

No evidence for association between the –112G/A polymorphism of *UGRP1* and childhood atopic asthma

Z. Jian*, J. Nakayama*, E. Noguchi*, M. Shibasaki† and T. Arinami*

*Department of Medical Genetics, Institute of Basic Medical Sciences, University of Tsukuba, Ibaraki, Japan, and †Department of Pediatrics, Tsukuba College of Technology, Ibaraki, Japan

Summary

Background Susceptibility to asthma is known to involve genetic factors. Genome-wide screens have indicated that the chromosome 5q31–q34 region is linked to and/or associated with asthma. A new gene, named *UGRP1* and reported by Niimi et al., encodes uteroglobin-related protein and is expressed in the lung and trachea. Niimi et al. showed the –112G/A polymorphism of the *UGRP1* gene to be associated with asthma in a case–control study.

Objective The objective of the present study was to replicate this association and confirm the possible role of the *UGRP1* –112G/A polymorphism in the aetiology of childhood asthma in a Japanese population.

Methods and results We conducted a transmission disequilibrium test (TDT) in 131 families identified through paediatric patients being treated for asthma. A case–control study was also carried out by comparing the probands and 137 unrelated non-atopic non-asthmatic Japanese children and 211 unrelated healthy Japanese adults. The –112G/A polymorphism was genotyped by the PCR-RFLP method. The TDT revealed that the –112A allele was not preferentially transmitted to asthma-affected children ($P = 0.85$). Neither the presence of at least one A allele in an individual's genotype (sum of the G/A and A/A genotypes) nor the –112A allele was more prevalent among the asthma subjects than among the control subjects.

Conclusion Our findings indicate that the *UGRP1* –112G/A polymorphism does not play a substantial role in genetic predisposition to childhood asthma in this Japanese population.

Keywords association study, Asthma, genetics, polymorphism, *UGRP1*

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Introduction

Asthma is a complex disease with a major genetic component and is characterized by polygenic inheritance as well as genetic heterogeneity. Environmental factors contribute to the manifestation of this disease. In recent years, much work has been carried out to find the responsible gene(s). Special attention has been given to chromosome 5q31–q34 because many linkage studies and genome-wide screens have pointed to this region [1–3]. Numerous candidate genes such as the T helper type 2 cytokine gene cluster (IL-4, IL-13, IL-5, and IL-9), the colony stimulating factor 1 receptor gene (*CSF1R*), and the beta-2-adrenergic receptor gene (*ADRB2*) are harboured here [4, 5].

Niimi et al. [6] found a new gene, named *UGRP1* (MIM606531), that encodes uteroglobin-related protein. *UGRP1* is located on chromosome 5q31–q34 and is expressed in the lung and trachea. *UGRP1* mRNA is decreased in the lungs of mice with antigen-induced pulmonary inflammation [6]. In a Japanese case–control study, Niimi et al. [7] found

the –112A/–112A and –112G/–112A genotypes of the *UGRP1* gene to be significantly associated with asthma (odds ratio of 4.11, 95% CI of 1.51–11.17). In their study, prevalence of the A variant was 22.0% among affected subjects and 10% among control subjects ($P = 0.003$). They also reported that, in comparison to the –112G allele, the –112A allele is responsible for 24% reduction in promoter activity.

The report by Niimi et al. is important because, if the reported association were true, *UGRP1* would be a major candidate explaining the linkage between asthma and 5q31–q34, which has been found in several studies, including one of ours [3]. To confirm the role of the *UGRP1* polymorphism in the development of asthma among our families, in whom asthma is linked to 5q31–q34, we performed an association study using the transmission disequilibrium test (TDT). We also conducted a case–control comparison.

Materials and methods

Subjects

Probands of the families studied were asthmatic children who visited the Pediatric Allergy Clinic of the University Hospital of Tsukuba. A full verbal and written explanation of the

Correspondence: Tadao Arinami, MD, Department of Medical Genetics, Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, Ibaraki-ken 305-8575, Japan. E-mail: tarinami@md.tsukuba.ac.jp

study was given to all family members interviewed, and 131 families (466 individuals) gave informed consent and participated in the study. The mean age of the probands and their siblings was 10.6 years (range, 3–29 years); the mean age of the parents was 40 years (range, 26–72 years). The criteria used to identify asthma patients were as described previously [3]. The families examined in this study included the 47 families that participated in our genome-wide study [3]. We had obtained supportive evidence in these 47 families for linkage of asthma to human chromosome 5q31–q34, where *UGRP1* is located. In the present study, we examined only the proband ($n = 131$) and parents ($n = 262$) in each family represented. A control group was composed of unrelated children living near the University Hospital. We selected 137 control children who met all of the following criteria: (1) neither symptoms nor history of allergic disease such as asthma, atopic eczema, or allergic rhinitis, (2) no detectable dust-mite-specific IgE antibody, and (3) total serum IgE levels below the general age-adjusted population mean. For the adult controls, we selected 211 anonymous, unrelated, healthy Japanese. Adult control subjects were not evaluated for asthma. This study was approved by the Ethics Committee of the University of Tsukuba.

Methods

DNA was extracted from peripheral blood leucocytes collected in EDTA. The *UGRP1* –112G/A polymorphism was examined with the use of a PCR fragment amplified by the forward primer 5' CTG TGC AAG GGT TTA TGC AA 3' and reverse primer 5' ATA TGC CCA TTG AGG ACC TG 3'. PCR was performed in a final volume of 15 µL containing 50 ng genomic DNA, 2.5 mM deoxyribonucleotides, 5.0 pM each primer, 2 mM MgCl₂, 1.5 µL Gold Buffer, and 0.25 U *Taq* Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA). The PCR conditions were as follows: denaturing at 95 °C for 10 min, then 35 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, and final incubation at 72 °C for 3 min. The –112G/A polymorphism was identified by loss of the *HphI* restriction site (the G allele, 106, 37 and 43 bp fragments; the A allele, 143 and 43 bp fragments). The PCR products were digested with 2 U of *HphI* (New England BioLabs, Inc, Beverly, MA, USA) at 37 °C overnight and were electrophoresed on an agarose gel and visualized by ethidium bromide staining and ultraviolet transillumination. The accuracy of the genotyping was confirmed by the direct sequence of PCR products from 48 subjects randomly selected from asthmatic families.

The results of allelic and genotypic TDTs were assessed with the SIB-PAIR program (<http://www2.qimr.edu.au/david>). Case-control comparisons were made by Fisher's exact test. A P -value of <0.05 was considered statistically significant.

Results

Results of the TDT are shown in Tables 1 and 2. The TDT showed no preferential transmission of the A allele to asthmatic children ($P = 0.85$). Genotypic transmission did not deviate significantly from random proportions ($P = 0.96$). Genotype distribution did not deviate from Hardy–Weinberg expectancy among the parents in our study.

Table 3 shows the results of case-control comparisons. The frequencies of affected, child control, and adult control subjects with at least one A allele (G/A + A/A genotypes) were 40.5%, 33.6%, and 37%, respectively. The frequencies of the A allele in the affected, child control, and adult control subjects were 24.4%, 19.7%, and 20.1%, respectively. Our data did not show significant association between the –112G/A polymorphism and childhood asthma in the study population.

Discussion

The present study in Japanese children failed to replicate the association reported by Niimi et al. between the –112G/A polymorphism and asthma. There are several possible

Table 1. Allele-by-allele transmission disequilibrium test for the –112G/A polymorphism of the *UGRP1* gene in 131 Japanese asthmatic families

Allele	Transmitted	Not transmitted	P
A	52	55	0.85

Table 2. Genotypic transmission disequilibrium test for the –112G/A polymorphism of the *UGRP1* gene in 131 Japanese asthmatic families

	G/G	G/A	A/A	P
Observed transmission	21	26	7	0.96
Expected transmission	20.2	27.0	6.8	

Table 3. Genotypic distribution of the –112G/A polymorphism of the *UGRP1* gene in the case-control population

	n	Genotype counts (frequencies)			P^*	Allele counts (frequencies)		P
		G/G	G/A	A/A		G	A	
Asthmatic children	131	78 (0.60)	42 (0.32)	11 (0.08)		198 (0.76)	64 (0.24)	
Controls (children)	137	91 (0.66)	38 (0.28)	8 (0.06)	0.26	220 (0.80)	54 (0.20)	0.21
Controls (adults)	211	133 (0.63)	71 (0.34)	7 (0.03)	0.57	337 (0.80)	85 (0.20)	0.22

*Fisher's exact test was performed for G/G vs. G/A and A/A.

explanations for the apparent inconsistency between our findings and the findings of Niimi et al., both obtained in Japanese individuals. We examined asthmatic children, whereas Niimi et al. examined asthmatic adults. Although the overall features of asthma are similar in children and adults, there are numerous distinctions, for example, in presentation, pathogenesis, and treatment. Some studies showed that children are more sensitive to bronchial challenge agents than adults, and the proportion of lavage fluid recovered and the total number of cells harvested was less in paediatric asthmatics than in adult asthmatics [8, 9]. During the aging process, the lungs are exposed to infectious agents, antigens, and pollutants, each of which can have both acute and chronic effects on the M2 muscarinic receptors of the parasympathetic nerves supplying the lungs. Childhood asthma may be influenced by genes other than *UGRP1*, which may mask the –112A effect we were looking for in our families. Thus, it is possible that the findings of both studies are true, and that an association exists for adult asthma, but not childhood asthma.

The precise function of *UGRP1* is unknown, but it is clear that *UGRP1* is predominantly expressed in the lung and that it is a downstream target gene for the T/EBP/NKX2.12.1 homeodomain transcription factor [6]. Western blotting analyses have indicated that *UGRP1* is a secreted protein that may function as a homodimer, as is the case with uteroglobin/CCSP, which is believed to modulate lung inflammatory response to infection [10, 11]. The amino acid similarities between *UGRP1* and uteroglobin/CCSP family proteins are significant in the areas of signal peptide and antilamin. It is possible that the –112A allele could have a more significant effect on the development of the inflammatory aspect of the asthmatic reaction than on the sensitization patterns.

It is also possible that we obtained false-negative results. The power of the present study was above 99.9% for detection of an association with an odds ratio of 4.11. However, the low end of the 95% confidence interval of the odds ratio for association reported by Niimi et al. was 1.51, and the detection power of the present study drops to 39% with an odds ratio of 1.51. Therefore, it is possible that an association is present, but that it is weaker than initially reported and that it escaped our statistical calculations.

It is also possible that the association found by Niimi et al. was not a true association. This is a likely possibility because the Niimi et al. study was primarily a case-control study. Case-control studies are prone to false-positive results caused by inadequate control. The frequency of the –112A allele among control subjects (0.10) in the Niimi et al. study was significantly lower than among our control children (0.20) ($P=0.0075$) and among our control adults (0.20) ($P=0.0026$, Fisher's exact test). There is the possibility that the frequency of the –112A allele may differ between Tsukuba and Fukushima, which are approximately 100 km apart. However, the –112A allele frequency among 1498 chromosomes from Japanese (0.215) in the JSNP database (<http://snp.ims.u-tokyo.ac.jp/index.html>) (JSNP ID: IMS-JST025649) is almost the same as the frequency of our controls (0.20 and 0.20) and is not significantly different from

the –112A allele frequencies of patients with asthma in the Niimi et al.'s study (0.22). Therefore, it is possible that the control samples in the Niimi et al. study may have been biased.

In addition to the case-control comparison, we examined the association by TDT. This family-based association test overcomes the problems of stratification, which can lead to both false-positive and false-negative findings. Our TDT analysis indicated that the –112A allele was not preferentially transmitted to asthmatic offspring, and this is consistent with our case-control association study findings.

In conclusion, our results indicate that the –112A polymorphism does not exert a substantial influence on the inheritance of childhood asthma in the Japanese population.

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