

Lack of association between a polymorphism in the interleukin-13 gene and total serum immunoglobulin E level among nuclear families in Costa Rica

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Summary

Background IL-13 has been implicated in the pathogenesis of asthma and in the regulation of IgE synthesis in humans. Single nucleotide polymorphisms (SNPs) in the IL-13 gene have been associated with asthma and total serum IgE level in Caucasian populations.

Objective To test for genetic association between an SNP in exon 4 of the IL-13 gene (IL-13 + 2044 or Arg130Gln) and total serum IgE level and asthma-related phenotypes in a population with high prevalence of asthma living in Costa Rica.

Methods Family-based association study.

Results Among 83 Costa Rican school children with asthma and their parents (249 individuals), there was no evidence of linkage disequilibrium between the IL-13 + 2044 SNP and any of the outcomes of interest (total serum IgE level on a logarithmic scale, number of positive skin tests to aeroallergens, and asthma). These results were not significantly changed after adjustment for age and gender.

Conclusions No significant evidence of linkage disequilibrium between an SNP in exon 4 of the IL-13 gene and total serum IgE level, sensitization to allergens or asthma was found in a family-based association study in Costa Rica.

Keywords asthma, IL-13 gene, SNP, total serum IgE level

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Introduction

Asthma, a major public health problem, affects over 17 million people in the United States [1]. The prevalence of childhood asthma in Costa Rica, a Latin American country, is one of the highest in the world [2]. An estimated 32.1% of Costa Rican children aged 6 to 7 years experience current wheezing, according to parental report in written questionnaires from the International Study of Asthma and Allergies in Childhood (ISAAC) [3].

Total serum IgE level is a strong predictor of asthma, a complex disease resulting from the interaction between genetic and environmental factors [4–7]. Linkage to markers in chromosome 5q31-q33 has been reported for total serum IgE level in Caucasian [8–11] and Japanese [12] populations. Chromosome 5q31-q33 contains genes for cytokines implicated in the pathogenesis of asthma and the regulation of IgE synthesis, such as IL-13 [13].

In murine models, IL-13 plays an independent and critical role in the expression of airway hyper-responsiveness, a cardinal feature of asthma [14,15]. In humans, an increased

expression of IL-13 mRNA has been found in the airways of subjects with asthma [16], and current data suggest that IL-13 is an important mediator in IgE isotype switching [17]. Furthermore, single nucleotide polymorphisms (SNPs) in the IL-13 gene have been associated with altered regulation of IL-13 production [18,19], asthma [18,19] and total serum IgE level [20] in studies conducted in Caucasian [18–20] and Japanese populations [19]. We were interested in testing for genetic association between an SNP in exon 4 of the IL-13 gene (IL-13 + 2044) and total serum IgE level and other asthma-related phenotypes in the Costa Rican population, using a family-based study design.

Methods

Study site

Costa Rica, a mountainous Central American republic located north of Panama, has coasts on the Atlantic and Pacific Oceans. The country has an area of 19 575 square miles and in 1997 had a population of 3.6 million inhabitants. Temperatures average 15 °C in the Central Valley and 27 °C in the coastal areas.

Study population

The majority of the Costa Rican population live in a Central Valley separated from the Atlantic and Pacific Oceans by

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mountain ranges. From the initial Spanish settlement in 1569 through to late in the 19th century, the population of the Central Valley was separated politically and geographically from other Costa Rican regions [21]. Due to minimal immigration to the Central Valley until the late 19th century, most current residents of this area descend from the approximately 4000 individuals counted in the census of 1697 (2100 Spanish, 1400 Native Americans, 300 individuals of African descent and 160 Mestizos) [22]. Although Costa Rica received a substantial number of immigrants from 1880 to 1915, the vast majority were Afro-Caribbeans who settled on the Atlantic Coast. A more recent wave of immigrants from neighbouring Nicaragua settled in various areas of Costa Rica, including the Central Valley.

In 1998, 75 of 5000 educational centres registered in Costa Rica were selected for inclusion in the study by random stratification for geographical location, including urban and rural areas. Core questionnaires from phase I of ISAAC [23] were sent to the parents of 1500 10-year-old children enrolled in these schools; 1105 (73.7%) of these questionnaires were completed. Of these 1105 schoolchildren, 299 (27%) had parental report of wheezing in the previous 12 months, and a sample of 83 of these children (all of whom had physician-diagnosed asthma) were included in this study along with their biological parents.

Study procedures

Data were collected between November 1998 and November 1999. After written consent was obtained from the parents of participating children, the following procedures were carried out in accordance with protocols from Phase II of ISAAC [24]: (i) completion of a standardized questionnaire including questions on respiratory symptoms and respiratory health status, home environment and family history of asthma and allergic illnesses; (ii) skin testing for reactivity to eight aeroallergens, along with a negative and positive control; and (iii) collection of a blood sample for measurement of total serum IgE. In addition, blood samples were collected from each child and both of his/her parents for DNA extraction. The study was approved by the Institutional Review Boards of the Hospital Nacional de Niños in San José (Costa Rica) and the Brigham and Women's Hospital in Boston.

Genotyping

The 249 participating subjects were genotyped for the IL-13 + 2044 polymorphism (Arg130Gln) by PCR amplification and restriction enzyme digestion. The primer sequences used to generate the 236-bp product containing the polymorphism have been previously reported [20]. The PCR reactions were carried out in a total volume of 25 µL containing 50 ng of genomic DNA, 1.5 mmol/L magnesium chloride, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 10 mM deoxynucleotide triphosphate mixture, 0.3 µL of Taq Gold DNA polymerase, and 10 pM of each primer. The PCR conditions included a 10-min incubation period at 95 °C (Amplitaq Gold, Applied Biosystems, Foster City, CA, USA) followed by 33 cycles at 94 °C for 40 s, 56 °C for 40 s, and 72 °C for 50 s, and then a final extension at 72 °C for 10 min. The PCR product was digested by the addition of 1 µL of *Nla*IV and incubation at 37 °C for 3 h. *Nla*IV (New England BioLabs, Boston, MA, USA) digests 26 base pairs from the 5' end, which is a control for assessing whether digestion was

successful. If the G nucleotide is present, it also digests 32 base pairs from the 3' end to produce a 178-bp fragment; if the A nucleotide is present, then the fragment size is 210 base pairs. The digested PCR products were then electrophoresed on 3% agarose gels to separate the fragments. The genotyping results were confirmed in a random subset (10%) of samples.

Measurement of total serum IgE

Total serum IgE levels were determined by means of the Abbot IMx system (Abbott Diagnostics, Abbott Park, IL, USA). All values were then transformed to a log₁₀ scale for analysis, in accordance with the recognized logarithmic distribution of total serum IgE levels in the general population [25].

Allergy skin testing

Skin testing was performed according to the ISAAC phase II protocol [24]. In addition to histamine and saline controls, the following antigens were applied to the skin of the forearm: *Periplanetaria americana* (American cockroach), *Dermatophagoides pteronyssinus* (Der p 1), *Dermatophagoides farinae* (Der f 1), dog dander, cat dander, mixed tree pollen, mixed grass pollen and *Alternaria tenuis*. A test was considered positive if the maximum diameter of the weal was >3 mm after subtraction of the negative control.

Statistical methods

Analysis of variance using the SAS statistical software package (SAS Institute Inc., Cary, NC, USA) was used to compare the geometric mean total serum IgE levels and mean number of positive skin tests to aeroallergens by genotypes of participating children. The PedCheck program was used to exclude Mendelian inconsistencies in allele assignment in each nuclear family [26]. Linkage disequilibrium between the IL-13 + 2044 SNP and discrete traits was examined with the transmission disequilibrium test in the ANALYSE program [27]. Linkage disequilibrium between the IL-13 + 2044 SNP and quantitative traits (total serum IgE level on a logarithmic scale and number of positive skin tests to allergens) was examined with the quantitative transmission disequilibrium tests in the QTDT program [28,29].

Results

Subject characteristics

The characteristics of the participating children are summarized in Table 1. There were no statistically significant differences in total serum IgE level, number of positive skin tests to aeroallergens or allele frequencies between children born in the Central Valley ($n = 66$) and children born in coastal areas ($n = 17$) ($P > 0.2$ in all cases).

The geometric mean total serum IgE level of participating children was 427.0 IU/mL. The frequency of the G allele among the parents was 69% and the Arg130Gln genotypes were in Hardy-Weinberg equilibrium.

Asthma, total serum IgE level, skin test reactivity to aeroallergens and Arg130Gln genotypes

Among the 83 participating children, there was no significant difference in either geometric mean total serum IgE level or

Table 1. Characteristics of 83 Costa Rican school children participating in a family-based study of the association between an SNP in the IL-13 gene and asthma-related phenotypes

Variable	n (%)
Gender	
Female	34 (41.0)
Male	49 (59.0)
Age (mean $\pm$ SD)	11.2 $\pm$ 0.4
Area of residence	
Central Valley	66 (79.5)
Coastal areas	17 (20.5)
Highest level of education attained by either parent	
Did not complete high school	29 (35.4)
High school	29 (35.4)
College or postgraduate school	24 (29.2)
Maternal smoking during pregnancy	3 (3.6)
Breast-fed	74 (89.2)
Parental history of asthma	44 (53.0)
≥ 1 positive skin test to allergens	64 (77.1)
Total serum IgE ≥ 100 IU/mL	66 (79.5)

Table 2. Geometric mean total serum IgE level (IU/mL) and number of positive skin tests to allergens, by Arg130Gln genotypes among 83 children participating in a study of the association between an SNP in the IL-13 gene and asthma-related phenotypes

Variable	ArgArg	ArgGln	GlnGln	P
Geometric mean total serum IgE IU/mL)	411.8	437.1	444.6	0.9
Mean number of positive skin tests 95% confidence interval)	2.4 (0–5.9)	2.3 (0–5.9)	2.9 (0–6.9)	0.7

Table 3. Transmission disequilibrium test for association between the IL-13 + 2044 single nucleotide polymorphism and quantitative traits in 83 nuclear families in Costa Rica

Allele	d.f.	Sum	SD	T	P
Testing trait: total serum IgE level on logarithmic scale					
A	44	1.55	2.5	0.62	0.54
Testing trait: number of positive skin tests to aeroallergens					
A	41	–1.10	7.4	–0.15	0.88

number of positive skin tests to aeroallergens between Arg130Gln genotypes (Table 2).

Among 83 participating children and their parents, we found no evidence of significant linkage disequilibrium between the IL-13 + 2044 polymorphism and either total serum IgE level (on a logarithmic scale) or number of positive skin tests to aeroallergens after using the quantitative TDT (Table 3). These results were not significantly changed by adjustment for age and gender, nor by restriction of the analysis to the nuclear families of 19 children without skin test reactivity to allergens.

There was no evidence of linkage disequilibrium between the IL-13 + 2044 polymorphism and asthma, total serum IgE level >100 IU/mL, or skin test reactivity to any of the aeroallergens studied ($P > 0.2$ in all cases).

Discussion

Using a family-based study design to control for population stratification [30], we found no significant evidence of linkage disequilibrium between a polymorphism in exon 4 of the IL-13 gene (IL-13 + 2044) and any of three asthma-related phenotypes (total serum IgE level, sensitization to aeroallergens and asthma) among 83 Costa Rican school children with asthma and their parents (249 individuals).

Two previous studies found no association between polymorphisms in the IL-13 gene and total serum IgE level. In a case-control study conducted in British and Japanese adults [19], a polymorphism in exon 4 of the IL-13 gene (Arg130Gln) was associated with both atopic and non-atopic asthma, but not with total serum IgE level. In that study, asthma was defined as a combination of physician-diagnosed asthma, recurrent dyspnoea and chest tightness requiring treatment, current wheezing, and labile airflow obstruction. The total serum IgE levels of cases and controls were not reported. In a study conducted in the Netherlands [18] a polymorphism in the promoter region of the IL-13 gene (C to T exchange at position –1055) was associated with altered production of IL-13 and an increased risk of allergic asthma. Among 101 subjects with asthma, however, there was no association between the IL-13 –1055 polymorphism and either total or specific serum IgE levels. The definition of asthma, although 'based on American Thoracic Society criteria' [31], was not described in detail. The geometric mean total serum IgE level of these asthmatic subjects (237 IU/mL) was lower than that of our study participants (427 IU/mL).

A polymorphism in exon 4 of the IL-13 gene (IL-13 + 2044) was recently found to be associated with total serum IgE level in 1399 Caucasian children living in the United States and Germany [20]. The association between the IL-13 + 2044 polymorphism and total serum IgE level, however, was only significant among non-atopic subjects, and the investigators hypothesized that their negative findings among atopic subjects could be explained by differences in genetic regulation of basal and allergen-specific serum IgE.

We recognize several limitations to our findings. First, our sample size was relatively small. However, assuming that the IL-13 + 2044 SNP explained at least 10% of the total variation in total serum IgE level in our asthmatic probands, our study of 83 trios had a power of approximately 80% at an α of 0.05 to detect an association of the IL-13 + 2044 SNP with total serum IgE level. Therefore, our study had sufficient power to exclude an effect of the IL-13 + 2044 SNP explaining $\geq 10\%$ of variation in total serum IgE levels among asthmatic children in Costa Rica. As our study only included 19 families of non-atopic children with asthma, we cannot exclude an association between the IL-13 + 2044 and total serum IgE level in non-atopic Costa Rican children. Secondly, we did not examine stools for ova and parasites in the index children. However, the prevalence of parasitism among Costa Rican children is low and therefore unlikely to explain the high total serum IgE levels of children participating in our study. In a study of 268 Costa Rican school children in 1996, intestinal parasites (*Ascaris lumbricoides*, *Necator americanus*, *Trichuris trichiura*, *Hymenolepis nana*) were found in 5.4% of the stool samples examined, and only 1% of the children in the study were infected with two or more parasites [32]. Thirdly, we did not genotype additional

SNPs in the IL-13 gene region adjacent to IL-13 + 2044. However, we selected IL-13 + 2044 because this SNP was previously found to be both strongly associated with total serum IgE level and in tight linkage disequilibrium with four additional SNPs in the IL-13 gene in Caucasian children [20]. Thus, additional genotyping in the region of the IL-13 gene adjacent to IL-13 + 2044 to conduct haplotype analysis would be unlikely to change our results.

In summary, we found no evidence of linkage disequilibrium between a polymorphism in exon 4 of the IL-13 gene (IL-13 + 2044) and total serum IgE level, sensitization to allergens or asthma in a family-based association study in Costa Rica. Our findings may be due to true ethnic heterogeneity, to differences in environmental exposures and thus gene-by-environment interactions between populations, to a spurious association in previous population-based case-control studies due to population stratification [30], or to the lack of significant influence of the region adjacent to the IL-13 + 2044 SNP on asthma-related phenotypes such as total serum IgE level.

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