*Allergology International* (2004) **53**: 241–244

Original Articles

# Signal transducers and activators of transcription 6 (*Stat6*) variants in childhood and adult asthma

Michiko Suzuki,[1] Hirokazu Arakawa,[1] Yasuko Kobayashi,[1] Kazushi Tamura,[1] Hiroyuki Mochizuki,[1] Kenichi Tokuyama,[1] Mayumi Tamari,[2] X-Q Mao,[2,3] Taro Shirakawa,[2,3] Kenji Izuhara[4] and Akihiro Morikawa[1]

[1]Department of Pediatrics and Developmental Medicine Gunma University Graduate School, Maebashi, Gunma, [2]RIKEN SRC, Yokohama, [3]Department of Health Promotion and Human Behavior, Kyoto University Graduate School of Public Health, Kyoto and [4]Department of Biochemistry, Saga Medical School, Saga, Japan

## Abstract

**Background:** Signal transducers and activators of transcription 6 (Stat6) is a key transcription factor involved in interleukin (IL)-4 and IL-13-mediated biological responses. Recently, we reported the association between the dinucleotide (GT) repeat polymorphism in the first exon of *Stat6* and allergic subjects in a Japanese population. The aim of the present study was to evaluate whether this GT repeat polymorphism is associated with bronchial asthma, including childhood asthma and atopic and non-atopic adult asthma.

**Methods:** *Stat6* gene polymorphisms were genotyped by polymerase chain reaction (PCR) fragment length polymorphism analysis.

**Results:** In the first exon of *Stat6*, polymorphic PCR products were classified into six alleles (12–17 GT repeats). A significant difference was found in the genotypic frequency of the GT repeat polymorphism between controls and child asthmatics ($P = 0.015$), but not atopic or non-atopic adult asthma. The frequency of the 15 repeat allele (wild type) was lower in child asthmatics than in controls ($P = 0.0047$; odds ratio (OR) 1.6, 95% confidence interval (CI) 1.16–2.23), whereas shorter repeat alleles (12, 13 and 14 GT repeat) were higher in child asthmatics than in controls ($P = 0.0064$; OR (95%CI) 1.66 (1.15–2.39)).

**Conclusion:** Genetic variations in the *Stat6* gene may be associated with a predisposition for childhood asthma.

**Key words:** atopy, dinucleotide repeat polymorphism, interleukin-13, interleukin-4, signal transducers and activators of transcription 6 (Stat6).

## Introduction

Allergic diseases may be based on an inflammatory mechanism involving Th2 cytokines, such as interleukin (IL)-4 and IL-13.[1,2] When IL-4 or IL-13 engages its receptor complex, the phosphorylation, dimerization and nuclear localization of the signal transducer and activator of transcription 6 (Stat6) results in the transcription of several IL-4-inducible genes[3–5] that play a central role in IgE synthesis and the development of bronchial hyperresponsiveness.[2,6,7]

Recently, various genetic studies have shown that polymorphisms in genetic variations of Th2 cytokines are associated with atopic disorders in Western or Japanese populations.[8–11] Recently, we reported the association between the dinucleotide repeat polymorphism of the *Stat6* exon 1 and allergic subjects in a Japanese population.[12] However, other groups have not replicated this finding; it was demonstrated that the GT repeat did not show a linkage/association to asthma in a Caucasian sib-pair study.[13]

Correspondence: Dr Hirokazu Arakawa, Department of Pediatrics and Developmental Medicine, Gunma University Graduate School, Maebashi, Gunma 371-8511, Japan.
Email: harakawa@showa.gunma-u.ac.jp

Received 4 December 2003. Accepted for publication 12 February 2004.

The aim of the present study was to test whether the GT repeat polymorphism of the *Stat6* exon 1 relates to bronchial asthma, including childhood asthma and atopic or non-atopic adult asthma, in a Japanese population.

## METHODS

### Bronchial asthma and control subjects

Bronchial asthma and control subjects were collected in accordance with methods described previously.[10,11] Briefly, adult and child asthmatic subjects in a Japanese population were diagnosed by physicians specializing in asthma, as having: (i) recurrent breathlessness and chest tightness requiring continuing treatment; (ii) physician-documented wheeze; and (iii) documented labile airflow obstruction with variability in serial peak expiratory flow rates > 30%. Because wheezing is often associated with viral respiratory infection in young children, children older than 3 years of age were evaluated for childhood asthma. Specific IgE was detected by MAST (Hitachi, Tokyo, Japan) or CAP-RAST (Pharmacia, Uppsala, Sweden). Atopy was diagnosed as the presence of a high concentration of total serum IgE (> 250 IU/mL), a positive specific IgE titer (> 4.41 LC in MAST and > 0.70 UA/mL in CAP-RAST) against one or more of 15 highly purified aeroallergens or a combination of these two features. Control subjects were 166 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 Pediatrics, The University of Gunma.

### Molecular methods

DNA was extracted from peripheral blood leukocytes. Polymerase chain reaction (PCR) primer sequences in the first exon of the *Stat6* gene and the PCR conditions used were as described previously.[12] Briefly, PCR reactions were performed in a volume of 25 μL containing 50 ng genomic DNA, each dNTP at 125 μmol/L, 2 U *Taq* polymerase, *Taq* buffer and 10 pmol forward and reverse primer. 6-Carboxy fluorescein (6-FAM)-labeled forward primer was used for *Stat6* exon 1. Cycle conditions were 95°C for 5 min, followed by 40 cycles of 95°C for 30 s, 66°C 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, Norwalk, CA, USA). After PCR, a 1 μL aliquot of the products plus 0.5 μL Genescan 400HD molecular weight standard (Applied Biosystems, Foster City, CA, USA) were denatured in 12 μL formamide, separated in an Applied Biosystems Prism Genetic Analyzer (ABI PRISM™310) with performance optimized polymer 6 (POP6) polymer and fragment lengths determined.

### Statistical analysis

Data were analyzed by the Chi-squared test. $P < 0.05$ was considered significant.

## RESULTS

Children or adults with bronchial asthma and controls were genotyped in terms of the GT repeat polymorphism in the first exon of the *Stat6* gene. The allelic and genotypic distributions of the GT repeat polymorphism are shown in Tables 1,2. In the first exon of *Stat6*, polymorphic PCR products were classified into six alleles (12–17 GT repeats; Table 1). The frequency of the 15 GT repeat allele was significantly lower in child asthmatics compared with controls (odds ratio (OR) 1.6; 95% confidence interval (CI) 1.16–2.23; $P = 0.0047$), whereas the frequency of shorter repeat alleles (12, 13 and 14 repeats) were significantly higher in child asthmatics than in controls (OR (95%CI) 1.66 (1.15–2.39); $P = 0.0064$). However, there was no significant difference between atopic or non-atopic adult asthmatics and controls.

We designated an individual homozygous for the most commonly occurring allele (the 15 GT repeat allele) as a wild type, an individual without a shorter repeat allele as a mutant 1 type and an individual having one or two shorter repeat alleles as a mutant 2 type (Table 2). There was a significant difference in the GT repeat polymorphism in genotype frequencies between controls and

**Table 1** Allele frequency of GT repeat polymorphisms in *Stat6* exon 1

| No. GT repeats | Controls | Asthmatics | | |
|---|---|---|---|---|
| | | Children | Atopic adults | Non-atopic adults |
| 12 | 0 | 1 | 0 | 0 |
| 13 | 69 | 79 | 29 | 42 |
| 14 | 1 | 6 | 0 | 0 |
| 15 | 222 | 156** | 78 | 134 |
| 16 | 40 | 37 | 11 | 21 |
| 17 | 0 | 1 | 0 | 1 |
| Total | 332 | 280 | 118 | 198 |

**$P < 0.01$ compared with controls.

**Table 2** Association between genotypic distribution of GT repeat polymorphisms in *Stat6* exon 1 and asthma

| Phenotype | No. cases | Genotype of GT repeat | | | *P* | *P*† | OR† (95%CI) |
|---|---|---|---|---|---|---|---|
| | | No. wild type (%) | No. mutant 1 (%) | No. mutant 2 (%) | | | |
| Control | 166 | 76 (45.8) | 30 (18.1) | 60 (36.1) | | | |
| Asthmatics | | | | | | | |
| Children | 140 | 42 (30) | 26 (18.6) | 72 (51.4) | 0.011 | 0.0072 | 1.87 (1.18–2.96) |
| Atopic adults | 59 | 23 (39.0) | 8 (13.6) | 28 (47.5) | 0.300 | 0.126 | |
| Non-atopic adults | 99 | 45 (45.5) | 15 (15.2) | 39 (39.4) | 0.783 | 0.597 | |

†Wild type + mutant 1 compared with mutant 2.

Wild type, wild/wild homozygosity; mutant 1, longer/longer, longer/wild; mutant 2, shorter/shorter, shorter/wild, shorter/longer. (Wild: 15 GT repeats; shorter: 12, 13 and 14 GT repeats; longer: 16 and 17 GT repeats.) OR, odds ratio; CI, confidence interval.

child asthmatics ($P = 0.011$), but not atopic and non-atopic adult asthmatics (Table 2). Furthermore, a significant difference was observed between child asthmatics and non-atopic adult asthmatics ($P = 0.049$). The frequency of an individual with a mutant 2 type was significantly higher in child asthmatics than in controls (OR (95%CI) 1.87 (1.18–2.96); $P = 0.0072$). In contrast, no significant difference was observed between atopic or non-atopic adult asthmatics and controls.

## Discussion

We found that the GT repeat polymorphism in the first exon of *Stat6* was significantly associated with childhood asthma, but not with atopic or non-atopic adult asthma. The frequency of a shorter repeat allele in child asthmatics was significantly different from those of controls. Our data suggest that the GT repeat polymorphism in the *Stat6* gene may play an important role in the development of childhood asthma in the Japanese population.

Despite intensive efforts and advances in molecular biology and genetics, no gene has been identified with any certainty as being involved in the heritability of asthma. However, the results of several studies have provided an indication that multiple genes may be involved in its pathogenesis. Recently, we found a novel dinucleotide repeat polymorphism in the first exon sequences of the *Stat6* gene and identified a strong association between the 13/15 allele heterozygote and allergic diseases.[12] The present study further evaluated whether this GT repeat polymorphism is associated with bronchial asthma and we have found a significant difference in allelic and genotypic frequencies in the GT repeat polymorphism between child asthmatics and controls, when considered as a wild or mutant type, suggesting that the GT repeat variant of the *Stat6* exon 1 is associated with childhood asthma. Duetsch *et al.*[13] found that there was a significant association of the 16 repeats allele with an increase in the eosinophil cell count. However, they found that there was no linkage of any of the GT repeat alleles with the asthmatic phenotype.[13] The discrepancy between the results of Duetsch *et al.*[13] and the present study may be explained by the different allelic distributions between different ethnic groups and different phenotypes of asthmatic patients. In fact, the frequency of 13 GT repeat allele was shown to be 40.14% in a Caucasian population,[13] whereas it was 20.8 and 28.2% in controls and child asthmatics, respectively, in the Japanese population in the present study. Furthermore, we evaluated patients divided by age and the level of IgE, whereas Duetsch *et al.*[13] used sib-pair analysis.

In the present study, we found that the frequency of shorter repeat alleles of the *Stat6* exon 1 were significantly higher in child asthmatics than in controls. In addition, the genotypic frequency of an individual with a mutant 2 type was significantly higher in child asthmatics than controls. The GT repeat polymorphism of *Stat6* may, directly or indirectly, modulate *Stat6* gene expression. Perhaps the repeat number may be an important factor for this modulation (e.g. a shorter repeat allele than a wild type enhances the function, whereas the longer repeats do not, and vice versa).

Interestingly, in the present study we could not identify a linkage with either atopic or non-atopic adult asthma. In Japanese people, less than 20% of adult asthma is a type developed during childhood. There seems to be some difference in the pathogenesis (especially genetic background) between adult asthma developed during childhood and adult-onset asthma, although no conclusion has been reached. We need to extend the association studies further to ascertain any differences in pathogenesis between these two types of asthma. More

than 90% of childhood asthma is of the atopic type, whereas two-thirds of adult asthma consists of infectious or mixed types. Moreover, childhood asthma contributes anti-IgE antibodies to environmental allergens, such as house dust mites and animal dander. Thus, there are some features that make adult-onset asthma different to that seen in younger patients. Our results suggest that the relative contribution of genetic and environmental factors in the pathogenesis of asthma may vary according to age. It has been reported, with respect to the Ile50Val variant in the IL-4 receptor α, that Ile50 was associated with atopic asthma but not with non-atopic subjects and that this association was especially strong in children.[10] These results seem to be somewhat consistent with those of the present study showing that the *Stat6* gene variant was significantly associated with childhood asthma, although we could not identify a linkage with atopic adult asthma. We previously found that IgE production was not associated with the *Stat6* gene.[12] Furthermore, because Duetsch *et al*.[13] failed to demonstrate a relationship between the level of serum IgE and *Stat6* gene variants in Caucasian asthma patients,[13] it is unlikely that the *Stat6* gene variants promote the dysregulation of IgE synthesis.

Our findings suggest that the polymorphic microsatellite at the *Stat6* locus may be a useful marker for predicting future childhood asthma and, thus, allow for early therapeutic intervention in cases of high-risk infants. Because asthma is a multifactorial disease, characterized by high genetic heterogeneity between different ethnic groups, it is possible that the influence of *Stat6* on asthma differs according to the population. Additional genetic and epidemiological studies, as well as functional analysis of *Stat6*, are required to fully elucidate the role of this interesting gene in the development of asthma.

## ACKNOWLEDGMENTS

The authors thank Mrs Tomoko Endo and Mrs Chinori Iijima for their technical assistance.

## REFERENCES

1 Takeda K, Kishimoto T, Akira S. STAT6: Its role in interleukin 4-mediated biological functions. *J. Mol. Med.* 1997; **75**: 317–26.
2 Izuhara K, Shirakawa T. Signal transduction via the interleukin-4 receptor and its correlation with atopy. *Int. J. Mol. Med.* 1999; **3**: 3–10.
3 Hou J, Schindler U, Henzel WJ, Ho TC, Brasseur M, McKnight SL. An interleukin-4-induced transcription factor: IL-4 Stat. *Science* 1994; **265**: 1701–6.
4 Takeda K, Tanaka T, Shi W *et al*. Essential role of Stat6 in IL-4 signalling. *Nature* 1996; **380**: 627–30.
5 Malabarba MG, Rui H, Deutsch HH *et al*. Interleukin-13 is a potent activator of JAK3 and STAT6 in cells expressing interleukin-2 receptor γ and interleukin-4 receptor α. *Biochem. J.* 1996; **319**: 865–72.
6 Wills-Karp M, Luyimbazi J, Xu X *et al*. Interleukin-13: Central mediator of allergic asthma. *Science* 1998; **282**: 2258–61.
7 Akimoto T, Numata F, Tamura M *et al*. Abrogation of bronchial eosinophilic inflammation and airway hyperreactivity in signal transducers and activators of transcription (STAT) 6-deficient mice. *J. Exp. Med.* 1998; **187**: 1537–42.
8 Hershey GK, Friedrich MF, Esswein LA, Thomas ML, Chatila TA. The association of atopy with a gain-of-function mutation in the α subunit of the interleukin-4 receptor. *N. Engl. J. Med.* 1997; **337**: 1720–5.
9 Kawashima T, Noguchi E, Arinami T *et al*. Linkage and association of an interleukin 4 gene polymorphism with atopic dermatitis in Japanese families. *J. Med. Genet.* 1998; **35**: 502–4.
10 Mitsuyasu H, Yanagihara Y, Mao XQ *et al*. Cutting edge: Dominant effect of Ile50Val variant of the human IL-4 receptor α-chain in IgE synthesis. *J. Immunol.* 1999; **162**: 1227–31.
11 Heinzmann A, Mao XQ, Akaiwa M *et al*. Genetic variants of IL-13 signalling and human asthma and atopy. *Hum. Mol. Genet.* 2000; **9**: 549–59.
12 Tamura K, Arakawa H, Suzuki M *et al*. Novel dinucleotide repeat polymorphism in the first exon of the Stat6 gene is associated with allergic disease. *Clin. Exp. Allergy* 2001; **31**: 1509–14.
13 Duetsch G, Illig T, Loesgen S *et al*. STAT6 as an asthma candidate gene: Polymorphism-screening, association and haplotype analysis in a Caucasian sib-pair study. *Hum. Mol. Genet.* 2002; **11**: 613–21.