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. Thus, this study investigated the relationship between cord blood soluble FasL and long-term allergic outcomes in a follow-up birth cohort. **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 7-year-old children with allergic rhinitis (Odds ratio [OR] = 2.41, $p = .012$) and expiratory airway obstruction (the highest forced expiratory volume in 1 second/ forced vital capacity $< 90\%$, OR = 2.11, $p = .022$). The FeNO and Dermatophagoides pteronyssinus-specific immunoglobulin E levels of 7-year-old children were positively correlated with cord blood soluble FasL levels ($p = .006$ and .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] In this Prediction of Allergies in Taiwanese Children (PATCH) birth cohort study, we measured the soluble

FasL concentration in cord blood and investigated its association with allergic outcomes and lung function in 7-year-old 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 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 giving consent, 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 7 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 specific IgE to D. pteronyssinus

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). The *D. pteronyssinus* -specific immunoglobulin E (IgE) levels were measured by a fluorescent enzyme immunoassay (ImmunoCAP®; detection limit 0.1 kU/L; Phadia, Uppsala, Sweden). Atopy was defined as the value of total IgE $>$ 0.35 kU/L.[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 (ATS/ERS) 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 ATS/ERS 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.

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 7-year-old children. During the univariate logistic regression, variables with ap- 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 7 years old (Figure 1). Among these, 132 children, 10 (7.6%) were diagnosed with asthma, 77 (58.3%) with allergic rhinitis, and 9 (6.8%) with atopic dermatitis. Of the 10 asthma cases, 9 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 7 years old and the initial 258 children enrolled at birth (Supplementary table).

Association of cord blood soluble FasL levels with atopic outcomes at 7 years old

The mean values and standard deviations for the cord blood soluble FasL levels in 7-year-old children with or without specific allergic outcomes are shown in Table 1. We observed that the cord blood soluble FasL levels were significantly higher in 7-year-old children with atopy (p = .003), *D. pteronyssinus* sensitization (p = .003), and allergic rhinitis (p = .016). The cord blood soluble FasL levels of these 7-year-old children with asthma and atopic dermatitis were not elevated at birth.

The FeNO levels at 7 years were also positively correlated with the cord blood soluble FasL levels (r^2 = .077, p = .006, Figure 2A). Similarly, *Dermatophagoides pteronyssinus* -specific immunoglobulin E levels at 7 years were positively correlated with cord blood soluble FasL levels (r^2 = .057, p = .02, Figure 2B). The 7-year-old children with expiratory airway obstruction (FEV1/FVC < 90%) had significantly higher cord soluble FasL levels at birth (p = .022, Table 1).

Logistic regression analyses of cord blood soluble FasL and allergic outcomes at 7 years old

Table 2 shows the risk of having allergic rhinitis at 7 years old increasing by 2.41 times ([95 % CI, 1.14-5.09], p = .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. Cord blood soluble FasL levels were associated with FeNO and *Dermatophagoides pteronyssinus* -specific IgE levels at 7 years old. This indicates that soluble FasL at birth or during pregnancy has a long-term effect on the development of allergic diseases in children.

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.[20] 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]

Traditionally, the Fas-FasL interaction that induces apoptosis plays a role in the disease pathophysiology of conditions such as Steven-Johnson syndrome and toxic epidermal necrolysis.[21] However, soluble FasL also actively participates in inflammation. Patients with systemic lupus erythematosus (SLE) have higher soluble FasL levels. Unlike membrane-bound FasL, the soluble FasL from SLE patients failed to trigger apoptosis. Instead, the soluble FasL promoted T-cell migration and caused inflammation via the NF-κB and PI3K pathways.[9,22] Soluble FasL also promoted T-helper 17 lymphocyte migration[8] and induced the synthesis of proinflammatory cytokines and chemokines such as interleukin (IL)-6, IL-8, and C-X-C Motif Chemokine Ligand 1.[23] To the best of our knowledge, no literature has been able to investigate the detailed mechanisms

surrounding soluble Fas ligand in allergic diseases. Therefore, the link between cord blood soluble FasL with allergic outcomes is worthy of further investigation.

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.[24,25]

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. The diagnosis of asthma was also facilitated by the objective parameters derived from the lung function and FeNO tests.

In conclusion, higher cord blood soluble FasL levels at birth have a potential 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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Impact Statement: This is the first study demonstrating that increased soluble Fas ligand, even in cord blood, increased the risk of allergic rhinitis, atopy, and lung function in children.

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Table 1. Cord blood soluble FasL level and allergic outcomes at 7 years old

Allergic outcome		Cord blood sFasL (pg/mL)	*P*-value	*P*-value
Atopy	Yes	134.8 ± 119.4	.003	
	No	79.3 ± 39.3		
D. pteronyssinus sensitization	Yes	79.3 ± 39.3	.003	

Allergic outcome		Cord blood sFasL (pg/mL)	*P*-value	*P*-value
	No	77.3 ± 29.1		
Allergic rhinitis	Yes	121.8 ± 109.0	.016	
	No	83.3 ± 48.3		
FEV1/FVC<90%	Yes	140.1 ± 131.2	.022	
	No	85.8 ± 51.1		

All data are expressed as mean ± standard deviation

Table 2. The effect of cord blood soluble FasL on the allergic outcomes at 7 years old

Variables	Univariate analysis	Univariate analysis	Multivariate 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)	.04	2.41 (1.14 – 5.09)	.02
Paternal allergic rhinitis	2.44 (1.15 – 5.19)	.02	2.41 (0.97 – 6.02)	.06
Male	1.66 (0.82 – 3.33)	.16	2.32 (0.98 – 5.46)	.06
Atopy at 7 years old				
Cord blood sFasL level (pg/mL)	4.32 (1.55 – 12.07)	.005	4.56 (1.66 - 12.50)	.003
Male	3.35 (1.22 – 9.24)	.019	3.50 (1.38 – 8.86)	.008
D. pteronyssinus **sensitization**				
Cord blood sFasL level (pg/mL)	2.75 (1.23 – 6.76)	.03	2.85 (1.17 – 6.97)	.02
Male	2.43 (0.93 – 6.37)	.07	2.46 (1.03 – 5.90)	.04
FEV1/FVC < 90%				
Cord blood sFasL level (pg/mL)	2.28 (1.27 – 4.09)	.006	2.11 (1.24 – 3.58)	.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

Figure legends

Figure 1. The study population at birth and at 7-year follow-up.

Figure 2.

(A) Scatterplot of cord blood soluble FasL and 7-year-old FeNO levels.

***** FeNO: fractional exhaled nitric oxide

(B) Scatterplot of cord blood soluble FasL and 7-year-old IgE to*Dermatophagoides pteronyssinus.*

***** IgE: Immunoglobulin E