

Novel dinucleotide repeat polymorphism in the first exon of the STAT-6 gene is associated with allergic diseases

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Summary

Background T helper-type 2 cytokines, such as interleukin-4 (IL-4) and IL-13, may play a central role in allergic diseases. The protein known as 'signal transducers and activators of transcription 6' (STAT-6) is a key transcription factor involved in both IL-4- and IL-13-mediated biological responses.

Objective The objective of this study was to evaluate the possible role of the STAT-6 gene in modulating atopy in the Japanese population.

Methods We screened all 23 exons of the STAT-6 gene from 10 subjects for mutations by direct polymerase chain reaction (PCR) sequencing. The STAT-6 gene polymorphisms were genotyped by PCR fragment length polymorphism analysis and PCR-SSCP analysis. The IL-4 receptor Q576R polymorphism was also examined by PCR-SSCP analysis.

Results We found a novel dinucleotide repeat polymorphism in the first exon of the STAT-6 gene. The genotypes were classified into four groups according to the number of GT repeats present, from 13 to 16. The frequency of the A1 allele (326 bp, containing 13 repeats of GT) was higher in children with allergic diseases (bronchial asthma, atopic dermatitis and/or food-related anaphylaxis) than controls, although this was not statistically significant ($P = 0.0158$). In addition, a strong association between the A1 and A3 allele (containing 15 repeats of GT) heterozygote and allergic diseases was identified ($P = 0.0002$). However, the levels of IgE were not related to the GT repeat polymorphism in the allergic subjects. The GT repeat polymorphism was not associated with the polymorphism in the IL-4 receptor chain gene (Q576R) and there was no association between the G2964A variant and allergic diseases.

Conclusion This suggests that genetic variation in the STAT-6 gene may be associated with predisposition to allergic diseases.

Keywords: atopy, IL-4, IL-13, STAT-6, dinucleotide repeat, polymorphism, IgE

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Introduction

Allergic diseases may be based on an inflammatory mechanism involving T helper type 2 (Th2) cytokines, such as IL-4 and IL-13. Upon release of IL-4 or IL-13, binding of the cytokine to its receptor at the cell surface induces receptor dimerization and activation of the

cytoplasmic receptor associated with Janus tyrosine kinases (JAK) 1 and 3. The JAKs, now activated, phosphorylate a distal tyrosine on the receptor. This receptor phosphotyrosyl residue is subsequently recognized by the SH2 domain of STAT-6, drawing them into the receptor complex, where they are activated by tyrosine phosphorylation [1–3]. The activated STAT-6 are rendered dimerization, and nuclear translocation, leading to its specific binding to promoter sequences in the DNA [4,5]. STAT-6 DNA-binding sites have been identified in the promoter regions of several

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IL-4-inducible genes. This results in transactivation of the target genes, which has a central role in IgE synthesis and development of bronchial hyper-responsiveness [6–9].

It has been recently reported that genetic variants of Th2 cytokines or their receptors are associated with atopy [10–15]. A recent study has shown that Q576R polymorphism in the IL-4R α chain (IL-4R α) gene may be associated with atopy [10], whereas another group showed lack of an association between atopy/asthma and the IL-4R α gene in the Japanese population [14]. In the downstream part of the signalling pathway mediated by Th2 cytokines, only a few groups have studied the association between the polymorphism in the 3' untranslated region of the STAT-6 gene and atopy [16]. In this study, we report the novel dinucleotide repeat polymorphism, and the linkage and association between this STAT-6 gene polymorphism and allergic diseases in the Japanese population.

Materials and methods

Allergic and control subjects

Sixty children with major allergic disease including bronchial asthma (BA), atopic dermatitis (AD) and/or food-related anaphylaxis (FA), were studied. Among these allergic subjects, 34 children had BA, 36 had AD and 12 had FA. A physical examination of each subject was carried out by a paediatrician. The serum IgE levels were measured by enzyme-linked immunosorbent assays in the case of almost all of the subjects.

BA was diagnosed according to the criteria of the National Institutes of Health of the United States with minor modification [17]. Briefly, the diagnosis of BA was made based on the appearance of both of the following characteristics: two or more episodes of wheezing and shortness of breath during the past year and reversibility of the wheezing and dyspnoea, either spontaneously or after treatment with a bronchodilator. As wheezing is often associated with viral respiratory infection in young children, subjects more than 3 years old were evaluated for the asthma phenotype [14].

The diagnosis of AD was made based on the appearance of active skin disease, the distribution of skin lesions, and the clinical course of the disease according to the diagnostic criteria of Hanifin and Rajka [18].

The diagnosis of FA was made based on recognition of the typical manifestations and the patient's history, with regard to the reproducibility of the reaction, the timing of the reaction, and the response to the elimination of food from the diet. All FA patients had a positive RAST response to food to which they were susceptible.

The control subjects were 54 individuals with no history of major atopic disease. Informed consent was obtained

from all subjects or their parents. This study was approved by the Committee of Ethics at the Department of Paediatrics, the University of Gunma.

Molecular methods

DNA was extracted from peripheral blood leucocytes. We screened all 23 exons of the STAT-6 gene from 10 subjects for mutations by direct polymerase chain reaction (PCR) sequencing. PCR primers were designed to amplify a fragment containing each exon. We found an identical alteration in the first exon. This alteration showed the presence of GT repeat polymorphism. PCR primers of exon 1 were designed to amplify a fragment containing the dinucleotide repeat region. Another alteration in the 3' untranslated region of the STAT-6 gene has been previously reported, as G2964A variant of the STAT-6 gene [16,19]. For genotyping of G2964A variant and Q576R of the IL-4 α gene, PCR-SSCP analysis was done.

PCR primer sequence

Primers for STAT-6 exon 1

Forward: 5'-GAGGGACCTGGGTAGAAGGA-3'
Reverse: 5'-CACCCCCATGCACTCATAG-3'

Primers for STAT-6 G2964A variant

Forward: 5'-GGAGCCAATCCACTCCTTCC-3'
Reverse: 5'-CAGACTCCTCCTATGCTCCC-3'

Primers for IL-4R α

Forward: 5'-GCAGATCCTCCGCCGAAATG-3'
Reverse: 5'-GCCCAAACCCACATTCTC-3'

PCR conditions

PCR reactions were performed in a volume of 25 μ L containing 50 ng of genomic DNA, each dNTP at 125 μ M, 2 U of *Taq* polymerase, *Taq* buffer, and 10 pmol of forward and reverse primer. As for STAT-6 exon 1, 6-FAM-labelled forward primer was used. The cycle conditions were 95 °C for 5 min, and then 40 cycles of 95 °C for 30 s, 66 °C (STAT-6 exon 1) or 60 °C (G2964A variant and IL-4R α) for 30 s, and 72 °C for 30 s, with a final extension step of 7 min at 72 °C, in a GeneAmp 2400 thermocycler (Perkin Elmer, Foster City, CA, USA). After PCR, 1 μ L of the products plus 0.5 μ L of Genescan 400HD molecular weight standard (Applied Biosystems, Foster City, CA, USA) were denatured in 12 μ L of formamide, separated in an Applied Biosystems Prism Genetic Analyser (ABI

Table 1. Allelic distributions of the STAT-6 gene polymorphism in Japanese patients with allergic disease and controls

Allele	Size (bp)	Allergic subjects	Control subjects	<i>P</i> -value*
A1	326	35 (0.2917)	17 (0.1574)	0.0158
A2	328	1 (0.0083)	1 (0.0093)	0.9403
A3	330	72 (0.6000)	73 (0.6759)	0.2342
A4	332	12 (0.1000)	17 (0.1574)	0.1939
Total no.		120	108	

*Significant value was taken at the level of $P < 0.0125$, giving an overall significant level for the four tests of 0.05.

PRISMTM310) with POP6 polymer, and fragment lengths were determined.

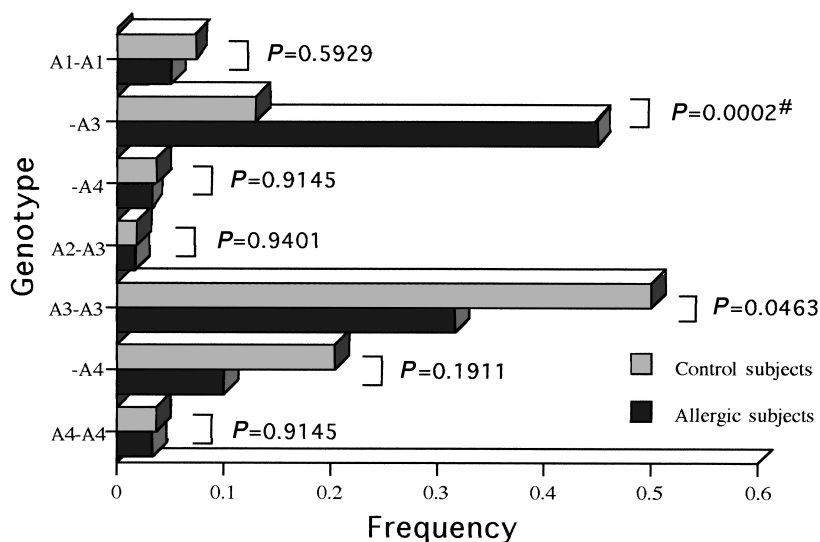
PCR-SSCP analysis

The amplified products of G2964A variant and IL-4R α were fractionated by electrophoresis on polyacrylamide gels, GeneGel Clean 15/24 (Amersham Pharmacia Biotech, Uppsala, Sweden), at 20 °C for 2 h. The gels were visualized by silver staining. Samples from two known homozygotic individuals and one heterozygotic individual, as confirmed by sequencing, were included in each reaction. Sequencing was performed using an automated sequencer (ABI PRISMTM310).

Statistical analysis

The data were analysed by the Fisher's direct method. Significant values were taken on the basis of numbers of tests.

Fig. 1. Comparison of frequency distribution of genotypes containing the GT repeat polymorphism at the STAT-6 gene locus between 60 subjects with allergic disease and 54 control subjects. (see Table 1 for explanation of each alleles). #Significant value was taken at the level of $P < 0.007$, giving an overall significant level for the seven tests of 0.05.



Results

The 60 subjects with allergic diseases and the 54 controls were genotyped in terms of GT repeat polymorphism in exon 1 of the STAT-6 gene. The polymorphic PCR products were classified into four alleles: A1 (326 bp, 13GT repeats) to A4 (332 bp, 16GT repeats). The distribution of the four alleles in the two groups is shown in Table 1. The frequency of A1 allele was higher in the allergic group than in the control group ($P = 0.0158$), although this was not statistically significant. There were no significant differences in the frequency of allele A2, A3 or A4 between the two groups. As for the genotypic distribution and frequency, the number of A1 and A3 heterozygotes was significantly greater in the allergic group than in the control group ($P = 0.0002$) (Fig. 1). In contrast, in the control group, the number of A3 homozygotes was greater than that in the allergic group, although the difference was not significant ($P = 0.0463$). The levels of IgE in allergic individuals, compared with those who were A1 and A3 heterozygotes and those who were not, are shown in Fig. 2. No significant difference in the levels of IgE was detected when comparing these two groups of allergic subjects. The frequencies of the R576 allele of the IL-4R α gene were similar in the two groups of the allergic subjects and the control groups (Table 2). There was no association between G2964A variant of the STAT-6 gene and allergic disease in the Japanese population (Table 3).

Discussion

This is the first report describing dinucleotide repeat polymorphism identified in the STAT-6 (signal transducer and activators of transduction 6) gene. In addition, our

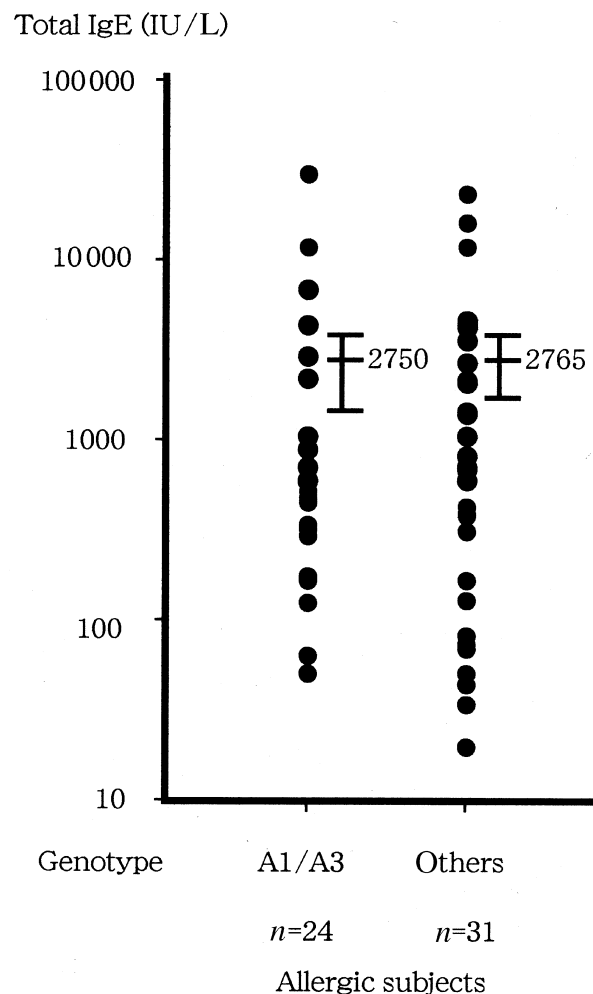


Fig. 2. Comparison of total IgE level in the group of allergic subjects that carried an A1/A3 heterozygote and the group that did not (others). The levels of total IgE are plotted. Values are means $\pm$ SE.

Table 2. Frequency of interleukin-4 receptor α alleles in subjects with allergic disease and control subjects

Group	No. of subjects	QQ	QR number	RR
Allergic subjects	60	42	17	1
A1/A3 heterozygote	28	18	9	1
Control subjects	54	35	18	1

The Q allele corresponds to 576Q and the R allele corresponds to 576R. QQ denotes homozygosity for the wild-type allele, RQ heterozygosity, and RR homozygosity for the mutant allele.

Table 3. Association between G2964A variant of the STAT-6 gene and allergic disease in the Japanese population

Group	No. of subjects	GG	GA + AA	P-value*
Allergic subjects	60	20	40	0.4963
Control subjects	54	17	37	

The G allele corresponds to 2964G and the A allele corresponds to 2964A. GG denotes homozygosity for the wild-type allele, GA heterozygosity, and AA homozygosity for the mutant allele.

*Significant value was taken at the level of $P < 0.05$.

findings suggest that GT dinucleotide repeat polymorphism in STAT-6 may play an important role in the development of allergic diseases in the Japanese population.

STAT-6 is a key transcription factor involved in IL-4 and IL-13 mediated biological responses. The human STAT-6 gene, being approximately 19 kb in length, is located at 12q13.3-q14.1 and it consists of 23 exons interrupted by 22 introns. The first 2 exons of the STAT-6 gene are non-coding regions. The AUG translation initiation codon, encoding the first amino acid, is located within exon 3. The DNA binding domain is encoded within exons 10–14 and the phosphorylated tyrosine residue critical for SH2 domain interaction is encoded within exon 17 of the STAT-6 gene [20]. In the present study we found a novel dinucleotide repeat polymorphism in the first exon of the STAT-6 gene. This exon is a non-coding region but it might play an important role in mRNA expression or mRNA stability.

In the present study we found that the frequency of A1 allele in the first exon of the STAT-6 gene was higher in the allergic group, although this was not statistically significant. Interestingly, heterozygosity for the A1 and A3 allele was very strongly associated with allergic diseases. However, there was no difference in the frequency of individuals homozygous for the A1 allele comparing the subjects in the allergic and those in the control group. Therefore, the A1 allele itself does not seem to influence the individuals who have this allele in terms of predisposing them to allergic disease. Alternatively, the penetrance of the A1 allele may be modified by other factors, including distinct genetic loci that impart susceptibility to or protection from atopy and environmental factors such as the level and duration of exposure to allergens [21–23].

We speculate that the heterozygosity for the A1 and A3 allele may be important in terms of the association with allergic disease. Buerger and coworkers have recently demonstrated that the number of CA repeats in intron 1 of the epithelial growth factor receptor (EGFR) gene is

correlated with the expression of EGFR *in vitro* and *in vivo*, suggesting that the function of microsatellites may be correlated with individual differences in gene expression [24]. Another explanation is that the dinucleotide repeat polymorphism identified in the present study may represent a marker mutation, or may be genetically linked to another polymorphism, which is associated with the development of allergic disease. We investigated the association of G2964A variant in the 3' untranslated region of the human STAT-6 gene previously reported [16,19] and allergic diseases. In the Japanese population, there was no association between this variant and allergic diseases. Further studies are needed to analyse the linkage of the STAT-6 genetic variants.

In the present study a significant predominance of GT repeat polymorphism in the first exon of STAT-6 was observed in the allergic subjects but a change in the level of IgE could not be detected. This polymorphism occurred independently of the phenotypes of allergic diseases (data not shown). Therefore, regulation of IgE production by this polymorphism is unlikely to predispose individuals to allergic diseases. Moreover, this polymorphism is not associated with the polymorphism in the IL-4R α (Q576R) gene, which is associated with atopy, as shown in a previous study [10]. Above this, we consider that the GT repeat polymorphism might promote allergic disease by the development of bronchial hyper-responsiveness or some other mechanisms. However, the true mechanism is unknown. Further analysis of different clinical expressions, such as hyper-IgE syndrome or non-atopic asthma, are needed to elucidate the role of the GT repeat polymorphism.

Our findings suggest that the polymorphic microsatellite at the STAT-6 locus may be a useful marker for predicting future allergic disease and for allowing early therapeutic intervention in the case of high-risk infants, although the number of samples investigated in this series might be small. Such epidemiological or genetic studies on the signal transducer will be expanded, and furthermore functional analyses of STAT-6 may be needed to elucidate the role of the STAT-6 gene in the development of allergic disease.

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