Association study of the *IL13* variant Arg110Gln in atopic diseases and juvenile idiopathic arthritis

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Background: It has previously been shown that various inflammatory diseases, such as diabetes mellitus, bronchial asthma, chronic inflammatory bowel diseases, and rheumatoid arthritis, are in some circumstances genetically linked to the same chromosomal regions. Consequently, common genes underlying the pathogenetics of these diseases have been proposed. Chronic inflammatory disorders can be subdivided by their predominant immune response, either T_H1 or T_H2. For example, juvenile idiopathic arthritis (JIA) is a T_H1 disease, and bronchial asthma is a T_H2 disease.

Objectives: The present study investigated the polymorphism Arg110Gln within the *IL13* gene, a strong T_H2 cytokine. We attempted to determine whether it is associated with these 2 diseases and whether this would reflect the T_H1/T_H2 paradigm.

Methods: Arg110Gln was typed in 4 different populations: asthmatic children, atopic children, children with JIA, and a control population. Statistical analysis was performed by using logistic and linear regression analysis of serum IgE levels and the Armitage trend test.

Results: The variant Gln110 was shown to be associated with increased total serum IgE levels in our atopic population (P = .006) and was weakly associated with bronchial asthma (P = .04). There was no association of the variant with JIA when compared with the control population. However, the variant Gln110 was significantly less frequent in children with JIA compared with its presence in children with bronchial asthma (P = .007).

Conclusion: This is the first study to compare the same gene variant in T_H1 and T_H2 chronic inflammatory diseases. The results suggest that the same gene variant might protect from one disease and make an individual susceptible to the other. (J Allergy Clin Immunol 2003;112:735-9.)

Key words: *Bronchial asthma, atopy, juvenile idiopathic arthritis, IL-13, association, genetic*

Abbreviations used
JIA: Juvenile idiopathic arthritis
RA: Rheumatoid arthritis
SPT: Skin prick test

During the last decade, much effort has been spent attempting to genetically dissect complex diseases, with linkage often shown to different chromosomal regions or association made to various candidate genes. Recently, the first complex disease genes found by means of positional cloning have been published.[1,2] Despite these successes, the global picture of investigations into complex diseases remains composite, and today the genetics of these diseases seem to be even more complicated than initially thought.

Some groups of complex genetic diseases might have a similar pathophysiology. Chronic inflammatory diseases, such as diabetes mellitus, chronic inflammatory bowel disease, rheumatoid arthritis (RA), systemic lupus erythematosus, and bronchial asthma, have been shown to possess some common genetic linkages.[3,4] This has led to the hypothesis that there might be common underlying genes for different inflammatory diseases.[4]

Chronic inflammatory disorders can be classified on the basis of their predominant immune response. Allergic diseases, such as bronchial asthma, atopy, and eczema, are typical T_H2 diseases and are characterized by a strong deviation toward the T_H2 immune response with an increase of typical T_H2 cytokines, such as IL-4 and IL-13. In contrast, juvenile idiopathic arthritis (JIA) is a T_H1-driven chronic inflammatory disease, and patients show a bias toward T_H1 cytokines, such as IFN-γ, in their affected joints.[5,6] The heritability of both diseases has been estimated to be approximately 50% to 60% on the basis of twin studies and segregation analyses.[7,8]

Recent epidemiologic studies have demonstrated that atopic diseases are significantly decreased in patients with RA, a T_H1-driven disease.[9-11] It has been speculated that RA might confer some protection from atopy because of the cytokine deviation toward T_H1 and thus away from T_H2.

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IL-13 is an important T_H2 cytokine. It is increased in the sera of individuals with atopic diseases and is also increased in the bronchoalveolar lavage fluid of asthmatic patients. The overexpression of IL-13 in airways leads to an increase in mucus production, bronchial hyperresponsiveness, and goblet cell hyperplasia.[12,13] Additionally, it has been suggested that IL-13 might inhibit the development of arthritis in animal models, and increased production of IL-13 significantly correlates with a reduction in pro-inflammatory cytokines. Consequently, the use of recombinant IL-13 as a medication for RA has been proposed.[14,15] Thus, *IL13* represents an ideal candidate gene for both diseases.

The role of *IL13* in the genetics of allergic diseases has already been widely investigated. Specifically, a single polymorphism within *IL13*, Arg110Gln, has been shown to be associated with bronchial asthma, atopic dermatitis, and increased IgE levels.[16-18] In addition, the variant is associated with heightened serum levels of IL-13.[19]

The aim of the present study was to test the association of the variant Arg110Gln in *IL13* with different inflammatory diseases, such as atopy, bronchial asthma, and, for the first time, JIA. Specifically, we investigated whether there was a difference in the allelic frequency of Arg110Gln in T_H1 and T_H2 diseases.

METHODS

Subjects

Three hundred twenty-one children (aged 5-18 years) with suspected asthma were recruited from the southwestern part of Germany between July 2000 and January 2003. The probands were characterized at the University Children's Hospital, Freiburg, Germany, by using a standardized clinical protocol. Participants were asked in advance to discontinue any asthma or allergy medication before the clinical testing. An extended medical history was recorded, including occurrence and duration of wheezing symptoms, previous and acute medications, severity of previous asthma attacks, previous allergic rhinitis or conjunctivitis, atopic dermatitis, and any family history of allergic diseases.

Skin prick tests

Skin prick tests (SPTs) to 17 common allergens and positive (histamine) and negative controls were performed. The following allergens were tested: house dust mites, different grass and tree pollens, *Aspergillus fumigatus, Alternaria alternata, Cladosporium herbarum,* and dog, cat, rabbit, duck, and horse dander. The wheal response diameters were recorded after 15 minutes. The SPT response was regarded as positive if the wheal produced by the test allergen was at least half the size of the wheal produced by the positive control.

Specific and total IgE

Specific IgE was detected by means of ELISA against 2 mixtures of grass pollens, mite allergens (*Dermatophagoides pteronyssinus and Dermatophagoides farinae,* respectively), cat and dog dander, and hazel and birch pollens (Magic Lite; Chiron Diagnostics, Fernwald, Germany). The cutoff point for a positive test result was 1.43 Magic Lite units.[20] Measurement of total serum IgE was carried out by using an enzyme allergosorbent test (Phadezym; Pharmacia, Uppsala, Sweden).

Pulmonary function tests

Pulmonary function tests were performed by using standard protocols. In addition, exercise-induced asthma was diagnosed by subjecting the probands to physical exercise for 6 minutes under standardized conditions. The first spirometry and peak flow measurement was performed after 2 to 3 minutes, and the second was performed after 5 to 6 minutes. After 10 more minutes, the children inhaled salbutamol, and a third spirometry and peak flow measurement was taken.

Inhalations with increasing doubling concentrations of histamine (from 0.125-8 mg/dL) were performed to test for bronchial hyperresponsiveness. Testing was stopped after a 15% decrease in FEV_1.

Asthma definition

Two hundred twenty-eight of the 321 recruited children were given a diagnosis of bronchial asthma. The diagnosis was based on a clear-cut history of asthmatic symptoms, the use of antiasthmatic medication, and at least some degree of bronchial hyperreactivity. The antiasthmatic drugs included typical betamimetika, such as salbutamol, and standard corticosteroids used in asthma treatment, such as budenoside. Bronchial hyperreactivity was defined as a decrease in FEV_1 of at least 15% in histamine testing or exercise provocation.

No child of the asthmatic population had JIA.

Control population

Two hundred seventy randomly chosen probands (aged 19-40 years) were used as control subjects. They originated from the same area in the southwestern part of Germany. No medical history was taken, and no medical testing was performed on control subjects.

Atopic population

The atopic population consisted of 267 individuals aged 6 to 22 years (mean, 15 years; 50% male and 50% female patients; 188 individuals with any specific sensitization and 79 control subjects; 168 patients with increased total serum IgE levels and 99 control subjects). These individuals are all second-generation members recruited from 2 different groups of nuclear families. Population 1 was recruited through a population-based study with 463 randomly contacted families from the southwestern part of Germany.[21,22] Sixty-four families with at least one member sensitized to common inhalant allergens participated. Population 2 consisted of 45 families recruited through our outpatient department. In both populations a medical history was recorded, SPTs were performed, and blood was drawn. In total, 75% of the individuals were atopic according to the definition stated below. Sixty-three percent had total IgE concentrations of greater than 100 kU/L and 70% specific sensitization to common inhalant allergens. Atopy was defined as any specific sensitization, an increased total serum IgE level, or both.

JIA population

Juvenile RA is a family of diseases with at least 7 definable forms. The forms can be differentiated by the pattern of onset, the number of joints involved, serologic findings, and extraarticular manifestations. The forms of JIA have been recently classified by the International League of Associations for Rheumatology.[23] The present study is focused on patients fulfilling the clinical criteria of chronic arthritis, which refers to arthritis of at least 6 weeks' duration and of unknown cause occurring in children less than 16 years old. In addition, all patients included in the study had positive test results for antinuclear antibodies in the serum (ie, titer >1:80). Eighty-six children fulfilling these criteria were included in this study. One child of this group had asthma but no increased IgE levels.

Genotyping

The polymorphism Arg110Gln was typed by means of RFLP with the primer pair 5′ TGG CGT TCT ACT CAC GTG CT 3′ and 5′ TTT CGA AGT TTC AGT GGA AC 3′ (annealing temperature of 55°C). After PCR, the product was digested with 2 units of *Nla*IV

TABLE I. Association analyses of Arg110Gln with different atopic phenotypes

	IgE >100 kU/L			Atopy		
Polymorphism	**Odds ratio**	**95% CI**	***P* value**	**Odds ratio**	**95% CI**	***P* value**
Arg110Gln	1.64	0.97-2.78	.06	1.06	0.57-1.96	.86
	Specific sensitization			**Log (IgE)**		
Polymorphism	**Odds ratio**	**95% CI**	***P* value**	**Parameter estimate**	**95% CI**	***P* value**
Arg110Gln	1.03	0.57-1.85	.93	0.222	0.063-0.381	.006

The estimate of 0.222 corresponds to the factor 1.67; that is, the IgE level of children bearing the mutation is, on average, 167% of the IgE level of children without the mutation.

(New England Biolabs) for 4 hours at 37°C, and the fragments were resolved on a 4% (wt/vol) agarose gel.

The genotyping was performed in a blinded manner by 2 investigators who were unaware of the phenotype.

Statistical analysis

Statistical analysis of the asthmatic and JIA population was performed with the Armitage trend test calculated by using the De Finetti program. The Armitage trend test is a statistical method that does not assume Hardy-Weinberg equilibrium to test for association between a single-nucleotide polymorphism and the disease of interest. Because deviation from Hardy-Weinberg equilibrium has been shown to inflate the chance of a false-positive association,[24] the Armitage trend test should be more useful than the usual χ^2 test.

The phenotypes IgE greater than 100 kU/mL, any specific sensitization, and atopy were considered as binary traits, and total serum IgE was considered as a continuous quantitative parameter on a logarithmic scale. Genotypes of *IL13* Arg110Gln were simply biallelic. Logistic and linear regression analyses were made following the method of Zeger and Liang,[25] which accounts for the correlation between siblings.[24] Therefore, generalized estimating equations were used (PROC LOGIST Statistical Analysis System; SAS Institute Inc, Cary, NC).[26]

Approval

The collection of serum and the subsequent DNA material and the experimental procedures were approved by the Ethical Commission of the University of Freiburg. A statement of informed consent was signed by all participants or signed by their parents in the case of children.

RESULTS

The polymorphism Arg110Gln within the *IL13* gene was genotyped in 4 different populations: asthmatic children, atopic children, children with JIA, and a control population.

In the atopic population the variant Gln110 was associated with increased total serum IgE levels (P = .006, see Table I).

The allelic frequency of Gln110 in the different populations are given in Table II, and the association results are given in Table III. The variant was weakly associated with bronchial asthma (F = 26.5% in asthmatic children and F = 21% in control subjects, P = .04). There was no association with JIA compared with control subjects (F = 16.5% in JIA, P = .19). However, the variant was significantly less frequent in patients with JIA compared with in patients with bronchial asthma (P = .007).

TABLE II. Allelic frequency of Arg110Gln in the different populations

Population	**Frequency of 110Gln (%)**
Patients with bronchial asthma	26.5
Patients with atopy	22.0
Control subjects	21.0
Patients with JIA	16.5

TABLE III. Pairwise association analyses by means of the Armitage trend test

	Control subjects	**Patients with JIA**
Patients with bronchial asthma	P = .042	P = .007
Patients with JIA	P = .194	–

The variant was in Hardy-Weinberg equilibrium in all populations, as calculated by the De Finetti program (data not shown).

DISCUSSION

The relationship between the occurrence of T_H2-mediated allergic diseases and T_H1-mediated autoimmune conditions, such as JIA, diabetes mellitus, or chronic inflammatory bowel diseases, is controversial and has been discussed in various epidemiologic studies. Recently, one study showed an increased risk of autoimmune disorders in persons with allergic diseases.[27] However, 2 other studies have shown the coexistence of T_H2 and T_H1 diseases, with common environmental factors being claimed as an explanation for the increase in the incidence of these disorders.[28,29] Benn et al[30] showed that these studies have some drawbacks, and most studies still support an inverse association between RA and bronchial asthma within the same individual at the same time.[9-11] Furthermore, an inverse association has also been shown for the T_H1 disorders multiple sclerosis[31] and diabetes mellitus[32] in relation to bronchial asthma.

To our knowledge, the current study is the first to concurrently perform association analyses of the same gene variant in populations of different chronic inflammatory

diseases. We have chosen bronchial asthma and atopy as typical T_H2-mediated diseases and JIA as a T_H1-mediated inflammatory condition. JIA is the most common rheumatic condition in children. Estimates of the incidence of JIA vary widely, but it is probably between 5 and 10 per 100,000 children (<16 years).[33,34] This low incidence means the population of children with JIA studied here is quite small, and this might explain the absence of an association between JIA with Arg110Gln when compared with control subjects. A larger study population will be needed to address this question.

However, the difference of the allelic frequency of Arg110Gln between children with asthma and JIA is highly significant (P = .007); the variant is much more common in asthmatic patients. It has previously been shown that Gln110 is associated with an increased serum level of IL-13, and computer modeling has suggested an enhanced binding of this variant to its receptor complex.[17] The variant leads to a markedly increased signaling of IL-13, which in turn results in an enhanced T_H2 immune response. Individuals bearing this variant will therefore be at an increased risk of T_H2-related chronic inflammatory diseases, such as bronchial asthma or atopy, as measured on the basis of increased IgE levels. The association of Gln110 with atopic phenotypes, as shown in this study and several previous studies, strengthens this conclusion.

In addition, a shift toward T_H2 immune responses might protect against the development of T_H1-mediated diseases, such as JIA, and it has been shown in animal models that IL-13 is beneficial in arthritis. Therefore, we propose that persons with greater serum IL-13 levels because of the variant might be at a decreased risk of JIA. This might explain why children with JIA possess Gln110 less frequently.

The description of T_H1/T_H2 as being in balance is an oversimplification of the immune response, and at least a third type of T cells, the so-called T_H3 cells, exist.[35] T_H3 cells have strong immunosuppressive properties, and it has been suggested that their cytokines could counterregulate the T_H2 response. The role of T_H3 cells in the T_H1/T_H2 balance has to be clarified, and this will certainly develop our understanding of the pathophysiology of chronic inflammatory diseases.

In addition, one should bear in mind that association studies can only be a hint for a possible pathophysiologic role of a gene product, and further studies are needed to strengthen the results. Also, one cannot exclude that another polymorphism in a different gene in linkage disequilibrium to Arg110Gln is the real disease-causing polymorphism.

Nevertheless, the results of the current study might be a first hint that different inflammatory diseases are indeed influenced by the same gene variant, as has been previously proposed on the basis of common genetic linkage. The association of *IL13* Gln110 with bronchial asthma and inverse association with JIA might also account for the clinical observation that both diseases are rarely seen in the same child.

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