

Letter to the Editor

Epidermal thymic stromal lymphopoietin predicts the development of atopic dermatitis during infancy

To the Editor:

To establish a primary prevention strategy for atopic dermatitis (AD), it is important to identify biomarkers that can predict the occurrence of AD. This study aimed to evaluate the expression level of epidermal proteins by using a tape stripping method to determine whether these proteins can be used as biomarkers predictive of AD development in infants.

In this prospective birth cohort study, we followed 75 infants in a risk group and 12 in a control group for 2 years (Fig 1). The control group consisted of infants with both parents who had neither allergy nor immediate skin test reactivity to 8 common inhalant allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, tree pollen mixture I & II, weed pollen mixture, grass pollen mixture, cat, and cockroach). The risk group was defined when at least 1 parent had both positive skin test response and a history of asthma or allergic rhinitis, or when at least 1 family member had AD. The diagnosis of AD was based on the Hanifin and Rajka criteria, and the severity was assessed using the

SCORing Atopic Dermatitis (SCORAD) method. Transepidermal water loss (TEWL) was measured on the volar surface of the forearm at age 2 months by using a Tewameter TM300 (Courage & Khazaka, Köln, Germany). Tape stripping was done on the volar surface of the forearm at 2 months, and epidermal protein levels were measured by mass spectrometry as previously described to evaluate the expression of filaggrin, alpha enolase, corneodesmosin, fatty acid-binding protein, serpin B3, transglutaminase 3, and thymic stromal lymphopoietin (TSLP).¹ Methods for mass spectrometry and statistics are described in this article's Online Repository at www.jacionline.org. This study was approved by the institutional review board (IRB) at Samsung Medical Center in Seoul (IRB no. 2011-11-072), and written informed consent was obtained from all the parents.

The cumulative incidence of AD was higher in the risk group than in the control group at 24 months (64.8% vs 16.7%; $P = .003$) (see Fig E1 in this article's Online Repository at www.jacionline.org). Eczema symptoms at age 12 months occurred mostly on the face (72.7%), but clinical AD did not appear on the forearm where tape stripping was conducted. The mean SCORAD score in affected infants was 13.3 ± 5.8 and 12.9 ± 5.4 at age 12 and

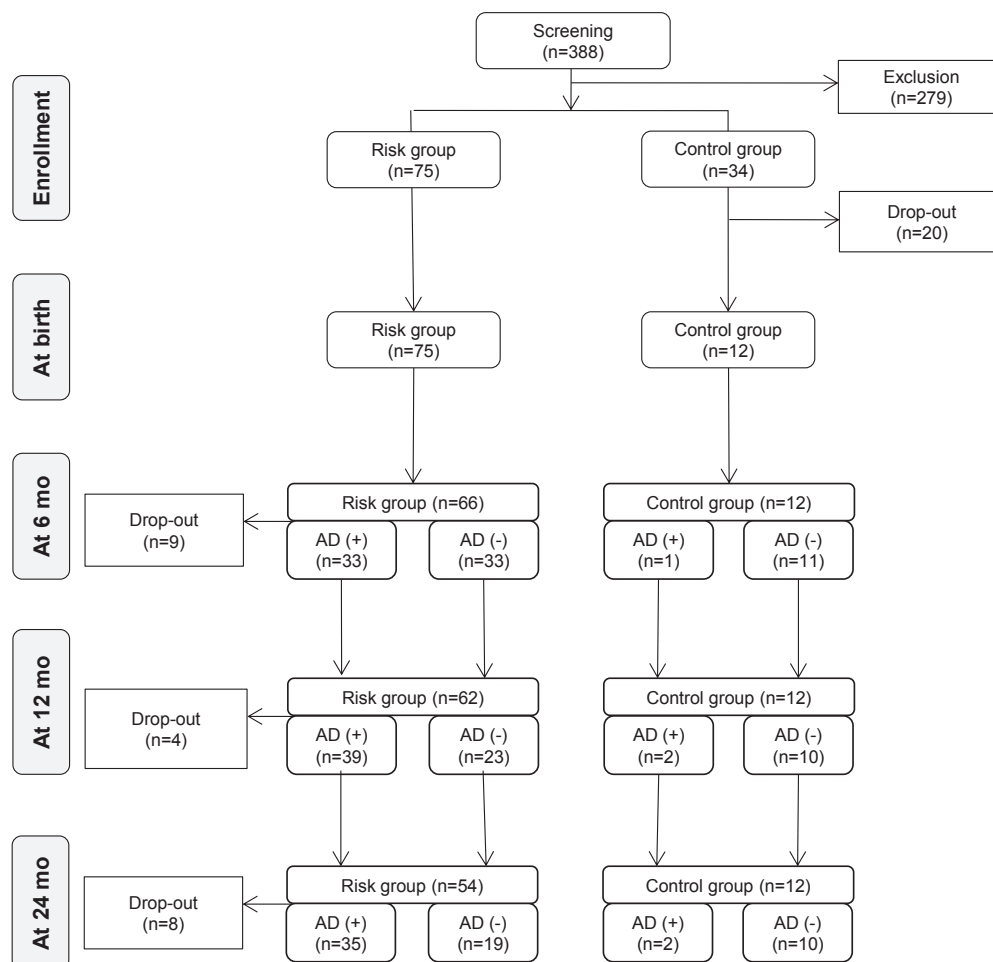


FIG 1. Flowchart of study population during the study period. The number of infants with AD indicates the cumulative number of patients who have developed AD at the time of evaluation. *mo*, Months.

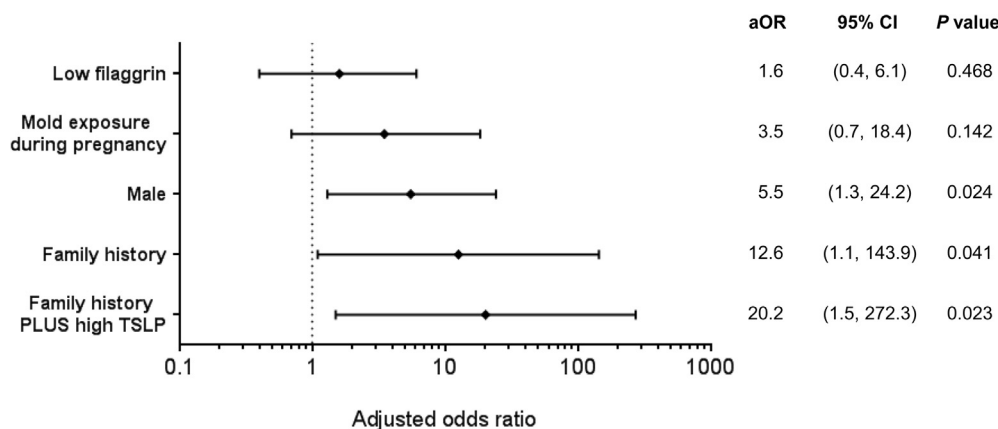


FIG 2. Multivariable analysis for factors influencing AD development by age 24 months. When TSLP expression was dichotomized into low or high levels according to its median value and added to family history, the predictive value for AD development was further increased in subjects with family history and high TSLP expression than in those with family history alone. Male gender was independently linked with AD development. Multivariable analysis was done after adjustment for factors with $P < .1$ in univariable analysis.

24 months, respectively. There was no difference between the risk group and the control group in sex, birth weight, intrauterine period, birth type, mother's age, family's monthly income, and maternal education levels (see [Table E1](#) in this article's Online Repository at www.jacionline.org).

To estimate risk factors predicting AD development by the age of 24 months, a logistic regression model was used. A multivariable analysis was done by adjusting for TSLP and other variables with P value of less than .1 in a univariable analysis. When TSLP expression was dichotomized into low or high levels according to its median value, 0.83 pmol/mg skin (range, 0.32-1.60 pmol/mg skin), TSLP expression at 2 months was 5.3 times more likely to develop AD by age 24 months (95% CI, 1.3-21.4). When TSLP expression was added to family history, adjusted odds ratio (aOR) for AD development was higher in subjects with family history and high TSLP expression (aOR = 20.2; 95% CI, 1.5-272.3) than in those with family history alone (aOR = 12.6; 95% CI, 1.1-143.9; [Fig 2](#)). Male gender was also independently related to AD development (aOR = 5.5; 95% CI, 1.3-24.2). In contrast, other variables such as birth type, passive smoking, mold exposure during pregnancy, exclusive breast-feeding, TEWL on the nonlesional area of the forearm at 2 months, and filaggrin level at 2 months showed no statistical significance in a logistic regression model. The expression of alpha enolase, corneodesmosin, fatty acid-binding protein, serpin B3, and transglutaminase 3 at 2 months was not predictive of AD development (see [Fig E2](#) in this article's Online Repository at www.jacionline.org).

Taken together, our birth cohort study first demonstrated that epidermal TSLP expression at as early as age 2 months is linked with AD later in life. In particular, the predictive value for AD development was further increased by high TSLP expression in subjects with family history which is the strongest risk factor. It indicates that epidermal TSLP level may be used as an early predictor of AD development.² TSLP is expressed in the horny layer of stratum corneum, promotes T_H2 -type inflammation, and downregulates filaggrin expression like other cytokines such as IL-4, IL-13, IL-17, IL-22, IL-25, IL-31, and IL-33.²⁻⁵ A recent study showed that the long isoform of TSLP exerts

proinflammatory activities, whereas the short isoform plays an anti-inflammatory role.⁶ Stratum corneum TSLP level is reduced by moisturizer application in AD patients,² and it may explain how AD development is prevented by the application of moisturizer to neonates.⁷ Although TSLP is an important biomarker in the Korean cohort, it is noteworthy that having a group of biomarkers, rather than 1 biomarker, is important because it may be different according to races and AD phenotypes.⁸

In an Irish birth cohort study, increased TEWL was found before the occurrence of AD and the authors suggested that measurement of TEWL at ages 2 days and 2 months was predictive of AD development at 1 year.⁹ However, we failed to find an association between TEWL at age 2 months and later development of AD in our Korean cohort. This may be explained by the extent of inflammation in the skin. Interestingly, the mean SCORAD score in the Irish study at 6 and 12 months was 21.54 ± 16.29 and 18.56 ± 14.92 , respectively, showing that the Irish infants had more severe AD than did ours. It is plausible that subclinical skin inflammation of the Irish study population at age 2 months could impair the skin barrier function as opposed to our cohort, although clinical AD was not apparent in both studies. Another possibility is that the pathobiology of AD is different in Korea versus Ireland.¹⁰ Indeed, levels of T_H17 - and T_H22 -related cytokines have been found to be higher in Asian than in European American AD skin biopsies.

In conclusion, epidermal TSLP protein expression may serve as an early biomarker to predict the development of infantile AD. To facilitate AD prevention studies, additional skin tape stripping studies are needed to identify biomarkers that predict infants at risk of developing AD in different racial groups.

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This study was supported by Samsung Biomedical Research Institute (grant no. GLI-B2-171-1). This work was supported in part by the Edelstein Family Chair of Pediatric Allergy-Immunology.

Disclosure of potential conflict of interest: D. Y. M. Leung has received grants from MedImmune. The rest of the authors declare that they have no relevant conflicts of interest.

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<http://dx.doi.org/10.1016/j.jaci.2015.12.1306>

METHODS

Study population

We tried to select the study population according to very strict criteria. We excluded parents with asthma or allergic rhinitis from the risk group unless they were sensitized to common inhalant allergens as well. Subjects with a history of AD were also excluded if eczema symptoms were not found on site. Likewise, all parents with asymptomatic sensitization were excluded from the control group even though they had never presented with asthma or allergic rhinitis symptoms. Finally, we excluded 279 subjects out of 388 after initial screening, and successfully followed 75 infants in the risk group and 12 in the control group for 2 years.

Sample collection, protein extraction, and mass spectrometry

Patients were directed to avoid showering on the day of the collection. A total of 20 consecutive D-Squame tape strips (22 mm diameter, CuDerm, Dallas, Tex) were applied on the volar surface of the right forearm at age 2 months. On application of the first tape disc, 4 marks were placed around the disc with a pen so that subsequent discs could be applied to the same location. Each tape disc was placed adhesive side up in its own 6-well plate and then frozen at -80°C .

Proteins were extracted by using a buffer composed of 0.01% 3-(3-[1,1-bisalkyloxyethyl] pyridin-1-yl)propane-1-sulfonate (Protein Discovery, Knoxville, Tenn) in 50 mmol/L ammonium bicarbonate with $1 \times$ HALT protease inhibitors, EDTA-free (Thermo Fisher Scientific, Rockford, Ill), and 50 mmol/L dithiothreitol (Bio-Rad, Hercules, Calif) and incubated on a rocker at room temperature for 1 hour. Extraction buffer was pooled from tape disc, transferred into polypropylene 1.5-mL microcentrifuge tubes, lyophilized, and stored at -80°C . Proteins were precipitated in 300 mL ice-cold precipitation buffer consisting of 0.1% formic acid in 80:20 methanol:water (VWR, West Chester, Pa, and Thermo Fisher Scientific, respectively). Samples were incubated at -20°C and vortexed for 30 seconds every 10 minutes for 1 hour, and then centrifuged at 18,000g at 4°C for 20 minutes. The supernatant was removed, and the protein pellet was resuspended in 8 mol/L urea (Sigma Ultra, St Louis, Mo) in 100 mmol/L TRIS HCl (pH 8.5; Thermo Fisher Scientific). Proteins were reduced with 5 mmol/L tris[2-carboxyethyl]phosphine (Thermo Fisher Scientific) for 20 minutes and alkylated with 500 mmol/L iodoacetamide (Bio-Rad, Hercules, Calif) for 15 minutes. Urea was diluted to 2 mol/

L with 100 mmol/L TRIS HCl (pH 8.5), and samples were incubated at 37°C overnight with 100 ng trypsin (Trypsin Gold; Promega, Madison, Wis). Samples were then cleaned by using PepClean C-18 spin columns (Thermo Fisher Scientific) following the manufacturer's protocol. All reagents were of the highest grade available for mass spectrometry.

Mass spectrometry was carried out as previously described.^{E1} Samples were run in triplicate on an Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, Calif) and Agilent ETD ion trap (model 6340) mass spectrometer with an HPLC chip to evaluate the expression of filaggrin, alpha enolase, corneodesmosin, fatty acid-binding protein, serpin B3, transglutaminase 3, and TSLP.

Statistical analysis

Data were analyzed using SPSS for Windows (version 23.0, SPSS, Chicago, Ill). The Fisher exact test was applied to determine differences in the proportions of sex, delivery mode, parental history of allergic diseases, parental atopy, monthly income, maternal education levels, and the incidence of AD between the risk group and the control group. The Mann-Whitney *U* test was used to analyze differences in birth weights, intrauterine periods, and maternal ages between the risk group and the control group.

To estimate risk factors predicting AD development by the age of 24 months, univariable and multivariable logistic regression analyses were used. In a univariable analysis, candidate variables for adjustment included epidermal protein levels, sex, parental history of allergic diseases, delivery mode, maternal education levels, move to a new house during pregnancy, mold exposure during pregnancy, exclusive breast-feeding during the first 4 months of life, and TEWL at age 2 months. TSLP and epidermal protein levels were dichotomized according to their median values. In a multivariable regression analysis, TSLP and other variables with a *P* value of less than .1 in univariable analysis were selected and adjusted for each other. The combined effect of TSLP and family history was also evaluated using a multivariable logistic regression. A *P* value of less than .05 was considered to be significant.

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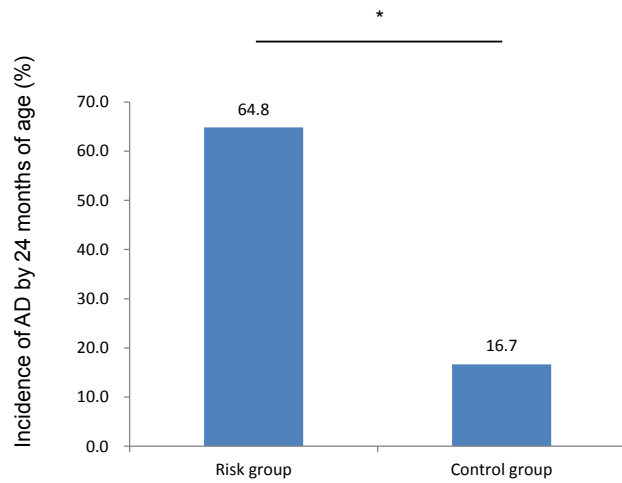


FIG E1. Comparison of the incidence of AD by age 24 months between the risk group and the control group. Statistical analysis was done using the Fisher exact test. * $P < .05$.

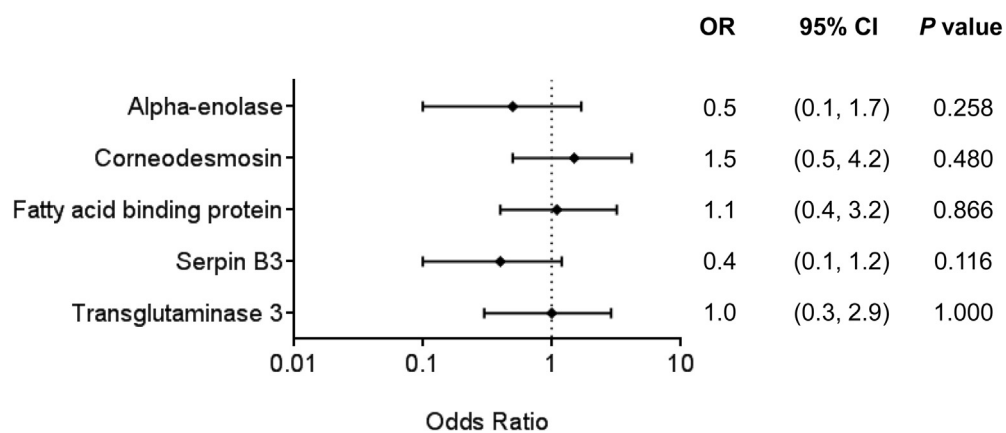


FIG E2. Univariable analysis for epidermal proteins influencing AD development by age 24 months. *OR*, Odds ratio.

TABLE E1. Characteristics of study participants

Characteristic	Risk group (n = 75)	Control group (n = 12)	P value
Sex: male	43 (57.3)	8 (66.7)	.754
Birth weight (kg)	3.2 ± 0.4	3.1 ± 0.5	.434
Intrauterine period (wk)	39.5 ± 6.1	39.4 ± 6.3	.696
Mother's age at child's birth (y)	32.1 ± 2.8	32.8 ± 3.3	.555
Birth type			.746
Vaginal delivery	53 (70.7)	8 (66.7)	
Cesarean section	22 (29.3)	4 (33.3)	
Paternal history of allergic diseases			
Atopic dermatitis	19 (25.3)	0 (0)	.041
Allergic rhinitis	25 (33.3)	0 (0)	.012
Asthma	5 (6.7)	0 (0)	.467
Maternal history of allergic diseases			
Atopic dermatitis	18 (24.0)	0 (0)	<.001
Allergic rhinitis	46 (63.0)	0 (0)	<.001
Asthma	3 (4.0)	0 (0)	.637
Paternal atopy*	46 (63.0)	0 (0)	<.001
Maternal atopy*	50 (66.7)	0 (0)	<.001
Monthly income (US \$)			.971
≤1799	9 (12.0)	1 (8.3)	
1800-3599	36 (48.0)	6 (50.0)	
3600-5399	22 (29.3)	4 (33.3)	
≥5400	8 (10.7)	1 (8.3)	
Maternal education			.546
College	4 (5.3)	0 (0)	
High school or less	71 (94.7)	12 (100.0)	

Values are n (%) or mean ± SD.

*Positive skin prick test result to common allergens.