

Short communication

IL15 gene variants are not associated with asthma and atopy

Background: Interleukin 15 (IL15) promotes activation and proliferation of CD8+ T cells and enhances the differentiation into Th2 cells. A previous study described five polymorphisms in the *IL15* gene to be associated with asthma in a haplotype analysis.

Aim: We selected HapMap tagging single nucleotide polymorphisms (SNPs) from *IL15* to systematically investigate these *IL15* associations in a large population-based sample.

Methods: Genotyping of seven *IL15* SNPs was performed using MALDI-TOF MS in a cross-sectional study population of 3099 children from Dresden or Munich (age 9–11 years). All children were phenotyped by standardized and validated protocols for atopic phenotypes. Effects of single SNPs and haplotypes were studied using SAS 9.1.3 and HAPLOVIEW. Equivalence tests were performed to prove the significance of negative results.

Results: Neither single *IL15* polymorphisms nor haplotype analyses showed associations with asthma or atopy after correction for multiple testing.

Conclusion: These results do not confirm previous case-control studies and suggest that *IL15* gene variants do not play an important role in the development for asthma or other atopic disorders.

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Only recently interleukin 15 (IL15) has been identified as an important player in allergic immune responses. Interleukin 15 mediates diverse biological responses, ranging from proliferation and differentiation to protection from apoptosis. Interleukin 15 binds to a trimeric receptor complex consisting of IL2R β , IL2R γ and IL15R α chains. This cytokine shares with IL2 IL2R β chain, and with other cytokines (IL2, IL4, IL9, IL13 and IL21) IL2R γ chain, while IL15R α is a specific receptor subunit for IL15. Produced by macrophages, epithelial cells and dendritic cells, IL15 activates peripheral T cells and recruits further proinflammatory cells to the site of inflammation (1). Dendritic cell-derived IL15 stimulates the proliferation of naive CD8+ T cells (2) and enhances the differentiation of naive T cells towards a Th2 phenotype in the presence of IL4 (3).

Kurz et al. (4) performed a mutation screening of the *IL15* coding region and reported five polymorphisms to be associated with asthma in haplotype analyses but not in single nucleotide proteins (SNP) analyses. Bierbaum et al. (5) showed the same association using different control subsamples selected from the multicentre allergy study cohort study (6). Thus, we investigated if the association between IL15 polymorphisms or haplotypes and atopic phenotypes could also be replicated in

an independent cross-sectional population sample of German children.

Methods

Population description for association studies

Between 1995 and 1996, a cross-sectional study was performed in Dresden and Munich as part of the International Study of Asthma and Allergy in Childhood (ISAAC phase II) to assess the prevalence of asthma and allergies in schoolchildren aged 9 to 11 years. All children of German origin with DNA available were included in this analysis ($n = 1940$ in Dresden and $n = 1159$ in Munich). Informed written consent was obtained from all parents of children included in the study. All study methods were approved by the local ethics committees. The populations and phenotyping methods have been described in detail before (7).

Phenotyping

Self-administered questionnaires included the ISAAC core questions on symptoms of asthma, hay fever and atopic eczema. Children whose parents reported a physician's diagnosis of asthma, recurrent spastic or asthmatic bronchitis were classified as having asthma ($n = 272/3099$; $n = 130$, 11.32% in Munich and $n = 142$, 7.45% in Dresden). Children's classification of hay fever or atopic eczema was based on a parental report of a physician's diagnosis.

The sensitivity to six common aeroallergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria tenuis*, cat dander, mixed grass and tree pollen) was assessed by using highly standardized extracts and lancets in a skin prick test (SPT) (ALK, Horsholm, Denmark). The presence of atopy was defined as a wheal reaction of at least 3 mm to one or more specific allergen after subtraction of the negative control ($n = 777/3099$; $n = 284$, 24.85% in Munich and $n = 493$, 26.52% in Dresden).

Genotyping

Genomic DNA was extracted from whole blood by a standard salting out method (8) and preamplified as previously described (9). DNA samples were genotyped using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Sequenom, Inc., San Diego, CA, USA). Polymerase chain reaction assays and associated extension reactions were designed using the SPECTRO-DESIGNER software (Sequenom, Inc.). All amplification and extension reaction conditions have been previously described (9) and details are available upon request from the corresponding author.

For quality control, Hardy-Weinberg calculations were performed to ensure that each marker was within the expected allelic population equilibrium. Selection of the SNPs in the *IL15* gene was performed using HapMap project data (<http://www.hapmap.org/>).

Statistical methods

Linkage disequilibrium (LD) patterns were assessed by HAPLOVIEW using HapMap samples with European ancestry Centre d'Etude du Polymorphisme Humain (CEPH) (10). All SNPs were tested for deviations from Hardy-Weinberg equilibrium (HWE) using the chi-square test, with expected frequencies derived from allele frequencies. Association between SNPs and dichotomous outcomes were tested using chi-square tests in an allelic and linear model. To correct for multiple testing we used a Bonferroni correction in every phenotype.

As failing the significance level does not prove the absence of association, equivalence tests were used to test the null hypothesis 'existence of association' against the hypothesis 'absence of association'. Equivalence is proved if the odds ratio (OR) ρ is in a predefined indifference zone with a certain probability of error expressed as:

$$H_0 : \rho \leq 1/(1 + \varepsilon) \text{ or } \rho \geq (1 + \varepsilon) \\ \text{and } H_1 : 1/(1 + \varepsilon) < \rho < (1 + \varepsilon),$$

where ρ is the OR and ε is a predefined value for the range of the indifference zone (11). Based on previous publications, we predefined the thresholds of ρ as an OR of 0.8 or 1.25 and ε as 0.25.

This test is not based on a *P*-value but determines a pair of integers $C1(s)$, $C2(s)$ for each genotype column total s using an algorithm. The test for equivalence is positive if and only if the frequency X of cases exhibiting genotype G falls between these limits, whereas for $X \leq C1(s)$ or $X \geq C2(s)$, the null hypothesis of a relevant association has to be accepted. A SAS procedure for this test (*bi2st*) is available at <http://www.zi-mannheim.de/wktsheq>.

Haplotype frequencies were estimated with an expectation-maximization (EM) algorithm (12) and associations of the common haplotypes ($\geq 3\%$) with asthma or atopy were calculated in children with complete genotype information for every genotyped SNP. All tests were two-sided and the differences were considered significant with $P < 0.05$. Calculations were carried out with SAS 9.1.3 or SAS GENETICS.

Results

All 20 HapMap SNPs in the *IL15* gene region on chromosome 4q31 were selected to determine LD patterns by HAPLOVIEW (Fig. 1). From these, five tagging SNPs were identified and genotyped. Additionally, two SNPs (C-80T = rs2254514, A13815T = rs1057972) described by Kurz et al. (4), which were not included in HapMap, were genotyped (Table S1). However, our analysis indicated that SNPs rs2254514 and rs4956407 were in LD ($r^2 = 0.98$), as well as SNPs rs2857261 and rs1057972 ($r^2 = 0.98$).

All tagging SNPs were successfully genotyped in a cross-sectional study population from Munich and Dresden. The two SNPs (C-80T = rs2254514 and A13815T = rs1057972) were only genotyped in Dresden but not in Munich as redundancy with tagging SNPs was identified by LD analysis. All polymorphisms showed call rates of at least 96% and no significant deviation from HWE in quality control procedures.

We assessed the relationship between genotypes of the tested SNPs and the primary phenotypes for asthma (doctor's diagnosis of asthma, current wheeze and bronchial hyperresponsiveness) and atopic diseases (atopic dermatitis and hay fever). Main results for asthma and atopy are shown in Table 1. No associations with these phenotypes were found and also no associations with hay fever or atopic eczema were identified (data not shown). For some SNPs the absence of association was proven by equivalence tests in an equivalence range for an OR from 0.8 to 1.25. A weak association with atopy for rs10833 (Table 1) did not remain significant after correction for multiple comparisons. Haplotype analyses did not yield additional information and no significant effects were detected in contrast to previous reports (Table 2).

Discussion

The present study contributes new knowledge to the asthma genetics field by systematically studying the role of *IL15* polymorphisms and haplotypes in atopic disorders including asthma, atopy, hay fever and atopic dermatitis. In our large population sample, genetic variations showed no significant association with asthma or further atopic disorders neither in one population (after correction for multiple comparisons) nor in both populations; in allelic and additive models (Armitage trend tests, as previously published); analyzing single gene effects and haplotypes. Furthermore, the lack of association was tested for its significance using equivalence tests. In these tests, the equivalence range was narrowly defined by $OR < 1.25$ to consider that genetic effects may often be quite subtle. Therefore, when a statistical absence of association is reported in our test, even very small effects are excluded. Single nucleotide polymorphisms not being rejected in the equivalence test and not

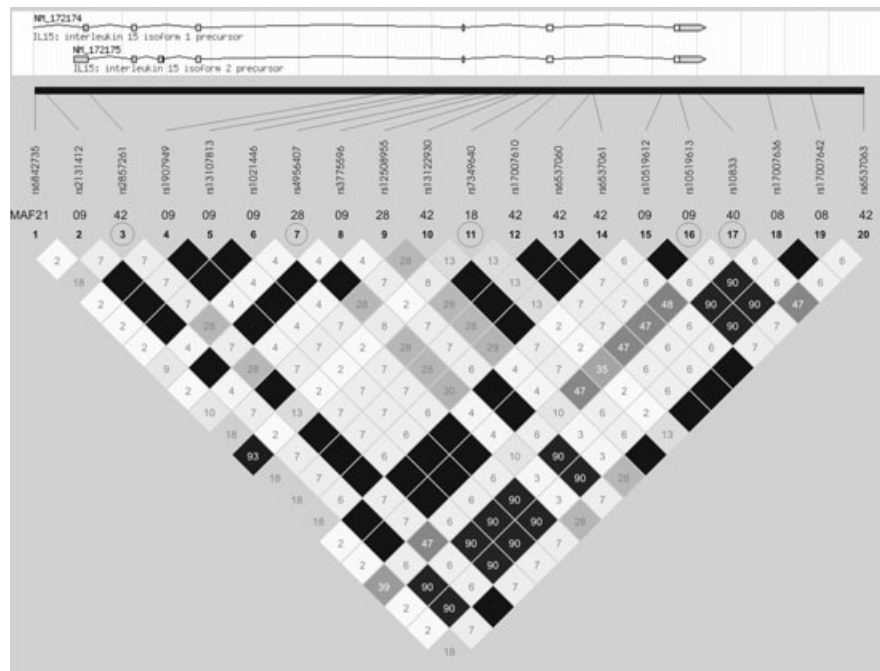


Figure 1. Description of the *IL15* gene structure on chromosome 4q31, identified *IL15* polymorphisms, linkage disequilibrium (LD) (r^2 plot), tagging single nucleotide proteins (SNPs) and minor allele frequencies (MAFs) of genotyped *IL15* polymorphisms. Linkage disequilibrium between *IL15* polymorphisms was determined using the information of the HapMap database. Colour code for the LD plot is given by HAPLOVIEW: white ($r^2 = 0$), shades of grey ($0 < r^2 < 1$) and black ($r^2 = 1$). *IL15* tagging SNPs are encircled. Minor allele frequencies are given in the line followed by the abbreviation MAF (%). All five tagging SNPs have been genotyped in the study population.

Table 1. Odds ratio (OR) (and 95% confidence intervals) for associations with asthma (doctor's diagnosis) or atopy (assessed by SPT) in the International Study of Childhood Asthma and Allergy (ISAAC) population from Dresden and Munich ($n = 3099$), and results of equivalence tests

	OR	Confidence limits (CLs) for the OR		<i>P</i> -value (Chi-square test)	Equivalence limits (using an OR of 0.8 to 1.25)		X	Equivalence decision
		Lower CL	Upper CL		C1(s)	C2(s)		
Asthma								
rs2857261	0.92	0.77	1.10	0.377	257	275	256	No
rs4956407	1.06	0.86	1.29	0.591	131	141	141	Yes*
rs7349640	0.85	0.64	1.12	0.240	70	74	63	No
rs10519613	0.91	0.68	1.22	0.526	56	59	53	No
rs10833	0.99	0.81	1.19	0.879	166	180	171	Yes*
Atopy								
rs2857261	0.91	0.81	1.02	0.117	724	794	733	Yes*
rs4956407	0.91	0.79	1.04	0.162	365	413	368	Yes*
rs7349640	0.92	0.77	1.10	0.363	194	214	193	No
rs10519613	1.11	0.92	1.33	0.279	156	170	174	No
rs10833	0.88	0.77	0.99	0.039*	468	526	464	No

*Significant by the respective test.

showing an association in our study also have a low probability to affect asthma. Taken together, these data suggest that no major genetic effect of most *IL15* SNPs is to be expected on asthma. This is in part in contrast to previous reports from Kurz et al. (4) and Bierbaum et al. (5) which, while also not finding consistent associations with asthma in single gene analyses, described associations of *IL15* haplotypes with childhood asthma.

The absence of polymorphisms affecting asthma development from *IL15* does not by itself dispute the role of *IL15* in asthma pathogenesis. While *IL15* expression may be necessary and important to develop atopy and asthma, our study only suggests that no common polymorphisms in the gene exist that change gene function in a way to modify disease development of atopic diseases. Also, while no polymorphisms in the *IL15* gene seem to be

Table 2. Associations between *IL15* haplotypes and asthma (doctor's diagnosis) or atopy (assessed by SPT)

Haplotype*		Asthma			Atopy		
		Estimated frequencies		<i>P</i> -value	Estimated frequencies		<i>P</i> -value
		Cases (%)	Controls (%)		Cases (%)	Controls (%)	
Hap 1	G-A-A-C-C	49.80	51.37	0.4982	49.49	51.79	0.1317
Hap 2	A-T-A-C-T	19.11	20.59	0.4187	19.80	17.55	0.0616
Hap 3	A-A-G-C-T	13.77	11.96	0.2565	13.75	12.84	0.3826
Hap 4	A-A-A-A-C	10.64	10.20	0.7537	10.25	11.42	0.2109
Hap 5	A-T-A-C-C	5.92	5.49	0.6925	5.94	5.76	0.8004
	All rare	0.76	0.39		0.77	0.64	

Haplotype frequencies for cases vs controls and *P*-values for the associations between individual haplotypes and outcomes.

*Based on five tagging single nucleotide proteins (SNPs): rs2857261, rs4956407, rs7349640, rs10519613 and rs10833.

relevant to the development of asthma, other genes involved in the *IL15* signalling pathway such as *IL15* receptor alpha, may be interesting candidate genes to be studied for their effect on atopic disorders in further association studies.

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Leonardo Araujo Pinto performed genotyping, data analysis and drafted the first version of the manuscript; Martin Depner con-

tributed and supervised data analysis, Lena Steudemann, Norman Klopp and Thomas Illig participated in genotyping; Erika von Mutius contributed to the collection of data and manuscript preparation; Michael Kabesch supervised all experiments, participated in the development of the study design, collection of data, data analysis and wrote the final version of the manuscript. This manuscript is part of the MD thesis of Lena Steudemann at the Ludwig Maximilian's University.

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Supplementary material

Additional Supporting Information may be found in the online version of this article:

Table S1. Position, minor allele frequencies (MAFs), rs numbers and tagging single nucleotide proteins (SNPs) of the *IL15* gene genotyped in the International Study of Childhood Asthma and Allergy (ISAAC) population.

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ONLINE SUPPLEMENT

Table S1 Position, minor allele frequencies, rs numbers and tagging SNPs of the *IL15* gene genotyped in the ISAAC population.

<i>IL15</i> Isoform 1	position to ATG	position in the gene	MAF	rs number	LD (r^2) with ...	...tagging SNP	genotyped SNPs
1	-1211	Intron / Prom	0.21	rs6842735	0.94	A10284G	
2	-973	Intron / Prom	0.09	rs2131412	1.00	C13467A	
**	-80	5' UTR	0.25	rs2254514	/		rs2254514
3**	20	Intron	0.42	rs2857261	Tag snp1	G20A	rs2857261
4	6705	Intron	0.09	rs1907949	1.00	C13467A	
5	7531	Intron	0.09	rs13107813	1.00	C13467A	
6	7939	Intron	0.09	rs1021446	1.00	C13467A	
7	8836	Intron	0.28	rs4956407	Tag snp2	A8836T	rs4956407
8	9196	Intron	0.09	rs3775596	1.00	C13467A	
9	9633	Intron	0.28	rs12508955	1.00	A8836T	
10	9795	Intron	0.42	rs13122930	1.00	G20A	
11**	10284	Intron	0.18	rs7349640	Tag snp3	A10284G	rs7349640
12	10647	Intron	0.42	rs17007610	1.00	G20A	
13	11435	Intron	0.42	rs6537060	1.00	G20A	
14	11512	Intron	0.42	rs6537061	1.00	G20A	
15	13083	Intron	0.09	rs10519612	1.00	C13467A	
16**	13467	3' UTR	0.09	rs10519613	Tag snp4	C13467A	rs10519613
**	13815	3' UTR	0.49	rs1057972	/		rs1057972
17	13929	3' UTR	0.40	rs10833	Tag snp5	C13929T	rs10833
18	15492	3'	0.08	rs17007636	0.90	C13467A	
19	16469	3'	0.08	rs17007642	0.90	C13467A	
20	17681	3'	0.42	rs6537063	1.00	G20A	

** SNPs genotyped by Kurz et al, *J Allergy Clin Immunol* 2004. In our population sample from Dresden we identified that SNPs rs2254514 and rs4956407 were in LD ($r^2=0.98$), as well as SNPs rs2857261 and rs1057972 ($r^2=0.98$)