

Original article

Association between interleukin-1 receptor antagonist gene and asthma-related traits in a German adult population

Background: A recent study in German and Italian families associated variants in the interleukin-1 receptor antagonist (IL1RA) gene with asthma. The aim of the present study was to further investigate the role of single nucleotide polymorphisms (SNPs) in the IL1RA gene in the development of atopy and lifelong asthma in a population-based study.

Methods: DNA samples from the German centres of the European Community Respiratory Health Survey were analysed for genetic variants in the IL1RA gene and the development of asthma, atopy and bronchial hyperreactivity.

Results: Carriers of the rare G allele of SNP rs447713 had a significantly increased risk of developing asthma ($P = 0.0013$) and allergic sensitization ($P = 0.0119$). Carriers of the rare C allele of SNP rs3087271 had an increased risk of asthma ($P = 0.0227$) and high immunoglobulin E (IgE) levels ($P = 0.0232$). A haplotype built from eight SNPs in the IL1RA gene (A-C-A-G-A-C-G-A) was associated with a higher prevalence of asthma ($P = 0.007$) and high total IgE ($P = 0.02$). Bronchial hyperreactivity was positively associated with the haplotype A-C-G-G-A-C-G-C ($P = 0.02$) and negatively with the A-C-G-G-A-C-T-C ($P = 0.03$).

Conclusion: A previously described association between IL1RA and asthma in families could be reproduced in a population-based sample. The genetic variants of IL1RA gene do not seem to affect asthma alone, but to act as modulators of asthma-related traits as well, where different haplotypes drive the development of different phenotypes.

C. Pattaro¹, J. Heinrich², M. Werner², R. de Marco¹, M. Wjst^{2,3}

¹Unit of Epidemiology and Medical Statistics, Department of Medicine and Public Health, Istituti Biologici II, University of Verona, Verona, Italy;

²GSF – National Research Centre for Environment and Health Institute of Epidemiology, Neuherberg;

³Institute of Epidemiology, Ludwig-Maximilians-University of Munich, Neuherberg, Germany

Key words: association study; asthma; atopic diseases; interleukin-1 receptor antagonist gene; single nucleotide polymorphism.

C. Pattaro
Unit of Epidemiology and Medical Statistics, DMSP
University of Verona
Istituti Biologici II
Strada Le Grazie, 8
37134 Verona
Italy

Accepted for publication 16 August 2005

Interleukin-1 receptor antagonist (IL1RA) polypeptide is fairly recognized to play an important role in a wide spectrum of diseases related to the response of the immunological system. The IL1RA polypeptide binds to the IL-1 cell receptors, in an attempt to reduce inflammatory responses (1). Thus, high or low amounts of interleukin-1 receptor antagonist gene (*IL1RN*) could produce two opposite situations: a too high level of IL1RA activity could result in an excessively reduced inflammatory response; on the contrary, a too low level of IL1RA activity could promote an excess of inflammation.

A recent study, based on a positional cloning approach, showed that *IL1RN* plays an important role in the genetics of atopic diseases: consistent results appeared both in a German and in an independent Italian population (2). The finding is supported by altered IL-1 levels in plasma (3) and nasal lavage (4). The levels of IL1RA were significantly higher in nonasthmatics. Functional mutations have not been identified so far (2), but results from other association studies may support an involvement of the gene (5–7).

The aim of the present study was to replicate our previous findings concerning the relationship between *IL1RN* and asthma in an unbiased population sample. In particular, we investigated if different genotypic patterns were related to different asthma-related traits. For this reason, a cross-sectional, population-based analysis has been performed and different atopic phenotypes have been investigated, in order to obtain more specific information on the role of *IL1RN*.

Materials and methods

Study population

This study includes individuals participating in the follow-up of the European Community Respiratory Health Survey (ECRHS) (8; see also <http://www.ecrhs.org>; accessed 31 August 2005), from the German cities of Hamburg (1 700 000 inhabitants) and Erfurt (200 000 inhabitants) (9, 10). The first survey of the ECRHS was performed during 1990–1992 (9, 10). All 731 participants from Erfurt and 900 of 1251 randomly selected participants from Hamburg were invited for the ECRHS-II survey during 2000–2001. Of these, 1220 agreed to a follow-up. The remaining 411 subjects

either could not be traced ($n = 257$), refused further participation ($n = 28$), moved out of the area ($n = 112$) or were no longer living ($n = 14$). From the sample of 1220 subjects, 590 participated in the follow-up study, which included an extensive interviewer administered questionnaire at the study centres. A total of 582 subjects took part in lung function measurements. The study protocol was approved by the local ethics committees.

Questionnaire, clinical data and phenotyping

Subjects underwent a standardized clinical interview administered by trained interviewers, lung function tests, methacholine challenge test for bronchial hyperreactivity (BHR) and allergological tests, in adherence to the ECRHS protocol (8). Methods and exclusion criteria have been described in detail elsewhere (9, 11). Total and specific immunoglobulin E (IgE) to *Dermatophagoides pteronyssinus*, timothy grass, *Cladosporium herbarum* and cat, were measured using the Pharmacia CAP system (Pharmacia Diagnostics, Uppsala, Sweden).

Four phenotypes were studied:

- Doctor-diagnosed asthma: subjects were considered asthmatics if they affirmatively answered both the following questions: ‘Have you ever had asthma?’ and ‘Was this (asthma) confirmed by a doctor?’.
- BHR: subjects were defined as bronchial hyperreactive when their $PD_{20} < 2000 \mu g$ (12).
- Allergic sensitization was considered present if a value $> 0.35 \text{ kU/l}$ (the lowest detection limit of the assay), for at least one specific allergen, was found.
- Total IgE, which has been dichotomized as $\leq 100 \text{ kU/l}$ and $> 100 \text{ kU/l}$ values.

Single nucleotide polymorphism genotyping

DNA was isolated from peripheral white blood cells using Qiamp (Qiagen, Hilden, Germany) or Puregene isolation kits (Gentra Systems, Minneapolis, MN). Genotyping was performed using MALDI-TOF mass spectrometry of allele-specific primer extension products, generated from previously amplified DNA sequences (MassARRAY; SEQUENOM Inc., San Diego, CA). Primers were obtained from Metabion GmbH (Planegg-Martinsried, Germany). A detailed description of assays and primers can be found in the article by Gohlke et al. (2).

Statistical analyses

All SNPs that showed a significant association in both the German and Italian samples of the cited work were considered for the analysis (2) (Fig. 1). Within these SNPs, we retain only those with a call rate $\geq 60\%$ (‘call rate’ was defined as the percentage of subjects for whose genotypes could be unequivocally determined). Hardy–Weinberg equilibrium (HWE) has been tested: SNPs for whose HWE hypothesis was not rejected ($1\% \leq \alpha < 5\%$) were considered only for single SNP association analysis but not for haplotype reconstruction. Linkage disequilibrium (LD) was studied estimating pairwise correlation and Lewontin’s D' statistic (13). Single nucleotide polymorphisms were tested for hidden subpopulation clusters by using the method of Pritchard et al. (14).

Single nucleotide polymorphism-trait association was studied after categorizing genotypes as carrier of the rare allele vs homozygous noncarrier. Three potential confounders were accounted for: age ($< 40, \geq 40$), gender (male, female) and centre (Erfurt, Hamburg).

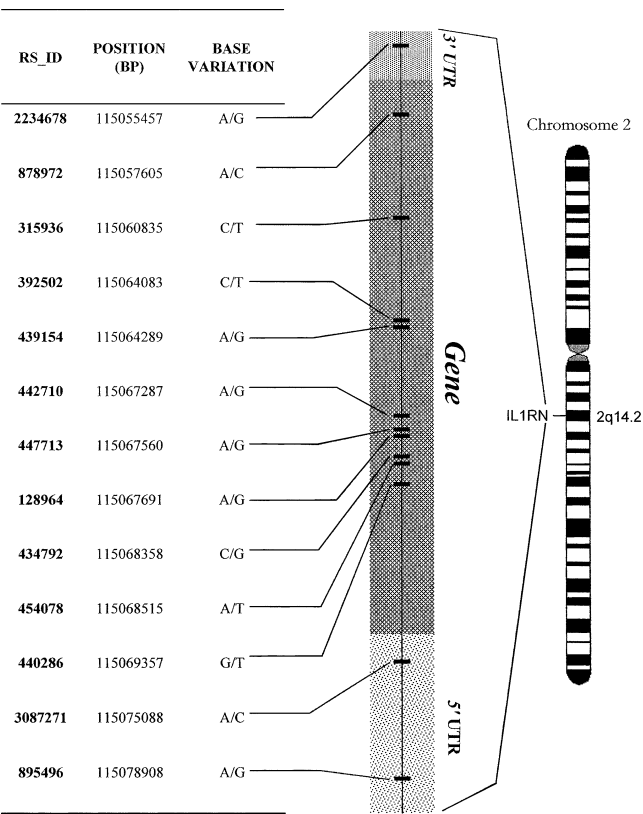


Figure 1. Genomic location of interleukin-1 receptor antagonist gene (*IL1RN*) on the second chromosome and of the 13 single nucleotide polymorphisms (SNPs) selected for the analysis (RS_ID: reference SNP identification number; BP: base pair).

Individual haplotypes and their sample frequencies were estimated with an algorithm for haplotype reconstruction (15, 16), where each subject was assigned the haplotype with the highest probability, and only haplotypes with expected frequency $> 2\%$ were considered for association analysis. For each haplotype, traits distributions within carriers (subjects with at least one copy of the haplotype) and noncarriers were compared by Fisher’s exact test.

False discovery rate (FDR) procedure was used to control for multiple testing within each phenotype. After sorting P -values in ascending order, the j th null hypothesis was rejected if j th $P \leq j \times \alpha / m$, where m was the number of tests, $j = 1, \dots, m$ and $\alpha = 0.05$ (17). The statistical software R 2.0.0 was used for the analyses (18).

The current analysis supersedes a previous communication at the Congress of the Human Society of Human Genetics 2003 (where SNP labels were wrongly assigned).

Results

For 551 subjects, we collected complete genotype and at least one phenotype information. Participants were equally distributed across the two cities (50.1% from Hamburg), 291 were males (52.6%) and 205 were < 40 years (37.2%).

Twenty-five subjects (4.5%) reported doctor-diagnosed asthma, 168 (30.8%) were sensitized, 121 (22.2%) had high total serum IgE level and 91 of 489 (18.6%) were affected by BHR.

Genomic position and base variation of selected SNPs are depicted in Fig. 1. Single nucleotide polymorphisms rs878972, rs454078, rs895496 and rs392503 were not in HWE, thus were not considered in the subsequent analyses. Single nucleotide polymorphism rs447713 was considered only for univariate association tests. The distribution of all SNPs is shown in Table 1.

Age and study centre did not affect the distribution of genotypes, while a significant association between gender and SNPs rs2234678, rs315936, rs128964 and rs434792 had been observed (P -value ranging from 0.003 to 0.03). Nevertheless, gender never affected the relationship between phenotypes and SNPs, thus only unadjusted associations were reported.

The rare G allele at position 115 067 560 (SNP rs447713) was associated with lower prevalence of doctor-diagnosed asthma ($P = 0.0013$) and allergic sensitization ($P = 0.0119$). Due to the low number of subjects with doctor-diagnosed asthma, we controlled the posterior power of the Fisher's test: it was almost close to 1 and 0.74 for the two tests respectively. At position 115 075 088 (SNP rs3087271), the rare C allele was associated with lower prevalence of doctor-diagnosed asthma ($P = 0.0227$) and total serum IgE high level ($P = 0.0232$). Power of the test was 0.58 and 0.65 respectively. No association was observed between single SNPs and BHR (Table 2).

Strong LD between SNPs was observed (Fig. 2): D' lies close to 1 for all the pairs of SNPs within the exon borders, while it was lower between SNPs inside and those outside exon borders, giving an overall block-type

structure. No subpopulations stratification could be observed (data not reported).

Haplotypes were estimated within SNPs rs2234678, rs315936, rs439154, rs442710, rs128964, rs434792, rs440286, rs3087271 and the distribution is reported in Table 3 together with the results of association analysis. Subjects carrying the haplotype A-C-A-G-A-C-G-A had a higher prevalence of doctor-diagnosed asthma (odds

Table 2. Distribution of disease traits across genotypes: prevalence (expressed as percentages) and Fisher's exact test P -values

SNPs		Doctor-diagnosed asthma		Allergic Sensitization		High level of total IgE		BHR	
rs_id	Genotype	%	P -value	%	P -value	%	P -value	%	P -value
2234678	A/A	5.9	0.1030	31.1	0.8522	23.0	0.6783	19.1	0.8153
	* /G	2.9		30.3		21.2		18.0	
315936	C/C	5.5	0.2059	31.1	0.8482	23.8	0.2411	20.1	0.3352
	* /T	2.9		30.2		19.3		16.1	
439154	G/G	4.8	0.8341	29.1	0.5663	20.4	0.4586	18.4	1.0000
	* /A	4.4		31.8		23.2		18.8	
442710	G/G	5.2	0.4103	32.4	0.3457	23.8	0.2954	17.9	0.6369
	* /A	3.6		28.4		19.8		19.7	
447713	A/A	6.0	0.0013	33.6	0.0119	23.3	0.3317	19.5	0.4203
	* /G	0.0		21.7		18.6		16.0	
128964	A/A	5.7	0.2175	32.2	0.4567	23.5	0.4690	19.0	0.9071
	* /G	3.2		29.0		20.6		18.2	
434792	C/C	5.6	0.2179	31.8	0.6415	23.4	0.4696	19.6	0.5601
	* /G	3.2		29.6		20.6		17.4	
440286	G/G	5.7	0.1421	31.0	0.9244	24.1	0.2053	20.7	0.1526
	* /T	2.8		30.4		19.2		15.3	
3087271	A/A	6.5	0.0227	34.0	0.0945	26.0	0.0232	18.5	1.0000
	* /C	2.3		27.1		17.8		18.7	

rs_id, reference SNP identification number; BHR, Bronchial Hyperreactivity. Values in bold indicate $P < 0.05$.

Table 1. Genotype distribution: the SNPs have been sorted by their position in the IL1RN gene

rs_id	Call rate (%)*	Rare allele frequency	Genotype distribution (%)**	HWE test†
2234678	98	0.25(G)	55/39/06	0.4273
878972	17	0.07(C)	90/05/05	<0.0001
315936	87	0.24(T)	59/36/06	0.9003
392503	94	0.30(C)	13/34/53	<0.0001
439154	96	0.41(A)	16/49/35	0.6524
442710	88	0.26(A)	06/39/55	0.6378
447713	61	0.20(G)	66/28/06	0.0166
128964	97	0.26(G)	54/41/06	0.3161
434792	97	0.26(G)	54/40/06	0.3164
454078	68	0.32(T)	55/26/19	<0.0001
440286	91	0.25(T)	57/37/06	0.9044
3087271	95	0.28(C)	50/43/06	0.1035
895496	41	0.38(A)	23/29/47	<0.0001

rs_id, reference SNP identification number; HWE, Hardy–Weinberg Equilibrium.

*Call rate: percentage of SNPs clearly recognized from the genotyping procedure.

**Could not sum to 100, due to rounding.

† P -value

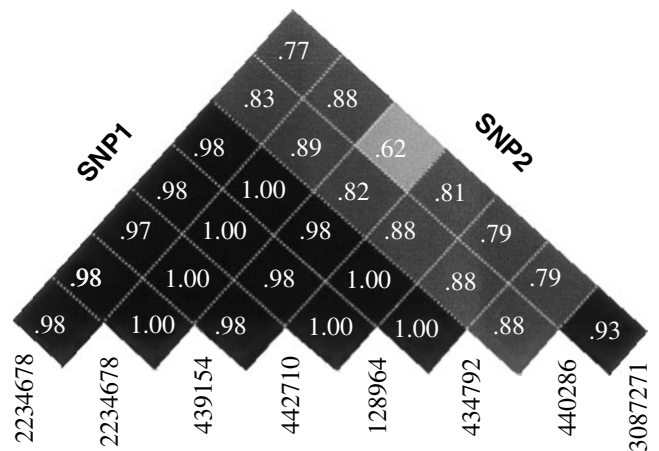


Figure 2. Linkage disequilibrium plot of the eight single nucleotide polymorphisms (SNPs) in Hardy–Weinberg equilibrium (HWE): SNP reference numbers are reported on the horizontal axis; each square represents the value of the Lewontin's D' statistics for the related couple of SNPs (black, $D' \geq 0.9$; grey, $0.7 \leq D' < 0.9$; light grey, $D' < 0.7$).

Table 3. Distribution of the most frequent, estimated haplotypes from the eight selected SNPs, and association with phenotypes

Haplotype*	Expected frequency (%)†¶	Observed frequency (%)‡	Doctor-diagnosed asthma			Allergic Sensitization			Total IgE			Bronchial Hyperreactivity		
			HNC	HC	P-value§	HNC	HC	P-value§	HNC	HC	P-value§	HNC	HC	P-value§
ACGGACGA	53.6	80.6	6.5	4.0	0.2985	34.9	29.8	0.3483	28.3	20.7	0.0922	17.5	18.9	0.8843
GTAAGGTA	21.3	39.7	6.0	2.3	0.0576	31.9	38.2	0.5078	24.6	18.4	0.0930	20.7	15.4	0.1548
ACAGACGA	13.5	24.0	3.1	9.1	0.0072	28.4	38.2	0.0393	19.8	29.8	0.0215	17.7	21.4	0.4141
ACGGACGC	3.6	6.9	4.7	2.6	1.0000	31.7	18.4	0.1017	22.6	15.8	0.4196	17.4	34.3	0.0220
ACGGACTC	2.2	4.2	4.7	0.0	0.6166	30.7	31.8	1.0000	21.9	27.3	0.6000	19.4	0.0	0.0340

HC, Haplotype Carriers; HNC, Haplotype Non-Carriers. Values in bold indicate $P < 0.05$.

*Only haplotypes with expected frequency $>2.0\%$ have been considered.

†Estimated with the method by Stephens et al. (15).

‡Percentage of subjects carrying at least one such haplotype.

§Fisher's exact test.

¶Total value = 94.22.

ratio [OR] = 3.12; 95%CI: 1.26–7.63), allergic sensitization (OR = 1.55; 95%CI: 1.00–2.39) and a high level of IgE (OR = 1.72; 95%CI: 1.07–2.74). Haplotype A-C-G-G-A-C-G-C was associated with a higher prevalence of BHR ($P = 0.0220$), while haplotype A-C-G-G-A-C-T-C was associated with a lower prevalence of BHR ($P = 0.0340$).

To better understanding the result of BHR, the joint distribution of BHR and haplotypes was studied: 34 subjects had at least one copy of the A-C-G-G-A-C-G-C haplotype (BHR prevalence = 0.35; 95%CI: 0.20–0.54), 19 had at least one copy of the A-C-G-G-A-C-T-C (none of them had BHR) and 435 had no one of the two haplotypes (BHR prevalence = 0.18; 95%CI: 0.15–0.22). Overall Fisher's exact test P -value was 0.004. The OR of having BHR for A-C-G-G-A-C-G-C carriers vs who had no one of the two haplotypes was 2.45 (95% CI: 1.06–5.43). Because we had no cases of BHR within the A-C-G-G-A-C-T-C carriers, OR for this haplotype could not be estimated.

When controlling for multiple testing we found that doctor-diagnosed asthma was associated to SNP rs447713 even under FDR restriction (minimum threshold for nine tests = 0.0055), but not to rs3087271 (threshold for the second of nine tests = 0.017). The association between asthma and haplotype A-C-A-G-A-C-G-A was confirmed.

Discussion

The results of this unselected population-based study highlight again that genetic variants of *IL1RN* are associated with asthma, and they are likely related to atopy and BHR. The *IL1RN* seems to act as a modulator of several associated traits: in fact, all the considered phenotypes were associated with different haplotypes. Our results, therefore, confirm the association previously reported (2).

The strength of our analysis was that we provided an unbiased sample of the general 20–44 years old German

population. The sample was obtained in the framework of the ECRHS (8), providing qualified information on asthma and related phenotypes.

We have found a significant relationship between the SNPs rs447713 and rs3087271 and allergy-related traits. The presence of the rare allele G of rs447713 was negatively associated with asthma and it seemed to be associated also with allergic sensitization. Although the high number of missing values and lack of HWE of this SNP might suggest that these associations might be spurious, the power of the test was high, thus suggesting that rs447713 may indicate the structural or functional involvement of *IL1RA*. The more rare C allele of rs3087271 was weakly related to asthma and high levels of total IgE. Thus, as frequently found with other complex diseases, the SNP by SNP analysis does not reveal strong association between traits and genotypes.

Haplotype A-C-A-G-A-C-G-A seems to play a role for atopic diseases with a major effect on asthma. The association with asthma was confirmed also after FDR adjustment, confirming the results of Gohlke et al. (2). A weaker but consistent, positive association was observed with allergic sensitization and IgE levels.

Bronchial hyperreactivity was negatively associated with the haplotype A-C-G-G-A-C-T-C and positively with the A-C-G-G-A-C-G-C. This finding would suggest – with some caution – that the same gene, depending from the specific pattern of its SNPs, could drive the development of very different traits. In particular case of BHR, both significant haplotypes had the pattern A-C-G-G-A-C*-C, where the SNP at the seventh position (rs440286) could drive to a risk (G) or protective (T) effect on BHR. This finding could highlight a kind of interaction that would not be obvious with single SNP analysis.

Results have been controlled for the FDR in order to better evaluate the strength of observed associations. In any case, the problem of multiple comparisons exists. Some authors (19) suggested to account for the number of tests on the genome on a world-wide scale, thus setting the significance levels at very small values, such as 2×10^{-5} or 2×10^{-8} . Thomson (20) noted that studies on complex

diseases have to be mainly regarded as exploratory data analyses, and multiple comparison correction could be inappropriate, because the goal is the identification of all possible susceptible loci, so that the control of false positives is not the main outcome criteria. A recent commentary (21) underlined that multiple comparison methodologies could lead to miss important findings. As a consequence, the FDR cut-off was not the only criteria for deciding in favour or against the validity of our results: other important information, like the relationship between different traits, was considered.

When testing a subset of 380 children in our recent family study (2) for serum levels of IL1RA (data not reported), none of the current SNPs had a significant increasing or decreasing effect on ILRA. This is could be because of different aspects.

First reason may be the true absence of any relationship between the SNPs that we considered and the serum levels, as the functional variant is currently not known. A second one could be the counter-regulative mechanism that is already in place, as the IL-1 systems are highly balanced. In fact, we analysed SNPs in *IL1RN* gene only: the combination with SNPs from the IL-1 α and IL-1 β would enable to study more complex haplotypes and taking into account the interaction effect that the three genes play on the inflammatory process.

A third reason is the complexity of the IL1RA, which includes one secreted (sIL1RA) and three intracellular isoforms (icIL1RA1, icIL1RA2 and icIL1RA3) (22) that have not been tested so far in this context. Their biological effects are not completely clear, but it is known that they can act in different ways. For example, the inhibition effect of icIL1RA3 on IL-1 is much weaker than the other two intracellular isoforms (23). In addition, it is worth noting that the finding of statistically significant different levels in the expression of IL1RA could not be enough for supporting an association with the disease: for the success of the inhibition process, the amount of IL1RA must be 100-fold greater than the amount of IL-1 (24).

Finally, any absent association can be explained simply by the low power of this pilot study that lead us to define only four general phenotypes. The phenotype which has the strongest association with the genotypes was 'doctor-diagnosed asthma', which included subjects who reported to have had asthma in their life and with a doctor diagnosis. But, with respect to the age of onset, asthma presents two different forms: the first one occurring in the

early childhood and affecting mainly males, and the second one occurring later in the life and affecting mainly females (25). Thus, the level of the IL1RA/IL-1 ratio should not be the same along all the course of the life and its relationship with the stable genotype in the *IL1RN* could sometimes be misleading. In addition, it has been observed that IL-1 is not so important in the already established situation but plays a critical role in the initiation of Th2 conditions (26). Gender-related differential levels of IL1RA, IL-1 β and IL1RA/IL-1 β were reported in a set of patients investigated for IgA nephropathy and Henoch-Schönlein nephritis (27).

As well as we could not distinguish early from late onset asthmatics, we could neither split the population into mutually exclusive phenotypes nor consider phenotypes derived from the combination of the four major ones (and, for example, disentangle atopic and nonatopic asthmatics). In a later step, this could be expanded in a further analysis, thus comparing and sorting the effects of gene mutations on the expression of different traits. We could expect that we could find much strong evidence of association of *IL1RN* with some of them, while this association would disappear with others.

In summary, SNPs and haplotypes of the *IL1RN* gene have an effect on asthma prevalence. Our results further confirm the importance of studying interaction between SNPs at different loci. In the special case of *IL1RN* gene, the additional finding that different haplotypes, based on the same, limited set of SNPs, were associated with different traits suggests that *IL1RN* acts as a modulator of different expressions of atopic diseases. Further research is necessary for exploring the different gametic patterns leading to different and more specific traits. In this sense, useful contribution could be derived from genetic epidemiology as well as from the study of biological interaction in the interleukin-1-induced inflammatory pathways.

Acknowledgments

We thank the study nurses M. Langelotz, H. Janiki and B. Manuwal for clinical examinations. We also wish to thank Prof. Helgo Mognussen, Dr Kai Richter and Prof. H. Erich Wichmann for the support. This study was supported by the German Research Foundation (DFG) contract numbers FR 1526/1-1, HE 3294/3-1, GSF FE 73922 and BMBF National Genome Network UW S1JT01.

References

1. Dinarello CA, Wolff SM. The role of interleukin-1 in disease. *N Engl J Med* 1993;**328**:106-113.
2. Gohlke H, Illig T, Bahnweg M, Klopp N, André E, Altmüller J et al. Association of the interleukin-1 receptor antagonist gene with asthma. *Am J Respir Crit Care Med* 2004;**169**:1217-1223.
3. Pillay V, Gaillard MC, Halkas A, Song E, Dewar JB. Differences in the genotypes and plasma concentrations of the interleukin-1 receptor antagonist in black and white South African asthmatics and control subjects. *Cytokine* 2000;**12**:819-821.

4. de Kluijver J, Grunberg K, Pons D, de Klerk EP, Dick CR, Sterk PJ et al. Interleukin-1beta and interleukin-1ra levels in nasal lavages during experimental rhinovirus infection in asthmatic and non-asthmatic subjects. *Clin Exp Allergy* 2003;**33**:1415–1418.
5. Mao XQ, Kawai M, Yamashita T, Enomoto T, Dake Y, Sasaki S et al. Imbalance production between interleukin-1beta (IL-1beta) and IL-1 receptor antagonist (IL-1Ra) in bronchial asthma. *Biochem Biophys Res Commun* 2000;**276**:607–612.
6. Joos L, McIntyre L, Ruan J, Connett JE, Anthonisen NR, Weir TD et al. Association of IL-1beta and IL-1 receptor antagonist haplotypes with rate of decline in lung function in smokers. *Thorax* 2001;**56**:863–866.
7. Joki-Erkila VP, Karjalainen J, Hulkkonen J, Pessi T, Nieminen MM, Aromaa A et al. Allergic rhinitis and polymorphisms of the interleukin 1 gene complex. *Ann Allergy Asthma Immunol* 2003;**91**:275–279.
8. Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. *Eur Respir J* 1994;**7**:954–960.
9. Nowak D, Heinrich J, Jorres R, Wassmer G, Berger J, Beck E et al. Prevalence of respiratory symptoms, bronchial hyperresponsiveness and atopy among adults: west and east Germany. *Eur Respir J* 1996;**9**:2541–2552.
10. Heinrich J, Nowak D, Wassmer G, Jorres R, Wjst M, Berger J et al. Age-dependent differences in the prevalence of allergic rhinitis and atopic sensitization between an eastern and a western German city. *Allergy* 1998;**53**:89–93.
11. Wassmer G, Jorres RA, Heinrich J, Wjst M, Reitmeir P, Wichmann HE. The association between baseline lung function and bronchial responsiveness to methacholine. *Eur J Med Res* 1997;**2**:47–54.
12. de Marco R, Bugiani M, Zanolin E, Verlato G, Rijken B. Doses and models in risk assessment analysis for bronchial hyperresponsiveness. *J Clin Epidemiol* 1998;**51**:19–28.
13. Ott J. Analysis of human genetic linkage, 3rd edn. Baltimore, MD: The Johns Hopkins University Press, 1999.
14. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics* 2000;**155**:945–959.
15. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 2001;**68**:978–989.
16. Stephens M, Donnelly P. A comparison of Bayesian methods for haplotype reconstruction. *Am J Hum Genet* 2003;**73**:1162–1169.
17. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B* 1995;**57**:289–300.
18. R Development Core Team. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2004.
19. Colhoun HM, McKeigue PM, Davey Smith G. Problems of reporting genetic associations with complex outcomes. *Lancet* 2003;**361**:865–872.
20. Thomson G. Significance levels in genome scans. In: Rao DC, Province MA, editors. Genetic dissection of complex traits. San Diego, CA: Academic Press, 2001:475–486.
21. Devlin B, Roeder K, Wasserman L. False discovery or missed discovery? *Heredity* 2003;**91**:537–538.
22. Arend WP, Guthridge CJ. Biological role of interleukin 1 receptor antagonist isoforms. *Ann Rheum Dis* 2000;**59**(Suppl. 1): i60–i64.
23. Malyak M, Smith MF, Abel AA, Hance KR, Arend WP. The differential production of three forms of IL-1 receptor antagonist by human neutrophils and monocytes. *J Immunol* 1998;**161**:2004–2010.
24. Arend WP, Welgus HG, Thompson RC, Eisenberg SP. Biological properties of recombinant monocyte-derived interleukin 1 receptor antagonist. *J Clin Invest* 1990;**85**:1694–1697.
25. de Marco R, Locatelli F, Cerveri I, Bugiani M, Marinoni A, Giammanco G. Incidence and remission of asthma: a retrospective study on the natural history of asthma in Italy. *J Allergy Clin Immunol* 2002;**110**:228–235.
26. Manetti R, Barak V, Piccinni MP, Sampognaro S, Parronchi P, Maggi E et al. Interleukin-1 favours the in vitro development of type 2 T helper (Th2) human T-cell clones. *Res Immunol* 1994;**145**:93–100.
27. Rauta V, Teppo AM, Tornroth T, Honkanen E, Gronhagen-Riska C. Lower urinary-interleukin-1 receptor-antagonist excretion in IgA nephropathy than in Henoch-Schonlein nephritis. *Nephrol Dial Transplant* 2003;**18**:1785–1791.