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Original Article

Cord blood soluble Fas ligand linked to allergic rhinitis and lung function in seven-year-old children

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Abstract *Background:* Serum or cord blood soluble Fas ligand (FasL) has been related to asthma, allergic rhinitis, and atopic dermatitis in cross-sectional and short-term follow-up studies. However, the association of cord blood soluble FasL with long-term allergic outcomes has seldom been investigated.
Methods: The Prediction of Allergies in Taiwanese Children birth cohort study recruited healthy newborns upon delivery. At birth, blood was collected from the umbilical cords of these children, and the cord blood soluble Fas ligand levels were measured. At the age of seven years, the allergic outcome of each child was diagnosed by pediatric allergists and pulmonologists. Tests were conducted to measure the specific immunoglobulin E, fractional exhaled nitric oxide (FeNO), and pulmonary function levels of each child.
Results: Cord blood soluble FasL levels were higher in seven-year-old children with allergic rhinitis (Odds ratio [OR] = 2.41, p = 0.012) and expiratory airway obstruction (the highest

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forced expiratory volume in 1 second/forced vital capacity < 90%, OR = 2.11, $p = 0.022$). The FeNO and *Dermatophagoides pteronyssinus*-specific immunoglobulin E levels of seven-year-old children were positively correlated with cord blood soluble FasL levels ($p = 0.006$ and 0.02, respectively).

Conclusion: In this birth cohort, the cord blood soluble FasL levels were associated with allergic rhinitis, obstructive-type lung function, FeNO, and house dust mite sensitization in 7-year-old children. The cord blood soluble FasL level might be used as a predictor for allergic diseases in children who are 7 years old.

Introduction

Environmental exposure and maternal status during pregnancy have a great impact on a child's health and susceptibility to diseases from childhood until adulthood.[1] Nutrition, pollution, microbiome, and other environmental factors that interact with the genetic background can reshape the fetal genetics and epigenetics.[2,3] These genetic and epigenetic factors can leave long-term influences on a patient's immunological, cardiovascular, and other systemic functioning and can lead to diseases later in life.[4,5] Many birth cohort studies have investigated the link between perinatal exposure and the outcomes of atopic diseases in an attempt to identify possible predictive biomarkers.[6,7]

Fas and Fas ligand (FasL) interaction is an important pathway in inducing apoptosis. However, soluble Fas ligand, cleaved from the membrane-formed Fas ligand by matrix metalloproteinases, participates in the inflammatory reactions of rheumatic and allergic diseases.[8,9] The concentration of soluble FasL in blood and bronchial lavage fluid has been noted to increase in asthmatic and allergic patients, especially during the allergy season.[10,11] Soluble FasL in cord blood has been associated with atopic dermatitis in children.[12]

The Prediction of Allergies in Taiwanese Children (PATCH) is one of the long-term follow-up birth cohorts in Taiwan. Those children were thoroughly evaluated at 7 years old, including physician examination, allergy tests, lung function tests, and fractional exhaled nitric oxide (FeNO). In this birth cohort study, we measured the soluble FasL concentration in cord blood and investigated its association with allergic outcomes and lung function in seven-year-old children. The purpose of this study is to investigate soluble FasL as a potential biomarker for the prediction of allergy in children.

Methods

Study design and study population

The recruitment for the PATCH study was conducted at Keelung Chang Gung Memorial Hospital from October 2007 to September 2010. The goal of the PATCH study is to identify biomarkers from umbilical cord blood and in early childhood to predict the development of asthma and other allergic diseases. The sample size is estimated to be at least 110 infants enrolled at birth when we set the power to 0.95 and alpha to 0.01. Because the lost-to-follow-up rate is usually high, we doubled the enrolling infants at birth. The comprehensive enrollment process, including the requirements for inclusion and exclusion, has been previously described.[13] Briefly, pregnant mothers were invited to enroll in the study at a gestational age of 32 weeks. After we obtained the written informed consent from each case's mother, the mother's precise prenatal information was collected. Upon delivery, healthy newborns more than 34 weeks of gestation were enrolled. Perinatal information and cord blood samples were collected at birth. At the ages of 6 months, 1 year, 1.5 years, 2 years, and annually thereafter, enrolled children came to the hospital for a follow-up visit. The study was approved by the Human Research Ethics Committee of Chang Gung Memorial Hospital (No. 100-0201B).

At the age of seven years, atopic diseases were diagnosed by pediatric allergists and pulmonologists. The diagnosis of asthma was based on the 2017 Global Initiative for Asthma guidelines.[14] Repeated pulmonary function tests were performed for ambiguous cases. Bronchodilator or methacholine challenge tests were also performed when appropriate. The diagnosis of allergic rhinitis was based on typical symptoms, physical examinations, and allergy tests.[15] As described by Hanifin, atopic dermatitis was diagnosed by the typical manifestations of recurrent pruritic eczema with exudates, dryness, or lichenification.[16]

Measurement of soluble Fas ligand and IgE

Cord blood soluble Fas ligand levels were measured using an enzyme-linked immunosorbent assay (Human Fas Ligand/TNFSF6 DuoSet ELISA; R & D Systems, Inc., Minneapolis, MN, USA). Total immunoglobulin E (IgE) and specific IgE levels were measured by a fluorescent enzyme immunoassay (ImmunoCAP®; detection limit 0.1 kU/L; Phadia, Uppsala, Sweden). We measured specific IgE to common aeroallergens and food allergens in Taiwan, including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, egg white, cow's milk, peanut, crab, shrimp, wheat, soy. Values of specific IgE ≥0.35 kU/L were indicative of allergic sensitization. Atopy was defined as the value of total IgE

≥100 kU/L along with at least sensitization to one allergen.[17]

Measurement of fractional exhaled nitric oxide

FeNO was measured using a handheld electrochemical analyzer (NIOX MINO®; Aerocrine AB, Sweden) according to the 2005 American Thoracic Society/European Respiratory Society recommendations for standardized single-breath online measurement.[18] Children were prevented from eating, drinking, and exercising at least 1 hour before the FeNO measurement; nitrate or nitrate-containing foods were avoided the night prior to testing.

Measurement of pulmonary function tests

Pulmonary function testing was performed through spirometry (Spirolab II®; Medical International Research, Roma, Italy), following the American Thoracic Society/European Respiratory Society recommendations.[19] Three acceptable tests were then recorded. Further, the highest forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC) were also documented. FEV1/FVC <0.9 was defined as expiratory airway obstruction in children.[14,20]

Statistical analysis

The relationship between cord blood soluble FasL and atopic outcomes was analyzed using an independent t-test. Univariate and multivariate logistic regression analyses were used to analyze the risk factors and confounding factors of allergic disease outcomes in seven-year-old children. During the univariate logistic regression, variables with a *p*-value less than 0.1 were included in the subsequent multivariate logistic regression. The relationships between cord blood soluble FasL and FeNO/*D. pteronyssinus*-specific IgE were analyzed by linear regression. All hypothesis testing was two-sided with *a priori* levels of significance set at $p < 0.05$. Statistical analyses were performed with IBM SPSS Statistics for Windows, version 20 (IBM Corp., Armonk, N.Y., USA).

Results

Subject characteristics

At birth, 258 healthy newborns were enrolled into the study. 132 children consistently followed up until seven years old. Among these, 132 children, 10 (7.6%) were diagnosed with asthma, 77 (58.3%) with allergic rhinitis, and nine (6.8%) with atopic dermatitis. Of the ten asthma cases, nine also had allergic rhinitis. All cases with atopic dermatitis also had allergic rhinitis. There was no overlap noted between cases with asthma and atopic dermatitis. There was no significant difference between the baseline demographic characteristics of the 132 children at seven years old and the initial 258 children enrolled at birth (Table 1).

Association of cord blood soluble FasL levels with atopic outcomes at seven years old

The mean values and standard deviations for the cord blood soluble FasL levels in seven-year-old children with or without specific allergic outcomes are shown in Table 2. We observed that the cord blood soluble FasL levels were significantly higher in seven-year-old children with atopy ($p = 0.003$), *D. pteronyssinus* sensitization ($p = 0.003$), and allergic rhinitis ($p = 0.016$). The cord blood soluble FasL levels of these seven-year-old children with asthma and atopic dermatitis were not elevated at birth.

The FeNO levels at seven years were also positively correlated with the cord blood soluble FasL levels ($r = 0.278$, $p = 0.006$, Fig. 1). Similarly, *D. pteronyssinus*-specific immunoglobulin E levels at seven years were positively correlated with cord blood soluble FasL levels ($r = 0.239$, $p = 0.02$, Fig. 2). The seven-year-old children with expiratory airway obstruction (FEV1/FVC < 90%)[14,20] had significantly higher cord soluble FasL levels at birth ($p = 0.022$, Table 2).

Logistic regression analyses of cord blood soluble FasL and allergic outcomes at seven years old

Table 3 shows the risk of having allergic rhinitis at seven years old increasing by 2.41 times ([95% CI, 1.14—5.09], $p = 0.02$) for every 100 pg/mL increase in the cord blood soluble FasL levels. By multivariate logistic regression, it was determined that higher cord blood soluble FasL levels also increased the risk of having atopy (Odds ratio = 4.56 [95% CI, 1.66—12.50]), *D. pteronyssinus* sensitization (Odds ratio = 2.85 [95% CI, 1.17—6.97]), and expiratory flow limitation (FEV1/FVC < 90%) (Odds ratio = 2.11 [95% CI, 1.24—3.58]). Male gender was also a risk factor in having atopy and *D. pteronyssinus* sensitization (Odds ratio = 3.50 [95% CI, 1.38—8.86] and 2.46 [95% CI, 1.03—5.90], respectively).

Discussion

In this study, we demonstrated that infants with higher cord blood soluble FasL levels had a higher risk of developing allergic rhinitis, atopy, and airway obstruction. Serum soluble FasL levels have been associated with allergic diseases in several previous cross-sectional studies. During the allergy season, serum soluble FasL levels were higher in children with allergic rhinitis. This was especially true in those with concomitant asthma.[11] During acute asthma exacerbations, children had significantly higher soluble FasL levels.[21] In adult asthmatic patients, Fas ligand expression was enhanced in bronchial lavage fluid-derived T-cells after a segmental allergen challenge.[10] Cord blood soluble FasL has also been associated with atopic dermatitis in 2-year-old children in a nested case-control study.[12] This study indicates that soluble FasL at birth or during pregnancy has a long-term effect on the development of allergic diseases in children.

Traditionally, the Fas—FasL interaction that induces apoptosis plays a role in the disease pathophysiology of

Table 1 Comparison of demographic data of children at age 7 years and enrolled initially.

Characteristics	Age 7 years (n = 132)		Enrolled (n = 258)		*p*-value
Family					
Maternal atopic diseases	53	(40.2%)	103	(40.1%)	0.985
Asthma	2	(1.5%)	11	(4.3%)	0.147
Rhinitis	35	(26.5%)	75	(29.2%)	0.535
Eczema	10	(7.6%)	19	(7.4%)	0.943
Paternal atopic diseases	70	(53.0%)	127	(49.4%)	0.501
Asthma	5	(3.8%)	10	(3.9%)	0.961
Rhinitis	49	(37.1%)	94	(36.6%)	0.923
Eczema	12	(9.1%)	18	(7.0%)	0.463
Smoking exposure					
Maternal smoking during pregnancy	1	(0.8%)	8	(3.1%)	0.154
Passive smoking	32	(24.2%)	77	(30.0%)	0.229
Older siblings	51	(38.6%)	113	(44.0%)	0.308
Household annual income					0.139
Low $\leq$ 500,000 NTD	40	(30.3%)	95	(36.8%)	
Medium 500,000–1,000,000 NTD	63	(47.7%)	115	(44.6%)	
High $>$ 1,000,000 NTD	29	(22.0%)	48	(18.6%)	
Children					
Sex, male	72	(54.5%)	131	(50.8%)	0.489
Delivery mode, vaginal	81	(61.4%)	159	(61.9%)	0.924
Gestational age (wk)	38.1 $\pm$ 1.8		38.1 $\pm$ 1.7		0.728
Birth body weight (gm)	3082 $\pm$ 501		3041 $\pm$ 413		0.480
Breastfeeding	94	(71.2%)	194	(75.2%)	0.396

Data shown are mean $\pm$ SD or number (%) of patients as appropriate.
NTD, New Taiwan Dollar; yr, years old; wk, week.

conditions such as Steven–Johnson syndrome and toxic epidermal necrolysis.[22] However, soluble FasL also actively participates in inflammation. Patients with systemic lupus erythematosus have higher soluble FasL levels. Unlike membrane-bound FasL, the soluble FasL from systemic lupus erythematosus patients failed to trigger apoptosis. Instead, the soluble FasL promoted T-cell migration and caused inflammation via the NF-κB and PI3K pathways.[9,23] Soluble FasL also promoted T-helper 17 lymphocyte migration[8] and induced the synthesis of proinflammatory cytokines and chemokines such as interleukin-6, interleukin-8, and C–X–C Motif Chemokine Ligand 1.[24]

It is interesting to find cord blood soluble FasL levels were associated with *D. pteronyssinus*-specific IgE levels and FeNO at 7 years old in this study. In adenoids removed from children with allergic rhinitis, FasL and CTLA4 expression were increased, especially from children with house dust mite sensitization.[25] In a cell model, Der p 2, a major allergen of *D. pteronyssinus* induced bronchial epithelial cells apoptosis via Fas/FasL interaction, which

Table 2 Cord blood soluble FasL level and allergic outcomes at 7 years old.

Allergic outcome		Case number	Cord blood sFasL (pg/mL)	*p*-value
Atopy	Yes	60	134.8 $\pm$ 119.4	0.003
	No	38	79.3 $\pm$ 39.3	
D. pteronyssinus sensitization	Yes	59	124.1 $\pm$ 112.3	0.003
	No	39	77.3 $\pm$ 29.1	
Asthma	Yes	6	71.8 $\pm$ 21.7	0.345
	No	98	108.0 $\pm$ 93.0	
Allergic rhinitis	Yes	61	121.8 $\pm$ 109.0	0.016
	No	43	83.3 $\pm$ 48.3	
Atopic dermatitis	Yes	6	189.4 $\pm$ 206.6	0.343
	No	98	100.8 $\pm$ 78.1	
FEV1/FVC $<$ 90%	Yes	35	140.1 $\pm$ 131.2	0.022
	No	90	85.8 $\pm$ 51.1	

All data are expressed as mean $\pm$ standard deviation.
Some parents refused to provide core blood at birth and allow children to receive lung function tests at 7 years old.

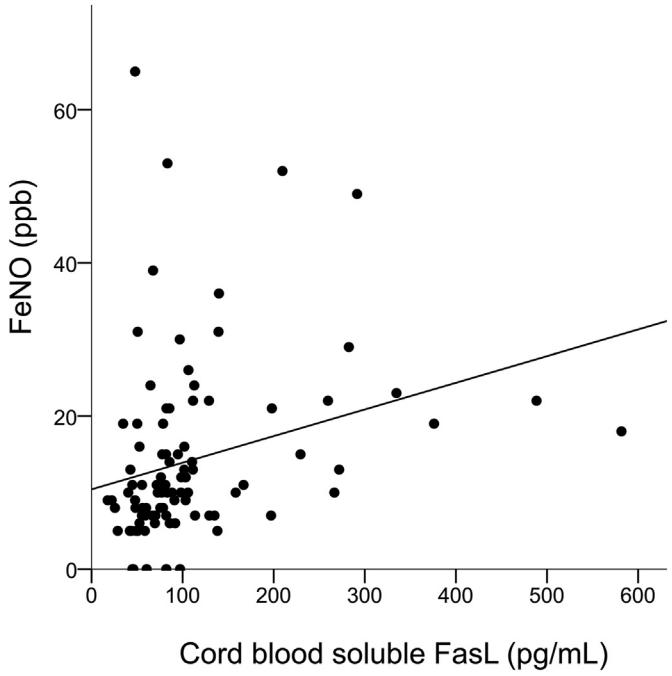

Figure 1. Scatterplot of cord blood soluble FasL and 7-year-old FeNO levels. FeNO: fractional exhaled nitric oxide.

indicated the direct effect of *D. pteronyssinus* on airway remodeling in asthma via Fas—FasL interaction.[26] Even though inducible nitric oxide synthesis and Fas/FasL interaction participated together in the apoptosis of coronary and pulmonary vascular diseases,[27,28] no literature demonstrated the interaction of nitric oxide and FasL in allergic diseases. Therefore, the link between cord blood soluble FasL with FeNO, house dust mite sensitization, and allergic outcomes is worthy of further investigation.

Many birth cohorts have demonstrated that environmental factors, such as antibiotics exposure, breastfeeding, and/or delivery modes, have an impact on the development of allergic diseases, compatible with the developmental origins of health and disease theory.[29,30] In this cohort, the prenatal status, perinatal conditions at

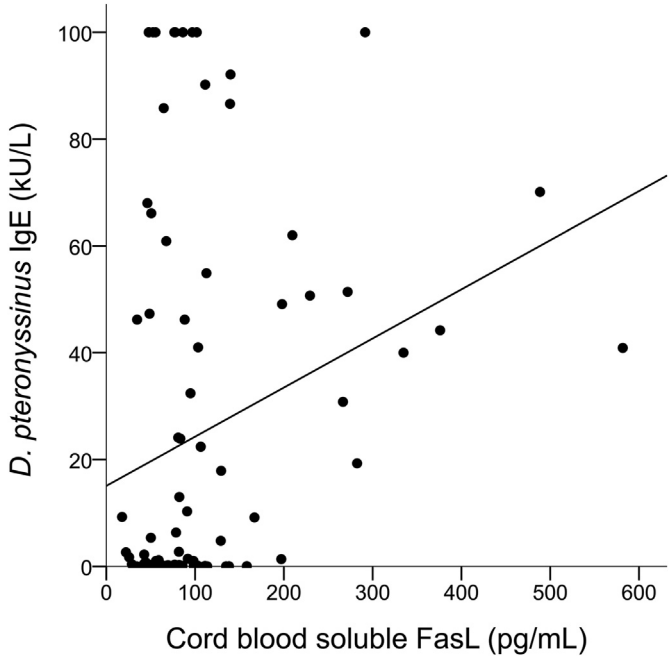

Figure 2. Scatterplot of cord blood soluble FasL and 7-year-old IgE to *Dermatophagoides pteronyssinus*. IgE: Immunoglobulin E.

Table 3 The effect of cord blood soluble FasL on the allergic outcomes at 7 years old.

Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
7 year-old allergic rhinitis				
Cord blood sFasL level (pg/mL)	2.02 (1.02—3.98)	0.04	2.41 (1.14—5.09)	0.02
Paternal allergic rhinitis	2.44 (1.15—5.19)	0.02	2.41 (0.97—6.02)	0.06
Male	1.66 (0.82—3.33)	0.16	2.32 (0.98—5.46)	0.06
Atopy at 7 years old				
Cord blood sFasL level (pg/mL)	4.32 (1.55—12.07)	0.005	4.56 (1.66—12.50)	0.003
Male	3.35 (1.22—9.24)	0.019	3.50 (1.38—8.86)	0.008
***D. pteronyssinus* sensitization**				
Cord blood sFasL level (pg/mL)	2.75 (1.23—6.76)	0.03	2.85 (1.17—6.97)	0.02
Male	2.43 (0.93—6.37)	0.07	2.46 (1.03—5.90)	0.04
FEV1/FVC < 90%				
Cord blood sFasL level (pg/mL)	2.28 (1.27—4.09)	0.006	2.11 (1.24—3.58)	0.006

FasL: Fas ligand, OR: odds ratio, 95% CI: 95% confidence interval.
FEV1: forced expiratory volume in the first second, FVC: forced vital capacity.
Multivariate logistic regression method: backward stepwise (conditional).
Adjusted by gender, mode of delivery, maternal and paternal allergic diseases, smoking during pregnancy and at home, gestational age, Cord blood sFasL level: every 100-increment.

birth, and longitudinal environmental exposure were collected. We ever reported that breastfeeding, cesarean section tobacco smoking exposure, and prenatal exposure to bisphenol-A, were associated with atopic diseases in the infant and preschool period.[31—34] However, we didn't find any environmental factors were associated with atopic outcomes at 7 years old during logistic regression analysis.

This study is limited by the fact that the PATCH birth cohort study is a relatively small cohort. The results found in this research need to be confirmed by larger birth cohorts. Another limitation is that a high percentage of participants either voluntarily withdrew from the study or have been lost to follow-up. The latter is a common challenge in many birth cohort studies. However, the follow-up rate of the PATCH birth cohort study was comparable to those of other birth cohorts that also included serial blood samplings and lung function testing.[35,36]

The strength of this study lies in being a longitudinal cohort that enrolled the general population rather than only newborns at high risk. Moreover, the atopic outcomes were ensured to be accurate since the diagnosis of asthma and other atopic diseases were based on long-term histories and made by pediatric allergists and pulmonologists. Children were predestined to be evaluated at 7 years old to prevent the influence of virus-inducing wheezing, which is common in preschool children.[37] We used spirometry as an objective parameter to improve the accuracy of the diagnosis of asthma. FeNO, a type 2 inflammatory marker of asthma and atopic diseases,[38] were also included as another outcome.

In conclusion, higher cord blood soluble FasL levels at birth have a potentially long-term effect on atopic diseases, allergen sensitization, and lung function. This association indicates that the cord blood soluble FasL level at birth may be used as a biomarker to predict the probable development of allergic diseases later in life.

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