

but not by preincubation with *A fumigatus* and *F culmorum* extracts. Similarly, mycoprotein extract could inhibit the reactions to *A alternata* and *C herbarum* but not the reactions to *A fumigatus*. This provides evidence of shared allergenic determinants at 15-16 kd between the mycoprotein, *A alternata*, and *C herbarum*; in contrast, the 31-kd band of *A fumigatus* seems to be unrelated to any mycoprotein band. It has to be emphasized that no allergen has yet been described with such molecular mass in *Cladosporium* extracts.³ In *Alternaria* extracts, the reduced form of Alt a 1 is known to be detected at such molecular mass,³ but inhibition experiments with recombinant Alt a 1 (Biomay, Vienna, Austria), in reduced and nonreduced form, demonstrated no link between the major allergen of *Alternaria* and the proteins recognized by our patient, which thus represent newly described allergens.

Until now, according to information obtained from the company itself, there have been 5 proven positive skin prick test results in individuals who have apparently reacted to mycoprotein in a manner suggestive of IgE involvement. In the literature, the immunologic status of 12 workers involved in mycoprotein production and 10 individuals who presented with vomiting or diarrhea after its ingestion has been investigated.⁴ Sensitization to the product has been demonstrated in 2 workers, but they had no symptoms. Data from the complaining consumers led the authors of that study⁴ to the conclusion that the mechanisms involved were probably not IgE-mediated. In the same study, RAST-inhibition experiments with mold-sensitized sera demonstrated immunologic cross-reactivity between mycoprotein and *A alternata*, *C herbarum*, and *A fumigatus*, which therefore indicated that patients allergic to molds might react to mycoprotein.

If food-pollen cross-reactivity and food-latex cross-reactivity are well-known syndromes,⁵ food-mold spores cross-reactivity is in comparison very poorly documented. A single case report has recently been published that describes cross-reactivity between mushroom and mold spores.⁶ This case and ours suggest the existence of mold spore-food cross-reactivity as a new inhalant-associated food allergy syndrome. In such syndromes, it is generally considered that inhalant allergy precedes and induces food allergy.⁵ The fact that our patient, to the best of her knowledge, had never previously eaten a Quorn product indicates a primary sensitization by inhaled mold spores. If this is the case, patients sensitized to mold spores (at least *Cladosporium* and *Alternaria*) could be at risk when eating such a product, even for the first time.

Although this new type of inhalant-associated food allergy seems to be rare and the kind of cross-reactivity presented here rather exceptional, special attention should be given to its occurrence and to the potential for a rise in its incidence as the consumption of products containing edible fungi or food ingredients derived from fungal fermentations continues to increase.

Paul Van Durme, MD^a

Jan L. Ceuppens, MD, PhD^{a,b}

Pascal Cadot, PhD^b

^aDivision of Allergy and Clinical Immunology

University Hospital Gasthuisberg
^bLaboratory for Experimental Immunology
Gasthuisberg O & N
B-3000 Leuven
Belgium

P. Cadot is supported by a grant from Levenslijn (FWO-Vlaanderen 7-0013-00).

REFERENCES

1. Rodger G. Production and properties of mycoprotein as a meat alternative. *Food Technology* 2001;55:36-41.
2. Voorhorst R. Perfections of skin testing technique. A review. *Allergy* 1980;35:247-50.
3. Kurup VP, Shen H-D, Vijay H. Immunobiology of fungal allergens. *Int Arch Allergy Immunol* 2002;129:181-8.
4. Tee RD, Gordon DJ, Welch JA, Newman Taylor AJ. Investigation of possible adverse allergic reactions to mycoprotein ('Quorn'). *Clin Exp Allergy* 1993;23:257-60.
5. Sicherer SH. Clinical implications of cross-reactive food allergens. *J Allergy Clin Immunol* 2001;108:881-90.
6. Dauby P-AL, Whisman BA, Hagan L. Cross-reactivity between raw mushroom and molds in a patient with oral allergy syndrome. *Ann Allergy Asthma Immunol* 2002;89:319-21.

doi:10.1067/mai.2003.1613

Association of thromboxane A2 receptor (TBXA2R) with atopy and asthma

To the Editor:

Human thromboxane A2 (TBXA2) is a potent constrictor of the airway smooth muscle and an inducer of acetylcholine release and of airway microvascular leakage.¹ It may play a part in the thickening and remodeling of the airway wall that may in turn contribute to airway hyperresponsiveness, a typical feature of asthma. The receptor for TBXA2, thromboxane A2 receptor (TBXA2R, MIM# 188070), is a member of the family of G protein-coupled receptors and performs an essential role in hemostasis by interacting with thromboxane. TBXA2R is located on chromosome 19p13.3¹ and contains 4 exons with total length of 15 kb, composed of 343 amino acids.² TBXA2 and its receptor (TBXA2R) are involved in the constriction of vascular and respiratory smooth muscles. Significantly, 1 polymorphism in the TBXA2R gene (*TBXA2R*+924T > C) was recently found to be associated with asthma and atopic asthma.^{3,4}

To assess whether genetic variants of the TXA2 receptor gene relate to the asthma phenotypes, we conducted an association study of genetic polymorphisms of TXA2 receptor (*TBXA2R*+795T > C and *TBXA2R*+924T > C) in a Korean asthma cohort (n = 721). In our article, we present a positive association of genetic polymorphisms with asthma and atopy development in the Korean population.

Subjects were recruited from the Asthma Genome Research Center, which consists of 4 tertiary hospitals in Korea (Soonchunhyang University Hospital, Ajou University Hospital, Choong-Ang University Hospital, and Ulsan University Hospital). Ethical approvals were obtained from the institutional review board of each hospital. All patients had clinical symptoms and a physical examination compatible with asthma. Each patient

showed airway reversibility as documented by an inhalant bronchodilator-induced improvement of more than 15% of FEV₁ or an airway hyperreactivity of less than 10 mg/mL of methacholine. Normal subjects were recruited from spouses of the patients and the general population who answered negatively to a screening questionnaire for respiratory symptoms and had an FEV₁ greater than 75% predicted, PC₂₀ methacholine greater than 10 mg/mL, and normal findings on a simple chest radiogram. Total IgE and specific IgE to *Dermatophagoides farinae* and *D pteronyssinus* were measured using the CAP system (Pharmacia Diagnostics, Sweden). Atopy was defined by the presence of an immediate skin reaction (histamine of > 3 mm in diameter) to 1 or more of 24 common aeroallergens (dust mites [*D farinae* and *D pteronyssinus*], cat fur, dog fur, cockroach, grass, tree pollens, and ragweed), or by the positive response (score ≥ 1) of specific IgE to *D pteronyssinus* or *D farinae*. (The clinical parameters are available at http://www.snp-genetics.com/user/news_content.asp?board_idx=141). Sequences of amplifying and extension primers for *TBXA2R*+795T > C and *TBXA2R*+924T > C were forward-5'-gagtgaccctggatctca-3', reverse 5'-gcgctctgtccacttct-3', extension-5'-cgcagcactgtctgggc-3' (*TBXA2R*+795T > C), and extension-5'-tcctggaccctgggtga-3' (*TBXA2R*+924T > C). A single base extension method was used for genotyping of single nucleotide polymorphisms (SNPs) in *TBXA2R* as described previously⁵ (available at http://www.snp-genetics.com/user/news_content.asp?board_idx=140). The genotype distribution of *TBXA2R* SNPs and haplotypes between (1) patients with bronchial asthma and normal subjects, and (2) atopic and nonatopic subjects were analyzed by logistic regression models controlling for age (continuous value), sex (male = 0, female = 1), and smoking status (categorized as nonsmoker = 0, exsmoker = 1, and smoker = 2) as covariables. Three SNPs have been reported previously in *TBXA2R* (*TBXA2R*+179(R60L) G > T, *TBXA2R*+795T > C, and *TBXA2R*+924T > C). But the nonsynonymous SNP in exon 1 (*TBXA2R*+179(R60L) G > T) was excluded in genotyping because of extremely low frequency in the Japanese population (freq = 0.003, n = 165).³ Two SNPs in *TBXA2R* (*TBXA2R*+795T > C and *TBXA2R*+924T > C) showed complete linkage disequilibrium (ID'1 = 1 and $d^2 \neq 1$), so only three haplotypes were constructed. *TBXA2R*-ht1[C-C] and *TBXA2R*-ht3[T-T] were equivalent with *TBXA2R*+795T > C and *TBXA2R*+924T > C, respectively. The allele frequencies of *TBXA2R*+795T > C, *TBXA2R*+924T > C, and *TBXA2R*-ht2[C-T] were 0.38, 0.20, and 0.17 in the Korean population (n = 721), respectively.

Genetic associations of two known variants in *TBXA2R* with asthma and atopy were analyzed. The statistical analyses are summarized in Tables I and II. In a comparison of genotype distribution in patients with asthma and normal controls, no significant differences were detected by single SNPs. And the frequency of *TBXA2R*-ht2[C-T] containing genotype in patients with asthma was lower than that in normal controls (28.8% vs 35.6%, $P = .004$). In subgroup analyses with atopic sub-

jects only, the T allele of *TBXA2R*+795T > C in patients with asthma was more frequent in them than in normal controls (42.5% vs 27.8%, $P = .004$). Also, a higher frequency of *TBXA2R*-ht2[C-T] containing genotype in patients with asthma was detected (69.2% vs 59.0%, $P = .007$). Although it was not statistically significant, *TBXA2R*-ht2[C-T] containing genotype in patients with asthma was also higher than in normal controls without atopy (75.9% vs 69.4%, $P = .31$). In analyses of association with atopy (Table II), the other SNP (*TBXA2R*+924T > C), which showed no signal for development of asthma, revealed a significant association with atopy in all subjects and asthma patients ($P = .006$ -.008). *TBXA2R*-ht2[C-T] also seemed to have weak signals with the same direction for atopy ($P = .05$ -.17) as in asthma development.

One of the genetic variants in the TXA2 receptor (*TBXA2R*+795T > C) was reported previously to have an association with serum IgE concentrations in atopic dermatitis,⁷ increasing susceptibility to asthma³ and atopic asthma⁴ developing. The results might be controversial, because the effects of *TBXA2R*+795T > C were statistically marginal in the Chinese population ($P = .04$) and in the Japanese population ($P = .01$ -.03).^{3,4} In this study, we could reproduce the association of genetic variants in *TBXA2R* with developing asthma (*TBXA2R*-ht2 and *TBXA2R*+795T > C) and with developing atopy (*TBXA2R*+924T > C) in the Korean population. Similarly, with study of the Chinese population,⁴ we could not find a significant relationship of *TBXA2R*+795T > C with serum total IgE (data not shown) reported in the Japanese population in patients with atopic dermatitis.⁷ Although asthma is a multifactorial condition, the strongest risk factor in the etiology of asthma is atopy. An atopic individual has a significantly greater probability of asthma developing, and persons with a family history of atopic disease are at greatest risk of asthma. It is accepted that an immunologic response to various allergenic stimuli, including pet dander, dust mites, cockroaches, fungi, and foods, is a major triggering factor in asthma symptom development. The allergic response to dust mites involves an immediate hypersensitivity reaction, including increases in specific IgE antibodies and T cells of the T_H2 phenotype. The associations of *TBXA2R* polymorphisms with asthma and atopy simultaneously in this study provide further evidence for a common genetic etiology of these two highly correlated phenotypes. The roles of *TBXA2R* in asthma and association results including this study suggest that *TBXA2R* polymorphisms might play a critical role in the development of asthma and atopy. In addition, positive signals from *TBXA2R* haplotype suggest that the effects of the *TBXA2R* variant could be the result of a combined genotype (haplotype) rather than single polymorphisms. A clinical study showed that responders and nonresponders to TBXA2 receptor antagonist exist in patients with asthma.⁶ This raises the possibility that *TBXA2R* polymorphism can influence the responsiveness to TBXA2 receptor antagonist in patients with asthma or that patients with asthma have a different pharmacoge-

TABLE I. Genotype distribution of *TBXA2R* SNPs in bronchial asthmatic and normal subjects in a Korean population

					P value*		
Locus	Group	Genotype			Co-dominant	Dominant	Recessive
All							
TBXA2R+795T>C	BA	T 217 (40.7%)	CT 240 (45%)	C 76 (14.3%)	.11	.13	.29
	NC	58 (35.4%)	79 (48.2%)	27 (16.5%)			
TBXA2R+924T>C	BA	T 338 (63.7%)	CT 169 (31.8%)	C 24 (4.5%)	.39	.44	.55
	NC	105 (64%)	52 (31.7%)	7 (4.3%)			
TBXA2R-ht2	BA	—/— 378 (71.2%)	—/ht2 135 (25.4%)	ht2/ht2 18 (3.4%)	.003	.004	.10
	NC	105 (64.4%)	49 (30.1%)	9 (5.5%)			
Atopy							
TBXA2R+795T>C	BA	T 157 (42.5%)	CT 156 (42.3%)	C 56 (15.2%)	.04	.004	.88
	NC	22 (27.8%)	49 (62%)	8 (10.1%)			
TBXA2R+924T>C	BA	T 248 (67.6%)	CT 103 (28.1%)	C 16 (4.4%)	.86	.57	.39
	NC	50 (64.1%)	27 (34.6%)	1 (1.3%)			
TBXA2R-ht2	BA	—/— 254 (69.2%)	—/ht2 97 (26.4%)	ht2/ht2 16 (4.4%)	.02	.007	.87
	NC	46 (59%)	29 (37.2%)	3 (3.8%)			
Nonatopy							
TBXA2R+795T>C	BA	T 60 (37%)	CT 82 (50.6%)	C 20 (12.3%)	.88	.28	.09
	NC	36 (42.4%)	30 (35.3%)	19 (22.4%)			
TBXA2R+924T>C	BA	T 89 (54.9%)	CT 65 (40.1%)	C 8 (4.9%)	.25	.09	.56
	NC	55 (64%)	25 (29.1%)	6 (7%)			
TBXA2R-ht2	BA	—/— 123 (75.9%)	—/ht2 37 (22.8%)	ht2/ht2 2 (1.2%)	.12	.31	.06
	NC	59 (69.4%)	20 (23.5%)	6 (7.1%)			

BA, Bronchial asthmatic; NC, normal.

*P values for logistic analyses of 3 alternative models (codominant, dominant, and recessive) are shown, controlling age and sex as covariables. *TBXA2R*-ht1 and -ht3 were not analyzed because of equivalent models with *TBXA2R*+795T>C and *TBXA2R*+924T>C.

nomic pattern according to *TBXA2R* polymorphism. In summary, polymorphisms (SNPs and haplotypes) in *TBXA2R* showed a significant relationship with asthma and atopy, although either the 795T > C or 924T > C variants do not confer an amino acid change. The frequency of the *TBXA2R*-ht2[C-T] containing genotype in patients with asthma was lower than that in normal controls (28.8% vs 35.6%, $P = .004$). In addition, *TBXA2R*+924T > C revealed a significant association with atopy in all subjects and asthma patients ($P = .006$ -.008). The results of this study could be helpful in understanding the important function of *TBXA2R* in asthma and atopy development, and also could be a useful target for drug development for asthma and atopy.

Hyoun Doo Shin, PhD^a
Byung Lae Park, PhD^a
Ji Hyun Jung, BS^a
Hoe Jin Wang, BS^a
Hae-Sim Park, MD^b
Byoung Whui Choi, MD^b
Soo-Jong Hong, MD^b

Young Mok Lee, MD^bYong Hoon Kim, MD^bChoon-Sik Park, MD^b^aDepartment of Genetic Epidemiology

SNP Genetics, Inc

Seoul, Korea

^bAsthma Genome Research Group

Soonchunhyang University, Ajou University,

Ulsan University, Choong-ang University Hospital

Korea

Supported by a grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (01-PJ3-PG6-01GN04-003).

REFERENCES

- Devillier P, Bessard G. Thromboxane A2 and related prostaglandins in airways. *Fundam Clin Pharmacol* 1997;11:2-18.
- Nusing RM, Hirata M, Kakizuka A, Eki T, Ozawa K, Narumiya S. Characterization and chromosomal mapping of the human thromboxane A2 receptor gene. *J Biol Chem* 1993;268:25253-9.
- Unoki M, Furuta S, Onouchi Y, Watanabe O, Doi S, Fujiwara H, et al. Association studies of 33 single nucleotide polymorphisms (SNPs) in 29

TABLE II. Genotype distribution of *TBXA2R* SNPs in atopic and nonatopic subjects in a Korean population

					<i>P</i> value		
Locus	Group	Genotype			Co-dominant	Dominant	Recessive
All							
<i>TBXA2R</i> +795T>C	No atopy	T 109 (36.7%)	CT 139 (46.8%)	C 49 (16.5%)	.81	.86	.82
	Atopy	218 (38.6%)	261 (46.2%)	86 (15.2%)			
<i>TBXA2R</i> +924T>C	No atopy	T 171 (57.2%)	CT 112 (37.5%)	C 16 (5.4%)	.01	.006	.49
	Atopy	376 (66.9%)	162 (28.8%)	24 (4.3%)			
<i>TBXA2R</i> - <i>ht2</i>	No atopy	—/— 212 (71.4%)	—/ <i>ht2</i> 76 (25.6%)	<i>ht2</i> / <i>ht2</i> 9 (3%)	.03	.05	.16
	Atopy	371 (66.1%)	164 (29.2%)	26 (4.6%)			
Asthmatic							
<i>TBXA2R</i> +795T>C	No atopy	T 60 (37%)	CT 82 (50.6%)	C 20 (12.3%)	.79	.39	.50
	Atopy	157 (42.5%)	156 (42.3%)	56 (15.2%)			
<i>TBXA2R</i> +924T>C	No atopy	T 89 (54.9%)	CT 65 (40.1%)	C 8 (4.9%)	.02	.008	.71
	Atopy	248 (67.6%)	103 (28.1%)	16 (4.4%)			
<i>TBXA2R</i> - <i>ht2</i>	No atopy	—/— 123 (75.9%)	—/ <i>ht2</i> 37 (22.8%)	<i>ht2</i> / <i>ht2</i> 2 (1.2%)	.04	.07	.11
	Atopy	254 (69.2%)	97 (26.4%)	16 (4.4%)			
Normal control							
<i>TBXA2R</i> +795T>C	No atopy	T 36 (42.4%)	CT 30 (35.3%)	C 19 (22.4%)	.83	.05	.04
	Atopy	22 (27.8%)	49 (62%)	8 (10.1%)			
<i>TBXA2R</i> +924T>C	No atopy	T 55 (64%)	CT 25 (29.1%)	C 6 (7%)	.51	.98	.11
	Atopy	50 (64.1%)	27 (34.6%)	1 (1.3%)			
<i>TBXA2R</i> - <i>ht2</i>	No atopy	—/— 59 (69.4%)	—/ <i>ht2</i> 20 (23.5%)	<i>ht2</i> / <i>ht2</i> 6 (7.1%)	.44	.17	.38
	Atopy	46 (59%)	29 (37.2%)	3 (3.8%)			

candidate genes for bronchial asthma: positive association with a T924C polymorphism in the thromboxane A2 receptor gene. *Hum Genet* 2000;106:440-6.

- Leung TF, Tang NL, Lam CW, Li AM, Chan IH, Ha G. Thromboxane A2 receptor gene polymorphism is associated with the serum concentration of cat-specific immunoglobulin E as well as the development and severity of asthma in Chinese children. *Pediatr Allergy Immunol* 2002;13:10-7.
- Makridakis NM, Reichardt JK. Multiplex automated primer extension analysis: simultaneous genotyping of several polymorphisms. *Biotechniques* 2001;31:1374-80.
- Tanaka H, Igarashi T, Saitoh T, Teramoto S, Miyazaki N, Kaneko S, et al. Can urinary eicosanoids be a potential predictive marker of clinical response to thromboxane A2 receptor antagonist in asthmatic patients? *Respir Med* 1999;93:891-7.
- Tanaka K, Roberts MH, Yamamoto N, Sugiura H, Uehara M, Mao XQ, et al. Genetic variants of the receptors for thromboxane A2 and IL-4 in atopic dermatitis. *Biochem Biophys Res Commun* 2002;292:776-80.

doi:10.1067/mai.2003.1641

Evidence for human mast cell nonreleaser phenotype

To the Editor:

Mast cells and basophils express high amounts of FcεRI. In basophils the concentration of anti-IgE or antigen required for maximal histamine release through FcεRI varies among donors, a concept termed "releasability." Some donors fail to release histamine in response to any concentration of IgE-mediated stimulus

(nonreleaser)¹⁻⁴ and were subsequently found to lack the protein tyrosine kinase Syk.⁵ Although the nonreleaser phenotype is common (ie, present in 10% to 20% of all donors), it is not known whether mast cells exhibit a similar IgE-mediated nonresponsiveness. We report here a lung mast cell preparation that did not respond to IgE-mediated stimuli.

Mast cells were dispersed from human lung tissue and enriched as described previously.⁶ This donor was undergoing a thoracotomy for lung cancer and was not receiving steroid therapy. These studies were approved by the Human Studies Internal Review Board at Virginia Commonwealth University. Mast cells were positively selected with mouse anti-c-kit receptor antibodies (YB5B8, a gift from Leonie Ashman) and bead-conjugated anti-mouse antibodies, as per the manufacturer's instructions (Miltenyi, Auburn, Calif). A total of 3.2×10^7 cells were isolated from 30 g of tissue. The purity of mast cells was 76%, as assessed with toluidine blue staining. The mast cells were cultured at 1×10^6 cells/mL in RPMI medium supplemented with 10% CPSR-1 (Sigma, St Louis, Mo), 10 μmol/L HEPES, 50 μmol/L 2-mercaptoethanol, 4 mmol/L L-glutamine, 1× MEM amino acids, 1× vitamin solution, 100 U/mL penicillin, 100 μg/mL streptomycin (Gibco, Gaithersburg Md), and 100 ng/mL recombinant human stem cell factor (Biosource, Camarillo, Calif).