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Genetic Association Studies of *IL13RA1* Polymorphisms in Asthma and Atopy

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Abstract

IL-13 plays a central role in asthma pathogenesis by binding to the IL-13 receptor, which is a heterodimer composed of the IL-13Rα1 and the IL-4Rα subunits. We characterized the genetic diversity in the *IL13RA1* locus on chromosome Xq24 and examined the association of identified polymorphisms with asthma and atopy phenotypes.

The promoter and the coding region of *IL13RA1* were screened for common genetic variants, and polymorphisms found were genotyped in a large cohort of 341 asthmatic Caucasian families (containing at least two asthmatic siblings) and 182 non-asthmatic control subjects. Genetic association was determined using case control, genotype-phenotype, genotype-haplotype, and TDT analyses.

Two common polymorphisms were identified, a newly found –281T>G SNP in the *IL13RA1* promoter, and the previously described 1365A>G variant in the *IL13RA1* proximal 3' UTR. No significant association of either –281T>G or 1365A>G with risk of asthma or atopy phenotypes was found, apart from a suggestive association between *IL13RA1* –281T/1365A haplotype and raised total serum IgE in adult female asthmatics.

These findings suggest that the *IL13RA1* –281T>G and 1365A>G polymorphisms do not contribute to asthma susceptibility or severity, although the *IL13RA1* locus might be involved in the control of IgE production.

Keywords: asthma; atopy; interleukin-13 receptor; genetics; polymorphism; IgE

Introduction

The Th2 cytokines IL-13 and IL-4 play a central role as effector molecules in asthma through multiple mechanisms including induction of IgE synthesis by B cells[1,2], airway eosinophilia[3], goblet cell metaplasia and mucus hypersecretion[4,5], and airway remodeling[6]. IL-13 elicits its biologic effects via a receptor complex which is composed of the heterodimeric proteins IL-13Rα1 and IL-4Rα[7]. The IL-13Rα1/IL-4Rα complex is also utilized by IL-4 as an alternative receptor, especially in non-hematopoietic cells that do not express the common gamma chain (IL-2Rγ)[8]. Based on data deposited at the Entrez Gene database, the *IL13RA1* gene maps to chromosome Xq24. The 5' flanking region of *IL13RA1* has been characterised and was found to lack both TATA and CCAAT boxes with a predicted transcription initiation site at –123 bp relative to the start codon[9]. IL-13Rα1 is expressed on both hematopoietic and non-hematopoietic cells including basophils, eosinophils, B cells, mast cells, fibroblasts, endothelial cells, smooth muscle cells, and airway epithelial cells[10]. Signaling of IL-4 and IL-13 through the IL-4Rα/IL-13Rα1 complex is thought to occur through IL-4Rα[11] leading to activation of several signaling molecules including STAT6, IRS-1, and IRS-2, that can translocate to the nucleus and bind to specific motifs in the promoter regions of responsive genes (e.g. MHC II, CD23, Iε, and IL-4Rα)[12].

Several studies have shown that genetic variants of *IL13*, *IL4*, and *IL4RA* confer susceptibility to atopy and asthma[13]. In contrast, there have been only two association studies of polymorphisms in the *IL13RA1* gene with asthma and atopy[14,15]. Ahmed and co-workers[14] screened the coding region of *IL13RA1* in a Japanese population and identified a rare C to T non-amino acid altering polymorphism at position 1050 relative to the translation initiation codon ATG. A low-powered association study by the same group of investigators found no association between the *IL13RA1* 1050C>T polymorphism and atopic asthma. Heinzmann and co-workers[15] screened the *IL13RA1* gene in British and Japanese populations and identified an A to G substitution at position 1365 relative to the translation initiation codon ATG, situated in the proximal 3' UTR of the gene, referred to as 1398A>G in this study. In the same study, the *IL13RA1*

1365A>G polymorphism was found to be associated with raised total serum IgE levels in male subjects in the British population.

On the basis of the central role of the IL-13/IL-4 pathway in atopy and asthma, we hypothesized that genetic variation in the *IL13RA1* gene may predispose to the development or/and predict severity of asthma and atopy. To test this hypothesis, we screened the promoter, coding region, and proximal 3' UTR of the *IL13RA1* gene for common genetic variants. Subsequently, we evaluated identified variants for evidence of association with asthma and atopy phenotypes in a large cohort of 341 asthmatic families and a cohort of 182 non-asthmatic control subjects using three methods: case-control analyses, phenotype-genotype and phenotype-haplotype association studies, as well as the transmission disequilibrium test (TDT). We report here a novel allelic variant in the *IL13RA1* promoter and two previously described SNPs of *IL13RA1*, as well as the association of the two common variants of *IL13RA1*, -281T>G and 1365A>G, with asthma and atopy phenotypes.

Materials and Methods

Subjects and clinical assessment

341 Caucasian families were recruited from the Southampton area, UK, containing at least two biological siblings (age 5-21 years) with a current physician diagnosis of asthma who were taking asthma medication on a regular basis (Table 1). Clinical phenotyping was based on a case report form and health survey questionnaire completed by each family member on the study day visit. This form included a list of inclusion and exclusion criteria, demographics, medical history, skin prick data, spirometry, challenge dose levels for the bronchial challenge, documentation of laboratory samples taken, and information on medicines taken in the last 12 months.

Asthma in the adults was defined as a positive response to the following three questions: "Have you ever had asthma?", and "Was this confirmed by a doctor?", and "Have you used any medicines to treat asthma, or any breathing problems, at any time in the last 12 months?". Baseline FEV_1 was obtained from pulmonary function testing. Three FEV_1 values within 5% of each other were obtained and the highest FEV_1 was recorded. Airway responsiveness was measured as the concentraction of inhaled metacholine required to reduce FEV_1 by 20% and was performed according to the ATS guidelines[16] using a Devilbiss 646 nebuliser in conjunction with a computerised system (KoKo Digidoser). Skin prick testing to 6 common aeroallergens was carried out: mixed grass, mixed trees, cat, dog, *Dermatophagoides pteronyssinus,* and *Alternaria* (Bayer Corporation, Spokane,WA), with a negative (saline) and a positive (histamine) control. Atopy was defined as either a positive skin prick test (> 3mm in wheal diameter) or a raised specific IgE (> 0.35 kUA/l) to one or more common allergens. Total IgE and specific IgE measurements were carried out by IBT laboratory (Kansas, MO, USA) using the Pharmacia CAP System™. Specific IgE was measured for the same allergens as for skin prick testing. Total IgE was adjusted for age by using the number of standard deviations (SD) away from the median for each age group. Severity scores for atopy and asthma were generated as previously described[17]. In addition, 182 non-asthmatic adult controls with no personal or family history of asthma were recruited from the same

Southampton area through blood donor clinics. Ethical approval for this work was granted by the Southampton and S.W. Hants Joint Research Ethics Committee.

Mutation screening

Using genomic DNA extracted from 20 male subjects (8 diagnosed with asthma) and 18 female subjects (4 diagnosed with asthma), a 2.2 kb fragment of the *IL13RA1* promoter (corresponding to –1584 to +610 relative to the translation initiation codon ATG) was generated by PCR. Due to the high GC content of the region, a combination of 0.1 U/μl Taq (Sigma-Aldrich, Poole, UK) and 0.0033 U/μl Pwo (Roche Applied Science, Ease Sussex, UK) was used for amplification of genomic PCR template (100 ng) in the presence of Pwo buffer, 5% DMSO, 1.5 mM $MgCl_2$, 0.2 μM of each primer (Table 2), 0.2 mM dNTPs containing a 3:1 ratio of dGTP to 7-deaza-dGTP, as well as fluorescent dCTP (R110) (ABI-Perkin Elmer, Warrington, UK) at a ratio of 1:100 ratio to unlabeled dCTP, to give a final reaction volume of 50 μl. Thermal cycling included a single soak at 95^{o}C for 3 min followed by 38 cycles of denaturation at 95^{o}C for 30 s, annealing at 63^{o}C for 30 s, extension at 72^{o}C for 2.5 min, and finally a 72^{o}C soak for 10 min, on a PTC-225 DNA Engine Tetrad (MJ Research Inc, Waltham, MA, USA). For mutation screening of the coding region of *IL13RA1*, spanning approximately 1.5 kb, total RNA was extracted from whole blood of 22 male subjects (11 diagnosed with asthma) and 25 female subjects (13 diagnosed with asthma) using the RNeasy blood kit (Qiagen, Crawley, UK) according to manufacturer's instructions. cDNA was generated using the Omniscript reverse transcription kit (Qiagen) as directed by the manufacturer using 2 μg of RNA template. To increase PCR yield, the coding region of *IL13RA1* was divided in three segments. Segment I spans from +38 to +301 relative to the translation initiation site, segment II from +238 to +1347, and segment III from +889 to +1443. PCR involved 2 μl cDNA template (from the 20 μl cDNA reaction), Jumpstart Taq (0.025 U/μl), standard PCR buffer, $MgCl_2$ (Table 2), 0.2 μM of each primer (Table 2), 0.2 mM dNTPs (including fluorescent dCTP (R110) 1:100 ratio of unlabeled dCTP) for segments screened using solid phase chemical cleavage), to give a final reaction volume of 20 μl. Thermal cycling included a single soak at 95^{o}C for 5 min followed by 35 cycles at 94^{o}C for 30 s,

annealing at a temperature indicated in Table 2 for 30 s, extension at 72°C for 60 s/kb, and finally a 72°C soak for 10 min. Due to its small size, segment I was screened using DHPLC (Transgenomic Ltd, Crewe, UK), whereas segments II and III were screened using solid phase chemical cleavage, essentially as previously described[18,19]. Positive samples were sequenced using dideoxy dye terminator cycle sequencing (BigDye Terminator Version 3.0, Applied Biosystems) on an ABI PRISM 377 DNA Sequencer (Applied Biosystems).

Genotyping

The *IL13RA1* –281T>G and the *IL13RA1* 1365A>G polymorphisms were genotyped using tetra-primer amplification refractory mutation system (ARMS)-polymerase chain reaction (PCR) assays[20] (Fig. 1). Each PCR reaction was carried out in a total volume of 15 μl containing 25 ng of template DNA, 0.2 mM dNTP, appropriate concentration of $MgCl_2$ (Table 3), 5% DMSO, Jumpstart Taq (0.05 U/μl), primers (15 μM of each inner primer and 3 μM of each outer primer) (Table 3) and standard PCR buffer. The PCR cycling conditions for both polymorphisms were: 95°C 5 min; then 10 cycles of 94°C 30 s, X°C 30 s (where X was initially 72°C, decreasing 1°C per cycle to 63°C), 72°C 30 s; then 31 cycles of 94°C 30 s, 63°C 30 s, 72°C 30 s; and finally 72°C 10 min. PCR products were resolved by micro-array diagonal gel electrophoresis (MADGE)[21] stained with Vistra Green (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) and visualized by a Fluorimager 595 (Molecular Dynamics, Sunnyvale, CA). Genotypes were scored using the Phoretix 1D gel analysis software (Nonlinear Dynamics, Newcastle upon Tyne, UK). Representative genotyping assays were confirmed by dideoxy dye terminator cycle sequencing (BigDye Terminator Version 3.0, Applied Biosystems) on an ABI PRISM 377 DNA Sequencer (Applied Biosystems).

Statistical methods

Case-control analysis. Case-control studies were conducted to evaluate association of the *IL13RA1* –281T>G and the *IL13RA1* 1365A>G polymorphisms with asthma. All comparisons were made between groups of same sex, since male subjects are hemizygous at *IL13RA1* due to the localization of the *IL13RA1* gene on the X chromosome. The groups of cases were: asthmatic mothers (n=100), asthmatic fathers (n=89), first affected female siblings (n=235), and first affected male siblings (n=271), whereas the control population were healthy Caucasians of the same area of residence, females (n=98) and males (n=86) (Table 1). Genotype frequencies for each group of cases were compared to the control population and analysed using χ^2 in SPSS version 11 (SPSS Inc; Chicago, IL, USA). Hardy-Weinberg equilibrium was confirmed using χ^2 with 1 degree of freedom.

Phenotype-genotype and phenotype-haplotype association studies. Data were analyzed in four asthmatic cohorts including first asthmatic female siblings, first asthmatic male siblings, female parents with a diagnosis of asthma, and male parents with a diagnosis of asthma. Statistical analysis was carried out using t-test for the male study groups and χ^2 for the female study groups after transformation of continuous phenotypes into categorical ones using appropriate cut off points on the following phenotypic markers: (i) total serum IgE (age corrected and $\log_{10}$ transformed to improve normality); (ii) FEV_1% predicted; (iii) slope of FEV_1 response to methacholine (transformed to [1/(least squares (LS) slope + 30)]∗1000] to improve normality and avoid negative values); (iv) atopy severity score; (v) asthma severity score. A *P*-value ≤0.05 was considered significant.

TDT analysis. Association of the *IL13RA1* –281T>G and the *IL13RA1* 1365A>G polymorphisms with asthma and asthma intermediate phenotypes was assessed using the Transmission Disequilibrium Test (TDT). This was implemented using STATA 6.0 (Stata Corporation, Texas, USA) and a TDT program written in STATA by David Clayton (MRC Biostatistics Unit, Cambridge, UK). Any test with a *P* value <0.05 was considered significant. Dichotomous variables analysed using TDT were: (i) asthma positive

on questionnaire ('Have you ever had asthma?', 'Was this confirmed by a doctor?' and 'Have you used any medicines to treat asthma, or any breathing problems, at any time in the last 12 months?)'; (ii) asthma with atopy (defined by raised specific IgE and/or positive skin prick test); (iii) asthma with raised total serum IgE levels (age corrected); (iv) asthma and PC_{20} methacholine <4 mg/ml (severe asthmatics); (v) asthma and PC_{20} methacholine ≤16mg/ml. Data analysis used the first affected sibling since transmissions to other siblings within the same family are not independent. Given that the TDT utilizes data from heterozygous parents, only maternal transmissions were analyzed since males are hemizygous at *IL13RA1*. Haplotype construction and haplotype frequency distributions were carried out using the ARELQUIN software (version 2.000).

Power calculations. The power of the TDT study to detect an important difference was calculated using a formula which assumes that the recombination fraction $\theta=0$ and there is no linkage disequilibrium[22]. The fact that the only informative transmissions were those from mothers, due to the X chromosome localization of *IL13RA1,* was taken into account by doubling the number of families given by the calculations. The power of the case-control study to detect a statistically significant difference was calculated using a statistical program (PS Program, Version 2.0, 1996, Vanderbilt University School of Medicine, Nashville, Tennessee, USA), which takes into account the alpha level (0.05), sample size, odds ratio, and polymorphism frequency in controls.

Results

Polymorphism identification

Mutation scanning of 2.2 kb of the *IL13RA1* promoter identified a novel T to G substitution at nucleotide position –281 relative to the ATG start, –281T>G (Fig. 2). Screening of the coding region and proximal 3' UTR of *IL13RA1* (approximately 1.5 kb) disclosed the presence of a previously described silent variant in the coding region of *IL13RA1* at position 1050 involving a C to T substitution[14], 1050C>T, as well as a previously reported SNP located in the proximal 3' UTR of the *IL13RA1* gene at position 1365 involving an A to G substitution[14], 1365A>G, described as 1398A>G in the original paper[15] (Fig. 2). The –281G allele of –281T>G was relatively abundant in our population (q=0.37 for affected female siblings and q=0.34 for affected male siblings). The 1365G allele of 1365A>G had a lower frequency in our cohort (q=0.17 for both affected female and male siblings). The *IL13RA1* 1050C>T variant was rare with a minor allele frequency of 0.040 in our cohorts. Distribution of the alleles in each group did not deviate from Hardy-Weinberg equilibrium when assessed by χ^2 analysis (data not shown).

Linkage disequilibrium and haplotype structure

Haplotype frequencies for the two *IL13RA1* polymorphisms were determined in multiple cohorts and among adult female asthmatics were found to be: –281T/1365A 0.67, -281T/1365G 0.17, and –281G/1365A 0.16, whereas for adult male asthmatics were: –281T/1365A 0.67, –281T/1365G 0.165, and -281G/1365A 0.165. No individuals were found with the –281G/1365G haplotype in this population. Linkage disequilibrium between *IL13RA1* –281T>G and *IL13RA1* 1365A>G was measured by calculating the r^2 value[23]. We have calculated r^2=0.406, meaning that around 40% of the information of one of the SNPs can be obtained from the other one, which indicates a moderate degree of linkage disequilibrium between the –281 and the +1365 SNP.

Genetic association studies

(i) *Case-control analysis:* We evaluated genetic association of the *IL13RA1* polymorphisms and asthma in the following study groups: asthmatic fathers (n=89), first affected male siblings (n=271), asthmatic mothers (n=100), and first affected female siblings (n=235) versus normal controls (n=184) of the same sex using χ^2 analysis. Distribution of genotypes and haplotypes of *IL13RA1* –281T>G and *IL13RA1* 1365A>G did not differ significantly between asthmatic subjects and normal controls (data not shown).

(ii) Phenotype-haplotype association analyses: within the asthmatic groups, we assessed potential associations of *IL13RA1* 2-allele haplotypes and various asthma intermediate phenotypes including total serum IgE, FEV_1% predicted, slope of FEV_1 response to metacholine, symptom score, and atopy severity score in asthmatic parents and first affected siblings. No significant associations between *IL13RA1* 2-allele haplotypes and asthma intermediate phenotypes were found in any of the study groups, apart from a borderline association between the –281T/1365A haplotype and raised total serum IgE in asthmatic mothers using χ^2 (T-A vs T-G haplotype OR=2.81, 95% CI 1.20-6.59, T-A vs G-A haplotype OR=1.07, 95% CI 0.50-2.28, *P*=0.049). (Table 4).

(iii) Phenotype-genotype association analyses: We investigated potential associations of the *IL13RA1* –281T>G and the *IL13RA1* 1365A>G polymorphisms with asthma intermediate phenotypes in asthmatic parents and first affected siblings. No significant associations were observed between different genotypes and phenotypes studied in any of the cohorts (data not shown).

(iv) TDT analysis: We further evaluated evidence for association of the *IL13RA1* –281T>G and the *IL13RA1* 1365A>G polymorphisms with asthma and asthma intermediate phenotypes using TDT. No alleles of either *IL13RA1* –281T>G or *IL13RA1* 1365A>G were found to be preferentially transmitted from heterozygous mothers to first affected siblings (Table 5). We also investigated the possibility of haplotype association due to the combined interaction of the *IL13RA1* –281T>G and the *IL13RA1*

1365A>G polymorphisms using TDT. Again, we found no significant association of the *IL13RA1* 2-allele haplotypes with either asthma or asthma intermediate phenotypes (data not shown).

Power calculations

We examined the hypothesis that the lack of association of the *IL13RA1* –281T>G and the *IL13RA1* 1365A>G polymorphisms alone with asthma and asthma intermediate phenotypes might have been due to insufficient power of our study to detect true associations. For the *IL13RA1* –281T>G polymorphism, TDT power calculations showed that the number of families necessary to obtain 80% power at a significance level of 0.05 and genotypic risk ratio of 2 was 106 families, whereas for a genotypic risk ratio of 1.5 316 families would be required. For the *IL13RA1* 1365A>G polymorphism, the number of families necessary to obtain 80% power for a genotypic risk ratio of 2 was 152 families, whereas for a risk ratio of 1.5 was 474 families.

The power of the case control study was calculated in the group of first affected female siblings, which was the most abundant study group with regard to the number of alleles analyzed, as well as in the group of asthmatic fathers, which was the less abundant study group. In the comparison of first affected female siblings (n=470 alleles) and female controls for association of the –281T>G SNP with asthma, the sample had a power of 80% to detect an odds ratio of 2 and a power of 35% to detect an odds ratio of 1.5. For association of the 1365A>G polymorphism with asthma in first affected female siblings, the power was 65% for an odds ratio of 2 and 24% for an odds ratio of 1.5. Comparing asthmatic fathers and male controls for association of the –281T>G variant in asthmatic fathers (n=89 alleles), this sample had a power of 60% to detect an odds ratio of 2 and a power of 25% to detect on odds ratio of 1.5. For association of the 1365A>G variant with asthma in asthmatic fathers, the sample had a power of 46% for an odds ratio of 2 and a power of 18% for an odds ratio of 1.5.

Discussion

In the present study, we have screened the promoter, coding region, and the proximal 3' UTR of the IL13RA1 gene for common genetic variants. We have identified a novel polymorphism in the *IL13RA1* promoter, –281T>G, in addition to two previously described variants, 1050C>T[14] and 1365A>G[15]. Using a case control study, phenotype-genotype and phenotype-haplotype association analyses, as well as a family-based approach we examined the two common *IL13RA1* polymorphisms, –281T>G and 1365A>G, for evidence of association to asthma and atopy phenotypes. There was no evidence to support a significant association of these variants with asthma or other atopy phenotypes apart from a borderline association between the *IL13RA1* –281T/1365A haplotype and raised total serum IgE in adult female asthmatics.

For SNP discovery in the 5' flanking region of *IL13RA1* 56 chromosomes were screened, whereas for the coding region of *IL13RA1* 47 X chromosomes were examined. The number of chromosomes screened provided adequate power to detect common SNPs with allelic frequency more than 0.05 in both regions. Mutation screening of *IL13RA1* was carried out in cohorts of healthy and asthmatic individuals using solid phase chemical cleavage and DHPLC, both shown to be very sensitive mutation detection methods[18,19].

The two common *IL13RA1* polymorphisms, –281T>G and 1365A>G, were found to be in moderate linkage disequilibrium (r^2=0.406)[23]. The 1365G allele had a frequency of 0.17, which is comparable with the frequency previously found in a British population[15] but lower than the frequency found in a Japanese population (≈0.40) in the same study. The rare 1050C>T variant had a minor allele frequency of 0.04 in our cohort, comparable with the frequency previously found in a Japanese population[14].

We found no association of either *IL13RA1* –281T>G or *IL13RA1* 1365A>G with asthma on both TDT and case-control analysis. The lack of association between the 1365A>G polymorphism and asthma is in accordance with the study of Heinzmann and co-workers[15]. In addition, none of the 2-allele haplotypes were associated with any asthma or atopy phenotypes, apart from a borderline association between the *IL13RA1* -281T/1365A haplotype and raised total serum IgE among adult female asthmatics.

The lack of association of this haplotype with total serum IgE in other study groups of our cohort might be due to the clear effects that sex and age have on allergic manifestation and total serum IgE levels. It has been found that males have a higher geometric mean total serum IgE levels than females throughout the entire age range of six to seventy-five or more years[24]. Moreover, total serum IgE levels reach a maximum at ten to fifteen years and then decline markedly with increasing age in both males and females, possibly due to gradually increasing suppressor T cell activity and progressive atrophy of the thymus[24]. Although these sex- and age-related effects on total serum IgE levels might explain the fact that the association between *IL13RA1* –281T/1365A haplotype and raised IgE was observed only among adult female asthmatics, we have to underline that the association was marginal and might have been reached by chance. It is also important to note that as families in this cohort were recruited on the basis of asthma there were too few atopic individuals without asthma to study the effects of *IL13RA1* polymorphisms on atopy alone.

In the study of Heinzmann and co-workers[15] the 1365A>G polymorphism (referred to as 1398A>G) was associated with raised total serum IgE in British male, but not in female, subjects[15]. The discrepancy between their study and ours might be due to differences in atopy severity or/and study design between the two cohorts. In addition, the –281T>G polymorphism was not evaluated in the study of Heinzmann and co-workers.

Power calculations demonstrated that our TDT study was well-powered to detect effects with odds ratio of 1.5 and above. The power of the case-control study to detect effects with odds ratio of 2 and above was adequate (60%-80%) for *IL13RA1* –281T>G and moderate (46%-56%) for *IL13RA1* 1365A>G, whereas the study was under-powered to detect an effect size less than 2 for both polymorphisms. The statistical power to detect a significant association depends on the size of the association and the frequency of the allele of interest. In a recent meta-analysis of 301 genetic association studies, most estimated odds ratios in follow-up studies were between 1.1 and 2[25]. It is likely that most genuine genetic associations in complex disease represent modest effects with odds ratios of 1.1-1.5[26]. Although this explains only 1-8% of the relative risk in the population, the additive effect of several

variants could make up the 20-70% of the overall disease risk that is attributable to genetic factors[26]. This highlights the challenge of recruiting larger cohorts of participants in order to detect modestly higher odds ratios. In our study, we may have missed small effects with odd ratio less than 1.5.

The *IL13RA1* 1050C>T polymorphism is located at the third nucleotide of codon 350, resulting in no amino acid alteration and has been previously described[14]. This polymorphism was not evaluated for association in our study due to the very low frequency (≈0.04) of the minor allele in our cohort. The –281T>G polymorphism in the *IL13RA1* promoter has not been previously described. This polymorphism may have a functional role by affecting transcriptional activation and gene production. Further in vitro studies are needed to demonstrate if this polymorphism directly affects transcription factor binding and transcriptional rate.

In conclusion, we have performed a mutation screen of the 5' flanking region, the coding region and the proximal 3' UTR of *IL13RA1*. We identified three polymorphic sites in *IL13RA1* including a novel one in the promoter region. We evaluated the two common variants of *IL13RA1* in a large cohort and found no evidence to support significant association of these polymorphisms with asthma or other atopy phenotypes, apart from a borderline association between the *IL13RA1* –281T/1365A haplotype and raised total serum IgE in adult female asthmatics. Further studies in additional cohorts are needed to determine whether variants of *IL13RA1* play a role in determining susceptibility to or modulating severity of asthma and atopy.

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Table 1. Phenotypic characteristics of study cohorts

Assessment	Male asthmatic parents (n=89)	Female asthmatic parents (n=100)	First asthmatic male siblings (n=271)	First asthmatic female siblings (n=235)	Controls (n=184)
Age (mean)	41.4	39.2	12.1	12.0	42.3
Age (range)	31.9 to 56.2	28.5 to 53.4	5.4 to 22.6	5.0 to 21.2	-
Gender (% male)	100	0	100	0	47.0
Asthma (%, doctor)	100	100	100	100	0
FEV_1 (% predicted)	91.10	96.8	95.68	94.19	ND[a]
BHR (1/LSlope+30)*1000	16.65	17.71	13.60	13.93	ND
Raised total IgE %[b]	65	51	82	75	ND

[a] ND: Not defined
[b] % of the cohort with raised age corrected total IgE

Table 2. Primers and PCR conditions used for mutation screening

Primers	Sequence 5'-3'	Temp.	Mg^{2+}	Amplicon size
IL13RA1 promoter				
Accession: AL606485[a]				
+610 to –1584[b]	Forward: CTCGCAAGAGCCACCCTTAGACA	63°C	1.5 mM	2194 bp
	Reverse: TTGCCCTTATCATTGCCCCTCAG			
IL13RA1 coding region				
Accession: Y10659				
+38 to +301	Forward: TGTGGGCGCTGCTGCTCT	66°C	2 mM	263 bp
	Reverse: TGGGACCCCACTTGCAGACAA			
+238 to +1347	Forward: CGGAAACTCGTCGTTCAATAG	58°C	2 mM	1109 bp
	Reverse: AACAAATGGAGAATGGGAAGA			
+889 to +1443	Forward: TCATGGTCCCTGGTGTTC	58°C	1.5 mM	554 bp
	Reverse: CGGTGCGCGACTCAACATAAA			

[a] Reference sequences were retrieved from GenBank.

[b] Position of primers is shown in relation to initiation codon ATG.

Table 3. Primers and PCR conditions used for genotyping

Polymorphism	Sequence 5'-3'	Temp.	Mg^{2+}	Amplicon
IL13RA1 –281T>G Accession: AL606485[a]	Forward inner primer (G allele): CTCCCGCGTCCGGTCTCTGACCGtAC[b] Reverse outer primer (G allele) [c]: CTGTCCTGGTGTCCAGCAGGGCACAGCC Reverse inner primer (T allele): TGGGCGGCGACGTGGTGGAAGAAGTTcTT Forward outer primer (T allele): CCTTCGCTCCCTCTTCCACTTCCCGGCTC	63°C	2 mM	172 bp (G allele) 256 bp (T allele)
IL13RA1 1365A>G Accession: AL606485*	Forward inner primer (A allele): TCTCCATTTGTTATCTGGGAACTTATTAA Reverse outer primer (A allele)[d]: CACCACTATCATCACTTTTTGCTTTGTCTTTGTC Reverse inner primer (G allele): ATGGTGCAGTAGTTTCAGTTTCCcTC Forward outer primer (G allele): CATACCCCTACGGTTCCATCCAC	63°C	2 mM	226 bp (A allele) 315 bp (G allele)

[a] Reference sequences were retrieved from GenBank.

[b] Deliberate mismatched nucleotides are denoted with a small letter.

[c] The control amplicon produced by the two outer primers of –281T>G is 373 bp long.

[d] The control amplicon produced by the two outer primers of 1365A>G is 423 bp long.

Table 4. *IL13RA1* –281T/1365A haplotype association with atopy and asthma phenotypes

Variable	n	Log age-corrected total IgE	FEV_1 (% predicted)	BHR slope	Atopy severity score	Asthma severity score
Asthmatic mothers, *P* value	100	0.049	0.753	0.420	0.700	0.120
Asthmatic fathers, *P* value	85	0.160	0.200	0.808	0.300	0.571
First affected female siblings, *P* value	216	0.768	0.832	0.870	0.640	0.830
First affected male siblings, *P* value	245	0.600	0.856	0.442	0.432	0.956

Table 5. Allelic association of the *IL13RA1* –281T>G and the *IL13RA1* 1365A>G variants with asthma and asthma intermediate phenotypes by TDT analysis

Variable	No of genotyped offspring	Variant	Observed	Expected	*P* value
Asthma diagnosis					
–281	328	T	66	71	0.401
		G	76	71	
+1365	333	A	43	44.5	0.750
		G	46	44.5	
Atopy + asthma					
–281	314	T	60	62	0.719
		G	64	62	
+1365	287	A	37	39	0.650
		G	41	39	
Raised IgE + asthma					
–281	292	T	63	65	0.725
		G	67	65	
+1365	294	A	38	39.5	0.735
		G	41	39.5	
PC_{20}* <4 + asthma					
–281	236	T	45	49.5	0.365
		G	54	49.5	
+1365	238	A	25	31	0.127
		G	37	31	
PC_{20} ≤16 + asthma					
–281	289	T	56	60	0.465
		G	64	60	
+1365	292	A	34	38	0.358
		G	42	38	

*PC_{20}=concentration of methacholine needed to provoke a fall in forced expiratory volume in 1 second of 20% or more.

Fig. 1. Genotyping of the *IL13RA1* –281T>G polymorphism using tetra-primer ARMS-PCR assay. Electrophoresis gel showing size markers in lane 1. In lanes 2, 3, and 8 the 373 bp control band and a 256 bp band are visible for homozygotes carrying two T alleles. In lanes 5 and 7, the control band plus a 172 bp band for homozygotes carrying two G alleles are shown. In lanes 4, 6, and 9 the control band plus two bands of 256 bp and 172 bp are visible for TG heterozygotes.

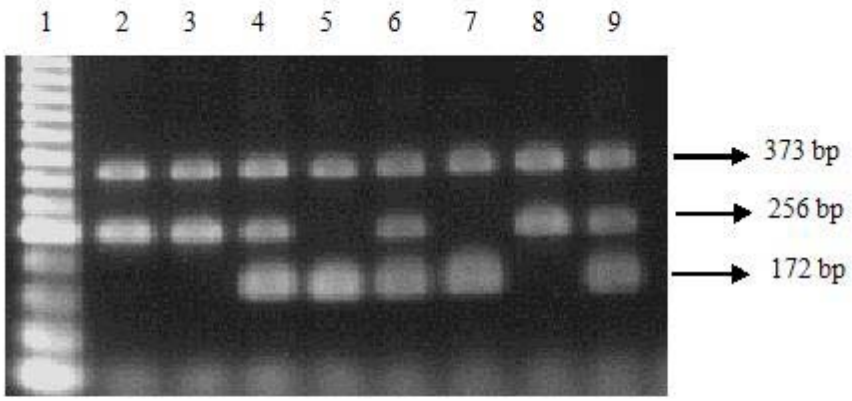
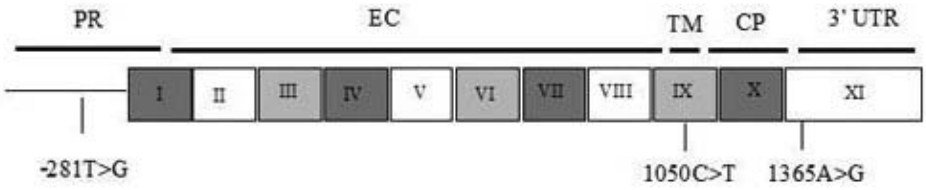


Fig. 2. Schematic representation of the human *IL13RA1* gene structure and position of polymorphisms. The eleven exons of the *IL13RA1* gene are represented as boxes. The promoter (PR), 5' UTR, extracellular domain (EC), transmembrane domain (TM), cytoplasmic domain (CP), 3' UTR as well as the position of the *IL13RA1* gene polymorphisms are also depicted.


PR
EC
TM
CP
3' UTR
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
-281T>G
1050C>T
1365A>G