

Measurement of specific IgE antibodies to Ses i 1 improves the diagnosis of sesame allergy

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Clinical & Experimental Allergy

Summary

Background The number of reported cases of allergic reactions to sesame seeds (*Sesamum indicum*) has increased significantly. The specific IgE tests and skin prick tests presently available for diagnosis of sesame allergy are all based on crude sesame extract and are limited by their low clinical specificity. Thus, oral food challenge (OFC) is still the gold standard in the diagnosis.

Objective The aim was to identify the allergen components useful to diagnose sesame-allergic children with the goal to reduce the number of OFCs needed.

Methods Ninety-two sesame-sensitized children were consecutively enrolled and diagnosed based on OFC or convincing history. Specific IgE to purified native 11S globulin (nSes i 11S), 7S globulin (nSes i 7S), 2S albumin (nSes i 2S), and two recombinant 2S albumins (rSes i 1 and rSes i 2) was measured by ELISA and/or ImmunoCAP (rSes i 1/streptavidin application).

Results Based on area under curve (AUC) values from receiver operating characteristic (ROC) analysis, rSes i 1 was shown to have the best diagnostic performance of the allergen components in ELISA. The experimental rSes i 1 ImmunoCAP test had larger AUC (0.891; 95% CI, 0.826–0.955) compared to the commercially available sesame ImmunoCAP (0.697; 95% CI, 0.589–0.805). The clinical sensitivity and specificity for the rSes i 1 ImmunoCAP test at optimal cut-off (3.96 kU_A/L) were 86.1% and 85.7%, respectively.

Conclusion and Clinical Relevance Sensitization to Ses i 1 is strongly associated with clinical sesame allergy. Measurement of specific IgE to rSes i 1 could reduce the numbers of OFCs needed.

Keywords 2S albumin, molecular-based allergy diagnostics, oral food challenge, Ses i 1, sesame

Submitted 6 April 2015; revised 30 July 2015; accepted 13 August 2015

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Cite this as: N. Maruyama,

T. Nakagawa, K. Ito, C. Cabanos, M.P. Borres, R. Movérare, A. Tanaka, S. Sato, M. Ebisawa, *Clinical & Experimental Allergy*, 2016 (46) 163–171.

Introduction

Sesame seed food allergy is an increasingly recognized health burden, especially in developed countries including European countries, Canada and United States [1]. Most cases occur in infancy and early childhood and the clinical presentation includes a significant numbers of patients with severe reactions [2]. Anaphylaxis has been reported in up to 30% of sesame-allergic children [3].

Sesame allergy is also present in adults and is sometimes preceded by *de novo* sensitization. Several reports

have shown a prevalence of sesame allergy in the range of 0.1–0.2% [1, 4]. Only in few studies, however, the gold standards of objective evidence have been used, that is assessment of sensitization and oral food challenge (OFC) [1]. The prevalence of sesame allergy seems to differ between communities due to different food habits [1]. Sesame allergy was reported to be the third most common allergy in Israeli children, preceded only by milk and egg allergy [5].

Sesame allergy is generally diagnosed on the basis of history, skin prick test (SPT), specific IgE measurement,

and OFC [1]. OFC is the gold standard for diagnosing food allergies but is expensive and potentially dangerous. On the other hand, the commercially available *in vitro* tests for sesame-specific IgE antibodies and SPTs based on crude sesame extract have low clinical specificity and cannot therefore sufficiently predict true sesame allergy [6–9]. Molecular-based allergy diagnostics is a promising approach to increase the diagnostic accuracy in food allergy [10, 11]. However, reports regarding sesame allergen components associated with clinical symptoms are limited.

Seven sesame allergen components have been registered by the WHO/IUIS Allergen Nomenclature Subcommittee, including two 2S albumins (Ses i 1 and Ses i 2) [12, 13], one vicilin-like 7S globulin (Ses i 3) [13], two oleosins (Ses i 4 and 5) [14], and finally two 11S globulins (Ses i 6 and Ses i 7) [15]. The 2S albumins, and 7S and 11S globulins are all seed storage proteins described as major allergens in many plant-derived foods including peanut, soya bean, and tree nuts [16–18]. The 2S albumins and 11S globulins are dominating seed storage proteins in sesame, whereas 7S globulin is a minor component [19, 20]. All together, they represent 80–90% of the total sesame seed proteins [19]. Motifs for N-glycosylation do not exist in the amino acid sequences of sesame 11S globulin (Ses i 6 and 7) and 2S albumin (Ses i 1 and 2), whereas 7S globulin (Ses i 3) has a potential N-glycosylation site.

In this study, we assessed the diagnostic value of measuring specific IgE antibodies to various sesame components in the diagnosis of children with suspected sesame allergy. The allergen components used in the study were native preparations of 2S albumin, 7S globulin, and 11S globulin purified from sesame seed and recombinantly expressed Ses i 1 and Ses i 2.

Methods

Subjects and oral food challenge

Children sensitized to sesame with symptoms from the skin and/or respiratory and gastrointestinal tract, and referred to Sagamihara National Hospital and Aichi Children's Health and Medical Center due to a suspicion of sesame allergy, were consecutively included in the study. In total, 92 children were included. At the entry, each child was evaluated with a focus on sesame allergy by both a detailed medical examination and medical history review. An oral sesame challenge test was performed in 68 cases. In the remaining 24 children, an oral sesame challenge test was not performed. Seven of them had a convincing positive history with high risk of a strong reaction on challenge testing, and 17 were considered as sesame tolerant after the anamnesis.

The OFCs were performed in accordance with the Japanese Pediatric Guidelines for Food Allergy [21]. Increasing doses, starting with 0.1–0.2 g of ground sesame seeds, were administered to patients every 15–30 min up to a cumulative dose of at least 3 g. A challenge was scored as positive when objective symptoms occurred. Rescue medications (antihistamine, glucocorticoid, nebulized β 2-agonist and/or adrenaline) were immediately administered in the event of an allergic reaction. A negative challenge was confirmed by the absence of any symptoms after ingestion of sesame products.

Ethical approval was obtained from the institutional review boards of Sagamihara National Hospital (Sagamihara, Japan) and Aichi Children's Health and Medical Center (Obu, Japan). Written informed consent was obtained from all children and/or children's parents prior to entry.

Preparation of native sesame seed allergens

Sesame seeds (*Sesamum indicum*) were ground in liquid nitrogen and subsequently defatted using *n*-hexane. Total seed proteins were extracted using phosphate buffer (0.4 M NaCl, 35 mM NaPi, pH 7.6, and protease inhibitors) and native 2S albumin, 7S globulin, and 11S globulin were separated by gel filtration using Superdex 200 pg (GE Healthcare Life Sciences, Uppsala, Sweden). Further purification of 7S globulin was performed using an anion exchange Mono Q column (GE Healthcare Life Sciences) and a linear sodium chloride gradient. Fractions that contained purified 2S albumin, 7S globulin, and 11S globulin were pooled and concentrated using a Vivaspin membrane filter (Sartorius, Hannover, Germany). Protein concentrations were determined using the Protein Assay Rapid Kit (Wako, Osaka, Japan) with bovine serum albumin (BSA) as standard. Purified 2S albumin, 7S globulin, and 11S globulin were designated as nSes i 2S, nSes i 7S, and nSes i 11S, respectively.

Preparation of recombinant Ses i 1 and Ses i 2

Total RNA was extracted from developing sesame seeds using RNAqueous kit (Life Technologies, Carlsbad, CA, USA). The following specific sense and antisense primers containing *Bam* HI and *Xho* I restriction sites were used to amplify the full-length sequences of the 2S albumin variants Ses i 1 (GenBank AF240005) and Ses i 2 (GenBank AF091841).

Ses i 1: 5'-TATTATAGGATCCCAGCAGAGCCAACAGT GCCG-3' and 5'-TAATTTTCTCGAGCTACACAAAGATAA CTCGGA-3'.

Ses i 2: 5'-TATTATAGGATCCTCCGCCATAAGACG GTGGT-3' and 5'-TAATTTTCTCGAGTCAGGCAAAGATT GGGCGCA-3'.

The amplified fragments were digested using *Bam* HI and *Xho* I and ligated with a pGEX vector (GE Healthcare Life Sciences). The sequences of the cloned cDNAs were identical to those in GenBank (Ses i 1, AF240005; Ses i 2, AF091841). The generated expression plasmids containing the Ses i 1 and Ses i 2 coding regions were transformed into Rosetta 2(DE3) *Escherichia coli* (*E. coli*) (Merck Millipore, Darmstadt, Germany). Recombinant proteins tagged with glutathione *S*-transferase were expressed in bacterial cells grown in Luria–Bertani medium containing 50 µg/mL carbenicillin. Cultures that had reached an OD₆₀₀ level of 0.4–0.8 were induced by adding isopropyl β-D-1-thiogalactopyranoside to a final concentration of 0.1 mM and then incubated for 16–24 h. Cells were harvested using centrifugation, suspended in phosphate-buffered saline (PBS) containing protease inhibitors, and sonicated. Supernatants were loaded onto a glutathione–Sephadex 4B column (GE Healthcare Life Science), and bound proteins were eluted using reduced glutathione. Recombinant fusion proteins were cleaved using the PreScission Protease (GE Healthcare Life Sciences) and separated using Superdex 200 pg gel filtration. The purified recombinant proteins were designated as rSes i 1 and rSes i 2, respectively.

Protein analysis

The purified sesame proteins were alkylated and digested with mass spectrometry grade trypsin (Thermo Scientific, Rockford, IL, USA). The digested proteins were analysed using the LC-MS/MS system QTOF G6520 (Agilent Technologies, Santa Clara, CA, USA) equipped with the HPLC-Chip Cube system G4240 (Agilent Technologies). Elution from the analytical column was performed by a solvent mixture composed of water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B). Mass spectra were processed and analysed using Mascot server (Matrix Science, London, UK).

One- and two-dimensional (2D) sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of the purified sesame proteins were performed using Mini-PROTEAN TGX (12% or Any kD) and Criterion TGX (18%) gels (Bio-Rad Laboratories, Hercules, CA, USA), respectively. IPG gel strips (11 cm, pH 3–11NL, GE Healthcare Life Science) were used for isoelectric focusing in 2D electrophoresis. The proteins were stained with Coomassie Brilliant Blue R-250.

Enzyme-linked immunosorbent assay

The levels of specific IgE to sesame seed extract or purified sesame components were measured using a

fluorescent enzyme-linked immunosorbent assay (ELISA). ELISA plates were coated overnight with proteins in 0.05 M carbonate buffer (pH 9.6) at 4°C, washed with PBS containing 0.05% (v/v) Tween 20 (PBS-T), and blocked with 1% (w/v) BSA in PBS-T. After washing the plates, serum samples diluted 1 : 10 in blocking buffer were added and incubated for 3 h at room temperature (RT). After three washes, a β-galactosidase-conjugated anti-human IgE antibody (Phadia, Uppsala, Sweden) diluted 1 : 10 in blocking buffer was added. After 2-h incubation at RT, the plates were washed three times and the substrate (0.2 mM 4-methylumbelliferyl β-D-galactopyranoside) was added. Fluorescence was measured with a microplate reader at 355 nm excitation and 460 nm emission. A pool of serum samples with known specific IgE levels (from ImmunoCAP analysis) were used to establish a standard curve, and the results were expressed as ELISA-based kilo units per litre (kU_E/L). Sera from 13 patients with other food allergies than to sesame and six nonatopic subjects were used as negative controls.

ImmunoCAP analysis

Total IgE and sesame- and rSes i 1-specific IgE antibodies were measured using ImmunoCAP (Phadia). An experimental rSes i 1 ImmunoCAP test was developed by biotinylation of rSes i 1 using EZ-link sulfo-NHS-LC-biotin (Thermo Scientific) and immobilization of the allergen to streptavidin ImmunoCAP tests following the recommendations by the manufacturer prior analysis. Samples with specific IgE values above the higher limit of quantitation of the assay (> 100 kU_A/L) were diluted 1 : 100 in sample diluent and re-analysed. Results below the lower limit of quantitation (< 0.10 kU_A/L) were assigned a value of 0.09 kU_A/L in the statistical calculations.

Statistical analysis

Statistical analyses were performed using the SPSS Statistics software ver. 17.0 (IBM, Armonk, NY, USA) and PRISM ver. 5.04 (GraphPad Software, La Jolla, CA, USA). The Mann–Whitney *U*-test and Fisher's exact test (both 2-tailed) were used to compare the continuous and categorical data between groups, respectively. Wilcoxon matched-pairs signed-rank test was used to compare continuous data between analysis parameters. Spearman rank correlation test was used in the analysis of associations between specific IgE concentrations. Diagnostic performance of tests was assessed using the area under curve (AUC) from receiver operating characteristic (ROC) analysis.

Results

Characterization of patients

Ninety-two sesame-sensitized children were divided into two groups according to their clinical reactivity to sesame seed based on challenge outcome and/or clinical history (Table 1). The symptomatic group consisted of subjects with a positive challenge ($n = 29$) and subjects with convincing history of a recent hypersensitivity reaction to sesame ($n = 7$) (Supporting Information Table S1). The latter were not challenged due to high risk of strong reactions. The asymptomatic group consisted of subjects with a negative challenge ($n = 39$) and subjects who, after the anamnesis, were considered as sesame tolerant which made a challenge unnecessary ($n = 17$).

The cumulative dose of ground sesame seeds to which those reacting in challenge varied from 0.1 to 18 g (median dose: 3 g). The children with a negative challenge did not exhibit any objective symptoms after ingesting a median dose of 10 g of sesame seeds. Skin symptoms were the most commonly observed symptoms during challenge (83%) followed by gastrointestinal symptoms (69%), respiratory symptoms (66%), cardiovascular symptom (4%), and other symptoms (17%). Symptom grade 2, according to Japanese Pediatric Guidelines for Food Allergy [21], in two or more organs was observed in 16 subjects (55%) among those reacting in food challenge. Symptom grade 3 or 4 in

respiratory and digestive organs, defined as anaphylaxis, was observed in 9 (31%) and 3 (10%) subjects, respectively.

Diagnostic performance of specific IgE testing with native sesame allergen components

Native 2S albumin, 7S globulin, and 11S globulin were prepared from defatted sesame seeds (designated as nSes i 2S, nSes i 7S, and nSes i 11S) and confirmed by SDS-PAGE (Fig. 1) and mass spectrometry (data not shown). Specific IgE to the protein preparations and sesame seed extract were analysed in sera from the 92 children using fluorescent ELISA (Table 2). Nearly, all patients in the study were sensitized to nSes i 7S and nSes i 11S irrespectively of their allergic status. However, the frequency of sensitization to nSes i 2S was significantly higher in the symptomatic group (91.7%) than in the asymptomatic group (42.9%). The serum levels of IgE antibodies to sesame extract and the three storage protein preparations were significantly higher in the symptomatic group than the asymptomatic group. ROC analysis showed that specific IgE to nSes i 2S had the largest AUC (0.868), followed by nSes i 7S (0.819), sesame seed extract (0.761), and nSes i 11S (0.643) (Table 3, Fig. 2). Mass spectrometry analysis of nSes i 2S revealed that the two known allergenic variants of 2S albumin in sesame seed named Ses i 1 and Ses i 2 were present in the preparation (data not shown). However, as indicated by 2D PAGE, Ses i 1 seems to dominate in the nSes i 2S preparation.

Diagnostic performance of specific IgE testing with rSes i 1 and rSes i 2

The rSes i 1 and rSes i 2 were produced using an *E. coli* expression system and purified as described. Their purity and identity were confirmed by SDS-PAGE (Fig. 1) and mass spectrometry (data not shown).

The frequency of sensitization to rSes i 1 and rSes i 2, as measured by ELISA, was significantly higher among the symptomatic children than the asymptomatic children, and the levels of specific IgE to the two recombinant allergens were significantly higher in the symptomatic group (Table 2). There was a correlation between the specific IgE levels to nSes i 2S and rSes i 1 ($r_s = 0.959$, $P < 0.001$) as well as rSes i 2 ($r_s = 0.774$, $P < 0.001$). Significantly, higher specific IgE levels to rSes i 1 than to rSes i 2 was observed in the symptomatic group ($P < 0.0001$). ROC analysis showed an AUC for specific IgE to rSes i 1 and rSes i 2 of 0.874 and 0.842, respectively (Table 3, Fig. 2). Any pairwise combination of the allergen preparations and/or recombinant components did not improve the diagnostic performance compared to using only rSes i 1

Table 1. Demographic, serologic, and clinical characterization of study subject

Patient characteristics	Symptomatic	Asymptomatic	<i>P</i> value
Number of patients	36	56	n/a
Sex, no of boys (%)	23 (63.9)	38 (67.9)	0.822
Number of diagnosis by OFC (%)	29 (80.6)	39 (69.6)	0.332
Total OFC dose, median with range (g)	3.0 (0.1–18.0)	10.0 (3.0–10.0)	n/a
Age, median with range (years)	5.9 (1.6–12.2)	4.7 (1.0–11.7)	0.179
Total IgE, median with range (kU/L)	914 (21–7374)	978 (62–11 409)	0.965
Sesame-specific IgE, median with range (kU _A /L)	22.0 (2.9–1883.2)	9.0 (0.8–1008.4)	0.001
Allergic complications			
Atopic dermatitis, no (%)	25 (69.4)	36 (64.3)	0.657
Bronchial asthma, no (%)	11 (30.6)	15 (26.8)	0.813
Other food allergies, no (%)	24 (66.7)	38 (67.9)	1.000

OFC, oral food challenge.

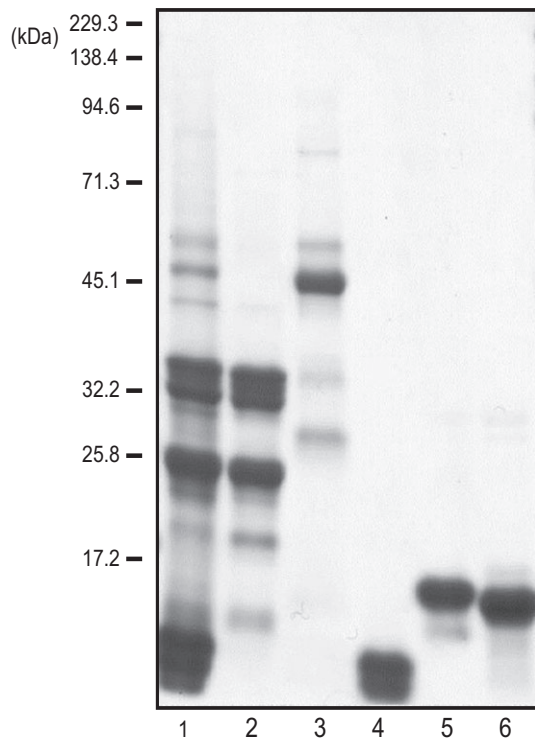


Fig. 1. SDS-PAGE analysis of purified and recombinantly expressed sesame allergens in the study. Lane 1, extract; lane 2, nSes i 11S; lane 3, nSes i 7S; lane 4, nSes i 2S; lane 5, rSes i 1; lane 6, rSes i 2.

Table 2. Frequency of sensitization (> 0.35 kU_E/L) and specific IgE levels (median with range) to sesame extract and allergen components analysed using fluorescent ELISA

IgE specificity	Symptomatic (<i>n</i> = 36)	Asymptomatic (<i>n</i> = 56)	<i>P</i> value
Sesame extract, no (%)	36 (100)	55 (98.2)	> 0.99
Concentration, kU _E /L	29.7 (8.9–179.6)	10.1 (0.2–75.2)	< 0.0001
nSes i 2S, no (%)	33 (91.7)	24 (42.9)	< 0.0001
Concentration, kU _E /L	26.0 (0.1–157.7)	0.2 (0.1–45.7)	< 0.0001
nSes i 7S, no (%)	36 (100)	52 (92.9)	0.15
Concentration, kU _E /L	24.5 (0.7–109.1)	4.2 (0.2–43.9)	< 0.0001
nSes i 11S, no (%)	36 (100)	54 (96.4)	0.52
Concentration, kU _E /L	11.7 (1.4–111.1)	6.9 (0.1–60.5)	0.021
rSes i 1, no (%)	33 (91.7)	18 (32.1)	< 0.0001
Concentration, kU _E /L	18.0 (0.1–143.5)	0.1 (0.04–33.2)	< 0.0001
rSes i 2, no (%)	24 (66.7)	9 (16.1)	< 0.0001
Concentration, kU _E /L	0.7 (0.1–74.7)	0.1 (0.04–31.1)	< 0.0001

kU_E/L, ELISA-based kilo units per litre.

(Table S2). None of the food-allergic or nonatopic control subjects had specific IgE antibodies to rSes i 1 [median concentration with range, < 0.1 kU_E/L (< 0.1 –0.12)] and rSes i 2 [< 0.1 kU_E/L (< 0.1 –0.28)].

The clinical usefulness of measuring IgE antibodies to rSes i 1 was confirmed using the ImmunoCAP technology. The rSes i 1 was biotinylated and immobilized to

streptavidin ImmunoCAP and compared with the commercially available sesame ImmunoCAP test. The specific IgE levels to rSes i 1 were significantly higher in the symptomatic group [median concentration with range: 20.2 kU_A/L (0.13–214.7)] compared to the asymptomatic group [0.3 kU_A/L (< 0.10 –38.1), $P < 0.0001$] when measured using ImmunoCAP (Fig. 3). A weak correlation between the specific IgE levels to sesame and rSes i 1 was observed using ImmunoCAP ($r_s = 0.640$, $P < 0.001$). ROC analysis showed that the rSes i 1 ImmunoCAP test had a larger AUC (0.891; 95% CI, 0.826–0.955) compared to the sesame ImmunoCAP test (0.697; 95% CI, 0.589–0.805) (Fig. 3). Similar results were obtained when excluding the 7 symptomatic and 17 asymptomatic patients diagnosed based on their clinical history, and without OFC, from the calculations (rSes i 1, AUC = 0.896; sesame ImmunoCAP, AUC = 0.714) (Supporting Information Figure S1).

Using the statistically optimal cut-off for specific IgE to rSes i 1 (3.96 kU_A/L), determined from the ROC analysis, a clinical sensitivity and specificity of 86.1% and 85.7% were obtained (Table 4). The sesame ImmunoCAP test had the same clinical sensitivity (83.3%) at the optimal cut-off (7.97 kU_A/L), but a much lower specificity (48.2%). Thus, the experimental rSes i 1 ImmunoCAP test was superior compared to the commercially available sesame ImmunoCAP test in this population of sesame-sensitized children.

Discussion

In this study, almost all sesame-allergic patients were sensitized to the seed storage proteins; 2S albumin, 7S globulin, and 11S globulin. Measurement of specific IgE to rSes i 1 (2S albumin) had the best diagnostic value among the sesame allergen components studied. An experimental rSes i 1 ImmunoCAP test was developed with a sensitivity and specificity of 86.1% and 85.7% at the optimal cut-off (3.96 kU_A/L) derived from ROC analysis. The extract-based sesame ImmunoCAP had an identical sensitivity (83.3%) but much lower specificity (48.2%) at the optimal cut-off (7.97 kU_A/L).

Our study is, to our knowledge, the largest sesame seed challenge study described with a patient cohort composed of 92 sesame-sensitized children of whom 36 were symptomatic and 56 asymptomatic. Twenty-nine of the sesame-allergic subjects reacted in OFC and seven had a convincing history of sesame allergy making OFC redundant. Forty-one per cent of the reacting subjects exhibit anaphylactic symptoms during sesame challenge. This is in line with the opinion that sesame allergy could be fatal. In a study by Dalal et al. [3], 30% of the children with sesame allergy presented symptoms of anaphylaxis. A correct diagnosis of sesame allergy is therefore crucial. Unfortunately, the

Table 3. Diagnostic performance of specific IgE measurement to sesame extract and allergen components calculated from ELISA data

	Sesame extract	nSes i 2S	nSes i 7S	nSes i 11S	rSes i 1	rSes i 2
AUC (95% CI)	0.761 (0.666–0.856)	0.868 (0.791–0.946)	0.819 (0.731–0.908)	0.643 (0.531–0.755)	0.874 (0.803–0.946)	0.842 (0.762–0.922)
Optimal cut-off (kU _E /L)	8.7	10.4	9.8	6.1	2.6	0.4
Sensitivity (%)	100.0	83.3	80.6	91.7	88.9	66.7
Specificity (%)	44.6	85.7	73.2	46.4	78.6	85.7
PPV (%)	53.7	79.0	65.9	52.4	72.7	74.2
NPV (%)	100.0	88.9	85.4	89.7	91.7	78.7

AUC, area under the curve; kU_E/L, ELISA-based kilo units per litre; PPV, positive predictive value; NPV, negative predictive value.

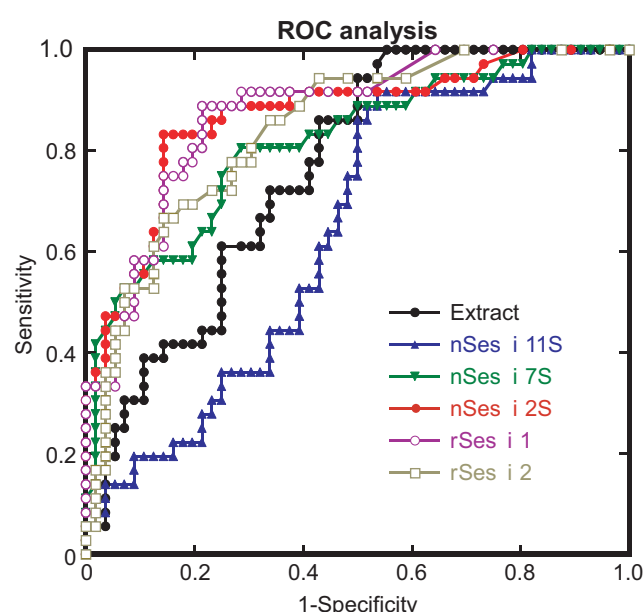


Fig. 2. ROC curves for specific IgE to sesame extract and allergen components based on ELISA analysis of sera from the symptomatic and asymptomatic groups.

commercially available *in vitro* tests for sesame-specific IgE antibodies, and SPTs with crude sesame extract, generally have low clinical specificity leading to a risk for overdiagnosis and unnecessary food restrictions and worries for the affected families [6–9].

We verified findings from others that specific IgE to sesame extract is a poor predictor of sesame allergy with an AUC of 0.761 in the ELISA evaluation. More interesting is our finding that specific IgE to rSes i 1 had the best diagnostic value among the sesame components studied with an AUC of 0.874. Similar diagnostic performances were observed in ROC analyses of specific IgE data obtained using the experimental rSes i 1 ImmunoCAP which was superior (AUC = 0.891) compared to the commercially available extract-based sesame ImmunoCAP test (AUC = 0.697).

The 2S albumins of peanut, various tree nuts, caster bean, oriental mustard, and buckwheat are in addition to the 2S albumins in sesame seed recognized as important allergens [17]. Recently, soya bean 2S albumin was also reported to be an important allergen and useful as a diagnostic tool in molecular-based allergy diagnostics [18, 22]. The degree of sequence homology among 2S albumins ranges from 14% to 40% [17]. The 2S albumins Ses i 1 and Ses i 2 have a similarity and identity of about 50% and 35%, respectively [13], and both have conserved cysteine residues related to disulphide bonds.

A number of studies have presented IgE reactivity data concerning sesame seed storage proteins based on immunoblotting results. Beyer *et al.* [13, 15] have documented that the majority of their 20–24 sesame-allergic patients exhibit obvious IgE binding to 7S and 11S globulins (around 75% and 67%, respectively). Ses i 2 was only recognized by 30% [13]. In a study by Pastorello *et al.* [12] including 10 Italian sesame-allergic patients, all patient sera were positive for IgE antibodies to 2S albumin. In contrast, Asero and co-workers reported that only one of their 10 Italian sesame-allergic patients had IgE to 2S albumin [23].

Identification of seed proteins corresponding to bands or spots on IgE immunoblots might be complicated, as several variants of seed storage proteins exist in seeds, and various allergens and fragments thereof could appear at similar positions in the immunoblot [13, 24]. Our study using purified native storage proteins and recombinant 2S albumins clearly indicates that the seed storage proteins are major allergens in sesame allergy. Sensitization to Ses i 1 and Ses i 2 was observed in 33 (91.7%) and 24 (66.7%) of the 36 symptomatic patients, respectively. The specific IgE levels, as analysed by ELISA, were higher to Ses i 1 than to Ses i 2. This all suggested that rSes i 1 was more allergenic than rSes i 2. When we analysed the nSes i 2S preparation by 2D PAGE, we found that Ses i 1 was the major protein. A

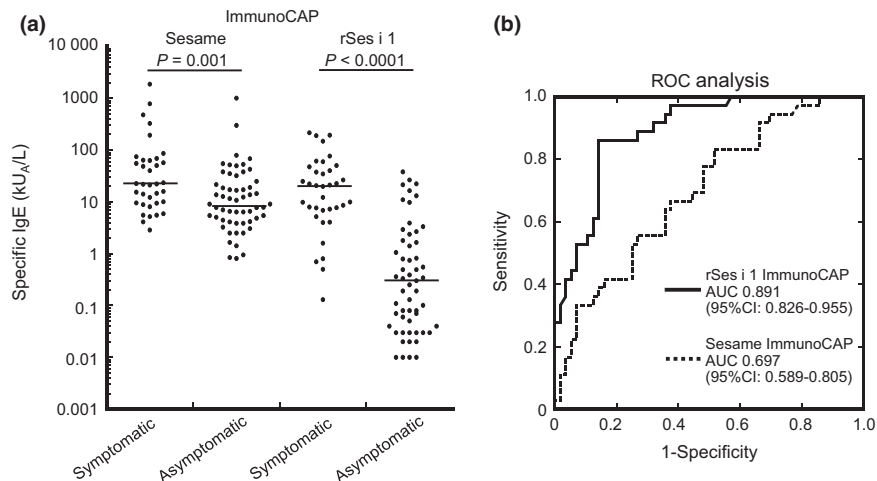


Fig. 3. Comparison between the experimental rSes i 1 ImmunoCAP test and the commercially available sesame ImmunoCAP test. (a) Specific IgE levels in the symptomatic and asymptomatic groups. Median values (solid lines) are indicated. (b) ROC curves for specific IgE to sesame extract and rSes i 1 based on ImmunoCAP analysis of sera from the symptomatic and asymptomatic groups.

Table 4. Diagnostic performance of specific IgE measurement to sesame extract and rSes i 1 calculated from ImmunoCAP data

Cut-off value (kU _A /L)	Sesame ImmunoCAP*		rSes i 1 ImmunoCAP†	
	0.35	7.97‡	0.35	3.96‡
Sensitivity (%)	100.0	83.3	97.2	86.1
Specificity (%)	0.0	48.2	55.4	85.7
PPV (%)	39.1	50.8	58.3	79.5
NPV (%)	100.0	81.8	96.9	90.6

PPV, positive predictive value; NPV, negative predictive value.

*Commercially available test.

†Experimental test where biotinylated rSes i 1 has been coupled to streptavidin ImmunoCAP.

‡Optimal cut-off calculated from ROC analysis.

higher amount of Ses i 1 than Ses i 2 in sesame might be the reason for the higher allergenicity of the former allergen. The high performance of rSes i 1-specific IgE measurement in the diagnosis of sesame allergy was confirmed using the ImmunoCAP technology after the development of an experimental rSes i 1 ImmunoCAP test based on biotinylated allergen and the streptavidin ImmunoCAP application which has been described elsewhere [25].

A great majority of the asymptomatic patients in our study had IgE antibodies to nSes i 7S and/or nSes i 11S. One explanation could be that these children in most cases were sensitized to peanut (data not shown). Peanut seeds contain both 7S globulin (Ara h 1) and 11S globulin (Ara h 3) common to sesame seed, and the homology of these proteins between peanut and sesame is high [13, 15]. Ses i 3 (7S globulin in sesame seed) exhibits 36% overall homology with Ara h 1, and there is up to 80% homology for the IgE-binding epitopes

[13]. IgE antibodies from peanut allergic patients bind to multiple epitopes of both Ara h 1 and Ara h 3 as shown by Lin et al. [26]. Therefore, IgE cross-reactivity between 7S and 11S globulins from sesame and peanut might be common in peanut-sensitized individuals, similar as between peanut and soya bean [27, 28]. This could affect the diagnostic values of these allergen components in the diagnosis of sesame allergy and could explain the high degree of asymptomatic sesame sensitization. On the other hand, peanut 2S albumin (Ara h 2) has about 27% of identity and 44% of similarity with Ses i 1, and sequence alignment shows that potentially important IgE-binding epitopes described for Ara h 2 have limited sequence identities with their counterparts in Ses i 1 (Supporting Information Figure S2) [29]. Clinicians should be aware of the risk for patients with peanut allergy to be falsely labelled as having sesame allergy if the diagnosis is solely based on SPT or specific IgE measurements based on crude sesame extract.

A potential limitation of our study is that open food challenges were used rather than double-blind placebo-controlled food challenges. However, only objective symptoms were used to define a positive OFC result, and the validity and sufficiency of using open challenges in young children has previously been independently confirmed [30]. Another limitation is that 24 of the 92 patients included were diagnosed based on anamnesis without OFC. However, when excluding them from the AUC calculations, no major differences were observed and the superiority of measuring Ses i 1-specific IgE was confirmed.

Furthermore, no comparison with skin prick testing with sesame extract was performed in this study. In a previous study conducted with American patients,

Permaul *et al.* reported an AUC for sesame-specific IgE and skin prick test wheal size of 0.56 and 0.67, respectively [6]. In a similar Australian study, skin prick testing with sesame extract showed a larger AUC (0.92) than that of sesame-specific IgE (0.76) [8]. These results suggest that skin prick testing has a higher diagnostic performance than measurement of sesame-specific IgE levels in the diagnosis of sesame allergy. However, skin prick tests are not routinely used in the diagnosis of food allergy in Japan. Therefore, improvement of specific IgE antibody measurements using allergen components is very important.

This study is the first report to show the diagnostic values of sesame allergen components, and we can confirm that the clinical specificity of specific IgE to crude sesame extract is low. Measurement of specific IgE to rSes i 1 using ImmunoCAP increased the clinical specificity in the diagnosis of sesame allergy in Japanese children compared to measurements using the commercially available sesame ImmunoCAP test. We conclude that measurement of Ses i 1-specific IgE antibodies will significantly improve the diagnosis of sesame allergy and might contribute to a reduction in the number of OFCs needed for patients with suspected sesame allergy.

Acknowledgements

This study was funded by a Health and Labor Science Research Grant for Research on Allergic Disease and Immunology from the Ministry of Health, Labour and

Welfare of Japan (to N.M., K.I., and M.E.), and by The Salt Science Research Foundation and JSPS KAKENHI (to N.M.). We thank Ms. Machiko Sawada (Kyoto University) for technical assistance. We thank Drs. Sigrid Sjölander and Peter Brostedt (Thermo Fisher Scientific) for critical reading of the manuscript. We are also grateful to Ms. Tsugumi Shiokawa and Dr. Hiroko Tada (Okayama University) for protein analysis and to Dr. Naoki Ogata (National Agriculture and Food Research Organization) for sharing sesame seeds.

Author contributions

Nobuyuki Maruyama, Tomoko Nakagawa, Komei Ito, Sakura Sato, and Motohiro Ebisawa were involved in study concept and design, analysis, and preparation of the article and gave final approval of the version to be published. Cerrone Cabanos was involved in preparation of components. Magnus P. Borres, Robert Movérare, and Akira Tanaka were involved in interpretation of the data, critically revision of the article, and final approval of the version to be published.

Conflicts of interest

Magnus P. Borres and Robert Movérare are employed at Thermo Fisher Scientific, Uppsala, Sweden. Akira Tanaka is employed at Thermo Fisher Scientific, Tokyo, Japan. All other authors have declared they have no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. ROC analysis.

Figure S2. Alignment of sesame and peanut 2S albumins. Potentially important IgE epitopes of Ara h 2 are shown in red letters (29).

Table S1. Characteristics of subjects with convincing positive history.

Table S2. Diagnostic values of pairwise combinations of sesame components calculated from specific IgE ELISA data.