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Original Article

Component-resolved diagnostics can be useful for identifying hazelnut allergy in Japanese children

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Abbreviations:

AUC, area under the curve;

CRD, component-resolved diagnosis;

DBPCFC, double-blind placebo-controlled

food challenge test; LTP, lipid transfer

protein; OAS, oral allergy syndrome;

OFC, oral food challenge; sIgE, specific IgE

ABSTRACT

Background: Cor a 9 and Cor a 14 are effective markers for predicting hazelnut allergy. However, there have been no reports on the component-resolved diagnostics (CRD) of hazelnut allergy using an oral food challenge (OFC) for diagnosis in Asia. We hypothesized that CRD would improve the accuracy of diagnosing hazelnut allergies in Japanese children.

Methods: We recruited 91 subjects (median age: 7.3 years) who were sensitized to hazelnuts and had performed a hazelnut OFC at the National Hospital Organization Sagami National Hospital between 2006 and 2017. All subjects were classified as allergic or asymptomatic to 3 g of hazelnuts. The sIgE levels (hazelnut/Cor a 1/Cor a 8/Cor a 9/Cor a 14/alder pollen) were measured using ImmunoCAP. We aimed to determine the predictive factors of hazelnut allergy.

Results: Nine subjects (10%) were allergic to ≤ 3 g of hazelnuts. Levels of sIgE for Cor a 9 in hazelnut-allergic subjects were significantly higher than those in asymptomatic subjects (4.47 vs. 0.76 kU_A/L, $p = 0.039$). Levels of sIgE to alder pollen and Cor a 1 in hazelnut-allergic subjects were significantly lower than those in asymptomatic subjects (<0.10 vs 13.0 kU_A/L, $p = 0.004$; <0.10 vs 5.03 kU_A/L, $p = 0.025$). The area under the receiver operating characteristics curve for hazelnut/alder/Cor a 1/Cor a 9 was 0.55/0.78/0.72/0.71, respectively, with $p = 0.651/0.006/0.029/0.040$, respectively.

Conclusions: The findings of a high sIgE level for Cor a 9 and a low sIgE level for Cor a 1 can improve the diagnostic accuracy to better identify Japanese children sensitized to hazelnuts.

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Introduction

Hazelnuts (*Corylus avellana*) belong to the same family of trees as birches and alders (family Betulaceae) and are commonly used in the food industry to prepare desserts such as pastries and chocolates. Hazelnuts are recognized as a common food allergen in Europe.^{1,2} In Japan, tree nuts are the eighth most common food that is associated with food allergies.³ If an allergy to a tree nut is suspected, the appropriate step would be to remove nuts from a

patient's diet even if they did not exhibit an allergic reaction or undergo an oral food challenge (OFC).⁴

Component-resolved diagnostics (CRD) can provide a more accurate diagnosis and assessment of the severity of the elicited symptoms, including for hazelnut allergy.^{5–7} Cor a 1, Cor a 8, Cor a 9, and Cor a 14 are the primary allergen components of sIgE antibodies and are used for diagnosing hazelnut allergy in Europe.^{8–10} In particular, sensitization to Cor a 9 (11S globulin storage protein) and Cor a 14 (2S albumin storage protein) has previously been reported as being associated with severe hazelnut allergy.^{6,11,12}

However, the sensitization pattern among hazelnut-allergic patients varies depending on their geographic backgrounds.¹³ To the best of our knowledge, there have been no studies that report on the utility of CRD for the diagnosis of hazelnut allergy based on an OFC in Asia. Therefore, the aim of our study was to investigate

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whether CRD can improve the accuracy in diagnosing hazelnut allergy in Japanese children.

Methods

Subjects

We retrospectively analyzed data from children sensitized to hazelnuts and who had performed a hazelnut OFC at the National Hospital Organization Sagamihara National Hospital (Kanagawa, Japan) between August 2006 and July 2017. Patients with an equivocal response to hazelnut ingestion during or after the OFC were excluded from the study due to lack of accurate clinical data. We also excluded patients with missing laboratory data (Fig. 1).

Oral food challenge

The challenge food was prepared either at the National Hospital Organization Sagamihara National Hospital or by a guardian. We prepared the following three types of roasted hazelnut challenge foods: pumpkin cake with ground and roasted hazelnuts, hamburger with ground and roasted hazelnuts, and whole roasted hazelnuts served alone. If patients found it difficult to eat whole roasted hazelnuts, the addition of chocolate or cookies that do not contain hazelnuts was allowed. During the last 72 h before the OFC, we encouraged the patients to discontinue the use of agents, such as antihistamines or other anti-allergic drugs, which could influence OFC results.

We performed the OFC openly and set the total challenge dose at 0.5, 3.0, and 10 g of hazelnuts (68, 408, and 1360 mg of hazelnut protein, respectively). According to prior risk assessments of hazelnut allergies, the OFC was performed at one of the three doses. We further instructed all patients to perform an OFC with a higher total challenge dose. In particular, for patients who had a negative OFC result with <3 g of hazelnuts, we encouraged them to increase hazelnut consumption to ≥ 3 g.

The hazelnut OFC was conducted in 5, 3, 2, or single portions (Supplementary Table 1). With 5 portions, 1/16 of the total amount was administered first, and then 1/16, 1/8, 1/4, and 1/2 of the total amount was administered at successive 15 min intervals. For those with 3 portions, 1/8 of the total amount was administered first, and then 3/8 and 1/2 of the total amount was administered at successive 30 min intervals. For those with 2 portions, 1/4 of the total amount was administered every 60 min. For those with a single dose, the total amount was administered at one time point. If symptoms were induced after administration of the first dose of an OFC test, the next administration was halted and appropriate treatment was started according to patient symptoms.

We assessed any symptoms induced by the OFC according to the grading of symptoms described in Supplementary Table 2.^{14,15} We judged an OFC as being positive when we observed objective symptoms greater than grade 2. We also regarded persistent subjective symptoms as likely positive symptoms. If grade 1 symptoms were not persistent, we continued the OFC and observed if other symptoms were induced. Anaphylaxis was defined according to the World Allergy Organization Anaphylaxis Guidelines.¹⁶ Patients were treated according to the European Academy of Allergy and Clinical Immunology's (EAACI) food allergy and anaphylaxis guidelines.¹⁷

Determination of hazelnut allergy

According to the OFC results and following any hazelnut ingestion that occurred at home after the OFC, the subjects were divided into two groups: 1) the allergic group, or children who reacted to ≤ 3 g of hazelnuts, and 2) the asymptomatic group, or children who did not react to ≥ 3 g of hazelnuts (Fig. 2).

Those with a positive OFC result with 0.5 g of hazelnuts were included in the allergic group ($n = 2$). Those with a negative OFC result with 0.5 g of hazelnuts were instructed to increase to ≥ 3 g of hazelnuts at home or encouraged to undergo an OFC with 3 or 10 g of hazelnuts. If any subject did not react to ≥ 3 g of hazelnuts, they were included in the asymptomatic group. If a subject reacted to ≤ 3 g of hazelnuts, they were included in the allergic group ($n = 7$).

Measurement of serum-specific IgE

The sIgE levels for hazelnut, Cor a 1, Cor a 8, Cor a 9, Cor a 14, and alder pollen were measured using the ImmunoCAP assay system (Thermo Fisher Scientific, Uppsala, Sweden). An sIgE level ≥ 0.35 kU_A/L was considered positive for an allergy. Blood samples were collected across the range of 1 year before to 1 year after the OFC.

Outcome measures

After assessing the subjects' backgrounds and their immunological test results, we compared the allergic and asymptomatic groups. We aimed to clarify predictors of hazelnut allergy by analyzing the participant characteristics, total IgE, hazelnut sIgE, and the sensitization pattern of the hazelnut allergen component of sIgE.

The background assessment of subjects included sex, age at the time of OFC, past history of immediate reaction to hazelnut, peanut, or any tree nut allergy, and any exhibiting any allergic complications at the time of the OFC. These data were collected from medical records and analyzed.

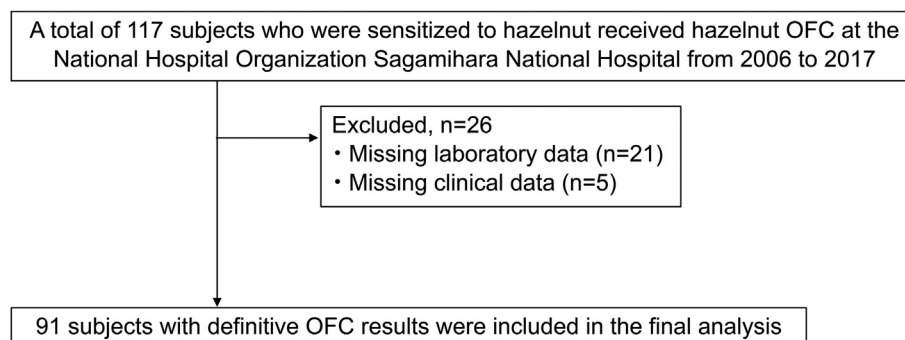


Fig. 1. Study flow chart. A total of 91 patients participated in the study after inclusion and exclusion criteria were applied.

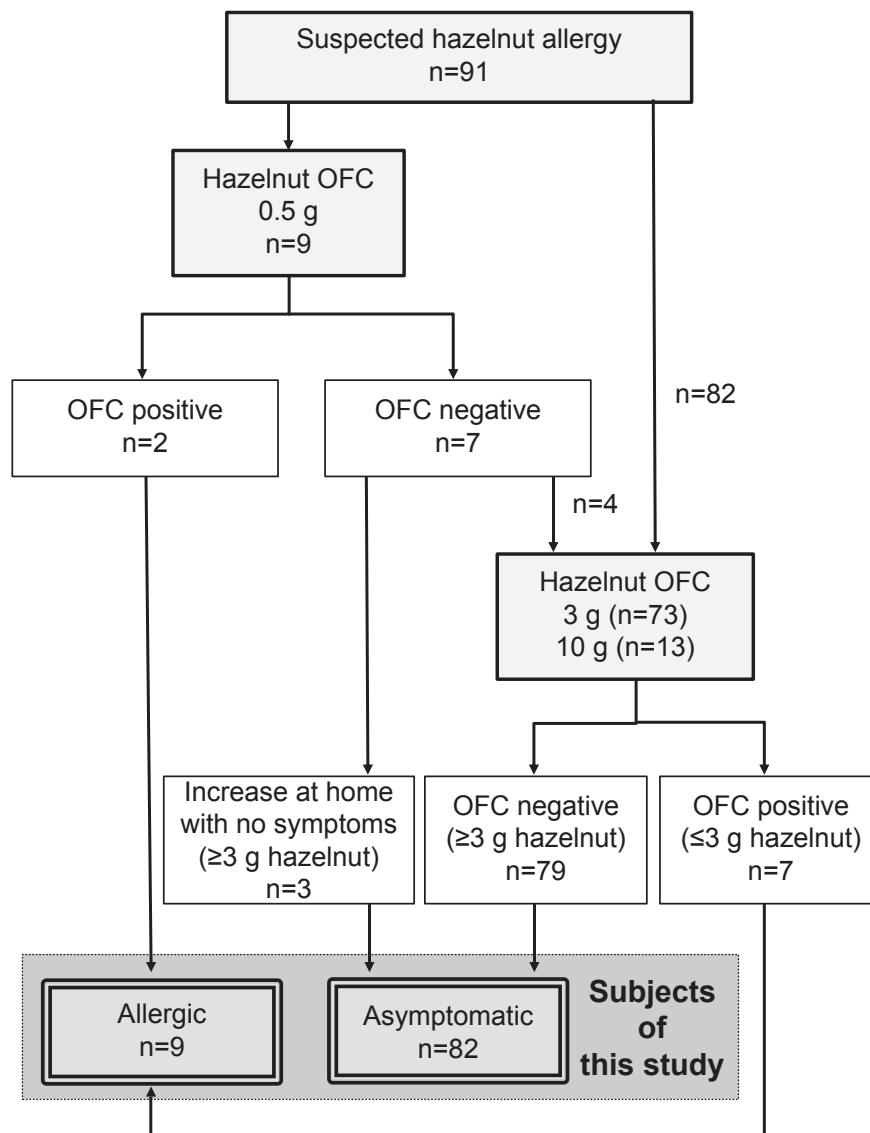


Fig. 2. Flow chart for diagnosing hazelnut allergy. We finally divided the patients into two groups: hazelnut-allergic group and asymptomatic group.

Statistical analysis

The results were expressed as medians, interquartile ranges, and 95% confidence intervals (CIs). The relationships between two variables were estimated using Spearman's correlation coefficients; p values < 0.05 were considered statistically significant. To compare differences between the two groups, we used Mann–Whitney U tests for continuous variables and Fisher's exact tests for categorical variables; p values < 0.05 were considered significant. Receiver operating characteristic curves (ROC) curves were generated for hazelnut sIgE, alder sIgE, Cor a 1 sIgE, Cor a 8 sIgE, Cor a 9 sIgE, and Cor a 14 sIgE. ROC curves were also generated for several ratios. Diagnostic performance of the variables was evaluated using the area under the curve (AUC). We calculated the hazelnut sIgE level for which approximately 5% or 10% of patients would have a clinical reaction to. This so-called 5% or 10% predictive decision point (PPV) is generally used as the value to decide whether an OFC should be performed. To identify the predictive factors for hazelnut allergy,

logistic regression analysis was performed using a logarithm of the sIgE values.

Antigen-sIgE antibodies levels of <0.35 kU_A/L were coded as 0.15 kU_A/L, <0.1 kU_A/L as 0.05 kU_A/L, and >100 kU_A/L as 101 kU_A/L. The sIgE values were analyzed by implementing the logarithm if necessary. Statistical analysis was performed with GraphPad Prism 7J[®] (GraphPad Software, Inc., CA, USA) and R version 3.4.1 (2017, The R Foundation, Vienna, Austria).

Ethical considerations

In accordance with the Declaration of Helsinki, all procedures performed in this study and the risk of symptoms following OFC were fully explained to the patients and their guardians both verbally and in writing. Written informed consent, for both OFC and publication of the data, was obtained from all participants. The study design was approved by the Institutional Review Board of the

National Hospital Organization Sagamihara National Hospital (approval no. 2016/16).

Results

Enrollment of subjects and characteristics

One hundred and seventeen patients who were sensitized to hazelnuts received OFCs with 0.5–10 g of roasted hazelnuts. We could not measure sIgE for the allergen component of hazelnut and alder pollen for 21 patients, and these patients were excluded due to missing laboratory data. Five patients were also excluded for missing clinical data. Therefore, our final analysis included 91 patients (Fig. 1).

Subject characteristics are summarized in Table 1. The median age was 7.3 years, and 69% were male. While 2 patients (2%) had a past history of immediate reaction to hazelnuts, neither of them had anaphylactic reactions. Thirty patients (33%) had a past history of immediate reaction to peanuts, and 31 patients (34%) had a history of immediate reaction to any tree nut except hazelnut.

Current comorbidities of atopic dermatitis, bronchial asthma, allergic rhinitis, and allergic conjunctivitis were present in 60 (66%), 34 (37%), 41 (45%), and 11 (12%) of all participants, respectively. The total IgE level was 1180 IU/mL, and the levels of antigen-sIgE antibodies for hazelnut, alder, Cor a 1, Cor a 8, Cor a 9, and Cor a 14 were 8.47 kU_A/L, 8.15 kU_A/L, 3.85 kU_A/L, <0.10 kU_A/L, 0.84 kU_A/L, and 0.10 kU_A/L, respectively (expressed as medians).

We also compared the characteristics of excluded subjects (n = 26) with those of the included subjects (n = 91) in Supplementary Table 3.

Diagnosis of hazelnut allergy

The final decision regarding determining a diagnosis of an allergy to hazelnuts is shown in Figure 2. Nine patients (10%) were classified as having a hazelnut allergy, while 82 patients (90%) were asymptomatic. The induced symptoms and their severities are summarized in Table 2. The most common symptoms were gastrointestinal symptoms, which were observed in all 9 allergic patients (100%). All of these patients had a localized reaction such as oral discomfort, but 2 exhibited more extensive gastrointestinal

Table 2

Symptoms and severity of the hazelnut allergic group. Data are expressed as n (%).

	Hazelnut allergic group (n = 9)
Symptoms	
Skin	4 (44%)
Gastrointestinal	9 (100%)
Respiratory	4 (44%)
Nervous	0 (0%)
Cardiovascular	0 (0%)
Anaphylaxis	3 (33%)
Severity	
Mild	4 (44%)
Moderate	4 (44%)
Severe	1 (11%)

symptoms including abdominal pain, diarrhea, and emesis. No patients developed neurological or cardiovascular symptoms.

Four patients (44%) had mild symptoms, which were mainly oral discomfort. Four (44%) had moderate symptoms. Only 1 patient (11%) had severe symptoms, which were generalized urticaria, repetitive cough, and throat tightness.

Anaphylaxis appeared in 3 patients (33%), with concomitant skin and respiratory symptoms in all cases; there were no patients with anaphylactic shock.

For treatment, oral antihistamine and β_2 -inhalation were used for 5 patients (56%) and 3 patients (33%), respectively. Only 1 patient (11%) used inhaled corticosteroids, which was based upon a decision by the patient's mother in order to relieve respiratory symptoms following an OFC at home. None of the patients used adrenaline.

A comparison of patient characteristics between patients with a hazelnut allergy and those who were asymptomatic is presented in Table 3. There were no significant differences among the patients in the hazelnut-allergic group vs. the asymptomatic group in terms of sex, age, past history of immediate reaction to any food, complications, and total IgE level. The observed doses of hazelnuts associated with a reaction were as follows: 3 patients reacted to 0.1–0.5 g of hazelnuts, 2 patients to 0.5–1 g, and 4 patients to 1–3 g. A comparison of sIgE in those with a hazelnut allergy vs. those in the asymptomatic group is shown in Figure 3. Hazelnut-allergic patients had significantly higher sIgE levels vs. those who were

Table 1

Characteristics of subjects. Values are reported as n (%), median (interquartile range) as appropriate (n = 91).

Total, n	91
Male, n (%)	63 (69%)
Age (years)	7.3 (5.9–10.5)
Past history of immediate reactions to	
Hazelnut	2 (2%)
Peanut	30 (33%)
Any tree nut except hazelnut	31 (34%)
Any food	80 (88%)
Complications	
Atopic dermatitis	60 (66%)
Bronchial asthma	34 (37%)
Allergic rhinitis	41 (45%)
Allergic conjunctivitis	11 (12%)
Total IgE (IU/mL) (n = 88)	1180 (507–2105)
sIgE (kU _A /L)	
Hazelnut	8.47 (3.30–23.0)
Alder pollen	8.15 (0.70–33.1)
Cor a 1	3.85 (<0.10–25.2)
Cor a 8	<0.10 (<0.10–0.16)
Cor a 9	0.84 (0.37–3.51)
Cor a 14	0.10 (<0.10–0.38)

sIgE, specific IgE.

Table 3

Characteristics of patients in the hazelnut asymptomatic or allergic group. Values are reported as n (%), median (interquartile range) as appropriate.

	Hazelnut asymptomatic group (n = 82)	Hazelnut allergic group (n = 9)	p value
Male, n (%)	57 (70%)	6 (67%)	0.565 [†]
Age (years)	7.3 (5.9–10.1)	10.3 (5.9–14.2)	0.254 [‡]
Past history of an immediate reaction to			
Hazelnut	1 (1%)	1 (11%)	0.189 [†]
Peanut	29 (35%)	1 (11%)	0.262 [†]
Any tree nut except hazelnut	27 (33%)	4 (44%)	0.484 [†]
Any food	74 (90%)	7 (78%)	0.257 [†]
Complications			
Atopic dermatitis	56 (68%)	5 (56%)	0.470 [†]
Bronchial asthma	31 (38%)	3 (33%)	>0.999 [†]
Allergic rhinitis	39 (48%)	2 (22%)	0.178 [†]
Allergic conjunctivitis	9 (11%)	2 (22%)	0.297 [†]
Total amount of reacting dose of hazelnut			
0.1–0.5 g	NA	3	NA
>0.5–1 g	NA	2	NA
>1–3 g	NA	4	NA
Total IgE (IU/mL) (n = 88)	1250 (523–2240)	771 (320–973)	0.068 [‡]

NA, Not Applicable.

[†] Fisher's exact test.

[‡] Mann–Whitney U tests.

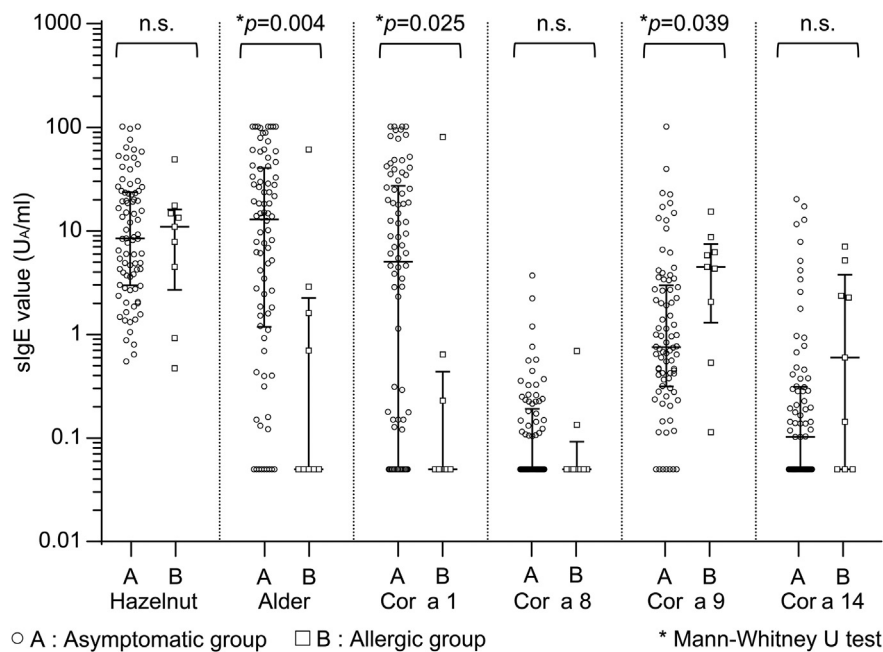


Fig. 3. Sensitization pattern and comparison of the sIgE levels in the hazelnut-allergic group and asymptomatic group. Almost all patients were sensitized to alder pollen, a major tree pollen, having cross-reactivity to Bet v 1 homolog Cor a 1.

asymptomatic for Cor a 9 sIgE (4.47 vs. 0.76 kU_A/L, $p = 0.039$), while patients with an allergy had significantly lower sIgE levels than asymptomatic patients for alder sIgE (<0.10 vs. 13.0 kU_A/L, $p = 0.004$) and Cor a 1 sIgE (<0.10 vs. 5.03 kU_A/L, $p = 0.025$). No significant differences were observed for hazelnut sIgE (11.0 vs. 8.43 kU_A/L, $p = 0.660$), Cor a 8 sIgE (<0.10 vs. <0.10 kU_A/L, $p = 0.358$), or Cor a 14 sIgE (0.60 vs. 0.10 kU_A/L, $p = 0.1318$).

Receiver operating characteristic analysis

The AUCs for hazelnut sIgE, alder sIgE, Cor a 1, Cor a 8, Cor a 9, and Cor a 14 are shown in Table 4, to discriminate between the hazelnut-allergic and asymptomatic groups. The AUC for alder sIgE was highest (0.78; 95% CI: 0.61–0.95, $p = 0.006$), followed by Cor a 1 (0.72; 95% CI: 0.55–0.90, $p = 0.029$) and Cor a 9 (0.71; 95% CI: 0.52–0.89, $p = 0.040$).

The AUCs for hazelnut sIgE, Cor a 8, and Cor a 14 were 0.55 (95% CI: 0.36–0.73, $p = 0.651$), 0.58 (95% CI: 0.39–0.78, $p = 0.406$), and 0.65 (95% CI: 0.44–0.86, $p = 0.151$), respectively.

The AUCs for several sIgE ratios are presented in Supplementary Table 4. The AUC for the sIgE ratio of hazelnut/alder was highest (0.83; 95% CI: 0.72–0.95, $p = 0.001$), followed by Cor a 9/alder

(0.80; 95% CI: 0.61–0.99, $p = 0.004$). The AUCs for these sIgE ratios were higher than that of alder sIgE alone.

Correlation between sIgE levels of allergen components

We analyzed the correlation coefficients between sIgE levels for hazelnut, alder, Cor a 1, Cor a 8, Cor a 9, and Cor a 14 (Supplementary Table 5). The sIgE levels for alder and Cor a 1 had a strong positive correlation ($R_s = 0.88$, $p < 0.001$). The sIgE levels associated with hazelnut and Cor a 1 ($R_s = 0.74$, $p < 0.001$) and hazelnut and alder ($R_s = 0.73$, $p < 0.001$) also had positive correlations.

Predictive factors for hazelnut allergy

We performed univariate logistic regression analysis using the logarithm of the sIgE levels for hazelnut, alder, Cor a 1, and Cor a 9 to identify the predictive factors for hazelnut allergy. The calculated crude odds ratios of sIgE levels for hazelnut, alder, Cor a 1, and Cor a 9 were 0.72 (95% CI: 0.22–2.36, $p = 0.583$), 0.38 (95% CI: 0.19–0.75, $p = 0.002$), 0.45 (95% CI: 0.22–0.93, $p = 0.013$), and 2.38 (95% CI: 0.91–6.24, $p = 0.070$), respectively (Table 5).

sIgE levels for predicting hazelnut allergy

The sIgE levels that predict hazelnut allergy were calculated from the results of the logistic regression analysis. Regarding the

Table 4

ROC curve predicts hazelnut allergy. In the table, areas under curve (AUC), including the 95% CI, are shown for several sIgEs ($n = 91$).

	AUC	95% CI	p value
sIgE (kU _A /L)			
Hazelnut	0.55	0.36–0.73	0.651
Alder	0.78 [†]	0.61–0.95	0.006*
Cor a 1	0.72 [†]	0.55–0.90	0.029*
Cor a 8	0.58	0.39–0.78	0.406
Cor a 9	0.71	0.52–0.89	0.040*
Cor a 14	0.65	0.44–0.86	0.151

sIgE, specific IgE.

*Significant p value.

[†] Values are lower in the hazelnut allergic group.

Table 5

Factors predicting a hazelnut allergy.

	OR (95% CI)	p value
Log (Hazelnut sIgE)	0.72 (0.22–2.36)	0.583
Log (Alder sIgE)	0.38 (0.19–0.75)	0.002
Log (Cor a 1 sIgE)	0.45 (0.22–0.93)	0.013
Log (Cor a 9 sIgE)	2.38 (0.91–6.24)	0.070

OR, odds ratio; sIgE, specific IgE.

hazelnut allergy outcome, the 5% and 10% PPVs for sIgE levels for alder, Cor a 1, and Cor a 9 were 7.68 and 1.32 kU_A/L, 4.67 and 0.53 kU_A/L, and 0.23 and 1.69 kU_A/L, respectively. Furthermore, the 10% PPV for sIgE for hazelnut was 7.03 kU_A/L, while the 5% PPV was not applicable (it was >101 kU_A/L, which was beyond our observation) (Supplementary Table 6).

Discussion

We found that a high sIgE level for Cor a 9 and a low sIgE level for alder and Cor a 1 may indicate actual hazelnut allergies from a hazelnut-sensitized population.

The AUCs for sIgE to alder, Cor a 1, and Cor a 9 were high; therefore, determining sIgE for each of these parameters would improve diagnostic performance for hazelnut allergy, while using the overall sIgE level for hazelnut may not in fact be a reliable marker. We also calculated the crude odds ratio to describe probability curves for alder, Cor a 1, and Cor a 9. Although we expect probability curves to be useful for assessing symptoms of hazelnut allergy, these curves did not seem to be practical for predicting hazelnut allergy due to the low odds ratios value.

Although Cor a 9 has been identified as a predictive marker for severe symptoms of hazelnut allergy in Europe, our novel finding was that Cor a 1 may also inversely be a reliable marker in Japanese children sensitized to hazelnuts.^{5,11,12} We hypothesized that the discrepancy between different markers used for diagnosing hazelnut allergy may come from variation in hazelnut sensitization patterns within a study population.

Cor a 1 can cross-react with the birch pollen allergen Bet v 1.^{11,18} In parts of Japan, including Kanagawa, alder pollen is more commonly found than birch pollen, except in Hokkaido and Nagano, where birch is mainly found. Figure 3 shows that our population consisted of alder pollen-sensitized patients. As confirmed by the strong correlation between sIgE and alder and Cor a 1 and hazelnut (Supplementary Table 5), we found that our study population had an elevated hazelnut sIgE level for alder-related cross-reactivity. This was not due to the primary sensitization to hazelnut itself.

The rate of hazelnut allergy in our study was lower than in previous studies. Previous studies have reported that 20%–40% of children who performed an OFC exhibited symptoms, though the characteristics of these populations differed from ours.^{5,19–21} Additionally, the severity of the induced symptoms in our study was milder than in prior studies, and anaphylaxis was less frequent. Regarding anaphylaxis elicited by hazelnut OFC, Brandström *et al.*¹⁹ reported that hazelnut double-blind placebo-controlled food challenge tests (DBPCFC) induced anaphylactic symptoms affecting multiple organs in 50% of the participants and anaphylactic shock in 12.5% of the participants. In addition, 25% of the patients required treatment upon admission.

The discrepancy in results may be explained as follows: we used roasted hazelnuts so the allergenicity of hazelnuts could be reduced. Some studies reported that Cor a 1 is a heat- and digestion-labile PR-10 protein, whose allergenicity is reduced when subjected to the roasting process.^{1,22,23} In Japan, it is not customary to eat raw hazelnuts. Thus, while some previous studies have used raw hazelnuts,^{5,19–21} we used roasted hazelnuts in our study. Therefore, the rate of confirmed hazelnut allergy and the severity of the induced symptoms in our study may be different from those reported in previous studies. Additionally, not only the influence of cross-reactivity with pollen but also the accumulative dose of hazelnuts may help explain the difference in findings between ours and those of previous reports. The previous studies set the accumulative hazelnut dose higher than our study did (in particular, ranging from 6 to 32 g of raw hazelnuts).^{5,19–21} As described later in this section, in our study, few patients consumed more hazelnuts

after the OFCs. The discrepancy in results may be attributed to the small accumulative dose of hazelnuts.

Although Cor a 8, a lipid transfer protein (LTP), has been associated with severe hazelnut allergy in Mediterranean areas, it was not a reliable diagnostic marker of hazelnut allergy in our study.^{9,10} Such discrepancies in the effectiveness of Cor a 8 as a marker for diagnosing hazelnut allergy may be due to the differences in geography, ethnicity, and eating habits, which can influence the potential of sensitization to LTP. From a standpoint of LTPs, Pru p 3 in peach allergy resembles Cor a 8 in hazelnut allergy, which is not an effective marker to diagnose allergy in Japan but an effective marker to predict allergy in Mediterranean areas.^{24,25} For Mediterranean patients allergic to LTP, peach has been implicated as the primary sensitizer.²⁵ Conversely, few Japanese patients with peach allergy are sensitized to LTP (Pru p 3).²⁴ We speculate that as most Japanese children are sensitized to pollen, while few children are sensitized to LTP, both LTPs (Pru p 3 and Cor a 8) are not useful as diagnostic markers of peach and hazelnut allergies.

Hazelnut allergy varies from mild oral symptoms to severe systemic reactions.^{1,5,13} In fact, some patients in our study experienced anaphylaxis at a constant rate. We hypothesize these severe symptoms may be attributed to sensitization to Cor a 9.

However, in our study, most patients with a hazelnut allergy had mild or moderate symptoms. Additionally, the rate of a hazelnut allergy diagnosis was not as high as in previous European studies.

Therefore, in Japan, we recommend that hazelnut-sensitized patients who have a history of immediate reaction to peanuts or any tree nuts need not simply eliminate hazelnuts from their diet unless it is confirmed that the patient is actually suffering from a hazelnut allergy. As shown in Supplementary Table 6, we suggest that the hazelnut-sensitized patients with alder sIgE >7.68 kU_A/L, with Cor a 1 sIgE >4.67 kU_A/L, or with Cor a 9 sIgE <0.23 kU_A/L can safely consume hazelnuts.

However, consumption of hazelnuts may induce adverse reactions, including (in some cases) anaphylaxis. In light of this, patients, especially those outside the abovementioned parameters, should be encouraged to undergo an OFC using roasted hazelnuts (with ample consideration for patient safety).

There are some limitations in our study. First, we performed an open OFC, not a DBPCFC. Since subjective symptoms, such as those associated with oral allergy syndrome (OAS), were observed in those with positive OFC results, DBPCFCs would in theory be appropriate for diagnosing the allergy. However, most of the participants in our study had several concomitant conditions associated with food allergies. Hazelnut is not a common food in Japan, and an OFC is time-consuming for patients. These considerations and the incurred costs make it difficult to perform hazelnut DBPCFCs.

Second, the number of hazelnut-allergic patients was limited within our study compared to the number of asymptomatic patients. Therefore, this may have reduced the statistical power of our results. In addition, we could not stratify the participants by age. Some studies reported that primary sensitization to hazelnuts was more common in children than in adults and that children more often develop severe and systemic reactions to hazelnuts. In contrast, OAS has been reported to be associated with mild symptoms after ingestion of hazelnuts and is mainly observed in adults who are sensitized and have cross-reactivity to birch pollen.^{1,11}

Third, we classified patients as those with a hazelnut allergy vs. those who were asymptomatic according to their tolerance to 3 g of hazelnuts. Some studies have reported that patients with birch pollen-related hazelnut allergy may develop severe symptoms at a higher dose even in OAS cases, which generally exhibit only mild symptoms.^{18,23} Although we encouraged our patients to perform higher-dose OFCs or increase their dosage of hazelnuts at home, few patients consumed more hazelnuts according to our

instructions. We could not confirm all patients were tolerant to 10 g of hazelnuts. However, 3 g of hazelnuts would be a sufficient dose for daily consumption in Japan.

In conclusion, with the findings of a high sIgE level for Cor a 9 and a low sIgE level for Cor a 1, diagnostic testing can be improved for Japanese children sensitized to hazelnuts. Further prospective studies are needed to evaluate the validity of our results in another cohort with a larger sample size.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.alit.2019.10.001>

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

YI and SS designed the study and wrote the manuscript. YI, KT, and SS contributed to data collection. YI and KT performed the statistical analysis and interpretation of the results. SS, NY, HY, NS, and ME supervised the study and drafted the manuscript. All authors read and approved the final manuscript.

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Supplementary Table 1. Administration intervals and dividing methods in oral food challenge tests

Dosing method	Administration interval	Dose dividing method
Five portions	15 min	$1/16 \rightarrow 1/16 \rightarrow 1/8 \rightarrow 1/4 \rightarrow 1/2$
Three portions	30 min	$1/8 \rightarrow 3/8 \rightarrow 1/2$
Two portions	60 min	$1/4 \rightarrow 3/4$
Single dose	-	$1/1$

Supplementary Table 2. Symptom grading for hazelnut allergy

	1 (mild)	2 (moderate)	3 (severe)
Skin	Localized urticaria, exanthema, wheal, pruritus Swollen eyelid or lip	Generalized urticaria, exanthema, wheal, pruritus Swollen face	- -
Gastrointestinal tract	Pruritus of the throat or oral cavity Mild abdominal pain Nausea, emesis, diarrhea	Throat pain Moderate abdominal pain Recurrent emesis, diarrhea	Cramps Continuous emesis, loss of bowel control
Respiratory tract	Intermittent cough, nasal congestion, sneezing, rhinorrhea	Repetitive cough Chest tightness, mild wheezing	Persistent cough, hoarseness, “barky” cough Audible wheezing, dyspnea, cyanosis, saturation <92%, swallowing or speaking

			difficulties, throat tightness, respiratory arrest
Cardiovascular	-	Pale face, mild hypotension, tachycardia (increase >15 beats/min)	Hypotension, dysrhythmia, severe bradycardia, cardiac arrest
Neurological	Change in activity level, tiredness	“Light-headedness,” feeling of “impending doom,” somnolence, headache	Confusion, loss of consciousness, incontinence

Supplementary Table 3. Characteristics of excluded subjects (n=26); comparison with included subjects (n=91). Values are reported as n (%), median (Interquartile range) as appropriate.

		Excluded subjects (n=26)	Included subjects (n=91)	<i>p</i> value
Male, n (%)		18 (69.2%)	63 (69.2%)	>0.999 ¹⁾
Age (years)		8.4 (6.5-9.6)	7.3 (5.9-10.5)	0.999 ²⁾
Past history of immediate reaction to	Hazelnut	1 (3.8%)	2 (2.2%)	0.533 ¹⁾
	Peanut	10 (38.5%)	30 (33.0%)	0.643 ¹⁾
	Any tree nut except hazelnut	12 (46.2%)	31 (34.1%)	0.356 ¹⁾
	Any food	24 (92.3%)	80 (87.9%)	0.730 ¹⁾
Complications	Atopic dermatitis	15 (57.7%)	60 (65.9%)	0.491 ¹⁾
	Bronchial asthma	10 (38.5%)	34 (37.4%)	>0.999 ¹⁾
	Allergic rhinitis	11 (42.3%)	41 (45.1%)	0.827 ¹⁾

	Allergic conjunctivitis	6 (23.1%)	11 (12.1%)	0.206 ¹⁾
Total IgE (IU /mL) (n=21)		1450 (894-1910)	1180 (507-2105)	0.321 ²⁾
Hazelnut sIgE (kU _A /L) (n=23)		15.4 (5.40-24.4)	8.47 (3.30 -23.0)	0.441 ²⁾

¹⁾ Fisher's exact test, ²⁾ Mann-Whitney U tests

sIgE, specific IgE.

Supplementary Table 4. ROC curve predicts a hazelnut allergy. In the table, areas under curve (AUC), including the 95% CI, are shown for several ratios (n=91)

		AUC	95% CI	<i>p</i> value
sIgE ratio	Hazelnut/Alder	0.83	0.72-0.95	*0.001
	Hazelnut/Cor a 1	0.76	0.58-0.93	*0.012
	Cor a 9/Alder	0.80	0.61-0.99	*0.004
	Cor a 9/Cor a 1	0.76	0.57-0.95	*0.011

sIgE, specific IgE.

Supplementary Table 5. Correlation between sIgE and hazelnut, Alder, Cor a 1, Cor a 8, Cor a 9, and Cor a 14. Values are reported as Spearman's correlation coefficient (*p* value), n=91

	Cor a 1	Cor a 8	Cor a 9	Cor a 14	Hazelnut	Alder
Hazelnut	0.74 (<0.001)	0.33 (0.0012)	0.07 (0.4809)	0.10 (0.3699)	1.0000	0.73 (<0.0001)
Alder	0.88 (<0.001)	0.37 (0.0003)	-0.34 (0.0010)	-0.17 (0.097)	0.73 (<0.0001)	1.0000

Supplementary Table 6. Clinical efficacy of sIgE level for predicting hazelnut allergy

	5% PPV	10% PPV
Hazelnut (kU _A /L)	NA	7.03
Alder sIgE (kU _A /L)	7.68	1.32
Cor a 1 sIgE (kU _A /L)	4.67	0.53
Cor a 9 sIgE (kU _A /L)	0.23	1.69

NA, Not Applicable; PPV, positive predictive value; sIgE, specific IgE.