

Genetic and Environmental Factors in the Immunopathogenesis of Atopy: Interaction of *Helicobacter pylori* Infection and *IL4* Genetics

T. Pessi^a M. Virta^a K. Ådjers^a J. Karjalainen^b H. Rautelin^{c, d} T.U. Kosunen^d
M. Hurme^{a, e}

^aDepartment of Microbiology and Immunology, Medical School, University of Tampere, and ^bDepartment of Respiratory Medicine, Tampere University Hospital, Tampere; ^cHUSLAB, Helsinki University Central Hospital, and ^dDepartment of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Helsinki; ^eCentre for Laboratory Medicine, Tampere University Hospital, Tampere, Finland

Key Words

Helicobacter pylori · Interleukin-4 · Genotype · Polymorphism · Atopy · Skin prick test

Abstract

Background: Both genetic and environmental factors, e.g. early childhood infections, have a role in the pathogenesis of atopic diseases. **Objective:** To examine simultaneously the strength and possible interactions of two known such factors, *IL4* genetics and *Helicobacter pylori* infection, on the risk of atopy and asthma. **Methods:** Gene polymorphism analyses and skin prick tests (SPT) were determined in 245 adult asthmatics and 405 non-asthmatic controls of population-based case-control study. SPTs were used as an indicator of atopy. *H. pylori* infection was verified by detecting anti-*H. pylori* IgG antibodies in sera. **Results:** A significant negative association was seen between the presence *H. pylori* antibodies and SPT positivity (≥ 1 positive reactions) in both asthmatics and controls ($p = 0.002$ and $p = 0.025$, respectively) but the effect of *IL-4* polymorphism (SNP –590C/T) was nonsignificant in both groups ($p = 0.071$ and $p = 0.072$, respectively). However, *IL4* genetics had an effect on susceptibility to *H. pylori*: asthmatics carrying the *IL4*

–590 allele T had a diminished risk to be *H. pylori* infected (OR 0.485 95%CI 0.287–0.819). This effect was not seen in controls. Logistic regression analysis indicated that *H. pylori* and *IL4* effects on atopy risk are not inter-dependent. **Conclusions:** This study showed that the effect of *H. pylori* infection on atopy risk is stronger than that of *IL4* genetics. There is no interaction between these factors on the pathogenesis of atopy suggesting that these factors have distinct immunopathogenetic mechanisms. However, the genetic effect may modify the role of infective agents by effecting on susceptibility to disease.

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Introduction

Both genetic and environmental factors are known to have an effect on susceptibility to atopic diseases (such as atopic eczema, rhinoconjunctivitis and asthma). Of the environmental factors, childhood infections have an important role: there is epidemiological evidence showing that infectious diseases (especially gastrointestinal infections) during early childhood decrease the risk of development of atopic diseases in later life, and vice ver-

sa, the absence of infectious diseases increases the risk, i.e. the factor which probably explains the rapid increase of atopic diseases in the developed countries during the last decades (reviewed in [1]). This 'hygiene hypothesis' can be explained also at the cellular level. The T helper (Th) cells are divided into two different functional subsets, Th1 and Th2, based on the cytokine pattern they produce (reviewed in [2]). Th1 cells are mainly responsible for the cell-mediated immunity, while Th2 cells help the antibody formation, especially of the IgE class. Consequently, the Th1/Th2 balance seems to be shifted towards the Th2 direction in atopic diseases. Differentiation of the Th cells is strongly influenced by factors derived from infectious agents (e.g. bacterial lipopolysaccharide, LPS) and by the 'cytokine milieu' induced, the presence of these factors strongly favoring differentiation towards the Th1 direction. In the absence of these stimuli, the differentiation of the Th2 subset is preferred (reviewed in [3]).

The Th2 cytokines (interleukin (IL)-4, IL-5, IL-9, IL-10 and IL-13) have a decisive role in the pathogenesis of atopic diseases [4]. IL-4 is a major cytokine responsible for induction of IgE synthesis and promotion of differentiation to Th2 cells [5]. The gene encoding *IL4* is located on chromosome 5q31–33 that has been shown to be in linkage with atopic diseases in several studies [6–10]. A common single nucleotide polymorphism (SNP) consisting of a C to T exchange at position –590 in the promoter of *IL4* gene (*IL4* –590C/T) has been associated with asthma, skin prick test (SPT) positivity and elevated levels of total IgE [7–10]. Moreover, the same polymorphism has also been linked to pathogenesis of severe infections, e.g. progression of HIV infection [11] and protection against malaria [12].

Helicobacter pylori is the most common infectious gastrointestinal pathogen that infects gastric mucosa in early life and often leads to a life-long chronic gastritis (reviewed in [13]). It induces a vigorous Th1 mediated inflammatory response [14]. Several studies [15–17] have demonstrated that *H. pylori* infection is associated with lower prevalence of atopy.

Very little is known about the interactions of genetics and environmental factors in the pathogenesis of atopy and asthma. We now analyzed the effect of two known factors, *IL4* –590C/T and *H. pylori* infection, on the prevalence of atopy and asthma in cohort of 245 adult asthmatics and in their 405 controls. Atopy was defined by more than one positive skin prick test and *H. pylori* infection by the presence of IgG anti-*H. pylori* antibodies.

Table 1. Characteristics of study groups

Variable		Asthmatics (n = 245)	Controls (n = 405)
Age, years	mean ± SD	59 ± 11	60 ± 11
Gender	female/male	152/93	254/151
Smoking	non/ex/current	123/80/42	237/89/79
Atopy ¹	yes	139	154
<i>H. pylori</i> antibodies	yes	115	205

¹ One or more positive reactions in skin prick test.

Methods

Subjects

Asthmatic and control subjects were participants in a Finnish population-based case-control study aimed at identifying risk factors and predictors of the outcome of adult asthma. Inclusion criteria for asthmatic subjects were age over 30 years and entitlement to special reimbursement for asthma medication from the Social Insurance Institution of Finland. The entitlement is granted if the criteria for persistent asthma are fulfilled as certified by a chest specialist. Typical history, clinical features and course of asthma must be documented. At least one of the following physiological criteria is required for diagnosis: (a) a variation of $\geq 20\%$ in diurnal PEF recording (reference to maximal value); (b) an increase of $\geq 15\%$ in PEF or FEV₁ with β_2 -agonist; (c) a decrease of $\geq 15\%$ in PEF or FEV₁ in exercise testing. Moreover, at least a 6-month period of continuing regular use of anti-asthmatic medication must have elapsed by the time of the decision. This method of case ascertainment has been described in detail and evaluated [18]. One to two controls matched for age, gender and area of residence not suffering from asthma or chronic obstructive pulmonary disease were initially selected for each subject through a register covering the entire population. The ethnic origin of patients and controls was the same (Finnish Caucasian). The basic characteristics of patient and control groups are presented in table 1. Approval for this study was obtained from the ethical committee at Tampere University Hospital. All subjects gave informed consent to participate.

Skin Prick Tests

Skin prick tests were performed by specially trained nurses with a panel of 22 common allergen extracts, including dog, cat, birch, cow dander, horse, mugwort, alder, meadow foxtail, timothy, barley, oats, wheat, rye, *Alternaria alternata*, *Acarus siro*, *Aspergillus fumigatus*, *Cladosporium herbarum*, *Der. farinae*, *Der. pteronyssinus*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae* and to both a negative control (saline) and a positive control histamine (ALK-Abello, Hørsholm, Denmark). The patient was considered prick test positive if at least one allergen elicited a weal with a diameter at least 3 mm larger than that of the negative control. Allergy testing by the skin prick method was carried out on 99.1% of asthmatic (93 males and 150 females) and 99.3% of control subjects (150 males and 252 females). Positive reactions to specific allergens and differences between asthmatics and nonasthmatic controls have been published earlier [19].

Table 2. Effect of *H. pylori* serology and *IL4* –590C/T allele carrier status on skin prick test positivity (SPT+) in asthmatics and controls

		SPT+		SPT–		p	OR	95% CI
		n	%	n	%			
<i>H. pylori</i> serology								
Asthmatics								
Seronegative		82	60.7	42	40.4	0.002	2.28 1	1.35–3.85
Seropositive		53	39.3	62	59.6			
Controls								
Seronegative		84	55.6	108	44.1	0.025	1.59 1	1.06–2.39
Seropositive		67	44.4	137	55.9			
<i>IL4</i> genetics								
Asthmatics								
<i>IL4</i> allele T noncarrier		48	35.5	49	47.1	0.071	1 1.61	0.96–2.72
<i>IL4</i> allele T carrier		87	64.5	55	52.9			
Controls								
<i>IL4</i> allele T noncarrier		63	41.7	125	51.0	0.072	1 1.46	0.97–2.19
<i>IL4</i> allele T carrier		88	58.3	120	49.0			

SPT+ = One or more positive skin prick test reaction; allele T noncarrier = CC genotype; allele T carrier = CT and TT genotypes.

p value calculated in 2 × 2 table by χ^2 test.

Serum Samples and *H. pylori* Serology

Serum samples and citrated whole-blood samples were collected from each patient. Samples were stored at –70°C until testing. The sera were assayed for helicobacter IgG antibodies. *Helicobacter* IgG antibodies were measured by using the enzyme-linked immunosorbent assay (Pyloriset EIA-G III, Orion Diagnostica, Espoo, Finland). Titres of 30 or higher were considered positive for *H. pylori* antibodies. With this cut-off value, a sensitivity of 99% and a specificity of 90% were demonstrated in a separate series of 16- to 91-year-old dyspeptic patients (gastroscooped at primary care level in Vammala, Finland, n = 561, median age 56 years, *H. pylori* infection prevalence 32.3%) used for the validation of the test (culture and histology as reference methods; data not shown). *Helicobacter* IgG antibody measurement was carried out on 98.8% of asthmatic (92 males and 150 females) and 98.8% of control subjects (151 males and 249 females).

DNA Extraction and Genotyping

DNA specimens from citrated whole-blood samples were prepared using standard methods. The region which contains the *Ava*II polymorphic site at position –590 (C to T base exchange) of the *IL4* gene was amplified by PCR [8]. The oligonucleotides 5' TAA ACT TGG GAG AAC ATG GT 3' and 5' TGG GGA AAG ATA GAG TAA TA 3' were used as primers. Fragments were analyzed by electrophoresis on 3% agarose stained with ethidium bromide. The genotype distribution of the *IL4* gene studied followed the Hardy-Weinberg equilibrium. *IL4* genotyping was carried out on 99.2% of asthmatic (92 males and 151 females) and 99.0% of control subjects (151 males and 250 females).

Statistical Analysis

To test the fit of genotype frequencies with the Hardy-Weinberg equation the exact test using Markov chain algorithm in Arlequin software was used (Arlequin program, ver. 2.0. A software for population genetics data analysis, Schneider S, Roessli D, Excoffier L, Genetics and Biometry Laboratory, Geneva, Switzerland). χ^2 test calculations were carried out with Statistica software (ver. Win.5.1D, StatSoft, Tulsa, OK). Odds ratios (OR) with 95% CI were calculated by CIA software (ver. 1.1, copyrighted by M.J. Gardner and the *British Medical Journal*, 1989). Binary logistic regression analysis of SPSS was used to analyze the interaction between *H. pylori* infection and *IL4* genetics as well as simultaneously significance of these factor on atopy risk. Findings were considered statistically significant at $p < 0.05$. Altogether 97.6% of asthmatics and 97.8% of controls had all required measurements (skin prick tests, serological and genotype results) and were used in statistical calculations.

Results

The effects of *H. pylori* serology and *IL4* genetics on SPT positivity were tested. Our data confirmed the earlier data [15–17] on the association of SPT positivity rates and *H. pylori* serology. In both asthmatics and controls SPT positivity rate was lower in subjects with *H. pylori* antibodies (table 2). A trend of association was observed between *IL4* gene polymorphism and SPT positivity rates

in asthmatics and controls ($p = 0.071$ and $p = 0.072$, respectively; χ^2 test, d.f. = 1). Allele T carriers (i.e. subjects having genotypes CT or TT) had slightly increased the risk of SPT positivity (table 2).

These two factors, *IL4* gene polymorphism and *H. pylori* serology, were then put in a logistic regression model to evaluate simultaneous significance and independency of these factors. The model showed that *H. pylori* serology was more significant predictor of SPT positivity than *IL4* polymorphism in both asthmatics and controls (table 3). These factors were independent. There was no interaction between seropositivity and *IL4* gene polymorphisms on SPT results in asthmatics and controls ($p = 0.685$ and 0.709 , respectively).

To evaluate the effect of *H. pylori* serology and *IL4* genetics on plurisensitization to allergens SPT-positive patients were divided into monosensitized (= one positive SPT reaction) and plurisensitized (= more than one positive SPT reaction) subgroups. As seen in table 4, in both asthmatics and controls the number of plurisensitized subjects was diminished in seropositive group ($p = 0.0005$ and $p = 0.004$, respectively; χ^2 test, d.f. = 1).

Table 3. Simultaneous effect of *H. pylori* serology and *IL4* -590C/T allele carrier status on skin prick test positivity (SPT+) in asthmatics and controls

	n	OR	95% CI	p
<i>Asthmatics</i>				
<i>H. pylori</i>				0.004
Seronegativity	124	2.16	1.272–3.668	
Seropositivity	115	1		
<i>IL4</i> polymorphism				0.195
<i>IL-4</i> allele T noncarrier	97	1		
<i>IL-4</i> allele T carrier	142	1.43	0.834–2.440	
<i>Controls</i>				
<i>H. pylori</i>				0.033
Seronegativity	192	1.56	1.036–2.353	
Seropositivity	204	1		
<i>IL4</i> polymorphism				0.094
<i>IL-4</i> allele T noncarrier	188	1		
<i>IL-4</i> allele T carrier	208	1.42	0.941–2.145	

Factors put into a logistic regression model. Allele T noncarrier = CC genotype; allele T carrier = CT and TT genotypes.

Table 4. Effect of *H. pylori* serology and *IL4* -590C/T allele carrier status on mono [SPT+(m)] and plurisensitization [SPT+(p)] to allergens in asthmatics and controls

	SPT+ (p)		SPT+ (m)		SPT–		SPT– vs. SPT+ (m), p	SPT– vs. SPT+ (p), p
	n	%	n	%	n	%		
(a) <i>H. pylori</i> serology								
Asthmatics								
Seronegative	70	64.2	12	46.2	42	40.4	0.59	0.0005
Seropositive	39	35.8	14	53.8	62	59.6		
Controls								
Seronegative	61	61.0	23	45.1	108	44.1	0.89	0.004
Seropositive	39	39.0	28	54.9	137	55.9		
(b) <i>IL4</i> genetics								
Asthmatics								
<i>IL4</i> allele T noncarrier	38	34.9	10	38.5	49	47.1	0.43	0.069
<i>IL4</i> allele T carrier	71	65.1	16	61.5	55	52.9		
Controls								
<i>IL4</i> allele T noncarrier	41	41.0	22	43.1	125	51.0	0.306	0.091
<i>IL4</i> allele T carrier	59	59.0	29	56.9	120	49.0		

SPT + (m) = One positive skin prick test reaction; SPT + (p) = more than one positive skin prick test reaction; allele T noncarrier = CC genotype; allele T carrier = CT and TT genotypes. p value calculated in 2×2 table by χ^2 test.

Table 5. Effect of *IL4* allele carrier status on *H. pylori* seropositivity in asthmatics and in controls

		Seropositive		Seronegative		p	OR	95% CI
		n	%	n	%			
Asthmatics								
<i>IL4</i> allele T noncarrier		57	49.6	40	32.3	0.007	1	0.287–0.819
<i>IL4</i> allele T carrier		58	50.4	84	67.7		0.485	
Controls								
<i>IL4</i> allele T noncarrier		103	50.5	85	44.3	0.220	1	0.525–1.16
<i>IL4</i> allele T carrier		101	49.5	107	55.7		0.779	

Allele T noncarrier = CC genotype; allele T carrier = CT and TT genotypes.
p value calculated in 2 × 2 table by χ^2 test.

Whereas there was only a trend of association between *IL4* gene polymorphism and plurisensitization in asthmatics and controls ($p = 0.069$ and $p = 0.091$, respectively; χ^2 test, d.f. = 1).

There was an association ($p = 0.007$; χ^2 test, d.f. = 1) between *IL4* gene polymorphism and *H. pylori* seropositivity among asthmatics (table 5). Allele T carriers had diminished risk for *H. pylori* infection (OR 0.485 95% CI 0.287–0.819). The effect was independent on atopic status of the subject (data not shown). In controls, the association between *IL4* gene polymorphism and *H. pylori* seropositivity was not significant ($p = 0.220$; χ^2 test, d.f. = 1).

The effect of these two factors on asthma risk was also evaluated. *H. pylori* seropositivity rates were similar in asthmatics and controls ($p = 0.370$, χ^2 test, d.f. = 1, see table 1). For the asthmatics, the frequencies were 0.41 for allele T noncarriers and 0.59 for allele T carriers at –590 of the *IL4* gene. For controls respective values were 0.48 and 0.52. No difference in carrier frequencies was observed between asthmatics and controls ($p = 0.093$; χ^2 test, d.f. = 1).

Discussion

In here interaction of two known atopy risk factors (*H. pylori* infection and *IL4* genetics) was studied. The result showed that the protective effect of *H. pylori* was not dependent on *IL4* genetics. Recently McIntire et al. [20] observed that hepatitis A infection protects individuals from atopy only if they carry a certain allele of the *TIM1* gene (a gene expressed on activated Th cells during dif-

ferentiation to Th2 direction and serving as a receptor for the virus). This allele did not have any effect on the infection rate, but obviously modified the effect of the virus on the Th cell differentiation. In here, *IL4* genetics and *H. pylori* infection were independent risk factors. The atopy protective effect of *H. pylori* infection observed here may be explained by the increased Th1 activity [21, 22]. *IL4* –590 T allele has been shown to be associated with a stronger transcription of *IL4* than allele C [11, 23], and consequently, the presence of this allele leads to preferential differentiation of the Th cells towards the Th2 direction. Our data suggest that *IL4* polymorphism and *H. pylori* infection have distinct pathways to regulate the pathogenesis of atopy.

Our study showed that *IL4* genetics had effect on infection rate: absence of allele T was a risk factor for this infection. IL-4 has a central role in the antibody production. *H. pylori* infection was evaluated by measuring specific IgG antibodies. One possibility is that *IL4* allele C, low IL4-producing allele, is involved in total IgG antibody production, contrast to IgE promoting allele T [11, 23]. Moreover, our observation is line with the associations observed with *IL4* –590C/T and other infectious agents (such as malaria antigens and HIV) [11, 12], explained by this increased Th1 activity.

Rockman et al. [24] have recently observed that allele frequency variation of the *IL4* SNP –590 in different populations is too great to be explained by simple genetic drift. They suggested that natural selection, e.g. various infections, have had an effect on the allele frequencies. In here the *IL4* –590 polymorphism was associated with susceptibility to a bacterial infection as well as there was a trend of association to atopy risk. This does not give di-

rect evidence for hypothesis that allergies are just by-products of the effective immune defense against microbes, a capacity which has had a positive selective advantage during evolution.

IL4 allele T has been linked to asthma in many studies [6–10]. However, contradictory results have been reported as well [25, 26]. Our study showed a weak association of *IL4* genetics and asthma risk but only in seronegative patients ($p = 0.03$; table 5). This might indicate that genetic risk is stronger in the absence of certain environmental factors. Obviously, more data are needed on the interactions of these genetic and environmental factors.

We observed that the protective effect of *IL4* polymorphism on *H. pylori* infection was seen in asthmatics but not in controls (table 5). The reason to this can only be speculated, but obviously asthmatics have some other factors modulating the effect of the *H. pylori* infection. One candidate is the anti-inflammatory cytokine IL-10. It has been observed that the severity of asthma is associated with the presence of a low IL10 producing haplotype [27], which then allows uninhibited production of pro-inflammatory cytokines and thus a more efficient shift of the Th1/2 balance towards the protective Th1 direction.

In this study, presence of *H. pylori* antibodies had a clear inverse association to the incidence of atopy. This finding is in line with previous reports from our country [15] and from elsewhere [16, 17], thus confirming the role of *H. pylori* infection as a strong atopy-risk modulating factor (according to the hygiene hypothesis, see introduction). The effect of *H. pylori* seropositivity was strikingly

different in subjects with one positive SPT (monoallergic) vs. several positive SPTs (pluri allergic), i.e. *H. pylori* infection only protected against plurisensitization. This might indicate that *H. pylori* infection does not affect the susceptibility per se but rather on the severity of the atopic disease. However, as *H. pylori* exposure is stronger in poor hygienic conditions [28, 29], we cannot exclude the possibility that *H. pylori* is just a surrogate marker of, e.g. a general bacterial LPS exposure.

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