



SHORT COMMUNICATION

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Investigation of the sensitization rate for gibberellin-regulated protein in patients with Japanese cedar pollinosis

Yuji Mori, MD^a, Fumiko Okazaki^b, Keiko Momma^c, Hiroshi Narita^{d,e} PhD, Yasuto Kondo^{a*}

^aDepartment of Pediatrics, School of Medicine, Fujita Health University, Aichi, Japan

^bDepartment of Food Science and Human Nutrition, Faculty of Agriculture, Ryukoku University, Shiga, Japan

^cDepartment of Food and Nutrition, Kyoto Women's University, Kyoto, Japan

^dKyoto College of Nutritional and Medical Sciences, Kyoto, Japan

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Abstract

Background: Pollen-food allergy syndrome (PFAS) usually manifests as an itching sensation in the mouth and throat immediately after eating fresh fruits and vegetables. However, some patients with PFAS experience systemic symptoms including anaphylaxis. In Europe, cypress gibberellin-regulated protein (GRP) has been noted to cause allergenicity and exhibit cross-reactivity with peach GRP. Japanese cedar (*Cryptomeria japonica*), classified in the cypress family, is the primary causative substance among all environmental allergens in Japan. However, studies on the prevalence of GRP sensitization in patients with cedar pollinosis are lacking.

Objective: This study examined the prevalence of GRP sensitization in patients with Japanese cedar pollinosis.

Methods: We enrolled 52 patients who had requested sublingual immunotherapy treatment with mild-to-severe rhinitis during spring, and had a JCP-specific immunoglobulin E (IgE) levels of $>0.7 \text{ U}_A/\text{mL}$. Peach GRP was purified using affinity chromatography with a monoclonal antibody column. Specific IgE levels to peach GRP were measured using an enzyme-linked immunosorbent assay. Samples exhibiting absorbance at 450 nm of over mean plus three standard deviations of the negative control value were defined as positive. Sera from three patients with severe peach allergy were used as positive controls.

Results: Eleven sera from 52 patients with JCP-induced allergic rhinitis were positive for peach GRP.

Conclusion: Twenty percent of patients with cedar pollinosis were sensitized to peach GRP. Well-powered studies are needed to clarify whether these patients are at an increased risk for systemic symptoms or whether they primarily demonstrate only localized symptoms.

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*Corresponding author: Yasuto Kondo, MD, PhD, Department of Pediatrics, School of Medicine, Fujita Health University, 3-6-10 Otobashi, Nakagawa, Nagoya, Aichi 454-8509, Japan. Email address: ykondo@fujita-hu.ac.jp

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Introduction

Pollen-food allergy syndrome (PFAS) usually manifests as an itching sensation in the mouth and throat immediately after eating fresh fruits and vegetables. However, patients with PFAS sometimes present with systemic symptoms including anaphylaxis.¹ Recent studies have revealed that the sera of some patients who are allergic to the pollen of the Mediterranean cypress (*Cupressus sempervirens*) react with gibberellin-regulated protein (GRP), can trigger a severe allergy to peach.² (2). The Japanese cedar (*Cryptomeria japonica*) belongs to the cypress family (*Cupressaceae*), and its pollen is the most common environmental allergen in Japan.³ The prevalence of GRP sensitization in patients with cedar pollinosis and the associated symptoms in GRP-positive individuals upon peach ingestion are unknown. In this study, we aimed to determine the prevalence of sensitization to GRP in patients with Japanese cedar pollinosis.

Methods

According to the Japanese rhinitis guidelines, sublingual immunotherapy is widely recommended as a fundamental treatment for allergic rhinitis in mild-to-severe cases. Our hospital had about 500 outpatients with cedar pollinosis who developed mild-to-severe rhinitis in the spring, living in the Chubu region of Japan from June 2018 to December 2019.

Inclusion criteria: all patients who have requested sublingual immunotherapy treatment and consented to blood sampling for the study.

Exclusion criteria: patients who were already receiving sublingual immunotherapy or who did not opt for sublingual immunotherapy during the study period.

Their levels of immunoglobulin E (IgE) specific to commercially available Japanese cedar pollen were measured using ImmunoCAP assays (Thermo Fisher Scientific, Uppsala, Sweden) and were of $>0.7 \text{ U}_A/\text{mL}$. Sera from four nonatopic individuals without food or pollen allergies served as negative controls. Sera from two patients (P1 and P2) with severe peach allergies who participated in a previous study acted as positive controls.⁴ Patients found to be sensitized to GRP were confirmed for any allergic symptoms when consuming peaches telephonically.

The study protocols were approved by the institutional ethics committee of Fujita Health University (approval number: HM19-394) and was conducted according to the principles of the Declaration of Helsinki. All participants provided written informed consent for the use of their sera.

Peach GRP was purified using affinity chromatography with a monoclonal antibody column,⁴ and was dissolved in phosphate-buffered saline (PBS) to a concentration of $1 \mu\text{g}/\text{mL}$. About $100 \mu\text{L}$ of the resulting solution was placed in each well of a 96-well Nunc-Immuno Plate I (Nunc; Roskilde, Denmark) and was incubated overnight at 4°C . Later, the wells were emptied, and $150 \mu\text{L}/\text{well}$ of PBS-based SuperBlock Blocking Buffer (Thermo Scientific; Rockford, IL, USA) was added. The plate was then reincubated at room temperature (approximately 25°C) for 1 hour, and the wells were washed with $200 \mu\text{L}/\text{well}$ of PBS-Tween 20

solution (Fujifilm Wako Pure Chemical Corporation; Osaka, Japan). The patient and control sera were diluted at a ratio of 1:4 with the SuperBlock Blocking Buffer were added, and the plate was reincubated overnight at 4°C . After incubation the plate was rewashed, with the SuperBlock Blocking Buffer and $100 \mu\text{L}/\text{well}$ of mouse anti-human IgE-biotin solution (1:1,000; Southern Biotechnology Associates; Birmingham, AL) was added, and was reincubated at room temperature for 1 hour. After another washing step, $100 \mu\text{L}/\text{well}$ of horseradish peroxidase-conjugated streptavidin solution (1:5,000; Southern Biotechnology Associates) was added, and the plate was left undisturbed at room temperature for 1 hour. After the final washing process, $100 \mu\text{L}/\text{well}$ of 3,3',5,5'-tetramethylbenzidine solution (MP Biomedicals; Illkirch-Graffenstaden, France), was added, and the plate was finally incubated under a light shield at room temperature for 30 minutes. Then the reaction was seized using $100 \mu\text{L}/\text{well}$ of 1 M hydrochloric acid (Fujifilm Wako Pure Chemical Corporation).

Absorbance was measured at 450 nm using a microplate reader (iMark Microplate Absorbance Reader, Bio-Rad, Hercules, CA). Values that were three standard deviations higher than the mean value of the negative control were considered positive.

Results

Of the 52 patients with Japanese cedar pollinosis (median age, 10.5 years; age range, 5-65 years; 26 females), 11 (21%) showed reactivity to peach GRP (Figure 1). Telephone surveys of the 11 patients revealed that two had experienced oral symptoms after eating fruits including peaches, seven were asymptomatic after eating peaches, and two had not tried eating peaches. The patient whose serum showed the strongest reaction to peach GRP (patient no. 8 in Figure 1) was unwilling to try consuming peaches because she had experienced severe oral symptoms after eating other fruits.

Discussion

The sensitization rate in our study was slightly lower than that reported by Sénéchal et al.,⁵ (37%) among patients with cypress pollen allergies in Southern France.

In a previous study, the two positive control subjects (P1 and P2) who exhibited severe systemic peach allergies were representative of patients who showed absorbance values of three standard deviations greater than the mean.⁴ The 11 patients who were sensitized to peach GRP in this study developed absent or mild symptoms, and none of the patients had systemic reactions. In our previous study,⁴ we selected patients with severe peach allergies, regardless of pollinosis. However, the present study, only focused on patients with Japanese cedar pollinosis, regardless of peach allergy.

Although sublingual immunotherapy is recommended to all patients with the mild-to-severe disease, one cannot deny the possibility of bias in patients who wish to receive sublingual immunotherapy. The variable inclusion and exclusion criteria could explain the observed differences in the severity of peach allergies secondary to

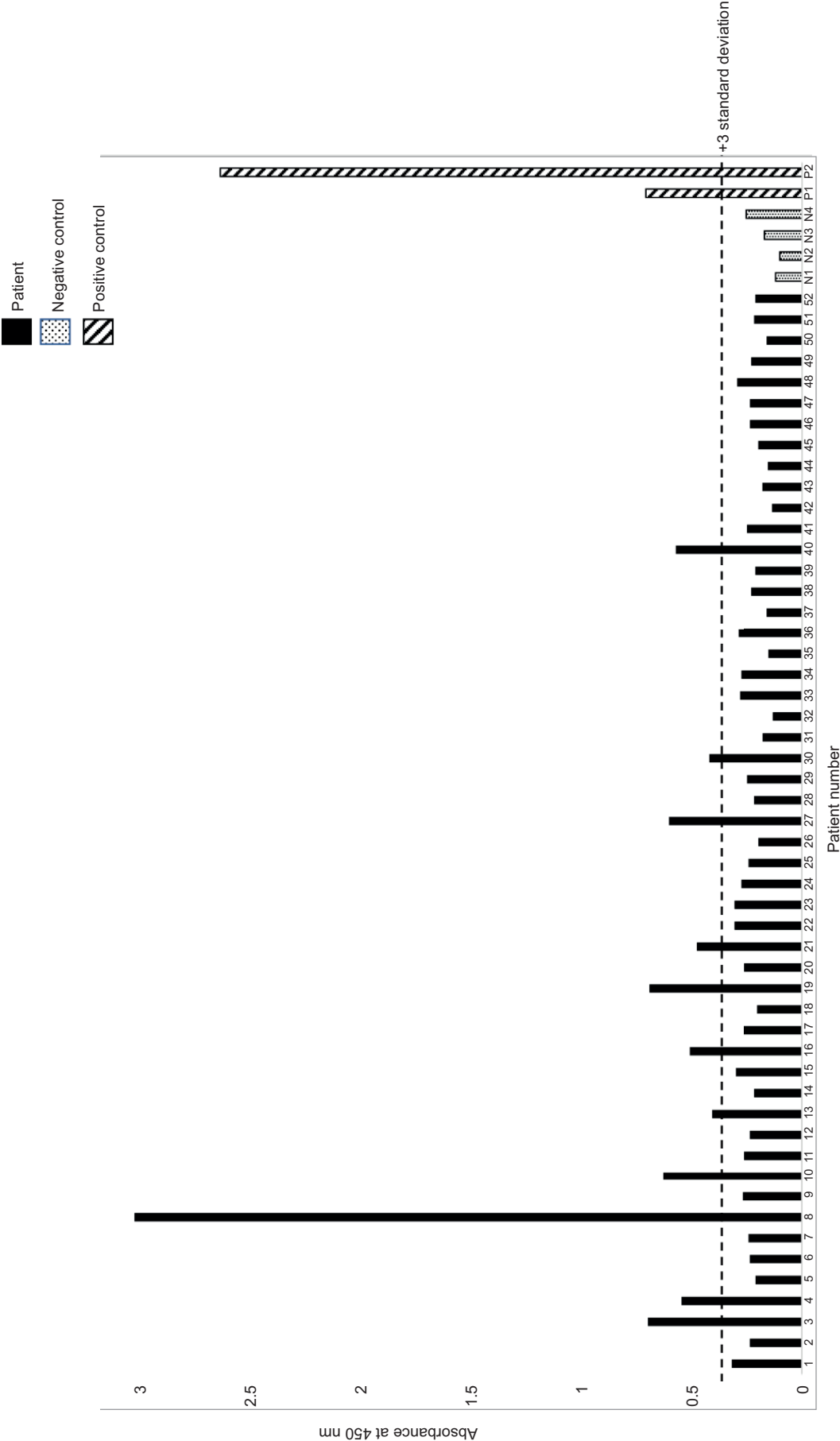


Figure 1 Prevalence of serum sensitization to peach gibberellin-regulated protein in patients with Japanese cedar pollen allergies. The dashed line represents the absorbance threshold for a positive result.

different sensitization routes. Physicians should examine the cross-reactivity of patients' sera to specific GRPs found in pollen, vegetables, and fruits.

In conclusion, 20% of patients with Japanese cedar pollinosis were sensitized to peach GRP. Even though this research was the first study to report these findings in Japan. They still have some limitations. First, the sensitization rate is based on a small group of people in a limited number of facilities. Second, the possibility of GRP-induced symptoms because of Bet v 1 sensitization cannot be ruled out as the presence or absence of fruit allergies was confirmed over a telephone survey rather than a food challenge test.

Well-powered studies should be conducted in the future to clarify whether this patient population is at an increased risk for systemic symptoms or whether they primarily demonstrate only localized symptoms.

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