

Original article

Association study of polymorphisms within interleukin-18 in juvenile idiopathic arthritis and bronchial asthma

Background: Interleukin-18 (IL-18) plays an important role in the regulation of TH1 as well as TH2 immunologic responses and thus in the development of chronic inflammatory diseases. Positive association studies of polymorphisms in IL-18 with different diseases have underlined the involvement of IL-18 in the pathogenetic processes. Our interest was to test polymorphisms of IL-18 for association with a typical TH1-mediated disease – juvenile idiopathic arthritis – and the TH2-mediated disease bronchial asthma in Caucasian children.

Methods: We genotyped five polymorphisms that were in association with chronic inflammatory diseases (–607C, –137C, 113G, 127T, and –133G). This was performed by restriction fragment length polymorphism in populations of asthmatic children, control individuals, and children with antinuclear antibodies (ANA)-positive juvenile idiopathic arthritis. Statistical analysis was performed by the Armitage trend test; haplotypes were calculated by the Arlequin program.

Results: No significant association was found between any single nucleotide polymorphism or any haplotype and bronchial asthma or ANA-positive juvenile idiopathic arthritis.

Conclusion: We conclude that the effect of IL-18 in the immunologic context of diseases like bronchial asthma or juvenile arthritis might be too complex to be reflected in a simple one-way association study. Furthermore, the polymorphisms under investigation might be nonfunctional.

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Interleukin (IL)-18 is a pleiotropic cytokine, which is often constitutively expressed and potentially secreted by a variety of different cell types including keratinocytes, colon epithelial cells, synoviocytes, macrophages, and Kupffer cells. Since its description in 1995 as interferon-gamma-inducing factor (1), its role in the immunologic network has been investigated extensively. Interestingly, IL-18 can regulate TH1- and TH2-mediated immune responses, and thus is involved in the pathogenesis of TH1 and TH2 chronic inflammatory diseases.

Allergic asthma represents a typical TH2-mediated disease and the contribution of IL-18 to asthma has been studied in human beings and mice. In human beings, the level of serum IL-18 is elevated during acute asthmatic exacerbations (2) and secretion of IL-18 by peripheral blood mononuclear cells is increased in asthma and atopic dermatitis (3). Controversially, another study demonstrated that in bronchoalveolar lavage levels of IL-18 are reduced in asthma when compared with healthy controls as well as patients with sarcoidosis (4). Finally, reduced expression of IL-18 mRNA in atopic dermatitis has also been described (5).

Similar conflicting results have been obtained in mouse models of allergic asthma: gene transfer of IL-18 to mice prevents asthma and even reverses established

allergen-induced airway hyperreactivity, especially when co-administered with IL-12 (6, 7). Furthermore, IL-18 knockout mice show enhanced allergen-induced eosinophilia (8). Thus, IL-18 seems to play some protective role in the development of bronchial asthma. However, other studies have demonstrated that the sole administration of IL-18 to lungs of sensitized mice, enhances antigen-induced eosinophil recruitment (9), serum IgE, and TH2 cytokine levels (10).

One explanation for these seemingly conflicting results is that IL-18 stimulates TH1 or TH2 responses depending on its cytokine milieu (11, 12). When IL-18 is given alone, it exhibits pro-allergic functions like stimulating IL-4 and histamine release from basophils; however, when it is given in a TH1 milieu – that is together with IL-12 – it prevents the development of allergic reactions (7, 13).

An important role of IL-18 has also been found in rheumatoid arthritis – a typical TH1-mediated disease. IL-18 is elevated in serum and synovial tissue of patients with arthritis (14, 15). In addition, administration of IL-18 together with IL-12 is important in sustaining TH1 responses and monokine production (16). Expression of IL-18 is associated with that of IL-1 β and tumor necrosis factor alpha and with local inflammation in the synovial tissue of patients with rheumatoid arthritis. Furthermore,

synovial IL-18 expression correlates with the acute-phase response (17).

Polymorphisms within IL-18 have been found in association with different chronic inflammatory diseases like Crohn's disease (18), diabetes mellitus (19), and adult onset of Still's disease (20). Furthermore, their impact on the development of lung diseases like sarcoidosis (21) and asthma (22) have been described. In our atopic population from southwestern Germany, IL-18 polymorphisms have been shown to be in association with specific sensitization, allergic rhinitis, and high total IgE levels (23).

As IL-18 is a very important cytokine for both, TH1- and TH2-mediated immunologic response, we sought to investigate common polymorphisms within IL-18 in children with a TH1-mediated disease – juvenile idiopathic arthritis (JIA) – and with bronchial asthma as TH2-mediated disease.

Material and methods

Subjects

A total of 321 children with suspected asthma (aged 5–18 years) 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, 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 test

Skin prick tests 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*, dog, cat, rabbit, duck, and horse dander. The wheal response diameters were recorded after 15 min. The skin prick test was regarded as positive if the wheal of the test allergen was at least half the size of the wheal of the positive control.

Specific and total IgE

Specific IgE was detected by enzyme-linked immunosorbent assays against two mixtures of grass pollens, mite allergens (*Dermatophagoides pteronyssinus* and *D. farinae*), cat and dog dander, hazel, and birch pollens (Magic Lite; Chiron Diagnostics, Fernwald, Germany). The cut-off point for a positive test was 1.43 ML units (24). Measurement of total serum IgE was carried out by an enzyme allergosorbent test (Phadezym; Pharmacia, Uppsala, Sweden).

Pulmonary function tests

Pulmonary function tests were performed using standard protocols. In addition, exercise-induced asthma was diagnosed by subjecting the probands to physical exercise for 6 min under standardized

conditions. The first spirometry and peak flow measurement were performed after 2–3 min and the second after 5–6 min. After 10 more minutes the children inhaled with salbutamol and a third spirometry and peak flow measurement were taken.

To test for bronchial hyperresponsiveness inhalations with increasing, doubling concentrations of histamine were performed (from 0.125 to 8 mg/dl). Testing was stopped after a 15% fall in FEV_1 .

Asthma definition

Of the 321 recruited children, 228 were diagnosed as positive for bronchial asthma. The diagnosis was based on a clear-cut history of asthmatic symptoms, the use of anti-asthmatic medication and at least some degree of bronchial hyperreactivity. The anti-asthmatic drugs included typical betamimetika such as salbutamol and standard corticosteroids used in asthma treatment such as budesonide. Bronchial hyperresponsiveness was defined as a fall in FEV_1 by at least 15% in histamine testing or exercise provocation. No child of the asthmatic population has JIA.

Control population

The randomly chosen probands (270) used as controls (aged 19–40 years) originated from the same area in the southwestern part of Germany. No medical history was taken and no medical testing was performed on controls.

Juvenile idiopathic arthritis population

The forms of JIA have been recently classified by the International League of Associations for Rheumatology (25). The present study focuses on patients fulfilling the clinical criteria of chronic arthritis, which refers to arthritis of at least 6 weeks duration of unknown cause occurring in children less than 16 years old. In addition, all patients included in the study tested positive for antinuclear antibodies (ANA) in the serum, i.e. titer > 1 : 80. Eighty-six children fulfilling these criteria were included in this study. One child of this group had asthma, but no elevated IgE levels.

Genotyping

Genotyping was performed as described previously (23). Genotyping was performed by two investigators blinded to the phenotype.

Statistical analysis

Statistical analysis was performed using the Armitage trend test calculated by the De Finetti program. $P < 0.01$ was regarded as significant (due to multiple testing). The Armitage trend test is a statistical method that does not assume Hardy–Weinberg equilibrium (HWE) to test for association between a single nucleotide polymorphism and the disease of interest. As deviation from HWE has been shown to inflate the chance of false-positive association (26), the Armitage trend test should be more useful than the usual chi-square test. Haplotypes were calculated by means of the Arlequin program (27).

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 in the case of children signed by their parents.

Results

Genotyping

The five polymorphisms were genotyped in three different populations: asthmatic children, children with JIA, and a control population. The variants were in HWE in all populations as calculated by the De Finetti program (see Table 1).

Association studies

The genotype distribution and the allelic frequencies of the five polymorphisms are given in Table 2 and the association results in Table 3. None of the polymorphisms was associated with asthma or with ANA positive JIA. Furthermore, there was no significant difference in the allelic frequencies between the asthmatic and rheumatoid populations.

Table 1. Hardy–Weinberg equilibrium (HWE) as calculated by the De Finetti program

Polymorphism	HWE asthma	HWE controls	HWE JIA
–607C	0.38	0.68	0.62
–137C	0.68	0.17	0.75
113G	0.68	0.23	0.75
127T	0.68	0.23	0.75
–133G	0.94	0.29	0.68

JIA, juvenile idiopathic arthritis.

Values indicate probability.

Table 2. Frequencies and genotype distribution of the polymorphisms in the different populations

Polymorphism	Population	Homozygote		Homozygote MU	Frequency WT
		WT	Heterozygote		
–607C	Asthma	63	121	46	0.54
	Controls	90	134	45	0.58
	JIA	36	37	12	0.64
–137C	Asthma	117	93	21	0.71
	Controls	133	118	17	0.72
	JIA	48	31	6	0.75
113G	Asthma	117	93	21	0.71
	Controls	133	118	18	0.71
	JIA	48	31	6	0.75
127T	Asthma	115	92	21	0.71
	Controls	133	118	18	0.71
	JIA	43	31	6	0.75
–133G	Asthma	109	99	23	0.69
	Controls	120	125	24	0.68
	JIA	43	36	6	0.72

WT, wild type; MU, mutation; JIA, juvenile idiopathic arthritis.

Table 3. Association analysis by the Armitage trend test

Polymorphism	Controls – asthma	Controls – JIA	Asthma – JIA
–607C	0.13	0.18	0.02
–137C	0.76	0.42	0.34
113G	0.71	0.39	0.34
127T	0.79	0.39	0.32
–133G	0.79	0.32	0.44

JIA, juvenile idiopathic arthritis.

Values indicate probability, with $P < 0.01$ being significant.

Table 4. Frequencies of the haplotypes in the populations

Haplotype	Asthma	Controls	JIA
1 1 1 1 1	0.510	0.550	0.613
1 1 1 1 2	0.020	0.035	0.030
1 2 2 2 2	0.005	0.000	0.000
2 1 1 1 1	0.172	0.130	0.101
2 1 1 1 2	0.004	0.000	0.000
2 2 2 2 1	0.002	0.000	0.000
2 2 2 2 2	0.286	0.283	0.256

JIA, juvenile idiopathic arthritis.

Haplotype analysis

Three major haplotypes were found (see Table 4 for their frequencies in the three populations). There was no association of the haplotypes with the diseases.

Discussion

The role that IL-18 plays in the pathophysiology of both – TH1- and TH2-mediated – chronic inflammatory diseases has been extensively studied. These results have been underlined by genetic investigations showing association of polymorphisms within the gene with these diseases, thereby dedicating a further pathogenetic effect to this gene.

We recently identified new polymorphisms within IL-18 and found association of four polymorphisms with mainly atopic phenotypes like elevated IgE levels and sensitization to common allergens (23). In this study, we screened these five polymorphisms in populations of asthmatic children, children with JIA, and controls.

In contrast to our first association study with atopic phenotypes and another study reporting association with asthma (22), we did not find any association of the five polymorphisms with neither bronchial asthma nor JIA. Such conflicting results are often observed in association studies using populations with different ethnic backgrounds. However, the populations of our earlier study on atopic phenotypes and of the study presented here have been recruited in the same area of southwestern Germany. Therefore, it seems unlikely that the contrary results are due to a different genetic background.

IL-18 represents a pleiotropic cytokine with varying effects in inflammatory diseases that are mainly influenced by the overall cytokine milieu and exhibits some protective role in the development of bronchial asthma (especially in conjunction with IL-12) (7). At the same time, it might initiate bronchial asthma through enhanced antigen-induced eosinophil recruitment (9) and enhanced serum IgE and TH2 cytokine levels (10) if administered solely.

Bronchial asthma and JIA represent complex and varying clinical pictures with varying cytokine milieus, depending on the respective activity of the diseases. It seems reasonable to assume that the opposite effects that IL-18 might exhibit on the asthma phenotype are due to the varying cytokine milieus in the course of the asthmatic disease. The same accounts for JIA.

This in turn would lead to different and even opposite pathophysiologic effects of the very same genetic variants. The same variant that enhances the process in one stage might turn it down in a later stage.

Our first study on atopic phenotypes covered a clear immunologic situation, the predominance of the TH2

cytokine milieu. Our second study covered a much more complex phenotype comprising TH2 as well as TH1 aspects, depending on the clinical stage. This could lead to a false-negative result with the genetic variants exhibiting opposite effects on the same clinical phenotype.

In fact, our negative association study cannot exclude the significance of IL-18 variants on the asthmatic or JIA phenotype but might be just influenced by their opposing effects. In other words, further functional studies are necessary to evaluate the functional effect of these IL-18 variants and their meaning in the context of complex inflammatory diseases.

We conclude from our data that the effect of IL-18 in the immunologic context of diseases like bronchial asthma or juvenile arthritis might be too complex to be reflected in a simple one-way association study.

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