Present address

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SUPPORTING INFORMATION

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Activation of the reward system ameliorates passive cutaneous anaphylactic reaction in mice

To the Editor,

Positive emotional states are often associated with better physical health outputs, including cancer survival.[1] Some allergic diseases may also benefit from positive emotional states, as a high placebo effect has frequently been observed in clinical trials of allergic diseases such as allergic rhinitis.[2] However, the causal link between positive emotional states and allergic reaction has not yet been investigated.

The brain's reward system, consisting of the dopaminergic neurons (DNs) in the ventral tegmental area (VTA) and the dopaminergic projections from the VTA to components of the limbic system, mediates positive emotions, expectations, and motivation,[3] which may contribute to the clinical benefit of placebo effects.[4] Given the close relationships between positive emotional states and the reward system, we hypothesized that activation of the reward system may affect allergic reactions. To test this hypothesis, we examined the effects of chemogenetic, spontaneous, and pharmacological activation of the brain reward system on the passive cutaneous anaphylactic (PCA) reaction, a classical model of IgE/mast cell-mediated allergic skin reaction, in mice.

Six-week-old male C57BL/6 mice and tyrosine hydroxylase (Th)-Cre mice housed under 12-hour light/12-hour dark conditions with ad libitum access to food and water were used. All animal experiments were approved by the Institutional Review Board of the University of Yamanashi. For more information, see the Supplementary Methods section.

First, we used the designer receptor exclusively activated by designer drugs (DREADD) system to specifically control reward system activity as previously described.[5] We injected the VTA of Th-Cre mice with a viral vector encoding DREADDs, mutated muscarinic receptors that respond to the synthetic ligand clozapine-*N*-oxide (CNO) but not their endogenous ligand, and the fluorescent reporter mCherry (AAV-hM3Dq); control mice were injected with a viral vector encoding mCherry but lacking DREADDs (AAV-control) (Figure 1A). Five weeks after the viral injections, the mice were treated intraperitoneally with CNO and subjected to the PCA reaction (Figure 1B).

After AAV injection, we assessed the efficacy of virus expression in the VTA by immunohistochemistry for Th, a marker for DNs, as well as mCherry, an indicator of virus expression. DREADDs were specifically expressed in the VTA, but not in the substantia nigra pars compacta (SNc), which also contains DNs, as judged by the expression levels of mCherry and Th (Figure 1C). DREADD (AAV-hM3Dq) expression was observed in >60% of Th-positive area in the VTA, while DREADD expression was less than 10% of Th-positive area in the SNc (Figure S1). The expression levels of mCherry in the VTA were comparable between AAV-control injected mice and AAV-hM3Dq-injected mice (Figure 1C). DNs in the VTA were activated in response to CNO in AAV-hM3Dq-injected but not AAV-control-injected mice, as judged by c-fos expression (an indicator of neuron activation) (Figure 1D). Interestingly, the extent of PCA reaction was significantly smaller in VTA-activated mice than in control mice (Figure 1E). These results suggest a causal link between activation of the brain's reward system and amelioration of the PCA reaction.

Next, we aimed to determine whether natural reward would affect allergic reactions. Rodents exhibit preferential saccharin intake when they are given an ad libitum two-bottle choice between

saccharin solution and water, in association with activation of the VTA and the nucleus accumbens (NAcc), a dopamine projection area from the VTA.[6] We used this experimental system for the present study (Figure 2A). Voluntary saccharin drinking markedly activated DNs in the VTA, as judged by c-fos expression (Figure 2B). The extent of PCA reaction was significantly smaller in mice that engaged in voluntary saccharin drinking than in control mice (Figure 2C). Importantly, administration of similar amounts of saccharin by gastric tube did not affect the extent of PCA reaction (Figure 2D). These results suggest that VTA activation triggered by voluntary saccharin drinking ameliorates the PCA reaction.

Finally, we examined the effects of the dopamine precursor levodopa (L-DOPA) on PCA reaction (Figure S2, A). L-DOPA is converted to dopamine in the brain and is to replenish depleted levels of dopamine in patients with Parkinson's disease. Normally,

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Highlights

The activation of the brain's reward system by chemo-genetics, natural reward, or pharmacological approaches ameliorates IgE/mast cell-mediated allergic reaction in mice. These findings suggest a functional connection between a mood-regulating neuronal circuit and allergic reaction.
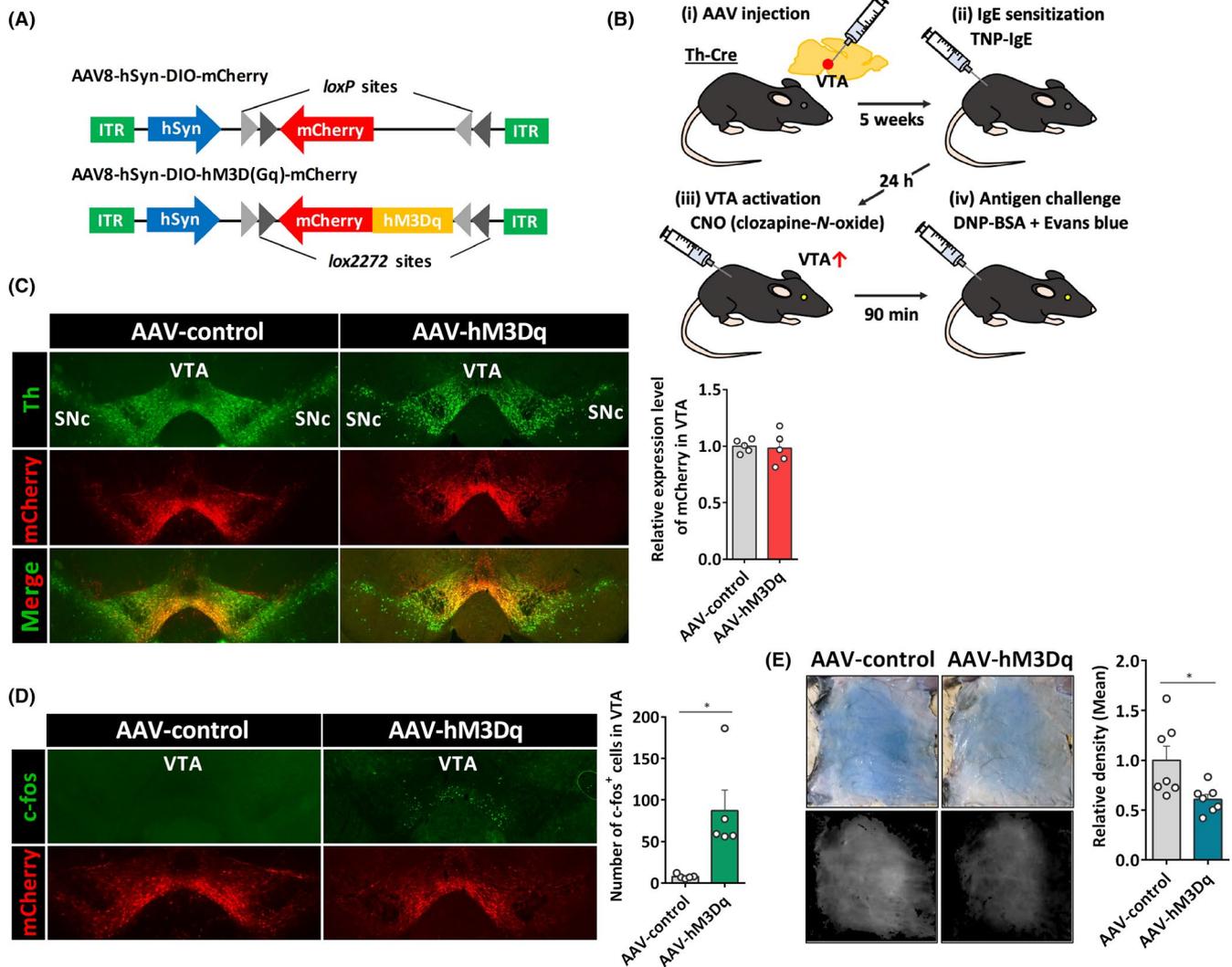

FIGURE 1 Amelioration of PCA reaction by activation of DNs in the VTA using DREADDs. A, Construction of the Cre-dependent DREADD AAV. B, Schematic illustration of the experimental design. C, Representative images of DREADD expression (mCherry: red) in VTA neurons (Th: green) in control (AAV-control) and VTA-activated mice (AAV-hM3Dq) (*left*). Quantitative analysis of mCherry expression in the VTA in control mice (AAV-control) and VTA-activated mice (AAV-hM3Dq) (n = 5) (*right*). D, Representative images of c-fos expression in VTA neurons 90 min after CNO administration in control (AAV-control) and VTA-activated mice (AAV-hM3Dq) (*left*). Quantitative analysis of c-fos⁺ neurons in the VTA of control mice (AAV-control) and VTA-activated mice (AAV-hM3Dq) (n = 5) (*right*). E, Representative images of PCA reaction in the skin of control mice (AAV-control) and VTA-activated mice (AAV-hM3Dq) (*upper panels*), and digitized images used for density value evaluations (black and white) (*lower panels*) **(left)**. Quantitative analysis of the images of PCA reaction **(right)**. Graph shows relative density (n = 7). C-E, Values are shown as means ± SEM. Data were analyzed by Student's t test; *P < .05

(A)

water or 0.05% saccharin
(one-bottle phase)

water-water or water-0.05% saccharin
(two-bottle phase)

anti-TNP IgE

DNP-BSA
with Evans blue

40 min

Assessment

Day 0   Day 4   Day 17 Day 18

(B)

(C)
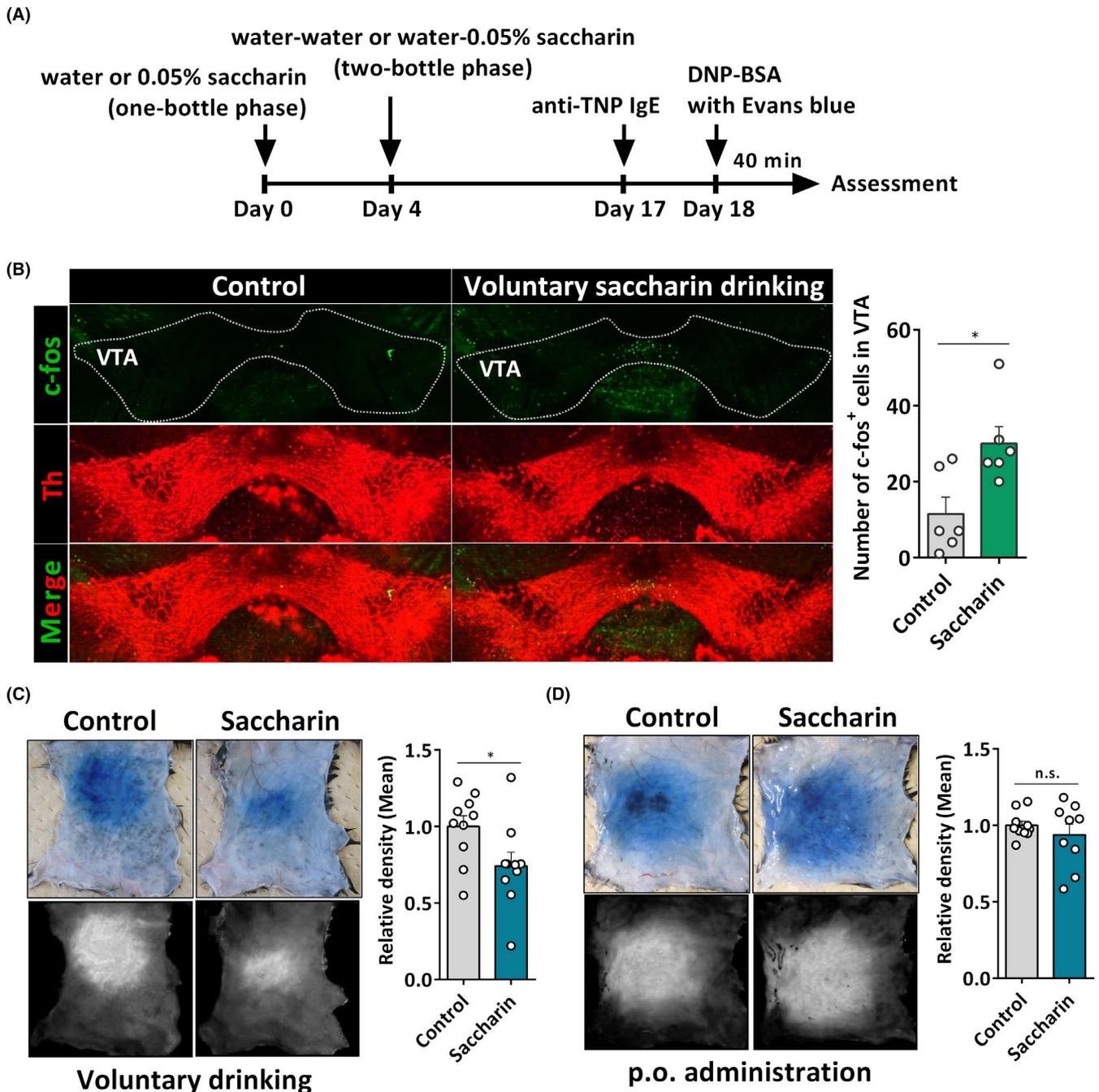

Voluntary drinking

(D)

p.o. administration

FIGURE 2 Amelioration of PCA reaction by spontaneous activation of the reward system with voluntary saccharin drinking. A, Schematic illustration of the experimental design. B, Representative images of c-fos expression in VTA neurons 3 days after voluntary saccharin drinking with a two-bottle choice (*left*). Quantitative analysis of c-fos+ neurons in VTA in control mice and voluntarily saccharin-drinking mice (*right*) (n = 6). C, Representative images of PCA reaction in the skin of control mice and voluntarily saccharin-drinking mice (*upper panels*), and digitized images used for density value evaluations (black and white) (*lower panels*) (*left*). Quantitative analysis of images of the PCA reaction (*right*). (n = 9-10). D, Representative images of PCA reaction in the skin of control mice and mice that received the same amount of saccharin by gastric tube (*left*). Quantitative analysis of images of the PCA reaction (*right*). (n = 9-10). B-D, Values are shown as means ± SEM. Data were analyzed by Student's *t* test; *$P < .05$

L-DOPA is administered with a dopamine decarboxylase inhibitor such as benserazide to prevent conversion of L-DOPA to dopamine in the bloodstream,[7] as dopamine cannot cross the blood-brain barrier. We found that administration of L-DOPA with benserazide decreased the extent of the PCA reaction relative to control mice (Figure S2B). Administration of L-DOPA or benserazide alone did not affect the extent of PCA reaction (Figure S2C,D), suggesting that dopamine levels in the brain, but not in the periphery, are important for suppression of the PCA reaction.

Several studies have reported relationships between dopamine and IgE/mast cell-mediated allergic reactions, including the PCA reaction. However, most of these studies focus on the

peripheral, but not brain (central), activity of dopamine/dopamine receptors, and some of their results are controversial. For instance, Mori et al reported that bone marrow-derived mast cells (BMMCs) express D1-like dopamine receptors and that the antagonist SCH23390 inhibited IgE-mediated degranulation in BMMCs and the PCA reaction in mice,[8] suggesting that dopamine promotes allergic reactions. By contrast, Casale et al reported that intradermal or intravenous dopamine suppressed histamine-mediated cutaneous responses in humans,[9] suggesting that dopamine antagonizes allergic reaction. We wish to emphasize that in this study, we used several independent approaches—DREADDs, spontaneous saccharine drinking, and L-DOPA with benserazide—that selectively or preferentially target the reward system in the mouse brain. This allows us to exclude peripheral or net (ie, both peripheral and central) effects of dopamine/dopamine receptors on the PCA reaction.

The mechanisms linking the reward system and allergic reaction remain to be determined. We observed that plasma histamine levels upon PCA reaction were comparable between VTA-activated and control mice, as well as between voluntarily saccharin-drinking mice and control mice (Figure S3A,B), suggesting that the reward system might affect PCA reaction downstream of mast cell activation. Consistently, activation of the brain's reward system by voluntary saccharin drinking slightly but significantly suppressed plasma extravasation by intradermal injection of histamine in the skin (Figure S4). In addition, we did not find any significant effects of 6-hydroxydopamines (6-OHDA) that chemically ablate peripheral catecholaminergic neurons of the sympathetic nervous system (SNS) on the inhibition of PCA reaction in VTA-activated mice by voluntary saccharin-drinking mice (Figure S5). 6-OHDA did not affect basal PCA reaction in mice (Figure S5). These data suggest that the brain's reward system might affect PCA reaction in part through inhibition of histamine-induced vascular permeability, independent on the SNS.

The extent of control PCA reaction appears to be smaller in virus-injected mice than mice without virus-injected mice (Figure 1E, Figure 2C,D, Figure S6). Thus, viral injection might affect PCA responses perhaps because viral injection affects innate immune response.

The limitations of this study are as follows;

1. Relationships between the reward system and positive emotional states are complex. In addition, the reward function associated with emotions is difficult to measure/interpret reliably in animals. Thus, we emphasize that it is the effects of VAT activation (the reward system activation), but not positive emotion, on PCA reaction that are directly demonstrated in this study.
2. Dopamine neurotransmission exerts different influences on neuronal processes and behaviors at different timescales. Thus, VTA activation by DREADDs, voluntary saccharin drinking, and L-dopa treatment will also affect other brain or physiological system at different time scales, which can also contribute to the observed effects.
3. Affective, cognitive, and reward-related brain circuits that activate VTA and inhibit PCA reaction should be investigated in future studies since the identification of such variables are relevant to real world.

In summary, this study shows that activation of the reward system in the brain by chemogenetic, natural reward, or pharmacological approaches can ameliorate PCA reaction in mice. These findings suggest a functional connection between a mood-regulating neuronal circuit, specifically the reward system, and type I allergic reactions.

KEYWORDS

dopamine, mast cells, passive cutaneous anaphylactic (PCA) reaction, reward system, ventral tegmental area (VTA)

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REFERENCES
1. Pressman SD, Jenkins BN, Moskowitz JT. Positive affect and health: what do we know and where next should we go? *Annu Rev Psychol.* 2019;70:627-650.
2. del Cuvillo A, Sastre J, Bartra J, et al. Placebo effect in clinical trials involving patients with allergic rhinitis. *J Investig Allergol Clin Immunol.* 2011;21(Suppl 3):40-45.
3. Russo SJ, Nestler EJ. The brain reward circuitry in mood disorders. *Nat Rev Neurosci.* 2013;14:609-625.
4. de la Fuente-Fernández R, Ruth TJ, Sossi V, Schulzer M, Calne DB, Stoessl AJ. Expectation and dopamine release: mechanism of the placebo effect in Parkinson's disease. *Science.* 2001;293:1164-1166.
5. Ben-Shaanan TL, Azulay-Debby H, Dubovik T, et al. Activation of the reward system boosts innate and adaptive immunity. *Nat Med.* 2016;22:940-944.
6. Dudek M, Abo-Ramadan U, Hermann D, et al. Brain activation induced by voluntary alcohol and saccharin drinking in rats assessed with manganese-enhanced magnetic resonance imaging. *Addict Biol.* 2015;20:1012-1021.
7. Shimozawa A, Fujita Y, Kondo H, et al. Effect of L-DOPA/Benserazide on propagation of pathological α-synuclein. *Front Neurosci.* 2019;13:595.
8. Mori T, Kabashima K, Fukamachi S, et al. D1-like dopamine receptors antagonist inhibits cutaneous immune reactions mediated by Th2 and mast cells. *J Dermatol Sci.* 2013;71:37-44.
9. Casale TB, Shelhamer JH, Parrillo JE, Kaliner MA. Dopamine inhibition of histamine-mediated cutaneous responses. *J Allergy Clin Immunol.* 1984;73:837-841.

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Dysregulation of microRNA expression in the skin during cutaneous adverse drug reactions

To the Editor,

The diagnosis of drug reaction with eosinophilia and systemic symptoms (DRESS), is challenging due to its heterogeneous clinical presentation and complex natural course.[1] It is therefore sometimes difficult, at an early stage, to differentiate between maculopapular exanthema (MPE) and DRESS due to overlapping clinical presentations.[2,3]

To better characterize cutaneous adverse drug reactions (cADRs), several approaches have been proposed. MicroRNAs (miRNAs) are short noncoding RNA molecules usually composed of 18-25 nucleotides. Their binding to target mRNAs usually results in degradation or translational inhibition. Multiple studies have explored the potential usefulness of miRNA expression profiles as biomarkers for the diagnosis and prognosis of cancers and inflammatory and allergic diseases.[4]

Serum miR-18a-5p expression has been described as a biomarker of severity,[5] and serum miR-124 has been proposed as a disease activity marker[6] of toxic epidermal necrolysis, a severe cADR. However, there is no study using miRNA-based approaches in MPE and DRESS. We aimed to identify miRNAs differentially expressed in the skin and blood of patients with MPE and DRESS compared to healthy controls (HCs) to assess whether miRNA expression was able to accurately distinguish DRESS and MPE and to identify molecules potentially implicated in their pathogenesis.

Detailed information on the included patients, experimental methodology, different steps of miRNA filtering and normalization, and statistical methods is available in Appendix S1. Briefly, skin and blood samples were obtained from MPE (n = 6) patients, DRESS (n = 6) patients and HCs (n = 6). The diagnosis of DRESS was defined according to RegiSCAR criteria with a score > 5 (definite case).[3] Culprit drug, clinical and biological characteristics, and skin tests are reported in Table 1.

For skin samples, total RNA was extracted, allowing quantification of 754 miRNAs. Quantitative PCR analysis of skin expression levels between MPE and DRESS patients compared to HCs was based on fold change (FC = $2^{(-\Delta\Delta CT)}$). Principal component analysis (PCA) of the final 631 detectable miRNAs showed that the skin miRNA profile clearly separated HCs from MPE and DRESS patients, but MPE and DRESS samples were not completely distinguishable using expression of the 631 miRNAs (data not shown). Using non-supervised hierarchical clustering analysis, we identified 34 differentially expressed miRNAs between DRESS patients and HCs, with a fold change <2 or >2 and adjusted *P*-value < .05 (Figure 1A). Of these, 24 were overexpressed and 10 were downregulated. Conversely, 4

Abbreviations: cADR, atopic dermatitis; DRESS, drug reaction with eosinophilia and systemic symptoms; HC, healthy controls; MiRNA, microRNA; MPE, maculopapular exanthema; PCA, principal component analyses.