Cord blood soluble Fas ligand and pediatric atopic dermatitis

Kuan-Wen Su, M.D.,[1] Pau-Chung Chen, M.D, Ph.D.,[2] and I-Jen Wang, M.D, Ph.D.[1,3,4]

Affiliations: From [1]Department of Pediatrics, Taipei Hospital, Department of Health, Taipei, Taiwan; [2]Institute of Occupational Medicine and Industrial Hygiene, National Taiwan University College of Public Health, Taipei, Taiwan; [3] College of Public Health, China Medical University, Taichung, Taiwan; [4]College of Medicine, Fu Jen Catholic University, Taipei, Taiwan

Dr. Su and Chen contributed equally to this article.

Funding Sources: This work was supported by the National Science Council of Taiwan (NSC 97-2314-B-192 -001 -MY2).

Running title: Cord blood sFasL and atopic dermatitis

Keywords: atopic dermatitis, cord blood, sFasL, IgE, nested case-control study, birth-panel cohort, apoptosis, keratinocyte, ISAAC questionnaire, biomarker.

Address correspondence and reprint requests to: Dr. I-Jen Wang

Department of Pediatrics, Taipei Hospital, Department of Health,

No. 127, Su-Yuan Road, Hsin-Chuang Dist., New Taipei City 242, Taiwan.

Phone: 886-2-2276-5566 ext. 2532

Fax: 886-2-2998-8028

E-mail: r92846001@ntu.edu.tw

ABSTRACT

Background: Keratinocyte apoptosis is a key pathogenetic mechanism in atopic dermatitis (AD). Fas and Fas ligand (FasL) interaction is an important pathway to induce apoptosis. However, the relationship between early life soluble FasL (sFasL) and AD is not yet clear.

Objective: To evaluate if sFasL is associated with the development of AD in children.

Methods: We performed a nested case-control study within a prospective Taiwan birth-panel cohort study. Umbilical cord blood and maternal plasma samples were gathered at birth. During follow-up, by the International Study of Asthma and Allergies in Childhood questionnaires, we identified 40 AD cases, which we matched to 80 unaffected controls chosen from this cohort. The concentrations of sFasL and immunoglobulin E (IgE) in plasma were determined by enzyme-linked immunosorbent assay (ELISA). The relationship of sFasL levels and AD was estimated by mix model. Receiver-operating-characteristic (ROC) curves were generated to see how well sFasL could predict AD.

Results: Cord-blood sFasL levels were significantly higher in the AD patients than in the controls (p=0.003). The concentration of sFasL in the cord blood was higher than in the maternal blood (p<0.001). There also existed a correlation between the concentration of sFasL in the maternal blood and the cord blood (r=0.23, p=0.01). The subjective severity of AD was positively correlated with sFasL levels (r=0.34, p=0.02). Cold blood sFasL may be a ~~better~~ biomarker ~~than IgE~~ in detecting pediatric AD (area under the ROC curve = 0.64).

Conclusion: Our results demonstrated a relation between cord blood sFasL and the development of AD in children.

INTRODUCTION

Atopic dermatitis (AD) is a common skin disease, and ~~which is usually the first step in the atopic march, followed by asthma and allergic rhinitis.~~[1] ~~It~~its prevalence has doubled, even tripled, in developed countries over the past 30 years. AD affects approximately 15 to 30 % of children.[1,2] Pediatric AD often starts in early infancy. A total of 45 % AD cases develop during the first six months of life, 60 % begin during the first year, and 85 % of cases start by five years of age.[3] However, more than 50 % of AD children, affected during their first two years of life, do not have immunoglobulin E (IgE) sensitization.[4] Traditionally, the primary defect of AD was regarded as an immunological disturbance causing IgE-mediated sensitization. Today, increasing evidence suggested that barrier dysfunction of epithelial function was an intrinsic defect of AD,[5] while local inflammation and immune response were subsequent reactions. Disruption of skin barrier function also predisposed AD patient to bacterial superinfection, especially *Staphylococcus aureus*. *Staphylococcus aureus* superinfection was related to IgE level in AD patients, and its endotoxin screwed the immune system toward a Th2 response.[6] Treatment of skin barrier dysfunction and cutaneous infection was equally important to food or other allergen avoidance.[7] Recent studies showed that loss-of-function mutations of filaggrin gene resulted in skin barrier dysfunction and the development of AD. However, only 30 % of patients with AD had defects in the filaggrin gene.[8] Other unknown intrinsic epithelial defects may be critical for the development of early-onset of AD.

Fas, officially named CD95, belongs to the tumor necrosis factor (TNF) superfamily. It is a type I membrane protein. Fas ligand (FasL), also known as the CD95-ligand, or CD178, is a type II membrane protein.[9] The Fas-FasL interaction is an

important cell apoptosis pathway, terminating lymphocyte proliferation and maintaining homeostasis of the immune system. As with many other members of the TNF family, Fas ligands have two forms: a membrane-bound form and a soluble variant.[10] Soluble FasL production occurs by proteolytical cleavage. Matrix metalloproteinase (MMP) cleaves FasL from cell membranes.[11] For both membrane and soluble FasL (sFasL), a spontaneous trimerization is necessary for death signal transmission via Fas.[12]

T-cell-mediated, FasL-induced keratinocyte apoptosis was found to play a key role in pathogenesis of AD.[13] In one clinical study, sFasL levels were found significantly elevated in severe cases of AD.[14] However, whether sFasL can serve as a predictor for AD is yet not clear. In this study, we evaluated if sFasL was associated with the development of AD in children ~~and assessed its relationship with intrinsic and extrinsic types of AD~~. Furthermore, the correlation between sFasL in maternal and cord blood was also investigated.

METHODS

Study Population

This was a nested case-control study conducted from 2004 to 2006 as part of a prospective Taiwan birth-panel cohort study. Subjects were recruited from medical centers, regional hospitals, local hospitals, and clinics in Taiwan in 2004. Potential environmental exposure and nationwide representation were considered as recruitment criteria. Pregnant women in their third trimester of pregnancy, who had prenatal examinations in the selected hospitals, were invited to join. Exclusion criteria included multiple gestation (e.g. twins, triplets), delivery by caesarian section, inability to answer questions in Chinese, and plans to move out of the area before delivery. In total, 328 mother-and-newborn pairs were recruited. Informed consent forms were obtained, and the study was approved by the Joint Institution Review Board in Taiwan.

After 2 years of follow-up, we identified 40 AD cases by the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaires. Two control subjects selected from the cohort were matched to each case subject by gender, age (within 5 months), and enrollment time (within 3 months). Control subjects were apparently healthy study participants who remained AD free during follow-up. ~~Patients with extrinsic AD were defined as having a positive history of intermittent allergic rhinitis, or asthma, or elevated total IgE, while intrinsic AD was defined by the absence of the above manifestations.~~ IgE and sFasL concentrations in cord blood and maternal plasma, and total IgE at 2 years of age were checked.

Questionnaire Survey

The parents were asked by home interview questionnaires after delivery, including

birth year, education level, occupation, and family income, history of atopic diseases, active smoking, and exposure to environmental tobacco smoke (ETS). Neonatal health data at birth obtained from the records of cooperating hospitals, included head circumference, birth body weight, height, weeks of gestation, and type of delivery.

We gathered post-natal information included duration of breast feeding and infant formula, early consumption of highly allergenic foods (such as egg, soy beans, or shrimp before 1-year of age), the presence of furry pets and older siblings at home or fungi on the house walls, ETS exposure, and respiratory and gastrointestinal tract infection.

When children were at two years of age, ISAAC questionnaires were performed. We identified AD cases by the following three questions from the ISAAC questionnaires: "Has your child ever had an itchy rash which was coming and going for at least 6 months at any time?" "Has the itchy rash been coming and going over elbows, knees, face, wrists, or generalized (4 or more localizations)?" and "Has your child ever had atopic dermatitis diagnosed by a doctor?" As described elsewhere, a dermatologist examined a subgroup of the participating infants, and a combination of answers that resulted in the greatest sum of sensitivity and specificity was determined.[15-17] Since being kept awake at night because of the itchy rash was used to represent a subjective parameter for the severity of AD, the answers for the following question in the ISAAC questionnaires were recorded: "In the last 12 months, how often has your child been kept awake at night by the itchy rash?".[18]

Laboratory Methods

After gathering maternal and cord blood, we immediately centrifuged specimens, collected plasma, and stored samples at -80 °C. Blood samples were taken from healthy

control subjects at time points corresponding to those of the disease group.

Determination of sFasL levels

Soluble FasL concentrations in plasma samples were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Medical & Biological Laboratories, Japan). All samples were checked three times to reduce the influence of unavoidable inter-assay variance.

IgE antibody analysis

Total IgE concentrations were determined with Pharmacia UniCap IgE assay test system (Pharmacia Diagnostics, Uppsala, Sweden). IgE levels were considered as increased at values greater than 100 kU/L. Concentrations below 0.35 kU/L were defined as absent or undetectable IgE.

Statistical analysis

Baseline characteristics were compared between cases and control subjects, applying a mixed-effect model for continuous variables and conditional logistic regression for categorical variables. To assess the correlation of maternal and cord blood sFasL levels, and the association of frequencies of being kept awake at night by itchy rashes and sFasL levels, we performed the Pearson Correlation. Receiver-operating-characteristic (ROC) curves were generated to assess sFasL prediction sensitivity and specificity toward subsequent AD disorders, and to determine the cut-off point that would provide the highest positive predictive value. All hypothesis testing was two-sided, with a significance level of 0.05 and was performed with SAS software version 9.1.

RESULTS

During follow-up, we identified 40 AD cases. Eighty matched control subjects were available. There were no significant differences in characteristics such as maternal, child, and environmental factors (Table 1), between the AD cases and the control group. Maternal age was slightly higher in the AD group than in the controls, but not to a statistically significant degree.

Compared with the control group, the mean level of sFasL in cord blood was significantly higher in the AD group (p=0.003, Table 2). There was no significant difference between the AD group and the control group in mean IgE concentration in cord blood or maternal plasma (p=0.48, Table 2). The mean maternal blood level of IgE was higher than in the cord blood (p<0.001, Table 2). The mean sFasL level in maternal blood was also higher in the AD cases. However, this failed to achieve statistical significance (p=0.06, Table 2). The mean sFasL level in cord blood was higher than in maternal plasma (p<0.001, Table 2). Cord blood sFasL levels positively and significantly correlated with maternal blood sFasL levels (r=0.23, p=0.01, Fig. 1). The frequencies of being kept awake at night by itchy rashes were positively correlated with sFasL levels (r=0.34, p=0.02). ~~However, there was no significant difference of cord blood sFasL between patients with intrinsic (191.84 ± 27.21 pg/ml) and extrinsic (178.49 ± 40.83 pg/ml) types of AD (p=0.23).~~

Because cord blood sFasL showed significant increases in AD subjects, we generated ROC curves to see how well cord blood sFasL levels could predict subsequent AD disorder. Cord blood sFasL may be a ~~better~~ biomarker ~~than IgE~~ in predicting pediatric AD (area under the ROC curve, AUC = 0.64). The highest positive predictive value occurred with a cut-off point of 184.60 pg/ml, with sensitivity of 69 % and specificity of

68 %.

DISCUSSION

This was the first study to investigate sFasL and its relationship with the development of AD. Through this prospective cohort, we found that cord blood sFasL levels were associated with the development of AD~~. We discovered that cord blood sFasL might serve as a biomarker to detect individuals at risk of AD~~, and its level was correlated with the subjective severity of AD. Few studies have ever reported an association between sFasL and AD. Sohn *et al*. reported that sFasL levels were significantly elevated in severe cases of AD and were correlated with serum interleukin-18 levels.[14] They also found that Staphylococcal enterotoxin B, an important aggravating factor in AD patients, stimulated peripheral blood mononuclear cells to produce sFasL.[19] Furthermore, Trautmann *et al*. demonstrated that under interferon-gamma stimulation, keratinocytes expressed high levels of Fas.[12] Through Fas/FasL pathway, sFasL induced keratinocytes apoptosis, broke down epithelial integrity, led to skin barrier dysfunction, and initiated the development of AD.[13,20] Moreover, FasL triggered the expression of proinflammatory cytokines, chemokines, and adhesion molecules.[21] Soluble FasL itself was also a strong chemotactic factor for inflammatory polymorphonuclear cells, which led to further skin inflammation and AD.[22]

Two possible mechanisms result in significant elevation of cord blood sFasL in AD children. First, children with AD may bare certain single nucleotide polymorphisms (SNPs), which lead to increased concentrations of sFasL. Evidence has shown the association of FasL genetic polymorphisms with Stevens-Johnson syndrome and toxic epidermal necrolysis.[23] It is also reported that patients with certain SNP had higher levels of FasL, leading to functional difference in cell apoptosis.[24] Another

possible mechanism is that elevated transcriptional factors may induce sFasL expression. Transcriptional factors, such as nuclear factor-κB (NF-κB), interferon regulatory factor 1 (IRF-1), and the nuclear factor of activated T-cells (NFAT), may regulate FasL expression.[25] These transcriptional factors were elevated in a Nishiki-nezumi Cinnamon/Nagoya (NC/Nga) mouse AD model and in AD patients.[26] Furthermore, matrix metalloproteinase, the key enzyme to produce sFasL by cleaving FasL from cell membrane, also increased in the skin and the plasma of AD patients.[27] Therefore, genetic susceptibility, FasL expression induced by transcriptional factors, or sFasL release caused by matrix metalloproteinase may all account for the elevation of cord blood sFasL in AD children.

It was interesting to discover that cord blood sFasL levels were positively correlated with maternal blood sFasL concentrations. The literature contains no reports until now, as to why cord blood sFasL levels positively correlate with sFasL in maternal blood. The precise role of Fas/FasL during pregnancy, and at the maternal-fetal interface, remains obscure. The possible explanation was that sFasL might cross the placenta, or that the infant might inherit the ability to produce sFasL from the mother. In addition, we demonstrated that mean cord blood sFasL levels were greater than maternal plasma sFasL levels. A study performed by Sarandakou and colleagues also showed that neonatal serum sFasL levels were significantly higher than maternal concentrations. This implicated that newborns had higher metabolic and apoptosis rates than adults.[28]

The frequencies of being kept awake at night by itchy rashes were positively correlated with sFasL level in our study. Sohn *et al*. also reported that sFasL levels was correlated with the severity of AD.[19] This implicated that sFasL may get involved not only in the pathogenesis of AD but also in the severity of AD. ~~However, we failed to~~

~~find significant difference in cord blood sFasL levels between patients with extrinsic and intrinsic. Until now, only few reports about sFasL levels between extrinsic and intrinsic asthma were found and the results were inconsistent. Some studies reported an increase of sFasL in extrinsic asthma patients, and such increases were associated with the type of anti inflammatory therapy and the kind of allergens.[27,28] Another study demonstrated a decrease of sFasL in an extrinsic asthma group.[29]~~ Further large-scaled study is necessary to evaluate if sFasL levels are associated with IgE related allergic diseases.

Consistent with previous studies,[29] we did not find significant correlation between cord blood IgE and AD. In past decades, many studies attempted to identify putative predictors of atopy in human cord blood, including cord blood IgE. Cord blood IgE was once thought to be a predictor of atopic diseases in infancy, although doubts about its predictability persisted for years. From previous reports, the sensitivity toward atopy ranged only between 10 % and 20 % depending on the cut-off level for cord blood IgE.[29] Moreover, Edenharter *et al.* also indicated that the predictive capacity of cord blood IgE was not high enough to recommend it as screening instruments for primary prevention.[30] Taken all together, cord blood IgE was not a reliable predictor for AD, and cord blood sFasL levels may serve as a surrogate.

The strength of our study included the nested case-control design in the prospective cohort study. Nested case-control studies can reduce recall bias and temporal ambiguity. They can also reduce costs, save time, and confirm causal relationships. Because cord blood sFasL concentrations were higher in neonates born by elective caesarean section than in those born vaginally,[28] we excluded those born by caesarean section from the study cohort. In this way, stress-induced elevation of cord

blood sFasL would not confound our results. Our limitation was that cord blood sFasL levels were checked only ~~once~~ at birth. This might not actually represent an individual's predisposition to sFasL levels throughout gestation and early life. Further serial measurements of sFasL, taken during pregnancy and after birth, may provide a more reliable picture of the child's status and AD pathogenesis. Another limitation was that not all cases of AD were diagnosed by dermatologists in this study. However, case ascertainment by questionnaires in the present study has been previously validated versus clinical examination in other large prospective cohort studies.[15-17]

In conclusion, our results demonstrated a relation between cord blood sFasL and the development of AD in children. ~~Cord blood sFasL may serve as a surrogate for IgE in identifying children with risk of AD.~~ A further large-scaled, long-term follow-up study is necessary to assess if genetic variation, type, and severity of AD have any effect on sFasL levels. Through a more detailed understanding of the Fas/FasL pathway in atopic diseases, we may develop an improved strategy to prevent the development of AD.

REFERENCES

1. Williams H, Flohr C. How epidemiology has challenged 3 prevailing concepts about atopic dermatitis. J Allergy Clin Immunol 118:209-213, 2006.

2. Stensen L, Thomsen SF, Backer V. Change in prevalence of atopic dermatitis between 1986 and 2001 among children. Allergy Asthma Proc 29:392-396, 2008.

3. Kay J, Gawkrodger DJ, Mortimer MJ, et al. The prevalence of childhood atopic eczema in a general population. J Am Acad Dermatol 30:35-39, 1994.

4. Illi S, von Mutius E, Lau S, et al. The natural course of atopic dermatitis from birth to age 7 years and the association with asthma. J Allergy Clin Immunol 113:925-931, 2004.

5. Weidinger S, Illig T, Baurecht H, et al. Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. J Allergy Clin Immunol 118:214-219, 2006.

6. Simpson AB, Yousef E, Hossain J. Evaluation of the relationship between IgE level and skin superinfection in children with atopic dermatitis. Allergy Asthma Proc 31:232-237.

7. Greenhawt M. The role of food allergy in atopic dermatitis. Allergy Asthma

Proc 31:392-397.

8. Sandilands A, Terron-Kwiatkowski A, Hull PR, et al. Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. Nat Genet 39:650-654, 2007.

9. Nagata S, Golstein P. The Fas death factor. Science 267:1449-1456, 1995.

10. Janssen O, Qian J, Linkermann A, et al. CD95 ligand--death factor and costimulatory molecule? Cell Death Differ 10:1215-1225, 2003.

11. Vargo-Gogola T, Crawford HC, Fingleton B, et al. Identification of novel matrix metalloproteinase-7 (matrilysin) cleavage sites in murine and human Fas ligand. Arch Biochem Biophys 408:155-161, 2002.

12. Holler N, Tardivel A, Kovacsovics-Bankowski M, et al. Two adjacent trimeric Fas ligands are required for Fas signaling and formation of a death-inducing signaling complex. Mol Cell Biol 23:1428-1440, 2003.

13. Trautmann A, Akdis M, Kleemann D, et al. T cell-mediated Fas-induced keratinocyte apoptosis plays a key pathogenetic role in eczematous dermatitis. J Clin Invest 106:25-35, 2000.

14. Sohn MH, Lee KE, Kim KE. Interleukin-18 is associated with increased severity of atopic dermatitis in children. Allergy Asthma Proc 25:181-184,

2004.

15. Benn CS, Benfeldt E, Andersen PK, et al. Atopic dermatitis in young children: diagnostic criteria for use in epidemiological studies based on telephone interviews. Acta Derm Venereol 83:347-350, 2003.

16. Chan HH, Pei A, Van Krevel C, et al. Validation of the Chinese translated version of ISAAC core questions for atopic eczema. Clin Exp Allergy 31:903-907, 2001.

17. Yamada E, Vanna AT, Naspitz CK, et al. International Study of Asthma and Allergies in Childhood (ISAAC): validation of the written questionnaire (eczema component) and prevalence of atopic eczema among Brazilian children. J Investig Allergol Clin Immunol 12:34-41, 2002.

18. Odhiambo JA, Williams HC, Clayton TO, et al. Global variations in prevalence of eczema symptoms in children from ISAAC Phase Three. J Allergy Clin Immunol 124:1251-1258 e1223, 2009.

19. Sohn MH, Kim JW, Kim WK, et al. Staphylococcal enterotoxin B upregulates fas-mediated apoptosis of peripheral blood mononuclear cells in childhood atopic dermatitis. Scand J Immunol 57:62-67, 2003.

20. Cork MJ, Robinson DA, Vasilopoulos Y, et al. New perspectives on epidermal

barrier dysfunction in atopic dermatitis: gene-environment interactions. J Allergy Clin Immunol 118:3-21, 2006.

21. Farley SM, Dotson AD, Purdy DE, et al. Fas ligand elicits a caspase-independent proinflammatory response in human keratinocytes: implications for dermatitis. J Invest Dermatol 126:2438-2451, 2006.

22. Ottonello L, Tortolina G, Amelotti M, et al. Soluble Fas ligand is chemotactic for human neutrophilic polymorphonuclear leukocytes. J Immunol 162:3601-3606, 1999.

23. Ueta M, Sotozono C, Inatomi T, et al. Association of Fas Ligand gene polymorphism with Stevens-Johnson syndrome. Br J Ophthalmol 92:989-991, 2008.

24. Zhang B, Sun T, Xue L, et al. Functional polymorphisms in FAS and FASL contribute to increased apoptosis of tumor infiltration lymphocytes and risk of breast cancer. Carcinogenesis 28:1067-1073, 2007.

25. Kavurma MM, Khachigian LM. Signaling and transcriptional control of Fas ligand gene expression. Cell Death Differ 10:36-44, 2003.

26. Tenda Y, Yamashita M, Kimura MY, et al. Hyperresponsive TH2 cells with enhanced nuclear factor-kappa B activation induce atopic dermatitis-like skin

lesions in Nishiki-nezumi Cinnamon/Nagoya mice. J Allergy Clin Immunol 118:725-733, 2006.

27. Devillers AC, van Toorenenbergen AW, Klein Heerenbrink GJ, et al. Elevated levels of plasma matrix metalloproteinase-9 in patients with atopic dermatitis: a pilot study. Clin Exp Dermatol 32:311-313, 2007.

28. Sarandakou A, Protonotariou E, Rizos D, et al. Soluble Fas antigen and soluble Fas ligand in early neonatal life. Early Hum Dev 75:1-7, 2003.

29. Hide DW, Arshad SH, Twiselton R, et al. Cord serum IgE: an insensitive method for prediction of atopy. Clin Exp Allergy 21:739-743, 1991.

30. Edenharter G, Bergmann RL, Bergmann KE, et al. Cord blood-IgE as risk factor and predictor for atopic diseases. Clin Exp Allergy 28:671-678, 1998.

Table 1. Study population characteristics

Characteristic	Case (N=40)	Control (N=80)
Mother		
Maternal age (years old)		
Mean±SD	31.83±4.12	27.56±5.27
Maternal education No.(%)		
High school and below	20 (50.0)	45 (56.3)
College and above	18 (45.0)	34 (42.5)
Missing	2 (5.0)	1 (1.3)
Maternal history of atopy N(%)	8 (20.0)	12 (15.0)
Children		
Birth weight (gm)		
Mean±SD	3245.10±498.18	3146.78±434.08
Gestational age (wks)		
Mean±SD	38.60±1.96	38.56±1.52
Environmental factors		
Duration of breast feeding ≥ 6 months N(%)	11 (27.5)	26 (32.5)
Pet raising N(%)	7 (17.5)	21 (26.9)
Older siblings ≥ 2 N(%)	18 (45.0)	45 (56.3)
Ever had respiratory tract infection N(%)	22 (55.0)	37 (46.3)
Fungi at house wall N(%)	11 (27.5)	18 (22.5)
Carpets at home N (%)	4 (10.0)	11 (13.8)
Postnatal ETS exposure N(%)	12 (30.0)	26 (32.5)
Family income per year (NT dollars) N(%)		
< 600,000	7 (17.5)	20 (25.0)
600,000–1500,000	21 (52.5)	48 (60.0)
> 1500,000	10 (25.0)	10 (12.5)
Missing	2 (5.0)	2 (2.5)

Abbreviations: ETS, environmental tobacco smoke; NT dollars, New Taiwan dollars; SD, standard deviation

Table 2 . Comparison of sFasL and IgE levels in cord and maternal blood in case and control

	Number	Cord blood	Maternal blood
		sFasL mean ± SD (pg/ml)	
Total	120	205.12±53.26	116.00±30.10
AD	40	215.09±58.05	119.24±33.04
Controls	80	185.17±34.91	109.52±22.11
P value†		0.01*	0.10
P value‡		0.003*	0.06
		IgE mean ± SD (KU/1)	
Total	120	1.54± 9.33	73.65± 109.67
AD	40	2.03± 11.54	92.65± 101.12
Controls	80	0.65± 1.23	64.67± 113.96
P value†		0.51	0.21
P value‡		0.48	0.21

* $P < 0.05$
† P value was obtained by independent t-test
‡P value was obtained by mixed model
Abbreviations: SD, standard deviation; sFasL: soluble Fas ligand

FIGURE

Figure 1. Scatter plot correlation of maternal and cord blood sFasL

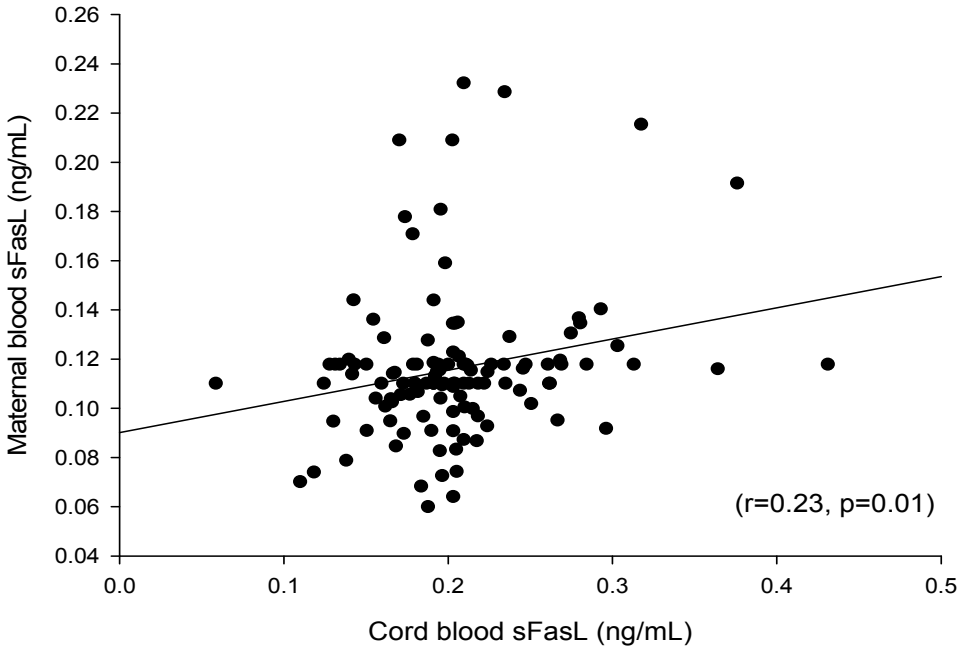


Figure 2. ROC curves of pediatric AD predicted by sFasL in cord blood. The ROC area is 0.64 (95% C.I. 0.54-0.74) for cord blood sFasL. AUC, area under the curve; CI, confidence interval.

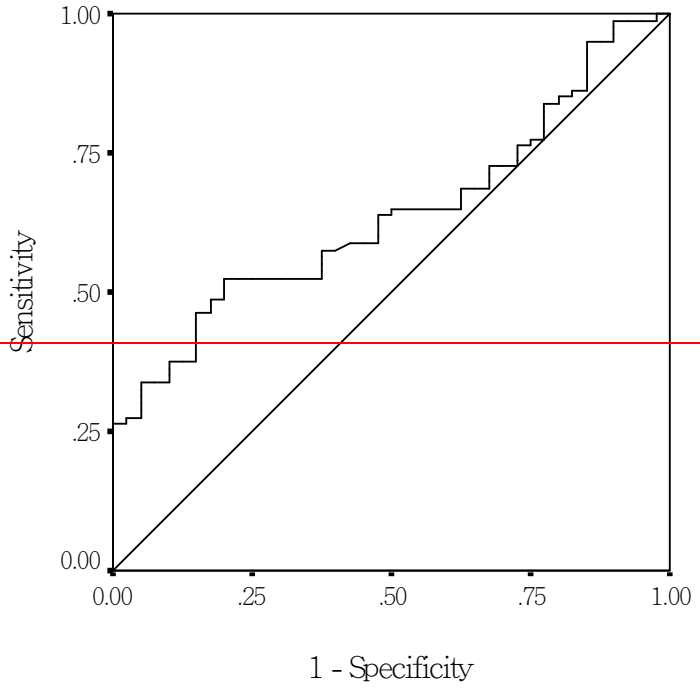


Table 1. Study population characteristics

Characteristic	Case (N=40)	Control (N=80)
Mother		
Maternal age (years old)		
Mean±SD	31.83±4.12	27.56±5.27
Maternal education No.(%)		
High school and below	20 (50.0)	45 (56.3)
College and above	18 (45.0)	34 (42.5)
Missing	2 (5.0)	1 (1.3)
Maternal history of atopy N(%)	8 (20.0)	12 (15.0)
Children		
Birth weight (gm)		
Mean±SD	3245.10±498.18	3146.78±434.08
Gestational age (wks)		
Mean±SD	38.60±1.96	38.56±1.52
Environmental factors		
Duration of breast feeding ≥ 6 months N(%)	11 (27.5)	26 (32.5)
Pet raising N(%)	7 (17.5)	21 (26.9)
Older siblings ≥ 2 N(%)	18 (45.0)	45 (56.3)
Ever had respiratory tract infection N(%)	22 (55.0)	37 (46.3)
Fungi at house wall N(%)	11 (27.5)	18 (22.5)
Carpets at home N (%)	4 (10.0)	11 (13.8)
Postnatal ETS exposure N(%)	12 (30.0)	26 (32.5)
Family income per year (NT dollars) N(%)		
< 600,000	7 (17.5)	20 (25.0)
600,000–1500,000	21 (52.5)	48 (60.0)
> 1500,000	10 (25.0)	10 (12.5)
Missing	2 (5.0)	2 (2.5)

Abbreviations: ETS, environmental tobacco smoke; NT dollars, New Taiwan dollars; SD, standard deviation

Table 2 . Comparison of sFasL and IgE levels in cord and maternal blood in case and control

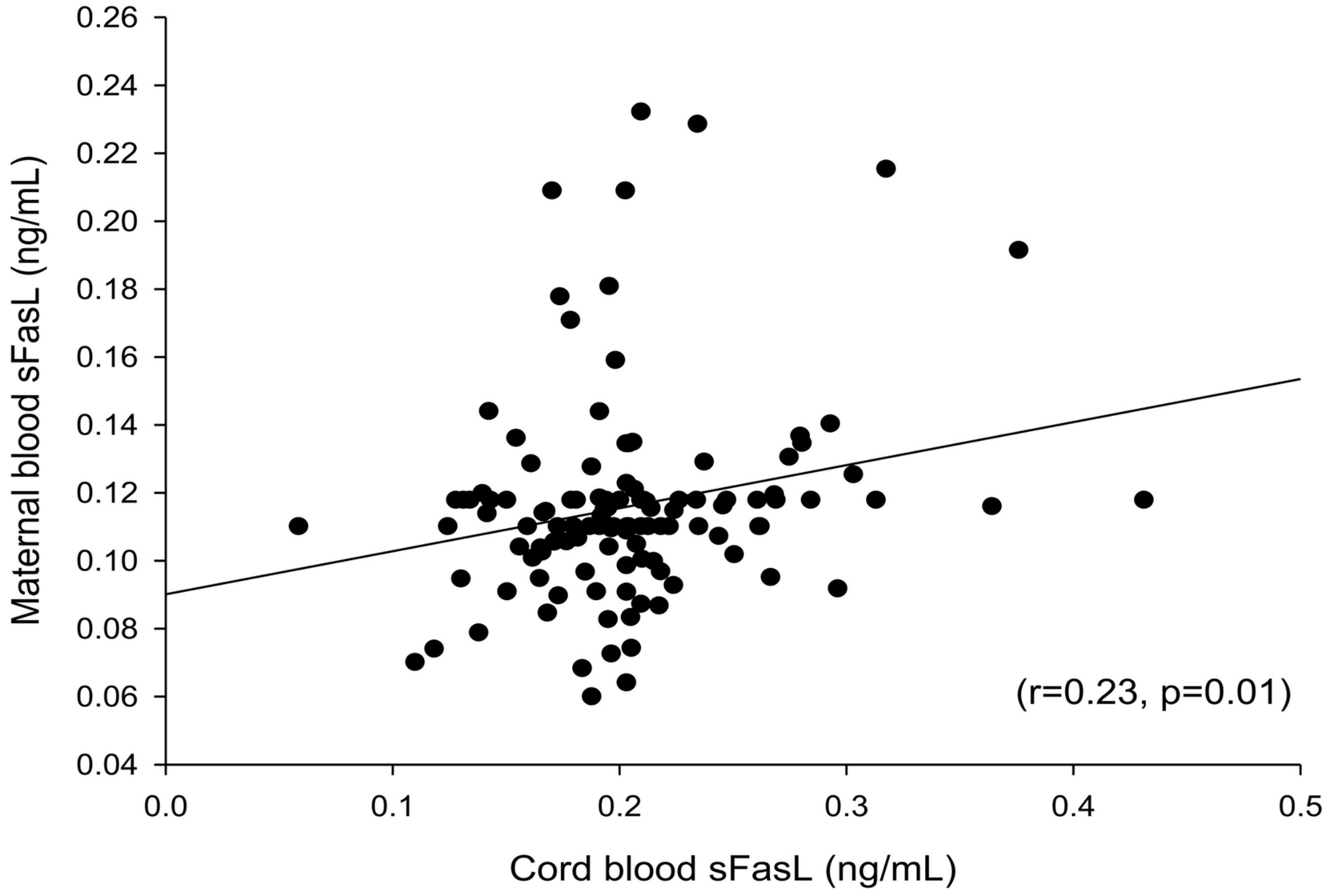
	Number	Cord blood	Maternal blood
		sFasL mean ± SD (pg/ml)	
Total	120	205.12±53.26	116.00±30.10
AD	40	215.09±58.05	119.24±33.04
Controls	80	185.17±34.91	109.52±22.11
P value†		0.01*	0.10
P value‡		0.003*	0.06
		IgE mean ± SD (KU/1)	
Total	120	1.54± 9.33	73.65± 109.67
AD	40	2.03± 11.54	92.65± 101.12
Controls	80	0.65± 1.23	64.67± 113.96
P value†		0.51	0.21
P value‡		0.48	0.21

* $P < 0.05$

† P value was obtained by independent t-test

‡P value was obtained by mixed model

Abbreviations: SD, standard deviation; sFasL: soluble Fas ligand


Maternal blood sFasL (ng/mL)
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.0
0.1
0.2
0.3
0.4
0.5
Cord blood sFasL (ng/mL)
(r=0.23, p=0.01)