(see Fig E3, A–C). Heterodimerization of PAR1 and PAR4 is pre- cedent, providing a mechanism for thrombin bound to PAR1 through exosite 1 to cleave PAR4 (which cannot bind) more effi- ciently.6 The formation of a ternary complex would thus render ROS generation sensitive to antagonism of both receptors and imply that the main effector of Der p 1–stimulated ROS production might be PAR4, which is notably associated with epithelial- mesenchymal transition in airway cells.

Hitherto, PAR1 and PAR4 have not been considered activatable by group 1 HDM allergens,7 but in revealing the Der p 1–depen- dent cleavage of prothrombin we have identified their canonical activation with subsequent intracellular ROS formation via ATP release. Extracellular ATP is elevated in asthma, which is note- worthy because it stimulates dendritic cells and triggers the release of IL-33, which is genetically linked to asthma suscepti- bility and a key activator of cytokine production by iH2 nuocytes.8 Thrombin is present in airway surface liquid in asthma at levels sufficiently elevated to drive cell proliferation and is also increased following respiratory virus infection.9 Although it is generally assumed that these changes are associated with tissue repair following inflammation, our data implicate thrombin-mediated signaling as both an innate strategic initiator and an effector-perpetuator of allergic sensitization through its direct generation by inhaled Der p 1.

That the Toll-like receptor 3 ligand poly i: c operates ROS generation through a mechanism that converges with Der p 1 signaling at pannexons is interesting because interactions be- tween allergens and respiratory viruses precipitate exacerbations of asthma and allergy-polarizing transcription factors are redox sensitive. PAR1 contributes to the pathogenesis of influenza A.7 PAR1 and Toll-like receptor 3 are both upregulated by respiratory virus infections.8 ATP promotes TNF2immunity, and P2X7 expression is upregulated in asthma.5 It will therefore be of interest to investigate the operational role of pannexons as a signaling nexus in allergic sensitization and the triggering of disease exacerbations.

The sensitivity of Toll-like receptor 3–mediated activation to argatroban or PAR1 antagonists (see Fig E2, E–F) suggests that events downstream of pannexon opening involve the endogenous activation of thrombin, creating a cyclical process. These findings reveal a surprising primary trigger for thrombin production that further emphasizes its contribution to inflammatory lung re- sponses. Although an oral thrombin inhibitor, albeit with bioavailability and protein binding which may preclude significant airway access from the systemic circulation, has only mod- erate improving effect on HDL-induced pathology in a murine model,1 our data suggest that it would be of interest to explore similar effects of ADIs, especially as these molecules have been optimized with the pharmacological credentials for inhaled delivery.

Additional information is available (see this article’s Methods, Results, and References sections in the Online Repository at www.jacionline.org).
So far, few cytokine-based biomarker platforms have been developed. Therefore, the detection of KD still mainly relies on the judgment of clinical physicians based on patients’ symptoms. However, infectious diseases by pathogens may also result in symptoms similar to those of KD, making it difficult to accurately diagnose KD and leading to a delay in optimal and timely treatment of KD.

Previous studies have shown that blood miRNAs can be used as disease biomarkers. So, we enrolled 70 fever control (FC, denoting patients without KD with fever) and 50 subjects with KD (see this article’s Methods section in the Online Repository at www.jacionline.org). Among them, 37 FC and 31 KD subjects comprised the training set, used for developing the KD biomarker panel with next-generation sequencing (NGS) and/or quantitative PCR (qPCR) assays. The remaining 33 FC and 19 KD subjects comprised the blind test set, used for examining the performance of the developed KD biomarker panel.

We collected the FC set subjects when the patients without KD had fever. In addition, all subjects, including FC and KD, did not suffer from other diseases except for the ones mentioned in this table. CALs were defined if any of the following findings were seen on 2-dimensional echocardiography: coronary artery internal diameter of greater than 3 mm in children younger than 5 years, or greater than 4 mm in children older than 5 years, or if the internal diameter of a segment was more than 1.5 times greater than that of an adjacent segment. Of the 50 patients with KD included in this study, 15 patients (30%) had positive CAL findings on 2-dimensional echocardiography and 2 patients (4%) were IVIG resistant. CAL, Coronary artery lesion; IVIG, intravenous immunoglobulin.

**TABLE I. Demographic data of subjects in this study**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>FC (N = 70)</th>
<th>KD (N = 50)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex: male, n (%)</td>
<td>33 (47.1)</td>
<td>25 (50)</td>
<td>.853</td>
</tr>
<tr>
<td>Clinical features</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper respiratory tract infection (28)</td>
<td></td>
<td>Oral mucosal change: 46 (92%)</td>
<td></td>
</tr>
<tr>
<td>Lower respiratory tract infection (27)</td>
<td></td>
<td>Lymphadenopathy: 23 (46%)</td>
<td></td>
</tr>
<tr>
<td>Gastroenteritis (3)</td>
<td></td>
<td>Swelling/peeling of hands and feet: 36 (72%)</td>
<td></td>
</tr>
<tr>
<td>Urticaria (3)</td>
<td></td>
<td>Rash: 43 (86%)</td>
<td></td>
</tr>
<tr>
<td>Septic arthritis (2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dengue fever (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scarlet fever (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic lupus erythematosus (1)</td>
<td></td>
<td>CALs: 15 (30%)</td>
<td></td>
</tr>
<tr>
<td>Henoch-Schönlein purpura (1)</td>
<td></td>
<td>IVIG resistance: 2 (4%)</td>
<td></td>
</tr>
<tr>
<td>Atopic dermatitis (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute hepatitis (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary tract infection (1)</td>
<td></td>
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</tbody>
</table>

We randomly selected the 12 FC and 12 KD RNA samples from the training set, followed by evenly pooling RNAs to form 2 pooled FC and 2 pooled KD libraries. The pooled RNA libraries were subject to NGS to determine miRNA expression profiles. The heat map showed that the pooled FC and KD libraries were distinguishable on the basis of miRNA expression profiles. Only the miRNAs with more than 100 transcripts per million in either library and with greater than 1.5-fold change were included for clustering analysis. We used qPCR to validate the expression profiles by NGS in all RNA samples from the training set. Among the 10 illustrated miRNAs, 8 are significantly differentially expressed between control and disease samples. The C and D prefixes in the labels of X-axis denote FC and KD samples, respectively. ****P < .0001, ***P < .001, **P < .01, and *P < .05. The miRNA expression abundances were presented in the unit of ΔCt with U6 as the internal control.
According to miRSeq result, almost 80% of the initial reads belonged to clean read, the sequence read with 3′ adaptor recognized and trimmed. In addition, most clean reads were 22-nt long (see Fig E1 in this article’s Online Repository at www.jacionline.org), reflecting high performance of the sample preparation procedures and implying the dominance of miRNAs, which was proven by Fig E2 in this article’s Online Repository at www.jacionline.org.

To examine whether the FC and KD samples were distinguishable on the basis of miRNA expression profiles, we conducted clustering analysis. The heat map showed that the duplicated libraries had similar and consistent patterns (Fig 1, A). Moreover, there were more than 20 miRNAs able to distinguish FC from KD samples so that they can serve as biomarkers of KD.

For further validation with qPCR in the training set, 37 FC and 31 KD subjects, we selected 10 miRNAs with NGS abundance larger than 1000 transcript per million in at least 1 library and with average fold change larger than 1.25 between FC and KD libraries. As a result, we observed 10 miRNAs whose expression preferences by qPCR were consistent with the ones by NGS (see Table E2 in this article’s Online Repository at www.jacionline.org). Fig 1, B, demonstrates that 8 of the 10 examined miRNAs were significantly differentially expressed. Although the remaining 2 miRNAs were not statistically significant, the expression preferences can be observed.

Because males have a higher KD incidence rate than do females, we further investigated whether the 10 miRNAs are significantly differentially expressed between male and female subjects, causing bias when served as a disease biomarker. Although the expression of hsa-miR-941 is significant between males and females (P = .0282), the difference between control and disease is much more significant than that between sex (P = 6.7319 × 10^-07 vs P = .0282). The expressions of the remaining 9 biomarker miRNAs were not sex specific. Therefore, we used the ΔCt values of the 10 miRNAs to develop a KD biomarker panel, training a Support Vector Machine (SVM) classification model, and diagnosing KD.

SVM is a machine learning algorithm and is good in dealing with binary classification problems, treatment versus control or disease versus normal. We used the ΔCt values of the 10 panel miRNAs (Table E2) to train the SVM classification model with 5-fold cross-validation specified to eliminate overfitting and underfitting. As shown in Fig 2, A, the final SVM classification model has a sensitivity of 83.3% and a specificity of 92.5%, leading to an overall accuracy of 87.9% and a 0.9 area under receiver operating characteristic (auROC) value.

An independent cohort, composed of 33 FC and 19 KD subjects, was applied to conduct a blind test. We determined the miRNA ΔCt values of the 52 blind test subjects (see Table E3 in this article’s Online Repository at www.jacionline.org). After SVM model alignment, 16 of the 19 KD samples were diagnosed as KD and 27 of the 33 FC samples were diagnosed as not KD, demonstrating a sensitivity of 84.20% and a specificity of 81.8%. The SVM performance examined with the blind test set was similar to the one examined with the training set, reflecting the robustness and reliability of the SVM classification model. In addition, it reflected that the overfitting and underfitting problems were slight in our SVM classification model.

To facilitate the early detection of KD, we built the Web-based user-friendly interface, Kawasaki disease Detection Platform (KDP, Fig 2, B), accessible via http://formosa3.nchc.org.tw:50190/KDP/index.php. To detect KD, the physicians or researchers may submit the ΔCt values of the 10 panel miRNAs onto the KDP. KDP calls the SVM alignment and then reports the detection result in both graphical (Fig 2, B) and text formats (see Fig E3 in this article’s Online Repository at www.jacionline.org). In summary, our biomarker panel provides a useful tool for researchers and physicians, facilitating early detection of KD.
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Ho-Chang Kuo, MD, PhD
Kai-Sheng Hsieh, MD
Mindy Ming-Huey Guo, MD
Ken-Pen Weng, MD
Luo-Ping Ger, PhD
Wen-Ching Chan, PhD
Sung-Chou Li, PhD

From the Department of Pediatrics, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan; the Kawasaki Disease Center, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan; the Department of Pediatrics, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan, and National Yang-Ming University, Taipei, Taiwan; the Department of Medical Education and Research, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan; the Genomics and Proteomics Core Laboratory, Department of Medical Research, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Kaohsiung, Taiwan; and Center for Research Informatics, The University of Chicago, Chicago, IL. E-mail: wenching.char@gmail.com.

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Peripheral blood gene expression predicts clinical benefit from anti–IL-13 in asthma

To the Editor:

Asthma is the most prevalent chronic disease of children in industrialized countries. However, the immunopathology of asthma is most commonly described in adults in whom the airways are characterized by heterogeneous eosinophilic airway inflammation. In moderate-to-severe asthma in adults, biologic therapies directed against type 2 inflammatory cytokines are currently under development; meaningful clinical benefit from these interventions is enriched in subjects selected on the basis of noninvasive biomarkers of type 2 "high" asthma, for example, serum periostin and blood or sputum eosinophils.1

In a study of 277 pediatric subjects with asthma, serum periostin and peripheral blood eosinophils were significantly negatively correlated with age and serum periostin was substantially elevated in pediatric patients with asthma compared with adult patients with asthma.2 These data suggest that factors other than type 2 inflammation may influence serum periostin and blood eosinophil levels in children with asthma. The ability of these biomarkers to predict clinical benefit from type 2 pathway–directed therapies in pediatric asthma is currently unknown. Therefore, alternative biomarkers of type 2 inflammation may need to be evaluated in children and adolescents with asthma.

We hypothesized that gene expression correlated with eosinophil count in asthma patients’ peripheral blood (1) would be enriched for genes whose expression levels predict treatment benefit from anti–IL-13 therapy, (2) would include genes whose expression was independent of age, and (3) whose cellular origin of expression is associated with type 2 inflammatory cells and/or type 2 cytokine signaling. To develop an eosinophil-related gene signature (ERGS), we evaluated genomewide transcript levels in stabilized RNA extracted from peripheral blood from multiple clinical studies of well-characterized patients with asthma. The clinical characteristics of subjects with asthma included in this analysis are summarized in Table E1 in this article’s Online Repository at www.jacionline.org.

We assessed peripheral blood gene expression in 298 subjects (12-75 years old) from the EXTRA study by microarrays (see this article’s Methods section in the Online Repository at www.jacionline.org). Although this study was not designed nor powered to detect an elevation in serum periostin or blood eosinophil count in adolescent (<18 years old, N = 15) versus adult (≥18 years old) patients with asthma, we observed median elevations of 37 ng/mL periostin (P < .05) and 20 eosinophils per μL (not statistically significant, respectively) (see Fig E1 in this article’s Online Repository at www.jacionline.org). Six hundred twenty-five genes were significantly correlated with blood eosinophil counts (adjusted P value < .05; 50 genes with the lowest adjusted P value are tabulated in Table E2 in this article’s Online Repository at www.jacionline.org). Regression analysis (Fig 1, A) reveals a skewed toward genes that are positively correlated with absolute blood eosinophil count, suggesting that blood eosinophil count is an important source of expression variation. CCL23, SIGLEC8, PTGDR2, CACNG6, IDO1, and HSD3B7 were among the most positively correlated genes with absolute blood eosinophil count (Fig 1, A; Table E2; see Fig E2 in this article’s Online Repository at www.jacionline.org) and selected as candidate genes for subsequent assessment due to (1) minimal evidence of age dependency across a wide range of age (12-75 years old; Fig E2), (2) biological plausibility, and (3) technical robustness of quantitative PCR assay development on a clinically amenable assay platform, the COBAS system (data not shown). Using this assay platform, we then characterized the expression of these genes on the basis of their ability to predict eosinophilic airway inflammation, blood eosinophil count, and clinical response to