Genetic variants of IL-13 signalling and human asthma and atopy


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Asthma and atopy show epidemiological association and are biologically linked by T-helper type 2 (Th2) cytokine-driven inflammatory mechanisms. IL-4 operates through the IL-4 receptor (IL-4R, a heterodimer of IL-4Rα and either γc or IL-13Rα1) and IL-13 operates through IL-13R (a heterodimer of IL-4Rα and IL-13Rα1) to promote IgE synthesis and IgE-based mucosal inflammation which typify atopy. Recent animal model data suggest that IL-13 is a central cytokine in promoting asthma, through the stimulation of bronchial epithelial mucus secretion and smooth muscle hyper-reactivity. We investigated the role of common genetic variants of IL-13 and IL-13Rα1 in human asthma, considering IgE levels. A novel variant of human IL-13, Gln110Arg, on chromosome 5q31, associated with asthma rather than IgE levels in case–control populations from Britain and Japan (peak odds ratio (OR) = 2.31, 95% CI 1.33–4.00); the variant also predicted asthma and higher serum IL-13 levels in a general, Japanese paediatric population. Immunohistochemistry demonstrated that both subunits of IL-13R are prominently expressed in bronchial epithelium and smooth muscle from asthmatic subjects. Detailed molecular modelling analyses indicate that residue 110 of IL-13, the site of the charge-modifying variants Arg and Gln, is important in the internal constitution of the ligand and crucial in ligand–receptor interaction. A non-coding variant of IL-13Rα1, A1398G, on chromosome Xq13, associated primarily with high IgE levels (OR = 3.38 in males, 1.10 in females) rather than asthma. Thus, certain variants of IL-13 signalling are likely to be important promoters of human asthma; detailed functional analysis of their actions is needed.

INTRODUCTION

Atopy is a common immune disorder characterized by raised IgE levels. It is a key predisposition to bronchial asthma between 3 and 20 years of age (1,2). Bronchial hyper-responsiveness (BHR), an exaggerated bronchospastic response to specific and non-specific substances, represents a physiological hallmark of asthma induced by T-helper type 2 (Th2) cytokines such as IL-4, -5, -9, -10 and -13 (1,2). The studies on Th2 cytokines in relation to asthma, however, have focused on IL-4 and IL-5. This is due to the crucial role of these two cytokines in generation of Th2 responses in a variety of animal models: IL-4 is essential for the maturation of naïve T cells towards Th2 cells, and the production of IgE (2,3), whereas IL-5 regulates activation and tissue recruitment of eosinophils (4). However, a variety of studies have

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shown that IL-4 and IL-5, alone and in combination, cannot fully account for the development of asthma (5,6); in BALB/c mice, BHR to methacholine is mediated by T cells, but is independent of IL-4 and IL-5. Other molecules must be involved in the development of BHR, and hence asthma.

IL-13 is a 12 kDa protein product and shares several biological profiles with IL-4 (1,2), including IgE production, CD23 and MHC class II expression, inhibition of antibody-dependent cell-mediated cytotoxicity with downregulation of IgG type I receptor (FcγRI), and suppression of type I interferon. Although IL-4 and IL-13 possess many similar biological activities (1,2), IL-13 shows some unique activities. Unlike IL-4-deficient mice, IL-13-null mice fail to generate goblet cells, responsible for mucus overproduction in asthma, fail to recover basic IgE levels after stimulation with IL-4 and fail to expel helminths (7). IL-13 operates through IL-13R, a heterodimer of IL-4Rα and IL-13Rα1 chains (1–3). Transgenic mice, with the promoter of the Clara cell 10 kDa protein (CC10) driving IL-13 expression selectively in the lungs, exhibit BHR to methacholine in addition to bronchial eosinophil prominence, epithelial cell hyperplasia, mucus cell metaplasia, hyperproduction of nucuc, deposition of Charcot–Leyden-like crystals and subepithelial airway fibrosis (8); these features are typical of T2 inflammation-induced asthmatic airways. These findings point to the importance of IL-13 in human asthma (9,10). Significantly higher IL-13 levels have been found in asthmatic patients with and without atopy (11,12). One report has related a polymorphism within the promoter region of IL-13 with allergic asthma in a Dutch population (13).

Here we report a variant of the human IL-13 gene, Gln110Arg, which specifically associates with both allergic and non-allergic asthma in case–control studies in both British and Japanese subjects. Our computer modelling suggests that residue 110 is relevant to the internal constitution of the ligand, and is likely to play a crucial role in ligand–receptor interaction. Using immunohistochemical techniques we show that functional IL-13Rα1 is specifically expressed on bronchial smooth muscle and epithelium in human asthmatic airways. These findings point to the importance of IL-13 in human asthma. In contrast, we show that a variant of IL-13Rα1, A1398G, on chromosome Xq13 (14) associates primarily with high IgE levels in the British subjects, a result that parallels our previous findings for variants of IL-4Rα in Japanese (15,16) and German (17) subjects. Thus, certain variants of IL-13 may promote the development of atopy, with high IgE levels, and asthma of diverse types across different ethnic groups.

**RESULTS**

**Genetic association study of an IL-13 variant in case–control populations**

A single-strand conformation polymorphism (SSCP) analysis among >200 atopic subjects identified different electrophoretic patterns in exon 4 of IL-13 (Fig. 1A); subsequent direct sequencing identified an A4464G variant (18) (Fig. 1B) indicating replacement of Arg by Glu at position 110 of the mature protein. Despite intensive screening we could not find any further common variants. We went on to test for a genetic association between Gln110Arg and clinical asthma and IgE levels in two populations (Table 1). The genotype frequencies were concordant with Hardy–Weinberg equilibrium. No significant differences in genotype frequencies were seen between two control populations: $P_{Arg} = 0.92$, $P_{Gln} = 0.08$ in a British population, and $P_{Arg} = 0.90$, $P_{Gln} = 0.10$ in a Japanese population. In a case–control study of British subjects, the Gln110 significantly associated with asthma, especially chronic unremitting asthma. In a second case–control study of Japanese subjects, Gln110 associated with atopic [odds ratio (OR) = 1.85, 95% CI: 1.05–3.24, $P = 0.033$] and also non-
Table 1. Odds ratio (95% CI) for IL-13 and its receptor genes, IL-4R and IL-13RA1, by asthma, total serum IgE, ASE and atopy in the Japanese and the British populations

<table>
<thead>
<tr>
<th></th>
<th>IL-13</th>
<th>IL-4Rα</th>
<th>IL-13Rα1</th>
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<tr>
<td></td>
<td>Gln110Arg</td>
<td>He50Val</td>
<td>A1398G</td>
</tr>
<tr>
<td></td>
<td>%Gln</td>
<td>OR (95% CI)</td>
<td>P value</td>
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<tr>
<td>Japanese</td>
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<tr>
<td>Controls</td>
<td>43</td>
<td>18.0</td>
<td>22.0</td>
</tr>
<tr>
<td>Atopic asthma</td>
<td>63</td>
<td>1.85 (1.05–3.24)</td>
<td>0.033</td>
</tr>
<tr>
<td>Non-atopic asthma</td>
<td>62</td>
<td>1.77 (1.01–3.10)</td>
<td>0.047</td>
</tr>
<tr>
<td>Asthmatics</td>
<td>62.5</td>
<td>1.81 (1.11–2.93)</td>
<td>0.013</td>
</tr>
<tr>
<td>Low serum IgE</td>
<td>56.3</td>
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<tr>
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<td>1.18 (0.72–1.90)</td>
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<tr>
<td>ASE (–)</td>
<td>52.7</td>
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<tr>
<td>ASE (+)</td>
<td>63.9</td>
<td>1.59 (1.00–2.53)</td>
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</tr>
<tr>
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<td>52.3</td>
<td>17.0</td>
<td></td>
</tr>
<tr>
<td>Atopic</td>
<td>64.6</td>
<td>1.66 (1.01–2.57)</td>
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<tr>
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<tr>
<td>Controls</td>
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<td>34.6</td>
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<tr>
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<td>2.14 (1.28–3.60)</td>
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<td>Severe asthmatics</td>
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<td>2.31 (1.33–4.00)</td>
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<tr>
<td>Low serum IgE</td>
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<td>31.3</td>
<td></td>
</tr>
<tr>
<td>High serum IgE</td>
<td>38.3</td>
<td>1.49 (0.92–2.42)</td>
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<tr>
<td>ASE (–)</td>
<td>30.0</td>
<td>31.2</td>
<td></td>
</tr>
<tr>
<td>ASE (+)</td>
<td>38.3</td>
<td>1.27 (0.77–2.10)</td>
<td>0.351</td>
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<tr>
<td>Non-atopic</td>
<td>27.2</td>
<td>30.2</td>
<td></td>
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<tr>
<td>Atopic</td>
<td>36.3</td>
<td>1.52 (0.90–2.58)</td>
<td>0.118</td>
</tr>
</tbody>
</table>

^aOR in females = 1.10 (95% CI:0.81–2.81, Fisher’s exact test, P = 0.765), in males = 3.39 (95% CI 1.04–9.35, Fisher’s exact test, P = 0.015 (see text).
atopic (‘intrinsic’) asthma (OR = 1.77, 95% CI 1.01–3.10, \( P = 0.047 \)); the overall OR for asthma was 1.81 (95% CI 1.11–2.93, \( P = 0.017 \)). There was no association between Gln110 and serum IgE levels in either population.

Figure 2. Ribbon diagrams of the IL-13 computer model. (A) The effect of replacement at position 110 on the internal constitution of the IL-13 ligand. (Left) Wild-type, Arg110; (right) mutant, Gln110. The blue and pink portions represent \( \alpha \)-helices or \( \beta \)-sheets, respectively. Numbering is based on the mature peptide. (B–E) Modelling of IL-13 in relation to its receptors, IL-4R\( \alpha \) and IL-13R\( \alpha_1 \) chains. Two models (1 or 2) are shown; the differences between the two depend on whether the IL-13 helix AC interfaces with IL-4R\( \alpha \) [model 1 (B and C)] or IL-13R\( \alpha_1 \) [model 2 (D and E)]. \( \beta \)-sheets are shown in blue, and \( \alpha \)-helices in red. (B) R110 of the IL-13 is predicted to interact with loop BC of the IL-4R\( \alpha \) (magenta). (C) Close-up of the interaction: R110 faces H131 of IL-4R\( \alpha \). (D) R110 is predicted to interact with loop C'E of the IL-13R\( \alpha_1 \) (magenta). (E) Close-up of the interaction: R110 faces E267 of IL-13R\( \alpha_1 \). Note that backbone atoms of V270 are also shown.
Genetic association study of the IL-13 variant in a general population in relation to serum IL-13 levels

To test the relationship between the Gln110Arg variant of IL-13 and serum IL-13 levels, we conducted a population-based survey of genotype frequencies among Japanese schoolchildren aged between 12 and 13 years (19). A significantly higher frequency of the Gln110 allele was seen among asthmatic children than that among non-asthmatic subjects. Those homozygous for Gln110 had significantly higher levels of serum IL-13 than those homozygous for Arg110.

Genetic association study of an IL-13Rα1 variant in case–control populations

We have searched for single nucleotide polymorphisms (SNPs) by SSCP in the coding region of IL-13RA1 on the X chromosome (14); all three SNPs discovered were silent and only one of them, A1398G, was relatively common (20). This variant showed marginal association with high IgE levels (OR = 2.88, 95% CI = 1.11–7.86, P = 0.026) but not with asthma in the British population; no association was seen with either high IgE levels or asthma in the Japanese population (15, 16); in our German population, atopy is also mainly related to IL-4R variants (OR = 3.39, P = 0.0042 for the combination with Pro478 and Arg551) (17).

Computer modelling of IL-13 and its receptor

To address the biological activity of the Gln110 variant of IL-13, molecular modelling was conducted on the basis of sequence alignment between IL-4 and IL-13 (20). Firstly, the modelling suggested that the replacement of Arg with Gln at position 110 may allow Arg10 to become closer to Gln110 with electrostatic interaction within IL-13 itself (Fig. 2A); this may result in subsequent change of electrostatic potential around glutamic acids at positions 11 and 14, and the former is believed to be important for IL-13 binding to the receptor on the basis of IL-4 homology (20).

Further molecular modelling focused on the interaction of IL-13 with IL-4Rα and IL-13Rα1, and was conducted on the basis of multiple alignment of cytokine receptors (Fig. 2B–E). Two alternative models (model 1, Fig. 2B; model 2, Fig. 2D) showed that Arg110 is likely to have direct interaction with one or other of the component receptor chains. Closer inspection of Arg110 in model 1 showed that this residue was located in proximity to His131 of IL-4Rα1, which might interact in the development of asthma and atopy in either the British or the Japanese populations (Table 2). In the development of asthma, the Gln110 variant of IL-13 is a significant factor in both populations; atopy associates with either an IL-13RA1 variant in the British population or an IL-4R variant in the Japanese population (15, 16); in our German population, atopy is also mainly related to IL-4R variants (OR = 3.39, P = 0.0042 for the combination with Pro478 and Arg551) (17).

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genetic factor</th>
<th>Variant</th>
<th>F value (df = 2)</th>
<th>British P value</th>
<th>Japanese P value</th>
</tr>
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<tbody>
<tr>
<td>Asthma</td>
<td>IL-4</td>
<td>-590C/T</td>
<td>0.511</td>
<td>0.600</td>
<td>0.490</td>
</tr>
<tr>
<td></td>
<td>IL-13</td>
<td>Gln110Arg</td>
<td>4.133</td>
<td>0.017</td>
<td>4.283</td>
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<tr>
<td></td>
<td>IL-4Rτ</td>
<td>Ile50Val</td>
<td>2.511</td>
<td>0.083</td>
<td>2.614</td>
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<tr>
<td></td>
<td></td>
<td>Arg551Gln</td>
<td>1.121</td>
<td>0.328</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>IL-13Rτ1</td>
<td>A1398G</td>
<td>2.704</td>
<td>0.069</td>
<td>1.134</td>
</tr>
<tr>
<td>Atopy (IgE levels)</td>
<td>IL-4</td>
<td>-590C/T</td>
<td>0.229</td>
<td>0.795</td>
<td>0.748</td>
</tr>
<tr>
<td></td>
<td>IL-13</td>
<td>Gln110Arg</td>
<td>0.935</td>
<td>0.394</td>
<td>1.494</td>
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<tr>
<td></td>
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<td>Ile50Val</td>
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<td>0.697</td>
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<td></td>
<td></td>
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<td>0.290</td>
<td>0.206</td>
</tr>
<tr>
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<td>A1398G</td>
<td>4.288</td>
<td>0.015</td>
<td>0.829</td>
</tr>
</tbody>
</table>

We found no linkage disequilibrium between the IL-4 (-590C/T) and the IL-13 (Gln110Arg) variants in either the British (t = ∆SE = –0.43) or the Japanese (t = ∆SE = –1.29) population.
Immunohistochemical assay with bronchial specimens

Only in murine studies has the existence of IL-13R been demonstrated in airways (1,2). Therefore, we conducted immunohistochemistry on pulmonary specimens from normal controls and asthmatic subjects, using monoclonal antibodies to both IL-13Rα1 and IL-4Rα (Fig. 3A–G). Both components were present prominently in smooth muscle cells and epithelial cells of the bronchus, but not in fibroblasts (Fig. 3A–D). Within alveolar tissue, neither epithelial cells nor pulmonary macrophages showed either component (Fig. 3A–E). Specificity of signal was confirmed by demonstration that the signal was not elicited by the treatment without the primary antibody (Fig. 3). Thus, the data suggest that functional IL-13R is specifically expressed in epithelial and muscle cells of the human bronchus.

DISCUSSION

Our data suggest an important role for genetic variants of IL-13 in the development of asthma, independently of IL-4, in humans. Many groups have identified linkage of both asthma and IgE levels to chromosome 5q31 (21,22) where IL-4, IL-13 and IL-5 cluster is localized. Although IL-4 is crucial for the development of T_{H}2 cells (1–3), and hence high IgE levels (23–26), IL-4 may not be a sufficient inducer for asthma itself. No functional polymorphism in IL-5 has been identified in relation to either asthma (27) or eosinophilia (28).

We have identified a novel coding variant of the human IL-13, Gln110Arg, that associates with asthma in both British and Japanese populations. Our computer modelling suggests that Gln at position 110 impacts on ligand–receptor interaction, through enhanced charge attraction to IL-13R. This in turn may enhance signalling, but detailed functional studies are now needed to test this. The genetic association of IL-13 with asthma across different ethnic populations, which is independent of IL-4, supports the candidacy of IL-13 as a major locus for asthma on chromosome 5q31. Moreover, we found a genetic association of the Gln110Arg variant of IL-13 with both atopic and non-atopic (intrinsic) asthma—a finding concordant with clinical reports on the significant elevation of IL-13 levels in both types of asthmatic subject (11,12). These findings extend the observation of association between a promoter polymorphism of IL-13 and allergic asthma in a Dutch population (13), and support the contention that IL-13 may be a key promoter of bronchial asthma in humans.

There is strong evidence that IL-13 is crucial for the induction of an asthma-like phenotype in animal models, independent of IL-4 (9,10), but which is dependent on IL-4Rα, a common component of IL-4R and IL-13R. T cell-deficient mice are capable of inducing an asthma-like phenotype on administration...
of IL-13 (9), suggesting that IL-13 may operate through mechanisms other than those that are classically implicated in Th2 cell-induced immune reactions (10). One possible explanation is that IL-13R is predominantly expressed in bronchial tissues, and that higher production of IL-13 in high risk genotypes induces hypertrophic change of the bronchial smooth muscle, subepithelial fibrosis and goblet cell hyperplasia through IL-13R.

To investigate this possibility, we stained airway specimens from normal controls and asthmatic subjects with anti-IL-13Rα1 and anti-IL-4Rα monoclonal antibodies. In asthmatic airways, in contrast to alveolar tissue, both components of IL-13R are significantly expressed. To date, two types of specific IL-13
receptor unit have been identified: IL-13Rα1 (29) and IL-13Rα2 (30). IL-13Rα2 is considered to be a ‘decoy’ receptor (31,32), whereas the heterodimer consisting of IL-13Rα1 and IL-4Rα acts as the functional receptor for human IL-13 (1,2). Human IL-4Rα is constitutively expressed in airway epithelial cells (33,34), and is essential for mucus production (7–9). Although human IL-4 acts as the functional receptor for human IL-13 (1,2). Human IL-4R is the regulating or coding parts of linkage disequilibrium with so far unidentified polymorphisms in the structure of the receptor, leading to altered phosphorylation of signal molecules, and hence lower IgE levels (17).

We now show that a variant of IL-13Rα1 on the X chromosome (14) showed association with IgE levels, but not with asthma, in British male subjects; significantly, the OR for high IgE levels was 3.38 in males, but 1.10 in females. This is the first indication of X-linked inheritance of an atopic immune disorder with high IgE levels, and further illustrates the heterogeneity of its genetic basis. It may also, in part, explain the previously noted transmission of atopy through non-affected mothers (37,38). Another cytoplasmic variant of IL-4Rα, Arg551Gln, associates with atopy (17); in our German population this variant was in tight linkage disequilibrium with Pro478Ser and the latter may change cellular IgE synthesis when tested by transfection into B cell lines. These combinations of primers covered 739 bp of sequence in the IL-13 promoter region were: 5′-GGG CAG CAT TGC AAA-3′ and 5′-GGG CAG CAT TGC AAA-3′; and 5′-GGG CAG CAT TGC AAA-3′ and 5′-GGG CAG CAT TGC AAA-3′. The annealing temperature was 60°C in all cases. Primers for the SSCP analysis for human IL-13 and IL-13Rα1 were as follows: searching for polymorphisms in the four exons of IL-13, we used the following primers: 5′-AAG CTT CCA CAA GAC GCC AA-3′ and 5′-GCC TGC TCA TGA CCT CAT CT-3′ for exon 1; 5′-GCA TTC TGC TCA TCT CT-3′ and 5′-AAG ATG GGG CTG AGA TGC CT-3′ for exon 2; 5′-CAC AAA AGG CAG CTG CCG AA-3′ and 5′-GTGT GGA CAC ACA CCA TGG AT-3′ for exon 3; and 5′-TGG CGT TCT ACT CAC GTG CT-3′ and 5′-CAG CAC AGG CAG CTG AAG TCT GT-3′ for exon 4.

SSCP analysis and direct sequencing
SSCP analysis for human IL-13 and IL-13Rα1 was done as follows: searching for polymorphisms in the four exons of IL-13, we used the following primers: 5′-AAG CTT CCA CAA GAC GCC AA-3′ and 5′-GCC TGC TCA TGA CCT CAT CT-3′ for exon 1; 5′-GCA TTC TGC TCA TCT CT-3′ and 5′-AAG ATG GGG CTG AGA TGC CT-3′ for exon 2; 5′-CAC AAA AGG CAG CTG CCG AA-3′ and 5′-GTGT GGA CAC ACA CCA TGG AT-3′ for exon 3; and 5′-TGG CGT TCT ACT CAC GTG CT-3′ and 5′-CAG CAC AGG CAG CTG AAG TCT GT-3′ for exon 4. The annealing temperature was 60°C in all cases. Primers for the IL-13 promoter region were: 5′-GCA ACA TAG TGA GAC CCC AT-3′ and 5′-GCT ATG GGA ATT TGG GGA GT-3′; 5′-TAA GAG ACT GTG TCA TCG AA-3′ and 5′-TTA AT TCCA GCG GCA GGC AA-3′; and 5′-GGG CAG CAT TGC AAA TCG CA-3′ and 5′-GAT TGA GCA GCG GAT GCA TA-3′. These combinations of primers covered 739 bp of sequence upstream from the ATG codon. The annealing temperature for the

**MATERIALS AND METHODS**

**Populations studied and phenotypes**
Three distinct populations were studied, comprising a total of 890 subjects to whom uniform criteria were applied for assignment as clinical asthma or atopy: (i) the British case–control group included 150 young adult subjects with clinical asthma and atopy, and 150 healthy controls, all from the Oxford region (39); (ii) the Japanese case–control group included 100 young adults with clinical asthma and atopy, 100 adults with clinical asthma but no atopy (non-atopic or intrinsic asthma) and 100 young adults attending a ‘well man’ clinic as controls, all drawn from the Osaka region (40); (iii) a general childhood Japanese population of 290 schoolchildren from Wakayama Prefecture who showed negative tuberculin responses at 6 and 12 years old, of whom 46.8% showed atopy and 13.4% clinical asthma (19). All the asthmatic subjects had specialist physician-diagnosed asthma with: (i) recurrent breathlessness and chest tightness requiring on-going treatment; (ii) physician-documented wheeze; and (iii) documented labile airflow obstruction with variability in serial peak expiratory flow rates >30%. There were no heavy smokers (>20 cigarettes/day) among the subjects. Allergenspecific IgE (ASE) was detected by the CAP ELISA system (Pharmacia, Uppsala, Sweden) and the criteria for a positive titre were as used previously (39,40). A high IgE titre (CAP system) was taken as >2 SD above published normal values (39,40). Atopy was defined as high IgE levels, by the presence of a high concentration of total serum IgE, or a positive ASE against one or more highly purified Aero-allergens.
first set was 63°C, whereas for the latter two was 55°C. When searching for polymorphisms in the IL-13Rα1 seven sets of primers were used for SSCP: 5'-TCC GAG CGC AGA GCC TGC AT-3' and 5'-CAC TGG GAC CCC ACT TGC AG-3' (60°C); 5'-GTA TTT TAG TCA TTT TGG CG-3' and 5'-AGT TAG TGT CGG GAC TGG TA-3' (60°C); 5'-GCA CAA CTT GAG CTA CAT GA-3' and 5'-TT CAC AGC CGA AGT TAA AGG-3' (60°C); 5'-AAA ATT AAA CCA TCC TTC AA-3' and 5'-GGA CCA TGA AAC AAG ATG TA-3' (50°C); 5'-TGA GAA TCC AGA ATT TGA GA-3' and 5'-TAA TCT TGA GCC TTT TTA GA-3' (45°C); 5'-TCA TCG TCG CAG GTG CAA TC-3' and 5'-AAT GGA GAA TGG GAA GAA TC-3' (50°C); and 5'-TCA GTG ATG GAT GTA ATT TA-3' and 5'-ATA AGA TTA ACT CCA CCA CT-3' (55°C). The annealing temperature is shown in parentheses. The amplified products were resolved on non-denaturing polyacrylamide gels under four different conditions: 10% polyacrylamide gel containing 0 or 10% glycerol at 10 or 20°C for 2 h. The gels were visualized by silver staining. Sequencing was conducted with the ‘big-dye system’ (Applied Biosystems, Warrington, UK) using downstream primers for IL-13 and IL-13Rα1, and the image was visualized in the commercial POP-6 gel using an automated sequencer (ABI Prism 310 Genetic Analyser).

**Serum IL-13 assay**

Serum IL-13 was immunoassayed in the Mitsubishi Kagaku BCL laboratories by means of a commercial kit (19). To limit circadian variation in cytokine production, blood samples were obtained between 09:00 and 10:00 h. The minimal detectable level was 3.1 pg/ml.

**Genotyping**

DNA samples were extracted using the IsoQuick kit (Microprobe, Garden Grove, IL). For genotyping Gln110Arg of IL-13, PCR primers were: 5'-TGG CGT TCT ACT CAC GTG CT-3' and 5'-TTT CGA AGT TTC AGT AC-3' (underlined bases were mutated to incorporate a restriction site). Amplified products were digested with SacI at 25°C. For genotyping IL-13Rα1, primers were: 5'-TCA GTG ATG GAT GTA ATT TA-3' and 5'-TGA GCT GCC TGT TTA TAA AT-3'. Amplification products were digested with Msel. Genotyping Ile50Val and Arg551Gln of the IL-13α1 gene were conducted using the extracellular domain of IL-13Rα1 specifically in immunohistochemistry.

**Computer modelling**

To assess the effect of replacement at position 110 on the internal constitution of IL-13 ligand, the modelling was conducted using the Homology module of the graphic program Insight 98 (Biosym, 1998) on a Silicon Graphic OCTANE workstation. The co-ordinate of 2.25 Å crystal structure of IL-4 (Protein Data Bank accession no. 1reb) was used as a template for homology modelling of IL-13. The Gln110Arg variant of the IL-13 was built up using the Biopolymer module on the basis of IL-13 model structure. The two complete structures were further minimized by heating to 300 K, equilibration for 1 ps, 200 steps of steepest gradient minimization and 10 000 steps of conjugate gradient minimization to optimize the hydrogen bonds, ion pairs and hydrophobic interactions. The minimization was performed by the Discover 3 program with the CVFF forcefield.

To investigate interaction between ligand and receptors, the three-dimensional structures of IL-4Rα and IL-13Rα1 were generated by application of restraint- and structure-based homology modelling techniques (42). The extracellular part of IL-4R consists of two cytokine receptor domains. In contrast, IL-13Rα1 has an additional fibronectin type II domain at its N-terminus. The shorter extracellular sequence of IL-4Rα and comparison with the structures of the human growth hormone (hGH) and the EPOR (erythropoietin receptor) complexes indicate that, in the complex including IL-4Rα1, IL-13 must bind to domains 2 and 3 of IL-13Rα1. These two domains also show the cytokine receptor-specific cysteine/tryptophan/proline pattern. The structures of hGH (43), EPOR (44) and gp130 (45) served as templates for the modelling. The crystal structure of IL-4 bound to IL-4Rα has been published recently (46), but the co-ordinates have not been released yet. However, the information given about loop conformation, residue exposure and inter-protein orientation was taken into account for modelling of IL-4Rα and the complex. For IL-13, an earlier prediction was considered (47). The models were refined by initial minimization (5000 steps), short molecular dynamics (40 ps) and final minimization using the Amber forcefield. Functional IL-13R is composed of IL-4Rα and IL-13Rα1 (1–3). Current knowledge about the ‘standard’ structures of Class I cytokine receptor complexes suggests that there are two faces on a cytokine interacting with its two receptors chains. One face is made up of residues predominantly located on helices A and C of the cytokine and interacts with one of the receptor chains. The other face consists of amino acids in the two long loops (loops AB and CD) and helix D of the cytokine. As it was not known whether the AC face of IL-13 interacts in the complex with IL-13Rα1 or IL-4Rα, two models were built. In model 1 the AC face interacts with IL-4Rα (Fig. 2B and C), while interacting with IL-13Rα1 in model 2 (Fig. 2D and E).

**Immunohistochemical assay**

Monoclonal antibodies to human IL-13Rα1 were established using the extracellular domain of IL-13Rα1 as antigen. One was designated as UU15 and is an IgG2a isotype. We immunostained human B cells, DND39 cells and human IL-13Rα1-transfected DND39 cells with UU15. It has been confirmed that DND39 cells do not express mRNA of human IL-13Rα1. The transfectedants showed strong staining, whereas parental cells did not give rise to any signal. These results confirmed that UU15 recognizes human IL-13Rα1 specifically in immunohistochemistry. Monoclonal antibody to human IL-4Rα was purchased from Genzyme (Cambridge, MA). Fresh human lung tissues were obtained and embedded in paraffin from patients undergoing surgery; informed consent was obtained. Asthmatic specimens were obtained from autopsy lungs. The sections were probed with UU15 by the same protocol used for IL-4Rα. Immunohistochemical staining of the sections by demonstration that: (i) the signal was not elicited by the treatment without the primary antibody; and (ii) adding excess IL-13Rα1 or IL-4Rα to the reaction diminished the signal.

**Statistics**

Contingency table analysis, ORs, 95% CIs and significance values were calculated by computerized methods (SPSS program v8). If the number in the column was <10, Fisher’s exact method was used. Probability values were corrected for multiple comparison by multiplying the P values by the number of loci.
compared (Bonferroni correction). ANOVA and multivariate analyses were also performed using this program; two-, three-, four- and five-way step interactions were tested. Linkage disequilibrium was calculated as described (48).

Sequence database

Sequences used here for modelling were derived from the database four- and five-way step interactions were tested. Linkage compared (Bonferroni correction). ANOVA and multivariate analysis.


