ONLINE MUTATION REPORT

A common IL-1 complex haplotype is associated with an increased risk of atopy

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The interleukin (IL)-1 gene cluster within chromosome 2 contains genes coding for both anti- and proinflammatory cytokines, including IL-1alpha, IL-1beta, IL-1Ra, and several novel cytokines designated IL-1F5 - IL-1F10.[1] Proinflammatory cytokines IL-1alpha and beta are involved in the enhancement of inflammation and host defence. Anti-inflammatory IL-1Ra counteracts the function of IL-1alpha and IL-1beta. These cytokines are produced by a variety of cell types, for example, monocytes, magrophages, and keratinocytes. All IL-1 family genes are polymorphic and several of these polymorphisms have been shown to be associated with either susceptibility to or severity of inflammatory conditions and diseases.[2]

Atopy is a familial syndrome underlying atopic eczema, allergic rhinoconjuctivitis, and bronchial asthma. The skin prick test (SPT) is used to examine specific IgE mediated allergic responses and the results are generally in line with anamnestic data on atopy.[3] Both IL-1alpha and beta have been shown to play a role in the regulation of allergen induced early and late reactions.[4 5] IL-1alpha has also been found to accelerate cutaneous inflammation,[6] whereas IL-1beta is involved in the regulation of contact sensitisation.[7] In asthmatic bronchial epithelium, the expression of both IL-1beta and IL-1Ra is increased.[8]

Cytokines function as a network and thus the net effect on allergic inflammation may be dependent on allele combinations of individual cytokine genes. Recently in our population based case control study, we showed that *IL1A* (SNP +4845) genotype influences the SPT results.[9] The carriers of the rarer allele 2 had a significantly lower SPT positive rate than that of non-carriers. Now we have expanded the study by analysing two additional SNPs (*IL1B* SNP +3954 C>T and *IL1RN* VNTR in intron 2) in the IL-1 gene cluster from the same cohort.

MATERIALS AND METHODS

Altogether 254 females and 151 males (mean age 60 years, age range 31-89 years) participated in the study. The subjects studied were the controls of a Finnish population based case-control study conducted to investigate the risk factors of adult asthma. More detailed information on the subjects is described elsewhere.[9] All subjects gave informed consent for participation. Approval for this study was obtained from the ethical committee of Tampere University Hospital.

Atopy was determined by skin prick test 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 with both a negative control (saline) and a positive control (histamine) (ALK A/S, Copenhagen, 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 99.3% of subjects (252 females and 150 males).

Key points

- We have recently shown that a single nucleotide polymorphism (SNP) at position +4845 (G>T) of the interleukin 1A (*IL1A*) gene is associated with reactivity in the skin prick test (SPT); the carriers of the rarer allele (allele 2) had a significantly lower SPT positive rate than that of non-carriers.

- To examine whether the allele combinations or haplotypes of this complex would mediate functionally different responses, we now genotyped two additional polymorphisms of the IL-1 gene complex.

- It was shown that in addition to *IL1A**1 homozygosity, *IL1B**1 homozygosity, and *IL1RN**2 homozygosity were associated with a higher risk of SPT positivity (OR 1.62 (95% confidence intervals 1.070 to 2.460) and 2.69 (1.320 to 5.470) respectively). The genotype combination containing homozygotes of *IL1A**1, *IL1B**1, and *IL1RN**2 was associated with the highest risk of SPT+ (OR 4.07 (1.720 to 9.630)). Moreover, haplotype analysis with expectation maximisation algorithm showed that *IL1A**1, *IL1B**1, and *IL1RN**2 belonged to the same haplotype and its presence increased the risk of SPT positivity (OR 1.80 (1.290 to 2.530)).

- We conclude that the *IL1A**1/*IL1B**1/*IL1RN**2 haplotype contains the decisive allele or allele combinations which increase the risk of atopy.

Genomic DNA was isolated from EDTA whole blood samples using standard methods. *IL1A* SNP+4845 (G/T polymorphism in exon 5; G is marked as allele 1 and T is marked as allele 2), *IL1B* SNP +3954 (C/T polymorphism; C is marked as allele 1 and T is marked as allele 2), and *IL1RN* VNTR (86 bp repeat sequence in intron 2; dup 2-5) were analysed as described previously.[9–11]

To detect a possible deviation of the genotype frequencies from the Hardy-Weinberg equation, the exact test using the Markov chain algorithm was used (Arlequin program, version 2.0. *A software for population genetics data analysis.* Schneider S, Roessli D, Excoffier L, Genetics and Biometry Laboratory, Geneva, Switzerland). The haplotypes and their frequencies were calculated according to genotyped data using the expectation maximisation (EM) algorithm (Arlequin program). This approach has been shown to have high predictive value and it is in good concordance (90-98%) with pedigree data or simulated sample set analysis.[12 13] The genotype distributions

Table 1 Genotypes of the IL-1 complex genes (*IL1A* SNP +4845, *IL1B* SNP +3954, and *IL1RN* VNTR) in skin prick test negative (SPT−) and skin prick test positive (SPT+) subjects. *IL1A* SNP +4845 (allele 1 is G, allele 2 is T), *IL1B* SNP +3954 (allele 1 is C, allele 2 is T), *IL1RN* VNTR (IVS2, 86 bp dup 2–5)

	All No (%)	SPT− No (%)	SPT+ No (%)	OR (95% CI)
IL1A				
1.1	184 (46.3)	99 (40.1)	85 (56.7)	**1.93 (1.280 to 2.900)**
1.2	166 (41.8)	116 (47.0)	50 (33.3)	**0.60 (0.367 to 0.852)**
2.2	47 (11.8)	32 (13.0)	15 (10.0)	0.74 (0.387 to 1.420)
IL1B				
1.1	224 (56.3)	128 (51.8)	96 (63.6)	**1.62 (1.070 to 2.460)**
1.2	143 (35.9)	98 (39.7)	45 (29.8)	**0.65 (0.419 to 0.994)**
2.2	31 (7.8)	21 (8.5)	10 (6.6)	0.76 (0.349 to 1.670)
IL1RN				
1.1	200 (50.4)	129 (52.4)	71 (47.0)	0.81 (0.541 to 1.220)
1.2	146 (36.8)	93 (37.8)	53 (35.1)	0.90 (0.587 to 1.370)
2.2	35 (8.8)	14 (5.7)	21 (13.9)	**2.69 (1.320 to 5.470)**
1.3	13 (3.3)	8 (3.3)	5 (3.3)	1.02 (0.328 to 3.190)
2.3	3 (0.8)	2 (0.8)	1 (0.7)	0.81 (0.073 to 9.080)

No=number of patients, CI=confidence intervals.

did not deviate from expected under the Hardy-Weinberg equation (HWE), which is a prerequisite for EM algorithm based haplotype analysis. Odds ratios and their 95% confidence intervals (CI) were calculated using CIA software (version 1.1, copyrighted by M J Gardner and British Medical Journal, 1989). Linkage disequilibrium (LD) between each locus was calculated as described previously.[14] The theoretical maximum D′ values between loci with alleles A/a and B/b (allele frequencies C_A, C_a, C_B, and C_b and haplotype frequencies C_{AB}, C_{Ab}, C_{aB}, and C_{ab}) were obtained by dividing C_{AB}-$C_A C_B$ by min ($C_A C_b$, $C_a C_B$), if D>0, and min ($C_A C_B$, $C_a C_b$), if D <0. The likelihood ratio test was used to assess the significance of the linkage between loci.

RESULTS

The percentage of skin test positive subjects was 38.0. Participants were divided into SPT+ subjects (that is, ≥1 positive reaction) and SPT− subjects. The genotypic data of these three SNPs is shown in table 1. *IL1A* genotype 1.1, *IL1B* 1.1, and *IL1RN* 2.2 were associated with an increased risk of SPT positivity, whereas *IL1A* genotype 1.2 and *IL1B* 1.2 were associated with diminished risk. After combining *IL1A*, *IL1B*, and *IL1RN* genotypes, it appeared that the distribution of genotype combinations differed significantly between SPT− and SPT+ groups (p<0.001, chi-square test, df=18). The genotype combination 1.1, 1.1, 2.2 markedly increased the risk of skin test positivity (OR 4.07 (95 % CI 1.720 to 9.630)). The opposite

Table 2 Genotype combinations and haplotypes of (*IL1A* SNP +4845, *IL1B* SNP +3954, and *IL1RN* VNTR) in skin prick test negative (SPT−) and skin prick test positive (SPT+) patients. *IL1A* SNP +4845 (allele 1 is G, allele 2 is T), *IL1B* SNP +3954 (allele 1 is C, allele 2 is T), *IL1RN* VNTR (IVS2, 86 bp dup 2–5). Genotype combinations containing 1.1, 1.2 and 2.2 (but not 1.3) are presented

Genotype combinations			All		SPT−		SPT+		
IL1A	*IL1B*	*IL1RN*	No	%	No	%	No	%	OR (95% CI)
1.1	1.1	1.1	72	18.9	43	18.2	29	20.1	1.13 (0.670 to 1.910)
1.2	1.1	1.1	18	4.7	11	4.7	7	4.9	1.05 (0.396 to 2.760)
1.1	1.2	1.1	3	0.8	0	0.0	3	2.1	–
1.2	1.2	1.1	69	18.2	51	21.6	18	12.5	**0.52 (0.289 to 0.928)**
2.2	1.2	1.1	14	3.7	8	3.4	6	4.2	1.24 (0.421 to 3.650)
1.2	2.2	1.1	2	0.5	2	0.8	0	0.0	–
2.2	2.2	1.1	22	5.8	14	5.9	8	5.6	0.93 (0.381 to 2.280)
1.1	1.1	1.2	67	17.6	39	16.5	28	19.4	1.22 (0.713 to 2.090)
1.2	1.1	1.2	19	5.0	14	5.9	5	3.5	0.57 (0.201 to 1.620)
2.2	1.1	1.2	2	0.5	2	0.8	0	0.0	–
1.1	1.2	1.2	3	0.8	2	0.8	1	0.7	0.82 (0.074 to 9.110)
1.2	1.2	1.2	46	12.1	29	12.3	17	11.8	0.96 (0.505 to 1.810)
2.2	1.2	1.2	3	0.8	3	1.3	0	0.0	–
1.1	2.2	1.2	1	0.3	1	0.4	0	0.0	–
1.2	2.2	1.2	1	0.3	0	0.0	1	0.7	–
2.2	2.2	1.2	4	1.1	3	1.3	1	0.7	0.54 (0.056 to 5.270)
1.1	1.1	2.2	26	6.8	8	3.4	18	12.5	**4.07 (1.720 to 9.630)**
1.2	1.1	2.2	7	1.8	5	2.1	2	1.4	0.651 (0.294 to 8.030)
2.2	1.2	2.2	1	0.3	1	0.4	0	0.0	–
Haplotypes									
1	1	1	327	41.0	206	41.8	121	40.3	0.94 (0.701 to 1.260)
1	**1**	**2**	**180**	**22.9**	**92**	**18.7**	**88**	**29.3**	**1.80 (1.290 to 2.530)**
2	2	1	180	22.8	122	24.8	58	19.3	0.73 (0.511 to 1.030)
2	1	1	44	5.7	27	5.4	17	5.8	1.03 (0.554 to 1.930)
2	**1**	**2**	**24**	**2.9**	**21**	**4.2**	**3**	**1.0**	**0.227 (0.0670 to 0.766)**
1	1	3	14	1.8	8	1.6	6	2.0	1.23 (0.424 to 3.590)
2	2	2	10	1.2	8	1.6	2	0.06	0.41 (0.085 to 1.920)
1	2	1	9	1.1	5	0.9	4	1.3	1.32 (0.351 to 4.940)

No=number of patients (in genotype combination)/chromosomes (in haplotypes), CI=confidence intervals.

effect was observed with the genotype combination –1.2, 1.2, 1.1 (table 2).

It has been shown that IL-1 complex loci are in strong linkage disequilibrium.[15] As expected, this was also true in our material. All the linkages between the pairs of loci (*IL1A-IL1B*, *IL1A-IL1RN*, *IL1B-IL1RN*) were significant in both groups. When LDs were compared between SPT+ and SPT– groups, a significant difference in *IL1A-IL1RN* linkage was observed (p<0.001, chi-square test, df=3). The linkage between *IL1A* and *IL1RN* was significantly stronger in SPT+ (D′= 0.87) than in SPT– (D′=0.40) groups, indicating that these three loci form haplotypes. When the haplotype frequency analyses were carried out with the EM algorithm, the haplotype distribution differed significantly between SPT– and SPT+ groups (p<0.001, chi-square test, df= 10). SPT positivity was associated with a common haplotype, *IL1A**1/*IL1B**1/*IL1RN**2 (table 2). A rare (2.9%) haplotype *IL1A**2/*IL1B**1/*IL1RN**2 markedly decreased the risk of SPT positivity.

DISCUSSION

In this study, we showed that the IL-1 gene complex on the whole has an effect on the risk of atopy. This report extends our previous finding[9] concerning the association of *IL1A* with the skin test responses for common allergens.

In the present study the alleles associated with an increased susceptibility for atopy, that is, *IL1A**1, *IL1B**1, and *IL1RN**2 were shown to belong to the same IL-1 gene complex haplotype. With respect to IL-1 family gene analysis, the analysis of haplotypes is essential. Owing to the unique structure of the IL-1 gene complex, each single haplotype will contain a distinct combination of alleles from genes with proinflammatory (*IL1A*, *IL1B*) and anti-inflammatory (*IL1RN*) significance. It is thus probable that each distinct haplotype will mediate inflammatory responses with individual strength and nature, and in the presence of co-dominance the net response of a subject will be dependent on the inherited combination of maternal and paternal haplotypes. However, at present the possibility that the observed effect is the result of an allele of an unknown gene locus located in this haplotype cannot be excluded.

The biological function of the individual IL-1 gene complex alleles studied here has been analysed extensively. In vitro findings have shown that allele 2 in *IL1A* (SNP –889 which is >99% disequilibrium with SNP +4845) increases the production of IL-1beta in healthy subjects,[16] whereas *IL1RN**2 has been associated with both high[17] and low[18] IL-1beta release depending on the study. In addition, *IL1RN**2 is associated with high levels of IL1Ra in plasma[17] but low levels produced by epithelial cells.[19] In these studies, however, the genetic linkage of IL-1 gene family members has not been taken into account. The biological significance of *IL1A* (SNP +4845), *IL1B* (SNP +3954), and *IL1RN* VNTR haplotype/genotype combination cannot be deduced from the published data.

It should be noted that although the odds ratio associated with the presence of the *IL1A**1, *IL1B**1, and *IL1RN**2 haplotype is low (1.80), it may be a substantial risk factor because of the high prevalence of this haplotype (22.9%). The risk of atopy in genotype combination was also shown to be greater than that of haplotypes. This is probably because of the dose effect. A protective haplotype was found in a small subgroup of subjects (2.9%). The importance of this rare haplotype, however, is doubtful owing to its low frequency in the general population.

According to current dogma, atopy is caused by an excessive Th2 response and its increase in developed countries has been attributed to the lack of Th1 inducing microbial contacts (the hygiene hypothesis). Yazdanbakhsh *et al*[20] have recently presented a model where the pro- versus anti-inflammatory axis is decisive in the formation of regulatory T cells which keep the inflammatory T cells (both Th1 and Th2) under control. This model explains some of the inconsistencies of the simple Th1/Th2 balance model. IL-1 is known to be one of the key inflammatory mediators in primary response to microbes. The genetic variability within the IL-1 gene complex could therefore affect this agonist/antagonist balance directly or via stimulation of other inflammatory cytokines.[21]

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