

IL13 variants are associated with total serum IgE and early sensitization to food allergens in children with atopic dermatitis

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Increased total and specific serum immunoglobulin E (IgE) levels are common characteristics of atopic diseases and their basal production is proposed to be under strong genetic control. Interleukin 13 (*IL13*) variants have been consistently associated with total serum IgE levels in white populations with a strongest association in non-atopics. The aim of this study was to test the *IL13* p.R130Q and c.1-1111C > T variants in children with atopic dermatitis (AD) for associations with total serum IgE and early sensitization to common food and inhalant allergens and with asthma. We included 453 children with AD [participants of the Early Treatment of the Atopic Child (ETAC) study] that were followed from the age of 12–24 months for 3 yr. Total and specific IgE were determined at four time points. We genotyped the *IL13* p.R130Q and c.1-1111C > T variants by melting curve analysis. In children up to 4 yr of age, the 130Q allele was related to slightly higher total IgE levels compared to heterozygotes and 130R homozygotes. More importantly, both *IL13* variants were significantly associated with sensitization to food allergens, with most significant results for sensitization to egg ($p = 0.0001$). Although early sensitization to hen's egg represents a strong risk factor for subsequent sensitization to inhalant allergens and asthma, the investigated *IL13* variants were not associated with these phenotypes at the age of 48–60 months. In summary *IL13* variants contribute to elevated levels of total serum IgE in young atopic children and are strongly associated with sensitization to food allergens, particularly to hen's egg. These findings suggest that *IL13* variants play a major role not only in non-cognate but also in allergen specific IgE synthesis.

Increased total serum immunoglobulin E (IgE) levels and specific IgE antibodies to food or inhalant allergens are characteristic findings in atopic dermatitis (AD), asthma and allergic rhinitis. Total serum IgE levels are presumably under strong genetic control, most likely regulated by a complex array of genes. Many linkage and association studies on total IgE levels have been published, but replication has been a major problem (1). However, a functional variant within the fourth exon of interleukin 13 (*IL13*) leading to

an arginine by glutamine exchange at codon 130 (*rs20541*, c.389G > A, p.R130Q) showed repeatedly associations with total IgE levels in Caucasian and Asian populations (2–6). Additionally, a functional *IL13* promotor variant (*rs1800925*, c.1-1111C > T; also referred to as c.1-1055C > T) has also been associated with increased total serum IgE levels (2, 3). Initial linkage and association studies on total serum IgE indicated a stronger effect of chromosome 5q31 markers (7) and *IL13* variants (2, 3) in non-atopic individuals.

Simona Eva Zitnik^{1,2}, Franz Rüschendorf³, Sebastian Müller^{1,4}, Claudia Sengler¹, Young-Ae Lee^{1,3}, Rupino W. Griffioen⁵, Paolo Meglio⁶, Ulrich Wahn¹, Heiko Witt⁷ and Renate Nickel¹

¹Department of Pediatric Pneumology and Immunology, Charité – Universitätsmedizin Berlin, Berlin, Germany, ²University Children's Hospital, Ljubljana, Slovenia, ³Max-Delbrück-Centrum for Molecular Medicine, Berlin, Germany, ⁴Department of Pediatric Nephrology, Charité – Universitätsmedizin Berlin, Berlin, Germany, ⁵Department of Pediatrics, Academic Medical Centre, Amsterdam, The Netherlands, ⁶Department of Pediatrics, University 'La Sapienza', Rome, Italy, ⁷Department of Gastroenterology, Charité – Universitätsmedizin Berlin, Berlin, Germany

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Simona Eva Zitnik Charité, campus Virchow, Augustenburger Platz 1, 13353 Berlin, Germany
Tel.: +49-30-450566182
Fax: +49-30-450566931
E-mail: rene.nickel@charite.de

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To test whether *IL13* variants also influence total and specific IgE levels in atopic children, we genotyped 453 participants of the ETAC (ETAC = Early Treatment of the Atopic Child) trial for two *IL13* variants, p.R130Q and c.1-1111C>T.

Materials and methods

The ETAC study was described in detail previously (8). Briefly, 795 Caucasian children with AD participated in a double-blinded placebo controlled trial to test whether cetirizine could prevent or delay the onset of asthma. After 18 months of treatment, follow-up continued for another 18 months. Follow-up visits were conducted every 3 months and included standardized interviews, physical examinations with standardized assessment of AD severity and asthma symptoms. Blood samples were obtained at baseline and at four timepoints thereafter for the determination of total IgE and specific IgE to egg, milk, peanut, house dust mite, grass pollen, and cat dander (CAP-RAST FEIA; Pharmacia, Uppsala, Sweden). Since total IgE levels increase with age we subgrouped IgE levels according to the age when determined: 12–23, 24–35, 36–47, 48–60 months. In children with more than one determination of total IgE within 1 yr, mean total IgE levels were calculated prior to subgroup analyses. Sensitization was defined as serum IgE to respective allergens equal to or greater than 0.35 kU/l at least once during the follow-up period. Control subjects for respective sensitization were selected based on non-detectable specific IgE antibodies to the tested allergen (<0.35 kU/l). Asthma was defined as three episodes separated by at least seven days, either of nocturnal cough with sleep disturbance lasting for at least three consecutive nights, three episodes of wheezing, or a combination of both symptoms.

Genotyping

In 453 children blood samples for genetic analyses and informed consent of the parents was available. The study was approved by the local Ethics Committee. Genomic DNA was extracted from peripheral blood leukocytes by standard techniques. Primers and fluorescence resonance energy transfer (FRET) probes were synthesized based on the published nucleotide sequence (GenBank No. U_31120.1). Primer sequences used for PCR were: 5'-TGGGGGTTCTGGAG-GACT-3' and 5'-AGCCTTAGTCCAGGTCA-GAGAT-3' (promotor); 5'-TTTGTAAGGA-CCTGCTCTTACA-3' and 5'-GCACAGGCT-

GAGGTCTAAGCTA-3' (exon 4). We performed PCR using 0.5 U AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA), 400 µM dNTPs, 1.5 mM MgCl₂ and 0.1 µM of each primer in a final volume of 25 µl. The reaction mix was denatured at 95°C for 12 min followed by 40 cycles of denaturation at 95°C for 20 s, annealing at 52°C for 40 s, elongation at 72°C for 90 s, and a final extension step for 2 min at 72°C in a thermocycler.

Genotyping was carried out by melting curve analysis with FRET probes in the LightCycler instrument (Roche Diagnostics, Mannheim, Germany). For detection of the c.1-1111C>T (rs1800925) variant the sequence of the anchor fluoresceine labelled probe was 5'-CCCCTGCA-GCCATGTCGCCTT-FL and that of the sensor LC Red 705 labelled probe was 5'-LC 705-CCT-GCTCTTCCCTCATTTTCCTAG-ph. For detection of the c.389G>A (p.R130Q) (rs20541) alteration the sequence of the anchor fluoresceine labelled probe was 5'-CAGGTCCTGTCTCTG-CAAATAATGATGCTTTTCGA-FL and that of the sensor LC Red 640 labelled probe was 5'-LC 640-TTTCAGTTGAACCGT-CCCTCGC-ph. FRET probes were designed and synthesized by TIB MOLBIOL (Berlin, Germany).

All genetic alterations are numbered at the cDNA level, indicated by a 'c.' before the number. Position +1 corresponds to the A of the ATG translation initiation codon located at nucleotide 57 in the NM_002188.2 *IL13* mRNA reference sequence.

Statistical analyses

Total IgE levels were log transformed to approximate a Gaussian distribution. The Kolmogorov–Smirnow test was used to test for a normal distributions. ANOVA with *IL13* genotypes and time as fixed factors and age as covariate was used to evaluate the relation of log transformed IgE values and *IL13* genotypes. Chi-square tests (2 × 3 table, two degrees of freedom) were performed to test for associations of *IL13* variants (genotype based) and sensitization to food and inhalant allergens and asthma (Software: SPSS 14.0; SPSS Inc., Chicago, IL, USA). Linkage disequilibrium and haplotype analyses were calculated with HaploView.

Results

Both variants were in Hardy–Weinberg equilibrium. The allele frequencies were as follows: *IL13* 130R 0.753, 130Q 0.247; *IL13* c.1-1111C 0.771, c.1-1111T 0.229.

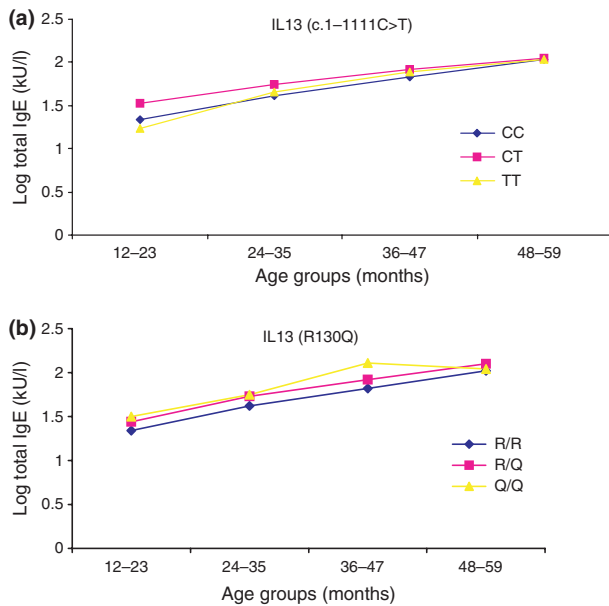


Fig. 1. Mean total serum IgE levels (log) across genotypes for the *IL13* variants. c.1-1111C>T (a) and p.R130Q (b) in different age groups.

The relationship between total serum IgE levels and the two *IL13* variants are shown in Fig. 1a,b. Homozygotes for the 130Q allele displayed slightly higher total IgE levels compared to heterozygous carriers or subjects bearing the wild-type within the three first age groups (factor genotypes $p = 0.013$, interaction effect time*genotypes $p = 0.91$). In contrast to previous observations, total IgE levels in individuals homozygous for the c.1-1111T genotype did not differ significantly compared to the wild-type genotype. However, slightly higher total IgE levels were seen in c.1-1111 heterozygotes in all age groups (factor genotypes $p = 0.039$, interaction effect time*genotypes $p = 0.68$).

Univariate analyses for single age groups revealed only significant results for *IL13*-1111 genotypes at age 12–23 months ($p = 0.011$).

A total of 246 children were sensitized to hen's egg, whereas 211 subjects showed no detectable specific antibodies against egg at any time point. The *IL13* 130Q and c.1-1111T alleles were significantly more frequent in children sensitized against egg compared to non-sensitized individuals. A weaker association was also seen for sensitization against milk (only for *IL13* 130Q genotypes) and peanut (Table 1). No association was observed for sensitization to inhalant allergens or asthma (data not shown). The *IL13* SNPs are tightly linked ($r^2 = 0.255$; $D' = 0.53$). No single haplotype resulted in lower p-values compared to the single SNP analyses with regard to sensitization to food allergens.

The results (total and specific IgE) did not differ between children that received cetirizine and those that received placebo.

Discussion

In the present study, we analyzed the effect of two functional *IL13* variants (p.R130Q and c.1-1111C>T), in a large population of white children with AD and a positive family history of asthma (ETAC study). The *IL13* R130Q variant showed statistically significant effects on total serum IgE levels with highest values in 130Q homozygous subjects up to the age of 4 yr. However, the differences were marginal.

Both *IL13* alleles were strongly associated with sensitization to food allergens, particularly to hen's egg.

Allele frequencies for both *IL13* variants were similar to those reported previously in white populations (2–4, 9).

Table 1. Genotype distribution and associations of *IL13* c.1-1111C>T and p.R130Q genotypes with sensitization to food allergens

Food allergen		Egg		Milk		Peanut	
Variant	Genotype	Cases (n = 237)	Controls (n = 202)	Cases (n = 230)	Controls (n = 209)	Cases (n = 171)	Controls (n = 268)
p.R130Q	R/R	119 (50.2%)	137 (67.8%)	115 (50.0%)	141 (67.5%)	89 (52.1%)	167 (62.3%)
	R/Q	91 (38.4%)	58 (28.7%)	91 (39.6%)	58 (27.8%)	63 (36.8%)	86 (32.1%)
	Q/Q	27 (11.4%)	7 (3.5%)	24 (10.4%)	10 (4.8%)	19 (11.1%)	15 (5.6%)
		$\chi^2 17.7/p = 0.0001$		$\chi^2 14.7/p = 0.0006$		$\chi^2 6.7/p = 0.04$	
c.1.1111C>T	CC	124 (51.9%)	145 (70.4%)	132 (57.6%)	137 (63.4%)	94 (53.7%)	175 (64.8%)
	CT	93 (38.9%)	55 (26.7%)	79 (34.5%)	69 (31.9%)	65 (37.1%)	83 (30.7%)
	TT	22 (9.2%)	6 (2.9%)	18 (7.9%)	16 (4.6%)	16 (9.1%)	12 (4.4%)
		$\chi^2 18.2/p = 0.0001$		$\chi^2 2.7/p = 0.27$		$\chi^2 7.2/p = 0.03$	

Marsh et al. first described linkage of genetic markers within chromosome 5q31-33 with total IgE levels. Most significant results were observed for a marker within the *IL4* gene in Amish families. When atopic and non-atopic subjects were analysed separately, linkage was seen in non-atopics only (7). Graves et al. reported highly significant associations of total serum IgE with *IL13* c.1-1111T and 130Q alleles in three independent paediatric study populations from Germany and the US comprising 1399 children. The strongest association was observed for *IL13* 130Q variant in skin test negative subjects (2). These results were confirmed by Liu et al. in 823 unrelated German children [Multicentre Atopy Study (MAS)-birth cohort] (3), implying that *IL13* variants regulate IgE production in a non-antigen-specific fashion.

In atopic diseases, highest levels of total IgE are seen in patients suffering from AD. As expected, log transformed total IgE levels in ETAC children were markedly higher than those observed in children of the German MAS birth cohort at the same respective age (2). However, the differences of mean (log) total IgE between *IL13* genotypes were far more pronounced in the MAS cohort than in ETAC children. In atopics, total IgE levels comprise varying amounts of specific IgE. With regard to our present observations it can be hypothesized that the low level of significance is still present in young children whereas increasing amounts of specific IgE mask the effect of *IL13* variants on non-cognate IgE synthesis in older children.

A highly significant association was observed for sensitization against food allergens, particularly egg allergen in the ETAC population. This is of particular interest, since sensitization to egg is a strong risk factor for subsequent sensitization to inhalant allergens (10) as well as for asthma later in life (11, 12). These findings confirm earlier results from the MAS cohort (13). Previous studies that described the strongest effects for *IL13* SNPs for total serum IgE in non-atopic subjects had not addressed sensitization to food allergens in childhood. It requires allergen testing in very young children because sensitization to food allergens is frequent, but often transient. However, we cannot exclude that gene-by-gene interactions (e.g. *IL13/IL4RA/IL4*) play a role in sensitization to food allergens.

An association of *IL13* variants with AD *per se* in the ETAC population is unlikely since allele and genotype frequencies are not statistically different from non-atopic white children of the German Multicentre Atopy Study (MAS-90; data not shown) (3, 13). Association of AD and

the *IL13* 130Q allele was described in 185 Japanese and 326 Danish children, but no influence on serum IgE level was observed in these studies (14, 15).

Associations of *IL13* variants with asthma or asthma-associated phenotypes have also been reported (4, 16), however, results are not as consistent as in studies on *IL13* variants and total IgE. In addition, there is strong evidence that interaction of genes within the *IL4/IL13* pathway influence the risk for asthma (9, 17). In our study, the two *IL13* variants were not associated with asthma. However, asthma was assessed at an very early time point (age 4–5 yr), therefore we cannot exclude a type I error (more children will likely be affected later in life). Furthermore, we did not test for gene-by-gene interactions.

In summary, our study supports the role of *IL13* in the regulation of total and specific IgE.

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