopathic tendencies in children: A two-stage genome-wide association study of pooled DNA. J Child Psychol Psychiatry 2010; 51: 780-788.
5) Medina I, Montaner D, Bonifaci N, Pujana MA, Carbonell J, Tarraga J, Al-Shahrour F, Dopazo J. Gene set-based analysis of polymorphisms: Finding pathways or biological processes associated to traits in genome-wide association studies. Nucleic Acids Res 2009; 37: W340-344.
6) Kicic A, Hallstrand TS, Sutanto EN, Stevens PT, Kobor MS, Taplin C, Pare PD, Beyer RP, Stick SM, Knight DA. Decreased fibronectin production significantly contributes to dysregulated repair of asthmatic epithelium. Am J Respir Crit Care Med 2010; 181: 889-898.
7) Fujita A, Sato JR, Rodrigues Lde O, Ferreira CE, Sogayar MC. Evaluating different methods of microarray data normalization. BMC Bioinformatics 2006; 7: 469.
8) Szklarczyk D, Franceschini A, Kuhn M, Simonovic M, Roth A, Minguez P, Doerks T, Stark M, Muller J, Bork P, Jensen LJ, von Mering C. The string database in 2011: functional interaction networks of proteins, globally integrated and scored. Nucleic Acids Res 2011; 39: D561-568.
9) Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using david bioinformatics resources. Nat Protoc 2009; 4: 44-57.
10) Berriz GF, Beaver JE, Cenik C, Tasan M, Roth FP. Next generation software for functional trend analysis. Bioinformatics 2009; 25: 3043-3044.
11) Yamaya M. Virus infection-induced bronchial asthma exacerbation. Pulmonary Med 2012; 2012.
12) Busse WW, Lemanske Jr RF, Gern JE. The role of viral respiratory infections in asthma and asthma exacerbations. Lancet 2010; 376: 826.
13) Simon-Nobbe B, Denk U, Pöll V, Rid R, Breitenbach M. The spectrum of fungal allergy. Int Arch Allergy Immunol 2008; 145: 58-86.
14) Yasuda H, Suzuki T, Zayasu K, Ishizuka S, Kubo H, Sasaki T, Nishimura H, Sekizawa K, Yamaya M. Inflammatory and bronchospastic factors in asthma exacerbations caused by upper respiratory tract infections. Tohoku J Exp Med 2005; 207: 109-118.
15) Nadeem A, Chhabra SK, Masood A, Raj HG. Increased oxidative stress and altered levels of antioxidants in asthma. J Allergy Clin Immunol 2003; 111: 72-78.
16) Misso N, Powers K, Gillon R, Stewart G, Thompson P. Reduced platelet glutathione peroxidase activity and serum selenium concentration in atopic asthmatic patients. Clin Exp Allergy 2006; 26: 838-847.
17) Mak JCW, Chan-Yeung MMW. Reactive oxidant species in asthma. Curr Opin Pulm Med 2006; 12: 7-11.

18) Ahuja-Jensen P, Johnsen-Soriano S, Ahuja S, Bosch-Morell F, Sancho-Tello M, Romero FJ, Abrahamson M, Van Veen T. Low glutathione peroxidase in rd1 mouse retina increases oxidative stress and proteases. Neuroreport 2007; 18: 797-801.

19) Jensen GE, Gissel-Nielsen G, Clausen J. Leucocyte glutathione peroxidase activity and selenium level in multiple sclerosis. J Neurol Sci 1980; 48: 61-67.

20) Devereux G, Seaton A. Diet as a risk factor for atopy and asthma. J Allergy Clin Immunol 2005; 115: 1109-1117.

21) Heinrich J. Air pollution, asthma and allergies in children. Occup Environ Med 2010; 67: 290-291.

22) Bošnjak-Petrović V. Drug induced asthma. Acta Clinica 2007; 7: 43-50.

23) Short PM, Williamson PA, Lipworth BJ. Effects of hydrocortisone on acute beta-adrenoceptor blocker and histamine induced bronchoconstriction. Br J Clin Pharmacol 2012; 73: 717-726.

24) Matsuno O, Ueno K, Hayama Y, Honda H, Yamane H, Saeki Y. Deterioration of asthma in a patient with diffuse panbronchiolitis (DPB) after macrolide therapy. J Asthma 2010; 47: 486-488.

25) Goncharova E, Goncharov D, Zhao H, Penn R, Krymskaya V, Panettieri Jr R. beta2-adrenergic receptor agonists modulate human airway smooth muscle cell migration via vasodilator-stimulated phosphoprotein. Am J Resp Cell Mol Biol 2012; 46: 48-54.

26) Singh D, Petavy F, Macdonald AJ, Lazaar AL, O'Connor BJ. The inhaled phosphodiesterase 4 inhibitor GSK256066 reduces allergen challenge responses in asthma. Respir Res 2010; 11: 26

27) Aich J, Mabalirajan U, Ahmad T, Khanna K, Rehman R, Agrawal A, Ghosh B. Resveratrol attenuates experimental allergic asthma in mice by restoring inositol polyphosphate 4 phosphatase (INPP4A). Int Immunopharmacol 2012; 14: 438-443.

28) Xu J, Meng Q, Chong Y, Jiao Y, Zhao L, Rosen E, Fan S. Sanguinarine inhibits growth of human cervical cancer cells through the induction of apoptosis. Oncol Rep 2012; 28: 2264-2270.

29) Niu X, Fan T, Li W, Xing W, Huang H. The anti-inflammatory effects of sanguinarine and its modulation of inflammatory mediators from peritoneal macrophages. Eur J Pharmacol 2012; 689: 262-269.