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***IL13* gene polymorphism association with cord serum immunoglobulin E**

Alireza Sadeghnejad¹, Wilfried Karmaus¹, S. Hasan Arshad², and Susan Ewart³

¹ *Department of Epidemiology and Biostatistics, University of South Carolina, Columbia, South Carolina, USA*

² *The David Hide Asthma and Allergy Research Centre, Isle of Wight, UK*

³ *National Food Safety and Toxicology, Michigan State University, East Lansing, Michigan, USA*

Abstract

Interleukin-13 (IL-13) has a pivotal role in the pathway of immunoglobulin E (IgE). Cord serum IgE has been suggested to be associated with allergy later in life, yet less affected by environmental exposures. We investigated the association of the interleukin-13 gene (*IL13*) polymorphisms on cord serum IgE. In the Isle of Wight birth cohort (UK, 1989–1990), cord serum IgE was measured using the ULTRA EIA® kit and was dichotomized at 0.5 kU/l (n = 1358). Five single nucleotide polymorphisms (SNPs: rs1800925 in promoter, rs2066960 in intron 1, rs1295686 in intron 3, rs20541 in exon 4 and rs1295685 in exon 4) in *IL13* were genotyped by pyrosequencing method. Linkage analysis using Haploview software revealed that rs1295686, rs20541 and rs1295685 were in strong linkage disequilibrium. Logistic regression and Armitage-Cochran test were used and gene association analysis included 798 children. Confounders were maternal age; maternal smoking, household dog, and household cat during pregnancy; season of birth; sex; position of child in family; and birth weight. SNP rs1295685 was associated with raised cord serum IgE (p = 0.031). This is the first report that shows an association between *IL13* polymorphism and cord serum IgE.

Keywords

interleukin-13; polymorphism; immunoglobulin E

Allergic mechanism has long been attributed to immunoglobulin E (IgE)-mediated reactions and cord serum IgE has been suggested to be a biomarker for atopic diseases later in life (1, 2).

Compared with serum IgE later in life, cord serum IgE seems to be a better marker for genetic association studies as it is not affected by postnatal environmental factors. However, there is limited information with regard to the effect of genes on cord serum IgE. Chang et al. (3) reported an association of cytotoxic T-lymphocyte antigen-4 (CTLA-4) polymorphisms with raised cord serum levels that was restricted to girls (3). Interleukin-13 (IL-13) is an important cytokine involved in the IgE pathway (4). Several studies have shown an association between *IL13* gene polymorphism and IgE levels (5–9) but there is no report for the effect of the *IL13* gene on IgE at birth.

In the current study, we investigated the association of *IL13* gene polymorphisms and raised cord serum IgE (≥ 0.5 kU/l) controlling prenatal factors that may affect IgE levels. The data

are from the Isle of Wight birth cohort, established in 1989 in order to study the risk factors for atopy and its development during childhood.

Population and methods

Study population

After approval of the local Research Ethics Committee and parental informed consent 1456 children born on the Isle of Wight, UK between 1 January 1989 and 28 February 1990 were enrolled at birth. This predominantly Caucasian birth cohort (99%) was followed up to 10 years of age.

Birth records, exposures during pregnancy and family history

Data were obtained from hospital records on neonatal complications (pre-term delivery, foetal distress, newborn infection, jaundice, low blood sugar, hypothermia and congenital abnormalities), antenatal complications (pregnancy-induced hypertension, gestational diabetes, infections needing antibiotic therapy, intrauterine growth retardation or 'miscellaneous') and method of delivery (non-instrumental vaginal and instrumental vaginal or caesarean section).

Parents were asked about their age, maternal smoking and pet-keeping during pregnancy, history of asthma, hay fever and atopic eczema.

IL13 genotyping

IL13 is a relatively small gene (2.9 kb) and only a few single nucleotide polymorphisms (SNPs) were needed for a reasonable assessment of genetic associations. The *IL13* gene and its variants were assessed using the SNPper (snp-per.chip.org) and Applied Biosystems (<http://www.appliedbiosystems.com>) databases. Polymorphism selection was based on SNP location, minor allele frequency and function. Selected SNPs had minor allele frequencies $\geq 19\%$ and were distributed throughout the *IL13* gene. Five SNPs from the *IL13* gene were used in this study, rs1800925 in the 5' promoter region; rs2066960 in intron 1, rs1295686 in intron 3, rs20541 in exon 4 [a coding variant: the common allele (G) coding for arginine and the minor allele (A) encoding glutamine]; and rs1295685 in the 3' untranslated region (3' UTR) of exon 4.

Genotypes were determined on DNA isolated from whole-blood samples obtained from 921 cohort children at age 10. Genomic DNA was isolated using QIAamp DNA Blood Kits (Qiagen, Valencia, CA, USA) or ABI PRISM™ 6100 Nucleic Acid PrepStation (Applied Biosystems, Foster City, CA, USA). Commercially available fluorogenic 5' nuclease chemistry polymerase chain reaction (PCR) assays (Taqman; Applied Biosystems) cycled on a 7900HT Sequence Detection System were initially used to determine genotypes for all the SNPs (rs1800925, ABI assay C_8932056; rs2066960, ABI assay C_15862743; rs1295686, ABI assay C_8932053; rs1295685, ABI assay C_8932052; rs20541, ABI custom assay). Some samples failed to amplify using these assays, in which case additional genotype data were generated by biotin-streptavidin-based pyrosequencing assays (see footnote of Table 2) performed on PSQ-96 instrumentation (Biotage AB, Uppsala, Sweden) or in the case of rs20541 by restriction digest (5).

Cord serum IgE determination

ULTRA EIA® kit (Pharmacia Diagnostics AB, Uppsala, Sweden), designed to measure IgE between 0.2 and 50 kU/l on 0.1 ml of serum, was used to perform duplicate measurements of IgE on cord serum obtained from the umbilical vein. This method is a solid-phase enzyme immunoassay that uses monoclonal anti-human IgE, with a high degree of correlation with the

Phadebas IgE PRIST® method ($r = 0.99$) (10). Cord IgA was measured on samples with an IgE level >0.3 kU/l. Any sample with IgA > 10 mg/l was considered to be contaminated by maternal blood and excluded from the analysis (10).

Statistical analysis

We used Haploview 3.2 software to test Hardy-Weinberg equilibrium for each SNP and to investigate pair-wise linkage disequilibrium (LD) between SNPs (11). For SNPs that were in strong LD based on D' and r^2 (12), one SNP was chosen as the representative marker for the block. Statistical analysis, using SAS/STAT® version 9.1, was performed on the data from children who had complete information on *IL13* genotypes and cord serum IgE. Logistic regression analysis and Armitage-Cochran tests for trend (assuming an additive model for minor allele) were conducted to test the association between covariates, *IL13* polymorphisms and raised cord serum IgE (≥ 0.5 kU/l). We also used logarithmic transformation of cord serum IgE as the outcome in a linear regression model. Potential confounders were: maternal age (categorized as 16–23, 24–33, ≥ 34 yr); maternal atopy (yes, no); paternal atopy (yes, no); maternal smoking during pregnancy (yes, no); household dog present during pregnancy (yes, no); household cat present during pregnancy (yes, no); season of birth (spring March–May; summer June–August; fall September–November; winter December–February); sex; birth order (first born, older siblings); and birth weight (≥ 2500 g, < 2500 g). We also investigated whether antenatal complications (yes, no); non-instrumental delivery (yes, no); and neonatal complications (yes, no) had an effect on cord serum IgE levels.

Results

Data on *IL13* genotypes and cord serum IgE were available for 798 children. These children had younger mothers and also less exposure to maternal smoking in comparison with all children who had information on cord serum IgE (results not shown). Among covariates, maternal atopy, being born in the fall, and being first born were risk factors for raised cord serum IgE (Table 1).

All five *IL13* SNPs were in Hardy-Weinberg equilibrium. Three of the SNPs (rs1295686, rs20541 and rs1295685) were in strong LD (minimum pair-wise r^2 and D' , 0.78 and 0.93, respectively). Therefore, one SNP (rs1295685) of this block was used for subsequent analysis.

Assuming an additive effect for a minor allele of rs1295685, *IL13* polymorphism at this locus was associated with raised cord serum IgE (Armitage-Cochran p value = 0.03; Table 2). Considering a dominant effect for the rare allele and including potential confounders did not change this association (odds ratio, OR = 1.61, Table 2). Analyzing logarithmic transformation of cord serum IgE in a linear regression model showed a similar effect (p value = 0.02 for the effect of minor allele, data are not shown).

Discussion

Using the data from the Isle of Wight birth cohort, this study investigated the association of five SNPs in the *IL13* gene and cord serum IgE. *IL13* gene polymorphism rs1295685 appeared to have a possibly dominant effect on cord IgE level ≥ 0.5 kU/l. To the best of our knowledge this is the first investigation identifying an association between *IL13* and IgE at birth.

Complete information was available for about 87% of the children who provided blood for genotyping. Although these children appeared to have statistically significant younger mothers and also less exposure to maternal smoking in comparison with all children who had information on cord serum IgE, the distribution of the outcome variable, cord serum IgE, was not different in the original sample and the sample used in the analysis. Therefore, estimates

were not affected by the selection process (13). With regard to the genotypes, the presence of a selection bias could result in a violation of the Hardy-Weinberg equilibrium. The genotypes of the five SNPs examined were in Hardy-Weinberg equilibrium and their allele frequencies were comparable with the equivalent SNP in other Caucasian populations (5,7). Hence, with regard to the SNPs a selection bias is unlikely.

Contamination of cord blood with maternal blood was assessed using IgA as a biomarker. This led to the exclusion of 86 infants from further analysis (10). The current analyses also revealed that antenatal complications, mode of delivery or neonatal complications did not seem to affect cord serum IgE. Hence, a potential misclassification of cord blood IgE is not likely. Cord serum IgE was below the detection limit of the assay (0.2 kU/l) in 60% of the children, very few had levels more than 1.0 kU/l and the rest had levels between 0.2 and 1.0 kU/l. Owing to this highly skewed distribution, logarithmic transformation could not normalize cord serum IgE. Most of the previous studies had analysed cord serum IgE as a dichotomized variable and the majority of these studies had used a cut-off point of 0.5 kU/l. We chose to use the cut-off point of 0.5 kU/l as it has been used in other studies (2,3) as well as in previous analyses of the Isle of Wight birth cohort cord serum IgE (1). In addition, to approximate a linear association, we used logarithmic transformation of cord serum IgE level. The results from linear regression models were comparable with those applying a cut-off of 0.5 kU/l in logistic regression. However, as the normal distribution requirement for linear regression was not fulfilled, we focused on results from logistic regression models.

Several studies have shown an association between *IL13* polymorphisms and serum IgE (5–9). However, there is no report for the effect of *IL13* polymorphisms on cord serum IgE. The SNP that we observed to be associated with raised cord serum IgE (rs1295685) was in strong LD with two other SNPs (rs1295686, rs20541). SNP rs20541 is a coding variant with common allele (G) coding for arginine and the minor allele (A) encoding glutamine. There is very limited information on the association of genes and cord serum IgE (3). An alternate explanation for our finding can be an indirect effect of maternal genotype through intrauterine environment on cord serum IgE. We did not have information on the mothers' genotypes, but maternal IgE levels were measured within the first week after delivery (14). However controlling for this marker did not change the results. Although the authors cannot exclude the possibility for findings to be false-positive, due to several similar reports in independent populations, this possibility is unlikely.

In the current study, maternal atopy, season of birth and position of child in the family appeared to affect cord serum IgE levels. We included these factors in the model. The levels of cord serum IgE may have been affected by prenatal environmental factors such as the season of birth. However, using serum IgE at birth, compared with IgE determinations later in life, provides an opportunity to avoid the impact of postnatal factors that may influence IgE. As cord serum IgE has the advantage of not being affected by numerous postnatal conditions, it should be considered as a preferable outcome marker for genetic association studies in comparison with IgE later in life.

In summary, our results corroborate prior reports that polymorphisms in the *IL13* gene and IgE are associated. In addition, the study demonstrates that using cord serum IgE facilitates the use of more parsimonious explanatory models in genetic epidemiology, as we do not have to control for a large number of postnatal exposures.

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Table 1
Non-adjusted odds ratios, logistic regression, for covariates on cord serum immunoglobulin E (IgE)

Covariate	Cord serum IgE		OR	95% CI	p value
	<0.5	≥0.5			
Maternal age ^a ≥24, <34	67.6% (700/1035)	65.2% (101/155)	0.72	0.39, 1.31	0.28
Maternal age ≥34 ^a	28.0% (130/465)	21.7% (15/69)	0.89	0.63, 1.28	0.54
Maternal atopy	31.4% (368/1170)	40.5% (70/173)	1.48	1.07, 2.06	0.02
Paternal atopy	25.6% (299/1166)	31.4% (53/169)	1.32	0.93, 1.88	0.12
Maternal smoking	24.8% (291/1173)	24.9% (43/173)	1.00	0.69, 1.45	0.99
Household dog	32.2% (376/1168)	33.5% (58/173)	1.06	0.76, 1.49	0.73
Household cat	29.7% (347/1168)	34.1% (59/173)	1.22	0.87, 1.72	0.24
Born in fall ^b	38.6% (246/637)	49.5% (50/101)	1.56	1.02, 2.37	0.04
Born in spring ^b	41.5% (277/668)	42.7% (38/89)	1.05	0.67, 1.64	0.82
Born in summer ^b	40.8% (270/661)	40.7% (35/86)	0.99	0.63, 1.57	0.98
Boy	51.0% (603/1183)	53.4% (93/174)	1.10	0.80, 1.52	0.54
First vs. higher born	39.8% (374/939)	50.3% (72/143)	1.54	1.07, 2.17	0.02
Birth weight (<2500 g) *	3.9% (45/1155)	2.9% (5/170)	1.34	0.52, 3.42	0.54
Antenatal complications	10.1% (120/1184)	6.9% (12/174)	0.66	0.35, 1.22	0.18
Non-instrumental delivery ^c	15.8% (150/950)	16.2% (24/148)	1.03	0.64, 1.65	0.89
Neonatal complications [†]	13.5% (160/1184)	15.5% (27/174)	1.18	0.75, 1.83	0.47

Reference groups:

^a maternal age <24;

^b born in winter;

^c instrumental vaginal delivery and caesarean section.

* Consists of: pregnancy-induced hypertension, gestational diabetes, infections needing antibiotic therapy and intrauterine growth retardation.

[†] Consists of: pre-term delivery, foetal distress, newborn infection, jaundice, low blood sugar, hypothermia and congenital abnormalities.

OR, odds ratio; CI, confidence interval.

Table 2
Association between interleukin-13 gene variants and cord serum immunoglobulin E (IgE)

SNP [†]	Genotype	Cord serum IgE ≥0.5 kU/l	Unadjusted OR* (95% CI)	Adjusted OR* (95% CI)	p value**
rs1800925 (C – 1112T)	CC	10.7% (55/514)	1.00	1.00	0.078
	CT	13.8% (35/253)	1.41 (0.91, 2.17)	1.28 (0.80, 2.05)	
	TT	19.3% (6/31)			
rs2066960 (C + 515A)	CC	11.8% (76/643)	1.00	1.00	0.980
	AC	11.8% (16/136)	0.99 (0.57, 1.74)	0.87 (0.47, 1.62)	
	AA	14.3% (1/7)			
rs1295685 (G + 2525A)	GG	10.1% (52/517)	1.00	1.00	0.031
	AG	15.9% (39/245)	1.67 (1.09, 2.58)	1.61 (1.01, 2.56)	
	AA	14.7% (5/34)			

* Considering a dominant effect for the rare allele, minor allele homozygous and heterozygous genotypes were combined and major homozygous was used as the reference for each single nucleotide polymorphism (SNP). Odds ratios were adjusted for maternal age; maternal smoking, household dog and household cat during pregnancy; season of birth; sex; position of child in family; and birth weight.

** Armitage-Cochran test for trend was used considering an additive model and having the rare allele as the risk allele.

[†] Primer and probe sequences for pyrosequencing assays: SNP rs1800925: Forward primer, 5'-GGGGTTTCTGGAGGACTTCT-3'; Reverse primer, 5'-GCAGAAATGAGTGTCTGTGGAG-3'; Probe, 5'-TTCTGGAGGACTTCTAGG-3'. SNP rs2066960: Forward primer, 5'-GCATTGGCCAACTGGATT-3'; Reverse primer, 5'-GGCAAGGAGCGGACTTCTAC-3'; Probe, 5'-AAGGGCGGGCCTAT-3'. SNP rs1295685: Forward primer, 5'-AGTGTGTTTGTCACCGTTGG-3'; Reverse primer, 5'-GGGCCCTGACTCTCTGAAC-3'; Probe, 5'-GGGGAAGACTGTGGC-3'.