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IL-1 signaling pathway molecules as key markers in childhood asthma

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Running title: Implication of IL-1 signaling in childhood asthma

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i. **Conflict of interest**

The authors declare that they have no conflict of interest.

ii. **Financial support**

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iii. **ABSTRACT**

Objective: The prevalence of childhood asthma has been increasing in recent years. This study aims to investigate the involvement of the key molecules of IL-1 (interleukin-1) signaling pathways in pediatric patients with asthma.

Methods: Differentially expressed genes (DEGs) associated with IL-1 signaling pathways were identified with RNA-seq from peripheral blood samples collected from asthmatic or healthy children and were further verified in clinical peripheral blood samples. Cellular models and asthmatic mice were subsequently developed to validate the identified asthmatic markers.

Results: Among the DEGs identified by RNA-seq, eight signal transducers associated with the IL-1 signaling network, namely IL-1RN, IL-1 β , IL-1RAP, IRAK3, IL-1R1, MYD88, IRAK2 and PELI1, were found to be substantially upregulated in children with asthma. Interestingly, a significant serially increased expression of four genes (IL-1RN, IL-1RAP, IRAK3 and PELI1) were observed in healthy subjects, patients with chronic persistent asthma and patients with acute exacerbation asthma. In particular, these four genes were continuously overexpressed in recurrent patients. A significant induction of the above four genes was then observed in house dust mite (HDM)-stimulated peripheral blood mononuclear cells (PBMCs) and ovalbumin (OVA)-induced asthmatic mice. In addition, a time-dependent induction of IL-1RAP and PELI1 was also detected in HDM-treated THP-1 cells, an acute monocytic leukemia cell line.

Conclusions: These results demonstrate that IL-1RN, IL-1RAP, IRAK3 and PELI1, which are signal transducers of the IL-1 signaling pathway, could serve as biomarkers for the pathogenesis of childhood asthma and for potential therapeutic targets of asthma.

Keywords: Childhood asthma; Asthmatic markers; IL-1 signaling; IL-1RN; IRAK3; IL-1RAP; PELI1

Key Message: We identified IL-1RN, IL-1RAP, IRAK3 and PELI1 as novel biomarkers during childhood asthma.

iv. Main text

INTRODUCTION

Recently, the prevalence of asthma continues to rise, especially in children (1). Children with asthma are at higher risk of developing chronic obstructive pulmonary disease (COPD) (2). In addition to acute inflammation caused by stimuli such as inhalation of allergens, other factors including microenvironments variation and viral infections can stimulate the lung structural cells and cause a strong secretion of pro-inflammatory mediators, cytokines, chemokines and proteases (3).

Theoretically, antigen stimulation causes an increase in IL-1 α and IL-1 β , the typical innate immune cytokines involved in the initiation and persistence of inflammation, in peripheral blood mononuclear cells (PBMCs) during the exacerbation of asthma (4). Furthermore, elevation of IL-1 β has been demonstrated to be implicated in the activation of the IL-1 signaling pathway, which plays an important role in the pathogenesis of various autoimmune diseases (5).

Airway inflammation and immune factors play an important role in the pathogenesis of asthma. It has been reported that IL-1 β induction as well as the activation of IL-1 signaling in sputum are implicated in airway neutrophilia and inflammasome activation during asthma (6). In adult, the upregulation of IL-1 pathway mediators, which was tested in sputum, is associated with frequent exacerbations of asthma (6,7). However, the involvement of IL-1 β and its downstream IL-1 signaling pathway in the peripheral blood of childhood patients with asthma need to be further investigated. Interestingly, a genome-wide association study (GWAS) of a child with asthma identified several loci encoding IL-1 signaling components including IL-33 and IL-1RL1 (8). However, research on the roles of these components in childhood asthma remains in its infancy.

In this study, we attempted to identify key molecules involved in the IL-1 signaling pathway in childhood asthma that contribute to revealing the pathogenesis of asthma and to investigate their implication in the airway immune response.

METHODS

1. Subjects

Asthma can be divided into three phases (acute exacerbation, chronic persistent and clinical remission) and the clinical manifestation grade of asthma can be divided into 1 to 5 steps according to the Global

Initiative for Asthma (GINA) guidelines. Pediatric patients with asthma and healthy controls were recruited from Children's Hospital of Soochow University and The Affiliated Hospital of Yangzhou University. Inclusion criteria: 1) definition of asthma was based on the GINA guidelines; 2) excluding persons with congenital heart or lung diseases, immunodeficiency diseases (such as chronic granuloma, hypogammaglobulinemia, ataxia telangiectasia and isolated IgA deficiency), or genetic diseases. Healthy controls were from children undergoing regular physical examinations. The study was approved by the Ethics Committee of the Children's Hospital of Soochow University and The Affiliated Hospital of Yangzhou University, and informed consent was obtained from guardians of children with asthmatic and healthy children.

2. RNA-seq and data analysis

Peripheral blood samples were collected from three pediatric patients who were clinically diagnosed with acute asthma exacerbation and from three healthy children who received routine physical examinations to serve as control. There are 2 boys and 1 girl with mean of 7 years old in both groups. No significant differences in age or weight were observed between the two groups. A Ribo-Zero rRNA Removal Kit (Illumina, USA) was used to remove ribosomal RNA. The libraries were generated and prepared using a TruSeq Stranded Total RNA Library Prep Kit (Illumina, USA). The quality and yield of the prepared libraries were assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, USA). Analyses were performed using Cuffdiff, from which the FPKM (fragments per kilobase of exon model per million mapped reads) values were calculated for estimating gene expression levels.

3. Clinical samples

Using the same inclusion criteria, we recruited another 90 children with asthma (48 with acute exacerbation and 42 in the chronic persistent stage) and 40 healthy children from routine physical examinations to validate the candidate differentially expressed genes (DEGs). The age, gender, weight, fractional exhaled nitric oxide (FeNO), blood test and inhaled corticosteroid (ICS) results were recorded (Table 1). Human peripheral blood samples were collected when they attended to clinic, and the PBMCs were separated with Human Lymphocyte Separation Medium (LTS1077, Tianjin Hao yang Biological Products Technology Co., Ltd.) within 4 h.

4. Asthmatic mouse model

The asthmatic mice model used the classic method referring to (9, 10). Briefly, asthmatic or control mice were euthanized by cervical dislocation after terminal anesthesia by intraperitoneal injection of 0.25 ml 3% chloral hydrate. 1 ml blood was then collected from the retro-orbital venous sinus. PBMCs were collected using a mouse lymphocyte separation medium within 4 h of blood collection. After three rinses with 0.5 ml saline through the left lung, 1.5 ml bronchoalveolar lavage fluid (BALF) was harvested.

5. Complete blood cell counts and white blood cell subsets in mice peripheral blood

Blood samples collected from mice were evaluated with an XS-800i (Sysmex, Japan) automatic analyzer for routine blood tests following the manufacturer's instructions.

6. H&E staining

After being rinsed with saline, the right lung tissues of mice were immediately fixed in 4% paraformaldehyde. Then the tissues were embedded in paraffin, cut into 3 μ m-thick sections and flattened in water at 56°C. Slides were then incubated in oven for 30 min at 80°C. Afterwards, the slides were stained with hematoxylin and eosin, and then dried with neutral resin. Photos were taken with a Nikon Eclipse Ti-U fluorescence microscope.

7. Immunofluorescence

After being rinsed with saline, the right lung tissues of mice were immediately fixed in 4% paraformaldehyde and blocked in PBS-Tween 0.05% containing 20% FBS. This was followed by incubation with IL-1 β primary antibody (12242, Cell Signaling Technology) for 2 h at room temperature. After being washed with PBS, the slides were incubated with Alexa Fluor 488 dye-conjugated chicken anti-mouse secondary antibody for 1 h at room temperature. The slides were then mounted with DAPI Fluoromount G (Southern Biotech) for 10 min after PBS washing. Finally, images were acquired with a Nikon Eclipse Ti-U fluorescence microscope, and analyzed with Image J.

8. Cells and reagents

Primary human PBMCs from healthy volunteers were stimulated with house dust mite (HDM) (XPB70D3A25, GREER, USA) (2 μ g/ml) for 24 h. Human monocytic THP1 cells were purchased from ATCC and stimulated with HDM (4 μ g/ml) for different lengths of time: 0, 6, 12, 18, 24, 48, or 72 hours.

9. RNA preparation and reverse transcription quantitative PCR

Total RNA was extracted with Trizol (Life science). The PrimeScriptTM RT reagent kit (Takara, Japan)

was used to synthesize cDNA from total RNA. Afterwards, cDNA products were applied as templates for quantitative PCR (qPCR) with SYBR Green PCR Kit (Takara, Japan), and qPCR was performed with an Applied Biosystems™ 7500 system (Life Technologies). Primers used in this study are listed in Supplementary Table 1 and 2.

10. Western blot analysis

Proteins were harvested with RIPA and the concentrations were measured using a Pierce™ BCA Protein Assay Kit (Thermo Scientific; Rockford, IL). The proteins were then transferred, washed and revealed with ECL kit (Amersham). The antibodies include anti-IL-1RN (AB124962, Abcam), anti-IL-1RAP (ab256461, Abcam), anti-IRAK3 (ab32394, Abcam), anti-PELI (ab199336, Abcam) and α -Tubulin (TU-02) (sc-8035, Santa Cruz).

11. Statistical analysis

Data were analyzed using GraphPad and SPSS software. Graphs are presented with standard deviation (SD) as errors bars. The student's *t*-test was used to determine the *P*-value. * for $P < 0.05$, ** for $P < 0.01$ and *** for $P < 0.001$.

RESULTS

1. IL-1 signaling pathway was activated in children with asthma

To identify the DEGs in asthma, we performed an unbiased RNA-Seq transcriptomic analysis using the peripheral blood samples from three children with acute asthma exacerbation (experimental group) and three healthy children (control group). We primarily screened 4658 DEGs (1760 upregulated and 2898 downregulated) in total and we were then interested in the IL-1 signaling pathway associated genes who had not been well studied in childhood asthma. Results showed that the mRNA expression levels of 5 genes were downregulated and 16 genes were upregulated, among which 8 of them—interleukin 1 receptor antagonist (IL-1RN), interleukin 1 beta (IL-1B), interleukin 1 receptor accessory protein (IL-1RAP), IL-1 receptor-associated kinases (IRAK3), type I IL-1 receptor (IL-1R1), myeloid differentiation primary response 88 (MYD88), IL-1 receptor-associated kinases 2 (IRAK2), and pellino E3 ubiquitin protein ligase 1 (PELI1)—showed statistical significance (Figure 1a). All of the 5 downregulated and 16 upregulated IL-1 signaling related DEGs together with their fold changes and *P* values were shown in Supplementary Table 3.

To validate the RNA-seq data, the eight significant DEGs that were annotated to the IL-1 signaling network were selected for qRT-PCR analysis in another cohort of patients, which included 90 children with asthma (48 with acute exacerbation and 42 in chronic persistent stage) and 40 healthy children. Results showed that the mRNA levels of the 8 genes were significantly increased in the patients with acute exacerbation as compared with the healthy control, which were consistent with the results from RNA-Seq (Figure 1b-i). Moreover, the expression levels of four genes, namely IL-1RN, IL-1RAP, IRAK3 and PELI1, were highest in the patients with acute exacerbation, followed by the patients in chronic persistent stage, and finally lowest in the healthy controls (Figure 1b, 1d, 1e, 1i). Besides, the decreasing tendency of leukocyte and percentage of neutrophil as well as increasing trend of lymphocyte, eosinophilic and basophilic were observed in these stages of asthma (Table 1). Interestingly, expression of IL-1RN, IL-1RAP, IRAK3 and PELI1 showed obvious positive correlation with FeNO and negative correlation with Forced expiratory volume in 1 second (FeV1) in both exacerbation and persistent groups (Supplementary Figure 1).

2. Continuous induction of IL-1 signaling components in recurrent patients

The reoccurrence of asthma attacks was documented within three months of follow-ups. Eleven patients from the acute exacerbation group (22.9%) and only one patient from the chronic persistent group (2.4%) experienced recurrence of an asthma attack (Table 1). The genes expression profiles of the 11 patients with recurrent asthma (named recurrence) and 37 patients without (named non-recurrence), all from the acute exacerbation group, were further analyzed using qRT-PCR with new collected peripheral blood samples. Surprisingly, the levels of IL-1RN, IL-1RAP, IRAK3 and PELI1 were still significantly overexpressed in the recurrent group (Figure 2). Altogether, these data indicate that IL-1 signaling pathway molecules are strongly implicated in clinical childhood asthma.

3. Induction of IL-1 signaling components upon HDM treatment in PBMC

We then tested whether the implication of IL-1 signaling in children with asthma could be verified in cultured cells. The HDM-stimulated and non-stimulated primary PBMCs from healthy volunteers were analyzed, and the results showed that the expression levels of IL-1 β , IL-1RN, IL-1RAP, IRAK3, and PELI1 were upregulated (Figure 3a-e). To further investigate the time-dependent induction of IL-1 signaling in asthma, human monocytic cell line THP1 cells were stimulated with HDM for 6, 12, 18, 24,

48, or 72 h and then analyzed using WB. Results showed that the HDM stimulation induced the over-production of IL-1RAP from 48 h and PELI1 as early as 12 h; but no obvious alternations of IL-1RN and IRAK3 were observed post HDM treatment (Figure 3f).

4. Induction of IL-1 signaling components in asthmatic mice

To verify our observations from clinical samples and *in vitro* studies, an *in vivo* mouse asthma model was developed (Supplementary Figure 2a) according to (10). The lung tissues collected from asthmatic and control mice were subjected to H&E staining (Supplementary Figure 2b). To test the inflammation of the asthmatic mice, the expression levels of IL-4, -5, and -13 in the BALF of OVA asthmatic mice were measured and showed significant elevation compared with the control group (Supplementary Figure 2c-e). Furthermore, immunofluorescence staining of total IL-1 β was then performed in right lung tissues, and results indicated that total IL-1 β expression was strongly induced in asthmatic mice (Supplementary Figure 2f). Collectively, these data demonstrated that the asthmatic mice model was successfully developed.

We were then interested in if the IL-1 signaling components could be induced in asthmatic mice. Blood test showed that the asthmatic mice had significant higher neutrophil, lower leukocyte, lymphocyte and platelet expression comparing with control group (Table 2). We then checked the expression levels of IL-1 signaling components, including IL-1 β , IL-1RN, IL-1RAP, IRAK3, and PELI1, in the PBMCs of asthmatic mice, and the results showed that their levels were significantly higher than in PBMCs of mice treated with normal saline (Figure 4a-e). These findings were consistent with the results obtained from clinical samples.

DISCUSSION

In this study, we demonstrated that IL-1 signaling pathway molecules, in particular IL-1RN, IL-1RAP, IRAK3, and PELI1, are key markers in the exacerbation of childhood asthma. Our data supported the potential application of IL-1 signaling pathway molecules as bio-markers for the prediction of exacerbation in childhood asthma. A challenge ahead will be to investigate the roles, functions, and therapeutic applications of these components in asthma.

The IL-1 signaling pathway has been verified as an important player in various autoimmune diseases (11). The members of the IL-1 family are not only the initiators but also the amplifiers of immune and inflammatory responses (12). Inhibiting the IL-1 signaling network has been used for the treatment of inflammatory cytokine-caused autoimmune diseases and has shown promising treatment efficacy (13).

The PELI1 mRNA level has been reported to be increased in sputum from patients with recurrent asthma and COPD (14). Here we report the differential expression of PELI1 in the PBMCs of asthma patients, mice, and *in vitro* cells. In addition, a time dependent induction of IL1-RAP and PELI1 but not IL-1RN and IRAK3 were observed in HDM induced THP-1 cells. The differences may due to the alternations of protein activity in distinct conditions or their different locations during signaling transduction cascade, which need to be further investigated. Currently, sputum samples are widely used to study asthma. However, it is difficult to collect such samples from children as they normally cannot master spitting or are unwilling to cooperate with sucking. Therefore, it would be much easier and more effective to extract PBMCs from blood samples, which are primarily used for routine blood monitoring of children with asthma. Furthermore, compared with the widely used Lipopolysaccharide (LPS) stimulation (15), HDM is a more common allergen for asthma attacks in humans and in animal models (16, 17). However, there are several limitations of this study. First, the quantity of samples used for RNA-Seq was small, which may result in ignoring potential molecules due to the large data variations. Second, as we mainly analyzed the IL-1 signaling, it should be noted that other cascades may also be involved in asthma (18-20). Finally, the roles and functions of the identified IL-1 signaling molecules in asthma need to be further investigated.

In conclusion, we demonstrated the over-production of several pro-inflammatory IL-1 signaling pathway components during childhood asthma, which might serve as biomarkers and contribute to the pathogenesis of children with asthma. Additionally, our study provides new insights for improving transcriptome analysis and expanding the theoretical understanding of patients with bronchial asthma. Further studies are needed to investigate the activation of IL-1 signaling, as well as the effects and roles of these factors during asthma.

v. Acknowledgements

The authors thank all the members in the Department of Respiratory Medicine, Children's Hospital of Soochow University and Department of pediatrics, The Affiliated Hospital of Yangzhou University for useful discussion.

vi. Impact statement

We identified IL-1RN, IL-1RAP, IRAK3 and PELI1 are novel biomarkers during childhood asthma.

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Table 1 Demographics of asthmatic or healthy individuals involved in this study (N=130).

Demographic	Exacerbation (n = 48)	Persistent (n = 42)	Healthy (n = 40)	P value
Age (mean)	7.5 (5-13)	7.2 (5-13)	7.5 (5-14)	NS
Gender (m/f)	23/25	20/22	19/21	NS
Weight (mean, kg)	27.2 (17-52)	26.6 (17-45)	26.3 (17-52)	NS
ICS (y/n)	21/23	23/19	N/A	NS
Asthma duration (year)	1.5	1.8	N/A	NS
GINA step (mean)	2.25	2.07	N/A	NS
Leukocyte (mean)	12.5 (6.3-22.3)	9.8 (3.8-21.1)	7.3 (3.5-13.4)	<0.01
Neutrophil (%)	88 (56.4-96.2)	72 (45.6-85.9)	58 (31.5-80.1)	<0.001
Lymphocyte (%)	8.4 (1.2-35.2)	20.8 (6.7-48.2)	32.1 (13.9-58.5)	<0.001
Monocyte (%)	3.3 (0.1-8.8)	6.1 (2.9-12.0)	7.1 (2.5-13.8)	NS
Eosinophili (%)	0.7 (0-3.1)	1.3 (0-5.6)	2.5 (0-7.5)	<0.05
Basophili (%)	0.1 (0-0.3)	0.2 (0-0.6)	0.4 (0-1.1)	<0.01
Erythrocyte	4.3 (3.0-5.3)	4.5 (3.5-5.4)	4.3 (2.7-5.4)	NS
Hemoglobin	126.0 (45-146)	128.7 (111-146)	125.7 (85-154)	NS
Platelet	277.8 (27-524)	303.2 (109-596)	267.1 (63-466)	NS
FEV1 (% predicted)	50.8 (30-71)	75.5 (70-88)	92.7 (85-100)	<0.001
FeNO (ppb)	30.5 (20-61)	24 (20-31)	12.7 (10-25)	<0.001
Reoccurrence (y/n)	11/37 (22.9%)	1/41 (2.4%)	N/A	NS

Table 2 Blood test data of control or asthmatic mice (N=16).

Demographic	Control (n = 6)	Asthma (n = 6)	P value
Leukocyte (mean)	7.0 (4.3-8.5)	5.3 (4.1-8.1)	<0.05
Neutrophil (%)	7.3 (4.9-11.7)	16.3 (10.3-22.1)	<0.001
Lymphocyte (%)	82.8 (66.2-96.4)	66.4 (48.2-77.2)	<0.01
Monocyte (%)	8.2 (3.6-10.7)	14.6 (0.4-28.9)	NS
Eosinophili (%)	2.7 (0.4-11.7)	2.5 (0.7-6.6)	NS
Basophili (%)	0.2 (0-0.6)	0.1 (0-0.2)	NS
Erythrocyte	10.1 (9.6-10.7)	9.8 (8.5-10.7)	NS
Hemoglobin	155.1 (147-161)	149.6 (132-162)	NS
Platelet	1058.0 (626-1336)	551.0 (100-1070)	<0.01

Figure Legends

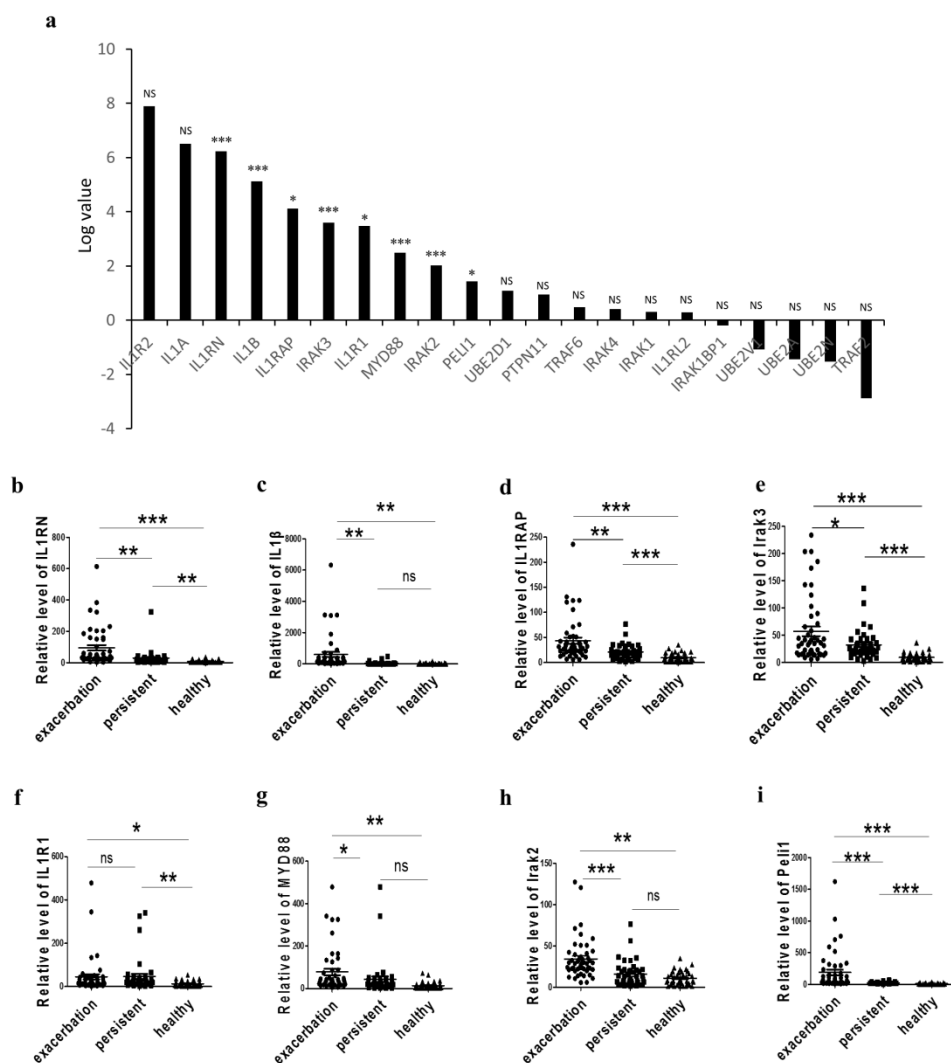
Figure 1 IL-1 signaling pathway molecules were activated in children with clinically diagnosed asthma. (a) RNA-seq transcriptomic analysis was performed to identify the DEGs associated with the IL-1 signaling pathway using peripheral blood samples from three children with acute exacerbated asthma exacerbation and three healthy children. Results are shown in log value and are ranked according to their expression compared to the healthy group. (b-i) Blood samples from another cohort of patients including 48 in acute exacerbation, 42 in persistent, and 40 in healthy control were tested for IL-1RN, IL-1B, IL-1RAP, IRAK3, IL-1R1, MYD88, IRAK2, and PELI1 expression using qRT-PCR. Relative expression was calculated based on the control group. Statistical analyses were performed with student's *t*-test (ns for not significant, * for $P < 0.05$, ** for $P < 0.01$, *** for $P < 0.001$).

Figure 2 Continuous induction of IL-1 signaling components in recurrent patients. Blood samples from 11 patients with recurrent asthma and 37 patients without, all from the acute exacerbation group, were further analyzed using qRT-PCR, and the mRNA expression levels of (a) IL-1RN, (b) IL-1RAP, (c) IRAK3, and (d) PELI1 are shown. Statistical analyses were performed with student's *t*-test (** for $P < 0.01$, *** for $P < 0.001$).

Figure 3 Induction of IL-1 signaling components upon HDM treatment. The HDM-stimulated and non-stimulated primary PBMCs were analyzed using qRT-PCR, and the expression of (a) IL-1 β , (b) IL-1RN, (c) IL-1RAP, (d) IRAK3 and (e) PELI1 is shown. Human monocytic cell line THP1 was stimulated with HDM for different lengths of time, and the expression levels of IL-1RN, IL-1RAP, IRAK3 and PELI1 were analyzed using WB (f). The experiments shown are representative of at least three biological replicates. Statistical analyses were performed with student's *t*-test (* for $P < 0.05$, ** for $P < 0.01$, *** for $P < 0.001$).

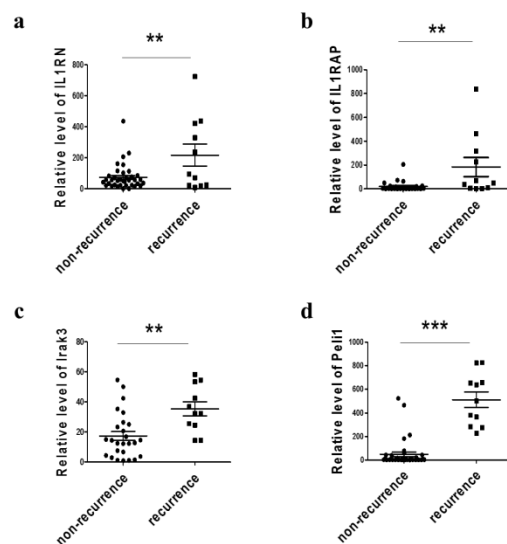
Figure 4 Induction of IL-1 signaling components in asthmatic mice. Using qRT-PCR, blood samples from asthmatic or control mice were tested for the levels of (a) IL-1 β , (b) IL-1RN, (c) IL-1RAP, (d) IRAK3, and (e) PELI1. Statistical analyses were performed with student's *t*-test (* for $P < 0.05$, ** for $P < 0.01$, *** for $P < 0.001$).

Fig 1. Wei et al.



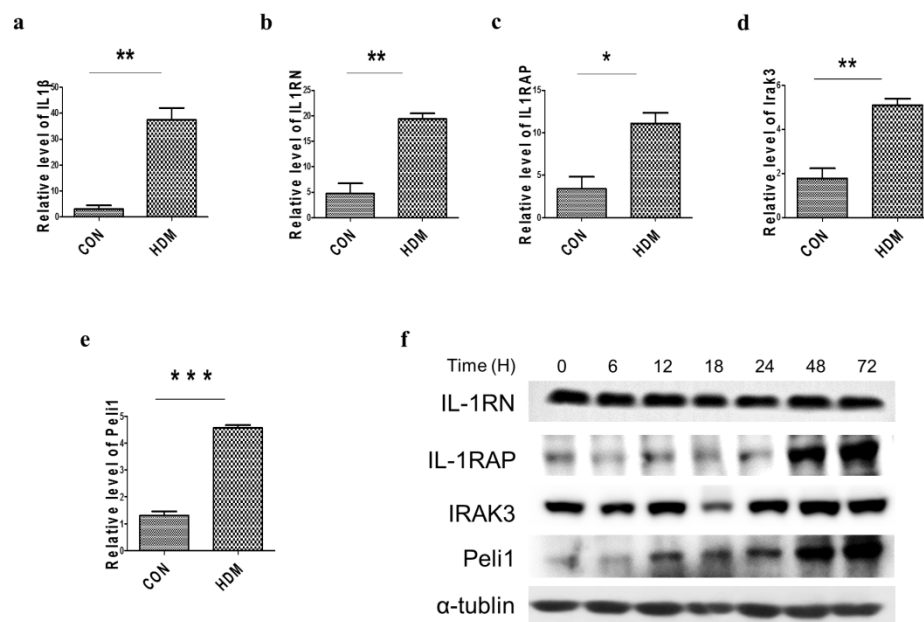
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Fig 2. Wei et al.



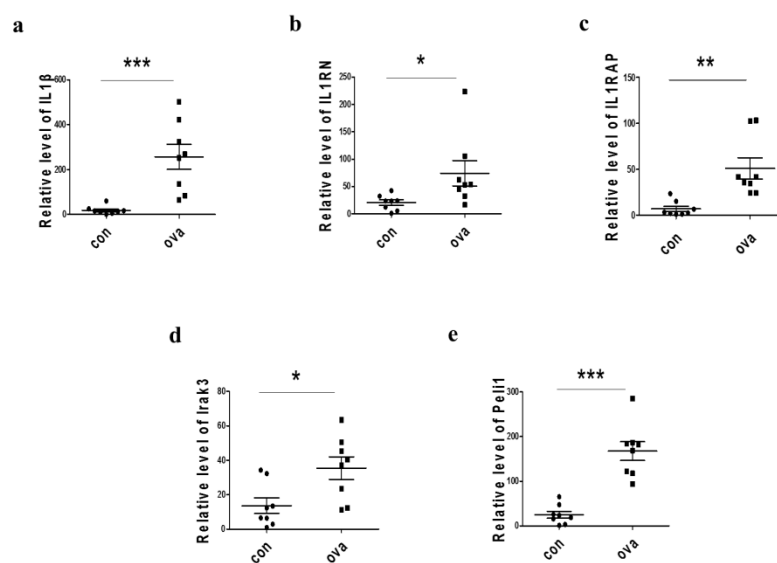
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Fig 3. Wei et al.



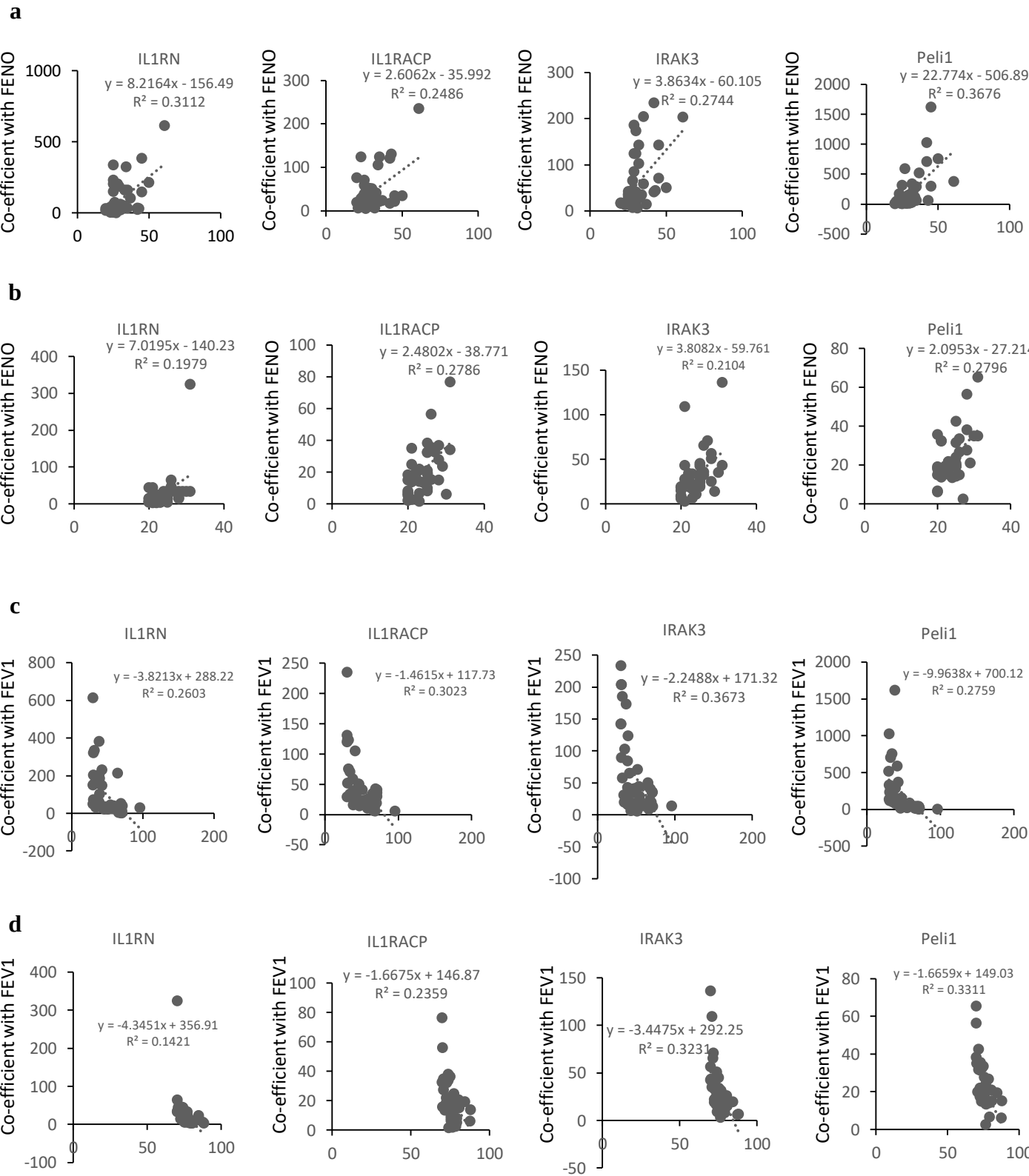
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Fig 4. Wei et al.



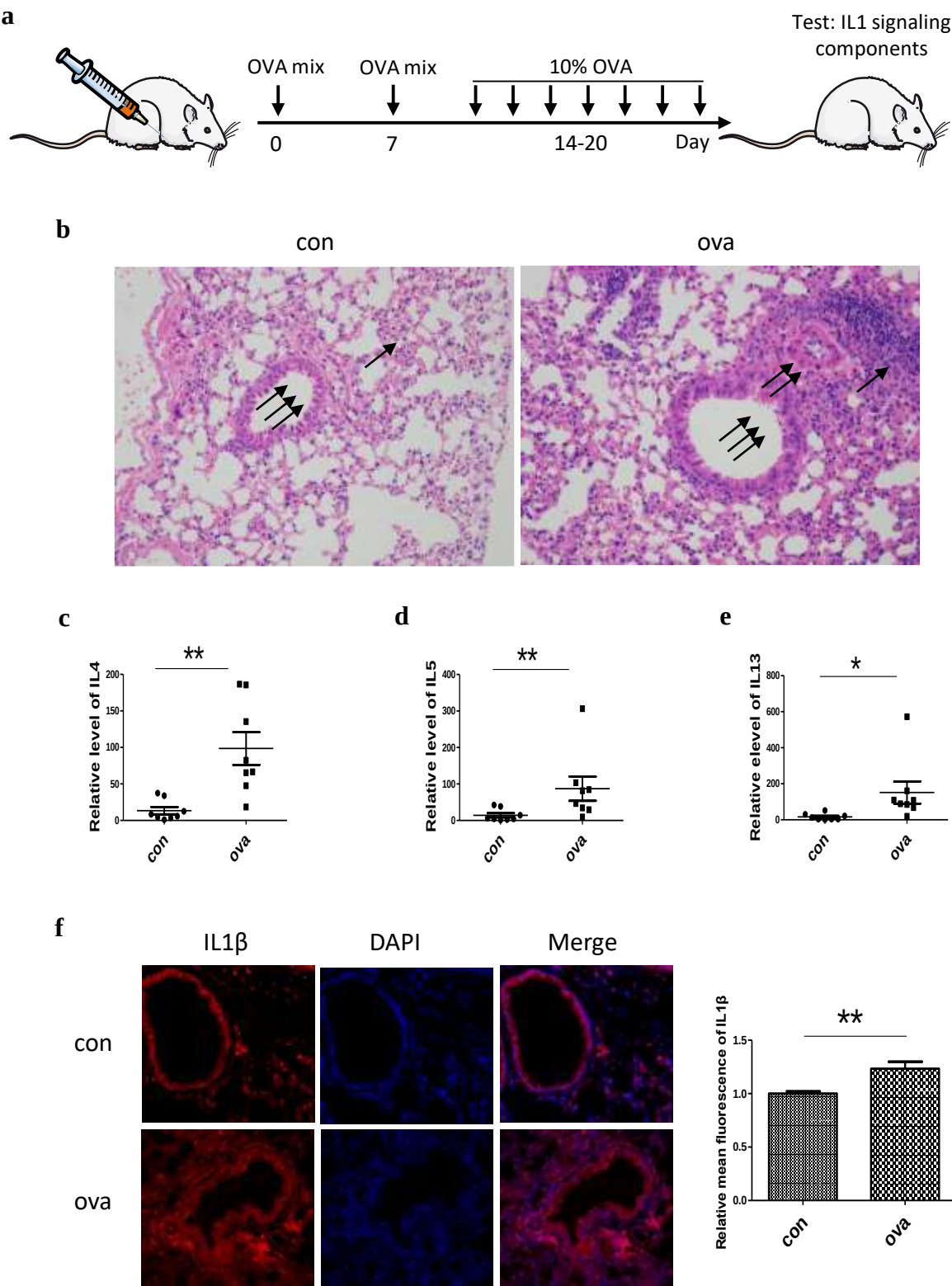
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Supplementary Figure 1, Wei et al.



Supplementary figure 1 Coefficient of correlations between IL-1RN, IL-1RAP, IRAK3, PELI1 mRNA level and FENO/FEV1 in both exacerbation and persistent group. (a) Coefficient of correlations between IL-1RN, IL-1RAP, IRAK3, PELI1 and FENO in exacerbation group. (b) Coefficient of correlations between IL-1RN, IL-1RAP, IRAK3, PELI1 and FENO in persistent group. (c) Coefficient of correlations between IL-1RN, IL-1RAP, IRAK3, PELI1 and FEV1 in exacerbation group. (d) Coefficient of correlations between IL-1RN, IL-1RAP, IRAK3, PELI1 and FEV1 in persistent group.

Supplementary Figure 2, Wei et al.



Supplementary figure 2 Establishment of asthmatic mice model. (a) Scheme depicting the development of asthmatic mice model. C57BL/6 mice were sensitized using OVA intraperitoneally on days 1 and 7 and then, after day 14, were challenged daily with 10% OVA via the airway for 7 days. Control group received saline instead of OVA. The behaviors were checked and IL-1 signaling molecules were then tested using qRT-PCR. (b) H&E staining was applied for lung tissues collected from asthmatic and control mice. The structures of terminal bronchioles, alveoli, and ciliated epithelium were intact, and no obvious inflammatory cell infiltration was observed in the control mice. In contrast, thickened bronchial walls, narrowing and tissue destruction in the bronchial lumen, shredded airway epithelial cells, as well as numerous inflammatory cells (lymphocytes, eosinophils, neutrophils) infiltrating the bronchial and blood vessels were observed in the asthmatic mice. (c-e) BALF from asthmatic or control mice were tested for IL-4, IL-5 and IL-13 using qRT-PCR. (f) Immunofluorescence staining of right lung tissues from asthmatic or control mice using anti-IL1 β antibody was performed. Representative pictures as well as fluorescence quantification are shown. Statistical analyses were performed with Student's t-test (* for $P < 0.05$, ** for $P < 0.01$).

Supplementary Table 1, Wei et al.

Gene symbol	Forward primers	Reverse primers
β-actin	GTGGCCGAGGACTTTGATTG	CCTGTAACAACGCATCTCATATT
Irak2	CTTAGATGCTGCCACCCCAA	CCTCGGCCAACACTATTCCA
Irak3	GTACATCAGACAGGGGAAACTTT	GACATGAATCCAGGCCTCTC
IL1β	CCTGAGCTCGCCAGTGAAAT	AGGACATGGAGAACACCACTTG
IL1r1	AGCGGCAGGAATGTGACAA	ACG TTCCTTG CATT TATCAGCC
IL1RN	CCTCCGCAGTCACCTAATCA	AGTGATGT TAACTGCCTCCAGC
IL1RAP	CATCAGCAGGCCCTAGAAGC	TGCTGCAATATGTAGTG TTCCTTA
PELI1	CCTCCACTAGCAGCAGCG	TGGGAGAGACCCATTATACCCTA
MYD88	TACATACGCAACCAGCAG	GGCAGTAGCAGATGAAGG

Supplementary Table 1 Sequences of human primers used for RT-qPCR.

Supplementary Table 2, Wei et al.

Gene symbol	Forward primers	Reverse primers
β-actin	AACAGTCCGCCTAGAAGCAC	CGTTGACATCCGTAAAGACC
Irak2	ATTGTCTTGAGCTGGCCCAT	GTCCTCAGTTGCTTGGACGA
Irak3	ACATGGGGGCATCAACGAGC	GGAAGCTGATAGGGGTTTTCTG
IL1β	GAAGAAGTGCCCATCCTCTG	AGCTCATATGGGTCCGACAG
IL1r1	AATGCTGTCCTGGGCTGACT	TGAGGCAGTAAGTTGAGTTTCTTAC
IL1RN	CCATAGACACTGCCTGGGTG	TCAGTGATGTAACTTCCTCCAGC
IL1RAP	AGAAAGTCACCCCGGAGGAT	AGGGTCTCATCTGTGACAATTCC
PELI1	TCAGGCTGCCAAGGCAATAA	GTA CTGACTGCGTGTCGGAA
MYD88	TGTTCTTGAACCCTCGGACG	TTCTGGCAGTCCTCCTCGAT

Supplementary Table 2 Sequences of mouse primers used for RT-qPCR.

Supplementary Table 3, Wei et al.

Gene symbol	Gene ID	Fold change (Log 2)	P value
IL-1R2	ENSG00000115590	7.88598	0.05355
IL-1A	ENSG00000115008	6.51161	0.12795
IL-1RN	ENSG00000136689	6.23228	0.00005
IL-1B	ENSG00000125538	5.12775	0.00005
IL-1RAP	ENSG00000196083	4.12482	0.03935
IRAK3	ENSG00000090376	3.59385	0.00005
IL-1R1	ENSG00000115594	3.47967	0.0451
MYD88	ENSG00000172936	2.49498	0.00005
IRAK2	ENSG00000134070	2.02843	0.00015
PELI1	ENSG00000197329	1.43342	0.0264
UBE2D1	ENSG00000072401	1.07998	1
PTPN11	ENSG00000179295	0.949355	0.17615
TRAF6	ENSG00000175104	0.48187	1
IRAK4	ENSG00000198001	0.408707	0.4367
IRAK1	ENSG00000184216	0.310529	0.53845
IL-1RL2	ENSG00000115598	0.288047	1
IRAK1BP1	ENSG00000146243	-0.18703	1
UBE2V1	ENSG00000244687	-1.07806	0.133
UBE2A	ENSG00000077721	-1.44605	1
UBE2N	ENSG00000177889	-1.53658	0.06035
TRAF2	ENSG00000127191	-2.8756	0.061

Supplementary Table 3 Values of the IL-1 signaling related DEGs screened from RNA-seq.