

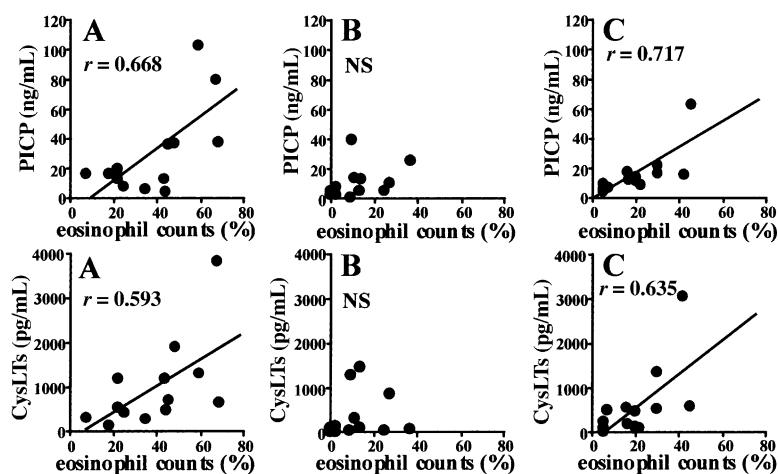


FIG 2. Correlation between eosinophil counts and concentrations of PICP and CysLTs in 13 montelukast-responders before (A) and on medication (B) with montelukast, and after its cessation (C). NS, Not significant.

attracting eosinophils into airways and inducing collagen production in fibroblasts. Further studies are required to confirm the current finding, because this is a pilot study.

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Association between TARC C-431T and atopy and asthma in children

To the Editor:

Asthma is one of the most common chronic illnesses in children. The International Study of Asthma and Allergies in Childhood found that asthma affected 7% to 10% of Hong Kong schoolchildren.¹ Overproduction of T_H2-related cytokines is an important feature of asthmatic airway inflammation.² Thymus and activation-regulated chemokine (TARC) is a key chemokine for attracting T_H2 lymphocytes into sites of allergic inflammation.^{3,4} Our research group has recently found plasma TARC concentrations to be related to chronic and acute asthma in children.^{5,6} TARC was also released into the bronchoalveolar lavage fluid of asthmatic patients who underwent segmental allergen bronchoprovocation.³ The gene encoding TARC (*TARC*) is located on chromosomal region 16q13.⁷ Polymorphism screening of the coding and promoter regions of *TARC* revealed 3 single nucleotide polymorphisms (SNPs).⁸ Among these, the mutant -431T in the *TARC* promoter (accession no. DDBJ; AB088475 and HGV base; SNP001743394) was detected in up to 40% of Japanese subjects. This polymorphism was associated with plasma TARC concentration. Nonetheless, the authors failed to show any association between this SNP and susceptibility to asthma or atopic dermatitis. However, they did not measure other detailed

TABLE I. Demographics and clinical characteristics in 282 asthmatic patients and 105 control subjects

Characteristics*	Asthmatic patients	Control subjects
Age (y)	10.1 ± 3.8	10.6 ± 4.9
Male sex (n [%])	182 (65)	64 (61)
Serum log-transformed total IgE concentration (kIU/L)	2.59 ± 0.62†	1.83 ± 0.79
Atopy (≥1 specific IgE) (n [%])	252 (89)†	48 (46)
Sensitization (specific IgE) to aeroallergens		
<i>D pteronyssinus</i> (n [%])	247 (88)†	46 (44)
Mixed cockroaches (n [%])	79 (28)‡	16 (15)
Cat (n [%])	41 (15)§	3 (3)
PB eosinophils (% of total leukocytes)¶	7.5 ± 4.7†	3.5 ± 3.1
Spirometric variables		
FEV ₁ (% predicted)	97 ± 20	ND
FEV ₁ /FVC (%)	78 ± 11	ND

PB, Peripheral blood; ND, not done; FVC, forced vital capacity.

*Expressed as means ± SD unless stated otherwise.

† $P < .0001$.

‡ $P < .01$.

§ $P < .005$.

¶Results on eosinophil counts were not available for 26 subjects.

markers of atopy (eg, eosinophil count and total and allergen-specific IgE level).⁸

To assess whether C-431T of the *TARC* gene is related to asthma and atopy phenotypes, we conducted a case-control association study of this SNP in 282 asthmatic and 105 control children in Hong Kong. In this article we present a significant association of *TARC* C-431T with the susceptibility to atopy and peripheral eosinophilia in these Chinese children.

Subjects aged 5 to 18 years of age were recruited from pediatric clinics of a university teaching hospital in Hong Kong. All patients were unrelated ethnic Chinese who were given a diagnosis of asthma according to the American Thoracic Society guidelines. Age- and sex-matched nonallergic control subjects were selected from a group of children attending the hospital for minor nonrespiratory complaints. All subjects were free from any symptom of infection for 4 weeks before the study. Subjects or their parents provided written consent, and the clinical research ethics committee of our university approved this study.

Eosinophils in peripheral blood were enumerated with a Coulter STKS counter (Beckman-Coulter Instruments Co, Miami, Fla). Serum total IgE concentration was measured by means of microparticle immunoassay (IMx analyzer; Abbott Laboratories, Abbott Park, Ill), and specific IgE antibodies to *Dermatophagoides pteronyssinus*, cat, and mixed cockroaches were measured by means of fluorescent enzyme immunoassay (AutoCAP system; Pharmacia Diagnostics AB, Uppsala, Sweden). Results on serum total IgE concentrations were compared with age-specific reference values (upper limits of normal,

120 kIU/L for 5 to 6 years, 160 kIU/L for 6 to 7 years, and 180 kIU/L for ≥8 years) established in our laboratory and expressed after log transformation. A specific IgE concentration of 0.35 kIU/L or greater was considered positive. Atopy was defined by the presence of 1 or more allergen-specific IgE in serum. TARC concentration in EDTA-anticoagulated plasma was measured by means of ELISA (R&D Systems, Minneapolis, Minn). The sensitivity of this kit was 7 pg/mL. Asthmatic patients also underwent spirometric assessment (COMPACT II; Vitalograph, Buckingham, England) to measure their FEV₁ and forced vital capacity values.

TARC C-431T genotypes were determined in genomic DNA by means of PCR and RFLP. The promoter region of *TARC* was amplified by using the following primers according to the published method⁸: forward, 5'-GGC AGA TAA AGC ATG GAT CTC-3'; reverse, 5'-GAG AGC ATC CTT CAT GCA TG-3'. Briefly, PCR reactions were carried out in a total volume of 30 µL containing 100 ng of DNA, 3 µL of 10 × PCR Gold Buffer, 0.25 mmol/L MgCl₂, 200 µmol/L of each deoxynucleotide triphosphate, 0.4 mmol/L of each primer, and 1 unit of AmpliTaq Gold polymerase (Roche Molecular Systems, Alameda, Calif). Samples were denatured at 95°C for 10 minutes, followed by 35 cycles of 95°C for 60 seconds, 65°C hybridization temperature for 60 seconds, and 72°C for 60 seconds and a final extension for 10 minutes at 72°C. The 260-bp PCR product was digested with 3 units of *SalI* (New England Biolabs, Hitchin, Herts, United Kingdom) and incubated overnight at 37°C. This enzyme digested the PCR product into 199- and 61-bp fragments when the C allele was present. The digested products were visualized on 1.5% (wt/vol) agarose gel. A positive control with known C-431T genotype was included in every run of restriction enzyme digestion. The results of RFLP assay were also confirmed by means of direct sequencing of the *TARC* promoter with BigDye Terminator Cycle sequencing kits with an ABI-3100 autosequencer (Applied Biosystems, Foster City, Calif) in 20 randomly selected samples. The allele frequencies of -431T in our patients and control subjects were 29% and 27%, respectively ($P = .536$), and the genotype-allele distribution is in Hardy-Weinberg equilibrium ($P = .241$ for patients and $P = .469$ for control subjects).

The demographics and pattern of allergic sensitization in our subjects is summarized in Table I. Associations between *TARC* C-431T genotypes with different dichotomous asthma and atopy phenotypes were analyzed by using multiple logistic regression to adjust for age and sex, and the results are summarized in Table II. Two alternative models (recessive and codominant) were fit for each outcome. For all outcomes except cockroach sensitization, a higher percentage of the subjects with the outcome had the TT genotype. However, an excess percentage of the CT genotype was only observed for those with susceptibility to peripheral eosinophilia. Briefly, the TT genotype of *TARC* C-431T was significantly associated with the presence of allergen-specific IgE (ie, atopy), sensitization to *D pteronyssinus* and cat

TABLE II. Association between asthma and atopy phenotypes and *TARC* C-431T genotypes

Phenotypes	n	<i>TARC</i> -431 genotypes			<i>P</i> values*	
		CC (%)	CT (%)	TT (%)	Recessive	Codominant
All physician-diagnosed asthma	282	147 (52)	105 (37)	30 (11)		
with control children	105	54 (51)	46 (44)	5 (5)	.077	.478
Asthma with atopy†	252	130 (52)	93 (37)	29 (12)		
with control children	105	54 (51)	46 (44)	5 (5)	.058	.385
Subjects with increased total IgE	261	137 (52)	98 (38)	26 (10)		
with normal total IgE	126	64 (51)	53 (42)	9 (7)	.420	.896
Subjects with atopy†	300	156 (52)	110 (37)	34 (11)		
with nonatopic subjects	87	45 (52)	41 (47)	1 (1)	.027	.254
Sensitization to aeroallergens						
<i>D pteronyssinus</i> IgE positive	293	151 (52)	109 (37)	33 (11)		
<i>D pteronyssinus</i> IgE negative	94	50 (53)	42 (45)	2 (2)	.027	.195
Cockroach IgE positive	95	51 (54)	40 (42)	4 (4)		
Cockroach IgE negative	292	150 (51)	111 (38)	31 (11)	.040	.230
Cat IgE positive	44	23 (52)	13 (30)	8 (18)		
Cat IgE negative	343	178 (52)	138 (40)	27 (8)	.049	.366
Subjects with increased PB eosinophils‡	206	98 (48)	84 (41)	24 (12)		
with normal eosinophils	155	91 (59)	59 (38)	5 (3)	.007	.004

PB, Peripheral blood.

*Analyzed by means of logistic regression analyses of recessive and codominant models, controlling for age and sex as covariates.

†Defined as 1 or more allergen-specific IgE.

‡Eosinophil count 5% or more of all peripheral blood leukocytes.

allergens, and susceptibility to peripheral eosinophilia (ie, $\geq 5\%$ peripheral blood leukocytes). Linear regression models were also fitted with the transformed continuous outcome variables, natural logarithm of total IgE, and square root of the eosinophil percentage. For log-transformed total IgE, the covariate corresponding to the recessive model was positive and significant ($P = .038$, adjusting for age and sex), indicating that subjects with the TT genotype had a significantly higher adjusted mean IgE level. The recessive model covariate was also positive and significant ($P = .014$) for the model with the square root of eosinophil percentage as the outcome, indicating that those with the TT genotype had significantly higher eosinophil percentages. The covariate corresponding to the codominant model was not significant for log IgE but was positive and significant ($P = .030$) for the square root of the eosinophil model.

The median plasma TARC concentrations were 142 pg/mL (interquartile range [IQR], 72–222 pg/mL) for 280 patients and 100 pg/mL (IQR, 65–172 pg/mL) for 91 control subjects whose plasma samples were available for testing ($P = .010$, Mann-Whitney U test). In the subgroup of 280 asthmatic patients, plasma TARC concentrations showed significant association with *TARC* C-431T ($P = .012$ for trend, Kruskal-Wallis test). Patients homozygous for the T allele had significantly higher plasma TARC levels compared with those with the CT and CC genotypes, with their median values being 188 pg/mL (IQR, 106–327 pg/mL), 148 pg/mL (IQR, 85–218 pg/mL), and 129 pg/mL (IQR, 61–208 pg/mL), respectively ($P = .004$ for TT vs CC and $P = .046$ for TT vs CT).

The genetic variant C-431T in the *TARC* promoter is a recently identified functional polymorphism, and

subjects with the T allele had increased plasma TARC concentrations. However, this polymorphism was not associated with asthma or atopic dermatitis in the adult Japanese population.⁸ This study is limited mainly by the relatively small number of subjects recruited, as well as the fact that atopy and related phenotypes (eg, serum IgE level and peripheral eosinophilia) were not assessed. In contrary, the present study confirmed that C-431T was associated with plasma TARC concentrations in our asthmatic children. Subjects with –431T were susceptible to atopy, sensitization to *D pteronyssinus* and cat allergens, and peripheral eosinophilia during childhood. Another possibility is that *TARC* C-431T might actually be a marker for a broader haplotype block, with linkage to SNPs in either *TARC* or the adjacent genes that regulates the expression of *TARC* or the susceptibility to asthma and atopy development.

Our results support the hypothesis that TARC, being a T_H2 -specific chemokine, plays a crucial role in the pathogenesis of atopy. Our previous study suggests that plasma TARC concentration is increased in children with chronic stable asthma⁵ and related to the severity of asthma exacerbation,⁶ and increased TARC production is associated with a state of systemic allergic inflammatory response. The associated T_H2 activation results in increased IgE production and recruitment of eosinophils from bone marrow under the actions of the important T_H2 cytokines IL-4 and IL-5, respectively.² The present study, as well as a previous report,⁸ suggest that the T allele increased TARC production. On the basis of the published sequences, this C-to-T change at –431 of *TARC* would alter a putative binding site for activator protein 4 transcription factor.⁹ Enhanced TARC expression could

also be the link for increased specific IgE production and peripheral eosinophilia. In the recessive genetic model, a higher percentage of subjects with the TT genotype have atopic asthma, and this supports the importance of TARC in asthma pathogenesis.³⁻⁶ Asthmatic patients homozygous for the T mutant had significantly higher plasma TARC concentrations compared with the CT and CC genotypes, which is consistent with the finding that TARC C-431T was significantly associated with various atopy phenotypes under the recessive but not codominant model. Thus we hypothesize that 2 T alleles have to be present for this SNP to exert any influence on TARC expression. Functional TARC reporter assay is necessary to answer this question.

The allele frequencies of the TARC C-431T allele in this study is lower compared with that seen in Japanese subjects⁸: the allele frequency of T was 27% in our control subjects and 36% in Japanese adult control subjects. The significant difference in genetic epidemiology of TARC C-431T between our southern Chinese subjects and the closely related Japanese subjects is similar to that reported for IL13 R130Q.¹⁰ This finding highlights the importance of replicating genetic association studies in each population rather than generalizing these findings when one tries to establish the relationship between susceptibility genes and complex human diseases, such as asthma and atopy.

In summary, the C-431T polymorphism in the TARC promoter shows a significant association with atopy, house dust mite, and cat sensitization and peripheral eosinophilia in Chinese children. The results of this study support that TARC plays an important role in enhancing T_H2-mediated allergic inflammation that results in IgE production and eosinophil recruitment.

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Exhaled air temperature and eosinophil airway inflammation in allergic asthmatic children

To the Editor:

The need to monitor airway inflammation in asthma, particularly in pediatric populations, has led to the development of a number of noninvasive methods of assessment,¹ including analysis of cells and cell products in sputum samples collected by means of induced sputum stimulation,¹⁻³ as well as the measurement of exhaled markers¹⁻³ and soluble mediators obtained from exhaled breath condensates.¹⁻⁵ Recently, exhaled air temperature has also been suggested as a noninvasive method for the evaluation of airway inflammation in asthma.^{6,7} The present study was performed to determine the utility of measurement of exhaled air temperature changes in a group of mite-sensitive asthmatic children as a predictive marker of lung inflammation, as determined on the basis of eosinophil content in hypertonic saline-induced sputum samples from these children.

Thirteen mite-sensitized asthmatic children (8-15 years of age) were evaluated on the day of the entry to the residential house "Istituto Pio XII" (Misurina BL) at 1756 m in the Italian Alps, an environment free of mite allergen because of its location at a high altitude. The study was approved by the Istituto Pio XII Ethics Committee, and informed consent was obtained from the children, as well as their parents. The children had been living with their families for at least a 3-month period before their admission to the residential home. An initial baseline evaluation was performed in September (T0) on entry to the residential home, and a second assessment was performed in December (T1). The study consisted of measurements of exhaled air temperature and sputum induction and was performed out of the pollen season to avoid the confounding effects of outdoor allergens.