Allergy Asthma Immunol Res. 2020 Jul;12(4):626-640
https://doi.org/10.4168/aair.2020.12.4.626
pISSN 2092-7355·eISSN 2092-7363

Allergy, Asthma & Immunology Research AAIR

Original Article

Genetic Signatures of Acute Asthma Exacerbation Related With Ineffective Response to Corticosteroid

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Received: Oct 15, 2019
Revised: Feb 17, 2020
Accepted: Feb 18, 2020

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ABSTRACT

Purpose: Acute exacerbation (AE) is an important domain of asthma management and may be related with ineffective response to corticosteroid. This study aimed to find mechanisms of AE using genome-wide gene expression profiles of blood cells from asthmatics and its perturbation by *in vitro* dexamethasone (Dex)-treatment.
Methods: We utilized lymphoblastoid B cells from 107 childhood asthmatics and peripheral blood mononuclear cells from 29 adult asthmatics who were treated with inhaled corticosteroids. We searched for a preserved co-expression gene module significantly associated with the AE rate in both cohorts and measured expression changes of genes belong to this module after Dex-treatment.
Results: We identified a preserved module composed of 77 genes. Among them, expressions of 2 genes (*EIF2AK2* and *NOL11*) decreased significantly after Dex-treatment in both cohorts. *EIF2AK2*, a key gene acting antiviral defense mechanism, showed significantly higher expressions in asthmatics with AE. The protein repair pathway was enriched significantly in 64 genes which belong to the preserved module but showed no expression differences after Dex-treatment in both cohorts. Among them, *MSRA* and *MSRB2* may play key roles by controlling oxidative stress.
Conclusions: Many genes belong to the AE rate-associated and preserved module identified in blood cells from childhood and adults asthmatics showed no expression changes after *in vitro* Dex-treatment. These findings suggest that we may need alternative treatment options to corticosteroids to prevent AE. *EIF2AK2*, *MSRA* and *MSRB2* expressions on blood cells may help us select AE-susceptible asthmatics and adjust treatments to prevent AE.

Keywords: Asthma; blood; symptom flare up; gene expression; steroids

INTRODUCTION

Acute exacerbation (AE) of asthma can be defined as worsening of symptoms requiring a use of systemic corticosteroids to prevent serious outcomes.[1] It was reported that about

Disclosure

There are no financial or other issues that might lead to conflict of interest.

15% of patients on the Global Initiative for Asthma step 3 treatment (low-dose ICS and long-acting beta2 agonist combination)[2] experienced AE for 1 year, and this frequency increased as asthma became severe.[3] Treatment compliance, genetic background, and various environmental factors, such as viral infection, smoking, and air pollution, are known to be important factors contributing to AE.[4-9]

A cell line model combined with genome-wide gene expression analysis was proposed to be a useful tool to understand drug response *in vitro*.[10,11] In asthma, previous reports showed that profiling of genes expressed in peripheral blood mononuclear cells (PBMCs) could predict corticosteroid responses.[12,13] In addition, genome-wide gene expression profiling of *in vitro* drug perturbations has proven to be useful for drug discovery and elucidation of drug mechanisms.[14,15]

In this study, we hypothesized that ineffective response to ICSs, a mainstay treatment of asthma, might be related to AE. To find out mechanisms underlying this ineffectiveness, we first investigated co-expressed gene modules associated with the AE rate using genome-wide gene expression profiles on blood cells from childhood and adult asthmatics treated with ICSs. Then we measured expression changes of the genes belong to the identified module after *in vitro* dexamethasone (Dex)-treatment to search transcriptomic signatures associated with AE via modifying corticosteroid responses.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board of the corresponding institution and informed consent forms were obtained from all study participants (Brigham and Women's Hospital [2002-P-00331/41] and the Seoul National University Hospital [H-1408-051-601]). The overall study design is presented in **Fig. 1**.

Study populations

Patients with asthma from 2 independent cohorts were enrolled. The first cohort consisted of non-Hispanic white children randomized to budesonide treatment in the Childhood Asthma Management Program trial.[16] They were treated by low-dose ICSs and followed for a mean of 4.3 years. The second cohort was drawn from adult asthmatics at Seoul National University Hospital, Seoul, Korea. They were treated by low, medium or high doses (based on the Global Initiative for Asthma guideline[2]) of ICS plus long-acting $beta_2$ agonist combinations to achieve asthma control and followed for 1 year and longer. The 2 analyzed cohorts differed in a variety of characteristics, including age, sex, race, lung function at baseline, ICS dose, and exacerbation rate. The definition of AE included a use of systemic corticosteroids for at least 3 days, or a hospitalization or emergency room visit due to symptoms aggravation.[17] As follow-up periods of participants were different, the number of AE per year (AE rate) was calculated for the comparison.

Gene expression arrays

All blood samples were drawn from participants in a stable state of asthma. As previously described[18] lymphoblastoid B cell lines (LCLs) derived from childhood asthmatics were cultured in RPMI 1640 medium (Sham-treated LCLs) and treated with Dex (10^{-6} M) for 6 hours (Dex-treated LCLs). PBMCs from adult asthmatics were cultured in the same conditions, that is, RPMI 1640 medium for 6 hours (Sham-treated PBMCs) and Dex (10^{-6} M)

https://doi.org/10.4168/aair.2020.12.4.626

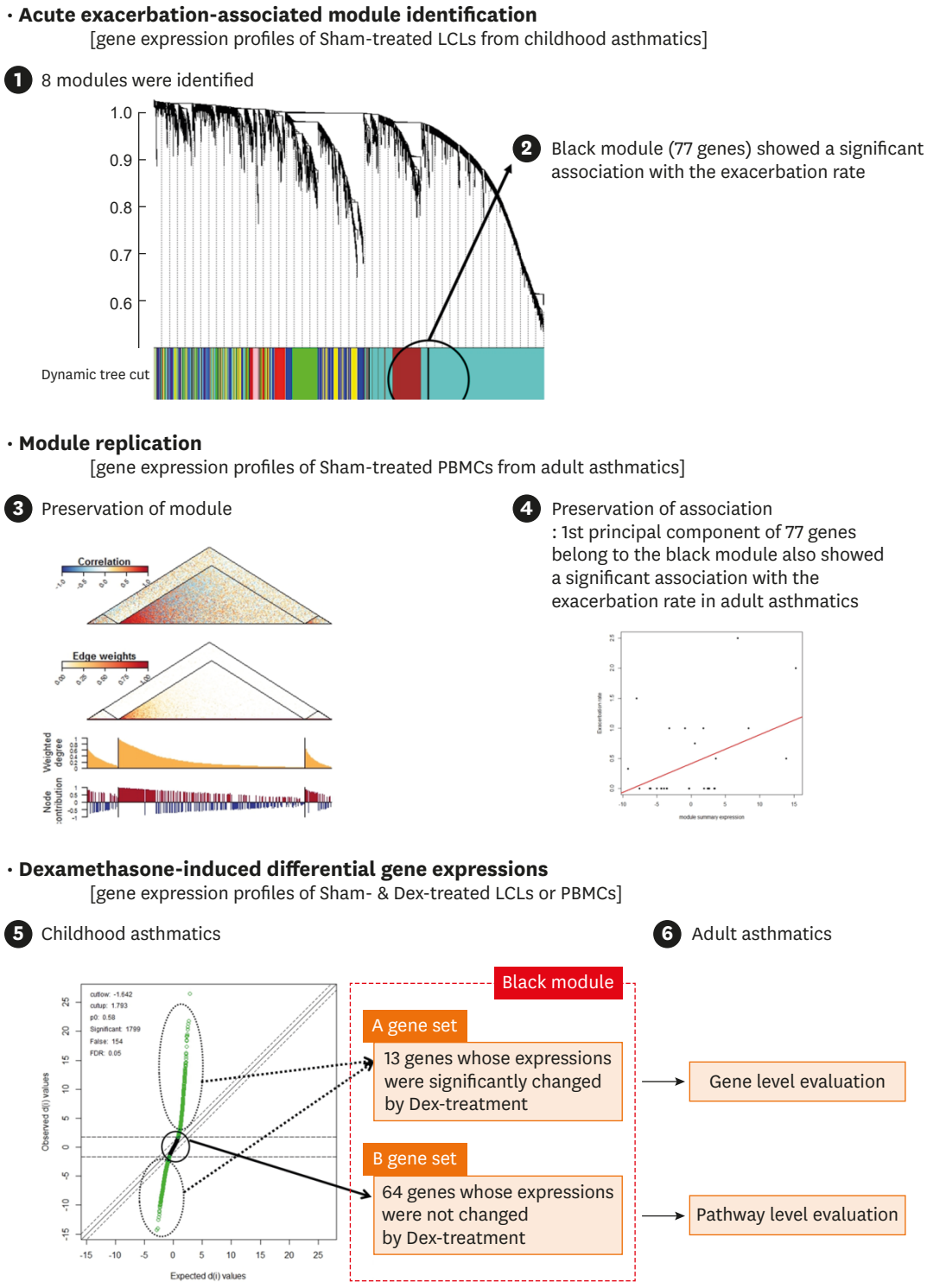

Fig. 1. Overall study design.
LCL, lymphoblastoid B cell line; PBMC, peripheral blood mononuclear cell; Dex, dexamethasone.

for 6 hours (Dex-treated PBMCs). Gene expression levels were measured using the Illumina HumanRef8 v2 BeadChip (Illumina, San Diego, CA, USA) for childhood asthmatics and the Affymetrix GeneChip Human Gene 2.0 ST (Affymetrix, Santa Clara, CA, US) for adult

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asthmatics. We removed probes with bad chromosome annotation, and probes in X or Y chromosome. We then did variance stabilizing transformation and quantile normalization respectively to reduce the effects of technical noises and to make the distribution of expression level for each array closer to normal distribution. We guessed that Sham-treated LCLs from childhood asthmatics or Sham-treated PBMCs from adult asthmatics might represent intrinsic genetic traits of participants and *in vitro* perturbation by Dex might give us an insight to understand responses to corticosteroid. We attempted to search genes or gene modules associated with AEs using both transcriptomic datasets. The expressions of genes of interest were relatively compared using raw intensity values and validated using real-time polymerase chain reaction (PCR).

Statistical analysis

To allow comparability of data from different microarray platforms, we first calculated the mean expression value of each gene in 2 expression profiles of Sham-treated LCLs and PBMCs after collapsing probes by genes. We then correlated the mean expression level of genes between 2 expression profiles and selected top-5,000 genes in common with the highest correlation.

Using these 5,000 genes in gene expression profiles of Sham-treated LCLs from childhood asthmatics, we first performed weighted gene co-expression network analysis with the R package "WGCNA" (R Foundation, Vienna, Austria).[19] Modules were defined as groups of highly interconnected genes.[19] We computed eigengene values of modules identified and performed multivariate linear regression analysis adjusted by baseline age, gender, atopy, forced expiratory volume in 1 second (FEV1) % predicted value and FEV1 over forced vital capacity (FVC) ratio to find a module whose eigengene value was significantly associated with the AE rate. We then checked whether modules identified in gene expression profiles of Sham-treated LCLs from childhood asthmatics were replicated in gene expression profiles of Sham-treated PBMCs from adult asthmatics. Replication was assessed in 2 ways: preservation of module (the consistency of network module structure across gene expression profiles) and preservation of association (association between corresponding eigengene values and the AE rate).[20,21]

Module preservation was measured by the R package "NetRep" (R Foundation).[22] NetRep can quantify preservation of gene co-expression modules across different datasets and produce unbiased *P* values based on a permutation approach to score module preservation without assuming data are normally distributed.[22] To check the preservation of association, we calculate the first principal component of expressions of genes belong to the preserved module in adult asthmatics and performed multivariate linear regression analysis adjusted by age, gender, atopy, FEV1% predicted value, FEV1/FVC ratio and dose of ICSs to check an association between the first calculated principal component and the AE rate. We called the module which satisfied the replication criteria, the AE-associated common gene module.

We next investigated the effects of *in vitro* perturbation by Dex-treatment on gene expressions of the AE-associated common module. We evaluated differential expression patterns of genes belonging to the AE-associated common module using gene expression profiles of Sham- and Dex-treated LCLs and PBMCs. A significant change in gene expression was defined when the false discovery rate (FDR) *P* value was lower than 0.05.

Finally, to assign biological meaning to interpretability of the gene module identified, we performed pathway enrichment analyses using the web interface of ConsensusPathDB (http://

cpdb.molgen.mpg.de) which is a meta-database that integrates different types of functional interactions from heterogeneous interaction data resources.[23]

RESULTS

Module construction and association

Table 1 summarizes the characteristics of 107 childhood and 29 adult asthmatics enrolled. Applying WGCNA to the 5,000 genes in gene expression profiles of Sham-treated LCLs from childhood asthmatics, we identified 8 modules of various sizes ranging from 71 in the pink module to 2,266 genes in the turquoise module (**Fig. 1**). A total of 16 genes could not be assigned to a module as a membership gene and were grouped into the grey module which was not considered for further analysis. To emphasize the impact of strong correlations over weak ones in the network construction, we chose an empirical soft threshold of 6, representing a strong model fit for scale-free topology ($R^2 > 0.97$, **Supplementary Fig. S1**). Eigengene values of the black module of 77 genes showed a significant association with the AE rate in multivariate linear regression analysis ($P = 0.04$) (**Fig. 2A**). Among the 8 modules identified in childhood asthmatics, 2 modules (yellow and black modules) were significantly preserved in the adult asthmatics (**Fig. 3**). Module preservation statistics and *P* values are presented in **Supplementary Table S1**. In addition, multivariate linear regression analysis showed that the first principal component value of the genes belonging to the black module were also significantly associated with the AE rate in adult asthmatics ($P = 0.03$) (**Fig. 2B**). Given that it was associated and preserved with AE in both childhood and adult asthmatics, we thus defined the black module as the AE-associated common gene module.

Corticosteroid mediated genes within the common module

We then identified the subsets of 1,799 genes differentially expressed between Sham- and Dex-treated LCLs and of 1,154 genes between Sham- and Dex-treated PBMCs (FDR *P* value < 0.05, **Supplementary Fig. S2**). Among the 77 genes belonging to the AE-associated common gene module, 13 were also differentially expressed between Dex and Sham in LCLs and were categorized as the A gene set (**Table 2**). The other 64 genes in the black

Table 1. Characteristics of the asthmatics enrolled

Characteristic	Childhood asthmatics* (n = 107)	Adult asthmatics† (n = 29)
Gender, male	64 (59.9)	10 (34.5)
Age (yr)	8.6 ± 2.2	56.7 ± 11.1
Ethnicity		
Non-Hispanic white	107 (100)	0 (0)
Asian	0 (0)	29 (100)
Atopy, Yes (%)	94 (87.8)	15 (51.7)
FEV1 (mL)	1,622.9 ± 463.3	1,917.6 ± 742.2
FEV1 (%pred)	95.2 ± 14.9	78.3 ± 21.6
FEV1/FVC ratio	79.3 ± 8.8	68.1 ± 1.8
Medication		
Low dose‡ ICS	0 (0)	5 (17.2)
Medium dose‡ ICS	107 (100)	12 (41.4)
High dose‡ ICS	0 (0)	12 (41.4)
Exacerbation, yes	74 (69.2)	11 (37.9)

Values are presented as number (%) or mean ± standard deviation.
CAMP, Childhood Asthma Management Program; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; ICS, inhaled corticosteroid; %pred, % predicted value.
*Characteristics measured at enrollment of the CAMP; †Characteristics measured at enrollment of the present study; ‡Based on the Global Initiative for Asthma guideline.[2]

https://doi.org/10.4168/aair.2020.12.4.626

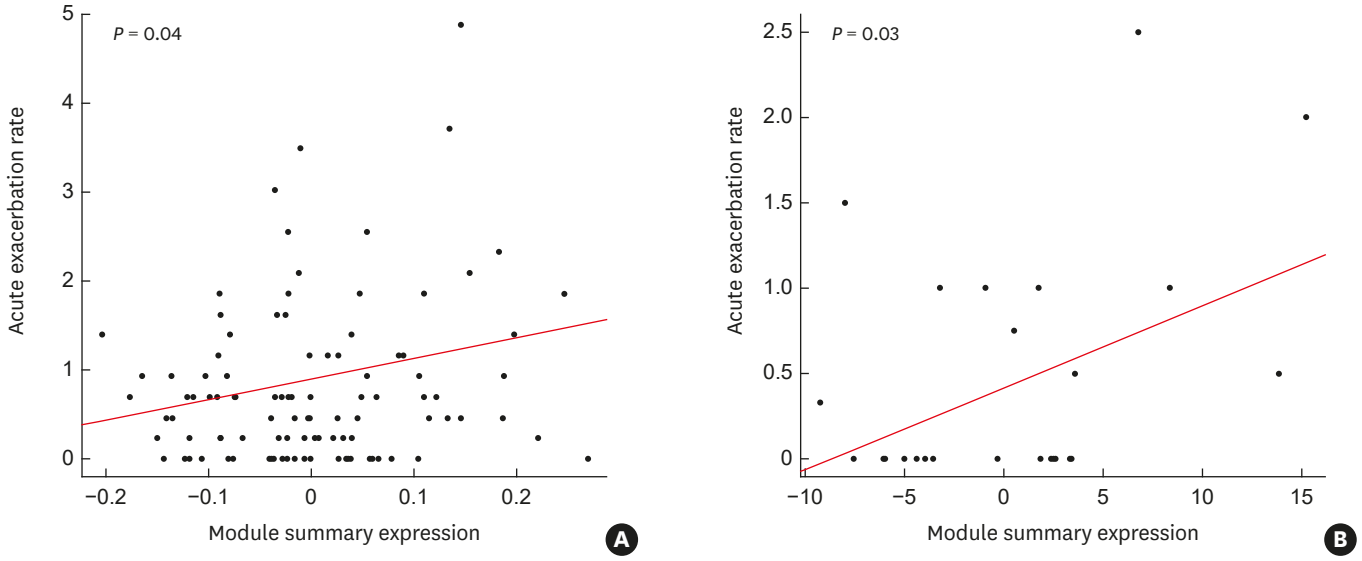

Fig. 2. Correlations between the eigengene value of the preserved gene module (black module) and the AE rate. (A) Childhood asthmatics. (B) Adult asthmatics. Both *P* values were adjusted ones.
AE, acute exacerbation.

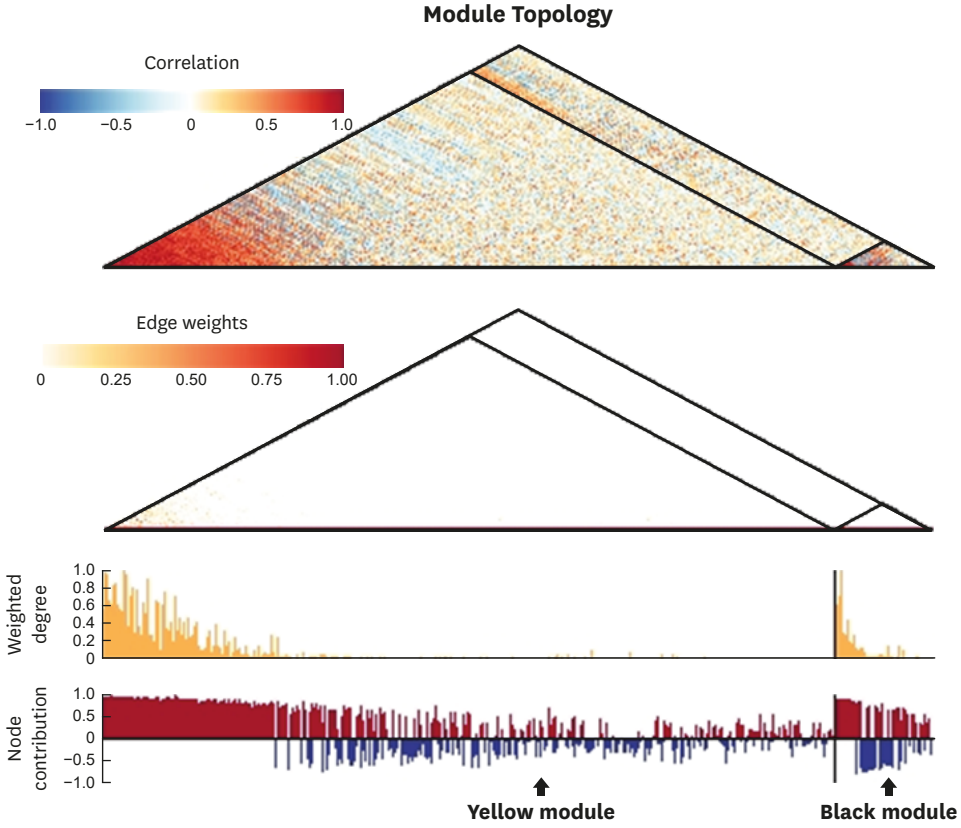

Fig. 3. Preservation of gene modules identified in LCLs from childhood asthmatics in PBMCs from adult asthmatics. The first (top) panel shows a heatmap of pair-wise correlations among the genes comprising the turquoise, magenta, and purple modules. The second panel shows a heatmap of the edge weights (connections) among the genes comprising the 3 modules. The third panel shows the distribution of scaled weight degrees (relative connectedness) among the genes comprising the 3 modules. The fourth panel shows the distribution of node contributions (correlation to module eigengene) among the genes comprising the 3 modules. Genes are ordered from left to right based on their weighted degree in the discovery cohort so as to highlight the consistency of the network properties in the replication cohort.
LCL, lymphoblastoid B cell line; PBMC, peripheral blood mononuclear cell.

https://doi.org/10.4168/aair.2020.12.4.626

module were classified into the B gene set (**Table 2**). Among 13 genes belonging to the A gene set, 2 also showed significant differential expression in PBMCs from adult asthmatics (**Table 2**). As shown in **Fig. 4**, expressions of eukaryotic translation initiation factor 2-alpha kinase 2 (*EIF2AK2*) in Sham-treated LCLs and PBMCs were significantly higher in asthmatics with AE compared to those without AE. These changes were validated using real-time PCR (**Supplementary Fig. S3**). Meanwhile, the 59 genes belonging to the B gene set showed no significant differential expression changes in PBMCs (**Table 2**). As shown in **Fig. 5**, expressions of methionine sulfoxide reductase A (*MSRA*) and methionine sulfoxide reductase B2 (*MSRB2*) of Sham-treated LCLs and PBMCs were significantly lower in asthmatics with AE compared to those without AE and Dex-treatment showed no significant expression changes in *MSRA* and *MSRB2*. These changes were validated using real-time PCR (**Supplementary Fig. S3**). The structures of the AE-associated common gene module based on expression correlations (co-expression networks) showed different connections between Sham-treated and Dex-treated LCLs from childhood asthmatics (**Supplementary Fig. S4**).

Table 2. Genes belonging to the acute exacerbation-associated common gene module and their expression differences between Sham- and Dex-treated blood cells

Gene	LCLs			PBMCs		
	Fold change*	Raw *P* value	Adjusted *P* value	Fold Change*	Raw *P* value	Adjusted *P* value
A gene set						
CALD1	1.060933149	1.4996E-20	**6.46378E-19**	1.0554004	0.759530296	0.909317997
CPOX	0.97263836	8.03415E-20	**3.11401E-18**	0.971923423	0.997767819	0.99919353
EIF2AK2	0.973333718	1.13199E-09	**1.58335E-08**	0.96965243	0.001204344	**0.01062032**
NEXN	0.966314429	1.71367E-08	**1.98112E-07**	0.967349306	0.936257622	0.977318445
NOL11	0.984302863	5.61941E-08	**5.9654E-07**	0.983734232	0.005400716	**0.035069586**
OXR1	0.981110354	1.71286E-07	**1.70603E-06**	0.980770783	0.673246792	0.865133375
DDX5	0.98299549	1.11695E-06	**9.78067E-06**	0.981860055	0.971922243	0.989008159
CEP290	0.984639854	2.44887E-05	**0.000166364**	0.980545301	0.011496491	0.06275377
GLT1D1	1.015204775	0.000255983	**0.001400346**	1.019586373	0.018962813	0.091874096
ZBED2	0.980105373	0.000455575	**0.002331498**	0.980723854	0.143664468	0.375691599
AMPH	1.004154065	0.000587485	**0.002911225**	1.004043207	0.120804068	0.338008025
MAP4K5	0.990445124	0.005850812	**0.02147875**	0.990246636	0.218321105	0.487759396
ST3GAL6	1.020664765	0.011757524	**0.0393755**	1.018811631	0.233623817	0.50699613
B gene set						
GLIS3	1.002342887	0.020643971	0.063637395	1.001197059	0.489962645	0.752795396
VPS72	0.99014686	0.036891865	0.104214308	0.990337803	0.314070853	0.597594631
PROK2	1.003166612	0.062428712	0.15852898	1.004287449	0.284094187	0.5648485
SSTR2	0.995620448	0.212456166	0.39624636	0.998636211	0.865144341	0.952058192
TM7SF2	0.991787778	0.213456324	0.397358444	0.994150099	0.660230898	0.859004551
FARS2	1.00431458	0.220293714	0.406719043	1.009103699	0.292267408	0.572175819
SPINK1	0.998768344	0.251495484	0.445124999	1.00021141	0.900256072	0.966977521
CDKN2B	0.998517994	0.253426808	0.446645767	0.999879523	0.96177239	0.985920656
DMTF1	1.006813917	0.271343402	0.466065617	1.008507968	0.541612206	0.789481043
MTAP	0.995104477	0.300381439	0.501471517	0.993046445	0.434041635	0.711203311
NPC2	1.004223832	0.31879817	0.523536915	1.004515485	0.611714403	0.830907909
TTC14	0.995484106	0.323569317	0.528708035	0.999535927	0.961912344	0.985920656
HIST1H4L	1.001237368	0.325956314	0.531046455	1.000701274	0.806025468	0.932509886
CACHD1	0.999078871	0.349467421	0.557008959	0.998805257	0.560437993	0.800982122
KIAA1524	0.99391181	0.362450994	0.570849805	0.993662723	0.609585907	0.830498512
MSRA	1.002891578	0.376649213	0.584859026	1.004743979	0.471966844	0.740921263
RPL28	1.00408898	0.378247216	0.586248011	1.004284506	0.616847859	0.833101437
RAB8B	0.997950857	0.389600526	0.596814532	0.996016675	0.404928403	0.684108444
NET1	1.002528311	0.399001733	0.605281755	1.001790531	0.758602049	0.909094866
NCOA6	1.002579232	0.441215777	0.641835735	1.003610076	0.629324162	0.838875183
DDX23	0.996413565	0.453040382	0.65298412	0.997614799	0.798379528	0.930573811
MSRB2	1.002896633	0.471116279	0.6690092	1.004005109	0.588816986	0.819166648
SPG21	0.997844152	0.475926594	0.673926075	0.995116221	0.468010928	0.738141808
ADAM23	0.996411727	0.492363414	0.686890923	0.99792013	0.804792845	0.93250926

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Table 2. (Continued) Genes belonging to the acute exacerbation-associated common gene module and their expression differences between Sham- and Dex-treated blood cells

Gene	LCLs			PBMCs		
	Fold change*	Raw *P* value	Adjusted *P* value	Fold Change*	Raw *P* value	Adjusted *P* value
ANK3	0.99896128	0.497331308	0.690648682	0.998149839	0.624036142	0.835352718
SLAMF8	1.000774252	0.500059469	0.692565882	0.999939814	0.975658576	0.990717482
EPC1	1.003662756	0.505954367	0.697321437	1.004501741	0.000784188	**0.007540268**
AHSA2	1.00585843	0.512405774	0.702888578	1.005613901	3.30E-05	**0.000555592**
INPP4B	0.997864074	0.54511216	0.731497799	1.000276169	0.004916367	**0.032583738**
MARVELD1	1.002603031	0.58430186	0.762398043	1.007447365	0.487930588	0.750893488
DNAH1	0.998768006	0.620516116	0.785463438	0.999086443	0.87040701	0.953766174
ERGIC1	1.002724993	0.632600708	0.793528234	1.00305354	0.816898955	0.937240656
RNASE1	1.001069693	0.63871757	0.798197414	1.001927988	0.657167036	0.857591728
RFX3	1.001661611	0.660370112	0.815575817	1.005170608	0.513437752	0.772424741
NKTR	1.002763795	0.687023254	0.83510105	1.000038788	4.39E-06	**9.62E-05**
PTBP2	1.001724458	0.703431462	0.848939732	0.999546231	0.955500677	0.983326779
OTUD4	1.001451825	0.725614327	0.860299311	0.999887116	0.989306706	0.996481372
OR56B1	0.999210704	0.745311391	0.871709229	1.000304066	0.956188543	0.98368206
PYGL	0.998878481	0.746741007	0.872972886	0.994452447	0.447354347	0.720787851
OR9A4	0.999394631	0.757012646	0.879461511	1.001475646	0.725773418	0.895048917
SERPINA1	1.000814698	0.762283405	0.880726347	1.001109082	0.824383314	0.942152359
NDUFA6	0.999065729	0.768535257	0.885817493	0.999029997	0.892314521	0.964039025
ATP6V1F	1.001440687	0.768775807	0.885890535	1.005957674	0.514667305	0.773278005
XPO1	0.998681558	0.772165242	0.887342268	0.995757109	0.628965426	0.838844259
TAF5L	1.002041816	0.775331753	0.889345897	1.008763354	0.546022324	0.79219529
CCL7	0.999276412	0.81666118	0.913286938	0.999107069	0.892829246	0.964178451
SFTPA2	0.999001319	0.828512118	0.919294688	1.002608616	0.801033129	0.931650534
PDE4DIP	0.999141373	0.82980626	0.91976819	1.000431229	0.961329739	0.985920656
CCDC14	1.000944199	0.831442751	0.920552205	1.000482159	0.957705998	0.984578458
SIPA1L3	0.999499003	0.835294575	0.922603288	0.999017432	0.844384083	0.947833478
MET	0.999554418	0.837011042	0.923647844	0.994889359	0.251389109	0.529359856
SELPLG	1.000835416	0.837194406	0.923647844	1.003529221	0.692652173	0.877600964
FCRL6	0.99915329	0.838797803	0.92440001	1.004774311	0.596940775	0.823623363
ITGB3	0.999627734	0.903512166	0.956151883	0.99603341	0.557545291	0.798677856
PPP3R1	0.998967761	0.913756019	0.962335991	1.002598969	0.902742594	0.96798197
NRIP3	1.000305095	0.938212507	0.974216306	1.000677275	0.000936725	**0.008689469**
SCFD2	1.000312055	0.93725572	0.974216306	1.002921629	0.747597418	0.904951305
CFH	1.000211059	0.943238885	0.976033615	1.002317965	0.703939041	0.884432298
CBX5	0.999762656	0.945996598	0.977064002	1.00211238	0.770255015	0.914140773
SIRPB1	0.999926251	0.957501043	0.982316839	1.001755353	0.523813854	0.778357992
SDC2	1.000136335	0.961597461	0.983832066	1.002481329	0.706725057	0.886207735
RAD17	1.000211856	0.967764869	0.985256299	0.99988785	0.992496141	0.99695365
PPBP	0.999898874	0.978076571	0.98935522	1.00380721	0.639730598	0.845757004
UTS2	1.000023252	0.994875001	0.996691549	1.001393036	0.847260891	0.948765239

Bold denotes adjusted *P* value less than 0.05.
A set, genes showing significant differential expressions between Sham- and Dex-treated LCLs; B set, genes showing insignificant differential expressions between Sham- and Dex-treated LCLs; Dex, dexamethasone; LCL, lymphoblastoid B cell line; PBMC, peripheral blood mononuclear cell.
*Log2 fold changes.

Pathway analysis

Pathway enrichment analyses using whole genes belonging to the AE-associated common gene module identified 3 Reactome biological pathways; Protein repair, Syndecan interactions and HATs acetylate histones (**Table 3**). All these 3 pathways were also identified in enrichment analysis using genes belonging to the B gene set. However, genes belonging to the A gene set provided no enriched biological pathway.

https://doi.org/10.4168/aair.2020.12.4.626

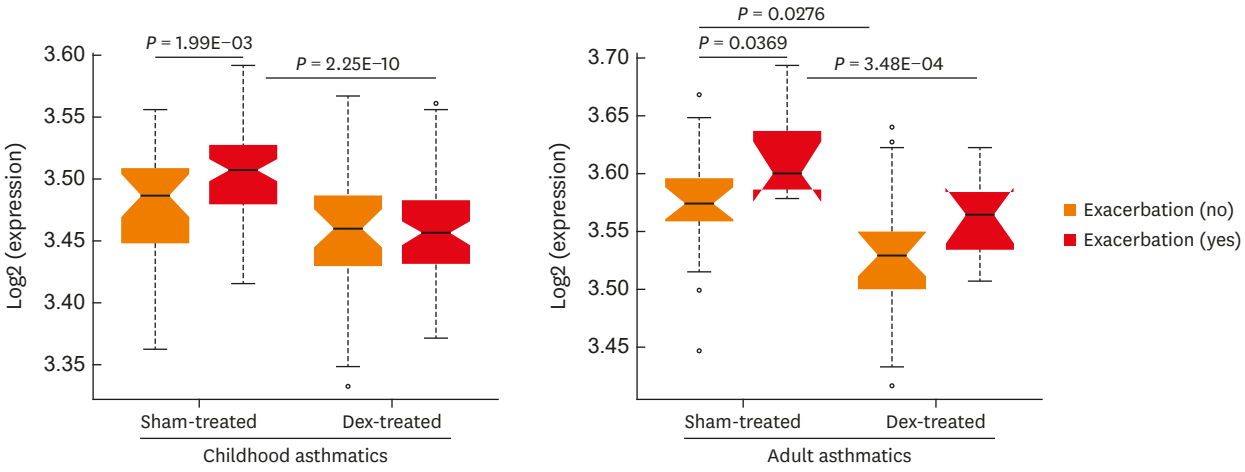

Fig. 4. Eukaryotic translation initiation factor 2-alpha kinase (*EIF2AK2*) expressions in lymphoblastoid cell lines form childhood asthmatics and peripheral blood mononuclear cells from adult asthmatics.
Dex, dexamethasone.

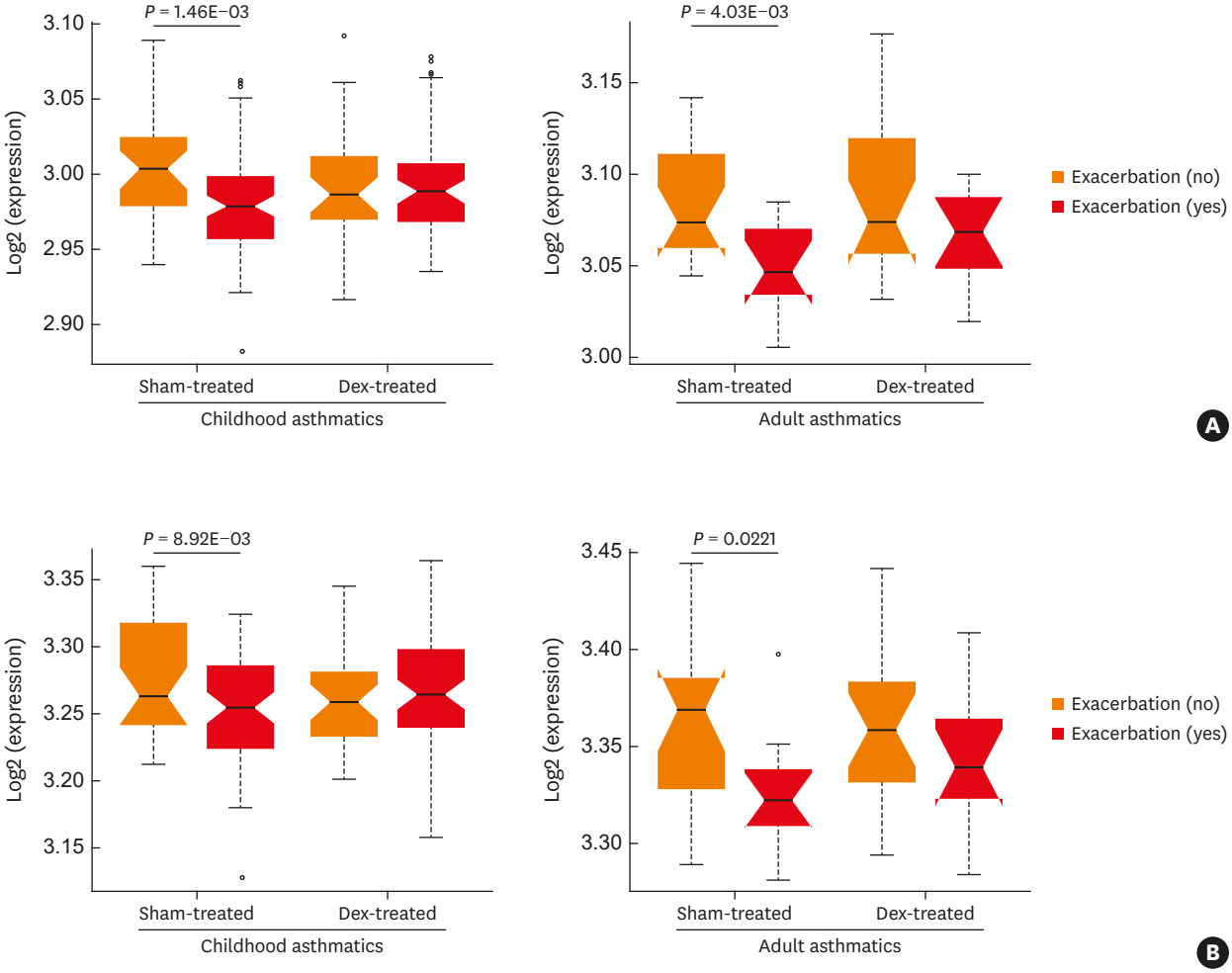

Fig. 5. Methionine sulfoxide reductase A (*MSRA*) and methionine sulfoxide reductase B2 (*MSRB2*) expressions in lymphoblastoid cell lines form childhood asthmatics and peripheral blood mononuclear cells from adult asthmatics. (A) *MSRA* gene expression. (B) *MSRB2* gene expression.
Dex, dexamethasone.

https://doi.org/10.4168/aair.2020.12.4.626

Table 3. Biological pathways enriched in the AE-associated common gene module (black module)

Pathway	Reactome_ID*	Raw *P* value	FDR *P* value†	Overlapped genes
Whole genes				
Protein repair	R-HSA-5676934	0.000285721	0.025429145	*MSRB2*; *MSRA*
Syndecan interactions	R-HSA-3000170	0.003477859	0.105783533	*ITGB3*; *SDC2*
HATs acetylate histones	R-HSA-3214847	0.003565737	0.105783533	*HIST1H4L*; *TAF5L*; *EPC1*; *VPS72*
Genes belong to B set				
Protein repair	R-HSA-5676934	0.000202316	0.015780612	*MSRB2*; *MSRA*
HATs acetylate histones	R-HSA-3214847	0.001895864	0.064446115	*HIST1H4L*; *TAF5L*; *EPC1*; *VPS72*
Syndecan interactions	R-HSA-3000170	0.002478697	0.064446115	*ITGB3*; *SDC2*
Peptide ligand-binding receptors	R-HSA-375276	0.00580197	0.113138409	*UTS2*; *PPBP*; *SSTR2*; *PROK2*
Transcriptional regulation by E2F6	R-HSA-8953750	0.007491007	0.116859712	*CBX5*; *EPC1*

Only depths 1–3 were presented.
AE, acute exacerbation; FDR, false discovery rate; HAT, histone acetyltransferase.
*Gene ontology biological pathway; †Benjamini-Hochberg FDR *P* value.

DISCUSSION

We searched for co-expressed gene modules associated with the AE rate using LCLs from childhood asthmatics and PBMCs from adult asthmatics. AE is a distinct domain of asthma management[2] and thus, is one of important target phenotypes of asthma pharmacogenomics studies.[5] As a result, we identified a gene module consisting of 77 genes showing significant associations with the AE rate in Sham-treated LCLs from childhood asthmatics and found that structures of this gene module were significantly preserved in gene expression profiles of Sham-treated PBMCs from adult asthmatics. In addition, we confirmed that this gene module also showed significant associations with the AE rate in adult asthmatics. We thus called this gene module "AE-associated common gene module".

Among the 77 genes belonging to the AE-associated common gene module, 13 genes showed significant changes in expressions between Sham- and Dex-treated LCLs derived from the pediatric cohort. Gene expression profiling of *in vitro* drug perturbations is useful for many biomedical discovery applications including drug repurposing. We assumed that differential connections in the AE-associated common gene module between Sham-treated and Dex-treated LCLs might help us see a genomic whole picture of acute asthma exacerbation related with ineffective response to corticosteroid.[24]

Since no biological pathway was enriched in this gene set, we focused on the individual genes for further analysis and found that 2 genes *EIF2AK2* and nucleolar protein 11 (*NOL11*) showed significant decreases in expressions after Dex-treatment in both childhood and adult asthmatics. *EIF2AK2*, also called the protein kinase R, is an interferon-inducible double-stranded RNA protein kinase with multiple effects in cells.[25,26] *EIF2AK2* actively contributes to the cellular response to numerous types of stress and plays a critical role in the antiviral defense mechanism of the host induced by interferons.[26,27] Expressions of *EIF2AK2* in Sham-treated LCLs and PBMCs were significantly higher in asthmatics with AE compared to those without AE (**Fig. 4**). As *EIF2AK2* expression is activated by virus infection as a host viral defense mechanism[28] and its increase was found in respiratory virus-infected airway epithelium even from subjects without respiratory illness,[29] our observation did not seem to be an etiology but a consequence of AE. Interestingly, corticosteroid-treatment significantly decreased *EIF2AK2* expression in both childhood and adult asthmatics with AE (**Fig. 4**). A previous report showed that mice given intranasal treatment with corticosteroids prior to influenza A virus infection developed more severe disease associated with amplified virus

replication.[30] Based on these findings, increased *EIF2AK2* expressions in blood cells from asthmatics may reflect previous AE. Although a confirmatory study is required, physicians would be cautious to prescribe corticosteroids when viral infection is suspected to be a cause of AE if patients have genetic variations affecting *EIF2AK2* expression.

Among 77 genes belong to the AE-associated common gene module, 64 showed no changes in gene expressions between Sham- and Dex-treated LCLs and these genes were categorized as the B gene set here. Three biological pathways, Protein repair, Syndecan interactions and HATs acetylate histones were enriched in this gene set significantly. The protein repair pathway maintains overall protein integrity by reduction or methyl group transfer.[31] It contains genes coding methionine sulfoxide reductases which can reduce methionine sulfoxide to methionine and restore the scavenging function of methionine.[32,33] Methionine sulfoxide reductases have wide tissue distribution and protects cells from oxidative-stress-induced cell injury.[33-35] However, its role in the airway has not yet been fully understood, although it is well known that AE of asthma is associated with increased oxidative stress.[36,37] *MSRA* and *MSRB2* expressions of Sham-treated LCLs and PBMCs were significantly lower in asthmatics with AE compared to those without AE and Dex-treatment showed no significant expression changes in *MRSA* and *MRSB2* (**Fig. 5**). Taken together, it is possible that asthmatics with decreased expressions of *MSRA* and *MSRB2* in blood cells are susceptible to AE. We thus may need a new treatment in addition to corticosteroids for the effective prevention of AE in these patients. An antioxidant supplementation based on the genetic susceptibility would be a way worth considering.[38]

Oxidative stress was associated with airway remodeling in patients with asthma[39] and also with smooth muscle remodeling in patients with chronic obstructive pulmonary disease by mitochondrial dysfunction.[40] Interestingly, syndecans, transmembrane heparan sulfate proteoglycans, play peculiar roles in development, tumorigenesis and inflammation, and there is growing evidence for involvement in tissue regeneration.[41] Recently, it has been reported that syndecan-1 promotes lung fibrosis by regulating epithelial reprogramming through extracellular vesicles.[42] Taken together, genes belonging to the Protein repair and Syndecan interaction pathway may collectively contribute to airway remodeling found in asthmatics and relative insensitivity to corticosteroid treatment.

Epstein-Barr virus-transformed LCL are widely used for human genomics study. However Epstein-Barr virus transformation itself can alter gene expression,[43] so it is controversial that gene regulation in LCLs recapitulates that of untransformed primary cells. Recently, it has been reported that genes involved in cholesterol metabolism are similarly regulated by statin between LCLs and primary B cells from the same donors.[44] Similarly, we observed that the Dex-regulated genes significantly overlapped in LCLs and primary B cells, and the expression of these genes showed significant correlations between treatment-naive LCLs and primary B cells.[45] Based on these findings, we used gene expressions in PBMCs to recapitulate changes in gene expressions in LCLs. However, PBMCs harbored various cell types, including B cells, although LCL gene expression showed little associations with the differential blood count.[46] We did not adjust PBMC gene expression by the differential blood count in this study and this point need to be considered before generalizing our observations.

The weakness of our study was that we utilized gene expression profiles on peripheral blood cells. Although previous reports showed that tissue-specific genes can be expressed in a non-tissue-specific manner[47,48] and peripheral blood cells express approximately over 80% of the

https://doi.org/10.4168/aair.2020.12.4.626

genes encoded by the human genome,[49] it is difficult to fully ascertain if peripheral blood cell can be a surrogate for airway cell biology. Given that peripheral blood is an easily accessible tissue, further studies are warranted to test a utility of gene expressions in blood cells to predict AE of asthma. A small number of participants, different ethnicity, and a relatively row rate of AE (especially in adult asthmatics) were another issues to be kept in mind before generalizing results from the present study. In addition, as a majority of asthmatics enrolled in this study was treated by low- to medium-dose ICSs, replicative studies performed in asthmatics treated by high-dose ICSs are warranted.

In summary, we identified a common gene module associated with the AE rate in both childhood and adult asthmatics using gene expression profiles of blood cells. The majority of genes belonging to the AE-associated common gene module showed no changes in expression after *in vitro* Dex-treatment, which suggested that we need a new treatment other than corticosteroids to prevent AE of asthma. In addition, *EIF2AK2, MSRA* and *MSRB2* expressions on blood cells may help us select asthmatics who are susceptible to AE and adjust treatments to prevent AE, although further studies confirming our results are needed.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health, US (R01 HL092197, R01 NR13391, U01 HL065899) to KGT, by the Seoul National University Hospital [0320140400 (2014-1194)] to PHW.

SUPPLEMENTARY MATERIALS

Supplementary Table S1

Module preservation statistics and *P* values in gene expression profiles of PBMCs from adult asthmatics

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Supplementary Fig. S1

Assessing scale-free model fitting in gene expression profiles of Sham-treated lymphoblastoid B cell lines from childhood asthmatics. The left panel shows scale-free topology plotted by soft threshold. The red horizontal line represents the cutoff for identifying a strong model fit. The right panel shows mean gene connectivity plotted by soft threshold.

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Supplementary Fig. S2

Differentially gene expressions between Sham- and Dex-treated blood cells (SAM plot). (A) Lymphoblastoid cell lines from childhood asthmatics. (B) Peripheral blood mononuclear cells from childhood asthmatics.

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Supplementary Fig. S3

Validation using real-time PCR (Sham-treated PBMCs from adult asthmatics). (A) *EIF2AK2*. (B) *MSRA*. (C) *MSRB2*.

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Supplementary Fig. S4

Structures of the acute exacerbation-associated common gene module (co-expression networks). (A) Sham-treated lymphoblastoid cell lines from childhood asthmatics. (B) Dex-treated lymphoblastoid cell lines from childhood asthmatics. For clarity, only the edges corresponding to the Pearson correlation coefficient > 0.8 were shown. The edge width is proportional to the Pearson correlation coefficient between 2 nodes. Reds nodes represented genes belonging to the A gene set. The network was visualized using qgraph R package.

Click here to view

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