

Predictive value of 7S globulin-specific IgE in Japanese macadamia nut allergy patients



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Clinical Implications

Assessment of macadamia nut 7S globulin allergen specific IgE in the serum can aid diagnosis of macadamia nut allergy in Japanese children.

Consumption of macadamia nut has increased recently in the United States and Europe with the change of dietary habits and increasing use in processed foods,^{1,2} underscoring the importance of the diagnosis of macadamia nut allergy. It is considered that macadamia 7S globulin, a vicilin-like protein, may contain antimicrobial peptides with allergenicity.³⁻⁵ However, no studies have reported on the relationship between the sensitization to macadamia 7S globulin and macadamia nut allergy from a diagnostic perspective. Hence, we determined whether the sensitization to macadamia 7S globulin specific IgE (sIgE) antibody is predictive of macadamia nut allergy in Japanese children.

A total of 111 pediatric participants suspected of macadamia nut allergy with a clinical history of immediate reaction to peanut and/or tree nuts, including macadamia nut, were recruited at the participating hospitals between July 2014 and December 2020. Twenty-five participants were excluded from the analyses owing to lack of clinical information or laboratory data, or discontinuation of hospital visit. Among the final 86 participants, 18 were diagnosed with macadamia nut allergy by pediatric allergists on the basis of a convincing history of immediate reactions accidentally induced by isolated ingestion of macadamia nut corresponding to the symptoms not less than grade 2 in the classification of anaphylaxis according to the Japanese Pediatric Guidelines for Food Allergy.⁶ They were classified as the patient group. The remaining 68 participants underwent an oral food challenge (OFC) test. Those with a positive OFC result were included in the patient group (n = 27) and those with a negative OFC result were classified as the control group (n = 41). OFC tests were performed in an open way with a total scheduled dose

of macadamia nut within 3.5 to 10 g, depending on the routine methods of the participating hospitals, according to the Japanese Pediatric Guidelines for Food Allergy.⁶ The total amount of the challenge food was divided into 3 portions at 30-minute administration intervals with gradual increase of the administered amount. The OFC result was considered positive when objective allergic reactions, defined as symptoms not less than grade 2 and/or localized skin reactions, were induced during the tests, whereas the OFC result was considered negative when no objective reactions were observed after completion of the challenged dose ingestion. Serum samples from the participants who underwent macadamia nut OFC test or the patients diagnosed with macadamia nut allergy on the basis of history were obtained within 1 year before or after the OFC test, or after the episode of immediate reactions induced by macadamia nut intake, respectively. Ethics approval was obtained from the ethics boards of all participating hospitals (accession no. 10488). Informed consent was obtained from all children and/or their legal representatives.

The basal characteristics of the patient (n = 45) and control groups (n = 41) were compared, without significant differences being observed in the total IgE levels and atopic comorbidities between them. Although commercially available serum crude macadamia nut sIgE levels (ImmunoCAP; Thermo Fisher Scientific, Waltham, Mass) were significantly higher in the patient group, they greatly overlapped between patients and controls (2.37 vs 0.836 kUA/L; $P < .01$) (Table 1).

We purified a whole macadamia 7S globulin recombinant protein using the database of *Macadamia integrifolia* 7S globulin mRNA sequence (GenBank: AF161883.1). The protein was expressed in *Escherichia coli* capable of producing disulfide bonds (SHuffle T7, New England Biolabs, Ipswich, MA) and biotinylated. The microplate coated with streptavidin was serially incubated with the biotinylated recombinant 7S protein and the participants' sera. Bound IgE was detected by chemiluminescence ELISA. Most of the serum samples in the patient group had macadamia 7S globulin sIgE (Fig 1). Receiver-operating characteristic (ROC) curve analysis using the data of macadamia 7S globulin sIgE levels in all analyzed patient and control groups revealed significantly higher area under the curve (AUC) than that based on crude macadamia nut sIgE levels (7S vs crude: AUC, 0.833 vs 0.660; $P < .01$) (see Fig E1 in this article's Online Repository at www.jaci-inpractice.org). These results were consistent with the results obtained from the subset of participants who underwent OFC (7S vs crude: AUC, 0.767 vs 0.639; $P < .01$). When the ROC analysis was conducted on cases with crude macadamia nut sIgE greater than or equal to 0.35 kUA/L (Patient group, n = 39; Control group, n = 28), the levels of macadamia 7S globulin sIgE showed significantly greater potential to differentiate between the 2 groups, compared with the ROC data with crude macadamia nut sIgE (7S vs crude: AUC, 0.823 vs 0.586; $P < .01$) (see Figure E2 in this article's Online Repository at www.jaci-inpractice.org).

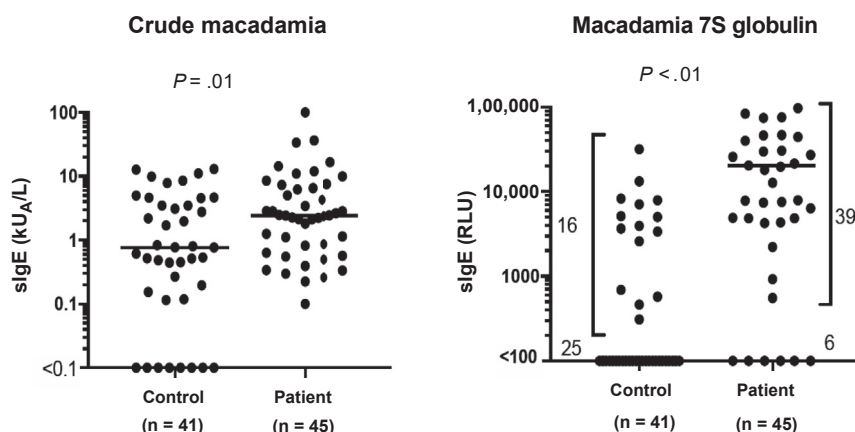
This is the first study to show a potentially predictive marker for the diagnosis of macadamia nut allergy. Interestingly, 6 patients who were all OFC-confirmed showed no macadamia 7S globulin sIgE. Because tree nut allergens belonging to 2S albumin are reportedly good predictive markers for tree nut

TABLE I. Clinical and serological features of the participants with and without macadamia nut allergy

Characteristic	Patients	Controls	P value*
No. of participants	45	41	
No. of males (ratio)	18 (0.4)	23 (0.56)	.71
Age (y), median (range)	6 (1-14)	6 (2-16)	.96
Serology data, median			
Total IgE (kU/L), median	555 (12-5,740)	715 (55-10,630)	.45
Crude macadamia sIgE (kUA/L), median (range)†	2.37 (<0.1-100)	0.836 (<0.1-13)	<.01
Atopic comorbidity (ratio)			
Atopic dermatitis	26 (0.57)	17 (0.41)	.13
Bronchial asthma	10 (0.22)	15 (0.37)	.22
Allergic rhinitis	16 (0.36)	12 (0.29)	.4

*Statistical significance was calculated by χ^2 or Mann-Whitney *U* test.

†Crude macadamia sIgE was measured by ImmunoCAP assay.

**FIGURE 1.** Sera IgE levels specific to recombinant macadamia 7S globulin and crude macadamia nut protein. sIgE was measured in all analyzed participants.

allergies,^{7,8} other component protein(s), including an unidentified macadamia 2S albumin allergen, may be allergenic for these patients. This might also suggest that the expression system could influence the folding of the allergen. In addition, optimal cutoff for our ELISA indicates a sensitivity of 80% with a specificity of 80.49%, still leaving a substantial overlap of control and patient values. Thus, the OFCs remain the criterion standard for the diagnosis of macadamia nut allergy although this component testing has the possibility to improve predictability of reactivity to macadamia nut.

Some limitations should be acknowledged in this study. The study cohort is relatively small, because macadamia allergy is not common in Japan. Because clinical presentations of macadamia nut allergy may vary with age, geographic region, and pollen sensitization, further studies in different geographical regions and populations with a larger sample size are needed. Second, the diagnostic value of the 7S globulin sIgE was not compared with that of skin prick test due to the lack of sufficient data. Third, this study includes the participants from various centers with diagnostic and testing methods depending on the centers, adding a factor of variability in the results.

In conclusion, we demonstrate that macadamia 7S globulin sIgE levels are potentially a better marker than crude macadamia

sIgE levels for the diagnosis of macadamia nut allergy in Japanese children.

Acknowledgments

We thank all the participants of this study, and Dr Tsutomu Iwata, Dr Shigetoshi Kobayashi, Dr Naoki Shimojo, Dr Yutaka Tanaka, Dr Shinsuke Miyamoto, Dr Sou Takada, Dr Masaki Shimizu, Dr Kenji Toyokuni, and Dr Yumiko Miyaji who helped to collect the serum samples. We thank the Promotion and Mutual Aid Corporation for Private Schools of Japan for funding. The data that support the findings of this study are available from the corresponding author on reasonable request.

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This study was partly funded by a Grant-in-Aid for Special Research in Subsidies for ordinary expenses of private schools from The Promotion and Mutual Aid Corporation for Private Schools of Japan, and the institutional research grants.

Conflicts of interest: The authors have no conflict of interest to declare.

Received for publication August 25, 2021; revised December 12, 2021; accepted for publication December 27, 2021.

Available online January 21, 2022.

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2213-2198

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<https://doi.org/10.1016/j.jaip.2021.12.039>

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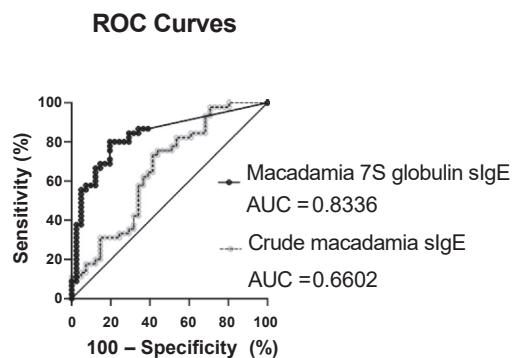


FIGURE E1. ROC curves for IgE to recombinant macadamia 7S globulin and crude macadamia nut protein on their ability to discriminate between all analyzed patient and control groups.

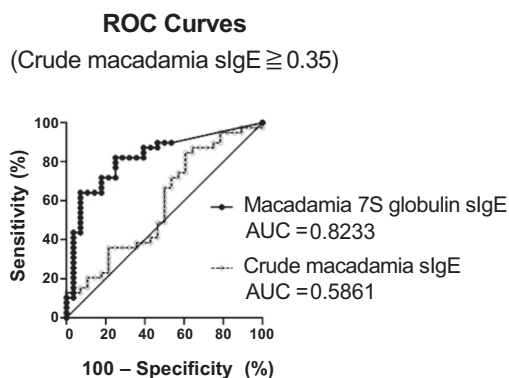


FIGURE E2. ROC curves for IgE to recombinant macadamia 7S globulin and crude macadamia nut protein on their ability to discriminate between all analyzed patient and control groups with crude macadamia nut IgE greater than or equal to 0.35 kU_A/L.