ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
June 2013; 12(2):107-114.

Raised Interleukin-13 Levels in Cord Blood Increases the Risk of Allergic Sensitization at 5 Years of Age

Nevin Uzuner [1], Arzu Babayigit Hocaoglu [1], Duygu Olmez Erge [1], Suna Asilsoy [2], Ozkan Karaman [1], Zekiye Sultan Altun [3], Abdullah Kumral [4], Ozlem Tufekci [1], and Huray Islekel [3]

[1] Dokuz Eylul University Hospital, Department of Pediatric Allergy, 35340, Balcova, Izmir, Turkey
[2] Baskent University Hospital, Department of Pediatric Allergy, Adana, Turkey
[3] Dokuz Eylul University Hospital, Department of Biochemistry, 35340, Balcova, Izmir, Turkey
[4] Dokuz Eylul University Hospital, Department of Neonatology, 35340, Balcova, Izmir, Turkey

Received: 16 May 2012; Received in revised form: 25 September 2012; Accepted: 8 October 2012

ABSTRACT

The identification of early markers of atopy in cord blood of newborns at delivery may offer prediction of future allergic sensitization. The aim of this study was to evaluate the relationship between cord blood interleukin-13 (IL-13) and interferon-gamma (IFN-γ) and development of allergic diseases during the first five years of life.

Umbilical cord blood samples were collected at the time of delivery from 62 newborns. The families of these newborns were asked to complete a questionnaire about age and education of parents, number of siblings, allergic diseases in family members, cigarette exposure during pregnancy and presence of pets in their house. The same subjects were evaluated when they were five years old. Venous blood samples were drawn and epidermal skin prick tests were performed. IL-13 and interferon-gamma (IFN-γ) levels were studied from the blood samples which were taken during birth and five years later.

There was no significant relationship between gender, type of delivery, educational levels of parents, exposure to cigarette smoke, atopy in parents, presence of pets in the house and IL-13 and IFN-γ levels in cord blood and at five years. Higher levels of IL-13 in newborns and five years olds, were found significantly related to skin prick test positivity (p=0.004 and p<0.0001, respectively) and presence of allergic diseases (p= 0.008 and p= 0.001, respectively). Levels of IFN-γ, both in cord blood and five years after, were not related with the future of allergic status of children.

Higher levels of IL-13 in cord blood may be a predictor of future development of allergic sensitization.

Keywords: Allergy; Cord blood; Interferon-gamma; Interleukin-13

Corresponding Author: Arzu Babayigit Hocaoglu, MD; Department of Pediatric Allergy, Dokuz Eylul University Hospital, 35340, Balcova, Izmir, Turkey. Tel: (+90 232) 4123660, E-mail: arbabayigit@yahoo.com

INTRODUCTION

Exposure to allergens before birth may influence the incidence of allergic diseases later in life.[1] Atopic

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

diseases are characterized by skewing of the T helper (Th)1/Th2 balance away from Th1 toward the Th2 cells.[2] While the Th1 pathway is necessary for cell-mediated immunity, Th2 pathway is essential for the humoral immunity.[3,4] Th2 type cytokines such as interleukin (IL)-4, IL-5 and IL-13 are primarily involved in the induction and maintenance of the IgE antibody production while Th1 type cytokines, interferon-gamma (IFN-γ) and IL-12, antagonize Th2 immune responses.[5]

During pregnancy, the fetomaternal interface is surrounded by high levels of Th2-like cytokines.[6] Prenatal cytokines are thought to play an important role in controlling the functional maturation of the developing fetal immune system.[7] There is evidence that maternal sensitization to allergens decreases the ability to produce the Th1 cytokine IFN-γ in newborn mice[8] and elevates production of the Th2 cytokine IL-13 in the infants.[9] Allergen-specific cord blood immune globulin (Ig) E may also be a predictor for atopic sensitization and allergic diseases later on in life.[10] Current literature reflects conflicting results about the relationship between cord blood cytokines and paediatric atopic diseases. The aim of this study was to evaluate the relation between cord blood Th1 (IFN-γ) and Th2 (IL-13) cytokines and development of allergic sensitization during the first five years of life in the offspring.

MATERIALS AND METHODS

Umbilical venous cord blood (5 ml total) samples were collected at the time of delivery from 62 newborns who were born in Dokuz Eylul University Hospital, Izmir. Newborns whose mothers had chronic diseases were excluded from the study.

IL-13 levels were studied with an ultrasensitive enzyme-linked immunosorbent assay (ELISA) (BioSource USA, Camarillo, CA) and IFN-γ with enzyme-amplified sensitivity immunoassay (EAISA) method in the serum samples which had been stored at -70°C.

The families of these newborns were asked to complete a questionnaire about age and education of parents, number of siblings, allergic diseases in family members, cigarette exposure during pregnancy and presence of pets in their houses. The same subjects were evaluated after five years. Five milliliters of venous blood samples were taken from all children for detection of IL-13 and IFN-γ levels. Epidermal skin prick tests were applied with common allergens using Allergopharma prick tests and evaluated according to Aas and Berlin criteria.[8] Sensitization to house dust mites, grasses, tree pollens, cereals, wild grass pollens, animal danders, moulds, cockroach, food and latex were evaluated with prick tests.

The study was approved by the Ethical Committee of Dokuz Eylul University and was carried out with the written informed consent of the parents.

Statistics

The calculations were made with the statistical package SPSS 14.0 for Windows (SPSS Inc, Chicago, IL, USA). Chi-square analyses were conducted among the subgroups to test for statistically significant differences. $P < 0.05$ was accepted as significant.

RESULTS

Among 62 newborns whose cord blood samples were taken and parents completed the questionnaire, only 40 (64.5%) finished the study. As 22 children and their parents migrated out of the city, they could not come for further evaluation performed five years later. Characteristics of 62 newborns enrolled into the first

Table 1. Demographic data of 62 newborns whose cord blood samples were taken

Demographic data	Number (Percentage)
Gender	
Female	28 (45.4 %)
Male	34 (54.8%)
Educational level of mother	
Primary school	22 (35.5%)
Secondary school	24 (38.7 %)
High school	6 (9.7 %)
University	10 (16.1 %)
Educational level of father	
Primary school	6 (9.7 %)
Secondary school	24 (38.7 %)
High school	29 (46.8 %)
University	3 (4.8 %)
Number of siblings (+/-)	28/34 (45.2/ 54.8 %)
Exposure to cigarette smoke during pregnancy (+/-)	20 /42 (32.3/67.7%)
Atopy in mother (+/-)	3/59 (4.8/95.2 %)
Atopy in father (+/-)	4/58 (6.5/93.5 %)
Presence of pets in house (+/-)	7/55 (11.3/88.7 %)

step of study were given in Table 1. Forty children were evaluated five years after birth and venous blood samples were taken and skin prick tests were performed. IL-13 and IFN-γ levels of cord blood and venous samples from 40 five-year olds are given in Table 2. No significant difference was found between IL-13 and IFN-γ levels in cord blood and venous blood samples which were taken over a five-year interval.

At the end of five year follow up, 25% (10/40) children had doctor-diagnosed symptoms of allergy whereas 75% (30/40) of infants were not diagnosed to have allergies. Of these, 12.5% (5/40) of infants had bronchial asthma, %5 (2/40) had allergic rhinitis and 2.5% (1/40) had atopic dermatitis. All patients with positive skin prick test had symptoms of allergic diseases. The relationship between all demographical data and cytokine levels were summarized in Table 3. There was no significant relationship between gender, type of delivery, educational level of parents, exposure to cigarette smoke during pregnancy, atopy in parents, presence of pets in the house and IL-13 and IFN-γ levels in cord blood after five years. Although no significant relationship was found between demographic data and cytokine levels, a significant relationship was detected between IL-13 levels and skin test positivity (Figure 1-2). Although higher levels of IL-13 in birth (Figure 1) and five years later (Figure 2) were found significantly related with skin prick test positivity (p= 0.004, p< 0.0001, respectively) and presence of allergic diseases (p= 0.008 and p= 0.001, respectively), no significant relationship was found between IFN-γ levels, skin prick test positivity of studied subjects and presence of allergic diseases (Table 3).

No significant difference was noted in terms of gender, type of delivery, educational level of parents, exposure to cigarette smoke during pregnancy, atopy in parents or the presence of pets in skin prick test positive and negative children (Table 4).

Table 2. Levels of IL-13 and IFN-γ in the cord blood and venous blood samples after five years.

	IL-13 (pg/ml)	IFN-γ (IU/ml)
Cord blood	3.82±8.98	3.72±6.52
	(0-37,1)	(0-22.42)
5 years old	2.96±9.59	4.43±3.12
	(0-42.2)	(1.2-14.2)
P-value	0.611	0.534

Data were presented as mean ± SD (minimum–maximum)

Table 3. Relationship between demograhic data and IL-13 and IFN-γ levels (*p* values).

	IL-13 (pg/ml) (Cord blood)	IFN-γ (IU/ml) (Cord blood)	IL-13 (pg/ml) (5^{th} year)	IFN-γ (IU/ml) (5^{th} year)
Gender	0.924	0.741	0.793	0.837
Type of delivery	0.516	0.215	0.698	1.0
Educational level of mother	0.782	0.760	0.257	0.448
Educational level of father	0.806	0.784	0.392	0.514
Exposure to cigarette smoke during pregnancy	0.103	0.215	0.604	0.940
Atopy in mother	0.494	0.049	0.445	0.082
Atopy in father	0.260	0.177	0.594	0.153
Presence of pets in house	0.310	0.454	0.267	0.392
Positivity of skin prick test	0.004*	0.603	0.000*	0.393
Presence of allergic diseases	0.008*	0.720	0.001*	0.493

*: p <0.05

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

Table 4. Demographical data of children according to skin test positivity.

Demographic data		Skin Prick Test		*P* value
		Positive (Number of children)	Negative (Number of children)	
Gender	Female	5	12	0.113
	Male	2	21	
Type of delivery	Vaginal	2	18	0.407
	Section	5	15	
Educational level of mother	Primary/secondary school	5	11	0.076
	High school/University	2	22	
Educational level of father	Primary/secondary school	4	14	0.383
	High school/University	3	19	
Exposure to cigarette smoke during pregnancy	Yes	0	6	0.567
	No	7	27	
Atopy in mother	Yes	0	2	0.677
	No	7	31	
Atopy in father	Yes	0	1	0.825
	No	7	32	
Presence of pets in house	Yes	0	4	0.448
	No	7	29	
Positivity of siblings	Yes	3	18	0.441
	No	4	15	

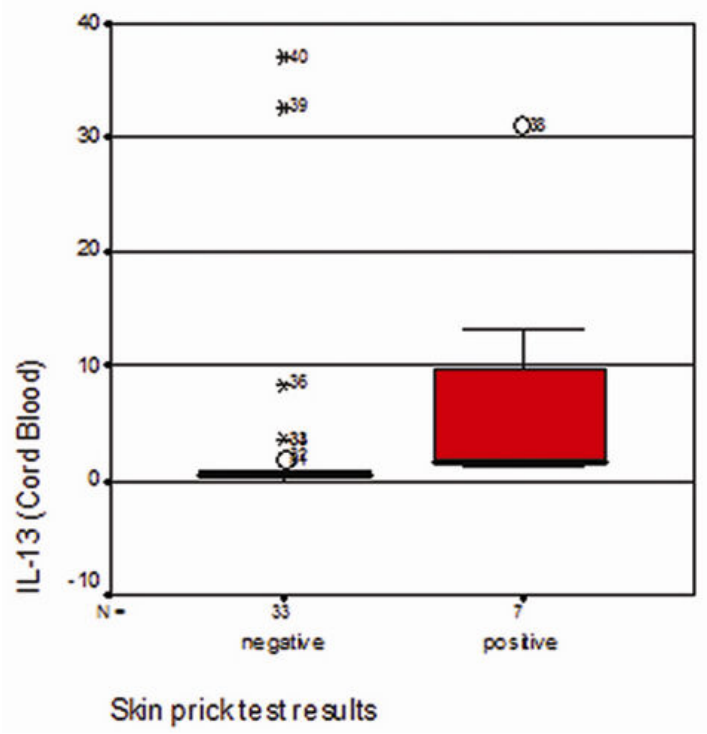

Figure 1. Cord IL-13 levels in skin prick test positive and negative children.

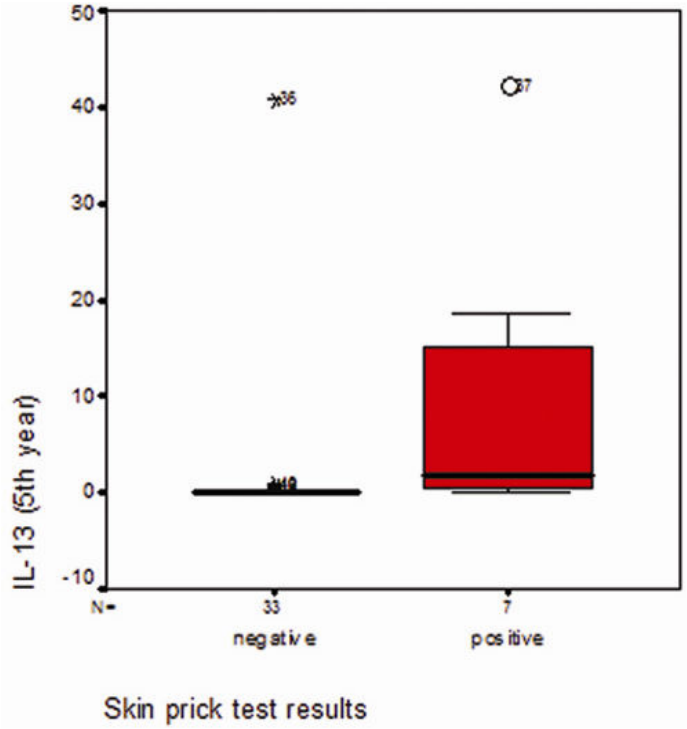

Figure 2. IL-13 levels (5th year) in skin prick test positive and negative children.

DISCUSSION

Immunological events driving Th development at cytokine level begin during the fetal period.[11] Fetal intra-uterine environment is characterized by a Th2 cytokine profile.[12,13] A characteristic feature of atopy is a Th2 immune response and its related cytokines are IL-4, IL-5 and IL-13.[14] It is shown that the fetus is able to produce IgE after the 22nd week of gestation.[15] Antenatal factors may be important in determining susceptibility to atopy. Prenatal cytokine environment may have an important role in controlling the functional maturation of the developing fetal immune system.[16] The identification of early markers of atopy in cord blood of newborns at delivery may offer the possibility of effective preventative strategies in subjects at high risk.

There are some studies evaluating the status of cytokines in cord blood in order to detect the risk of allergic diseases. Although a few of them found no relationship between cytokines in cord blood and allergic diseases, most of them suggest that there is an association between some cytokines in cord blood and future atopy. Macaubas *et al.*[17] reported that cord blood concentrations of IL-4 and IFN-γ were associated with lower risk of asthma and atopy (sensitization to some inhalant allergens) outcome in six year olds. A study reported by Kondo et al suggested that newborns with atopy risk had a lower IFN-γ producing capacity at birth.[18] In a cohort study, the predictive value of a cord serum screen test and possible subsequent development of allergic disease in infants is evaluated and it is reported that infants who had allergic diseases in the first year of life had significantly elevated IgE and significantly low IFN-γ levels in cord serum.[19] Herberth *et al.* also suggested that low levels of IFN- γ and high levels of IL-4-producing T cells at birth may enhance the risk of subsequent development of atopic dermatitis.[20] The results of a recently published prospective study indicate that low cord blood Treg numbers may predict early atopic dermatitis.[21]

Perinatal factors during delivery might be associated with the development of allergic diseases later.[22] Conflicting results were found in studies about the mode of delivery as a risk factor of later-developing allergic diseases.[23-25] Pistiner *et al.*[26] found that cesarean delivery is associated with allergic rhinitis and atopy among children with a parental history of asthma

or allergies. They suggested that this could be explained by lack of contact with the maternal vaginal/fecal flora or reduced/absent labor during cesarean delivery. It is known that cesarean section babies have a different gut flora,[27] which has been suggested to prolong immunological immaturity and thereby increase the risk of later development of allergic disease.[28] In a meta-analysis performed by Bager *et al*,[29] an association was found between delivery by cesarean section and a moderate risk increase for allergic rhinitis, asthma and perhaps food allergy, but not with inhalant atopy or atopic dermatitis. In our study, neither cytokine levels nor status of atopy were associated with the type of delivery.

IL-13 is a Th2 -type cytokine that is primarily produced by activated T cells,[30] with the highest levels derived from $CD4^+$ Th0 and Th2 T-cell clones. Human basophils and mast cells are also capable of producing IL-13.[31,32] IL-13 has been shown to be a critical mediator of the effector arm of the allergic response. Overproduction of this cytokine has been shown to induce airway hyperresponsiveness, eosinophilic inflammation, IgE production, mucus hypersecretion, and subepithelial fibrosis.[33] Williams *et al.*[16] found that IL-13 is spontaneously produced by fetal blood mononuclear cells. The detection of this Th2-type cytokine from as early as 27 weeks of gestation indicates that the fetal immune system is capable of cytokine synthesis and secretion. Prokesová *et al.*[34] tested cytokines IL-4, IL-5, IL-6, IL-10, IL-13, IFN-γ and transforming growth factor (TGF)-β in 21 healthy and 21 allergic mothers and their children of up to one year of age by taking samples at the time of delivery, four days post-partum and then after three, six and 12 months. They found that there were relatively high levels of IL-13 in cord blood, which showed a marked decrease till the fourth day after delivery. They suggested that the decrease could reflect the largely placental origin of newborn's IL-13. Some studies have shown an association between IL-13 gene polymorphism and IgE levels.[35,36] Sadeghnejad *et al.*[37] recently reported an important relation between IL-13 polymorphism and cord serum IgE for the first time in literature. In our study both cord blood and serum IL-13 levels in children in their five year of life were significantly and positively associated with allergic sensitization.

Improved hygiene practices may be partly responsible for the increased prevalence of atopy.[38] Exposure to infectious agents in childhood stimulates the immune system in favour of a Th1 response which inhibits Th2 cells.[39] It was shown that larger family size and domestic crowding had a protective effect on atopic sensitization.[40,41] According to the hygiene hypothesis, the presence of a large family size and older siblings protects the development of allergic disease by mediating infection acquired from contact with siblings.[40] In our study no association was found between the number of siblings and the presence of atopy. We also found no relationship between the presence of pets at home and allergic sensitization in the current study. In a study performed by Kuyucu *et al,*[42] higher paternal education level was the strongest risk factor showing a linear positive relationship with atopic sensitization. Also there are some studies showing positive associations between the skin test positivity and higher socioeconomic status[40,41] or parental education status in the literature.[43] We also found no relationship between the educational levels of the parents and the skin test results.

In summary, the results of this study showed that IL-13 levels in cord blood and venous samples in five year olds were significantly related with presence of allergic diseases and skin prick test positivity. Higher levels of IL-13 in cord blood may be used as a predictor for atopic sensitization later in life.

REFERENCES

1. Sandberg M, Frykman A, Ernerudh J, Berg G, Matthiesen L, Ekerfelt C, et al. Cord blood cytokines and chemokines and development of allergic disease. Pediatr Allergy Immunol 2009; 20(6):519-27.
2. Bidad K, Nicknam MH, Farid R. A Review of Allergy and Allergen Specific Immunotherapy. Iran J Allergy Asthma Immunol 2011; 10(1): 1-9.
3. Movérare R, Elfman L, Stålenheim G, Björnsson E. Study of the Th1/Th2 balance, including IL-10 production, in cultures of peripheral blood mononuclear cells from birch-pollen-allergic patients. Allergy 2000; 55(2):171-5.
4. Grünig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. Science 1998; 282(5397):2261-3.
5. Novak N, Bieber T. Allergic and nonallergic forms of atopic diseases. J Allergy Clin Immunol 2003;

112(2):252-62.
6. Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol Today 1993; 14(7):353-6.
7. Devereux G, Barker RN, Seaton A. Antenatal determinants of neonatal immune responses to allergens. Clin Exp Allergy 2002; 32(1):43–50.
8. Herz U, Ahrens B, Scheffold A, Joachim R, Radbruch A, Renz H. Impact of in utero Th2 immunity on T cell deviation and subsequent immediate-type hypersensitivity in the neonate. Eur J Immunol 2000; 30(2):714-8.
9. Kopp MV, Zehle C, Pichler J, Szépfalusi Z, Moseler M, Deichmann K, et al. Allergen-specific T cell reactivity in cord blood: the influence of maternal cytokine production. Clin Exp Allergy 2001; 31(10):1536–43.
10. Pfefferle PI, Sel S, Ege MJ, Büchele G, Blümer N, Krauss-Etschmann S, et al. Cord blood allergen-specific IgE is associated with reduced IFN-gamma production by cord blood cells: the Protection against Allergy-Study in Rural Environments (PASTURE) Study. J Allergy Clin Immunol 2008; 122(4):711-6.
11. Allam JP, Zivanovic O, Berg C, Gembruch U, Bieber T, Novak N. In search for predictive factors for atopy in human cord blood. Allergy 2005; 60(6):743-50.
12. Jones CA, Holloway JA, Warner JO. Fetal immune responsiveness and routes of allergic sensitization. Pediatr Allergy Immunol 2002; 13 Suppl 15:19-22.
13. Raghupathy R, Makhseed M, Azizieh F, Hassan N, Al-Azemi M, Al-Shamali E. Maternal Th1- and Th2-type reactivity to placenta antigens in normal human pregnancy and unexplained recurrent spontaneous abortions. Cell Immunol 1999; 96(2):122-30.
14. Leung DY, Boguniewicz M, Howell MD, Nomura I, Hamid QA. New insights into atopic dermatitis. J Clin Invest 2004; 113(5):651-7.
15. Lima JO, Zhang L, Atkinson TP, Philips J, Dasanayake AP, Schroeder Jr HW. Early expression of iepsilon, CD23 (Fcepsilon- RII), IL-4Ralpha, and IgE in the human fetus. J Allergy Clin Immunol 2000; 106(5):911-7.
16. Williams TJ, Jones CA, Miles EA, Warner JO, Warner JA. Fetal and neonatal IL-13 production during pregnancy and at birth and subsequent development of atopic symptoms. J Allergy Clin Immunol 2000; 105(5):951-9.
17. Macaubas C, de Klerk NH, Holt BJ, Wee C, Kendall G, Firth M, et al. Association between antenatal cytokine production and the development of atopy and asthma at age 6 years. Lancet 2003; 362(9391):1192-7.
18. Kondo N, Kobayashi Y, Shinoda S, Takenaka R, Teramoto T, Kaneko H , et al. Reduced interferon gamma production by antigen-stimulated cord blood mononuclear cells is a risk factor of allergic disorders – 6-year follow-up study. Clin Exp Allergy 1998; 28(11):1340-4.
19. Shah S, Bapat M. Cord serum screening test and the newborns allergic status. Indian Pediatr 2009; 46(4):295-9.
20. Herberth G, Heinrich J, Röder S, Figl A, Weiss M, Diez U, et al. Reduced IFN-gamma- and enhanced IL-4-producing CD4+ cord blood T cells are associated with a higher risk for atopic dermatitis during the first 2 yr of life. Pediatr Allergy Immunol 2010; 21(1 Pt 1):5-13.
21. Hinz D, Bauer M, Röder S, Olek S, Huehn J, Sack U, et al. Cord blood Tregs with stable FOXP3 expression are influenced by prenatal environment and associated with atopic dermatitis at the age of one year. Allergy 2012; 67(3):380-9.
22. Keski-Nisula L, Harju M, Järvelin MR, Pekkanen J. Vacuum-assisted delivery is associated with late-onset asthma. Allergy 2009; 64(10):1530-8.
23. McKeever TM, Lewis SA, Smith C, Hubbard R. Mode of delivery and risk of developing allergic disease. J Allergy Immunol 2002; 109(5):800-2.
24. Maitra A, Sheriff A, Strachan D, Henderson J; ALSPAC Study Team. Mode of delivery is not associated with asthma or atopy in childhood. Clin Exp Allergy 2004; 34(9):1349-55.
25. Renz-Polster H, David MR, Buist AS, Vollmer WM, O'Connor EA, Frazier EA, et al. Caesarean section delivery and the risk of allergic disorders in childhood. Clin Exp Allergy 2005; 35(11):1466-72.
26. Pistiner M, Gold DR, Abdulkerim H, Hoffman E, Celedón JC. Birth by cesarean section, allergic rhinitis, and allergic sensitization among children with a parental history of atopy. J Allergy Clin Immunol 2008; 122(2):274-9.
27. Grönlund MM, Lehtonen OP, Eerola E, Kero P. Fecal microflora inhealthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery. J Pediatr Gastroenterol Nutr 1999; 28(1):19-25.
28. Bjorksten B. Effects of intestinal microflora and the environment on the development of asthma and allergy. Springer Semin Immunopathol 2004; 25(3-4):257-70.
29. Bager P, Wohlfahrt J, Westergaard T. Caesarean delivery and risk of atopy and allergic disease: meta-analyses. Clin Exp Allergy 2008; 38(4):634-42.

30. Minty A, Chalon P, Derocq J-M, Dumont X, Guillemot J-C, Kaghad M, et al. IL-13 is a new human lymphokine regulating inflammatory and immune responses. Nature 1993; 362(6417):248-50.
31. Burd PR, Thompson WC, Max EE, Mills FC. Activated human mast cells produce interleukin-13. J Exp Med 1995; 181(4):1373-80.
32. Li T, Sim TC, Alam LR. Interleukin-13 released by and localised in human basophils. J Immunol 1996; 156(12):4833-8.
33. Wills-Karp M. IL-12/IL-13 axis in allergic asthma. J Allergy Clin Immunol 2001 ; 107(1):9-18.
34. Prokesová L, Lodinová-Zádníková R, Zizka J, Kocourková I, Novotná O, Petrásková P, et al. Cytokine levels in healthy and allergic mothers and their children during the first year of life. Pediatr Allergy Immunol 2006; 17(3):175-83.
35. Graves PE, Kabesch M, Halonen M, Holberg CJ, Baldini M, Fritzsch C, et al A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children. J Allergy Clin Immunol 2000; 105(3):506-13.
36. Leung TF, Tang NL, Chan IH, Li AM, Ha G, Lam CW. A polymorphism in the coding region of interleukin-13 gene is associated with atopy but not asthma in Chinese children. Clin Exp Allergy 2001; 31(10):1515-21.
37. Sadeghnejad A, Karmaus W, Hasan Arshad S, Ewart S. IL13 gene polymorphism association with cord serum immunoglobulin E. Pediatr Allergy Immunol 2007; 18(4):288-92.
38. Strachan DP. Hay fever, hygiene, and household size. BMJ 1989; 299(6710):1259–60.
39. Openshaw PJM, Walzl G. Infections prevent the development of asthma—true, false or both? J R Soc Med 1999; 92(10):495-9.
40. Strachan D. Family size, infection and atopy: The first decade of the hygiene hypothesis. Thorax 2000; 55 Suppl 1:S2-10.
41. Forastiere F, Agabiti N, Corbo GM, Dell'Orco V, Porta D, Pistelli R, et al. Socioeconomic status, number of siblings and respiratory infections in early life as determinants of atopy in children. Epidemiology 1997; 8(5):566-70.
42. Kuyucu S, Saraçlar Y, Tuncer A, Saçkesen C, Adalioglu G, Sümbüloglu V, et al. Determinants of atopic sensitization in Turkish school children: effects of pre- and post-natal events and maternal atopy. Pediatr Allergy Immunol 2004; 15(1):62-71.
43. Ring J, Kramer U, Schafer T, Abeck D, Vieluf D, Behrendt H. Environmental risk factors for respiratory and skin atopy: Results from epidemiological studies in former East and West Germany. Int Arch Allergy Immunol 1999; 118(2-4):403-7.