

values showed no change, and peanut was not introduced into the diet. For the 4 participants with worsened FAQLQ scores for either child/adolescent or parent, 3 of the children/adolescents had increased CRD values post-immunotherapy and 1 showed no improvement. Nonetheless, all 4 participants introduced peanut into their diets. Possible explanations include increased awareness of food allergy with age and experience of reactions during OFCs. However, 2 participants had improved FAQLQ scores despite no CRD improvement and not introducing peanut into their diets. Possible explanations include frequent visits to medical experts who provided comprehensive education, and the ability to observe, experience, and treat allergic reactions.

Limitations inherent in this project include small sample size and lack of a control group. The first FAQLQ was administered a year after treatment initiation, at VIPES completion, and before OLFUS-VIPES and the start of OFCs for the study. Perhaps more changes would have been noted if administered at baseline in VIPES. Additionally, questionnaires were not blinded to the study team, possibly impacting family responses.

This is the first investigation of patient and parent experiences after completing peanut immunotherapy trials. Overall, information gained from this project will help inform future studies and highlights the need to consider QoL when assessing food allergy therapies. We suggest that continued observation of patients after trial participation is essential to better understand long-term benefits and challenges with immunotherapy.

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Analysis of allergens in corn-dependent exercise-induced anaphylaxis



Since the first report of food-dependent exercise-induced anaphylaxis (FDEIA) in 1979,¹ many foods have been labeled as causative allergens. Although wheat and crustaceans are the most frequent allergens,² corn is also described as a rare allergen. In the current study, a case of FDEIA to corn is reported. In addition, focus is put into the analysis using proteomic approaches for identification of potential allergens.

A 7-year-old boy with atopic dermatitis was referred to our hospital after experiencing 2 episodes of anaphylaxis in the previous 3 months. The patient developed facial angioedema, cough, and dyspnea during exercise after eating popcorn. After 3 months, he experienced a similar reaction during exercise after eating a snack made of corn. His father was allergic to shrimp, and both his father and mother had allergic rhinitis. Until experiencing anaphylactic episodes, the patient was able to eat corn and wheat without any allergic reactions. Therefore, corn was suspected as the causative agent of FDEIA.

The current study was approved by the ethics committee of Toho University Ohashi Medical Center (H17023). Written informed consent was obtained from the patient's parents.

Serum levels of specific immunoglobulin E (IgE) antibodies to each allergen were measured using the ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). No positive IgE-specific antibodies were detected. A skin prick test (SPT) was performed using multiple commercial allergens (Torii Pharmaceutical Co, Ltd, Tokyo, Japan). Fresh-food SPTs using the prick + prick technique³ were performed with boiled corn, popcorn, and a snack made of corn using the bifurcated needle (Allergy Laboratories, Inc., Columbus, OH). Histamine at 10 mg/mL concentration and saline were used as positive and negative controls, respectively. A wheal with a mean diameter of 2 mm or larger that was visible 15 minutes after the test was defined as a positive reaction.³ Positive reactions were observed to histamine (11 × 14 mm), corn extract (5 × 5 mm), boiled corn (6 × 7 mm), popcorn (6 × 7 mm), and a snack made of corn (6 × 6 mm). The reaction to saline was negative (0 × 0 mm). A basophil activation test using flow cytometry determined the basophil CD203c expression, which was assessed after stimulating the reaction with boiled corn, using the Allergenicity Kit (Beckman Coulter, Fullerton, CA). A nonallergic subject was designated as a control. The extracted corn induced a concentration-dependent enhancement of CD203c expression in the patient, and the rates of activated basophils at 10, 100, and 1,000 µg/mL concentrations were 5.8%, 19.6%, and 68.7%, respectively. A corn extract

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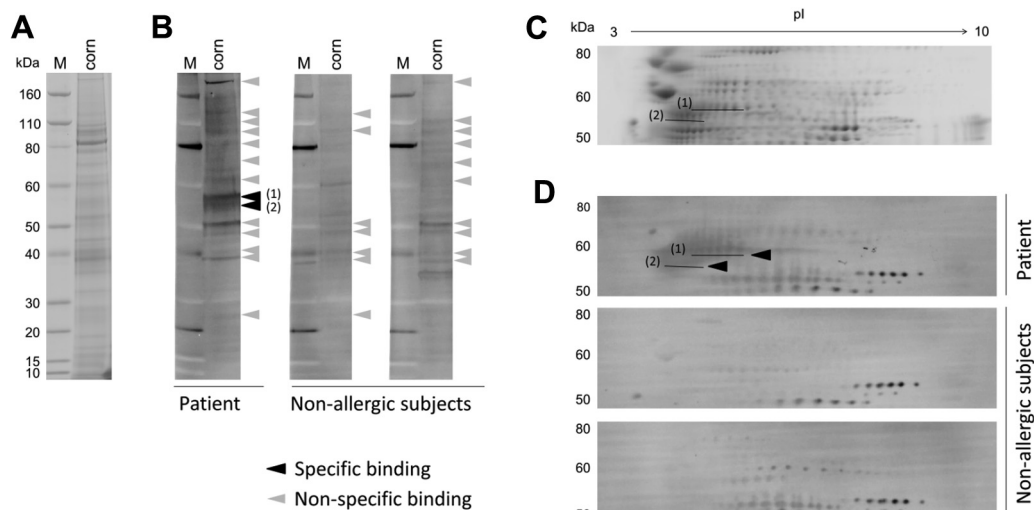


Figure 1. According to immunoblotting of corn protein separated by SDS (a, b, 4 μ g/lane) and 2D-PAGE (c, d, 50 μ g/lane), (1) GBSS I and (2) UDP-glucose pyrophosphorylase 2 were identified at approximately 60 kDa as the potential allergens. a), c): stained by Sypro Ruby; b), d): immunoblotting using sera. 2D, 2-dimensional; GBSS I, granule-bound starch synthase I; M: molecular weight standards; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.

concentration at approximately 420 μ g/mL was considered 50% activation. No activation was observed in the control. Furthermore, provocation tests were performed on admission. After premedication with aspirin and consumption of boiled corn, the exercise challenge test was performed according to the Bruce protocol.⁴ During the exercise challenge test, pulmonary function was measured (Spiro Sift SP370 HYPER PLUS, Fukuda Denshi, Tokyo, Japan). Blood pressure and pulse rate were monitored before and after the exercise challenge and during the allergic reactions. Within 5 minutes of initiating the exercise challenge test, the patient developed facial angioedema, cough, and dyspnea. A significant decrease in the percentage of peak expiratory flow rate, forced expiratory volume in 1 second, airflow rate at 50% vital capacity, and airflow rate at 25% vital capacity (14.8%, 17.7%, 16.0%, and 11.6%, respectively) was observed. However, no significant changes in the blood pressure or pulse rate were observed during the allergic reactions. All symptoms disappeared approximately 1 hour after exercise without medication.

Corn extract was subsequently prepared. Raw corn was purchased at a supermarket and steamed for 15 minutes. After homogenizing, the sample was mixed with 1:5 sucrose buffer (1 M sucrose; 2% [wt/vol] polyvinylpyrrolidone [Sigma-Aldrich, Co., St. Louis, MO]; 2 mM ethylenediamine-N,N,N',N'-tetraacetic acid disodium salt dehydrate; and 10 mM sodium N,N-diethyl dithiocarbamate trihydrate [Wako Pure Chemical Industries, Ltd., Osaka, Japan]), and the pH was adjusted to 9.5 to 10.0, using sodium hydroxide. After gently stirring the sample overnight at 4°C, the mixture was centrifuged at 14,000 rpm for 20 minutes and filtered through an MF-Millipore 0.45- μ m membrane (Merck Millipore, Billerica, MA). The supernatant was collected and used as the sample. Total protein in the sample was quantified using 2-D Quant Kit (GE Healthcare UK Ltd., Buckinghamshire, England) as a colorimetric method and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and 2-dimensional (2D)-PAGE. Immunoblotting was performed with the separated proteins using the patient's serum (Fig 1), and mass spectrometry was performed as previously reported.^{5–7} Nonallergic subjects were designated as controls. Proteins were identified according to the National Center for Biotechnology Information (NCBI) database (Zea). Finally, granule-bound starch synthase I (GBSS I) and UDP-glucose pyrophosphorylase 2 were identified at approximately 60 kDa as the potential allergens.

Manabe et al reported that the frequency of FDEIA in Japanese elementary school children was 0.0047%.⁸ A case of FDEIA to corn was previously reported, which, to the best of our knowledge, is the only report of corn-dependent exercise-induced anaphylaxis⁹; however, no potential corn allergen was identified in this study. In the current study, a case of FDEIA to corn is reported, and GBSS I and UDP-glucose pyrophosphorylase 2 are identified as the potential corn allergens for the first time. To prove the relevance of both proteins, one must further identify them in other patients with FDEIA to corn. Fonseca et al reported cross-reactions between these proteins and rice.¹⁰ In addition, the homology between both proteins and rice proteins is high: 82.32% and 93.45%, respectively (NCBI database accession no. ACJ86358.1 and XP_015611911.1). However, the patient in the current study was able to eat rice without any allergic reactions, suggesting that rice antigenicity may reduce after cooking it under high pressure. On the contrary, both proteins, acting as allergens in rice-allergic patients, may induce FDEIA to corn in the same individuals. The patient in the current study also suffered from atopic dermatitis, suggesting that epicutaneous sensitization induced by contact with corn may have led to the development of FDEIA to corn. Since then, the patient avoided eating corn before exercise and developed no further allergic symptoms.

In a previous report, the rare case of a patient experiencing an episode of anaphylaxis to corn without exercise 1 year after the development of FDEIA to corn was described.⁹ However, in FDEIA, cooling of the respiratory mucosa is essentially an important triggering factor both when inhaling cold air and when exercising after eating a causative food agent.

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Comparison of allergic rhinitis outcomes of the environmental exposure unit and nasal allergen challenge model



Traditional field studies are among the earliest study designs used to assess the clinical efficacy of allergic rhinitis (AR) medications.¹ However, accurately attributing clinical improvement to new treatments using this design can be difficult because of confounding factors such as variable weather conditions and airborne allergen levels.^{1,2} Controlled allergen challenge facilities (CACFs) such as the environmental exposure unit (EEU) and direct nasal allergen challenge (NAC) models were developed to provide allergen exposure under tightly controlled conditions. Situated in Kingston, Ontario, the EEU is capable of simultaneously exposing up to 120 study participants to a controlled quantity of airborne allergen while controlling environmental variables such as humidity and temperature.³ In comparison with airborne pollen grains, the NAC elicits robust AR symptoms by delivering a single pre-titrated dose of allergen extract intranasally to both nostrils. The NAC protocols can vary in terms of the type of extracts used, method of allergen delivery, and calculation of the dose needed to elicit adequate symptoms.⁴ Protocol customization and validation are needed before using NACs to assess treatment efficacy.

Validation methods for experimental AR models can include an assessment of symptom reproducibility and a comparison of challenge-induced symptoms with those elicited from natural settings. These studies have been conducted for the EEU and other CACFs.^{5,6} Recent validation studies showed reproducible clinical symptoms over multiple challenge visits using the NAC model.⁷ Correlating NAC symptoms with seasonal outcomes can be difficult because study participants are challenged once with a single pre-titrated dose to achieve a pre-specified symptom score. Instead, CACFs may serve as an alternative comparator to that of

seasonal outcomes. The aim of the current study was to directly compare nasal symptoms obtained using the NAC model with those generated in the EEU using the same allergen.

Eight birch-allergic and 4 nonallergic participants aged 18 to 65 years old who had completed a previous birch pollen EEU study⁸ were recruited into the current investigation. The study was reviewed and granted ethical clearance by the Queen's University and Affiliated Teaching Hospitals Research Ethics Board. All participants provided written informed consent before involvement. The exact methodology for the birch pollen EEU study has been previously published elsewhere.⁸ Briefly, participants attended a 4-hour birch pollen challenge with European white birch pollen (*Betula pendula*, Greer Laboratories, Lenoir, NC) in the EEU. For the NAC, participants first underwent a baseline titrated NAC to determine the challenge dose of white birch pollen extract (ALK-Abello, Mississauga, Ontario) that induced adequate symptoms.⁷ The cumulative dose (sum of all doses received during the titration) was delivered intranasally to both nostrils during the challenge visit. For both EEU and NAC studies, total nasal symptom score (TNSS; sum of rhinorrhea, nasal congestion, sneezing, nasal itching) and peak nasal inspiratory flow (PNIF) were recorded during the challenges.

Compared with non-allergics, birch-allergic participants experienced a significantly higher peak TNSS ($P = .004$) and % PNIF reduction from baseline ($P = .004$) after allergen challenge in the EEU. Similar findings were observed during the NAC (Fig 1A, 1B). Peak symptom severity was also comparable between the EEU and NAC for TNSS (8.12 ± 0.48 vs 7.50 ± 0.63 , $P = .47$) and % PNIF reduction ($-46.33\% \pm 5.89\%$ vs $-46.75\% \pm 6.18\%$, $P = .96$) (Fig 1A, 1B). Agreement between both models was observed with positive correlations for TNSS ($r = 0.70$, $P = .0132$) and % PNIF reduction ($r = 0.69$, $P = .0159$) (eFig 1A, 1B). These findings were replicated when mean symptom scores were assessed (TNSS, $r = 0.89$, $P = .0003$; % PNIF reduction, $r = 0.60$, $P = .0428$).

In the EEU, birch-allergic participants experienced a gradual increase in TNSS during the 4-hour pollen exposure period. This is contrary to the sharp increase in TNSS observed during the NAC and is reflected in the time taken to reach peak TNSS severity between both models (2.56 ± 0.43 hours vs 0.25 hours, $P = .0078$) (eFig 1C).

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