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Title

Hsa-mir-144-3p is increased in umbilical cord serum of infants developing atopic dermatitis

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Disclosure of potential conflicts of interest

None

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Key messages

- Hsa-miR-144-3p is elevated in the umbilical cord serum of children who develop atopic dermatitis at 1 year of age
- In keratinocytes, hsa-miR-144-3p increases the nuclear translocation of the NF- κ B p65 subunit in response to house dust mite and IL-17 stimulation
- These findings imply that hsa-miR-144-3p may not only herald the early onset of AD, but may also play a role in lowering the threshold for inflammation in keratinocytes

Capsule Summary

Elevated hsa-miR-144-3p levels in umbilical cord serum may lower the threshold for allergen-induced skin inflammation. This gives insight in to possible mechanisms

promoting early life atopic dermatitis and highlights the importance of skin barrier protection.

Keywords

atopic dermatitis, microRNAs, umbilical cord serum, keratinocytes

Abbreviations

AD	Atopic dermatitis
ABCA1	ATP-binding cassette transporter A1
CS	Cord serum
dCt	Delta cycle threshold
hBD	Human beta defensin
HDM	House dust mite
MiRNAs	MicroRNAs
MS	Maternal serum
NF- κ B	Nuclear factor kappa B
OPLS-DA	Orthogonal Projections to Latent Structures - Discriminant Analysis
SERPIN	Serine protease inhibitor
STAT3	Signal transducer and activator of transcription 3

To the Editor

Atopic dermatitis (AD) is a common chronic inflammatory disease of childhood. Up to 60% of cases appear during the first year of life and most resolve during the first few years (1). The skin in AD is characterized by several abnormalities in barrier function including altered lipid composition, decreased number of tight junctions, altered protease activity and altered anti-microbial activity (2). The dual exposure to allergen hypothesis suggests that tolerance to antigens occurs in the neonate through high-dose oral exposure and allergen sensitization occurs through low-dose cutaneous exposure (3). This makes AD a gateway for the allergic march highlights the importance of preventing infantile AD. However, little is known about the initial changes that specifically occur in keratinocytes, which lead to barrier dysfunction. MicroRNAs (miRNAs), which are short non-coding RNAs involved in post-transcriptional gene regulation, have been implicated in the pathogenesis of many diseases including allergic diseases (4). In this study, we aimed to understand whether serum miRNAs expressed in the peri-natal period could influence the development of AD in infancy.

The samples for this study were obtained from a birth cohort at high risk for developing allergic diseases (unpublished data). Details of the cohort study are provided in this article's Methods section in the Online Repository. To identify miRNA changes seen in maternal serum (MS) and umbilical cord serum (CS) of children with AD, we selected a nested case-control population of 36 children who did (AD) or did not develop AD (No AD) at 1 year of age and their mothers by propensity score matching. The baseline characteristics of the infants and their mothers in the nested case-control population were similar to those of the cohort population (see this article's tables E1 and E2 in the Online Repository at www.jacionline.org).

Firstly, we determined the expression levels of 179 miRNAs in CS and MS by qPCR. To understand the association between miRNAs in CS and MS and to distinguish between miRNAs that may or may not be associated with AD, the pool of dCt values obtained from qPCR was subjected to Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA). Figure 1, A shows the distinct expression patterns of miRNAs in CS and MS, describing 32.7% ($R^2X = 0.327$) of variation in X, 96.3% ($R^2Y = 0.963$) of the variation in Y and predicting 93.3% ($Q^2Y = 0.933$) of the variation Y. As OPLS-DA analysis could not distinguish miRNA profiles for AD and no AD groups, we analyzed miRNAs in CS and MS separately and identified 24 miRNAs in CS and 23 in MS with a VIP (Variable Importance in Projection) score of 1.5 or more that were independently associated with the development of AD (Fig 1, B and see this article's table E3 in the Online Repository at www.jacionline.org). As there was no association between miRNA expression in MS and CS, suggesting that maternal miRNAs may not have a direct effect on the baby (see this article's Fig E1 in the Online Repository at www.jacionline.org), we further evaluated the candidate miRNAs in CS. Comparison of their expression levels in AD and no AD groups showed that hsa-miR-144-3p (miR-144) was increased in CS of children who developed AD at 1 year of age. This change was not seen in MS nor in serum at 1 year of age (Fig 1, C). MiR-144 expression in CS was not associated with total IgE levels at 1 year of age (see this article's Fig E2 in the Online Repository at www.jacionline.org), that miR-144 might play a role in the pathogenesis of AD independent of the atopic status. Therefore, we focused on the role of miR-144 in keratinocytes.

Using web-based software (microRNA.org, TargetScan and miRBase), we identified ATP-binding cassette transporter A1 (ABCA1) as a target of miR-144. ABCA1 is involved in cholesterol efflux from cells and miR-144 has been shown to

downregulate ABCA1 expression leading to cholesterol accumulation within cells (4). To observe the effect of miR-144 on the expression of ABCA1 in keratinocytes, we transfected neonatal human primary epithelial keratinocytes (referred to as keratinocytes hereafter) with miR-144. Transfected keratinocytes showed decreased ABCA1 mRNA expression (see this article's Fig E3, A in the Online Repository at www.jacionline.org), decreased ABCA1 protein levels and cholesterol accumulation (see this article's Fig E3, B in the Online Repository at www.jacionline.org) demonstrating the role of miR-144 in disrupting the cholesterol composition of the cell membrane in keratinocytes.

ABCA1 knockout in macrophages has been shown to exaggerate cytokine responses to TLR agonists through nuclear factor kappa B (NF- κ B) and activator protein-1 signaling (5). In response to stimulation with house dust mite extract (HDM), miR-144 transfected keratinocytes increased the nuclear translocation of NF- κ B p65 subunit (Fig 2, A).

We next looked at molecules downstream of NF- κ B activation that may play a role in the pathogenesis of AD. As reported previously, stimulation of AD skin with HDM induces the expression of many cytokines and inflammatory markers including IL-17A and the anti-microbial peptide (AMP) hBD-2 encoded by *DEFB4A* (6). Although hBDs, at low concentrations, can eliminate cutaneous pathogens, at high concentrations they act as chemo-attractants to Th2 cells and promote mast cell degranulation (7). When we looked at the mRNA expression of several AMPs in keratinocytes, only hBD-2 mRNA levels showed a significant increase in response to miR-144 and HDM stimulation (Fig 2, B and see this article's Fig E4 in the Online Repository at www.jacionline.org). This was also seen in response to IL-17A stimulation (Fig 2, B and see this article's Fig E5 in the Online Repository at

www.jacionline.org) and was abrogated by co-incubation with JSH-23, a specific inhibitor of p65 translocation (Fig 2, B). We could not detect the mRNA expression of CXCL1 and CXCL2 with HDM or IL-17A stimulation.

To look at immune-related pathways activated by IL-17A, we evaluated the mRNA expression of STAT3, IL-23p19, SERPINB3 and SERPINB4. In response to miR-144 and IL-17A, SERPINB3 and SERPINB4 showed a significant increase in mRNA expression. This increase was also inhibited by JSH-23, showing that both molecules were downstream of IL-17A-mediated NF- κ B activation (Fig 2, C).

SERPINB4, a known enhancer of cell growth and inhibitor of apoptosis, has been shown to be important in initiating allergen-induced acute skin inflammation in a mouse model with more recent studies correlating serum SERPINB4 with AD severity and serum TARC levels in pediatric age groups (8, 9).

These results show that increased miR-144 can activate the NF- κ B pathway leading to the increase in hBD-2 and SERPINB4, two molecules that have a pro-inflammatory role in AD. Although this study is limited by being *in vitro*, these findings raise the possibility that miR-144 may be able to lower the threshold for skin inflammation and provides preliminary insight in to what prompts early-life AD.

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Figure legends

Figure 1. MiR-144 levels are elevated in the cord serum of infants who developed AD at 1 year of age. (A) The expression of 179 miRNAs in the CS and MS of each subject was characterized by qPCR. OPLS-DA was used to discriminate the expression patterns in CS and MS. (B) The expression levels of all miRNAs with a VIP score ≥ 1.5 in CS and MS are plotted against their VIP score. MiR-144-3p showed the highest expression in CS. (C) MiR-144 levels were estimated in CS, MS and serum at 1 year of age by qPCR. MiR-144 expression was increased in the CS of the AD group, but not in MS or serum at 1 year of age. CS, cord serum; MS, maternal serum; VIP score, Variable Importance in Projection score. $^{**}P < 0.01$, Mann-Whitney test. AD n = 18; No AD n = 18.

Figure 2. MiR-144 induces nuclear translocation of NF- κ B p65 subunit translocation. (A) Representative staining of the NF- κ B p65 subunit in keratinocytes. MiR-144 induced the nuclear translocation of p65 (green). This was enhanced by miR-144 translocation prior to HDM stimulation and abrogated by JSH-23 (p65 translocation inhibitor). Nucleus stained blue with DAPI. (B and C) MiR-144 increases hBD-2 expression in response to treatment with HDM and IL-17A. Keratinocytes were transfected with miR-144 for 48 hours and stimulated with HDM or IL-17A for a further 24 hours with or without JSH-23. The hBD-2 mRNA levels were quantified by qPCR. $^{*}P < 0.05$, $^{**}P < 0.01$, Kruskal-Wallis test. Means + SEMs pooled over 3 independent experiments are shown.

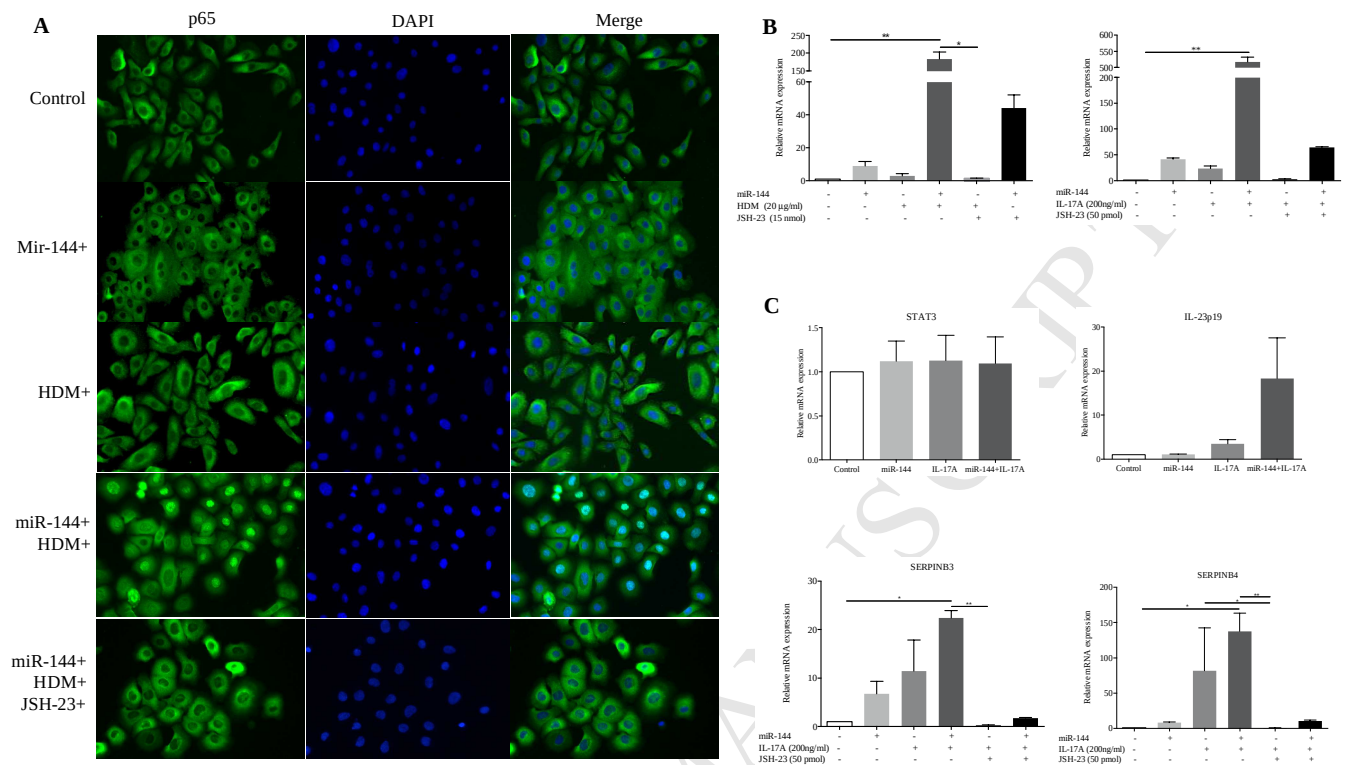
Figure 2

Table E1. Baseline characteristics of the infants and their mothers in the cohort population and the nested case-control population defined by propensity score matching. Maternal age, parity, BMI, gestational age, and birth weight were all compared by Student's t-test, and serum total IgE levels by Mann-Whitney test. Other variables were compared by Pearson's χ^2 test.

	Cohort population (n = 268)	Study population (n = 36)	P value
	Mean ($\pm$ SD), %	Mean ($\pm$ SD), %	
Maternal characteristics			
Age	33.51 ($\pm$ 4.67)	34.25 ($\pm$ 4.66)	0.357
Parity	1.55 ($\pm$ 0.71)	1.64 ($\pm$ 0.87)	0.769
History of AD	25.0	27.8	0.688
History of other allergic diseases	86.6	83.3	0.608
BMI before pregnancy	21.43 ($\pm$ 3.16)	21.6 ($\pm$ 2.83)	0.502
BMI during pregnancy	25.06 ($\pm$ 3.12)	25.29 ($\pm$ 2.96)	0.577
Total IgE	210.87 ($\pm$ 508.29)	104.94 ($\pm$ 135.07)	0.213
Affected by AD	1.5	2.8	0.470
Affected by bronchial asthma	2.2	8.3	0.078
Infant characteristics			
Gender (girls)	50.4	41.7	0.377
Cesarean section	29.5	30.6	1.000
Gestational age (weeks)	38.88 ($\pm$ 1.39)	38.61 ($\pm$ 1.23)	0.180
Birth weight (g)	3026 ($\pm$ 360.76)	3023.00 ($\pm$ 317.47)	0.830
Total IgE at birth	0.74 ($\pm$ 1.53)	0.4 ($\pm$ 0.46)	0.051
Total IgE at 1 year	45.22 ($\pm$ 84.56)	46.92 ($\pm$ 75.63)	0.849

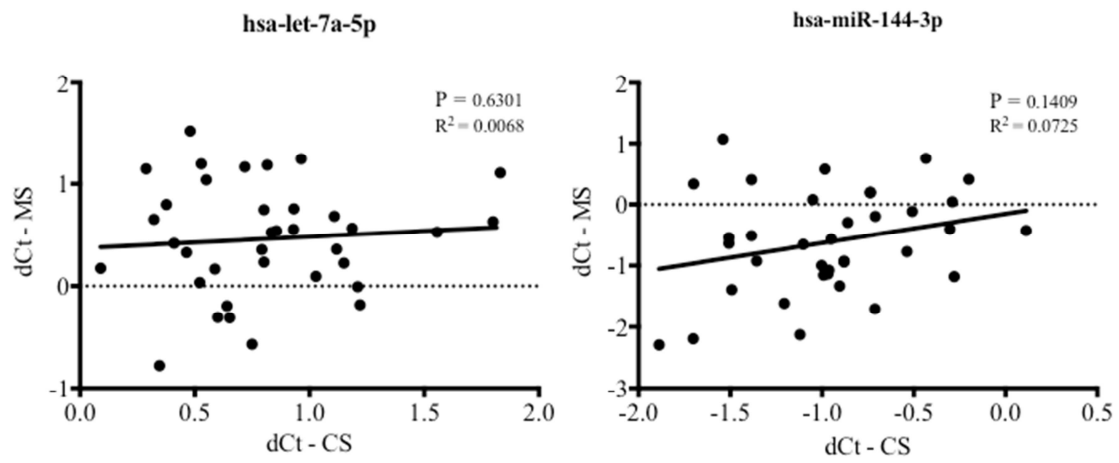
Table E2. Background information of the nested case-control population

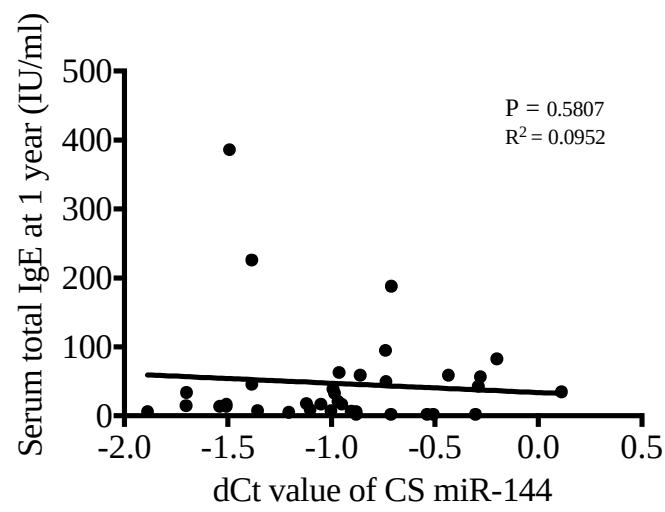
Case ID	Infants					Mothers					
	Gender	Gestational age (weeks)	Birth weight (g)	Total IgE (IU/mL)	AD	Maternal age (y)	History of AD	History of allergic disease	AD	Asthma	Total IgE (IU/mL)
C019	Boy	37	2710	0.32	+	40	-	+	-	-	305.0
C038	Girl	39	2848	0.19	-	26	-	+	-	-	12.8
C051	Boy	38	2660	0.09	-	36	-	+	-	-	2.5
C052	Girl	38	3180	0.64	+	31	-	+	-	-	136.0
C062	Boy	41	3244	0.08	+	36	-	+	-	-	6.8
C066	Boy	38	3012	1.34	+	30	+	+	+	-	80.4
C069	Girl	39	2956	0.10	+	26	+	+	-	-	11.3
C070	Boy	38	3380	0.13	-	35	-	+	-	-	10.0
K007	Boy	38	2952	0.04	-	40	-	-	-	-	2.5
K008	Girl	38	3190	0.26	+	25	-	+	-	+	62.5
K013	Boy	39	2706	1.98	+	39	-	-	-	-	48.4
K032	Boy	39	3082	0.28	+	31	-	-	-	-	87.8
K040	Boy	36	2464	0.31	-	37	-	+	-	-	31.1
K045	Boy	38	2412	0.29	-	36	-	+	-	-	131.0
K059	Girl	38	2990	0.04	-	38	+	+	-	-	9.6
K074	Girl	40	2912	0.18	+	33	+	+	-	-	48.0
K078	Boy	36	3110	0.45	+	39	-	+	-	-	104.0
K079	Boy	38	3390	0.10	-	38	-	+	-	-	13.2
K083	Girl	39	2642	0.04	-	30	+	+	-	-	13.6
K085	Boy	39	3712	0.70	+	39	-	-	-	-	123.0
K095	Boy	39	2984	0.13	+	36	-	+	-	-	11.4
K102	Boy	40	3508	0.30	-	35	-	-	-	-	144.0
K107	Boy	38	3522	0.50	-	42	+	+	-	-	187.0
K112	Boy	38	2604	0.25	+	30	-	+	-	-	126.0
K132	Boy	40	3202	0.17	+	38	-	+	-	-	42.3
K138	Boy	37	3274	0.22	+	27	+	+	-	-	113.0
K144	Boy	41	3536	0.11	+	34	-	+	-	+	27.6
K146	Girl	37	2802	0.30	-	30	-	+	-	+	304.0
K180	Girl	40	2890	0.62	-	28	-	+	-	-	688.0
K196	Girl	39	2832	0.04	+	40	-	-	-	-	2.5
K197	Girl	40	3190	0.23	+	37	+	+	-	-	104.0
K199	Girl	39	2940	0.65	-	39	-	+	-	-	255.0
K205	Girl	40	3468	0.26	-	36	-	+	-	-	14.2
K207	Girl	38	2770	1.15	-	35	+	+	-	-	313.0
K209	Boy	38	2904	1.67	-	31	+	+	-	-	165.0
K228	Girl	40	2850	0.16	-	30	-	+	-	-	41.4

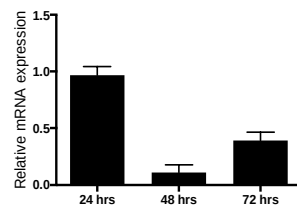
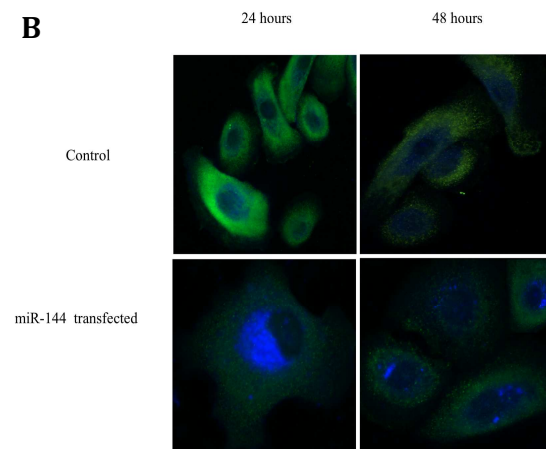
Table E3. MicroRNAs associated with the development of AD at 1 year.

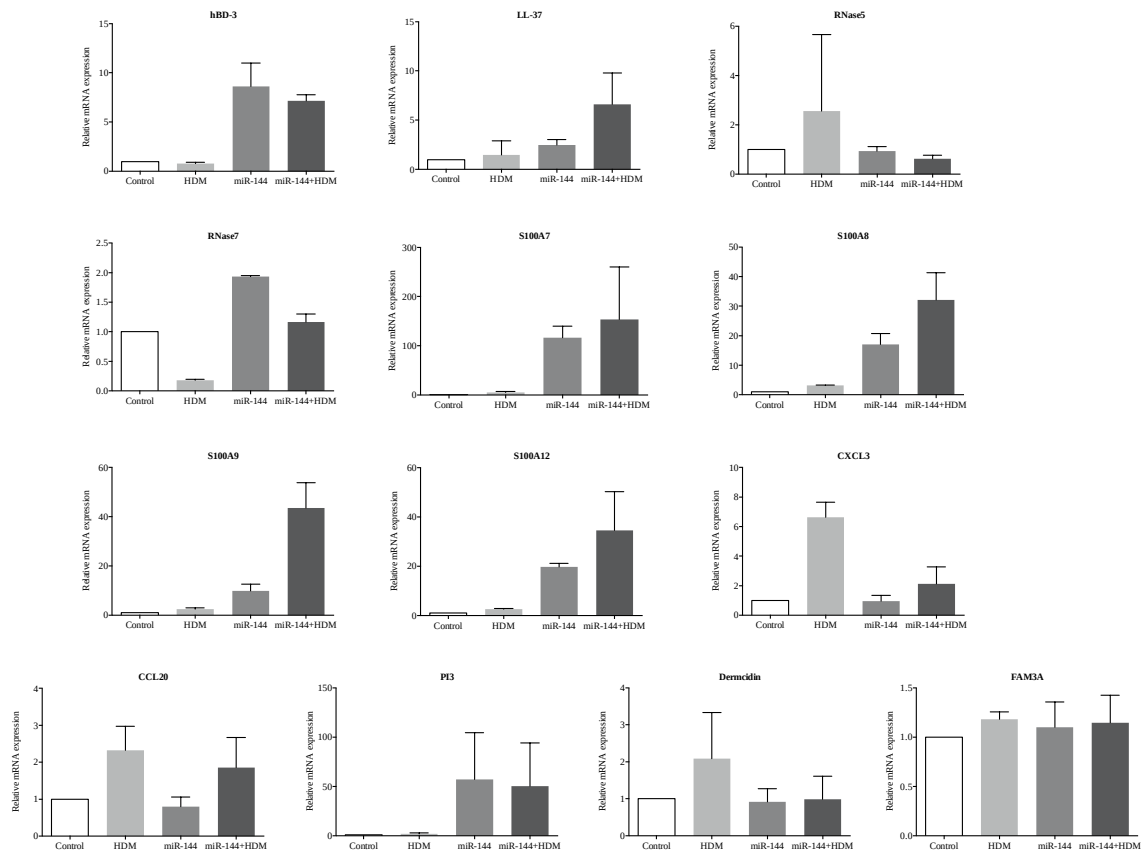
OPLS-DA was used to identify miRNAs associated with the development of AD at 1 year of age. The miRNAs in umbilical cord serum (CS) and maternal serum (MS) with a VIP score over 1.5 were selected as candidates for further analysis.

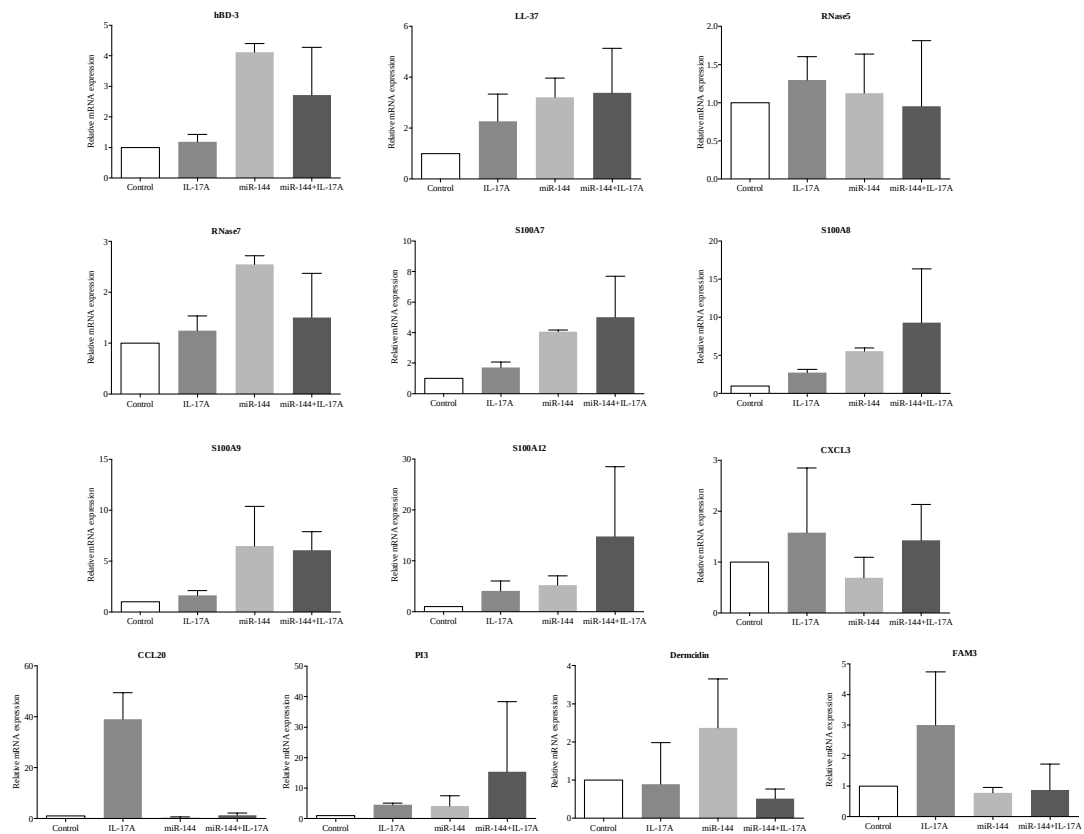
miRNAs in MB (n = 36)				miRNAs in CB (n = 36)			
	VIP score	VIP SE	Coefficient		VIP score	VIP SE	Coefficient
hsa-miR-301a-3p	2.854	1.594	0.051	hsa-miR-361-3p	2.512	1.726	0.046
hsa-miR-133b	2.732	0.713	-0.054	hsa-miR-144-3p	2.500	0.811	-0.037
hsa-miR-133a	2.676	1.019	-0.049	hsa-miR-195-5p	2.484	1.706	0.043
hsa-miR-424-5p	2.407	0.538	0.031	hsa-miR-150-5p	2.257	0.733	0.039
hsa-miR-27b-3p	2.256	1.341	0.030	hsa-miR-423-3p	2.191	1.405	0.042
hsa-miR-485-3p	2.196	0.437	0.020	hsa-miR-146b-5p	1.816	0.826	0.028
hsa-miR-148b-3p	2.146	0.760	0.031	let-7i-5p	1.803	0.515	0.034
hsa-miR-142-3p	1.992	0.749	0.021	hsa-miR-30d-5p	1.758	1.934	0.024
hsa-miR-101-3p	1.943	0.547	0.017	hsa-miR-107	1.749	1.398	-0.030
hsa-miR-15a-5p	1.937	0.967	0.020	hsa-miR-500a-5p	1.732	1.143	-0.030
hsa-miR-296-5p	1.933	0.914	-0.025	hsa-miR-151a-3p	1.717	2.235	0.014
hsa-miR-128	1.912	1.230	0.018	hsa-miR-200c-3p	1.706	1.556	0.039
hsa-miR-21-5p	1.866	0.984	0.010	hsa-miR-162-3p	1.699	1.518	0.025
hsa-miR-141-3p	1.854	1.329	0.019	hsa-miR-191-5p	1.688	1.618	0.025
hsa-miR-30d-5p	1.812	1.902	0.028	hsa-miR-532-3p	1.682	0.521	-0.020
hsa-miR-191-5p	1.785	0.715	-0.019	let-7g-5p	1.678	0.866	0.021
hsa-miR-320a	1.780	1.126	0.017	hsa-miR-181a-5p	1.660	1.341	0.022
hsa-miR-154-5p	1.775	0.892	-0.029	hsa-miR-342-3p	1.648	1.038	0.028
hsa-miR-382-5p	1.776	1.037	-0.026	hsa-miR-133a	1.593	1.996	-0.032
hsa-miR-126-3p	1.654	1.024	0.010	hsa-miR-505-3p	1.575	1.178	0.028
hsa-miR-215	1.654	0.630	0.016	hsa-miR-335-5p	1.544	2.076	0.018
hsa-miR-375	1.626	1.334	0.027	hsa-miR-376a-3p	1.510	2.177	0.025
hsa-miR-766-3p	1.618	1.255	-0.018	hsa-miR-605	1.509	2.093	-0.033
let-7i-3p	1.539	0.918	0.025				





A**B**





Supplementary data

METHODS

Study design

The Chiba High risk Birth cohort for Allergy (CHIBA) study of newborn infants with a family history of allergic disease was set up in Seikei-kai Chiba Medical Center and Chiba University Hospital in Chiba, one of the prefectures around the Tokyo region, from January 2010 to December 2011. This study protocol was approved by the Bioethics Review Committee, Faculty of Medicine, Chiba University, Japan (approval number 754).

Study participants

The study was advertised by use of a poster at the above hospitals and all women attending the above hospitals who were at 34 weeks of gestation, who, whose partner, and/or their children had any allergic diseases such as atopic dermatitis, asthma, allergic rhinitis, and/or food allergies were invited to participate in the study.

The eligibility criteria were:

- a. Japanese ethnicity
- b. Parent/s and/or sibling/s of the expected child has one or more allergic diseases
- c. Not having or expected to have any severe congenital abnormalities such as congenital heart disease

The mothers and their expected babies were enrolled in the study after obtaining informed consent. Following the birth of the study subjects, we reconfirmed the subject's

eligibility, and there were no babies excluded due to congenital abnormalities. We did not exclude babies having any perinatal abnormalities. The guardians were requested by letter to take the study subjects to each clinic at 1 month, 6 months, 1 year, and 2 years of age for physical examinations.

Questionnaires

At 1 month, 6 months, 1 year, and 2 years of age, the guardians of the study subjects were interviewed about the health of their children (symptoms or a diagnosis of allergic diseases), breast-feeding, exposure to second-hand tobacco smoke at home, and exposure to dogs or cats. Diagnosis of wheezing was made by history of 3 or more episodes of wheezing or by the subject's family doctor. Diagnosis of both allergic rhinitis and food allergies was made by the subject's family doctor.

Physical examination

Physical examinations were performed at 1 month and 6 months of age, by Dr. Naoki Shimojo at Chiba University Hospital or by Dr. Hiroko Suzuki at Seikei-kai Chiba Medical Center. Diagnosis of infantile eczema was made by physical examination and/or by the history of itchy eczema on the face by 6 months of age. Physical examination of all participants was repeated 1 year and 2 years of age, by Dr. Naoki Shimojo at Chiba University Hospital, and a diagnosis of AD was made using the guidelines for management of AD by the Japanese Dermatological Association.

Blood sampling and allergen-specific IgE assays

Blood samples were collected from the mothers at 36 weeks of gestation and cord blood, and blood samples at 1 year and 2 years of age were collected from the infants. The serum samples were stored at -30°C within 24 h of collection until measurement of allergen-specific IgEs. Levels of specific IgEs to *Dermatophagoides farinae* (Derf), cat dander and Japanese cedar pollen in the MS and those to egg white, milk, Derf, cat dander and Japanese cedar pollen in umbilical CS and the children's sera at 1 and 2 years of age were measured by ImmunoCAP®. A specific IgE level > 0.7 UA/mL was considered positive.

Cell culture

Neonatal human primary epithelial keratinocytes (referred to as keratinocytes hereafter) were obtained from CELLnTEC Advanced Cell Systems AG (Bern, Switzerland). The cells were cultured in CnT-Prime medium (CELLnTEC #CNT-PR). All cells tested negative for Mycoplasma.

MiRNA transfection

A human miR-144 mimic (C-300612-05-0002), a miRNA positive control for GAPDH (CP-001000-02-05) and a miRNA negative control (CN-001000-01-05) were purchased from Dharmacon (Horizon Discovery, UK). Keratinocytes were transfected with miRNAs using the LipofectamineTM RNAiMAX Transfection Reagent (Invitrogen, USA) by following the manufacturer's instructions. Briefly, keratinocytes were seeded to reach 60-80% confluency at transfection. For triplicates on a 24-well plate, the

LipofectamineTM RNAiMAX reagent was diluted in 50 µl of Opti-MEM[®] medium (Gibco) and added to 3 µl of the 10 mM miRNA diluted in 50 µl Opti-MEM[®], so that the final amount of miRNA in each well was 5 pmol. Cells were incubated with the miRNAs for 24-48 hours.

HDM and IL-17A stimulation of keratinocytes

Keratinocytes were stimulated with 25 µg/ml of freeze-dried HDM (*Dermatophagoides farinae*) or 200 ng/ml of recombinant human IL-17A (BioLegend Inc., San Diego, CA) for a further 24 hours after transfection and then used for qPCR and immunostaining. The freeze-dried HDM extract was kindly provided by Torii Pharmaceutical Co., Ltd. (Tokyo, Japan).

Total RNA extraction and qPCR

Total RNA was extracted from serum using the miRNeasy Serum/Plasma Kit (Qiagen, Hilden, Germany). The initial screening of samples for candidate miRNAs was done using the Exiqon Serum/Plasma Focus microRNA PCR Panel, 96-well (V4.MI) which contains assays for 179 individual miRNAs (Exiqon, Denmark). Total RNA was extracted from all cell types using the miRCURYTM RNA Isolation Kit – Cell & Plant (Exiqon, Denmark). Complementary DNA was synthesized using SuperScript[®] III First-Strand Synthesis SuperMix (Invitrogen, USA) and real-time PCR was performed

with the QuantiTect SYBR Green PCR kit (Qiagen, Germany) on a CFX Connect Real-Time System (Bio-Rad). Primer sequences used in the study are listed in table E4.

Fluorescence microscopy and filipin staining for cholesterol

Keratinocytes were grown to 60-70% confluence on glass coverslips and fixed in 4% paraformaldehyde for 10 minutes. The cells were permeabilized with 0.3% saponin for 20 minutes, washed and blocked with 3% BSA in PBS for 1 hour. The cells were stained overnight with a 1:200 dilution of rabbit polyclonal antibodies to ABCA1 (ab7360, Abcam, UK). Goat polyclonal Alexa Fluor[®] 488-conjugated antibodies to rabbit IgG (ab150077, Abcam, UK) were used as secondary antibodies and incubated for 1 hour at a 1:300 dilution. Staining for cholesterol was done for a further 30 minutes with a working solution of 0.05 mg/ml Filipin III (CAS 480-49-9, Cayman Chemical, USA) dissolved in DMSO and diluted in PBS. Imaging was done on a Keyence BZ-X700 (Keyence, Japan).

NF- κ B p65 subunit translocation inhibition assay

Keratinocytes were grown on coverslips as for fluorescence microscopy as mentioned above. Forty-eight hours following transfection, the cells were co-incubated for 24 hours with 25 μ g/ml of HDM extract and 15 nmol of the NF- κ B p65 inhibitor JSH-23 (Calbiochem[®], Merck, Germany) or 200 ng/ml of recombinant IL-17A (BioLegend Inc., San Diego, CA) and 50 nmol of JSH-23. The cells were stained overnight with a 1:200 dilution of rabbit monoclonal antibodies to the NF- κ B p65 subunit (#8242, Cell Signaling Technology, USA). Goat polyclonal Alexa Fluor[®] 488-conjugated antibodies to rabbit IgG (ab150077, Abcam, UK) were used as secondary antibodies at a 1:300 dilution

for 1 hour of staining. Cells were stained with a 300 nM solution of DAPI for 5 minutes. Imaging was done on a Keyence BZ-X700 microscope (Keyence, Japan).

Statistical analysis

When comparing the baseline characteristics of the study participants, maternal age, parity, BMI, gestational age and birth weight were compared by Student's t-test, and serum total IgE levels by Mann-Whitney test (two-sided). Other variables were compared by Pearson's χ^2 test. A *P* value less than 0.05 was considered significant. These analyses were performed using the SAS program (v.19.0, SPSS Inc.)

The Orthogonal Projections to Latent Structures-Discriminant Analysis (OPLS-DA) method was employed for discrimination between MS and CS and the AD and No AD groups using SIMCA 13 (Umetrics, Umeå, Sweden). *R*² and *Q*² values were calculated, where *R*² shows the dispersion of the data from the model and *Q*² shows the predicted variance in the data. The term *Q*² was calculated by 7 fold cross-validation. MiRNAs with a variable importance in projection (VIP) score >1.5 were considered to be influential for the separation of groups in OPLS-DA analysis.

Statistical analysis for results from *in vitro* experiments was done on GraphPad Prism® 6.0f. All data are shown as mean ± SEM. Mann-Whitney Test and the Wilcoxon test were used for comparison of groups (two-sided). All results shown are representative of 3 independent experiments.

Figure legends

FIG E1. MiRNAs in maternal serum (MS) and cord serum (CS) are not correlated.

The expression levels of 179 miRNAs in MS and CS were measured by qPCR. The levels of each miRNA did not show any correlation between MS and CS. The data shows 2 representative miRNAs including miR-144, which was investigated further in this study.

FIG E2. MiR-144 levels in cord serum are not related to the total IgE levels in serum at 1 year of age. The miR-144 levels in cord serum were plotted against the serum total IgE levels at 1 year of age. There was no correlation between the levels.

FIG E3. MiR-144 transfection decreases ABCA1 expression in keratinocytes.

(A) Keratinocytes were transfected with miR-144 for 24 – 72 hours. The ABCA1 mRNA levels were quantified by qPCR. **(B)** Keratinocytes grown in medium only or transfected with miR-144 for 24 and 48 hours were stained with Alexa Fluor 488 for ABCA1 (green) and filipin for cholesterol (blue).

FIG E4. Messenger RNA levels of AMPs induced by HDM stimulation of keratinocytes.

Keratinocytes were incubated with 25 µg/ml HDM for 24 hours with or without miR-144 transfection for 48 hours. The mRNA levels were quantified by PCR. Results show means + SEMs pooled over 3 independent experiments.

FIG E5. Messenger RNA levels of AMPs induced by IL-17A stimulation of keratinocytes. Keratinocytes were incubated with 200 ng/ml of IL-17A for 24 hours with or without miR-144 transfection for 48 hours. The mRNA levels were quantified by PCR. Results show means + SEMs pooled over 3 independent experiments.