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SCCA2 is a reliable biomarker for evaluating pediatric atopic dermatitis



To the Editor:

It was claimed before that there is no reliable biomarker for atopic dermatitis (AD).¹ However, a systematic review recently showed that thymus and activation-regulated chemokine (TARC)/chemokine C-C motif ligand 17, a chemokine involved in type 2 inflammation, is the most reliable biomarker indicating the disease severity of AD.² Nevertheless, TARC has a disadvantage in that infants and children show significantly higher TARC levels than adults do; TARC levels in infants <1 year old are almost 3 times those of adults (<1367 ng/mL vs <450 ng/mL).³ This indicates that we need to evaluate serum TARC values depending on the ages of the subjects and that high pathological TARC levels may be masked in younger subjects. Therefore, a novel biomarker for AD must be found and developed that provides reliable information, particularly in younger subjects.

We previously found that 2 squamous cell carcinoma antigen (SCCA) molecules, SCCA1/SERPIN B3 and SCCA2/SERPIN B4, are downstream molecules of IL-4 and IL-13, signature cytokines of type 2 inflammation,⁴ and that SCCAs are strongly expressed in the epidermis of AD patients.⁵ We furthermore established an ELISA system to distinguish SCCA1 and SCCA2 and found that expression of SCCA2 is predominant in AD patients.⁶

In this study, we performed a multicenter analysis to compare SCCA2, TARC, and IgE, a commonly used biomarker, in the sera of children without allergies and children with AD. From 2015 to 2017, we recruited 159 subjects without allergies and 176 AD patients, including ≥10 subjects in each year of age, ≤7 years old. Selection of the subjects and the distributions of ages and clinical severities of AD are shown in the supplemental data and Table E1, which are available in this article's Online Repository at www.jacionline.org. No psoriasis patient in which we previously showed high SCCA2 levels was included.⁷ This study was conducted at 3 hospitals—Mie National Hospital, National Center for Child Health and Development, and Fukuoka National Hospital—after approval by the local institutional review boards. Measurement of SCCA2 by ELISA and preparation of recombinant SCCA2 proteins were performed as previously described, with the modification of using not biotin-conjugated, but peroxidase-conjugated, SS8G.⁶

We first examined SCCA2, TARC, and IgE in children without allergies (n = 159). Both the median values of SCCA2 and TARC decreased as the ages went up ($P < .001$), whereas the median values of IgE went up as the age increased (see Fig E1 and Table E2 in this article's Online Repository at www.jacionline.org). Both the median levels of SCCA2 and TARC were invariant in males and females.

We then compared the levels of SCCA2, TARC, and IgE in the overall group of AD children and children without allergies (Fig 1, A). SCCA2, TARC, and IgE were all significantly higher in AD children than in children without allergies (median: SCCA2: 3.90 vs 0.75 ng/mL; TARC: 1073 vs 341 pg/mL; and IgE: 807 vs 39.4 IU/mL; $P < .001$). Presence of asthma did not affect SCCA2 in AD children (data not shown). SCCA2 was well correlated with TARC ($P < .001$, $r = 0.757$), and weakly with IgE ($P < .01$, $r = 0.237$) (see Fig E2 in this article's Online Repository available at www.jacionline.org). The area under the curve (AUC) by the receiver-operating characteristic curve was higher in SCCA2 (0.929) than in TARC (0.871) and IgE (0.820) (Fig 1, B, and see Fig E3 in this article's Online Repository available at www.jacionline.org). SCCA2 showed higher AUCs than TARC did in all age groups or 3 age groups—≤1 year, 2 to 6 years, and 7 to 15 years (Fig 1, B, and see Fig E4 in this article's Online Repository available at www.jacionline.org). These results demonstrate that SCCA2 corresponded more closely to diagnosis of AD than TARC and IgE in the children overall.

We next examined the correlation of each biomarker with objective Scoring Atopic Dermatitis (O-SCORAD) (Fig 2, A). SCCA2, TARC, and IgE all showed good correlations with O-SCORAD, with the highest association in SCCA2 (SCCA2: $r = 0.622$; TARC: $r = 0.491$; IgE: $r = 0.407$; $P < .001$). To estimate the ability to reflect clinical severity, we analyzed the SCCA2 and TARC levels in 3 groups—mild (O-SCORAD: <15, n = 56), moderate (O-SCORAD: 15–40, n = 60), and severe (O-SCORAD: >40, n = 60) (Fig 2, B).⁸ Both SCCA2 and TARC increased according to the existence or the clinical severity of AD (median: SCCA2: 0.75, 2.0, 3.5, and 10.8 ng/mL; TARC: 342, 885, 897, and 2530 pg/mL). SCCA2 showed higher AUCs than TARC in all 3 comparisons—control versus mild (SCCA2: 0.864; TARC: 0.822), mild versus moderate (SCCA2: 0.671; TARC: 0.571), and moderate versus severe (SCCA2: 0.783; TARC: 0.725) (Fig 2, C). It is of note that SCCA2, but not TARC, showed a statistical significance in the comparison

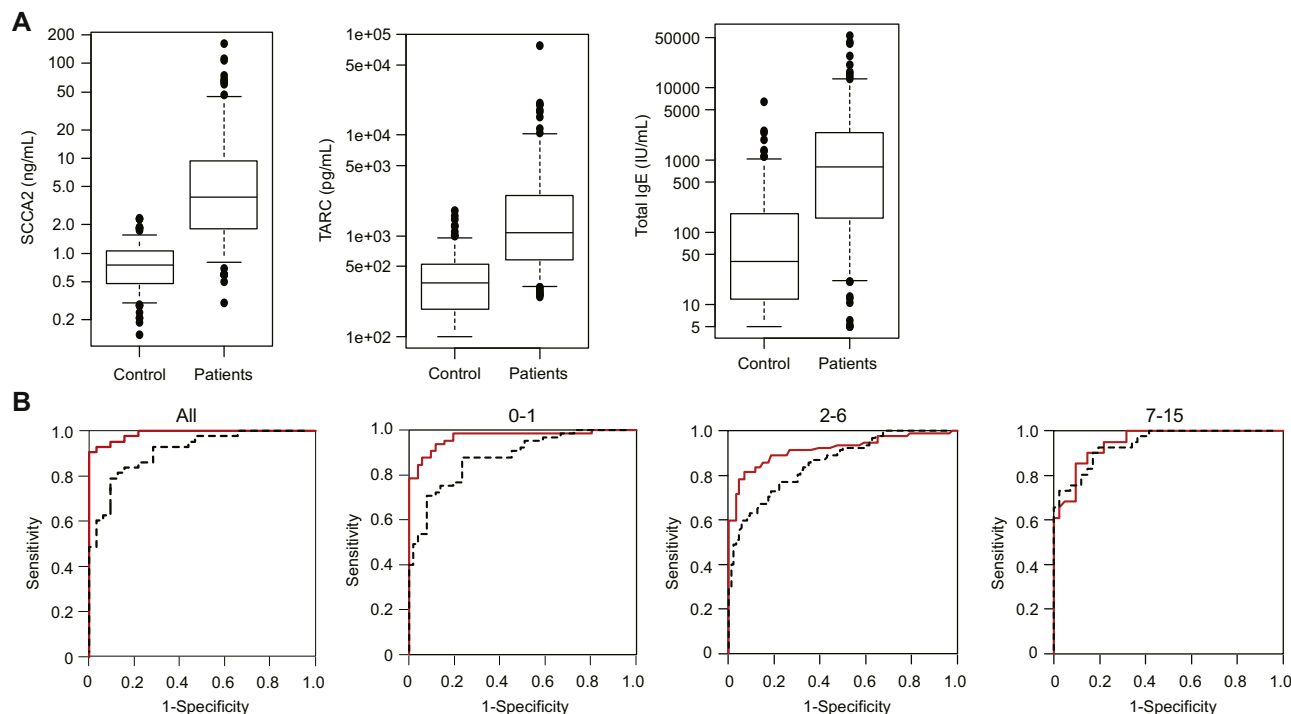


FIG 1. Correspondence of SCCA2, TARC, and total IgE to diagnosis of AD in children. **A**, Serum levels of SCCA2, TARC, and total IgE in control subjects ($n = 159$) and AD children ($n = 176$). **B**, Receiver-operating characteristic curve analyses of SCCA2 (red solid line) and TARC (black dashed line) in all and ≤ 1 year old, 2 to 6 years old, and 7 to 15 years old of control subjects and AD children.

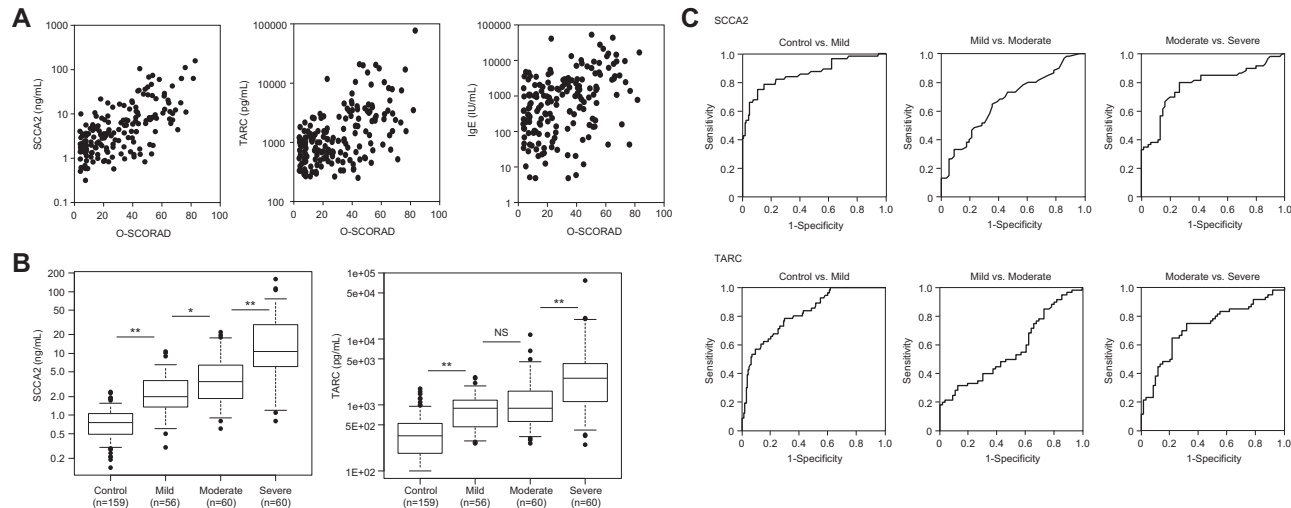


FIG 2. Association of O-SCORAD with SCCA2, TARC, or IgE in AD children. **A**, Associations of O-SCORAD with SCCA2, TARC, and IgE in the whole of AD children. **B**, Serum levels of SCCA2 (left) and TARC (right) in control subjects and each severity of AD children with varying AD severity. * $P < .01$; ** $P < .001$; NS, Not significant. **C**, Receiver-operating characteristic curve analyses of SCCA2 (upper) and TARC (lower) in control subjects versus mild AD children (left), mild AD children versus moderate AD children (middle), and moderate AD children versus severe AD children (right).

between mild and moderate patients. The fitted probability curve for the severities of AD by SCCA2 was steeper than that by TARC (see Fig E5 in this article's Online Repository at www.jacionline.org). SCCA2 showed better correlations than TARC in 6 intensity items (erythema, edema/papulation, oozing/crusts, excoriations, lichenification, dryness) (see Fig E6 in this article's Online

Repository at www.jacionline.org). Moreover, SCCA2 showed good correlation with other parameters for AD—Eczema Area and Severity Index and visual analog scale scores—in limited investigated numbers (data not shown). These results suggest that SCCA2 has a greater ability than TARC does to distinguish the clinical severities in AD children.

Finally, we estimated the ability of each biomarker to assess the effects of treatment with topical corticosteroids (see Fig E7 in this article's Online Repository at www.jacionline.org). O-SCORAD completely decreased according to the treatment (39.4 vs 1.8). Accordingly, both SCCA2 (7.4 ng/mL vs 1.5 ng/mL; $P < .001$) and TARC (2555 pg/mL vs 771 pg/mL; $P < .001$) significantly decreased, whereas the change in IgE was very slight (711.4 IU/mL vs 643 IU/mL). The change of O-SCORAD in TARC was almost the same as that in SCCA2, suggesting that TARC and SCCA2 have almost the same ability to estimate the effects of treatments.

In this study, we showed that SCCA2 is a more reliable biomarker than TARC to correspond to diagnosis of AD and to estimate the clinical severity of AD in children, TARC being the biomarker now assumed to be the most reliable. It was reported that combination of IL-17 and IL-22 upregulated in AD patients, particularly in Asian countries,⁹ enhances SCCA2 expression in keratinocytes,⁷ which may explain why SCCA2 is more reliable than TARC. It remains open how the cutoff values should be set for SCCA2; however, setting only 1 or 2 cutoff values depending on ages would be convenient for clinicians. In a simple way, only 1 cutoff value, 1.6 ng/mL, provided an acceptable result for corresponding to diagnosis of AD (79.5% of sensitivity and 95% of specificity across all ages) (Fig 1) and the difference of AUCs between 1 cutoff value and the cutoff values depending on age was not significant in SCCA2 compared with TARC (SCCA2: 0.042; TARC: 0.064) (see Table E3 in this article's Online Repository at www.jacionline.org), which suggests that the difference among ages is not big in SCCA2 compared with those in TARC. Please see the Online Repository for Tables E1 to E3 and Figs E1 to E7.

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Reprogramming immunity to food allergens



To the Editor:

Allergen immunotherapy is used to treat hypersensitivities to environmental or food allergens. It involves administering repeated and increasing doses of a soluble allergen until a maintenance dose is reached, at which symptoms are improved on allergen exposure.¹ Although widely used, several limitations exist, including the risk of severe adverse events (eg, fatal anaphylaxis), high costs of antigens and clinical monitoring, and requirement of many years of treatment.² Many patients remain refractory to treatment, and for peanut allergy, this strategy has a high risk of adverse reactions.³ Thus current immunotherapy protocols have distinct limitations, and there is a major need for improved approaches, particularly for food allergies.

When effective, the mechanisms of allergen immunotherapy are not well understood, although it is thought to involve a combination of antigen-induced desensitization of effector immune cells and development of tolerance. Skewing the T_H1/T_H2 profile toward a T_H1 direction could markedly reduce T_H2 -mediated pathologic responses to allergens. Thus attempts have been made to block production of T_H2 cytokines, to directly inhibit their action, or to favor T_H1 immunity by administering exogenous T_H1 -type cytokines, such as IL-12. For asthma treatment, exogenous IL-12 delivery was not effective, which

METHODS

Diagnosis of AD was based on the Japanese guidelines.^{E1} The clinical severity of AD was evaluated by using the O-SCORAD.^{E2} The AD patients were treated with topical steroids and emollients, and 49 patients were evaluated twice during the course of the treatment. Healthy children without allergies were eligible when the following criteria were met: (1) they or their caregivers answered “No” to all the questions concerning current symptoms of asthma, AD, and allergic rhinitis on the International Study of Asthma and Allergies in Childhood Questionnaire 3; (2) the clinical investigators diagnosed no allergic diseases in the subjects; (3) no acute infectious diseases

at enrollment and no history of autoimmune diseases or malignancies were identified. Caregivers of the subjects gave written informed consent.

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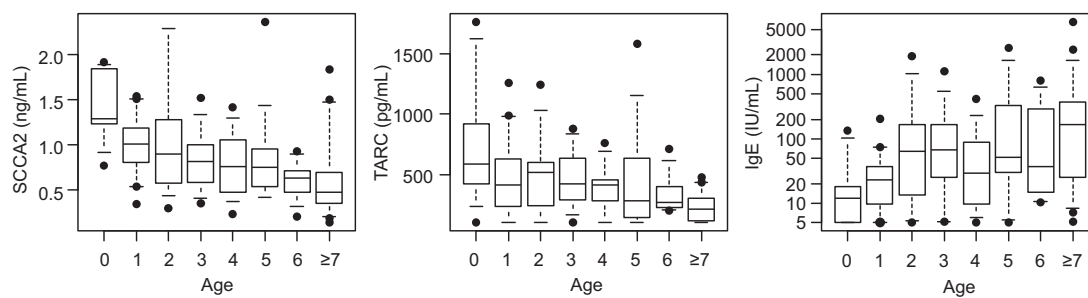


FIG E1. Serum levels of SCCA2, TARC, and total IgE in children without allergies. Serum levels of SCCA2, TARC, and total IgE in each age group of children without allergies.

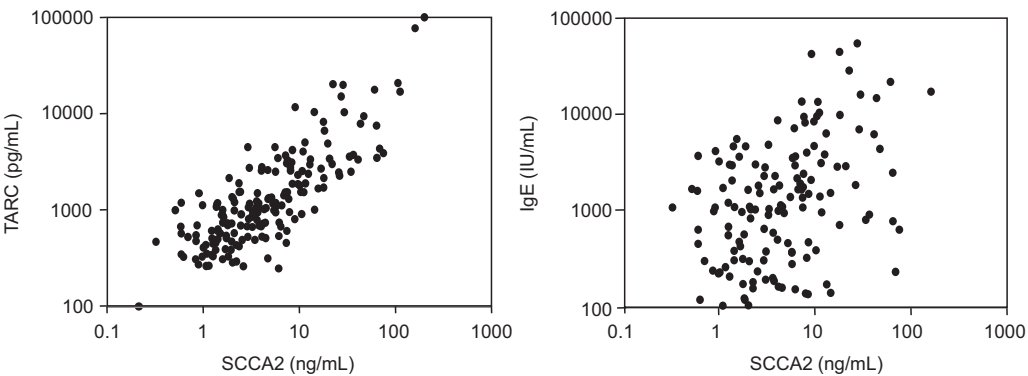


FIG E2. Association of SCCA2 with TARC or IgE in AD children. Associations of SCCA2 with TARC or IgE in AD children.

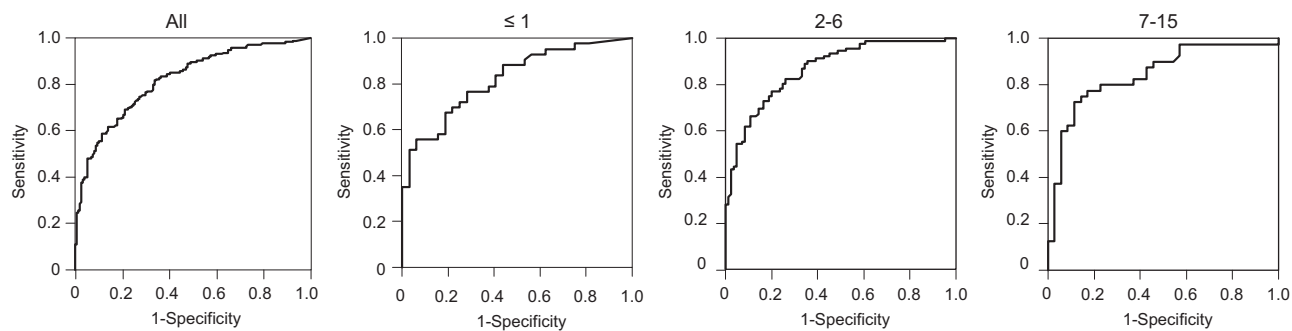


FIG E3. Receiver-operating characteristic (ROC) curve analyses of IgE depending on the ages of participants. ROC curve analyses of IgE in all and ≤ 1 year old, 2 to 6 years old, and 7 to 15 years old of control subjects ($n = 159$) and AD children ($n = 176$).

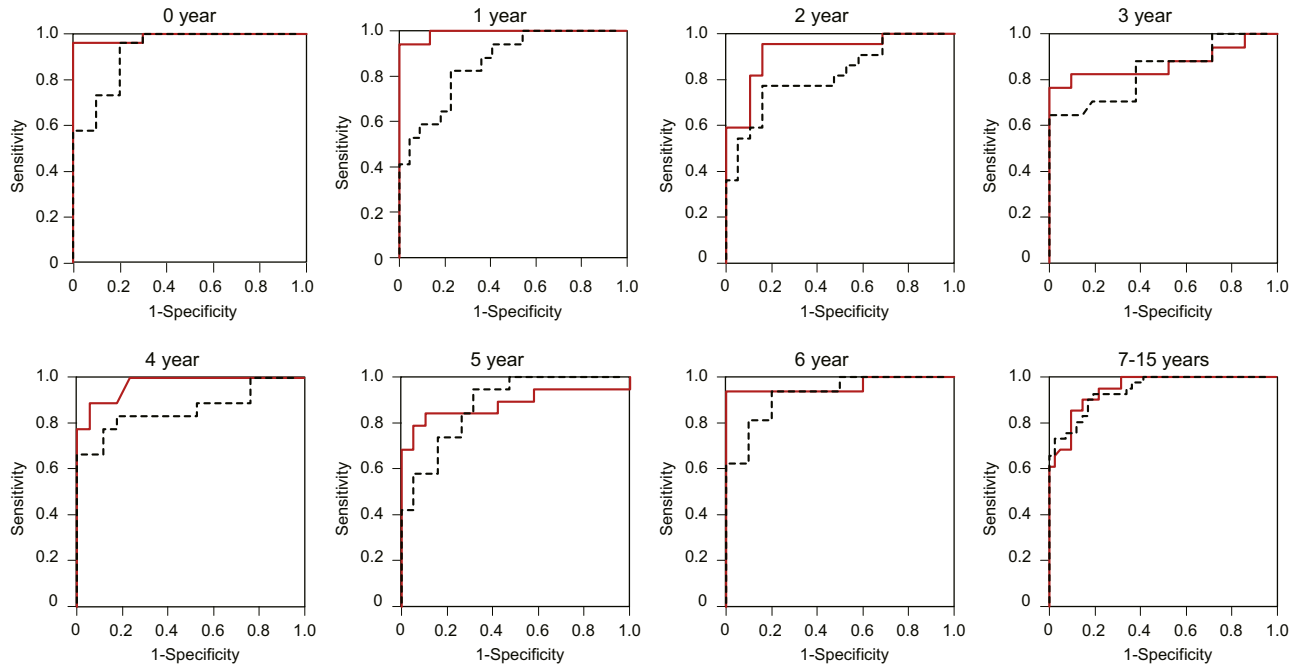


FIG E4. ROC curve analyses of SCCA2 and TARC in each age of AD children. ROC curve analyses of SCCA2 (red solid line) and TARC (black dashed line) in each age of children.

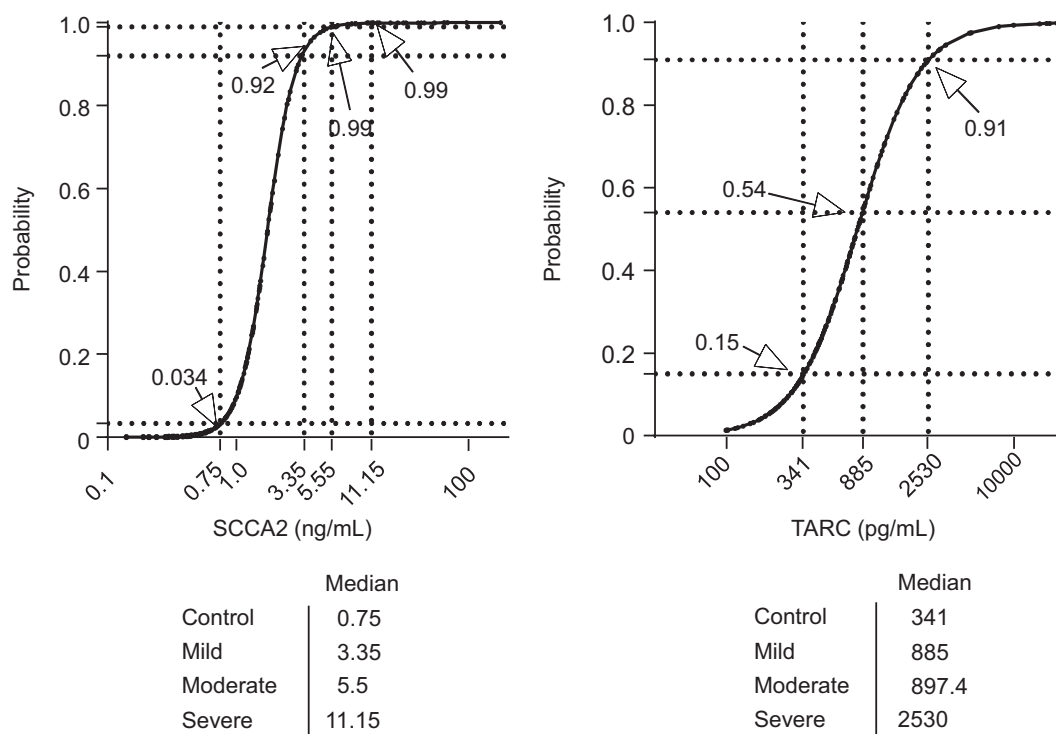


FIG E5. Predicted probability derived from logistic regression for the severities of AD. Estimated probability curves for the severities of AD at a given level of SCCA2 (*left*) and TARC (*right*) are depicted. Dotted lines indicate cutoff levels obtained from ROC analysis.

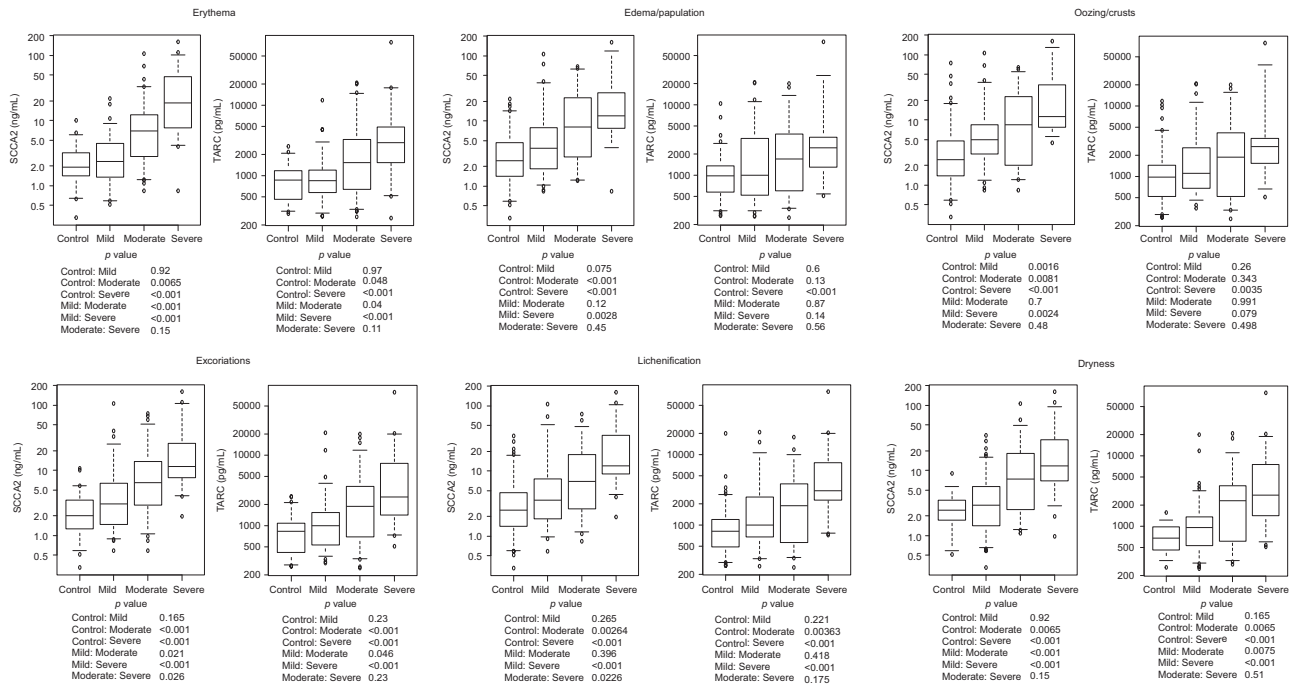


FIG E6. Association of each component of O-SCORAD with SCCA2 in AD children. Association of each component of O-SCORAD (erythema, edema/papulation, oozing/crusts, excoriations, lichenification, dryness) with SCCA2 or TARC. P values in each comparison are depicted.

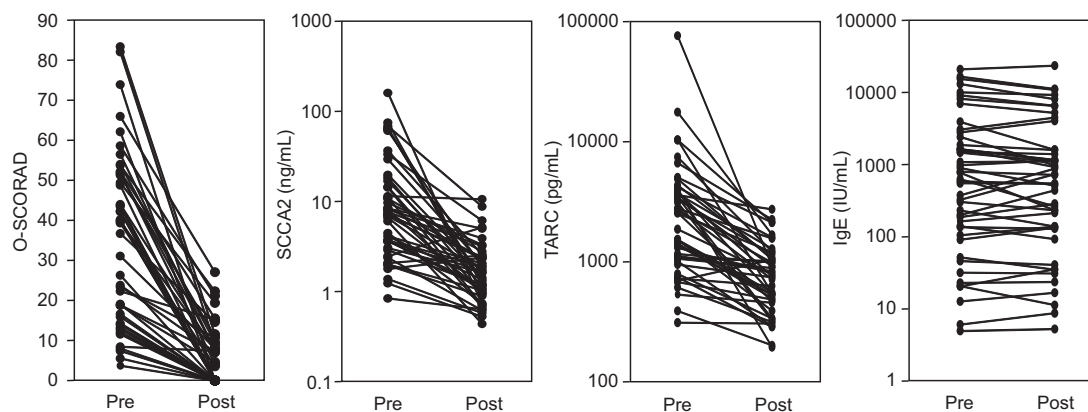


FIG E7. Association of SCCA2, TARC, and IgE with treatment for AD children. Changes of O-SCORAD, SCCA2, TARC, and IgE before and after the appropriate treatments in AD children are depicted.

TABLE E1. Distributions of ages in nonallergic children and AD children and clinical severities in AD children

Nonallergic children				AD children			
Age (y)	Male	Female	Total	Age (y)	Male	Female	Total
0	4	6	10	0	13	13	26
1	12	10	22	1	10	7	17
2	9	10	19	2	12	10	22
3	10	11	21	3	8	9	17
4	9	8	17	4	15	3	18
5	10	9	19	5	10	9	19
6	5	5	10	6	9	7	16
7-15	21	20	41	7-15	23	18	41
Total	80	79	159	Total	100	76	176
O-SCORAD	< 15			15-40	40 <		
Clinical severity	Mild			Moderate	Severe		
Subject no.	56			60	60		

TABLE E2. Serum levels of SCCA2, TARC, and total IgE depending on age in nonallergic children

Age (y)	0	1	2	3	4	5	6	7-15
SCCA2 median (ng/mL)	1.29	1.02	0.90	0.82	0.76	0.75	0.64	0.48
TARC median (pg/mL)	587	412	516	419	412	282	270	215
Total IgE median (IU/mL)	12.0	23.1	63.5	67.3	29.3	51.9	37.8	166

TABLE E3. Comparison of the abilities to diagnose AD of SCCA2 and TARC using single cut-off value and cut-off values depending on age

Cut-off value	Sensitivity	Specificity	AUC	95% CI lower limit	95% CI upper limit
SCCA2					
Single cut-off value 1.6 ng/mL	0.795	0.950	0.873	0.838	0.907
Cut-off values depending on age	0.886	0.943	0.915	0.885	0.944
TARC					
Single cut-off value 669 pg/mL	0.722	0.843	0.783	0.738	0.826
Cut-off values depending on age	0.858	0.836	0.847	0.808	0.886