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Genetic Variants of IL-13 and IL-4 in the Korean Population: Polymorphisms, Haplotypes and Linkage Disequilibrium

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Abstract

Asthma is an inflammatory airways disease characterized by bronchial hyperresponsiveness and airways obstruction, which results from a complex interaction of genetic and environmental factors. Interleukin (IL)-13 and IL-4 are important in IgE synthesis and allergic inflammation, therefore genes encoding IL-13 and IL-4 are candidates for predisposition to asthma. In the present study, we screened single-nucleotide polymorphisms (SNPs) in IL-13 and IL-4 and examined whether they are risk factors for asthma. We resequenced all exons and the promoter region in 12 asthma patients and 12 normal controls, and identified 18 SNPs including 2 novel SNPs. The linkage disequilibrium(LD) pattern was evaluated with 16 common SNPs, and haplotypes were also estimated within the block. Although IL-13 and IL-4 are localized within 27 kb on chromosome 5q31 and share many biological profiles, this region was partitioned into 2 blocks. One SNP and three SNPs were determined as haplotype-taggingSNPs (htSNPs) within IL-13 and IL-4 haplotype-block, respectively. No significant associations were observed between any of the SNPs or haplotypes and development of asthma in small number of Korean subjects. However, the genetic variants of IL-13 and IL-4 would provide valuable strategies for the genotyping studies in large population.

Keywords: Asthma, Haplotype, IL-4, IL-13, Linkage Disequilibrium

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Introduction

Asthma is a common respiratory disease characterized by intermittent airways obstruction and respiratory symptoms that are caused by acute and chronic bronchial inflammation (Renauld, 2001). The development of asthma appears to be determined by the interaction between host susceptibility and a variety of environmental exposures (Howard *et al.*, 2001). Linkage studies have mapped asthma susceptibility genes to a region on chromosome 5q31 containing a cluster of proinflammatory cytokines, including interleukin (IL)-4 and IL-13 (Howard *et al.*, 2001; Mansur *et al.*, 1998). The IL-13 gene is located 12 kb upstream of the IL-4 gene in a tail-to-head orientation. Both genes have been genetically and functionally implicated in the pathogenesis of asthma (Heinzmann *et al.*, 2000; Noguchi *et al.*, 2001; Walley and Cookson, 1996). These $CD4^+$ T-helper type 2 cytokines play crucial role in the allergic response by regulating the isotype switch of B cells from IgM to IgE synthesis (Punnonen *et al.*, 1994). They appear to share many, but not all, biological profiles, including IgE production, CD23 and MHC class Ⅱ expression, inhibition of antibody-dependent cell mediated cytotoxicity with downregulation of IgG receptor 1 (FcγRⅠ), and suppression of type Ⅰ interferon (Izuhara and Shirakawa, 1999; Shirakawa *et al.*, 2000). Although it has been previously demonstrated that genetic variants of IL-13 and IL-4 are associated with asthma or allergic disorders in some ethnic groups (Heinzmann *et al.*, 2000), real association with asthma in other population still remains to be elucidated. In this study, we screened genetic variants in IL-13 and IL-4, and examined their associations with development of asthma in the small number of Korean subjects.

Materials and Methods

Subjects

A total of 24 unrelated Korean individuals consisting of 12 asthma patients and 12 normal controls were used for SNP screening. All patients with asthma had currently one or more asthma symptoms and the physical examination compatible with asthma definition by the American Thoracic Society (1987). All studies were approved by the institutional review board of Korea

National Institute of Health, and written informed consent was obtained from all participants.

Resequencing and genotyping analysis

To identify common single nucleotide polymorphisms (SNPs) in IL-13 and IL-4, we resequenced the entire coding regions and approximately up to the ~0.5 kb proximal promoter regions in genomic DNA from 24 selected subjects. The each fragment amplified by PCR from genomic DNA was sequenced on both strands with an ABI Prism 3100 sequencer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. PolyPhred program (http://droog.gs.washington.edu/PolyPhred.html) was used to assemble the sequences and identify SNPs (Nickerson *et al.*, 1997). In addition to resequencing, we also selected 10 known SNPs of each IL-13 and IL-4 from NCBI dbSNP database and genotyped for the same subjects using TaqMan assay with an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The ethnic comparison of allele frequency was performed by searching JSNP database (http://snp.ims.u-tokyo.ac.jp/) and/or dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) database.

Statistical analysis

Deviation from Hardy-Weinberg expectancy was examined with χ^2 test. Lewontin's D' was used to determine whether the pairs of sites were in strong linkage disequilibrium (LD). Haplotypes were inferred using the PL-EM algorithm (http://www.people.fas.harvard.edu/~junliu/plem/) (Qin *et al.*, 2002). Program SNPtagger (http://www.well.ox.ac.uk/~xiayi/haplotype/index.html) was used to determine the haplotype-tagging SNPs (htSNPs) (Ke and Cardon, 2003). The frequencies of alleles, genotypes and haplotypes were compared between patients and controls by Fisher's exact test or χ^2 statistic, as appropriate, using SAS for windows V8 (SAS institute Inc., Cary, NC, USA). Statistical significance was inferred at a two-tailed value of $P < 0.05$.

Results and Discussion

Genetic variants of IL-13 and IL-4 in the Korean population

Resequencing and genotyping of IL-13 and IL-4 genomic DNA in 24 samples (including 12 asthma patients and 12 normal controls) identified 18 SNPs, including 2 novel SNPs, in the Korean population (Fig.1a and Table 1): 7 SNPs in IL-13 and 11 SNPs in IL-4, respectively. As shown in Table 1, there were substantial differences of allele frequencies among different ethnic groups. Furthermore, some SNPs with allele frequency up to 0.2 in Africans, selected from NCBI dbSNP database, showed monomorphic genotyping data in Koreans: rs3212145, rs2069746, rs2069749 and rs2069750 of IL-13; rs2243251, rs4986964 and rs2243286 of IL-4 (data not shown). These results reconfirmed that an extensive verification of public SNPs in a particular population studied should be undertaken prior to their association studies as previously reported (Lee *et al.*, 2003).

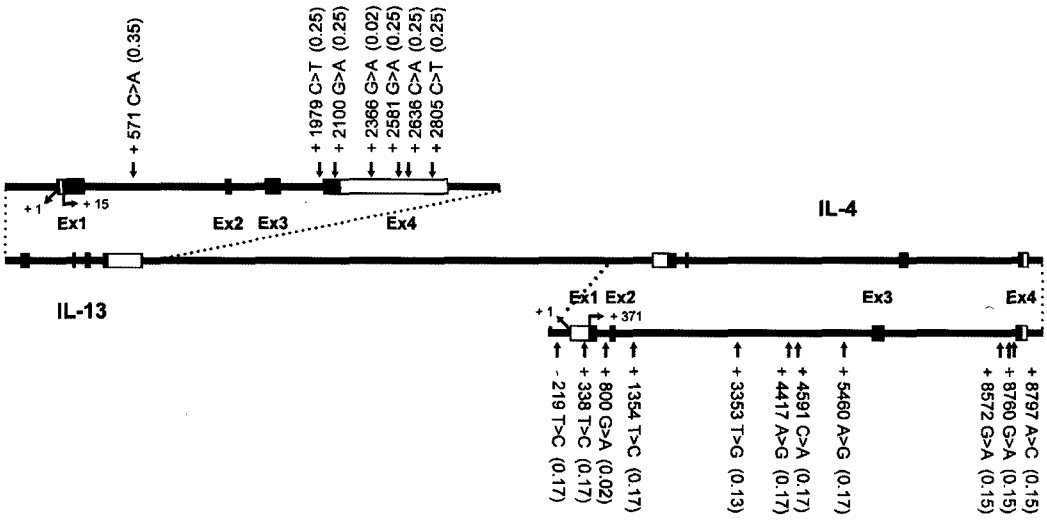
LD block structure and haplotypes of IL-13 and IL-4 in the Korean population

The result of LD analysis of IL-13 and IL-4 was summarized in Fig.1b. Since |D'| are strongly inflated for SNPs with rare alleles, high values can be obtained even when markers are in fact in linkage equilibrium (Ardlie *et al.*, 2002). Therefore, SNPs with frequency at least 5 % were used for LD analysis. Even if IL-13 and IL-4 are localized within 27 kb on the proximal portion of chromosome 5q31 and share many biologic activities, this region was decomposed into discrete 2 haplotype-blocks in the Korean population. Complete LD was observed between any 2 of 10 SNPs in IL-4, whereas there was no strong LD between the +571 C>A and the others in IL-13. From this result, we could deduce that there might be more than one haplotype-block in IL-13. Halotypes were constructed on the basis of the genotype data using PL-EM algorithm within each block. Two haplotypes, identified in IL-13, accounted for all observed haplotype diversity (Fig.1b). Any one of 5 SNPs within the IL-13 haplotype-block was determined as htSNP, because they were completely matched. In IL-4, four haplotypes were identified, and 2 dominant haplotypes with frequencies over 5 % accounted for 96 % of all haplotypes (Fig.1b). There were 3 htSNPs, which capture 100 % of the haplotype diversity observed within IL-4, as follows: htSNP1 was any one of -219 T>C, +338 T>C, +1354 T>C, +4417 A>G, +4591 C>A and +5460 A>G; htSNP2 was any one of +8572G>A, +8760 G>A and +8797 A>C; htSNP3 was +3353 T>G. In case of covering 2 common haplotypes, any one of 10 SNPs was identified as htSNP within IL-4.

No association of IL-13 and IL-4 genetic variants with asthma in the small number of Korean subjects

For each SNP, the genotype frequency was consistent with Hardy-Weinberg equilibrium ($P > 0.31$) and those of three alternative models, such as, co-dominant, dominant and recessive models, were not significantly different between patients and controls ($P > 0.59$) (data not

a. Gene Map of IL-13 and IL-4



b. Pairwise LD and Haplotypes of IL-13 and IL-4

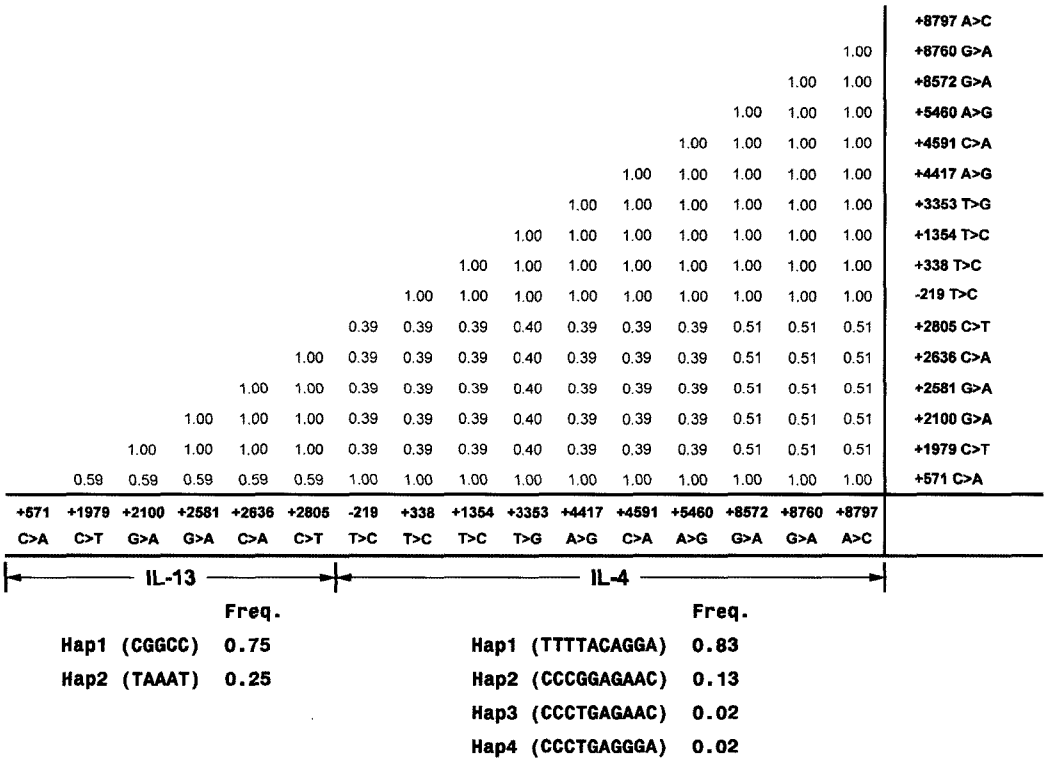


Fig. 1. Genomic structure, LD block pattern and haplotypes of IL-13 and IL-4.
(a): Gene structure and SNP map of IL-13 and IL-4. Coding exons were marked by shaded blocks and 5'- and 3'-UTR by white blocks. The minor allele frequencies of SNPs were shown (N=24). First base of the transcription start site is denoted as nucleotide +1 (reference sequences for IL-13 and IL-4 mRNA: NM_002188 and NM_000589).
(b): Pairwise LD and haplotypes of IL-13 and IL-4. Only common SNPs with frequency over 5 % were used for LD analysis. Haplotypes of IL-13 and IL-4 were shown below the LD block of each gene.

Table 1. Comparison of SNP allele frequency of IL-13 and IL-4 in diverse ethnic groups

Gene	SNP ID (rs# or new)	SNPs[1)]	Region	Amino acid change	Korean	Japanese[2)]	African[2)]	Caucasian[2)]	Ethnic difference[3)]
IL-13	rs2066960	+571 C>A	Intron 1		0.65 : 0.35		0.80 : 0.20	0.95 : 0.05	0.30
	rs1295686	+1979 C>T	Intron 3		0.75 : 0.25		0.33 : 0.66	0.74 : 0.26	0.41
	rs20541	+2100 G>A	Exon 4	Arg144Gln	0.75 : 0.25		0.88 : 0.12	0.73 : 0.27	0.15
	new	+2366 G>A	Exon 4	3'-UTR					
	rs1295685	+2581 G>A	Exon 4	3'-UTR	0.75 : 0.25		0.95 : 0.05	0.82 : 0.18	0.20
	rs848	+2636 C>A	Exon 4	3'-UTR	0.75 : 0.25		0.50 : 0.50	0.86 : 0.14	0.36
	rs847	+2805 C>T	Exon 4	3'-UTR	0.75 : 0.25		0.84 : 0.16	0.80 : 0.20	0.09
IL-4	rs2243250	-219 T>C	Promoter		0.83 : 0.17		0.71 : 0.29	0.17 : 0.83	0.66
	rs2070874	+338 T>C	Exon 1	5'-UTR	0.83 : 0.17	0.66 : 0.34	0.38 : 0.63	0.17 : 0.83	0.66
	new	+800 G>A	Exon 2	Val53Ile					
	rs734244	+1354 T>C	Intron 2		0.83 : 0.17	0.68 : 0.32	0.38 : 0.63	0.17 : 0.83	0.66
	rs2227284	+3353 T>G	Intron 2		0.88 : 0.13	0.79 : 0.21	0.85 : 0.15	0.28 : 0.72	0.59
	rs2243266	+4417 A>G	Intron 2		0.83 : 0.17	0.68 : 0.32	0.31 : 0.69	0.18 : 0.82	0.65
	rs2243268	+4591 C>A	Intron 2		0.83 : 0.17		0.24 : 0.76	0.11 : 0.89	0.72
	rs2243274	+5460 A>G	Intron 2		0.83 : 0.17		0.65 : 0.35	0.20 : 0.80	0.63
	rs2243288	+8572 G>A	Intron 3		0.85 : 0.15		0.65 : 0.35	0.18 : 0.82	0.67
	rs2243289	+8760 G>A	Intron 3		0.85 : 0.15	0.69 : 0.31	0.29 : 0.71	0.18 : 0.82	0.67
	rs2243290	+8797 A>C	Intron 3		0.85 : 0.15	0.68 : 0.32	0.31 : 0.69	0.17 : 0.83	0.68
Mean ethnic difference (minimum ~ maximum)									0.51 (0.09 ~ 0.72)

SNPs, single nucleotide polymorphisms; UTR, untranslated region

1) First base of the transcription start site was denoted as nucleotide +1.

2) Comparison of allele frequency was performed by searching JSNP database and dbSNP data with verified SNPs in the Korean population (24 Korean samples).

3) Ethnic difference in allele frequency was calculated by subtracting the lowest allele frequency from the highest allele frequency of minor allele among ethnic groups at each SNP site.

shown). No significant differences in allele frequencies were also observed between two groups (data not shown). We further performed association analyses between the haplotypes with frequency more than 5 % and development of asthma. However, no statistically significant associations were observed ($P > 0.64$) (data not shown). Although there were no significant associations between all single SNPs or common haplotypes and development of asthma, further studies would be needed to examine whether their association with asthma using a large disease samples with well-defined asthmatic phenotypes such as serum IgE level. Our results will be useful to select an optimal reference set of SNPs for any subsequent genotyping study in large population.

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