

BRIEF COMMUNICATION

Association between *IL-4* genotype and *IL-4* production in the Japanese population

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We have identified that there are only two *IL-4* gene haplotypes (I and II) in the Japanese population. There are significant differences among three genotypes (I/I, I/II and II/II) in the *IL-4* producing proportion of peripheral Th cells using intracellular cytokine detection assay. These results make it likely that *IL-4* genotype could influence the type of immune response.

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Within the Japanese population we have studied the distribution of a variable number of tandem repeat polymorphisms (VNTR) located within the third intron of the *IL-4* gene. Two alleles, B1 and B2, were characterized by two and three tandem repeats, respectively. We also found that there was a strong linkage disequilibrium between VNTR and single nucleotide polymorphisms (SNPs) within the *IL-4* gene promoter region. These results indicated that only two *IL-4* gene haplotypes, I and II, are present in the Japanese population. We also measured the proportion of peripheral Th cells which produced *IL-4* among each of the genotype I/I, I/II, and II/II. Interestingly, the percentage of II/II Th cells which produced *IL-4* was significantly lower than that of both I/I and I/II Th cells, suggesting that the haplotype I may allow for high *IL-4* production.

CD4⁺ helper T (Th) cells can be classified into two different subsets based on their patterns of cytokine production. Th1 cells produce mainly interferon- γ (IFN- γ) and interleukin-2 (IL-2) and promote cell-mediated immunity, whereas Th2 cells, which secrete *IL-4*, *IL-10* and *IL-13*, are associated with humoral immune responses and induce antibody production.¹ It has been demonstrated that an imbalance between Th1 and Th2 cytokine production is highly correlated with the induction and development of several autoimmune diseases. Correction of this Th1/Th2 imbalance has in fact led to the prophylaxis and therapy for such diseases in various models of autoimmunity.^{2–5}

During the differentiation of mouse naive CD4⁺ T cells, *IL-4* plays a necessary early role in the generation of *IL-*

4 producing effector cells *in vitro* and *in vivo*: *IL-4* is required for the subsequent appearance of *IL-4*-producing cells, and thus for Th2 lineage commitment.^{6–8} Furthermore Bix *et al*⁹ documented that *IL-4* gene allelic expression was regulated in a probabilistic manner and indicated the possibility that microenvironmental signals such as *IL-12* or *IL-4* could influence the prevalence of cells that express distinct cytokine patterns.

The *IL-4* gene is located on the long arm of chromosome 5, where it lies in close proximity to the genes for other Th2 cytokines such as *IL-5* and *IL-13*. We have studied the distribution among the Japanese population of a variable number of tandem repeat polymorphism (VNTR) with a unit size of 70 bp, located within the third intron of the *IL-4* gene.¹⁰ Allele-specific amplified DNAs were size-separated by 4% agarose electrophoresis and molecular genotyping of the VNTR polymorphism was performed. Two alleles were observed, B1 (183 bp) and B2 (253 bp), that are characterized by two and three tandem repeats, respectively. The allele and VNTR genotype frequencies in our population are strikingly different from those of the Caucasian population.^{11–14} The allele frequencies of B1 and B2 in the Japanese population as measured were 0.67 and 0.33, and the genotype frequencies for B1/B1, B1/B2, and B2/B2, were 0.45, 0.45 and 0.10, respectively (Table 1).

Within the *IL-4* promoter region the –590C/T and –34C/T SNPs were previously reported.^{15,16} Therefore we studied the association between VNTR polymorphism and these SNPs, and found that –590T, –34T and B1 are on haplotype I, while –590C, –34C and B2 are on the other (haplotype II). These results indicated that there are only two *IL-4* gene haplotypes within the Japanese population.

In order to assess the functional consequence of such haplotypes, we measured the *IL-4*-producing proportion of peripheral Th cells using an intracellular cytokine detection assay. The mean percentages of peripheral Th cells which produced *IL-4* were 3.83 ± 1.46 for I/I, 3.69 ± 0.87 for I/II, and 1.97 ± 0.65 for II/II. The value measured

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Table 1 Distribution of IL-4 VNTR allele and genotype in Japanese and other populations

	Japanese	Caucasian	Sardinian	Swedish Caucasian	Caucasian	French Caucasian
Allele						
B1	146 (0.67)	12 (0.11)	20 (0.12)	46 (0.22)	22 (0.09)	28 (0.14)
B2	70 (0.33)	116 (0.89)	144 (0.88)	168 (0.78)	224 (0.91)	180 (0.87)
Total	216 (1.00)	128 (1.00)	164 (1.00)	214 (1.00)	246 (1.00)	208 (1.00)
Genotype						
B1/B1	49 (0.45)	0	0	6 (0.05)	0	1 (0.01)
B1/B2	48 (0.45)	12 (0.19)	20 (0.24)	34 (0.32)	22 (0.18)	26 (0.25)
B2/B2	11 (0.10)	52 (0.81)	62 (0.76)	67 (0.63)	101 (0.82)	77 (0.74)
Total	108 (1.00)	64 (1.00)	82 (1.00)	107 (1.00)	123 (1.00)	104 (1.00)
Reference	This study	Vandenbroeck <i>et al</i> ¹¹		Huang <i>et al</i> ¹²	Cantagrel <i>et al</i> ¹³	Buchs <i>et al</i> ¹⁴

Values are numbers (frequencies).

for the II/II genotype is significantly lower than those of the other two. (Figure 1)

It was reported that -590T in the promoter region of the *IL-4* gene have been related to elevated of serum IgE; this locus has been associated with the diagnosis of asthma in some studies, but not in others.¹⁷⁻¹⁹ Burchard *et al*²⁰ have showed the association between -590T and asthma severity within the Caucasian population. This SNP is located upstream of all the known control element of IL-4, such as the negative regulatory element, the positive regulatory elements, the NF-Y recognition sequence, the OAP40 recognition sequence, the NF-P recognition sequence, and the TATA box, and affects IL-4 transcription activity.¹⁹ -590T promoter sequence showed greater

binding to nuclear transcription factors from allergen stimulated or Jurkat human T cells than that of -590C sequence, and alteration in electrophoretic mobility shift assay was observed.^{15,21}

On the other hand, there have been several reports of the association between the VNTR B1 allele and disease severity. For example, Vandenbroeck *et al*²² showed an association between B1 and delayed age at onset of multiple sclerosis (MS). Since cytokines produced by Th1 cells are known to promote the development of MS,²² it was suggested that increased responsiveness of IL-4 transcriptional activation due to the B1 allele may lead to overexpression of IL-4. Buchs *et al*¹⁴ demonstrated that the B1 allele may be a protective factor for severe joint destruction in rheumatoid arthritis (RA), showing that only patients with non-destructive arthritis had an increased carriage rate of the B1 allele. In fact local IL-4 treatment to collagen-induced murine arthritis prevents joint damage and bone erosion to an impressive extent.²³ Since these two reports have lacked -590SNP analysis the possibility of linkage disequilibrium between B1 and -590T cannot be excluded. It is possible that B1 may be nothing but the marker for -590T.

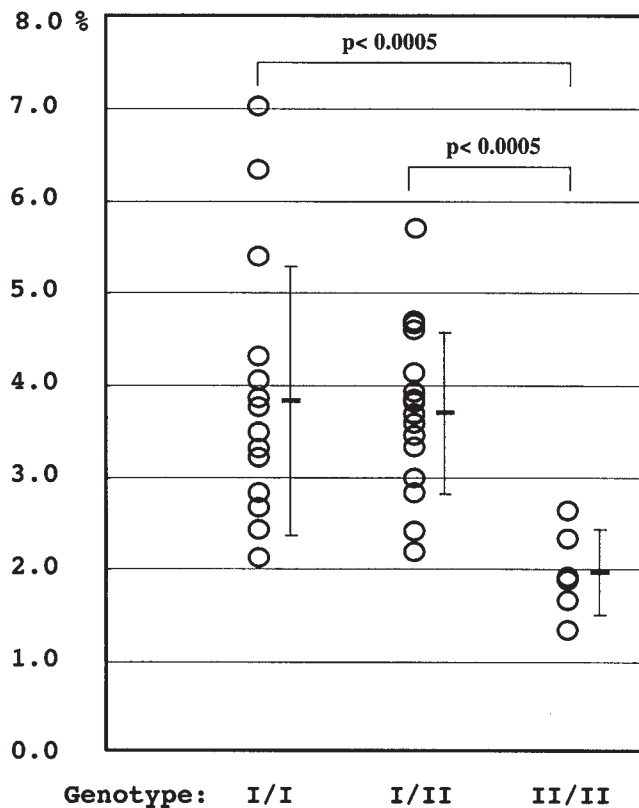


Figure 1 Relationship between IL-4 VNTR genotypes and the proportion of peripheral Th cells producing IL-4. Flow cytometric analysis of IL-4 expression in peripheral CD4⁺ T cells was performed as described previously.²²⁻²⁹ Briefly, aliquots (500 μ l) of heparinized whole blood were first stimulated with a combination of 25 ng/ml phorbol myristate acetate (PMA) and 1 μ g/ml of ionomycin in the presence of 10 μ g/ml of brefeldin A (Sigma, St Louis, MO, USA) and then cultured for 4 h at 37°C in a humidified incubator containing 7% CO₂. Activated cultures were stained with 20 μ l of peridinin chlorophyll protein conjugated CD4-specific monoclonal antibody (mAb) (Becton Dickinson, San Jose, CA, USA) for 15 min at room temperature, and then treated with 2 ml of FACS lysing solution (Becton Dickinson). After 5 min of incubation, the samples were centrifuged and treated with FACS permeabilization solution (Becton Dickinson) for 10 min at room temperature. The sample tubes were washed and incubated with phycoerythrin (PE)-conjugated IL-4 specific mAb (Becton Dickinson) for 30 min at room temperature. PE-conjugated mouse IgG1 were used as controls. After washing again, cells were resuspended in 1% paraformaldehyde and analyzed on a FACScan flow cytometer (Becton Dickinson). The resulting data files were analyzed using Cellquest software (Becton Dickinson). Significant differences by Student's *t*-test are shown.

A number of variables influence the type of immune response including antigen dose and mode of delivery, types of antigen presenting cells, the nature of stimulatory or costimulatory signals, and specific cytokines present in the developmental microenvironment.²⁴ The early IL-4 expression is a critical role in supporting the generation of IL-4-producing effector cells.^{25,26} The high IL-4 phenotype of BALB/c mice stems not from the elevated production of IL-4 per cell, but rather from an increase in the proportion of naive T cells that commit to IL-4 expression after T cell receptor activation.²⁵ The fact that a significance between genotypes was observed in the proportion of Th cells producing IL-4 makes it likely that *IL-4* genotype could influence the type of immune response.

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