

RESEARCH ARTICLE

Urinary lipid profile of atopic dermatitis in murine model and human patients

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Abstract

Atopic dermatitis (AD) is the most common inflammatory skin disease in children. The serum level of thymus and activation-regulated chemokine (TARC) is a useful AD index to reflect disease severity; however, it requires blood collection from young children. In comparison, urine samples are easier to collect in a pediatric clinical setting. Here, we analyzed the lipids excreted in urine to identify a diagnostic biomarker for AD. We generated a murine dermatitis model by repeated topical application of 2,4-dinitrofluorobenzene (DNFB) or tape-stripping the dorsal skin. Lipid metabolites excreted in the urine were comprehensively analyzed using liquid chromatography-tandem mass spectrometry. To corroborate our findings, we also analyzed urine samples from patients with AD. DNFB application induced AD-like skin lesions, including epidermal thickening, infiltration of eosinophils and T cells, and an increase in Th2 cytokine levels. Assessment of lipids excreted in urine showed a dominance of prostaglandins (PGs), namely, a PGF_{2α} metabolite (13,14-dihydro-15-keto-tetranor-PGF_{1α}), a PGE₂ metabolite (13,14-dihydro-15-keto-tetranor-PGE₂), and a PGD₂ metabolite (13,14-dihydro-15-keto PGJ₂). mRNA and protein expression of PGF_{2α}, PGE₂, and PGD₂ synthase was upregulated in DNFB-treated skin. The tape-stripping model also caused dermatitis but without Th2 inflammation; urine PGF_{2α} and PGD₂ metabolite levels remained unaffected. Finally, we confirmed that the urinary levels of the aforementioned PG metabolites, as well as PGI₂ metabolite, 6,15-diketo-13,14-dihydro-PGF_{1α} and arachidonic acid metabolite, 17-hydroxyeicosatetraenoic acid (17-HETE) increased in patients with AD. Our

Abbreviations: AA, arachidonic acid; AD, atopic dermatitis; AKR, aldo-keto reductase; CHS, contact hypersensitivity; COX, cyclooxygenase; CYP, cytochrome P450; DHA, docosahexaenoic acid; diHDoHE, dihydroxy-docosahexaenoic acid; DNFB, 2,4-dinitrofluorobenzene; EASI, eczema area and severity index; EPA, eicosapentaenoic acid; FA, food allergy; H&E, hematoxylin and eosin; HDoHE, hydroxy-docosahexaenoic acid; HETE, hydroxyeicosatetraenoic acid; HpETE, hydroperoxyeicosatetraenoic acid; IL, interleukin; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LOX, lipoxygenase; PG, prostaglandin; PGDS, PGD synthase; PGES, PGE synthase; PGFS, PGF synthase; SCCA, squamous cell carcinoma antigen; SCORAD, scoring of atopic dermatitis; TARC, thymus and activation-regulated chemokine.

Nanae Nagata, Yuta Hamasaki, Shinichiro Inagaki, and Tatsuro Nakamura contributed equally to this work.

data highlights the unique urinary lipid profile in patients with AD, which may provide insight into novel urinary biomarkers for AD diagnosis.

KEYWORDS

atopic dermatitis, human patient, lipid, murine model, urine

1 | INTRODUCTION

Atopic dermatitis (AD) is an inflammatory skin disease characterized by recurrent pruritus eczema. The estimated prevalence of AD in children and adults is 9.8%–13.2% and 2.5%–9.4% respectively.¹ Researchers have been studying AD for many years, however, the underlying mechanism for its onset and progression remains unclear. It is thought that T helper 2 (Th2)-type inflammation and skin barrier disruption are important in the pathogenesis of AD. In particular, in early stage AD, the Th2-type immune response is dominant. Th2 cytokines including interleukin (IL)-4, -13, and 31, stimulate the production of bioactive substances, such as immunoglobulin E (IgE), periostin, thymic stromal lymphopoietin (TSLP),² and thymus and activation-regulated chemokine (TARC).^{3,4} These substances promote pruritic inflammatory reactions, impair skin barrier function, and lead to more dryness and dysbiosis of the skin. The interaction between Th2 inflammation and barrier disruption is thought to be associated with the chronicity of AD.⁵

The diagnosis of AD is based on various clinical signs and symptoms. The severity of AD is assessed by serum biomarkers, such as TARC and squamous cell carcinoma antigen (SCCA).⁶ However, in some cases, it is difficult to assess the severity of AD using the existing biomarkers, as they have high baseline values in non-AD children.⁷ AD usually appears in infancy; therefore, noninvasive diagnostic biomarkers can help in the early detection of AD.

Lipid mediators are small signaling molecules that regulate the progression and resolution of inflammation. These bioactive molecules are derived from n-6 polyunsaturated fatty acids (PUFAs), such as arachidonic acid (AA), or n-3 PUFAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), via enzymatic or non-enzymatic oxidation processes. Since the production and metabolism of lipid mediators can vary depending on the type, stage, and tissue site of the disease, clarification of the lipid profile is useful for elucidating the disease pathophysiology and identifying biomarkers. Several reports have shown that some lipid mediators and their metabolites are excreted in urine and reflect the physiology of allergic diseases including asthma and anaphylaxis^{8,9}; thus, we hypothesized that some urinary metabolites of lipid mediators may also reflect skin inflammation in AD.

We attempted to clarify the profile of lipid metabolites in the urine of AD model mice in comparison with that of non-allergic dermatitis model mice. We then analyzed the lipid profile in the urine of children with AD.

2 | MATERIALS AND METHODS

2.1 | Animals

Male BALB/c mice were purchased from CLEA Japan (Tokyo, Japan). Mice were maintained in isolated cages with a 12-h light/12-h dark photoperiod. Water and food were available ad libitum. All experiments were approved by The University of Tokyo's Institutional Animal Care and Use Committee.

2.2 | Skin inflammation model

The DNFB-induced skin inflammation mice model was generated as described previously.¹⁰ Briefly, 3 days after depilatory the dorsal hair, the mice were sensitized on day 1 by applying 25 μ l 0.5% DNFB (w/v) in a mixture of acetone and olive oil (4:1) on the upper side of dehaired back skin. On days 5, 8, and 11, the mice were challenged with 100 μ l vehicle or 0.2% DNFB on lower side of back skin. After 24 h of the last DNFB stimulation, the skin specimen was sampled and used. In tape-stripping skin inflammation model, 3 days after depilatory the dorsal hair, the back of mice was stripped 20 times with ordinary adhesive tape.¹¹ After 9 days, the skin specimen was sampled. Scratching behavior of mice was counted for 10 min after stimulation. The severity of dermatitis of the back skin was evaluated by the total points scored as follows: 0, none; 1, mild; 2, moderate; and 3, severe by using the SCORAD index which was based on the severity of erythema, hemorrhage, edema, papules, excoriation, erosion, scaling, dryness, and lichenification.¹² The urines were serially collected from the same mice in metabolic cages and stored until used at -80°C .

2.3 | Patients

AD human patients ($N = 13$) were clinically diagnosed with AD by their primary doctors if they fulfilled the diagnostic

criteria proposed by Hanifin and Rajka¹³ between July 2016 and July 2017. As shown in the Table S1, mild to severe patients with AD were enrolled (SCORAD index ≥ 15). Eczema area and severity index (EASI) and serum levels of TARC and IgE were also obtained from the patients. Non-AD individuals without clinical symptoms were categorized as control ($n = 4$). Informed consent from all individual and/or their parents and ethical approval for the study was obtained prior to beginning the study by the institutional review board of the National Center for Child Health and Development (approval number: 838) and The University of Tokyo (approval number: 10586-1). Urine samples were collected in fasting state and stored until used at -80°C .

2.4 | Histology

Excised tissues were fixed in 4% paraformaldehyde (PFA) for 24 h and embedded in paraffin. Tissue sections ($4\text{ }\mu\text{m}$) were stained with hematoxylin and eosin (H&E), chloroacetate esterase (CAE), and May-Grunwald and Giemsa (MGG) as described previously.¹⁴ CAE-positive mast cells and MGG-positive eosinophils were counted in 10 randomly selected fields ($\times 400$ magnification) per section.

2.5 | Immunohistochemistry

Dissected tissues were fixed in 4% PFA and embedded in paraffin or OCT compound (Sakura Finetek Japan, Tokyo, Japan). Sections ($4\text{ }\mu\text{m}$ thickness) were incubated with methanol containing 0.3% hydrogen peroxidase for 30 min at room temperature (RT). After incubation with 0.1% TritonX-100 and 5% normal goat serum for 30 min at RT, primary antibodies were treated to sections at 4°C overnight and then, second antibodies were incubated for 2 h at RT (Table S2). After incubation with Avidin-biotin complex (VECTASTAIN ABC Kit, Vector Laboratories, Burlingame, CA, USA) for 30 min at RT, the sections colored by incubation with 0.2 mg/ml 3,3'-diaminobenzidine tetrahydrochloride (DAB, DOJINDO Laboratories, Kumamoto, Japan) and 0.03% hydrogen peroxidase. The images were captured using BZ-X710 microscope (KEYENCE, Tokyo, Japan).

2.6 | Quantitative RT-PCR

Total RNA was isolated from skin by using Trizol reagent (Molecular Research Center, Inc, Cincinnati, OH, USA) and reverse transcribed to cDNA using ReverTra Ace (TOYOBO, Osaka, Japan). Quantitative RT-PCR was performed using THUNDERBIRD SYBR qPCR Mix (TOYOBO)

and AriaMx Real-Time PCR System (Agilent Technologies Japan, Tokyo, Japan). The primer sequences are provided in the Table S3. The quantification was performed by delta Ct methods with 18S rRNA as an endogenous control.

2.7 | Comprehensive analysis of lipid metabolites

Two hundred microliters of urine was mixed with $300\text{ }\mu\text{l}$ of 0.1% formic acid (v/v) and $10\text{ }\mu\text{l}$ of the internal standard solution (Table S4). The mixed solutions were applied to solid phase extraction cartridge (OASIS HLB $\mu\text{Elution}$ plate, Waters, Massachusetts, USA) preconditioned with $200\text{ }\mu\text{l}$ acetonitrile and distilled water. After washing with $200\text{ }\mu\text{l}$ distilled water and $200\text{ }\mu\text{l}$ hexane, the lipid fractions were eluted with $100\text{ }\mu\text{l}$ methanol. The sample solution was injected to high performance liquid chromatography (Nexera 2, Shimadzu, Kyoto, Japan) equipped with mass spectrometer (LCMS-8060, Shimadzu, Kyoto, Japan). 138 types of metabolites, 3 types of PUFAs, and 15 types of internal standards were measured and analyzed by using LC/MSMS Method Package for Lipid Mediators version 2 with LabSolutions software (Shimadzu, Kyoto, Japan) as per manufacturer's instruction. Creatinine-corrected peak ratio was calculated using the following formula; (the peak area of each metabolite/the peak area of internal standards)/creatinine concentration. Urinary creatinine concentration was measured using a kit, LabAssayTM Creatinine (Wako, Osaka, Japan).

2.8 | Statistics

Statistical analyses were performed using BellCurve for Excel 2015 software (Social Survey Research Information Co., Ltd. Tokyo, Japan). Statistical differences were analyzed by Student's *t* test or Mann-Whitney *U* test for two-group comparisons, one-way ANOVA with Tukey's test or Kruskal-Wallis test with the Steel-Dwass test for multiple-group comparisons to evaluate the atopic scoring and urinary metabolites. The relationships between urinary metabolites and AD index were evaluated by the Spearman's rank correlation coefficient. Data are expressed as mean $\pm$ SEM. Statistical significance was defined as $*p < .05$ and $**p < .01$.

3 | RESULTS

3.1 | Application of DNFB caused allergic skin inflammation in mice

AD is characterized by recurrent skin inflammation with pruritus eczema driven by a type 2 immune response.¹

First, we generated a murine model of skin inflammation induced by repeated painting of DNFB on the dorsal skin. Mice were sensitized on day 1 by applying 0.5% DNFB and then challenged with 0.2% DNFB on days 5, 8, and 11 (Figure 1A).

DNFB application, which was performed thrice, induced dermatitis accompanied by hemorrhage, excoriation, papules, dryness, lichenification (Figure 1B), increased dermatitis score (Figure 1C), scratching (Figure 1D), and epidermal thickening (Figure 1E,F). Histological sections showed that eosinophils and neutrophils, but not mast cells, were remarkably increased in the dermis. This increase reached a plateau after the first DNFB application (Figure 1G). After the onset of severe dermatitis, mRNA expression of keratinocyte-derived cytokine thymic stromal lymphopoietin (*Tslp*), a key initiator of the Th2 immune response, and representative Th2 cytokine interleukin 4 (*Il4*) was significantly increased

in inflamed skin (Figure 1H). Moreover, immunostaining showed that CD4⁺ T cells infiltrated the skin lesions (Figure 1I). These results indicated that repeated DNFB application mimicked the pathological conditions of AD skin lesions.

3.2 | The urinary lipid profile of DNFB-treated mice

We comprehensively analyzed 138 types of lipid metabolites in mice urine samples from the first and third DNFB treatments. We focused on the metabolites detected in all urine samples from any of the three groups (pre, first, and third DNFB treatments). As shown in the Table S5, the levels of 13 types of metabolites derived from n-6 fatty acids (AA) were significantly increased in the urine samples. On the other hand, the levels of two

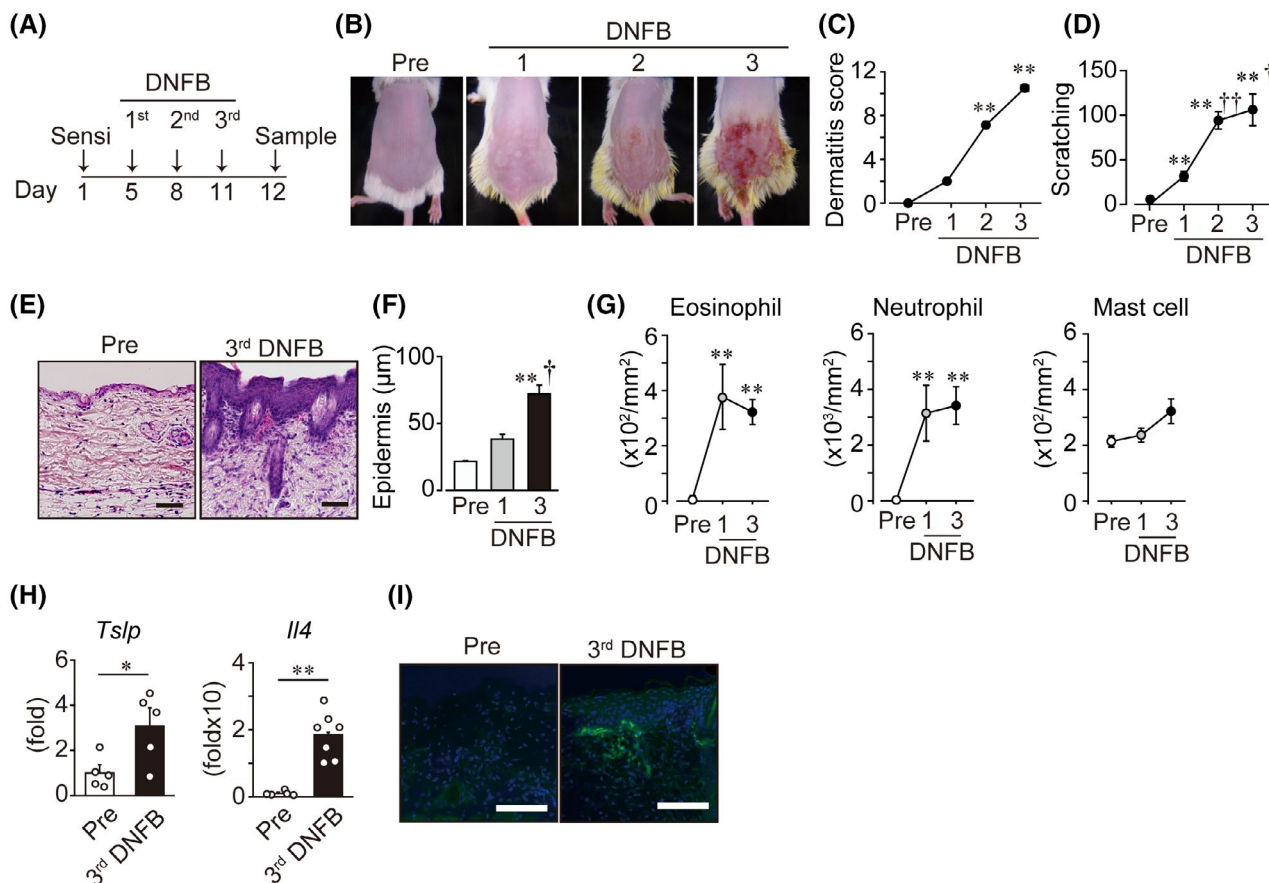


FIGURE 1 DNFB stimulations induced AD in mice. (A) DNFB-induced dermatitis protocol. (B) Dorsal skin view of DNFB treated mice before and 1st, 2nd, and 3rd stimulations. Change in (C) dermatitis and (D) scratching symptom score. Data are presented as mean $\pm$ SE ($n = 8$). ** $p < .01$, compared with before stimulation. † $p < .05$, †† $p < .01$, compared with 1st stimulation. (E) Representative pictures of H&E-stained dorsal skin. Scale bar, 50 μ m. (F) Change in epidermal thickness measured from H&E-stained images. Data are presented as mean $\pm$ SE ($n = 5-7$). ** $p < .01$, compared with before stimulation. † $p < .05$, compared with 1st stimulation. (G) The number of eosinophils, neutrophils, and mast cells in the lesional skin ($n = 5-8$). Data are presented as mean $\pm$ SE. ** $p < .01$. (H) Relative mRNA levels of *Tslp* and *Il4* mRNA from 3rd DNFB-treated skin. Data are presented as mean $\pm$ SE ($n = 5-7$). * $p < .05$ and ** $p < .01$, compared with before stimulation. (I) CD4 immunostaining. Scale bar: 50 μ m

n-3 fatty acid-derived metabolites (PGD₃ and resolvin D1) and one arachidonoyl ethanolamide (AEA) metabolite (PGE₁-EA) were decreased after the first DNFB treatment (Table S5).

PGF_{2α}, PGE₂, PGD₂, and PGI₂ accounted for nearly 69% (9/13 types, Figure 2A–I) of the increased metabolites. An increase in their levels was noted in the urine samples from DNFB-treated mice, suggesting that these PGs are associated with the progression of dermatitis. In particular, 13,14-dihydro-15-keto-tetranor-PGF_{1α} (Figure 2A), 13,14-dihydro-15-keto-tetranor-PGF_{1β} (Figure 2C), 13,14-dihydro-15-keto-tetranor-PGE₂ (Figure 2G), and 13,14-dihydro-15-keto-PGJ₂ (Figure 2F) were predominantly increased in the urine of mice with severe

dermatitis. A slight but noticeable increase in a urine PGI₂ metabolite, 6-keto-PGF_{1α} (Figure 2I), was also detected.

3.3 | Lipid synthase expression in DNFB-treated mice skin

Next, we assessed the mRNA and protein expression of each enzyme that produced lipid metabolites in DNFB-treated skin. PGF_{2α}, PGE₂, and PGD₂ are produced by the activity of cyclooxygenase (COX, type 1 and 2) and its specific synthase, that is, PGF synthase (aldo-keto reductase AKR1B3),¹⁵ PGE synthase (mPGES-1, mPGES-2, and cPGES), and PGD synthase (H-PGDS and L-PGDS)

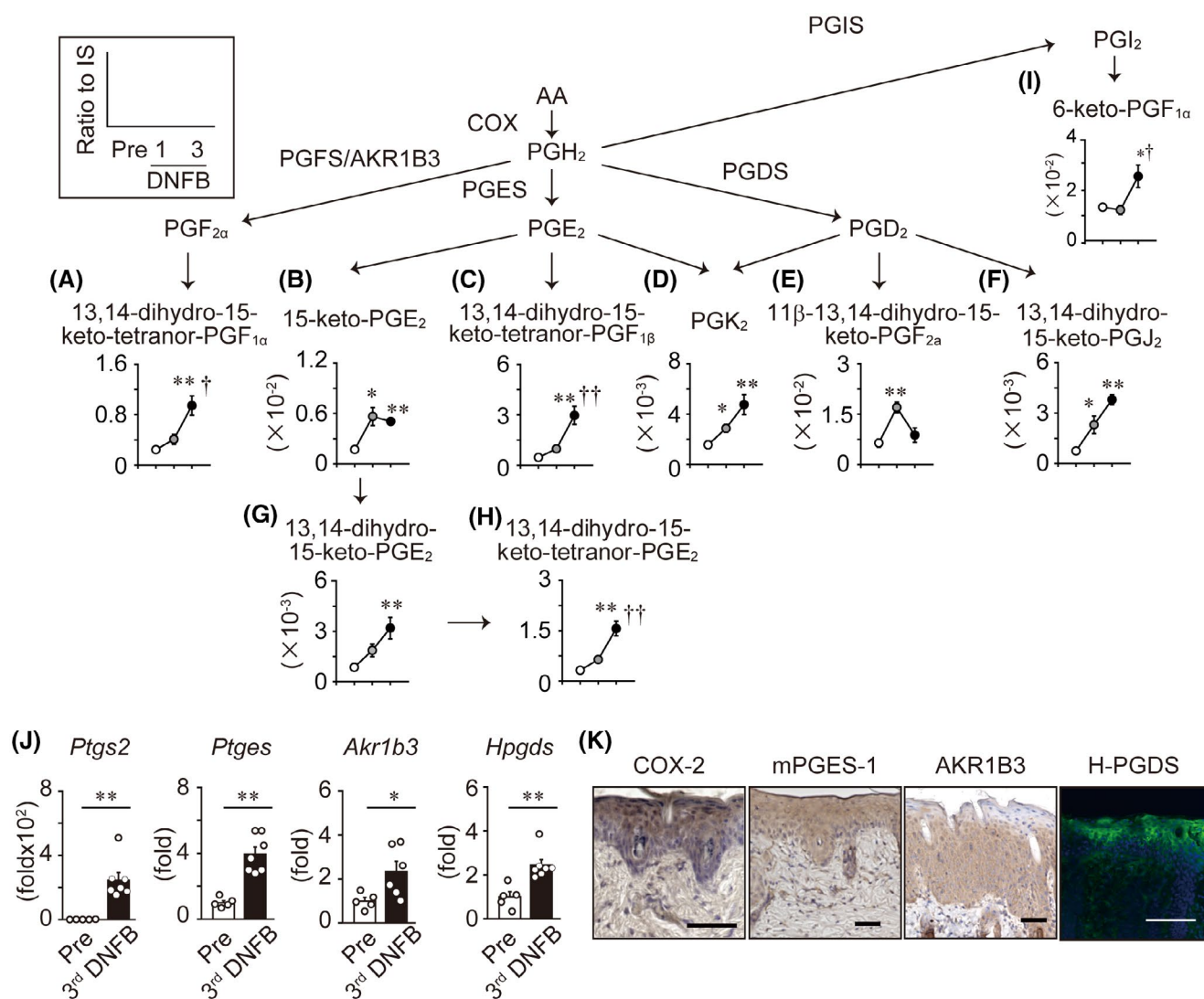


FIGURE 2 The urinary levels of lipid metabolites in AD murine model. (A–I) AA-derived lipid metabolite levels mediated by COX in the urine of AD mice. Data are expressed ratio to internal control and presented as mean $\pm$ SE ($n = 6–8$ each). * $p < .05$, ** $p < .01$, compared with before stimulation. $^{\dagger}p < .05$, $^{\dagger\dagger}p < .01$, compared with 1st stimulation. AA, arachidonic acid; COX, cyclooxygenase; PG, prostaglandin (J) Relative mRNA levels and (K) immunostaining for COX-2, mPGES-1, AKR1B3, and H-PGDS from 3rd DNFB-treated skin. Data are represented as mean $\pm$ SE ($n = 5–7$). * $p < .05$ and ** $p < .01$, compared with before stimulation. Scale bar: 50 μ m

respectively.¹⁶ The mRNA expression of *Ptgs2*, *Ptges*, *Akr1b3*, and *Hpgds* significantly increased in severely inflamed skin tissue (Figure 2J). However, the expression levels of *Ptgs1*, *Ptges2*, *Ptges3*, and *ptgds* mRNA were not affected by DNFB treatment (Figure S1A). Consistent with these results, COX-2, mPGES-1, AKR1B3, and H-PGDS proteins were highly expressed in the thickened epidermal layers (Figure 2K). COX-1 immunostaining was weak. Normal serum and vehicle-treated skin preparations showed no positive immune reactivity (Figure S1B). These observations suggest that DNFB-induced AD-like skin inflammation increased the expression of PG synthases in the epidermis, especially in thickened keratinocytes, which resulted in increased levels of PGE₂, PGD₂, and PGF_{2α} in urine.

3.4 | Tape-stripping caused non-allergic skin inflammation in mice

To verify the specificity of these lipid profiles in AD-like, Th2-biased skin inflammation, we generated a tape-stripped, non-allergic skin inflammation mouse model (Figure 3A). Nine days after tape stripping, remarkable epidermal thickening was observed (Figure 3B,C). Neutrophils and mast cells, but not eosinophils, infiltrated the dermis nine days after tape stripping (Figure 3D). *Tslp* mRNA expression tended to increase in severely inflamed skin tissue, whereas the mRNA expression of Th2 cytokines *Il4* and *Il13* remained unaffected by tape stripping (Figure 3E). These results indicate that tape-stripping induced epidermal thickening without Th2 inflammation.

3.5 | The urinary lipid profile of tape-stripped mice

Next, we analyzed the urine samples from days 1 and 9 after tape stripping. In this model, only two metabolites, PGF_{2α} and 13,14-dihydro-15-keto-tetranor-PGF_{1β} (PGE₂ metabolite), were significantly increased in the urine on day 1 (Figure 3F). As opposed to AD-model mice, the levels of these metabolites returned to basal values even in the presence of epidermal thickening (Figure 3B,F). The levels of other urinary metabolites of PGF_{2α} or PGE₂, which strongly increased in the AD model, did not change significantly between groups (Figure 3F). Supporting these findings, we demonstrated that tape stripping did not alter the mRNA expression of *Ptgs2*, *Akr1b3*, and *Hpgds*, except *Ptges* (Figure 3G). Immunostaining showed that it slightly increased the protein expression of AKR1B3 (Figure 3H), but only marginally compared to that induced due to DNFB treatment. These results suggest that

Th2 inflammation, rather than epidermal thickening, is required for the increased levels of PG metabolites in urine.

3.6 | The urinary lipid profile of patients with AD

Finally, we performed a comprehensive analysis of human urine from 13 patients with AD and four control volunteers with non-inflamed skin (Table S1). The mean age of patients with AD was 7.8 ± 3.5 years. The TARC levels, total IgE levels, EASI, and SCORAD index were 3622.6 ± 7664.5 pg/ml, 3561.6 ± 4224.6 IU/ml, 18.2 ± 12.4 , and 47.7 ± 22.8 , respectively.

We focused on metabolites detected at >70% of individuals in the two groups. Similar to the murine AD-like skin inflammation model, AA-COX metabolites (7/15 type) were dominant in the urine of patients with AD (Figure 4A–G) and n-3 fatty acid-derived metabolites were reduced (3/3 type) (Table S5). Notably, 13,14-dihydro-o-15-keto-tetranor-PGF_{1α} (PGF_{2α} metabolite) (Figure 4B), 13,14-dihydro-15-keto-tetranor-PGE₂ (PGE₂ metabolite) (Figure 4E), and 13,14-dihydro-15-keto PGJ₂ (PGD₂ metabolite) (Figure 4F) were significantly increased in the urine of patients with AD. An increase in the PGI₂ metabolite, 15-diketo-13,14-dihydro-PGF_{1α}, was also detected in the urine of patients with AD (Figure 4G). Additionally, PGE₂-metabolites, 20-hydroxy-PGE₂ (Figure 4C),¹⁷ and 17-hydroxyeicosatetraenoic acid (HETE) levels (Figure 4H), increased in the urine of human patients with AD, whereas they remained unchanged in the urine of AD mice. Correlation analysis between these metabolites and the symptom severity was performed. As shown in Table S6, only 15-keto-PGE₂ showed negative correlation with SCORAD in the AD patients. Although, the indexes of AD severity used in this study were not positively correlated with these metabolites, the current sample size was small. Further studies are needed to clarify these points. Taken together, our results suggest that the metabolites of PGD₂, PGE₂, PGF_{2α}, and PGI₂ might increase in the urine of AD patients with Th2 skin inflammation.

4 | DISCUSSION

Complex interactions between skin barrier dysfunctions, allergy/immunity, and pruritus are associated with AD.¹⁸ In this study, using urine from mice with DNFB-induced AD-like skin inflammation and children with AD, LC-MS-based lipidomic analyses demonstrated that AA-derived COX metabolites, especially PGD₂, PGE₂,

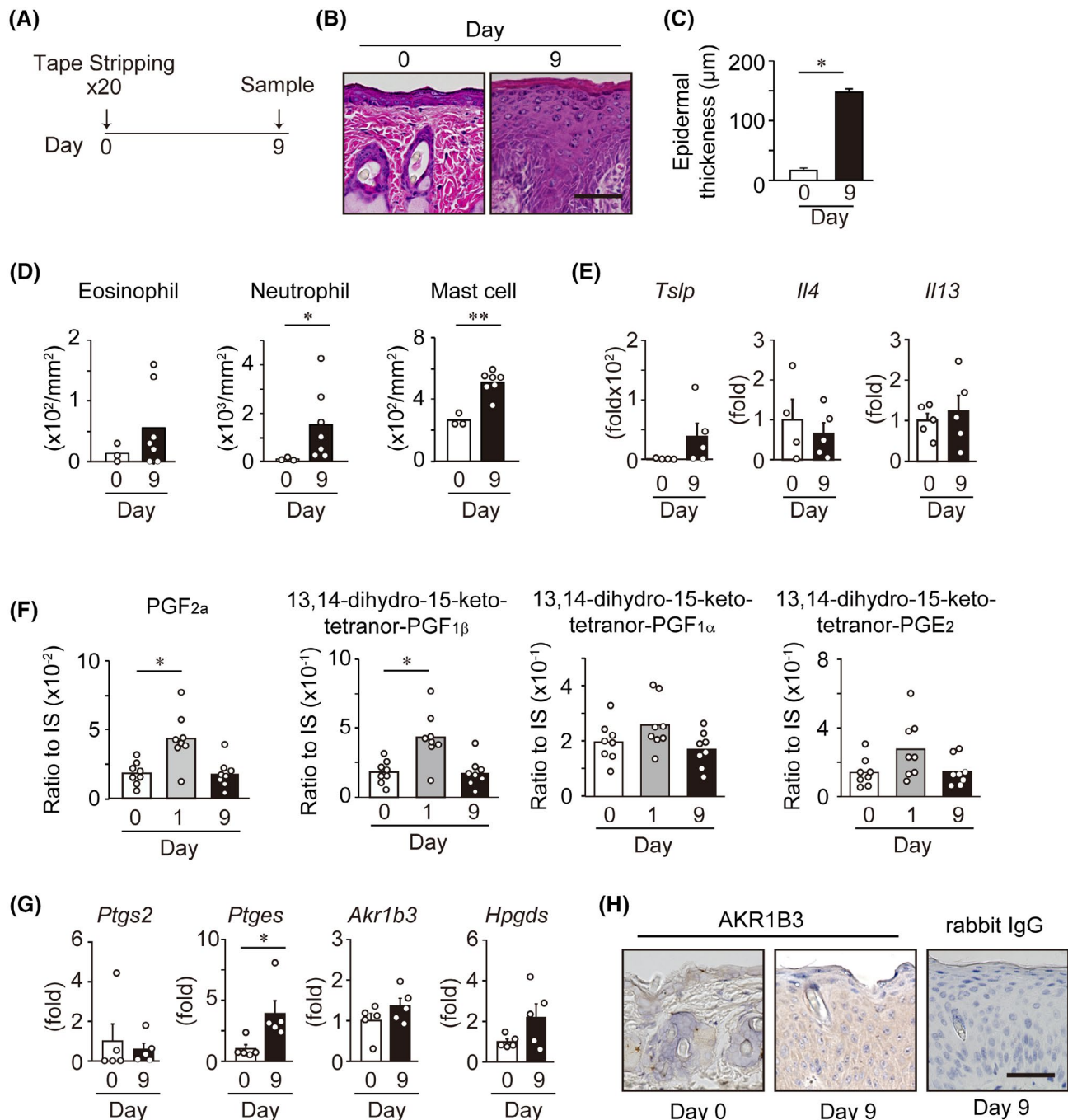


FIGURE 3 Tape-stripping skin inflammation model. (A) Protocol for the tape-stripped skin inflammation model. (B) Representative pictures of H% E-stained dorsal skin. Scale bar: 50 μm . (C) Change in epidermal thickness measured from H&E-stained images. Data are presented as mean $\pm$ SE ($n = 3-7$). * $p < .05$, compared with before stripping. (D) The number of eosinophils, neutrophils, and mast cells from tape-stripped skin. Data are presented as mean $\pm$ SE ($n = 3-7$ each). * $p < .05$, ** $p < .01$, compared with before stimulation. (E) Relative mRNA levels of *Tslp*, *IL4*, and *IL13*. Data are represented as mean $\pm$ SE ($n = 4-5$). (F) AA-COX metabolites in the urine of tape-stripped mice. Data are presented as mean $\pm$ SE ($n = 6-8$). * $p < .05$, compared with before stimulation. (G) Relative mRNA levels of *Ptgs2*, *Ptges*, *Akr1b3*, and *Hpgds*. Data are represented as mean $\pm$ SE ($n = 5$). * $p < .05$, compared with before stripping. (H) Immunostaining for AKR1B3 from tape-stripped skin. Scale bar: 50 μm

and $\text{PGF}_{2\alpha}$ metabolites were elevated in AD urine. These profiles were unique to AD and were not observed in the non-allergic dermatitis model. Although the mechanism by which the Th2 immune response upregulates the

expression of PG synthase is unknown, our results suggest that thickened keratinocytes with allergic (Th2-type) inflammation produced PGD_2 , PGE_2 , and $\text{PGF}_{2\alpha}$ and their metabolites, which were excreted in the urine of AD mice.

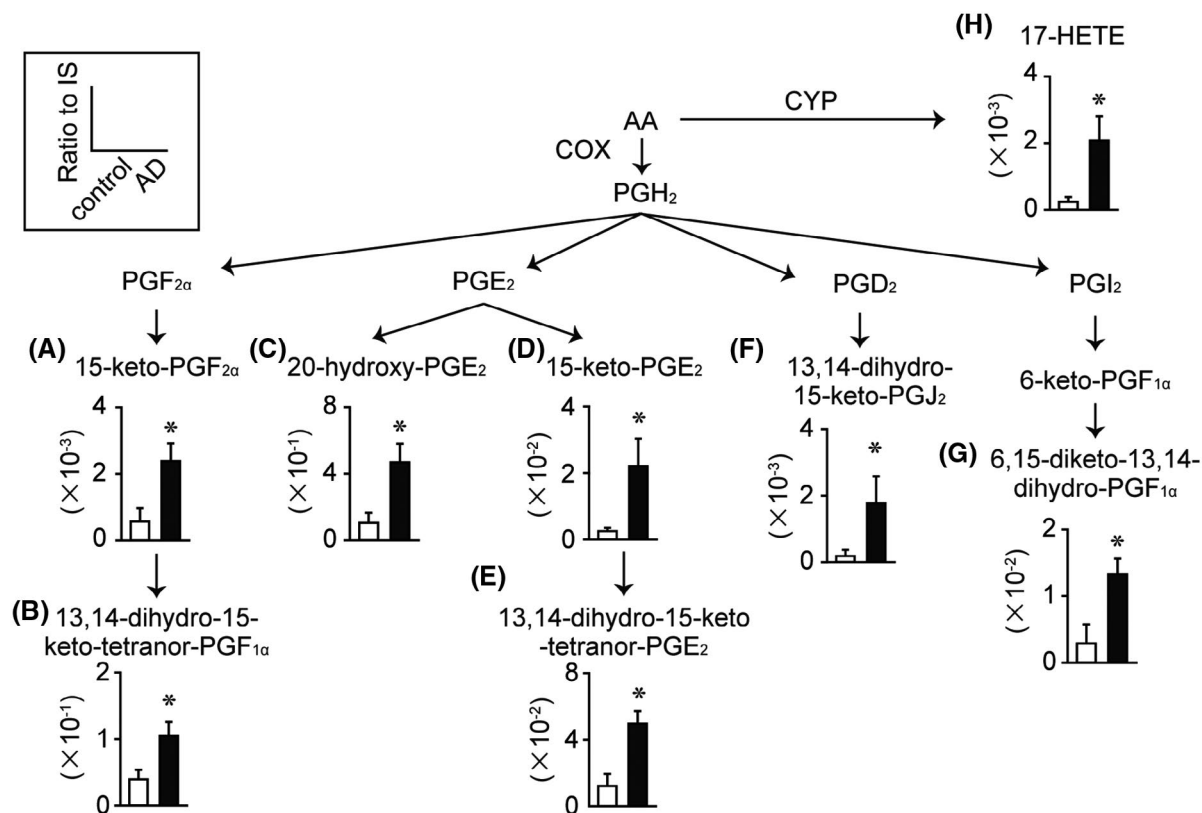


FIGURE 4 The urinary levels of lipid metabolites in patients with AD. The levels of AA-derived lipid metabolites metabolized by COX (A–G) and CYP (H) in the urine of patients with AD. Data are represented as mean $\pm$ SE. * $p < .05$, compared with individual control ($n = 3$ –4) and patients with AD ($n = 10$ –14). AA, arachidonic acid; COX, cyclooxygenase; CYP, cytochrome P450; PG, prostaglandin. HETE; hydroxyeicosatetraenoic acid

Some previous studies have reported the association of PGs with AD. Pentland et al¹⁹ showed that cultured human keratinocytes synthesized and secreted PGE₂, PGD₂, and PGF_{2α}. Fogh et al²⁰ also reported that PGE₂ levels were elevated in the skin biopsies of patients with AD. Moreover, Øymar and Aksnes²¹ reported that higher levels of 9α,11β-PGF₂ (11β-PGF_{2α}) were observed in the urine of children with severe AD than in those without atopy. 11β-PGF_{2α} is produced from PGD₂ via PGFS (AKR1C3),²² and previous studies found that AKR1C3 was upregulated in AD lesions.²³ Consistent with these reports, we showed that mRNA levels of *Pge2*, *Pgd2*, and *Pgf2α* synthases were increased in the skin of AD mice. Additionally, the PGE₂, PGD₂, and PGF_{2α} synthase proteins (mPGES-1, H-PGDS, and AKR1B3, respectively) were highly expressed in AD mouse keratinocytes. These facts suggest that thickened keratinocytes with Th2 inflammation contribute to the urinary lipid profile of AD by producing PGD₂, PGE₂, and PGF_{2α}.

We also detected an increase in PGI₂ metabolites in the urine of DNFB-induced AD mice (6-keto-PGF_{1α}) and human patients (6,15-diketo-13,14-dihydro-PGF_{1α}), but not in the urine of tape-stripped mice. Consistent with this, Sugimoto et al²⁴ reported that PGE₂, PGF_{2α}, and PGI₂

levels increased in the skin of AD mice. Furthermore, a separate study showed that the upregulation of COX-2 expression resulted in an induction of PGIS expression in mouse keratinocytes.²⁵ Since PGI₂ is a well-known inflammatory mediator which causes tissue edema and pain,²⁶ the produced PGI₂ may promote dermatitis.

Interestingly, we observed an increase in 20-hydroxy-PGE₂ and 17-HETE in AD patient urine but not in the urine of AD model mice. Although a previous study showed that 17-HETE inhibited proximal tubule ATPase activity,^{27,28} there is no report showing its involvement in AD pathology. 20-hydroxy-PGE₂ and 17-HETE are produced by CYP4F21¹⁷ and CYP1A1,²⁹ respectively, via sub-terminal (ω-n)-hydroxylation.³⁰ CYP1A1 enzymes are reported to be regulated by the aryl hydrocarbon receptor (AhR), a transcription factor expressed in all types of skin cells.³¹ AhR responds to external environmental factors, such as ultraviolet B exposure, dietary components, and environmental chemicals.³² Presumably, more diverse environmental factors affect patients with AD than the AD murine model, potentially resulting in these additional metabolites being observed in AD patient urine.

We also detected some other lipid metabolites, such as AA-LOX (lipoxygenase), in the urine of AD mice

(N-acetyl LTE₄) and human patients (5-HpETE). A previous study showed that activated eosinophils produce leukotriene C₄ (LTC₄), precursor of N-acetyl LTE₄, in the skin of patients with AD.³³ Neutrophils express 5-LOX, which produces metabolites such as 5-HETE, LTB₄, and cysteinyl leukotrienes.³⁴ Antigen stimulation triggers mast cells to release PGD₂, thromboxane (TX) B₂, 5-HETE, and LTB₄.³⁵ Thus, immune cells, which reside and/or infiltrate skin lesions, can be a source of lipid metabolites in AD urine.

We did not find a significant increase in *Tslp* and Th2 cytokine expression in skin with severe epidermal thickening nine days after stripping. These results suggest that tape stripping causes non-allergic skin inflammation. In the urine of these mice, metabolites of PGD₂, PGE₂, PGF_{2α}, and PGI₂ were not detected. Thus, tape-stripping-induced skin inflammation did not have a significant impact on lipid excretion into the urine.

We found that 13,14-dihydro-15-keto PGJ₂ was the major PGD₂ urine metabolite in human and mouse AD. In contrast, we previously showed that for food allergies, tetranor-PGDM was the major PGD₂ urine metabolite in both species.^{8,9} These findings suggest that lipid metabolism may be specific to disease pathophysiology, and further studies are needed to confirm this.

Here, we showed that an increase in PGF_{2α}, PGE₂, and PGD₂ metabolite levels was unique to the urine of mice with Th2-type skin inflammation and patients with AD. Additionally, we confirmed that urine PGI₂ metabolite and 17-HETE levels were significantly increased in patients with AD. In summary, these findings may help to reveal the pathophysiology of AD and develop noninvasive biomarkers for AD diagnosis.

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DISCLOSURES

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Tatsuro Nakamura and Takahisa Murata designed the experiments. Takahisa Murata and Yukihiro Ohya supervised the project. Nanae Nagata, Yuta Hamasaki, Daiki Horikami, and Koji Kobayashi performed experiments and analyzed the data. Shinichiro Inagaki, Kiwako Yamamoto-Hanada, Yusuke Inuzuka, Tatsuo Shimosawa, Masami Narita, and Yukihiro Ohya

provided patients' samples and clinical information. Nanae Nagata, Tatsuro Nakamura, and Takahisa Murata wrote the manuscript. All authors provided feedback on the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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