

Nonadverse Effects on Allergenicity of Isopentenyltransferase-Transformed Broccoli

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■ Abstract

Background: Genetically modified organisms (GMOs) provide modern agriculture with improvements in efficiency and the benefits of enhanced food production; however, the potential impact of GMOs on human health has not yet been clarified.

Objective: To investigate the allergenicity of isopentenyltransferase (*ipt*)-transformed broccoli compared with non-GM broccoli.

Methods: Sera from allergic individuals were used to identify the allergenicity of GM and non-GM broccoli. Immunoglobulin (Ig) binding of different lines of GM and non-GM broccoli was identified using immunoblotting, enzyme-linked immunosorbent assay, and the histamine release assay.

Results: Positive reactions to broccoli (*Brassica Oleracea*) were observed in 7.02% of individuals. Specific IgE to broccoli and total IgE from allergic individuals were well correlated. The different tests performed showed no significant differences in the allergenicity of conventionally raised and GM broccoli, indicating the absence of unexpected effects on allergenicity in *ipt*-transformed plants. Using Western blot analysis, we detected heterogeneous IgE-reactive allergenic components in broccoli-allergic sera, but no significant differences between GM and non-GM broccoli were observed in serum from the same patients.

Conclusions: Our study demonstrates that there are no differences between GM (*ipt*-transformed) broccoli and non-GM broccoli, as determined by specific IgE in sera from broccoli-allergic patients. This indicates that there were no unexpected effects on allergenicity in this GM broccoli.

Key words: Genetically modified organisms. Isopentenyltransferase gene. Broccoli allergenicity.

■ Resumen

Introducción: Los organismos modificados genéticamente (GM) dan lugar a mejoras en la agricultura moderna relacionados con la eficiencia y beneficios de la producción de alimentos, sin embargo el potencial impacto de estos en la salud humana no ha sido clarificado. El motivo de este estudio fue investigar la alergenicidad entre el brócoli modificado genéticamente mediante isopenteniltransferasa frente al no modificado.

Para ello se utilizó suero de pacientes alérgicos a brócoli para identificar la alergenicidad de ambos brócolis modificado o no modificado. Se estudió la unión de la IgE al brócoli de uno u otro tipo mediante inmunoblotting, ELISA y liberación de histamina.

Resultados: Los resultados mostraron que el 7.2% de los pacientes tenían reacciones a brócoli (*B. oleracea*). La IgE específica a brócoli y la IgE total de los sujetos sensibilizados mostraba una correlación. Mediante Western blot observamos una heterogeneidad en la IgE en el suero de los pacientes reactiva frente a los componentes alérgicos.

La alergenicidad de los brócolis, modificado o no modificado, no mostró diferencias significativas, lo cual indica que la transformación del brócoli no afecta a su alergenicidad.

Conclusiones: En nuestro estudio demostramos la ausencia de diferencias entre brócoli transformado genéticamente o no (isopenteniltransferasa), por lo tanto no son esperables efectos sobre la alergenicidad en el brócoli transformado.

Palabras clave: Organismos modificados genéticamente. Genes de la Isopentenil transferasa. Alergenicidad del brócoli.

Introduction

The term *genetically modified* (GM) foods is commonly used to refer to crop plants created for human or animal consumption using modern molecular biology techniques. These plants are modified in the laboratory to enhance desired characteristics such as increased resistance to insects and herbicides. To enhance the growth and production, and to ensure a constant supply of foodstuffs, numerous transgenic plants have been developed. Although GM organisms (GMOs) provide modern agriculture with improved efficiency and enhanced food production [1,2], their impact on human health continues to be investigated. Following the establishment of the 2003 Codex guidelines concerning the methods to evaluate the safety of GM foods [3], many institutes and countries introduced safety assessment principles and testing methods for GM products that investigate aspects such as toxicity, allergenicity, antinutritional effects, and unintended effects [4]. Similarly, safety requirements to ensure edibility and assessment standards for agricultural GMOs have been drawn up in many countries around the world, including China [5].

Genetic engineering with transcription factors is used to modify the genetic composition of certain foods in order to increase crop yield and quality. GM foods have been developed for resistance to insects or herbicides, or for improved growth and nutrient status. Such modifications, however, may affect the expression of multiple genes and have unintended effects on protein expression levels in GM foods. In order to conduct an appropriate safety assessment, it is important to evaluate the proteomics of transgenic plants, particularly with respect to allergenicity [6]. It has been reported that transgenic potatoes may express slightly higher levels of allergens, but their immunoglobulin (Ig) E-binding patterns have been found to be almost the same as those of control potatoes [7]. With respect to IgE-binding proteins, no qualitative differences have been found between GM and non-GM amago salmons, suggesting that endogenous allergen expression in this case is not altered by genetic modification [4].

Food allergy, which has an estimated prevalence of 2% to 3% in adults and of 4% to 5% in children, is an adverse immune response to food. There are approximately 3 cases of food-induced anaphylaxis per 100,000 population per year [8,9]. Although food allergy is most commonly triggered by what are known as major food allergens (milk, eggs, peanuts, tree nuts, shellfish, fish, wheat and soybeans), it can also be caused by less common foods, including maize, rice, and broccoli. Broccoli (*Brassica oleracea*) is grown throughout the world and is one of the most popular vegetables in the Brassicaceae family. IgE-mediated allergy to foods in this family has been reported [10,11]. Bra j 1A, a major allergen of mustard seeds (Brassicaceae), has been characterized as a seed storage protein belonging to the 2S albumin family with an approximate molecular weight of 14 to 16 kD [12]. The major allergen of *B oleracea*, Bra o 3, was identified as a lipid transfer protein (LTP), with specific IgE found in the sera of 86% of allergic patients [13]. Plant pathogenesis-related (PR) proteins are either constitutively expressed or produced in response to developmental signals, physical stress, or infection by bacteria. At least 14 groups of PR proteins have been identified in a variety of plants, and

some have been recognized as cross-reacting pan allergens that play an important role in oral allergic syndrome [14]. Since LTP belongs to the PR-14 family, it is of interest to investigate differences in allergenic components before and after genetic modification. Asthma and allergic rhinitis are highly prevalent worldwide, and sensitization to allergens is a risk factor for the development of allergic diseases [15]. It is therefore clinically important to identify food allergens in order to prevent allergic reactions. It is equally important to investigate the hazardous levels of allergenicity associated with GM crops.

The *ipt* gene coding for isopentenyl transferase (IPT), a key enzyme in the biosynthesis of cytokinin, has been reported to have effects on photosynthesis, sugar allocation, and nitrogen partitioning [16]. The first genetic engineering study of enhanced IPT expression in tobacco induced by *Agrobacterium tumefaciens* reported increased cytokinin concentration in leaves as well as increased stomatal opening and faster transpiration [17]. Senescence-inducible expression of IPT extends leaf life, increases drought stress resistance, and alters cytokinin metabolism in *ipt*-transgenic Cassava [18]. A growing number of researchers have been focusing efforts on constructing an *ipt*-transgenic plant and investigating the efficiency of drought stress resistance. However, the issue of whether or not the allergenicity of these plants is altered has not been determined yet.

There is growing concern among the scientific community and the general population about the potential health risk associated with the use of GM food crops in consumers and nontarget organisms. In vitro safety assessment of the genes to be used in transgenic plant development programs is highly recommended. Food allergy is usually mediated by IgE and most allergic reactions occur in the gastrointestinal tract, skin, or respiratory tract. The reliability of the safety assessment strongly depends on the monitoring of all possible allergic reactions triggered by GM products. In this study, we wished to evaluate whether effects on allergenicity were altered after GM with the *ipt* gene. Proteomic analysis, Western blotting, enzyme-linked immunosorbent assay (ELISA), and basophil histamine release assay with specific antibodies of allergens may all be useful methods when attempting to comprehensively compare allergens in GM and non-GM foods, especially with regards to potential allergenicity [4]. The specific objective of this study was to investigate allergenicity in GM and non-GM broccoli (*B oleracea*) using sera from allergic individuals to identify specific IgE-binding allergenic components.

Materials and Methods

Study Individuals

A total of 185 individuals who attended outpatient clinics belonging to the Division of Allergy, Immunology, and Rheumatology at the Taichung Veterans General Hospital were enrolled in this study. Blood samples were drawn and sera were stored for the measurement of specific IgE to broccoli and the house dust mite (HDM) *Dermatophagoides Pteronyssinus*. Based on the specific IgE results, 15 individuals were recruited for further analysis: 13 broccoli-allergic patients (A1-A13) and

2 healthy volunteers (N1-N2). In the broccoli allergy group, there were 7 men and 6 women, with a mean (SD) age of 28 (3) years. Patients were classified as allergic if they reported immediate adverse reactions related to broccoli ingestion and had IgE-positive responses to broccoli. Healthy volunteers with negative responses to broccoli and *D pteronyssinus* by specific IgE measurements were selected as negative controls. The Institutional Review Board of the Taichung Veterans General Hospital reviewed and approved this study (IRB No. C06193). Verbal and written informed consent was obtained from the study participants after the provision of detailed explanations.

Determination of IgE Antibodies in Sera Against Broccoli and HDM

Blood samples of 5 mL were collected in serum separator tubes (Kendall) and processed within 4 hours. The serum samples were stored at -20°C until analysis. Allergen-specific IgE was measured by ImmunoCap (Phadia CAP no. k77) using the UniCAP 250 system, following the supplier's instructions. IgE antibodies to broccoli and *D pteronyssinus* were measured using the Phadia ImmunoCap system. The assay procedure was performed automatically, and results were calibrated against the World Health Organization standard for IgE. A concentration greater than 0.35 kU/L was considered positive. Concentrations ranged from 0.35 to 100 kU/L. The titers of antigen-specific IgE were further classified as follows: class 1, 0.35 to 0.7 kU/L; class 2, 0.7 to 3.5 kU/L; class 3, 3.5 to 17.5 kU/L; class 4, 17.5 to 50.0 kU/L; class 5, 50 to 100 kU/L; and class 6, more than 100 kU/L. Classes 1 and 2 were considered low, classes 3 and 4 moderate, and classes 5 and 6 high.

Plant Materials and Preparation of Broccoli Extracts

Broccoli (*B oleracea*) varieties of inbred line (No.104) and Green King (GK) seeds were obtained from the Know-You Seed Company in Kaohsiung, Taiwan. *ipt*-transformed broccoli was developed by Dr Long-Fang Chen's laboratory in Taiwan. The preparation of *ipt*-transformed broccoli has been previously described [19]. In brief, transgenic plantlets with a retarding effect on postharvest yellowing in broccoli were generated via *A tumefaciens*-mediated transformation of the cytokinin-synthesizing *ipt* gene. The gene was constructed under the control of senescence-associated gene promoters from Arabidopsis in the forms of pSG529(+) and pSG766A, which were gifts from Dr RM Amasino at the University of Wisconsin in Madison in the United States. Evidence of transgene integration was confirmed by assays on neomycin phosphotransferase II (NPTII) activity of selection markers, polymerase chain reaction, and Southern hybridization. Three *ipt*-gene transformed broccoli inbred lines (No. 101, No. 102, No. 103), 1 nontransformed inbred line (No.104), and the nontransformed parental variety GK were planted in an isolated field at Taiwan Agricultural Research Institute (TARI). When ripe, the florets of broccoli were harvested, lyophilized, and homogenized into a powder as previously described [13]. Broccoli extract was prepared from broccoli powder, stored at -70°C before use. Protein concentration was quantified using the Bradford protein assay (Bio-Rad) and bovine serum albumin as a standard.

Preparation of HDM Extracts

The preparation of crude extract of *D pteronyssinus* has been described previously [20]. In brief, *D pteronyssinus* mites were cultured overnight at $25^{\circ}\text{C}/75\%$ relative humidity in a covered plastic cup containing a medium consisting of mouse chow. Separation of the mites from the medium was achieved by gently stirring the medium with a glass rod following overnight culture. The mites, which had migrated to the cover, were collected and frozen at -80°C . The collection included a high proportion ($>90\%$) of mites, with a minimum of feces and practically no medium. The frozen mites were homogenized and extracted with phosphate-buffered saline (PBS, pH 7.2). Protein concentration was determined with the Bradford protein assay and bovine serum albumin as a standard.

Western Blot Analysis

The protein solution of broccoli extract was mixed with $4\times$ sodium dodecyl sulfate (SDS) sample buffer and incubated for 10 minutes at 100°C before loading. Total protein samples (300 μg) were subjected to 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) for 1 hour at 70 V and for 1 hour 30 minutes at 120 V. The separated proteins were electrophoretically transferred onto a polyvinylidene difluoride membrane for 2 hours at 350 mA. The membranes were blocked with 3% skimmed milk in Tris-buffer solution containing 0.05% Tween-20 (TBS-T) for 1 hour at room temperature. The membranes were then incubated with the primary antibodies (serum from allergic patients) at a dilution of 1:10 in TBS-T overnight at 4°C . Next, the membranes were washed 4 times with TBS-T and incubated with secondary antibody for 1 hour at room temperature in TBS-T containing alkaline phosphatase conjugated anti-human IgE (Stressgen). After 4 rinses with a wash buffer, the membranes were developed in a substrate solution for 30 minutes and antigen-antibody binding was recorded photographically.

Antibody Detection in Serum by ELISA

Microtiter plates (Maxisorp, Nunc) were coated with broccoli extract (1 μg per well in 100 μL coating buffer, pH 9.6) and left overnight at 4°C ; they were blocked with 1% skimmed milk in PBST (room temperature, 2 hours). Serum samples were diluted at 1:10, 20, 40, 100 for IgE detection in PBST and incubated overnight at 4°C . After washing with PBST, human IgE was detected with alkaline phosphatase-conjugated anti-human IgE secondary antibody. *p*-nitrophenyl phosphate (Kirkegaard and Perry Laboratories Inc.) was used as an enzymatic substrate for signal generation and colorimetric readout. Plates were read at a wavelength of 405 nm using an ELISA reader.

Basophil Histamine Release Assay

Sera from 3 broccoli-allergic patients (A1, A3, and A9) and 1 healthy volunteer (N1) were selected for the basophil histamine release assay. Washed polymorphonuclear cells were obtained from the venous blood of nonatopic donors using Polymorphprep solution (Axis-Shield). The cells were resuspended in RPMI-1640 medium by adjusting to 2×10^6 cells/mL using trypan

blue staining. Passive sensitization of basophils with specific IgE from the sera of the 3 patients was performed for 4 hours at 37°C. The sensitized cells were triggered with 50 µg/mL of allergen for 30 minutes at 37°C; the supernatant was then collected and reacted with *O*-phthalaldehyde (OPA, 5 mM) for 7 minutes [21]. The reaction was stopped by adding H₂SO₄ (0.04 M). The histamine released into the supernatant was measured by a fluorescence spectrophotometer. The percentage of histamine release was calculated using the following formula: (stimulated released histamine – spontaneous released histamine)/total released histamine × 100%.

Statistical Analysis

SPSS Sample Power 2.0 software was used for power calculation analysis. Statistical comparisons of IgE levels between non-GM broccoli and GM broccoli were performed using the unpaired *t* test. Two-tailed *P* values of lower than .05 were considered to be statistically significant. Correlation between levels of broccoli-specific IgE and total IgE (IU/mL) in serum samples from patients with broccoli allergy (Allergic group: A1-A13) was assessed using the Spearman rank correlation test.

Results

Prevalence of Sensitization to Broccoli

We analyzed the prevalence of specific IgE to broccoli in the sera of 185 individuals who attended allergy, immunology, and rheumatology outpatient clinics. The data showed that the most predominant allergen was HDM, with a positive response to *D pteronyssinus* in 68.6% of individuals (127/185). Approximately 9.7% of individuals (18/185) had specific IgE to food (including soybeans, rice, maize, broccoli, and

peanuts). Total IgE and broccoli-specific IgE were measured in this group of patients. The results showed that 7.02% of the entire group (13/185) had positive reactions to broccoli (*B oleracea*). All of the broccoli-allergic individuals (A1-A13) had total IgE of over 500 IU/mL and broccoli-specific IgE of over 0.35 kU/L. The results showed higher specific IgE reactions to broccoli in the serum of these 13 patients than in that of the negative controls (N1-N2) (Figure 1A). The cutoff of 0.35 kU/L for broccoli-specific IgE and abnormal values of over 500 IU/mL for total IgE in serum samples from broccoli-allergic patients were calculated using the Spearman rank correlation test. Tests of IgE reactivity showed a significant positive correlation between levels of broccoli-specific IgE and total IgE ($R^2=0.87$, $P<.0001$) (Figure 1B).

Comparison of Allergenicity of GM and Non-GM Broccoli by ELISA

Sera with positive reactions to broccoli by ImmunoCAP were selected for confirmation of IgE reactivity to crude extracts of broccoli and comparison of the allergenicity of GM and non-GM broccoli using ELISA. Crude extracts of GM and non-GM broccoli were coated onto an ELISA plate prior to the addition of serum. Our preliminary test showed that the optimal level of absorption was obtained by coating a plate with 10 µg/mL of crude extract. For the remainder of the tests, thus, ELISA plates were coated with 10 µg/mL crude extract of GM or non-GM broccoli. Sera from 15 patients (A1-A13 and N1-N2) were selected to investigate differences in allergenicity between the different broccoli lines. The results showed similar specific IgE titers to broccoli extracts derived from GM lines (lines 101, 102 and 103) and non-GM lines (lines 104 and GK) (Table). The absence of significant differences in the allergenicity of GM and non-GM lines, indicates that the GM of broccoli with *ipt* did not have any unexpected effects on allergenicity in.

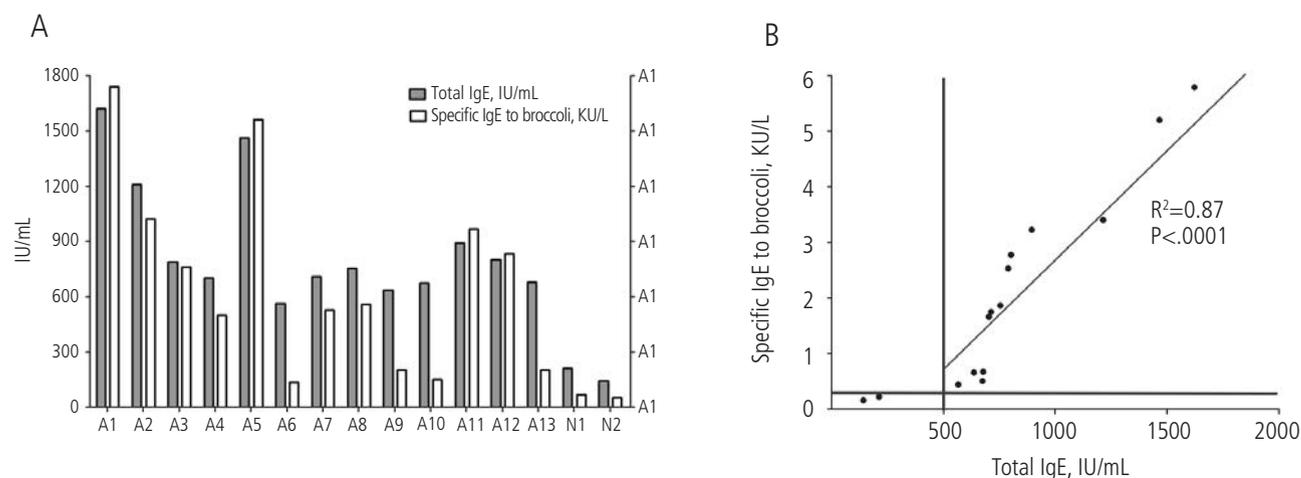


Figure 1. A, Serum concentrations of total immunoglobulin (Ig) E (IU/mL) and broccoli-specific IgE (kU/L) from broccoli-allergic and nonallergic individuals. A1-A13, serum from broccoli-allergic individuals; N1-N2, serum from nonallergic individuals. B, Correlation between levels of broccoli-specific IgE and total IgE (IU/mL) in serum samples from patients with broccoli allergy (A1-A13) using the Spearman rank correlation test. The straight line represents the cutoff of 0.35 kU/L for broccoli-specific IgE and abnormal values of over 500 IU/mL for total IgE. The control group included 2 healthy volunteers (N1-N2).

Table. Specific Immunoglobulin (Ig) E to Broccoli as Determined by ELISA^a

Serum Samples ^b	Broccoli Lines				
	GM-101 ^c	GM-102 ^c	GM-103 ^c	GM-104 ^c	Non-GM GK ^c
A1	2.13 (0.021)	2.16 (0.018)	2.14 (0.030)	2.21 (0.050)	2.17 (0.019)
A2	1.78 (0.015)	1.67 (0.012)	1.73 (0.023)	1.76 (0.021)	1.69 (0.012)
A3	1.57 (0.011)	1.61 (0.019)	1.49 (0.029)	1.58 (0.031)	1.53 (0.012)
A4	1.41 (0.023)	1.51 (0.034)	1.45 (0.031)	1.38 (0.028)	1.48 (0.037)
A5	2.01 (0.021)	1.98 (0.009)	2.02 (0.019)	1.89 (0.017)	1.92 (0.034)
A6	0.35 (0.038)	0.42 (0.041)	0.36 (0.023)	0.38 (0.034)	0.29 (0.045)
A7	1.67 (0.045)	1.58 (0.038)	1.62 (0.041)	1.72 (0.014)	1.55 (0.024)
A8	1.78 (0.034)	1.82 (0.051)	1.77 (0.024)	1.81 (0.051)	1.76 (0.034)
A9	0.44 (0.041)	0.37 (0.038)	0.42 (0.035)	0.35 (0.044)	0.41 (0.048)
A10	0.42 (0.021)	0.45 (0.031)	0.41 (0.028)	0.45 (0.021)	0.38 (0.023)
A11	1.77 (0.017)	1.69 (0.015)	1.73 (0.021)	1.81 (0.023)	1.82 (0.018)
A12	1.73 (0.009)	1.71 (0.016)	1.66 (0.012)	1.56 (0.014)	1.72 (0.023)
A13	0.34 (0.016)	0.28 (0.035)	0.29 (0.009)	0.35 (0.024)	0.26 (0.016)
N1	0.23 (0.002)	0.21 (0.018)	0.18 (0.012)	0.19 (0.021)	0.22 (0.017)
N2	0.17 (0.005)	0.24 (0.021)	0.23 (0.031)	0.22 (0.012)	0.17 (0.018)

Abbreviations: ELISA, enzyme-linked immunosorbent assay; GM, genetically modified.

^aAbsorbance was measured at an optical density of 450 nm.

^bA1-A13, serum from broccoli-allergic individuals; N1-N2, serum from nonallergic individuals.

^cData are presented as means (SD).

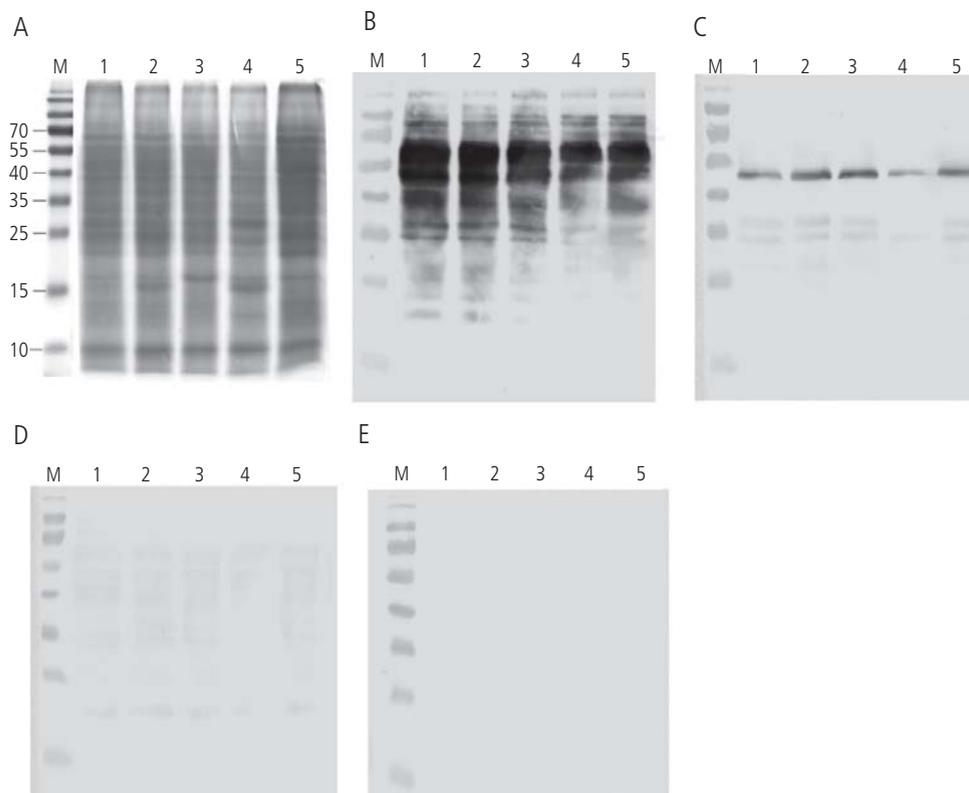


Figure 2. Comparison of allergenic components in conventionally raised and genetically modified (GM) broccoli. A, Protein profiles of extracts from GM line 101 (lane 1), GM line 102 (lane 2), GM line 103 (lane 3), non-GM line GK (lane 4), and non-GM (lane 5). B, Western blot analysis with serum from broccoli-allergic individual A1. C, Western blotting with serum from broccoli-allergic individual A3. D, Western blotting with serum from broccoli-allergic individual A9. E, Western blotting with serum from nonallergic individual, N1. GM indicates genetically modified; M, molecular marker.

Determination of Serum IgE Antibodies Against Crude Broccoli Extract by Immunoblot Analysis

To compare differences between allergenic components in GM and non-GM broccoli, we analyzed 3 lines of GM broccoli (lines 101, 102 and 103) and 2 lines of non-GM broccoli (lines 104 and GK) by Western blot analysis. Three serum samples that were positive to broccoli by ImmunoCAP (A1, A3, and A9) and 1 that was negative (N1) were used to analyze specific IgE-binding allergenic components in the 5 lines of broccoli; the results showed that there were some differences in IgE binding activity in patients with different levels of broccoli allergy (Figure 2A, B, and C). However, there were no differences in IgE responses to GM and non-GM broccoli with serum from the same patients (Figure 2). There were also no differences in the proteins identified by ELISA and Western blot analysis in the different GM lines (lines 101, 102 and 103) and non-GM lines (lines 104 and GK). This meant that there were no significant differences in the allergenic components of GM and non-GM broccoli.

Comparison of Allergenicity by Histamine Release Assay With Stimulation by Broccoli From Different Lines

Sera from 3 broccoli-allergic patients (A1, A3, and A9) and 1 healthy volunteer (N1) were selected for testing with an in vitro basophil histamine release assay with stimulation by broccoli. Passive sensitization of basophils was performed with washed polymorphonuclear cells obtained from nonatopic donors; these cells were then individually sensitized using sera from the 3 patients and the healthy volunteer. Following sensitization, the basophils were stimulated with different lines of broccoli. In the presence of broccoli, histamine release from basophils presensitized with serum from the broccoli-allergic individuals showed significantly increased changes (>20%), but the differences were not significant when compared with normal serum (N1) (<20%) (Figure 3). Histamine release resulting from basophil degranulation was higher in patients A1 and A3 (range, 60%-80%), and in patient A1 in particular (80%). On analyzing histamine release triggered by different

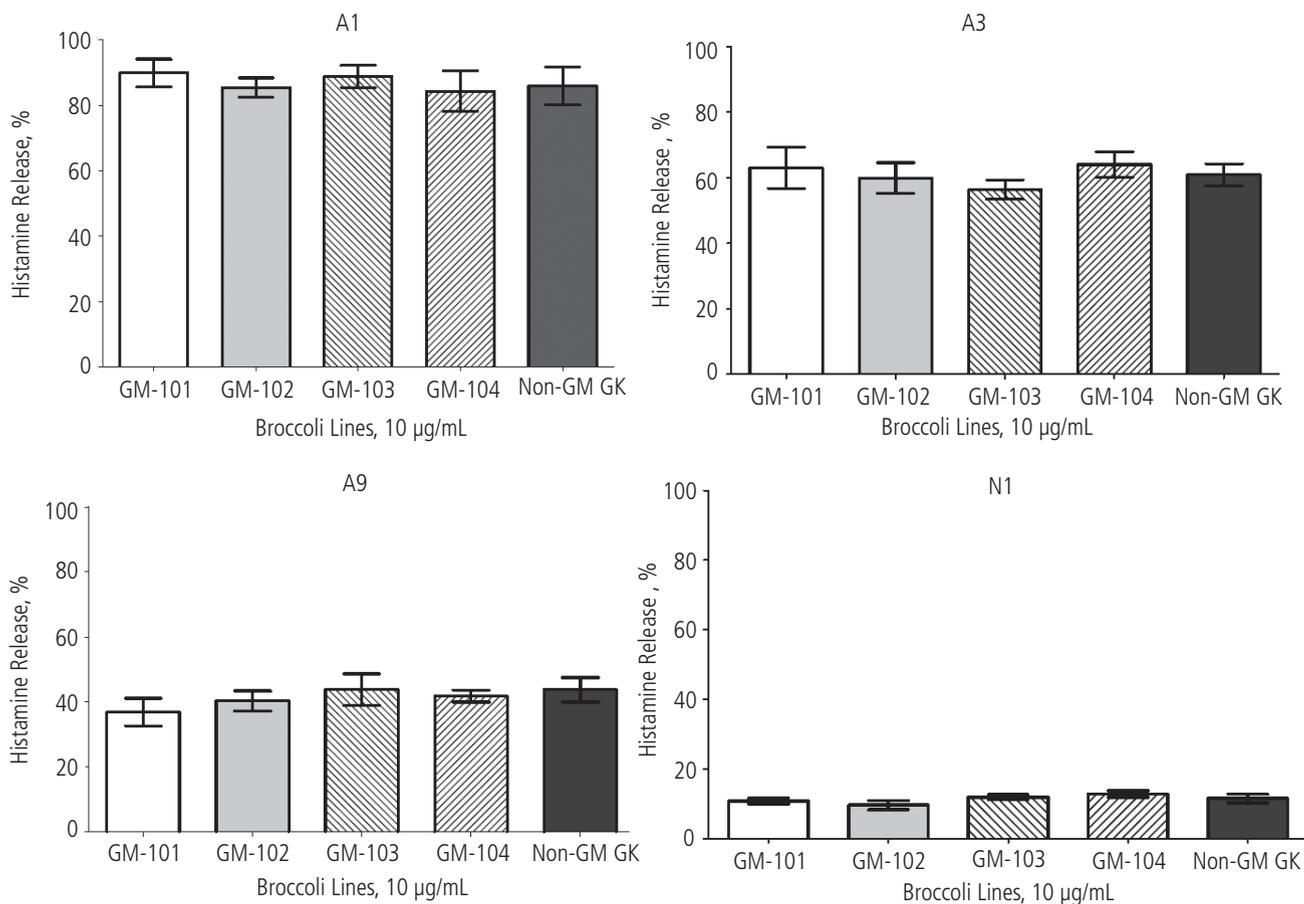


Figure 3. Histamine release from basophils shown as a percentage. The basophils were presensitized with sera from broccoli-allergic individuals (A1, A3, and A9) and a healthy volunteer (N1) and were then stimulated with different lines of broccoli at a concentration of 10 µg/mL.

lines of broccoli, no significant differences were found between GM and non-GM lines with serum from the same patient. This meant that there were no significant differences in the allergenic components of GM and non-GM broccoli.

Discussion

Adverse food reactions are associated with the ingestion of foods and classified as food intolerance or food allergy depending on the pathophysiological mechanism of the reaction. Food allergy refers to an abnormal immunologic response to food [22]. Food hypersensitivity reactions have become an increasingly serious problem in recent decades, affecting 4% to 5% of children and 2% to 3% of adults [23]. Food-induced allergic reactions are responsible for a variety of cutaneous, gastrointestinal, and respiratory symptoms that can be attributed to IgE-mediated and cellular mechanisms [24]. The most common allergies in children are induced by milk, eggs, wheat, and peanuts, whereas the most common allergies in adults are induced by fish, shellfish, fruit, and peanuts [24].

Although allergies tend to be triggered mainly by major allergens, such as milk, eggs, peanuts, fish, and shellfish, they can also be caused by minor allergens, such as fruit, vegetables, maize, and soybean [25]. There have been reports of many different food and vegetable allergens inducing respiratory or cutaneous allergic symptoms. The *Brassica* genus includes vegetables (broccoli) and condiments (mustard) containing allergic components [26]. Both IgE sensitization and IgE-mediated (type I) hypersensitivity reactions can occur in foods from this genus. Although allergy to broccoli has rarely been observed in the general public [11], several cases of dermatitis involving allergy due to occupational contact with broccoli have been reported; one of these cases involved an employee at a greengrocer's and another involved a person who had worked in a broccoli winter nursery for 7 years [27]. Overall, comprehensive research focusing on the prevalence of broccoli allergy has not been reported. The present study can thus serve as a background reference regarding the prevalence of broccoli allergy in Taiwan. Our findings show that the most predominant allergen in our study population was HDM (positive IgE to *D pteronyssinus* in approximately 68.6% of individuals). Approximately 9.7% of the population had specific IgE to food and 7.0% had positive IgE to broccoli (*B oleracea*).

Palacín et al [13] identified a 9-kD cabbage IgE-binding protein, Bra o 3, as an LTP and a major allergen in *B oleracea*, cross-reacting with mugwort pollen and other plant foods. The authors reported that 94% of cabbage-allergic patients (16/17) showed specific IgE to Bra o 3, detected by ELISA, and 86% (12/14) had a positive skin prick test to this allergen. LTPs are small molecules of approximately 9 to 10 kDa that belong to a family of structurally highly conserved proteins present in a wide range of fruit and vegetables. They have been reported as relevant allergens in most plant foods, such as Rosaceae fruit, nuts, and broccoli, and might represent a novel plant panallergen. Type I LTPs are believed to be involved in plant defense mechanisms. GM broccoli could therefore overexpress this protein to enhance crop yield. LTPs are the most frequent cause of primary food allergy, and IgE-mediated allergy to

foods in the Brassicaceae family has been increasingly reported in recent years. In the present study, there were no differences in IgE-binding components in the 9- to 10-kDa proteins in crude broccoli extracts analyzed by Western blot analysis, indicating that the expression of LTP-like proteins was not deregulated by GM. Nonetheless, we did not compare the binding activity of proteins in the crude extract with that of purified LTP. Such a comparison would be important in future studies.

The use of generic drugs has increased in the European Union in recent years. Hypersensitivity to generic drugs has been attributed to the allergenic component of soybean in these drugs. The allergenicity of soybean was greatly enhanced after GM and this component has caused anaphylaxis in patients after the ingestion of a generic omeprazole capsule [28]. In this study, we wished to evaluate whether GM of broccoli with the *ipt* gene had an effect on the allergenicity of this plant. Our results showed similar titers of specific IgE in GM and non-GM lines, measured by ELISA, suggesting the absence of such an effect. Testing of histamine release triggered by different lines of broccoli showed no significant differences with the same patient's serum, indicating no significant changes to the allergenic components of GM broccoli. Similar results were found by Western blot analysis. Our findings are limited by the small number of serum samples from patients with broccoli allergy. Any significant differences that might exist cannot be investigated until more sera have been collected.

In conclusion, in our series of individuals enrolled from allergy, immunology, and rheumatology outpatient clinics, 68.6% of individuals had positive reactions to HDM (*D pteronyssinus*) and 7.0% had positive reactions to broccoli (*B oleracea*). There were no significant differences between conventionally raised broccoli and *ipt*-transformed broccoli, as detected using specific IgE in the sera of broccoli-allergic patients. Our results indicate that there were no unexpected effects on allergenicity in *ipt*-transformed broccoli.

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