

EDITORIAL

Functional significance of polymorphisms of the interleukin-4 and interleukin-13 receptors in allergic disease

Introduction

Asthma, allergic rhinitis and atopic dermatitis are almost invariably accompanied by elevated levels of IgE, which results from a complex interaction of genetic and environmental factors. Linkage has been recently reported between serum IgE levels and chromosome 5q31-q33, a region rich in candidate genes involved in the IgE mediated inflammatory process, including especially the genes encoding interleukin-4 (IL-4) and IL-13 [1]. IL-4 and IL-13 play a crucial role in the development of allergic diseases. Genetic variants of the IL-4/IL-13 and their receptors influence the activities of these genes and their products, and are associated with genetic predisposition to increased serum IgE levels [2].

Genetic significance of IL-4 and IL-13 in allergic diseases (Fig. 1)

IL-4, which is the key cytokine involved in the development of atopy [3], is absolutely required for class switching of B cells to IgE production and also promoting eosinophil chemotaxis and adherence [4]. Mash *et al.* [1] have demonstrated in an inbred population that a polymorphic dinucleotide repeat in the second intron of IL-4, a region known to be functionally significant, was associated with elevated total serum IgE levels. Song *et al.* [5] reported that a nucleotide substitution within the OAP40 element located just upstream of the NF-AT site appears to be largely responsible for the increased expression of the IL-4 genes in humans, while Rosenwasser *et al.* showed a further polymorphism at position -589 of the IL-4 promoter which has been related to higher IgE levels and influenced transcriptional activities [6]. Furthermore, an association has been shown between this variant and total serum IgE levels in a Japanese population [7]. In this issue, Suzuki *et al.* reported a novel polymorphism, C + 33T in the promoter region of the IL-4 gene which was associated with elevated total serum IgE levels ($P < 0.05$). This report further explains genetic linkage or association between elevated total serum IgE levels and markers on chromosome 5q31 [8]. We have also found a significant association of the C + 33T polymorphism with asthma in another group of Japanese ($P < 0.006$), but not in British population [Robert *et al.* unpublished results].

IL-13 has come to be appreciated as a critical mediator in

the induction and maintenance of IgE production and IgE-dependent allergic responses [9,10]. Many linkage and association studies have identified genetic variants of IL-13 as being potentially important in terms of contributions to human asthma and atopy [2,11]. Graves *et al.* have identified seven novel polymorphisms in the gene of IL-13 and its promoter, these polymorphisms have been associated with total IgE in three separated populations — two in Germany; one in Arizona [12]. More recently, we have found the variant of human IL-13, Gln110Arg, associated with asthma rather than IgE levels in case-control populations from both Britain and Japan. A computer modelling analysis proposed 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; this may result in subsequent change of electrostatic potential around glutamic acids at position 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 [11]. Another variant at position -1055 in the promoter region of IL-13 has been found to be associated in a Dutch population with allergic asthma ($P < 0.002$), altered regulation of IL-13 production ($P = 0.002$), and increased binding of nuclear proteins to this region [13]. The same variant has been studied in both British and Japanese populations, which suggested that there was no association with atopy nor the asthma phenotype [Gao *et al.* unpublished results].

Genetic significance of the polymorphisms of IL-4 and IL-13 receptors (Fig. 2)

Interleukin-4 and IL-13 have overlapping roles. This is partly due to the shared use of the IL-4 receptor α chain (IL-4R α) in the multimeric IL-4 and IL-13 receptor complex [14]. IL-4 will bind to heterodimer receptors made up of the IL-4R α chain with the common lymphoid chain γ_c or IL-4R α with IL-13R α_1 chain. This shared hybrid receptor between IL-4R α and IL-13R α_1 shares signalling pathways that are involved in the common biologic features between IL-4 and IL-13, including IgE isotype switching, CD23 induction, stimulation of eosinophilic activity, mucus production and smooth muscle activity in the airway [2,11,15]. There is evidence that many of these shared pathways may involve activation through the transactivator and transcription factor, STAT6,

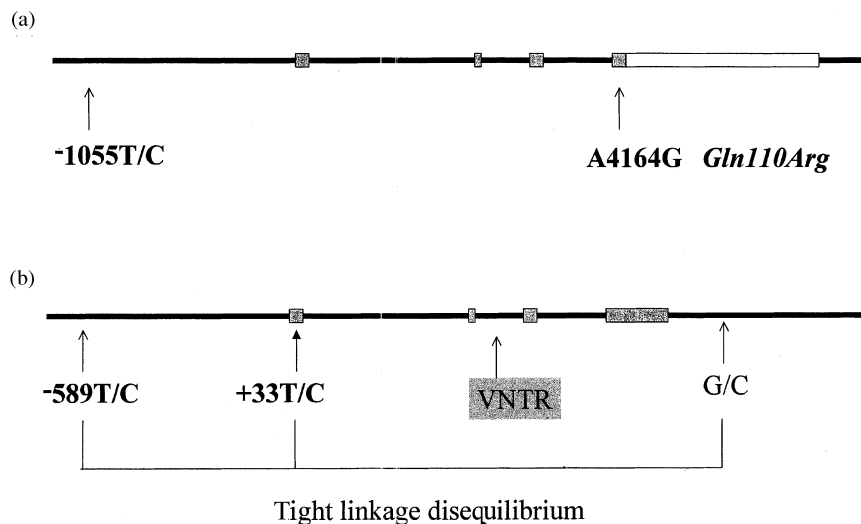
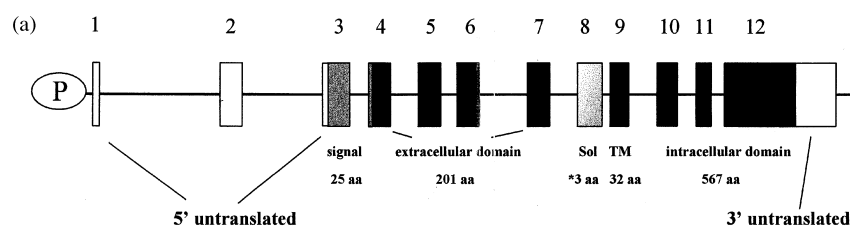


Fig. 1. Genomic structure of the human (a) IL-13 and (b) IL-4 genes with critical SNPs (single nucleotide polymorphism). A G/C variant in the 3'UTR is also in tight linkage disequilibrium with other two SNPs (Roberts and Mao *et al.* unpublished results).

insulin receptor substrate (IRS)-1 and (IRS)-2 of IL-4/IL-13R signalling pathways as well as the IL-13R α 1 gene itself [16]. To varying degrees, genetic variants in downstream receptors and signalling molecules are likely to affect susceptibility or severity and chronicity of allergic disorders. The IL-4R α gene (*IL4R*) is located on chromosome 16p21 and eight polymorphisms have been identified in the coding part of IL-4R α gene leading to amino acid changes in the gene products [2]. We found an extracellular variant, Ile50Val, that has been shown in relation to atopic asthma in a Japanese population [17]. Functional assays, based on transfection of cDNA into both human and murin B-cell lines, suggested that Ile50 upregulates receptor response to IL-4 leading to enhanced stat6 activation, increased TH2 cell secretion and IgE synthesis. Although

Ile50Val associated with atopic asthma in a Japanese population, we can not replicate this result across the different ethnic populations and explain the structural changes by the computer remodelling. The extracellular polymorphism may alter the ligand-binding affinity of IL-4R α [18] or through unknown splice variant mechanism. Arg551Gln, a polymorphism in the intracellular domain located in the stat6 binding region, enhances signalling by IL-4 and strongly associated with severe atopy through impairment of inhibitory molecule, SHP-1 to the receptor [19]. However, no excess of Arg551 was found in a large cohort of hyper IgE syndrome [20], and impairment of SHP-1 was not replicated [21]. The Pro478Ser, another intracellular polymorphism located in the IL-4R motif was in tight linkage disequilibrium with Arg551Gln in a



Human IL-4R α gene



Human IL-13R α 1 cDNA

Fig. 2. (a) Genomic structure of the human IL-4R α gene and (b) a cDNA of the human IL-13R α 1 gene. The arrow shows a silent SNP in IL-13R α 1. Note that Ile50Val and Arg551Gln are in exon 5 or exon 12, respectively.

German population, and changing the structure of the receptor, leads to altered phosphorylation patterns of signal molecules, and hence low IgE levels [22]. The recent work by Ober and colleagues [23] is particularly interesting. They studied asthma and atopy in the Hutterite population and in out-bred white, black and hispanic populations. Seven amino acid substitution variants in the coding region of the mature protein were investigated in patient and control groups, and analysed, both individually and in different combinations. All the groups showed significant associations of *IL4R* locus with both atopy and asthma.

The genomic location for the coding region of both IL-13R α 1 and IL-13R α 2 are on the X-chromosome in the region of human Xq13 which may be related to some of the maternal influence seen in the British population with linkage and association in atopy and asthma [11]. In contrast with IL-4R α , little is known about IL-13R [24]. We identified a novel single nucleotide polymorphism (SNP), A1398G, in the coding region of the IL-13R α 1 gene to be significantly associated with high serum IgE levels. The association of this variant with IgE levels was significantly greater in males than that in females in the British population [11]. As this is a silent variant, it seems possible that there is another variant, in linkage disequilibrium, of IL-13R α 1 which functionally impacts on allergic responses. It would be of great importance to clarify this point.

It is worth noting that stat6 plays an important role in the signal transduction of IL-4/IL-13. It has been shown to be prominent in the bronchial epithelium and increased in severe asthma. When stat6 or both IL-4 and IL-13 are deleted in mice, antigen induced BHR and goblet cell metaplasia are ablated along with the expected inhibition of TH2 function and IgE synthesis. The polymorphism in the IL-4R α has been shown to be associated with the activation of stat6 [18]. More recently, a genetic association has been found between the variant in a 3'-UTR of the *STAT6* and asthma with modest atopy phenotype in a Japanese population [25]. It would be of importance to search for another variant which influences activity or expression level of stat6.

Conclusion

Recent studies, including Suzuki *et al.* in this issue [8], point to the involvement of common variants of IL-4 and IL-13 signalling predisposition to asthma and allergy. IL-4 and the closely related cytokine IL-13 signal through a shared surface receptors IL-4R α and IL-13R α 1 that activate a specific transcription factor, STAT6. Moreover, an multivariate analysis shows that the association are independent among the variants of IL-4, IL-13, IL-4R α and IL-13R α 1 [11]. This implies that genetic aetiology of

allergic diseases is far more complex than we expected. These studies open up a new area not only for investigating the molecular genetic events but also for expanding our knowledge of the mechanism underlying the IL-4/IL-13 signalling pathway. More thorough understanding of the signalling events will offer an attractive target for future therapeutic intervention and preventive strategies. However, a great deal of work remains to be done.

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