# Genetic association studies of interleukin-13 receptor α1 subunit gene polymorphisms in asthma and atopy

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**ABSTRACT:** Interleukin (IL)-13 plays a central role in asthma pathogenesis by binding to the IL-13 receptor, which is a heterodimer composed of the IL-13 receptor α1 subunit (IL-13Rα1) and IL-4Rα. The genetic diversity at the IL-13Rα1 gene (*IL13RA1*) locus on chromosome Xq24 was characterised and the association of identified polymorphisms with asthma and atopy phenotypes examined.

The promoter and coding region of *IL13RA1* were screened for common genetic variants, and polymorphisms found were genotyped in a large cohort of 341 asthmatic Caucasian families (each containing at least two asthmatic siblings) and 182 nonasthmatic control subjects. Genetic association was determined using case–control and transmission disequilibrium test analyses.

Two common polymorphisms were identified, a newly found thymidine (T) to guanine (G) transition of nucleotide -281 (-281T>G) single nucleotide polymorphism in the *IL13RA1* promoter and the previously described 1365A>G variant in the *IL13RA1* proximal 3′ untranslated region. 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 the *IL13RA1* -281T/1365A haplotype and raised total serum immunoglobulin E levels in adult female asthmatics.

These findings indicate that the interleukin-13 receptor α1 subunit gene -281T>G and 1365A>G polymorphisms do not contribute to asthma susceptibility or severity, although the interleukin-13 receptor α1 subunit gene locus might be involved in the control of immunoglobulin E production.

**KEYWORDS:** Asthma, atopy, genetics, immunoglobulin E, interleukin-13 receptor, polymorphism

The type-2 T-helper cell cytokines interleukin (IL)-13 and IL-4 play a central role as effector molecules in asthma through multiple mechanisms, including induction of immunoglobulin (Ig)E synthesis by B-cells [1, 2], airway eosinophilia [3], goblet cell metaplasia and mucus hypersecretion [4, 5], and airway remodelling [6]. IL-13 elicits its biological effects *via* a receptor complex composed of the heterodimeric proteins IL-13 receptor α1 subunit (IL-13Rα1) and IL-4Rα [7]. The IL-13Rα1/IL-4Rα complex is also utilised by IL-4 as an alternative receptor, especially in nonhaematopoietic cells that do not express the common γ chain (IL-2Rγ) [8]. Based on data found on the Entrez Gene database [9], the IL-13Rα1 gene (*IL13RA1*) 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 base pairs relative to the start codon [10]. IL-13Rα1 is expressed on both haematopoietic and nonhaematopoietic cells, including basophils,

eosinophils, B-cells, mast cells, fibroblasts, endothelial cells, smooth muscle cells and airway epithelial cells [11]. Signalling of IL-4 and IL-13 through the IL-4Rα/IL-13Rα1 complex is thought to occur through IL-4Rα [12], leading to activation of several signalling molecules, including signal transducer and activator of transcription 6 and insulin receptor substrates 1 and 2, which can translocate to the nucleus and bind to specific motifs in the promoter regions of responsive genes (*e.g.* major histocompatibility complex class II, CD23, the IgE germline transcript and IL-4Rα) [13].

Several studies have shown that genetic variants of *IL13*, *IL4* and *IL4RA* confer susceptibility to atopy and asthma [14]. In contrast, there have only been two association studies of polymorphisms in the *IL13RA1* gene with asthma and atopy [15, 16]. AHMED *et al.* [15] screened the coding region of *IL13RA1* in a Japanese population and identified a rare cytosine (C) to thymidine (T) nonamino-acid-altering polymorphism (substitution) at position

1050 (1050C>T) relative to the translation initiation codon ATG. A low-power association study by the same group of investigators found no association between the *IL13RA1* 1050C>T polymorphism and atopic asthma. HEINZMANN *et al.* [16] screened the *IL13RA1* gene in British and Japanese populations and identified an adenine (A) to guanine (G) substitution at position 1365 relative to the translation initiation codon ATG, situated in the proximal 3′ untranslated region (UTR) of the gene, referred to as 1398A>G by the present authors. In the same study, the *IL13RA1* 1365A>G polymorphism was found to be associated with elevated 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, it was hypothesised that genetic variation in *IL13RA1* may predispose to the development and/or predict severity of asthma and atopy. In order to test this hypothesis, the promoter, coding region and proximal 3′ UTR of *IL13RA1* were screened for common genetic variants. Subsequently, the identified variants were evaluated for evidence of association with asthma and atopy phenotypes in a large cohort of 341 asthmatic families and a cohort of 182 nonasthmatic control subjects using three methods, case–control analyses, and phenotype–genotype and phenotype–haplotype association studies, as well as the transmission disequilibrium test (TDT). Here, a novel allelic variant of the *IL13RA1* promoter and two previously described single nucleotide polymorphisms (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 are reported.

## MATERIALS AND METHODS

### Subjects and clinical assessment

Caucasian families (n=341) containing at least two biological siblings (aged 5–21 yrs) with a current physician's diagnosis of asthma and who were taking asthma medication on a regular basis were recruited from the Southampton area of the UK (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, spirometric data, challenge dose levels for the bronchial challenge, documentation of laboratory samples taken and information on medicines taken during the last 12 months.

Asthma in the adults was defined as a positive response to the following three questions: 1) ''Have you ever had asthma?''; 2) ''Was this confirmed by a doctor?''; and 3) ''Have you used any medicines to treat asthma, or any breathing problems, at any time in the last 12 months?''. The baseline forced expiratory volume in one second (FEV1) was obtained from pulmonary function testing. Three FEV1 within 5% of each other were obtained, and the highest value was recorded. Airway responsiveness was defined as the concentration of inhaled methacholine required to reduce FEV1 by 20% (PC20), and was performed according to American Thoracic Society guidelines [17], using a DeVilbiss 646 nebuliser (Sunrise Medical, Inc., Carlbad, CA, USA) in conjunction with a computerised system (KoKo Digidoser; Ferraris Respiratory, Louisville, CO, USA). Skin-prick testing was carried out to the

| TABLE 1 | Phenotypic characteristics of study cohorts | | | | |
|---|---|---|---|---|---|
| | **Asthmatic family members** | | | | **Controls** |
| | **Father** | **Mother** | **First brother** | **First sister** | |
| **Subjects n** | 89 | 100 | 271 | 235 | 184 |
| **Mean age yrs** | 41.4 | 39.2 | 12.1 | 12.0 | 42.3 |
| **Age range yrs** | 31.9–56.2 | 28.5–53.4 | 5.4–22.6 | 5.0–21.2 | |
| **FEV1 % pred** | 91.10 | 96.80 | 95.68 | 94.19 | ND |
| **BHR#** | 16.65 | 17.71 | 13.60 | 13.93 | ND |
| **Raised total IgE¶ %** | 65 | 51 | 82 | 75 | ND |

All of the asthmatic family members had a current physician's diagnosis of asthma. The control group comprised 47% males and none had a current physician's diagnosis of asthma. FEV1: forced expiratory volume in one second; % pred: % predicted; BHR: bronchial hyperresponsiveness; Ig: immunoglobulin; ND: not determined. #: FEV1 response to methacholine (transformed to 1/(least squares slope+30) × 1,000; ¶: percentage of cohort with raised age-corrected total IgE levels.

following six common aeroallergens: mixed grass, mixed trees, cat, dog, *Dermatophagoides pteronyssinus*, and Alternaria (Bayer Corporation, Spokane, WA, USA), with a negative (saline) and a positive (histamine) control. Atopy was defined as either a positive skin-prick test result (>3 mm increase in weal diameter over control) or raised specific IgE (>0.35 kUA·L⁻¹) to one or more common allergens. Total and specific IgE measurements were carried out by IBT laboratory (Kansas, MO, USA), using the ImmunoCAP System (Phadia, Uppsala, Sweden). Specific IgE levels were measured for the same allergens as used for skin-prick testing. Total IgE was adjusted for age by using the number of SD away from the median for each age group. Severity scores for atopy and asthma were generated as previously described [18]. In addition, 182 nonasthmatic 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 & South West Hampshire Joint Research Ethics Committee (Southampton, UK).

### Mutation screening

Using genomic DNA extracted from 20 male subjects (eight diagnosed with asthma) and 18 female subjects (four diagnosed with asthma), a 2.2 kilobase (kb) fragment of the *IL13RA1* promoter (corresponding to nucleotides -1584–610 relative to the translation initiation codon ATG) was generated by PCR. Due to the high GC content of this region, a combination of 0.1 U·µL⁻¹ Taq (Sigma-Aldrich, Poole, UK) and 0.0033 U·µL⁻¹ Pwo DNA polymerase (Roche Applied Science, Lewes, UK) was used for amplification of the genomic PCR template (100 ng) in the presence of Pwo buffer, 5% dimethylsulphoxide (DMSO), 1.5 mM MgCl₂, 0.2 µM of each primer (table 2) and 0.2 mM deoxyribonucleoside triphosphates (dNTPs), containing a 3:1 ratio of deoxyguanosine triphosphate (dGTP) to 7-deaza-dGTP, as well as fluorescent (R110) deoxycytidine triphosphate (dCTP; Applied Biosystems, Warrington, UK) at a ratio of 1:100 to unlabelled dCTP, to give a

| **TABLE 2** | Primers and PCR conditions used for mutation screening of interleukin-13 receptor α1 subunit gene | | | | |
|---|---|---|---|---|---|
| | Accession number | Primers[#] | Annealing temperature °C | Mg$^{2+}$ mM | Amplicon size bp |
| **Promoter** | AL606485 | | | | |
| 610– -1584 | | CTCGCAAGAGCCACCCTTAGACA (F) | 63 | 1.5 | 2194 |
| | | TTGCCCTTATCATTGCCCCTCAG (R) | | | |
| **Coding region** | Y10659 | | | | |
| 38–301 | | TGTGGGCGCTGCTGCTCT (F) | 66 | 2.0 | 263 |
| | | TGGGACCCCACTTGCAGACAA (R) | | | |
| 238–1347 | | CGGAAACTCGTCGTTCAATAG (F) | 58 | 2.0 | 1109 |
| | | AACAAATGGAGAATGGGAAGA (R) | | | |
| 889–1443 | | TCATGGTCCCTGGTGTTC (F) | 58 | 1.5 | 554 |
| | | CGGTGCGCGACTCAACATAAA (R) | | | |

Reference sequences were retrieved from GenBank. Primer nucleotide position is shown relative to initiation codon ATG. bp: base pair; C: cytosine; T: thymidine; G: guanine; A: adenine; F: forward; R: reverse. [#]: 5′ to 3′.

final reaction volume of 50 μL. The thermal cycling included a single soak for 3 min at 95°C followed by 38 cycles of denaturation for 30 s at 95°C, annealing for 30 s at 63°C and extension for 2.5 min at 72°C, and, finally, a soak for 10 min at 72°C on a PTC-225 DNA Engine Tetrad (MJ Research, Inc., Waltham, MA, USA). For mutation screening of the coding region of *IL13RA1*, spanning ~1.5 kb, total RNA was extracted from whole blood from 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 the manufacturer's instructions. Complementary DNA (cDNA) was generated using an Omniscript reverse transcription kit (Qiagen) as directed by the manufacturer with 2 μg RNA template. In order to increase PCR yield, the coding region of *IL13RA1* was divided into three segments. Segment I spanned nucleotides 38–301 relative to the translation initiation site, segment II 238–1347 and segment III 889–1443. PCR involved 2 μL cDNA template (from the 20 μL cDNA reaction), Jumpstart Taq (0.025 U·μL$^{-1}$; Sigma-Aldrich), standard PCR buffer, MgCl$_2$ (table 2), 0.2 μM of each primer (table 2) and 0.2 mM dNTPs (including fluorescent (R110) dCTP at a 1:100 ratio to unlabelled dCTP for segments screened using solid-phase chemical cleavage), to give a final reaction volume of 20 μL. The thermal cycling included a single soak for 5 min at 95°C followed by 35 cycles for 30 s at 94°C, annealing for 30 s at the temperatures indicated in table 2 and extension for 60 s·kb$^{-1}$ at 72°C, and, finally, a soak for 10 min at 72°C. By virtue of its small size, segment I was screened using denaturing HPLC (DHPLC; Transgenomic, Crewe, UK), whereas segments II and III were screened using solid-phase chemical cleavage, essentially as previously described [19, 20]. 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 1365A>G polymorphisms were genotyped using tetra-primer amplification refractory mutation system PCR assays (fig. 1) [21]. Each PCR reaction was carried out in a total volume of 15 μL, containing 25 ng template DNA, 0.2 mM dNTP, 2 mM MgCl$_2$, 5% DMSO, Jumpstart Taq (0.05 U·μL$^{-1}$), 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 5 min at 95°C followed by 10 cycles of 30 s at 94°C, 30 s at 73–n°C (where n is cycle number) and 30 s at 72°C, and then 31 cycles of 30 s at 94°C, 30 s at 63°C and 30 s at 72°C, and, finally, 10 min at 72°C. PCR products were resolved by microplate-array diagonal-gel electrophoresis [22], stained with Vistra Green (Amersham Biosciences, Little Chalfont, UK) and visualised using a Fluorimager 595 (Molecular Dynamics, Sunnyvale, CA, USA). Genotypes were scored using Phoretix 1D gel analysis software (Nonlinear Dynamics, Newcastle upon Tyne, UK). Representative genotyping assay results were confirmed by dideoxy dye terminator cycle sequencing on an ABI PRISM 377 DNA Sequencer.

### Statistical methods

Case–control analysis

Case–control studies were conducted to evaluate association of the *IL13RA1* -281T>G and 1365A>G polymorphisms with asthma. All comparisons were made between groups of the same sex, since male subjects are hemizygous for *IL13RA1* due its localisation 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). The control population were healthy Caucasians of the same area of residence: females (n=98) and males (n=86; table 1). The genotype frequencies for each group of cases were compared to the control population and analysed using the Chi-squared test. The Hardy–Weinberg equilibrium was confirmed using a Chi-squared test with one degree of freedom.

Phenotype–genotype and phenotype–haplotype association studies

The data were analysed in four asthmatic cohorts, including first asthmatic female siblings, first asthmatic male siblings, female parents with a diagnosis of asthma and male parents
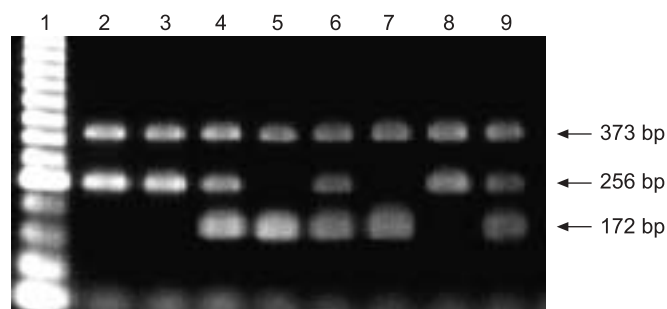
**FIGURE 1.** Genotyping of the interleukin-13 receptor α1 subunit gene polymorphism involving thymidine (T) to guanine (G) transition at nucleotide -281, using a tetra-primer amplification refractory mutation system PCR assay (lane 1: electrophoresis size markers). All lanes contain the 373-base pair (bp) control amplicon. In addition, lanes 2, 3 and 8 contain the 256-bp amplicon corresponding to the T allele and are from homozygotes carrying two T alleles, and lanes 5 and 7 contain the 172-bp amplicon corresponding to the G allele and are from homozygotes carrying two G alleles. Lanes 4, 6 and 9 are from TG heterozygotes and contain both amplicons.

with a diagnosis of asthma. Statistical analysis was carried out using an unpaired t-test for the male study groups and the Chi-squared test for the female study groups, following transformation of continuous phenotypes into categorical ones using appropriate cut-off points for the following phenotypic markers: 1) total serum IgE (age-corrected and logarithmically (base 10) transformed to improve normality); 2) $FEV_1$ (percentage of the predicted value); 3) slope of $FEV_1$ response to methacholine (transformed to $1/(\text{least squares slope}+30) \times 1,000$ to improve normality and avoid negative values); 4) atopy severity score; and 5) asthma severity score. A p-value of $\leqslant 0.05$ was considered significant.

Transmission disequilibrium test analysis

Association of the *IL13RA1* -281T>G and 1365A>G polymorphisms with asthma and intermediate phenotypes of asthma was assessed using TDT analysis. Any test with a p-value of <0.05 was considered significant. Dichotomous

variables analysed using the TDT were: 1) asthma-positivity on questionnaire (*i.e.* positive responses to the three questions detailed above); 2) asthma with atopy (defined by raised specific IgE levels and/or positive skin-prick test results); 3) asthma with raised total serum IgE levels (age-corrected); 4) asthma and $PC_{20}$ <4 $mg \cdot mL^{-1}$ (severe asthmatics); 5) asthma and $PC_{20}$ $\leqslant 16$ $mg \cdot mL^{-1}$. Data analysis was based on the first affected sibling since transmission to other siblings within the same family is not independent. Given that the TDT utilises data from heterozygous parents, only maternal transmission was analysed since males are hemizygous at *IL13RA1*. Haplotype construction and frequency distributions were also carried out.

Power calculations

The power of the TDT study to detect an important difference was calculated using a formula that assumes that the recombination fraction is 0 and there is no linkage disequilibrium [23]. The fact that the only informative transmissions were those from mothers, due to the X chromosome localisation 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 significant difference was calculated using a programme which takes into account the alpha level (0.05), sample size, odds ratio (OR) 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* (~1.5 kb) disclosed the presence of a previously described silent variant at position 1050, in the coding region of *IL13RA1,* involving a C to T substitution [15], 1050C>T, as well as a previously reported SNP located at position 1365, in the proximal 3′ UTR of *IL13RA1,* involving an A to G substitution [15], 1365A>G, described as 1398A>G in the original article (fig. 2) [16]. The -281G allele of -281T>G was relatively abundant in the present

| TABLE 3 | Primers and PCR conditions used for genotyping interleukin-13 receptor α1 subunit gene polymorphisms | | |
|---|---|---|---|
| **Polymorphism** | **Primers[#]** | | **Amplicon size bp** |
| | **Type** | **Sequence[#]** | |
| **-281T>G** | G allele: forward inner | CTCCCGCGTCCGGTCTCTGACCG<u>T</u>AC | 172 |
| | G allele: reverse outer | CTGTCCTGGTGTCCAGCAGGG<u>C</u>ACAGCC | |
| | T allele: reverse inner | TGGGCGGCGACGTGGTGGAAGAAGTT<u>C</u>TT | 256 |
| | T allele: forward outer | CCTTCGCTCCCTCTTCCACTTCCCGGCTC | |
| **1365A>G** | A allele: forward inner | TCTCCATTTGTTATCTGGGAACTTATTAA | 226 |
| | A allele: reverse outer | CACCACTATCATCACTTTTTGCTTTGTCTTTGTC | |
| | G allele: reverse inner | ATGGTGCAGTAGTTTCAGTTTCC<u>C</u>TC | 315 |
| | G allele: forward outer | CATACCCCTACGGTTCCATCCAC | |

Reference sequences were retrieved from GenBank (accession number AL606485). Deliberately mismatched nucleotides are underlined. The control amplicon produced by the two outer primers of -281T>G is 373 base pairs. The control amplicon produced by the two outer primers of 1365A>G is 423 bp. A $Mg^{2+}$ concentration of 2 mM was used and annealing was performed at 63°C. T: thymidine; G: guanine; A: adenine; C: cytosine. [#]: 5′ to 3′.

population (q=0.37 for affected female siblings and q=0.34 for affected male siblings). The 1365G allele of 1365A>G exhibited a lower frequency in the present cohort (q=0.17 for both female and male affected siblings). The *IL13RA1* 1050C>T variant was rare, with a minor allele frequency of 0.040 in the present cohorts. Distribution of the alleles in each group did not deviate from the Hardy–Weinberg equilibrium when assessed using Chi-squared 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 and found to be 0.67 for -281T/1365A, 0.17 for -281T/1365G and 0.16 for -281G/1365A, whereas those for adult male asthmatics were 0.67 for -281T/1365A, 0.165 for -281T/1365G and 0.165 for -281G/1365A. No individuals were found with the -281G/1365G haplotype in this population. Linkage disequilibrium between *IL13RA1* -281T>G and 1365A>G was measured by determining $r^2$ [24]. This was calculated to be 0.406, meaning that ~40% of the information of one of the SNPs can be obtained from the other, indicating a moderate degree of linkage disequilibrium between the SNPs at nucleotides -281 and 1365.

### Genetic association studies

Case–control analysis

The genetic association of the *IL13RA1* polymorphisms and asthma were evaluated 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 Chi-squared analysis. The distribution of genotypes and haplotypes of the *IL13RA1* -281T>G and 1365A>G did not differ significantly between asthmatic subjects and normal controls (data not shown).

Phenotype–haplotype association analyses

Within the asthmatic groups, potential associations of the *IL13RA1* two-allele haplotypes and various intermediate phenotypes of asthma, including total serum IgE level, FEV1 (% pred), slope of FEV1 response to methacholine, symptom score and atopy severity score, were assessed in asthmatic parents and first affected siblings. No significant associations between *IL13RA1* two-allele haplotypes and intermediate phenotypes of asthma were found in any of the study groups, apart from a borderline association between the -281T/1365A

haplotype and elevated total serum IgE levels in asthmatic mothers using the Chi-squared test (TA *versus* TG haplotype: OR 2.81 (95% confidence interval (CI) 1.20–6.59); TA *versus* GA haplotype: OR 1.07 (95% CI 0.50-2.28); p=0.049; table 4).

Phenotype–genotype association analyses

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

Transmission disequilibrium test analysis

Evidence for association of the *IL13RA1* -281T>G and 1365A>G polymorphisms with asthma and intermediate phenotypes of asthma were further evaluated using TDT. No alleles of either -281T>G or 1365A>G were found to be preferentially transmitted from heterozygous mothers to first affected siblings (table 5). The possibility of haplotype association due to combined interaction of the *IL13RA1* -281T>G and 1365A>G polymorphisms were also investigated using TDT. Again, no significant association of the *IL13RA1* two-allele haplotypes was found with either asthma or intermediate phenotypes of asthma (data not shown).

### Power calculations

The hypothesis that the lack of association of the *IL13RA1* -281T>G and 1365A>G polymorphisms alone with asthma and intermediate phenotypes of asthma might have been due to insufficient power of the present study to detect true associations was examined. 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, whereas, for a genotypic risk ratio of 1.5, 316 families would be required. For the 1365A>G polymorphism, the number of families necessary to obtain 80% power for a genotypic risk ratio of 2 was 152, whereas, for a risk ratio of 1.5, 474 families were required.



**FIGURE 2.** Schematic representation of the human interleukin-13 receptor α1 subunit gene (*IL13RA1*): structure and position of polymorphisms. The eleven exons of *IL13RA1* are represented by boxes. The promoter (P), extracellular domain (EC), transmembrane domain (TM), cytoplasmic domain (CP) and 3′ untranslated region (UTR), as well as the position of the *IL13RA1* polymorphisms, are also depicted.

| **TABLE 4** | Interleukin-13 receptor α1 subunit -281T/1365A haplotype association with atopy and asthma phenotypes in asthmatic family members | | | |
|---|---|---|---|---|
| | **Mother** | **Father** | **First sister** | **First brother** |
| **Subjects n** | 100 | 85 | 216 | 245 |
| **Log total IgE[#]** | 0.049 | 0.160 | 0.768 | 0.600 |
| **FEV1 % pred** | 0.753 | 0.200 | 0.832 | 0.856 |
| **BHR slope[¶]** | 0.420 | 0.808 | 0.870 | 0.442 |
| **Atopy severity score** | 0.700 | 0.300 | 0.640 | 0.432 |
| **Asthma severity score** | 0.120 | 0.571 | 0.830 | 0.956 |

Data presented are p-values, unless otherwise stated. Ig: immunoglobulin; FEV1: forced expiratory volume in one second; % pred: % predicted; BHR: bronchial hyperresponsiveness. [#]: age-corrected; [¶]: FEV1 response to methacholine.
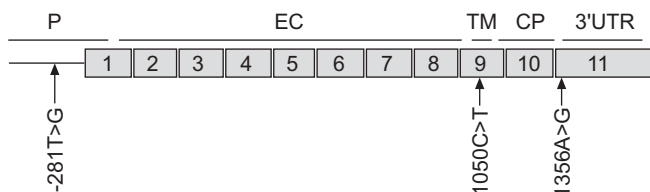
| TABLE 5 | Allelic association of the interleukin-13 receptor α1 subunit gene -281T>G and 1365A>G variants with asthma and intermediate phenotypes of asthma by transmission disequilibrium test analysis | | | | |
|---|---|---|---|---|---|
| | Genotyped offspring n | Variant | Observed n | Expected n | p-value |
| **Asthma** | | | | | |
| -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 | |
| **PC20 ⩽4 mg·mL$^{-1}$ + asthma** | | | | | |
| -281 | 236 | T | 45 | 49.5 | 0.365 |
| | | G | 54 | 49.5 | |
| 1365 | 238 | A | 25 | 31 | 0.127 |
| | | G | 37 | 31 | |
| **PC20 ⩽16 mg·mL$^{-1}$ + asthma** | | | | | |
| -281 | 289 | T | 56 | 60 | 0.465 |
| | | G | 64 | 60 | |
| 1365 | 292 | A | 34 | 38 | 0.358 |
| | | G | 42 | 38 | |

-281T>G: thymidine (T) to guanine (G) transition of nucleotide -281; Ig: immunoglobulin; PC20: provocative concentration of methacholine causing a ⩾20% fall in forced expiratory volume in one second; A: adenine; C: cytosine.

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 analysed, as well as in the group of asthmatic fathers, which was the least abundant study group. In the comparison of first affected female siblings (n=470 alleles) with female controls for determining association of the -281T>G SNP with asthma, the sample had a power of 80% for detecting an OR of 2 and a power of 35% for detecting an OR of 1.5. For association of the 1365A>G polymorphism with asthma in first affected female siblings, the power was 65% for an OR of 2 and 24% for an OR 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% for detecting an OR of 2 and a power of 25% for detecting on OR of 1.5. For association of the 1365A>G variant with asthma in asthmatic fathers, the sample had a power of 46% for an OR of 2 and a power of 18% for an OR of 1.5.

## DISCUSSION

In the present study, the promoter, coding region and proximal 3′ UTR of *IL13RA1* were screened for common genetic variants. A newly identified polymorphism in the *IL13RA1* promoter, -281T>G, along with two previously described variants, 1050C>T [15] and 1365A>G [16], were studied. Using a case–control study and phenotype–genotype and phenotype–haplotype association analyses, as well as a family-based approach, the two common *IL13RA1* polymorphisms, -281T>G and 1365A>G, were examined for evidence of association with 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 levels 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 for detecting common SNPs with allelic frequencies of >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 [19, 20].

The two common *IL13RA1* polymorphisms, -281T>G and 1365A>G, were found to be in moderate linkage disequilibrium ($r^2$=0.406) [24]. The 1365G allele had a frequency of 0.17, which is comparable with the frequency previously found in a UK population [16], but lower than that 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 the present cohort, comparable with the frequency previously found in a Japanese population [15].

No association of either *IL13RA1* -281T>G or 1365A>G with asthma was found on either TDT or case–control analysis. The lack of association between the 1365A>G polymorphism and asthma is in accordance with the study of HEINZMANN *et al.* [16]. In addition, none of the two-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 levels among adult female asthmatics. The lack of association of this haplotype with total serum IgE levels in other study groups in the present cohort might be due to the clear effects of sex and age on allergic manifestation and total serum IgE levels. It has been found that males show higher geometric mean total serum IgE levels than females throughout the entire age range of 6–⩾75 yrs [25]. Moreover, total serum IgE levels reach a maximum at age 10–15 yrs 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 [25]. Although these sex- and age-related effects on total serum IgE levels might explain the fact that the association between the *IL13RA1* -281T/1365A haplotype and raised IgE levels was observed only among adult female asthmatics, it should be emphasised that the association was marginal and might have occurred by chance. It is also important to note that, since families in the present 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 *et al.* [16], the 1365A>G polymorphism (referred to as 1398A>G) was associated with raised total serum IgE levels in UK male, but not female, subjects [16]. The discrepancy between their study and the present one might be due to differences in atopy severity and/or study design between the two cohorts. In addition, the -281T>G polymorphism was not evaluated in the study of HEINZMANN *et al.* [16].

Power calculations demonstrated that the present TDT study was well-powered for the detection of effects with ORs of $\geqslant 1.5$. The power of the case–control study to detect effects with ORs of $\geqslant 2$ was adequate (60–80%) for -281T>G and moderate (46–56%) for 1365A>G, whereas the study was under powered for the detection of an effect size of <2 for both polymorphisms. The power to detect a significant association depends upon 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 ORs in follow-up studies ranged 1.1–2.0 [26]. It is likely that most genuine genetic associations in complex disease represent modest effects, with ORs of 1.1–1.5 [27]. 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 overall disease risk that is attributable to genetic factors [27]. This highlights the challenge of recruiting larger cohorts of participants in order to detect modestly higher ORs. In the present study, small effects with ORs of <1.5 may have been missed.

The *IL13RA1* 1050C>T polymorphism is located at the third nucleotide of codon 350, resulting in no amino acid alteration, and has been described previously [15]. This polymorphism was not evaluated for association in the present study due to the very low frequency (~0.04) of the minor allele in the present cohort. The -281T>G polymorphism in the *IL13RA1* promoter has not been previously described. This polymorphism may have a functional role in affecting transcriptional activation and gene production. Further *in vitro* studies are needed to demonstrate whether or not this polymorphism directly affects transcription factor binding and transcriptional rate.

In conclusion, mutation screening of the 5′ flanking region, coding region and proximal 3′ untranslated region of the interleukin-13 receptor α1 subunit gene was undertaken. Three polymorphic sites were identified, including a novel one in the promoter region. The two common variants of the interleukin-13 receptor α1 subunit gene were evaluated in a large cohort, and no evidence was found to support significant association of these polymorphisms with asthma or other atopy phenotypes, apart from a borderline association between the -281 thymidine/1365 adenine haplotype and raised total serum immunoglobulin E levels in adult female asthmatics. Further studies in additional cohorts are needed to evaluate whether or not variants of the interleukin-13 receptor α1 subunit gene play a role in determining susceptibility to or modulating severity of asthma and atopy.

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