

## Original Paper

# A Comprehensive Analysis of Interleukin-4 Receptor Polymorphisms and Their Association with Atopy and IgE Regulation in Childhood

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## Key Words

Atopy · Childhood · Immunoglobulin E · Interleukin-4 receptor · Polymorphism · Single nucleotide polymorphism

## Abstract

**Background:** The interleukin (IL) 4/IL13 pathway is involved in the regulation of IgE production associated with atopic diseases. Numerous polymorphisms have been identified in the coding region of the IL4 receptor  $\alpha$  chain (IL4Ra) and previous association studies have shown conflicting results. Based on their putative functional role, polymorphisms A148G, T1432C and A1652G, located in the coding region of IL4Ra, were selected for association and haplotype studies in a large German population sample ( $n = 1,120$ ). **Methods:** Genotyping was performed using allele-specific PCR and restriction-enzyme-based assays. Haplotypes were estimated, and population-derived IgE percentiles (50% IgE >60 IU/ml, 66% IgE >115 IU/ml and 90% IgE >457 IU/ml) were calculated as outcome variables in a haplotype trend regression analysis. **Results:** In our population, only polymorphism T1432C showed a trend for a protective effect against atopic rhinitis (odds ratio, OR: 0.52, 95% confidence interval, CI: 0.26–1.02,  $p = 0.05$ ). When haplotypes were calculated, one haplotype was significantly associ-

ated with elevated serum IgE levels at the 50th percentile (OR 1.60, 95% CI 1.08–2.37,  $p = 0.02$ ). **Conclusions:** These data indicate that IL4Ra polymorphisms, although suggested to be functionally relevant by in vitro studies, have only a minor influence on IgE regulation in our large population sample.

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## Introduction

The switching of immunoglobulin production from IgM to IgE in activated B lymphocytes is common to all atopic diseases. Two of the most important cytokines involved in IgE switching and the persistence of the allergic reaction are the pro-inflammatory molecules interleukin (IL) 4 and IL13. Both IL4 and IL13 affect the expression of IgE by signaling through a common pathway. Binding of IL4 and IL13 is facilitated through a heterodimeric surface receptor consisting of the IL4 receptor  $\alpha$  chain (IL4Ra) and either the common  $\gamma$  chain (for IL4 and IL13) or the  $\alpha$  chain of the IL13 receptor (for IL13 only) [1–3]. On interaction with the receptor, intracellular signaling is initiated through the phosphorylation of signal transducer and activator of transcription 6 (STAT6) molecules which in turn penetrate the nucleus activating the transcription of target genes [4]. Located on chromo-

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some 16q12, IL4Ra has been associated initially with the hyper-IgE syndrome [5, 6], IgE regulation and the development of atopic diseases [7]. At least 14 polymorphisms have been described in the coding region of the gene, 10 of which lead to changes in the putative amino acid sequence [8]. However, association studies on the effect of single IL4Ra polymorphisms on IgE regulation and the development of atopic diseases have led to conflicting results [9–13].

Thus, we genotyped a large German population sample ( $n = 1,120$ ) for three common single nucleotide polymorphisms (SNPs) located in the extracellular domain and two in the intracellular domain of the  $\alpha$ -chain of the receptor, spanning the whole IL4Ra gene. Haplotype analysis was performed to investigate interactions between polymorphisms within the IL4Ra gene.

## Methods

The population used in this study has been described in detail previously [14–16]. Therefore, only methods pertaining to this analysis are given below. Informed written consent was obtained from all parents, and all study methods were approved by the ethics committees of the Bavarian Medical Council and the University of Leipzig.

### Subjects

In this study, only children of German origin, 9–11 years of age, from Munich (southern Germany) and Leipzig (eastern Germany) who had both DNA and IgE data available were included in the analysis ( $n = 1,120$ , Munich  $n = 528$ , Leipzig  $n = 592$ ). No significant differences between the study populations from both cities were found with respect to population characteristics [15]. The schools sent parental self-completion questionnaires on to the families. Children underwent skin prick testing, blood was collected for serum IgE measurements, and DNA extraction was performed from leukocytes. No selection bias was detected between those included and those who were not included in the study (data not shown).

### Questionnaire

Children whose parents reported a doctor's diagnosis of either asthma, recurrent spastic or recurrent asthmatic bronchitis were classified as having asthma. Children were classified as having allergic rhinitis or atopic dermatitis when parents reported a doctor's diagnosis of hay fever or atopic eczema, respectively.

### Skin Prick Test

In Munich and Leipzig, the sensitivity to six common aeroallergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria tenuis*, cat dander and mixed grass and tree pollens) was assessed using highly standardized extracts and lancets (ALK, Hørsholm, Denmark). A child was considered atopic if a wheal reaction of more than 3 mm occurred to at least one specific allergen after subtraction of the negative control.

### Total Serum IgE Measurements

Total serum IgE levels were measured using the Insulite system (DPC Biermann, Bad Nauheim, Germany). Based on the cross-sectional survey of children aged 9–11 years (International Study of Childhood Asthma and Allergy;  $n = 3,063$ ), IgE percentiles were calculated. For haplotype analysis, the 50th (60.4 U/ml), 66th (115 U/ml) and 90th percentiles (457 U/ml) for total serum IgE were used as outcome variables. Percentiles were chosen for our haplotype analysis as they may better reflect the distribution of IgE levels in the population. As no differences in IgE levels were observed between subjects included/not included in the study or between populations from Leipzig and Munich (data not shown), percentiles may be assumed to be representative for a general German population aged 9–11 years where 50th, 66th and 90th percentiles correlate with modestly, moderately and highly elevated serum IgE levels.

### Molecular Genetic Methods

Genomic DNA was extracted from whole blood by a standard salting out method [17]. All PCR reactions were carried out in a volume of 15  $\mu$ l containing 24 ng of genomic DNA using standard thermocyclers (Eppendorf, Cologne, Germany). For initial genotyping, allele-specific PCR and standard sequencing were used to genotype the study population for three specific polymorphisms in the IL4 receptor gene. Specific primer and assay conditions used for genotyping are given in table 1. The reproducibility of genotyping results was tested by restriction-endonuclease-based assays in at least 10% of random repeats. Hardy-Weinberg equations were calculated for all genotypes.

### Statistical Analysis

Standard analysis of variance (ANOVA) for quantitative traits and  $\chi^2$  tests for qualitative traits were used to compare means and proportions between groups. Total serum IgE levels were summarized descriptively with geometric means, and linear trends were tested in ANOVA models of log-transformed values. The haplotype trend regression model, where the estimated probabilities are modeled in a logistic regression for their association with a specific trait [18, 19], was used to calculate haplotype frequencies not directly observable in unrelated individuals. Two additional haplotyping methods were applied to confirm results, where the probabilities of each haplotype were dichotomized into binary variables using two predefined thresholds ( $Pr > 0.80$  and  $> 0.0$ ), followed by a  $\chi^2$  test for association [20]. Only results consistent over all three haplotyping methods were reported. All haplotype analyses were carried out using SAS/Genetics, version 8.2 [19].

## Results

The study population ( $n = 1,120$ ) was genotyped for the three putatively functional polymorphisms A148G, T1432C and A1652G in the IL4Ra gene. No selection bias was detected between those included and not included in the study (data not shown). Genotyping was successful in at least 95% of all samples: 1,092 samples for A148G, 1,074 for T1432C and 1,081 for A1652G. Allele frequencies were 0.57 for A at position 148, 0.87 for T at

**Table 1.** Primers and assay conditions

SNP	Forward (5') primer Reverse (3') primer	Product bp	Genotyping assay
IL4Ra A148G	ACTCCGCCCTGCTCACC TAGGCAGGTGTGAGGAG <u>T</u> ATC	409	<i>BciIV</i>
IL4Ra A148G	GCC TCC GTT GTT CTC AGG TA TGT GAG GAG CAT CCT TGG T <u>A</u> C	267	<i>RsaI</i>
IL4Ra A148G	GCC TCC GTT GTT CTC AGG TAT GCC TCC GTT GTT CTC AGG TAC ATC CTT GGT GCA TGT GGT AAG GCT TAT ACC CCT CTT CCC CAC ACG CCC CTC GTC ATC GCA G	257 439	asPCR
IL4Ra T1432C	GAC ACG GTG ACT GGC TCG AGG GCA TCG CCT GAG AGC AGC AG	373	<i>BseRI</i>
IL4Ra T1432C	GCT CTC TGG GAC ACG GTG ACT <u>G</u> GC TCT CGG ATG GGG GAG TCA TGC CTT CTT CCA <u>C</u> CC TCG G	259	<i>AvaI</i>
IL4Ra A1652G	GCT TAT ACC CCT CTT CCC CAC ACG CCC CTC GTC ATC GCA G CAC CGC ATG TAC AAA CTC CC CCC CCA CCA GTG GCT ATC A	439 288 189	asPCR asPCR asPCR
IL4Ra A1652G	CAA AGG TGA ACA AGG GGA CAG GGA CT <u>C</u> G GTC TCG GCC CCC ACC AGT GGC T <u>T</u> C	265	<i>AvaI</i>

asPCR = Allele-specific PCR. Underlined bases represent mismatches introduced for restriction-enzyme-based digestions.

**Table 2.** IL4Ra haplotypes based on the polymorphisms A148G, A1652G and T1432C and haplotype frequencies (haplotypes with frequencies < 3% were not considered)

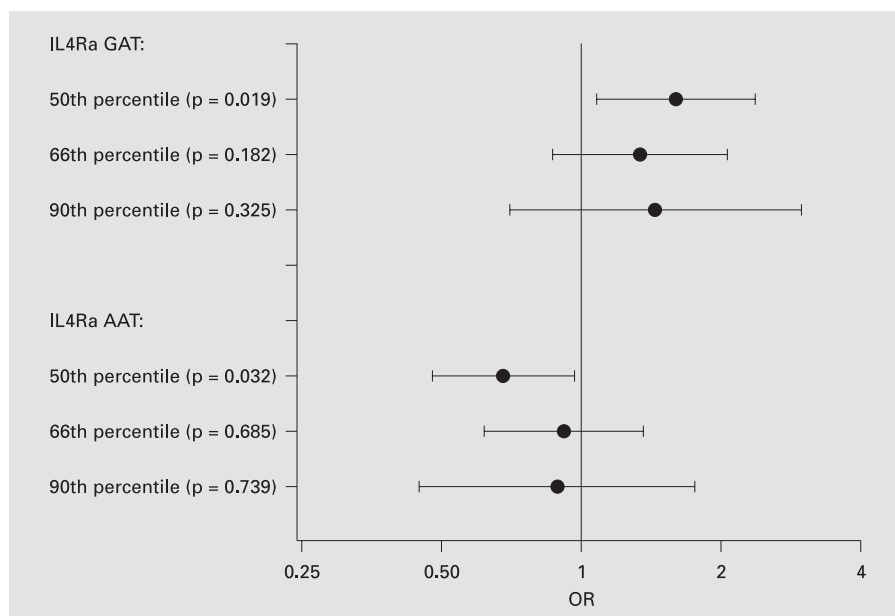
No.	Haplotypes	Frequencies
1	A-A-T	0.47
2	A-G-C	0.05
3	A-G-T	0.05
4	G-A-T	0.31
5	G-G-C	0.07
6	G-G-T	0.04

position 1432 and 0.80 for A at position 1652, respectively. No significant deviation from the Hardy-Weinberg equilibrium was observed with any polymorphism. Using a correlation coefficient ( $\Delta$ ), the linkage disequilibrium between the three SNPs was assessed to be low to moderate between all three SNPs ( $\Delta = 0.11$  for A148G and both other polymorphisms and  $\Delta = 0.66$  for T1432C vs.

A1652G. Allele frequencies for the genotyped IL4Ra polymorphisms were similar to previously reported frequencies in Europeans, but differed from those reported in non-Caucasian populations [8, 20–23].

To assess the role of IL4 receptor polymorphisms in the development of asthma and atopy, association studies were performed, and the main atopic phenotypes of asthma, atopic rhinitis, atopic dermatitis, atopy by skin prick test and serum IgE were assessed. Only polymorphism T1432C showed a trend for a protective effect against the development of atopic rhinitis (T1432C: 0.52, 95% confidence interval, CI: 0.26–1.02,  $p = 0.05$ ). Furthermore, for A148G a borderline significant trend towards elevated serum IgE levels was observed: geometric means for serum IgE (IU/ml) were 47.1 in individuals with the wild-type allele versus 55.5 in carriers of the G allele ( $p = 0.08$ ), respectively.

Next we performed a haplotype analysis comprising the three polymorphisms A148G, A1652G and T1432C. When haplotypes were estimated using the ER method in the 1,043 samples for which complete genotyping information was available, only six of eight possible haplotypes



**Fig. 1.** ORs and 95% CIs for IgE percentiles (50th, 66th and 90th percentiles) by IL4Ra haplotypes GAT and AAT (calculated using the haplotype trend regression model).

exceeded a frequency of 3% (table 2). For the haplotype analysis, percentiles for serum IgE levels based on our cross-sectional study in a German population were calculated instead of the widely used arbitrary cutoff point for elevated serum IgE levels of 100 IU/ml. As shown in figure 1, we observed a risk haplotype G-A-T with odds ratios of 1.60 (95% CI 1.08–2.37,  $p = 0.02$ ), for serum IgE levels above the 50th percentile, while the risk for elevated IgE at the 66th and 90th percentiles was not increased significantly. In contrast, the haplotypic combination A-A-T showed a protective effect against the development of elevated IgE levels with odds ratios of 0.68 (95% CI 0.48–0.97,  $p = 0.03$ ) for serum IgE above the 50th percentile. No associations between haplotypes and asthma, atopic diseases or atopy were observed (data not shown). All other haplotype combinations were too infrequent to perform adequate haplotype analysis (data not shown).

## Discussion

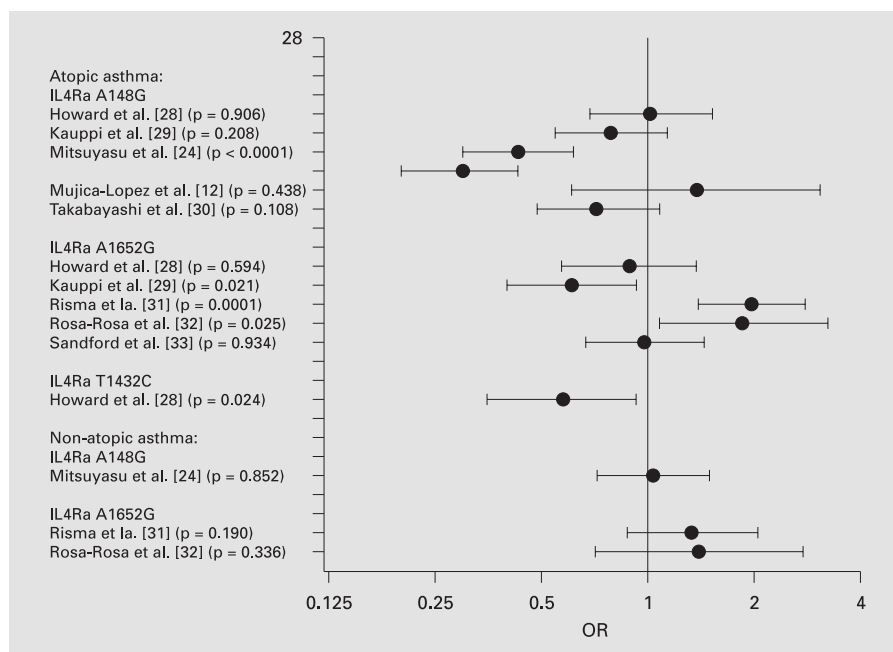
The three polymorphisms tested in our population sample lead to putative amino acid changes. A148G leads to an amino acid change at position 50 of the extracellular domain of the receptor chain (Ile50Val). In vitro studies have shown increased IL4-dependent signaling and elevated IgE production in the presence of the A (Ile) allele [24]. In our study however, these findings were not con-

firmed in our large study cohort. In contrast, carriers of the A allele showed a trend towards lower levels of serum IgE. Results from Japanese [24, 25] and European studies [23] were also conflicting. In summary, these results indicate that A148G may have only minor effects (if any) on the regulation of serum IgE levels or the development of atopic phenotypes.

Polymorphisms T1432C and A1652G lead to amino acid changes in Ser478Pro and Gln551Arg, respectively, in the intracellular domain of the IL4Ra chain. Thus, both polymorphisms have been suspected to influence the intracellular activation of the signaling cascade. As polymorphism A1652G is located one base pair from a STAT6-recruiting domain in IL4Ra, a functional relevance seemed possible. While Kruse et al. [1] showed an effect of the polymorphism on phosphorylation and signalling transduction, no such effect was observed in another functional study [26]. In association studies, conflicting results for both polymorphisms in different populations and ethnicities exist. While an association was initially observed in an inbred population of German ancestors (Hutterites) [20] and a study cohort from southern Germany [21], no such association was observed in our population sample or the German Multicenter Atopy Study [23].

To assess the effects of haplotypes in our cross-sectional study of unrelated subjects, haplotypes were estimated according to standard methods using the expectation maximization algorithm. Using this procedure, only weak

**Fig. 2.** Comparative analysis of respective IL4Ra polymorphism effects on asthma. Respective allele frequencies were taken from previous reports, and ORs were calculated and evaluated graphically. Mitsuyasu et al. [24] studied asthma in adults and children.



but significant associations between the regulation of serum IgE levels and IL4Ra haplotypes were detected. While the risk haplotype G-A-T conferred a risk for modestly elevated serum IgE levels (at the 50th percentile), the A-A-T haplotype was protective at the same level. As shown in figure 1, the same trends for associations were observed for the 66th and 90th percentiles. However, as fewer observations contribute to these associations, the statistical significance decreased.

Only three out of 14 known polymorphisms in the IL4Ra-coding region were genotyped in our population and included in the haplotype analysis. However, it has been shown previously that other polymorphisms in the IL4Ra gene and the IL4R promoter were either very infrequent or in strong linkage disequilibrium with the polymorphisms genotyped here [20, 22, 27]. Thus, it seems unlikely that further, non-genotyped polymorphisms in the IL4Ra gene may confer significant effects on IgE regulation or the development of atopic diseases.

Even though a large body of literature exists on the putative effects of genetic variations in the IL4Ra gene on the development of atopic diseases and the regulation of serum IgE levels, it was not possible to perform a meta-analysis of the current literature. This was due to a lack of standardization between reports in terms of the phenotypes assessed, statistical methods and the presentation of the results of genotyping. In figure 2, results from association studies accessible for a comparative analysis of the effects of poly-

morphisms A148G, T1432C and A1652G on asthma are summarized descriptively. As shown in this comparison, results vary greatly between different studies and populations. In our cross-sectional study, no significant association between IL4Ra polymorphisms and asthma was observed (fig. 2). However, small genetic effects requiring an even larger study population may have been missed.

In conclusion, our data suggest that IL4Ra polymorphisms exert only a minor effect on the regulation of serum IgE and do not contribute significantly to the development of atopic diseases in our large study cohort of German children. However, it cannot be excluded that IL4Ra polymorphisms interact with polymorphisms in other genes of the IL4/IL13 pathway or certain environmental factors that may have modifying effects on IgE regulation and the development of atopic diseases. While some aspects of these possible interactions, e.g. between IL13 and IL4Ra polymorphisms, have already been addressed [23, 28], further studies on the complete pathway including IL4, IL13, IL4Ra and the intracellular signal transducer STAT6 may be necessary to elucidate the complex genetic regulation of serum IgE levels.

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