Exploring the Potential Pathogenic Mechanisms of Asthma Deterioration Based on Modular Drivers

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Asthma is a complicated chronic airway inflammatory disease caused by the interaction of genetic susceptibility and environmental impact. Although biologists have explored the pathogenesis of asthma in various aspects, the exact molecular mechanism continues to be elusive. In this study, we conducted a modular study of asthma-related genes to explore their core pathogenic driving genes. Firstly, the expression profiles of normal, mild to moderate and severe asthma patients were analyzed to screen the differentially expressed genes. Secondly, differential genes of asthma were integrated, co-expressed and clustered into modules. Next, enrichment of GO function and KEGG pathway of module genes were analyzed. Finally, non-coding RNA (ncRNA) and transcription factors that regulate modules are predicted by hypergeometric test. In summary, we have obtained 14 co-expression modules, among which CDCA5, JUNB and other genes are significantly differentially expressed in asthmatic patients, and have an active regulatory role in dysfunction module, so they are recognized as asthma-driving genes. Enrichment results showed that module genes were significantly involved in cell growth, transcription factor activity, cellular response to drugs and the transport of various ions. In addition, they also radically regulate Wnt, TGF-beta, JAK-STAT and extracellular matrix signaling pathways. Finally, we identified significant regulatory dysfunction modules of ncRNA pivot (including miR-181a-5p and let-7d-5p) and TF pivot (including NFKB1, ESR1 and MYC). Overall, our work has uncovered a co-expression network involved in the regulation of core pathogenic genes of asthma. It helps to reveal the core dysfunction modules and potential regulatory factors of this disease, and to enhance our understanding of the molecular mechanisms of asthma-related diseases.

Key Words: Asthma, Differential Analysis, Co-expression Module, Driver Gene, Enrichment Analysis
Tob Regul Sci.™ 2021;7(4-1): 756-764
DOI: doi.org/10.18001/TRS.7.4.1.30

INTRODUCTION

Asthma is a chronic inflammatory airway disease characterized by heterogeneity [1]. In many parts of the world, the incidence and mortality of asthma are increasing, and asthma has become a global health problem [2, 3]. The key symptoms of asthma include cough, wheezing, dyspnea, chest tightness and increased mucus secretion. In addition to the above core symptoms, asthma is usually related to a variety of combined symptoms, including rhinitis, sinusitis, gastroesophageal reflux disease, obstructive sleep apnea, hormone disorders and psychopathology [5]. Studies have demonstrated that genetic, environmental, developmental and host factors and their interactions may contribute to the onset of asthma [6]. In addition, epidemiological studies have shown that asthma is closely associated with respiratory pathogen infections, including common respiratory viruses such as rhinovirus, human respiratory syncytium virus, adenovirus, coronavirus and influenza virus, as well as bacteria (including atypical bacteria) and fungi [7]. In genetics, interleukin-6 (IL6) is an inflammatory

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cytokine, which has been taken into account to play an important role in the pathogenesis of asthma, and IL-6 single nucleotide polymorphism (SNP) is associated with childhood and adult asthma [8]. In addition, 11 key SNPs were associated with eight loci of the recent GWAS genome-wide association study for asthma [9]. At this stage, scientists have succeeded in interpreting asthma from numerous perspectives. Among them, research on asthma has confirmed that lncRNA GAS5 plays an important role in the pathogenesis of asthma by regulating the microRNA-10A/BDNF signaling pathway to promote the proliferation of aortic smooth muscle cells [10]. At the same time, the expressions of microRNA-223-3p, microRNA-142-3p and microRNA-629-3p increased in sputum of patients with severe asthma and were involved in neutrophilic airway inflammation, suggesting that these microRNAs contribute to the inflammatory phenotype of asthma [11]. Circulating microRNAs may be a biomarker of the severity of asthma [12]. On the other hand, microRNA-221 was differentially expressed in asthmatic patients and control subjects, and the blockade of microRNA-221 resulted in a decrease in airway inflammation in OVA-induced asthmatic mice [13]. It is useful to noting that the up-regulation of endogenous microRNA-3162-3p level is accompanied by the decrease of the expression of beta-catenin and beta-catenin, which can effectively reduce the toxicity of endogenous beta-catenin in OVA-induced asthmatic mice, alleviate airway hyperresponsiveness and improve airway inflammation [14]. These findings deepen our understanding of the pathogenesis of asthma and is a primary consideration in further research.

Although a series of studies on asthma has been reported, the overall effect of asthma is still elusive. At present, regardless of the fact that there are available treatments, it is still unable to achieve adequate disease control of asthma to a large extent. In this study, we constructed a co-expression network of differentially expressed genes in patients with asthma by exploring the differentially expressed genes in mild to moderate asthma and severe asthma, and then identified the pathogenic genes and the core regulatory factors of asthma. Based on the analysis of multi-factor interaction network modules, this work not only reveals the relationship between modules and differential genes, but also provides additional guidance for biologists to further study.

RESULT

Differentially Expressed Genes between Asthma

Biologists have carried out many experiments and studies on the process of asthma, and thus identified the probable pathogenic genes of different degrees of asthma. However, the intricate molecular linkages and overall effects of these genes remain unclear. In order to observe the molecular changes in the course of different degrees of asthma, we performed differential expression analysis based on microarray data to identify the differential gene expression (DEG) between moderate and mild asthma and normal samples, as well as between severe asthma and normal samples. Setting P value < 0.05 as a meaningful threshold, we can get the genes that may lead to asthma. Two hundred and forty-five differentially expressed genes (Figure 1A) were generated, of which 1375 were down-regulated and 1120 were up-regulated. There were 3696 differentially expressed genes (Figure 1B) in normal-severe asthma, involving 1819 down-regulated genes and 1877 up-regulated genes. By comparing the two sets of differentially expressed genes (Figure 2), we found that 1079 genes such as UTS2, SLC22A16 were no longer differentially expressed, while 1416 genes such as CLCA1, CPA3 were still differentially expressed. In addition, 2280 genes, such as PHACTR3, KRT6A, TCN1, were added, which may hold the key genes regulating asthma and exacerbating the disease. Targeted regulatory therapy of these genes may effectively control the condition of asthma in order to avoid aggravation.

Figure 1. Volcanic maps of differentially expressed genes in mild and severe asthma. Red node represents up-regulation, blue node represents down-regulation.

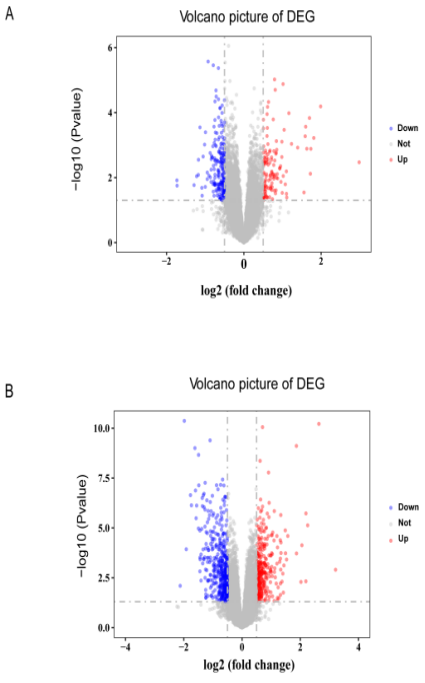
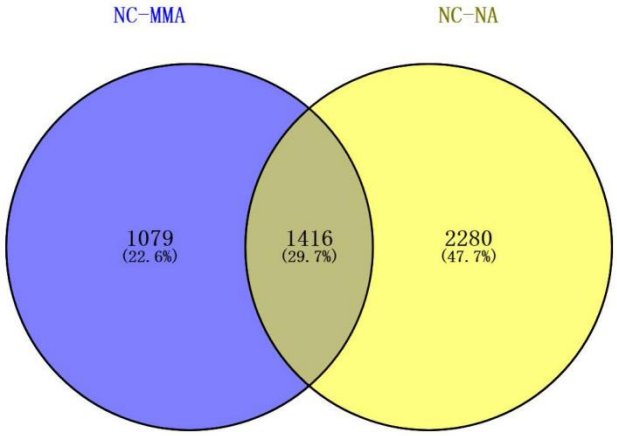


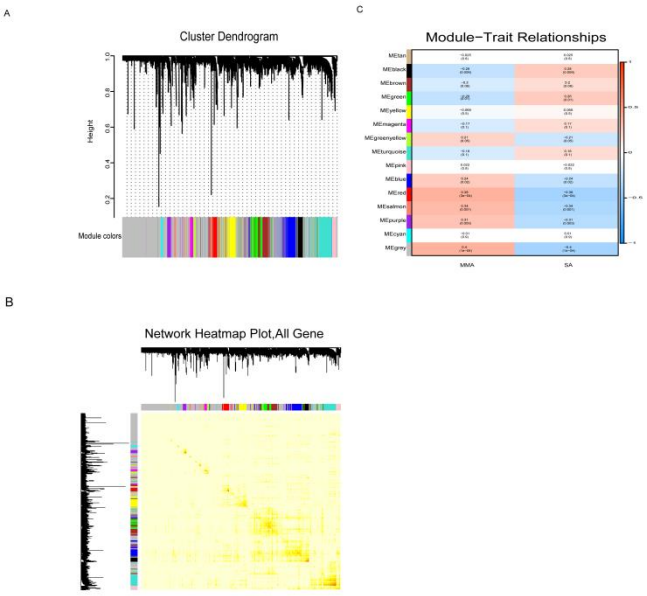
Figure 2. Differential gene comparison between moderate and severe asthma.



Co-expression Analysis of Differential Genes

Firstly, we integrated the differential genes of mild asthma and severe asthma, and got 4775 differential genes. Then, based on weighted gene co-expression network analysis (WGCNA), we observed significant grouping co-expression of these genes in disease samples. Clustering the expression and behavior of asthma in patient samples into modules is helpful for us to observe the complex synergistic relationship between these genes from the perspective of expressive behavior. Therefore, by identifying the co-expression group as an asthma-related module, we obtained 14 co-expression modules (Figure 3A, B). At the same time, core driving genes were further excavated for co-expression module, and core genes such as CDCA5 and JUNB were identified. In addition, according to the correlation analysis between modules and phenotypic data, we can see that MEred is related to MMA (Figure 3C).

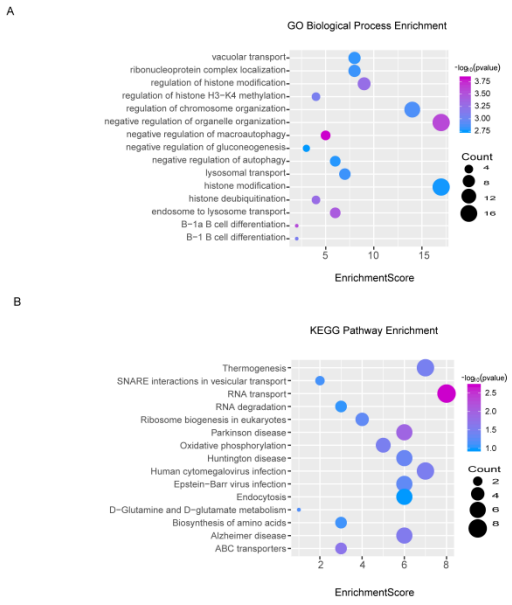
Figure 3. Synergistic expression of asthma differential genes in patient samples. A. Fourteen expression groups were identified as modules, and fourteen colors represented fourteen expression modules. B. The expression thermograms of all genes in the samples were clustered into 14 expression modules. C. Each row represents a module, and each column represents a phenotype. The color of each cell is mapped by the corresponding correlation coefficient. Number from - 1 to 1, color from blue to white, and then to red.



Functional and Pathway Enrichment

Function and pathway are crucial mediators of disease physiological response. Exploring the function and pathway of gene involvement in dysfunctional module is not only helpful to determine the upstream and downstream relationship of the same pathway gene in the module, but also helpful to establish the molecular bridge between module and disease in system biology, and to deepen the understanding of the potential molecular mechanism of disease. Therefore, we analyzed the enrichment of GO function and KEGG pathway in 14 modules, and obtained 31025 biological processes, 3800 cell components, 5760 molecular functions and 1816 KEGG pathways (Figure 4). It was found that these functions are mainly focused on biological processes such as cell growth, transcription factor activity, cellular response to drugs and the transport of various ions. On the other hand, enrichment of KEGG pathway reflects that asthmatic differential genes are mainly involved in Wnt, TGF-beta, JAK-STAT signal, eosinophils and extracellular matrix. These signaling pathways have been shown to be associated with the occurrence and development of asthma.

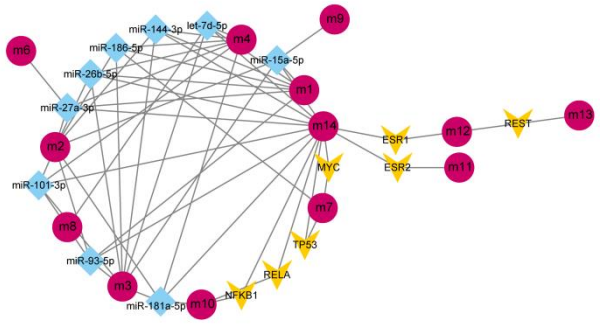
Figure 4. Functional and pathway enrichment analysis of modular genes (excerpts). A. Selection of GO functional enrichment analysis of module genes. From blue to purple, the enrichment increased dramatically. The larger the circle, the larger the proportion of module genes in GO functional entry genes. B. KEGG Pathway Enrichment Analysis of Modular Genes. From blue to purple, the enrichment increased markedly. The larger the circle, the larger the proportion of module genes to KEGG pathway entry genes.



Transcription Factors and Ncrna of Regulatory Dysfunction Module

From the perspective of systems biology and systems genetics, gene transcription and post-transcriptional regulation have been considered as key regulatory factors for the occurrence and progress of diseases. Although many biologists have paid attention to the regulation of a single or several TF and ncRNA on the pathogenesis of asthma, few studies have focused on their overall effects on dysfunction mechanisms and their bridging role in the development of asthma. Therefore, in this study, based on the targeting regulation relationship between TF and ncRNA and module genes, pivot analysis of co-expression modules was conducted to explore the key transcriptional regulators regulating the progression of asthma. The forecast results showed that 753 ncRNAs involved 1061 ncRNA-module regulatory pairs and 50 transcription factors involved 57 TF-module target pairs. In addition, the number of pivot regulatory modules was statistically analyzed, and ncRNA (miR-181a-5p, let-7d-5p, miR-101-3p, etc.) and TF (NFKB1, MYC, RELA, etc.) were the most regulated dysfunctional modules. These transcription factors and ncRNA may regulate the progression of asthma by mediating dysfunction modules. Therefore, we identified these potential regulators as dysfunctional molecules in the progression of asthma deterioration. Finally, by synthesizing nine core ncRNAs and seven core TFs (Figure 5) that drive the asthma co-expression module, we can intuitively see the relationship between the transcription and post-transcriptional regulation of diverse genes in asthma and the modules.

Figure 5. Modular synthesis views of regulated by key ncRNA and transcription factor. The purple circle represents the module, the blue parallelogram represents ncRNA, and the arrow represents the TF of the control module.



DISCUSSION

Asthma is a heterogeneous disease was characterised by variable airflow obstruction and bronchial hyperresponsiveness [15]. In the United States, about 4500 people die of asthma every year, a disease that seriously influences the quality of life of patients [16]. Although research on asthma is summarized in the NCBI database from various aspects, the pathogenesis of its deterioration is still unclear. More importantly, despite the fact that there are currently available treatments, asthma control cannot be fully achieved to a large extent [17]. In this study, we interpret the potential drivers of asthma deterioration from the perspective of co-expression modules, core genes, functional and pathway analysis, and regulatory analysis. At the module level, we first noticed that the genes of 14 modules involved in cell growth regulation, transcription factor activity and neuron death regulation in varying degrees. In addition, the genes of the module are also significantly involved in Wnt, TGF-beta, JAK-STAT signaling, eosinophils and extracellular matrix signaling pathways. The expression of TIPE2 can inhibit the development of asthma through Wnt/beta-catenin signaling pathway [18]. The changes of SMAD signal transduction, the over expression of TGF-beta gene and the change of transcription all lead to the over-secretion of TGF-beta and the increase of its expression in target cells, and induce the course of moderate-severe or severe asthma and the earlier

and faster progress of asthma [19]. At the same time, specific asthma is associated with high levels of Th2 cells, which involve many inflammatory reactions and JAK-STAT signaling pathways [20]. In addition, severe asthma is linked to a marked eosinophilic inflammatory process and often occurs in adolescents. The most severe asthma is involved in neutrophil-derived inflammation encountered in young children or adolescents [21]. In addition, excessive deposition of extracellular matrix (ECM) and airway remodeling of smooth muscle was associated with increased airway responsiveness and asthma severity [22].

On the other hand, at the molecular level, we excavated 14 core genes including JUNB, CDCA5 and NPBWR1 by co-expression analysis. These core genes are not simply differentially expressed, but also play an important regulatory role in dysfunction module. Among them, studies have confirmed that Th2 cells play a major role in the pathogenesis of allergic asthma, while mice with JunB deficient CD4 + T cells exhibit allergen-induced airway inflammation [23]. In addition, the inhibition of CDCA5 knockdown is mediated by the decreased expression of cyclic dependent kinase 1 (CDK1) and cycling B1, and the increased expression of CDCA5 in hepatocellular carcinoma is significantly related to the shortened survival of patients [24]. NPBWR1 is involved in regulation of feeding behavior, energy homeostasis, neuroendocrine function and inflammatory pain [25]. The hypothetical role of CDCA5, NPBWR1 and other core genes in asthma has not been reported. However, our analysis shows that they play an active role in the dysfunction module of regulating asthma deterioration, which can be used as candidates for further molecular experimental verification.

In addition, 753 ncRNAs were expected to participate in the deterioration mechanism of asthma through mediation modules, and their abnormal expression in asthma was verified by differential analysis. According to the statistical analysis, we determined that microRNA-181a-5p had momentous effects on six dysfunctional modules, while let-7d-5p, microRNA-101-3p, microRNA-144-3p and microRNA-15a-5p had significant effects on five modules. MiR-181a-5p promotes the progression of gastric cancer through activation of MAPK signal transduction mediated by RASSF6, and inhibits cell proliferation and migration by targeting Kras in non-small cell lung cancer A549 cells [26, 27]. No conceivable role of miR-181a-5p in asthma has been reported. However, the predicted results of this study clearly indicate that miR-181a-5p, as the ncRNA regulating the most dysfunctional module of asthma, may play a huge potential role in the pathogenesis and regulation of asthma, which can be used as a candidate molecule for further molecular experimental verification. Meanwhile, differentially expressed levels of microRNAs in plasma were found between AE-IPF and S-IPF. The combination of these two microRNAs may be a potential biomarker of AE-IPF from IPF [28]. MiR-101-3p can inhibit the proliferation and metastasis of HCC cells by interacting with LINC00052 to decrease SOX9 [29]. In addition, studies have shown that heme can prolong the regression of self-limiting inflammation, which is linked to the down-regulation of ALX/FPR2 mediated by heme-induced microRNA-144-3p [30]. On the other hand, microRNA-15a-5p, as a regulator of the expression of VEGFA, seriously affects the inflammation and fibrosis of peritoneal mesothelium cells [31]. The effects of ncRNAs on asthma are also found in the studies mentioned above, but our research demonstrates that they can lead to the occurrence and development of asthma, which needs further study. At the same time, other ncRNAs that substantially regulate asthma dysfunction modules may also participate in the basic process of asthma, which needs experimental confirmation. This will facilitate biologists to further explore its supervisory role in asthma.

Finally, we identified 50 transcription factors differentially expressed in varying degrees and considerably regulated asthma dysfunction modules. According to regulatory analysis, NFKB1, MYC, RELA, ESR2 and ESR1 significantly regulate two modules, which may play a significant role in asthma. In asthma experiments, the lungs of mice showed strong eosinophil infiltration, excessive Th2 polarization, significant airway hyperresponsiveness, simplified alveoli, decreased lung compliance and decreased pulmonary angiogenesis. These changes were related to the increased expression of RUNX3, alveolar cell apoptosis and reduced expression of anti-angiogenic factor GAX and pro-angiogenic factor NF-kappa B and VEGFR2 [32]. At the same time, NFKBIA/I-kappa B alpha is the central hub of transcriptional responses to prevalent pulmonary diseases in children, including respiratory syncytium virus infection, asthma, and bronchopulmonary dysplasia, while NFKBIA is the major negative regulator of NF-kappa B [33]. In addition, the application of iPSC-w/oc-Myc not only inhibits Th1 inflammation, but also has a therapeutic effect on systemic allergy and airway hyperresponsiveness. Therefore, transplantation of iPSC-w/oc-Myc may be a possibility way to treat allergic reaction and bronchial asthma [34]. More importantly, the prototype irreducible form of NF-kappa B is a heterodimer composed of NF-kappa B and RelA, both of which belong to the NF-kappa B/Rel family. They assist in immune-mediated diseases such as allograft rejection, rheumatoid arthritis and bronchial asthma [35]. On the other hand, several receptors

(EGFR, EGR1, ESR2, PGR), transcription factors (MYC, JAK), cytokines (IL8, IL6, IL1B), a chemokine (CXCL1), a kinase (SRC) and a cyclooxygenase (PTGS2) are described as being associated with the inflammatory environment and steroid resistance of asthma [36]. Finally, ESR1 gene mutations may affect the development of BHR, especially in female subjects, which may also lead to faster lung function loss in asthmatic patients and female subjects [37]. At the same time, other transcription factors that significantly regulate the dysfunction module of asthma may also participate in the elementary process of asthma, which needs experimental confirmation.

MATERIALS AND METHODS

Data Resources

NCBI Gene Expression Omnibus Database (GEO Dataset) [38] includes a wide range of classification of high-throughput experimental data, including single-channel and dual-channel microarray-based measurements of gene abundance, genomic DNA and protein molecular experimental data. We first collected a set of gene expression profiles of asthma from GEO, the number of which is GSE43696 [39]. The data set included 50 patients with moderate to mild asthma, 30 patients with severe asthma and 20 normal subjects. Then, we screened ncRNA-RNA (protein) interaction pairs with score >= 0.5 from RAID v2.0 database [40], including 431937 interaction pairs involving 5431 ncRNA. Meanwhile, all human transcription factor target data, involving 2492 transcription factors and 9396 interaction pairs, are downloaded and used in TRRUST V2 database [41].

Differential Expression Analysis

Differential expression analysis of gene expression profile data in this study was implemented by R language limma package [42-44]. Firstly, backgroundCorrect function is used for background correction and standardization. Secondly, normal Between Array function quantile normalization method was used to filter out the control probe and the low expression probe. Then, differentially expressed genes in datasets were identified based on lmFit and eBayes functions, and default parameters were utilized.

Co-Expression Analysis

In order to explore the pathogenic potential drivers of asthma deterioration, we analyzed the differences between normal and moderate asthma and severe asthma, and integrated the two results to obtain the differential gene expression profiles of asthma. At the same time, in order to study the co-expression of different genes in asthma, we used weighted gene co-expression network analysis (WGCNA) [45] to analyze the matrix of differential expression profiles in asthma, and to find the co-expression gene module. Firstly, the weighted value of correlation coefficient, i.e. the N power of gene correlation coefficient, is utilized to calculate calculate the correlation coefficient (Person Coefficient) between any two genes. The connection between genes in the network obeys scale-free networks, which make the algorithm more biologically meaningful. Then, a hierarchical clustering tree is built by the correlation coefficient between genes. Different branches of the clustering tree represent separate gene modules, and different colors represent different modules.

Functional and Pathway Enrichment Analysis

Exploring the function and signaling pathway of gene involvement is often advantageous to study the molecular mechanism of diseases. Enrichment of genes in dysfunctional modules is an effective means to explore the potential mechanism of asthma. Therefore, we used R language Cluster Profiler package [46] to enrich and analyze the Go function (pvalueCutoff = 0.01, qvalueCutoff = 0.01) and KEGG pathway (pvalueCutoff = 0.05, qvalueCutoff = 0.2) respectively. Cluster Profiler is a software package of Bioconductor, which can perform statistical analysis and visualization of functional clustering of gene sets or gene clusters.

Pivot Analysis and Prediction Module Transcription Regulator

Gene transcription and post-transcriptional regulation are often driven by non-coding genes (ncRNA) and transcription factors (TF). Therefore, we scientifically predict and test its role in asthma dysfunction module. Pivotal regulators are defined as regulators that have significant regulatory effects on modules during asthma exacerbation, including ncRNA and TF. We require that there are more than two control links between each regulator and each module, and that the significance of the enriched targets in each module based on the hypergeometric test calculation is p value < 0.01.

FUNDING

This work was supported by Outstanding Youth Project of Education Department of Hebei Province(YQ2014008)

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