

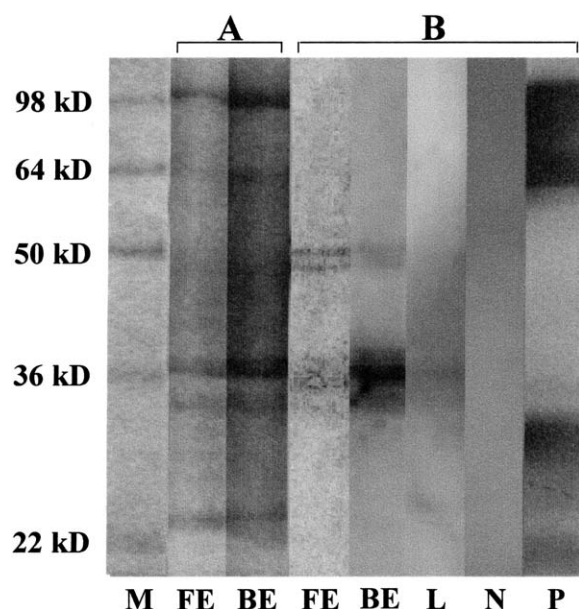


FIG 2. Identification of IgE-binding components with patient serum. Staining with Coomassie brilliant blue on gel electrophoresis (**A**) and IgE immunoblot (**B**) is shown. *M*, Marker; *FE*, 300 µg of fresh eggplant extract; *BE*, 300 µg of boiled eggplant extract; *L*, 125 µg of latex; *N*, boiled eggplant with pooled sera (*n* = 10) as a negative control; *P*, 100 µg of Der f 2 as a positive control.

Partial inhibition of fresh eggplant might come from lower antigenicity of the extract, as seen consistently in SPTs, ELISAs, and Western blotting, and therefore one might presume that thermal processing can modify the antigenicity of eggplant.

We identified one IgE-binding component with the same molecular weight (1 protein band between 22 and 36 kD) from eggplant and latex that is a candidate allergen to reveal cross-reactivity, as are some vegetables of the *Solanum* subfamily. However, the IgE-binding components of eggplant exhibit no cross-reactivity with potato (*Solanum tuberosum*).

We report, for the first time, that eggplant itself contains allergens in amounts that can elicit a specific IgE-mediated response in susceptible individuals and that eggplant allergens have cross-reactivity with some latex antigens.

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REFERENCES

1. Gil M, Hogendijk S, Hauser C. Allergy to eggplant flower pollen. *Allergy* 2002;57:652.
2. Blanco C, Carrillo T, Castillo R, Quiralte J, Cuevas M. Latex allergy: clinical features and cross-reactivity with fruits. *Ann Allergy* 1994;73:309-14.
3. Kim SS, Park HS, Kim HY, Lee SK, Nahm DH. Anaphylaxis caused by the new ant, *Pachycondyla chinensis*: demonstration of specific IgE and IgE-binding components. *J Allergy Clin Immunol* 2001;107:1095-9.
4. Sastre J. Occupational asthma due to different spices. *Allergy* 1996;51:117-20.
5. Reche M, Pascual CY, Vicente J, Caballero T, Martin-Munoz F, Sanchez S, et al. Tomato allergy in children and young adults: cross-reactivity with latex and potato. *Allergy* 2001;56:1197-201.
6. Seppala U, Palosuo T, Kalkkinen N, Yliralo L, Reunala T, Turjanmaa K, et al. IgE reactivity to patatin-like latex allergen, Hev b 7, and to patatin of potato tuber, Sol t 1, in adults and children allergic to natural rubber latex. *Allergy* 2000;55:266-73.
7. Seppala U, Alenius H, Turjanmaa K, Reunala T, Palosuo T, Kalkkinen N. Identification of patatin as a novel allergen for children with positive skin prick test responses to raw potato. *J Allergy Clin Immunol* 1999;103:165-71.
8. Tucke J, Posch A, Baur X, Rieger C, Raulf-Heimsoth M. Latex type I sensitization and allergy in children with atopic dermatitis. Evaluation of cross-reactivity to some foods. *Pediatr Allergy Immunol* 1999;10:160-7. doi:10.1016/j.jaci.2004.01.565

Combination of CC16, GSTM1, and GSTT1 genetic polymorphisms is associated with asthma

To the Editor:

Several studies indicated that oxidative stress impairs pulmonary function and can lead to asthma.^{1,2} Genome-wide searches and candidate gene approaches have identified many genes and regions that potentially influence asthma susceptibility.³⁻⁵

It is reported that glutathione S-transferases (GSTs) modulate reactive oxygen species (ROS) levels.⁶ There are well-defined genetic polymorphisms named *GSTM1-0* and *GSTT1-0* in the *GSTM1* and *GSTT1* genes, respectively, that are null alleles that result in nonfunctional protein. There is not as much information on the relationship between null genotypes of *GSTM1* and *GSTT1* and asthma.^{3,4} It has been shown that the null genotypes of *GSTM1* and *GSTT1* are associated with asthma.⁴ However, other studies did not replicate this association.³

CC16 is a 16-kD homodimeric protein secreted in large amounts in airways by the nonciliated bronchiolar Clara cells. CC16 has an important role in inflammation and immune modulation.⁷ Nucleotide sequence analysis of exon 1 of the gene revealed a nucleotide substitution from A to G at position 38 in the 5' promoter region. This substitution is strongly associated with asthma in a population of Australian children.⁵ However, studies on populations of Japanese and British adults did not replicate these associations.⁸ Taken together, the association between genetic polymorphisms of *GSTM1*, *GSTT1*, and CC16 and susceptibility to asthma is still an open question. Therefore the present study was performed.

This case-control study consisted of 85 patients (58 female and 27 male patients) with asthma (mean ± SD

age, 38.7 ± 14.7 years). A total of 85 healthy individuals matched with the patients for age (± 5 years) and sex were also studied as a control group (mean $\pm$ SD age, 39.6 ± 15.9 years). Both control and patient groups were unrelated Iranian Muslims. Patients were provisionally labeled as having asthma when they met at least 2 of the following 3 criteria: (1) typical history of attacks of breathlessness and/or wheezing, nocturnal cough, chest tightness either spontaneously or triggered by exercise, respiratory infections, or irritants; (2) reversibility if FEV_1 , defined as $[FEV_1 \text{ after} - FEV_1 \text{ before}] / FEV_1 \text{ before}$, greater than 15% after a standard dose of inhaled β_2 -agonist; and (3) diurnal variability in peak expiratory flow (PEF) rate of greater than 20% calculated from the following formula: $(PEF_{\text{highest}} - PEF_{\text{lowest}}) / PEF_{\text{highest}}$.⁹ Patients were included only if they had no history of exposure to agents known to cause interstitial lung disease or extrinsic allergic alveolitis. Jobs such as painting, woodworking, welding, farming, milling, fire fighting, and baking were not allowed. None of the patients worked in petroleum industries. Informed consent was obtained from all participants at the time of blood withdrawal. At the time of blood donation, participants completed a brief questionnaire that ascertained smoking status, age, and alcohol consumption. Both case and control groups had negative histories of alcohol consumption and smoking.

Genomic DNA for PCR was isolated from whole blood. The PCR conditions for determining *GSTM1* and *GSTT1* genotypes were the same as those reported previously.⁴ The absence of amplified product was consistent with the null genotypes. Successful amplification with β -globin-specific primers confirmed the proper function of the PCR reaction. The PCR condition for amplification of a fragment of exon 1 of *CC16* was the same as that reported previously.⁵ Amplification products were digested with *Sau* 96I.

Genotype was defined as AA (absence of restriction site on both alleles), GG (presence of restriction site on both alleles), and AG (heterozygous) after electrophoresis of digested DNA samples.

Because no statistical differences were observed between the sex groups for the frequencies of null genotypes of *GSTM1* and *GSTT1*, the sex groups were pooled. The frequency of the *GSTM1* null genotype is 23.5% and 49.4% in the healthy control subjects and patients, respectively. The *GSTM1* null genotype was more frequent in asthmatic patients compared with control subjects (odds ratio [OR], 3.17; 95% CI, 1.65-6.12; $P = .0004$). The frequency of the null genotype of *GSTT1* is 41.2% and 64.7% in control and patient groups, respectively. The null genotype of *GSTT1* was associated with an increased odds of asthma (OR, 2.62; 95% CI, 1.41-4.87; $P = .002$). To investigate whether the profile of GST genotypes might be associated with the risk of asthma, we examined the asthma risk associated with combinations of the genotypes. The reference group consisted of individuals with 2 putative low-risk genotypes (ie, the presence of *GSTM1* and *GSTT1* functional alleles). A significant association was observed for con-

TABLE I. Association between the combination of *CC16*, *GSTM1*, and *GSTT1* genotypes and asthma

Genotypes	Control subjects	Patients	OR	95% CI
Three putative low-risk genotypes	21	7	1.0	Reference
One putative high-risk genotype	44	30	2.05	0.77-5.41
Two putative high-risk genotypes	18	33	5.50	1.96-15.41
Three putative high-risk genotypes	2	15	22.5	4.08-123.48

χ^2 For linear trend for 0, 1, 2, and 3 putative high-risk genotypes is equal to 23.514 ($P < .00001$).

current lack of the *GSTM1* and *GSTT1* active genes and asthma (OR, 10.23; 95% CI, 3.58-29.29; $P < .00001$).

These results are consistent with those reported by Ivaschenko et al.⁴ In asthma numerous studies have shown that ROS are involved in the inflammatory process.² The enzymes encoded by *GSTM1* and *GSTT1* genes might be involved in developing asthma because the GSTs might influence the synthesis of eicosanoids through modulation of ROS levels.⁶ This view is supported by studies showing that individuals with reduced antioxidant capacity are at increased risk of allergic asthma and that decreased intake of antioxidants is associated with the asthma-related phenotypes.¹

The GG, AG, and AA genotypes of *CC16* were observed in 58, 23, and 4 healthy control subjects and 45, 31, and 9 patients, respectively. The frequencies of the 38A allele in the control and patient groups were 0.18 and 0.29, respectively.

The frequency of the 38A allele was significantly higher in asthmatic patients when compared with control subjects ($\chi^2 = 5.29$; $df = 1$; $P < .05$). Individuals having at least one 38A allele (AA and AG genotypes) compared with the GG genotype showed higher odds of having asthma (OR, 1.91; 95% CI, 1.01-3.61; $P = .047$). Previous research has provided conflicting results on the association between *CC16* polymorphism and asthma.^{5,8} *CC16* appears to function as an anti-inflammatory agent,⁷ and the substitution of 38G for 38A is associated with a significant decrease of the human concentration of *CC16*.¹⁰

Table I shows the potential effect of combinations of genotypes on asthma risk. The reference group consisted of individuals with 3 putative low-risk genotypes (ie, the presence of *GSTM1* and *GSTT1* functional genotypes and the *CC16* GG genotype). The presence of 2 putative high-risk genotypes resulted in an almost 5.5-fold risk of the disease (OR, 5.50; 95% CI, 1.96-15.41; $P = .0008$). The concurrence of the AA genotype, the AG genotype, or both of *CC16* and lack of both of the active genes of *GSTM1* and *GSTT1* posed more than a 22-fold odds of asthma (OR, 22.5; 95% CI, 4.08-123.48; $P = .00004$). χ^2 Analysis of linear trend for 0, 1, 2, and 3 putative high-risk genotypes is equal to 23.514 ($P < .00001$). It is

suggested that inability to detoxify ROS because of null genotypes of *GSTM1* and *GSTT1* and decreased capacity of the inflammatory effect of *CC16* because of the presence of the 38A allele through the low level of CC16 concentration should perpetuate the inflammatory process, activate bronchoconstrictor mechanisms, and precipitate asthma symptoms. Other studies are needed to elucidate the cooperative or possible antagonistic effect of these polymorphisms with other candidate genes, as well as the effect of environmental factors in asthma.

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REFERENCES

1. Soutar A, Seaton A, Brown K. Bronchial reactivity and dietary antioxidants. *Thorax* 1997;52:166-70.
2. Barners PJ. Reactive oxygen species and airway inflammation. *Free Radic Biol Med* 1990;9:235-43.
3. Fryer AA, Bionco A, Hepple M, Jones PW, Strange RC, Spiteri MA. Polymorphisms at the glutathione S-transferase GSTP1 locus. *Am J Respir Crit Care Med* 2000;161:1437-42.
4. Ivaschenko E, Sideleva G, Branaov S. Glutathione S-transferase macro and theta gene polymorphisms as new risk factor of atopic bronchial asthma. *J Mol Med* 2002;80:39-42.
5. Laing IA, Goldblatt J, Eber E, Hayden CM, Rye PJ, Gibson NA, et al. A polymorphism of the CC16 gene is associated with an increased risk of asthma. *J Med Genet* 1998;35:463-7.
6. Hayes JD, Strong RC. Potential contribution of the glutathione S-transferase supergene family to resistance to oxidative stress. *Free Radic Res Commun* 1995;22:193-207.
7. Mango GW, Johnston CJ, Reynolds SD, Finkelstein JN, Plopper CG, Stripp BR. Clara cell secretory protein deficiency increases oxidative stress response in conducting airways. *Am J Physiol* 1998;275:L348-56.
8. Gao PS, Mao XQ, Kawai M, Enomoto T, Sasaki S, Tanabe O, et al. Negative association between asthma and variants of CC16 (CC10) on chromosome 11q13 in British and Japanese populations. *Hum Genet* 1998;103:57-9.
9. Emad A, Rezaian GR. The diversity of the effects of sulfur mustard gas inhalation on respiratory system 10 years after a single, heavy exposure. *Chest* 1997;112:734-8.
10. Laing IA, Hermans C, Bernard A, Burton PR, Goldblatt J, Le Souef PN. Association between plasma CC16 levels, the A38G polymorphisms, and asthma. *Am J Respir Crit Care Med* 2000;161:124-7.

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late anaphylactic reactions without early-phase reactions are rarely reported in patients with food-induced anaphylaxis.¹ We here report the first case, to our knowledge, of IgE-mediated cutaneous, respiratory, and abdominal symptoms occurring 10 to 12 hours after consuming natto (ie, soybeans fermented by the bacteria *Bacillus natto* [subtilis]).

A 36-year-old man presented with a 2-month history of recurrent generalized urticaria and anaphylaxis. He had taken antihistamines, but had urticaria >2 to 3 days a week and more recently had at least 3 severe anaphylactic reactions accompanied by dyspnea, chest tightness, abdominal cramps, palpitation, vomiting, dizziness, and headache. These 3 episodes required emergency care. Symptoms resolved with intravenous antihistamines. His medical history revealed asthma in childhood. There was no family history of food allergy or of anaphylaxis.

He was treated with antihistamines such as loratadine and hydroxyzine daily and was instructed to keep a diet diary. Thereafter, he had 3 anaphylactic reactions over a period of 47 days. Symptoms resolved 1 to 3 hours after receiving dexchlorpheniramine. In 2 of 3 episodes, he ate dinner containing natto at 6 PM or 8 PM, respectively, and then anaphylactic reactions developed at 6 AM while sleeping. He had never realized that these reactions were elicited by something he had eaten, because he had 2 of these 3 episodes while sleeping. His diet diaries, however, revealed that he had ingested natto 10 to 12 hours before the onset of symptoms in all 3 instances, indicating an association between natto and these 3 anaphylactic events.

Total IgE concentration was 710 IU/mL (range for age, 1-300). Specific IgE antibodies were detected against egg white (a score of 2+), but not against soybean or the other foods, by CAP RAST (Pharmacia, Uppsala, Sweden). Responses to all skin prick tests (SPTs) were read at 15 minutes and interpreted according to European Academy of Allergy and Clinical Immunology guidelines.² All results of SPTs with multiple commercial foods allergens (eg, soybean, rice, and egg white; Torii Pharmaceutical Co, Tokyo, Japan) were negative. The vehicle control was negative. According to Dreborg and Foucard,³ the results of the prick-by-prick test with the raw ingredients included in the dinner the patient had eaten 12 hours before an episode that occurred while he was sleeping were negative for all ingredients except natto (Okamenatto; Takanofoods Co Ltd, Ibaragi, Japan). Results for natto revealed a mean wheal diameter of 5 mm and a mean flare diameter of 20 mm, whereas the 10 control subjects remained negative for wheals and flares. Subsequent SPTs with a saline solution containing *B natto* powder (50 mg/mL; Yuzo Takahashi Laboratory, Yamagata, Japan) and *B natto* cultivated on agar were negative. In addition, to test for allergy to natto, we prepared our own extract. A total of 25 mL 0.125 mol/L ammonium bicarbonate was poured over 1 g commercial natto (Okamenatto) followed by homogenization in blender for 30 minutes at 4°C until a smooth paste was achieved. After centrifugation at 3000 rpm for 30 minutes, the supernatant was dialyzed against saline and filtered through a 0.45-μm membrane. Then the natto

Late-onset anaphylaxis to *Bacillus natto*-fermented soybeans (natto)

To the Editor:

Onset of an anaphylactic reaction usually occurs within 30 minutes after exposure to the relevant allergen. Isolated