

Rice Allergy Demonstrated by Double-Blind Placebo-Controlled Food Challenge in Peach-Allergic Patients Is Related to Lipid Transfer Protein Reactivity

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Key Words

Rice allergy · Lipid transfer protein · Pru p 3 · Peach allergy · Double-blind placebo-controlled food challenge · Food challenge · Anaphylaxis

Abstract

Background: The risk factors for sensitisation to rice and the involved allergens are still partially unknown. In this study we evaluated the clinically relevant aspects of rice allergy in DBPCF-positive patients, the major rice allergens, the severity of peach- and rice-induced symptoms in respect to Pru p 3 sensitisation and the role of anti-rPru p 3 IgE levels as a risk factor for rice allergy. **Methods:** In 148 peach-allergic subjects, patients with allergic reactions to rice and rice-positive serum IgE were selected. Symptoms were verified by double-blind placebo-controlled food challenges (DBPCFCs), performed at a maximum dosage of 25 g. Rice allergens, identified by IgE immunoblotting, were characterised by N-terminal amino acid sequencing. The relationship between anti-rPru p 3, 1 and 4 IgE levels and rice symptoms were statistically analysed. **Results:** Eight out of 10 recruited rice-al-

lergic patients had positive DBPCFCs, while 2 patients were not challenged due to their previously documented severe reactions. All patients with rice-induced symptoms were Pru p 3 positive and presented with higher anti-rPru p 3 levels than the rice-sensitised but tolerant patients. A 9-kDa lipid transfer protein, which was highly homologous to Pru p 3, was identified as the major rice allergen and elicited a positive response in all of the patients. Five patients reacted to a putative 15- to 17-kDa rice allergenic protein, and 3 patients reacted to an [alpha]-amylase/subtilisin inhibitor that was approximately 20 kDa. **Conclusion:** Rarely, allergic reactions to rice can arise in patients with peach allergies who are sensitised to Pru p 3, particularly in patients with high anti-rPru p 3 IgE levels.

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Introduction

Rice (*Oryza sativa*) is a cereal obtained from the Liliopsida plant and belongs to the Poales order and the Gramineae family. Although approximately 80% of the

world's rice is produced in Asia, rice is grown and consumed all over the world.

In 2009, rice produced in Europe represented only 0.4% of worldwide production. Italy is the most prominent rice-producing country in Europe with approximately 1.5 million tons or 49.5% of European production. Italy is followed in rice production by Spain and Greece with 29.2 and 6.7% of European production, respectively. Italy is not only the European leading rice producer but is also one of the major non-Asian producers of rice after Brazil and the US.

In Italy, rice farming is mostly concentrated in the north in the Piedmont and Lombardy regions, where the climate is suitable for cultivation and where maximum rice consumption occurs. For these reasons, the clinical problems related to rice allergies are considered to be most relevant in northern Italy [1].

Rice is usually considered a safe food, but it can cause two immunologic syndromes: (1) the food protein-induced enterocolitis syndrome (FPIES) and (2) IgE-mediated food allergy. FPIES is a non-IgE-mediated hypersensitive disorder that can affect children younger than 12 months old very severely and cause vomiting, diarrhoea, shock and metabolic acidosis [2]. IgE-mediated allergies can affect children and adults, causing respiratory symptoms upon inhalation of raw rice dust or rice vapour when rice is boiled, as well as gastrointestinal and other systemic symptoms upon ingestion [3–8]. However, there are few reports in the literature of such reactions. Cases of exacerbation of atopic dermatitis caused by rice have been reported in Japan [9]. In India, Kumar et al. [10] described 165 patients with a history of respiratory allergies to rice. Of these patients, 20 with positive skin-prick tests were selected for open food challenge, and 10 of these patients with immediate positive reactions underwent a double-blind placebo-controlled food challenge (DBPCFC) that was positive in 6 cases. Wuthrich et al. [11] described a case of rhinitis and asthma after the ingestion of cooked rice, and Orhan [12] described a patient with acute gastrointestinal symptoms after eating cooked rice as well as urticaria and angioedema while sorting rice.

In rice grain, approximately 80% of the total protein content corresponds to acid-soluble and/or alkaline-soluble proteins (oryzenins). Of the remaining proteins, 15% represent salt-soluble proteins (globulins and albumins), and 5% include alcohol-soluble proteins (prolamins). The albumin proteins with molecular weights of 14–16 kDa belonging to the cereal α -amylase/trypsin inhibitor family have been found to be the major allergens in rice grain extracts [13]. Other known rice allergens in rice grains

include a novel type of plant glyoxalase I of 33 kDa and an α -globulin of 26–28 kDa [14–16]. Moreover, two studies identified a lipid transfer protein (LTP) as a potential allergen involved in symptoms induced by ingestion of cooked rice or inhalation of raw rice in patients who are allergic to Prunoideae fruits [7, 17]. Trcka et al. [18] identified a 56-KDa protein as a relevant allergen in patients with rice-induced anaphylaxis.

Recently, we have observed a small number of patients reporting rice-induced symptoms in a study population of subjects allergic to peaches, many of whom reported severe reactions to peaches caused by sensitisation to peach LTP [19]. Based on these reports, we investigated allergic reactions to rice in these patients to evaluate the clinical reactivity to rice using DBPCFC, to analyse major allergens involved by IgE immunoblotting and to identify allergens responsible for the association between rice- and peach-induced symptoms.

Methods

Study Population

A population of 148 patients recently selected for a study of the clinical role of sensitisation to the major peach allergens was screened with a standard questionnaire to determine each patient's likelihood of having an allergic reaction to rice [20]. All the peach-allergic patients were also screened for rice-specific IgE antibodies. In the patients reporting rice-induced symptoms, the diagnosis of food allergy to rice was confirmed by a DBPCFC with rice flour, except for patients with histories of rice-induced anaphylaxis or a severe systemic reaction who should not be challenged.

Pollen sensitisation profiles were studied in an earlier paper on peach allergies [19]. Serum samples from all patients and one negative control were used for IgE immunoblotting analyses to identify the major allergens in rice. The study protocol was approved by the Human Ethics Committee of the Niguarda Hospital, and all patients gave their written consent.

Rice DBPCFC Challenge Testing

All patients were on a rice-free diet and gave their informed consent. Preparation of the meal and the challenge procedure were performed as previously described [21].

Active and placebo test meals were administered on separate days, and doses were administered at 20-min intervals. A minimum starting dose of 100 mg of active meal was administered, after which the following doses were given (the cumulative dose is shown in parentheses): 500 mg (600 mg), 1 g (1.6 g), 1.5 g (3.1), 3.0 g (6.1), 6.0 g (12.1) and 12.9 g (25). We considered the final cumulative dose that solicited symptoms as the cumulative provocation dose. The maximum cumulative dose administered was 25 g of raw rice flour. Dosing continued until obvious allergic symptoms appeared. Subjective symptoms were confirmed with 3 subsequent doses of the test meal and the negative placebo meal before the test was considered to be positive [22].

Allergen-Specific IgE

Specific IgE levels to rice, peach and relative recombinant allergens (rPru p 1, 3 and 4) were determined using the ImmunoCAP system (Thermo Fisher Scientific Inc., Uppsala, Sweden) according to the manufacturer's instructions. A positive result was considered to be between 0.101–100 KUA/l.

Raw Rice Extract

The extract was prepared according to the methods of Sandiford et al. [23] and then lyophilised. The protein content of the rice extract was estimated using the Lowry [24] method.

Electrophoresis SDS-PAGE and Immunoblotting

Raw rice extract (0.26 mg/cm) was separated by SDS-PAGE (7.5–20% separation gradient gel) under reducing conditions [25] and stained with Coomassie brilliant blue R-250. After electrophoresis, the gel was electroblotted to a nitrocellulose membrane as previously described [26]. The IgE-binding components were determined using I²⁵-labeled anti-IgE antibodies (Radim, Rome, Italy). Visualisation of bound antibodies was completed using auto-radiographic film (Hyperfilm Hamersham) at –70°C for 4–7 days.

Amino Acid Sequencing

Bands corresponding to the IgE-binding rice proteins were excised from the 7.5–20% SDS-PAGE gradient gel, passively eluted by a slightly modified Zieske technique and microsequenced on a Procise 492 protein sequencer (Applied Biosystems, Foster City, Calif., USA) as previously described [27]. The amino acid sequences were compared to the UniProt KB database using BLASTP software (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). To identify the low molecular weight rice allergens, the peak containing a partial purification of the lyophilised rice extract as analysed by HPLC was separated by 10% SDS-PAGE, and the corresponding bands were microsequenced as described above.

Purification by HPLC

The rice proteins of approximately 9 kDa were purified using the following chromatographic steps (AKTA Purifier, Amersham Biosciences, Uppsala, Sweden). First, rice proteins were separated by a cation-exchange Resource-S column (volume = 1 ml) in 30 mM sodium citrate buffer (pH 6) with a linear gradient of 0–1 M NaCl. The concentrated fractions were then separated on a gel filtration Superdex 75 HR 10/30 column (Amersham Biosciences) in a 30 mM sodium citrate (pH 6), 150 mM NaCl buffer. SDS-PAGE and IgE/immunoblotting analyses for these three rice proteins were performed with pooled sera using pre-cast NuPAGE Novex Bis-Tris 10% Mini Gels (Invitrogen, Carlsbad, Calif., USA). The 9 kDa protein from peach extract was isolated and purified according to Pastorello et al. [28].

Immunoblotting Inhibition of the HPLC-Purified Rice Fraction

Chromatographically purified rice fractions of low molecular weight underwent immunoblotting inhibition [29]. Briefly, 500 µl of pooled serum from patients P4 and P6 were pre-incubated with 500 µl of purified peach LTP containing 4 µg protein. Then, the analysis was performed as in the immunoblotting protocol described above.

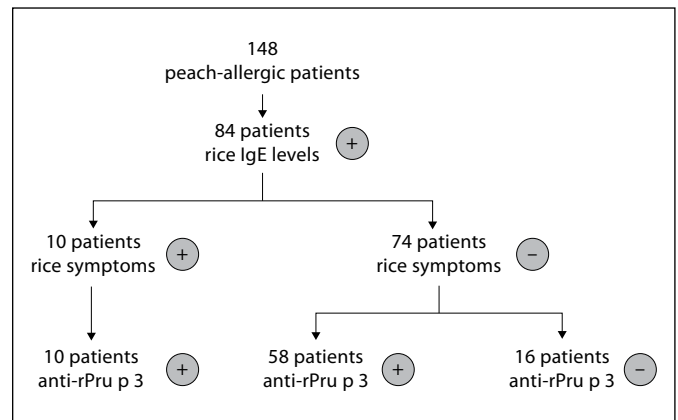


Fig. 1. Distribution of the anti-rice IgE-positive patients according to presence or absence of anti-rPru p 3 IgE antibodies and rice-induced symptoms.

Statistical Analysis

Variables were first validated and explored using the standard descriptive methods; comparisons of continuous variables were performed with Mann-Whitney U test, and categorical variables were analysed with Fisher's exact test. We evaluated the differences in anti-rPru p 3 IgE levels among rice-allergic patients and all of the investigated patients using the Mann-Whitney U test. Furthermore, among the rPru p 3-positive rice-sensitised patients, we used the Mann-Whitney U test to compare the anti-rPru p 3 IgE levels between patients with rice-induced symptoms and patients sensitised but clinically tolerant to rice. Then, we evaluated the existence of an association between positivity to rPru p 3 and the presence of symptoms to rice using the Mann-Whitney U test. Statistical significance was assumed at $p < 0.05$ and the data analysis was performed using Stata/SE 11.1 (Stata Corp Inc.).

Results

Patients

In our study population, 84 of 148 (56.8%) patients with peach allergies were classified as rice-sensitised based on IgE testing (rice-specific IgE level >0.101 kUA/l). As shown in figure 1, among these 84 patients, 68 were rPru p 3 positive, while 16 were negative. In patients presenting positive specific IgE to rPru p 3, only 10 reported rice symptoms and 58 of them tolerated rice consumption. In the rPru p 3-negative group, no patient reported clinical symptoms induced by rice. The clinical reactivity was confirmed by DBPCFC in 8 rice-allergic patients, while the other 2 patients were not challenged because of their documented severe rice-induced anaphylaxis. Among the 148 peach-allergic patients, we also found 3 patients who reported clinical symptoms upon rice inha-

Table 1. Patient data, rice allergy history and test results

Patient	Sex/ age	Rice history	CAP, kUA/l			Rice DBPCFC				Pollen sensitization	Other symptomatic foods
			rice	peach	rPru p 3	AC	PL	SYM	CPD, g		
1	F/38	AS	0.70	5.25	5.30	+	–	C	3.1	W	apricot, fennel, hazelnut, maize, peanut, walnut, tomato, soy
2	F/33	E, GI	3.59	17.30	17.10	+	–	E	25	B, G, M, R, W	peanut
3	M/32	ANA	37.80	27.1	25.3	Nt	Nt	–	–	G, M, R, W	apricot, cherry, pear, strawberry, garlic, onion
4	F/32	AS, GI	5.03	31.10	27.5	+	–	V, GI	25	G, M, R, W	cherry, maize, peanut, walnut, hazelnut, kiwi, celery
5	F/38	ANA	20.10	91.50	100	Nt	Nt	–	–	B, M, R, G, W	apricot, cherry, plum, pear, grape, peanut, walnut, hazelnut, tomato, fennel, lettuce
6	F/40	P, V, E	1.48	2.94	4.19	+	–	V, E	25	G, M, R	peanut, apple, cherry, plum, kiwi, tomato
7	M/48	AS	1.07	7.47	8.61	+	–	P, C	25	B, G, M, R, W	apricot, maize, plum, cherry, peanut, tomato
8	F/56	U, AE, P, V	8.96	37.40	10.3	+	–	E, U, V	12.1	G, W	cherry, maize, plum, apricot
9	F/62	V, P	1.46	8.92	11.6	+	–	GI	25	G	maize, walnut, peanut
10	F/58	OAS	0.15	5.12	11.2	+	–	OAS	25	G, R	cherry, fig, kiwi

AS = Asthma; E = erythema; GI = gastrointestinal; ANA = anaphylaxis; U = urticaria; AE = angioedema; P = pruritus; V = nausea/vomit; OAS = oral allergy syndrome; C = cough; AC = active meal; PL = placebo meal; SYM = challenge symptoms; CPD = cumulative provocation dose, Nt = not tested; B = birch; G = grass; M = mugwort; R = ragweed; W = wall pellitory.

lation but tolerated rice ingestion. Unfortunately, they did not present serum-specific IgE to rice, so they were not considered for the in vitro study.

Table 1 shows the demographic data, clinical histories and specific IgE levels to rice, rPru p 3 and peach, the results of the rice DBPCFCs, including the cumulative provocation dose (from 3.1 to 25 g), and the elicited symptoms following the challenge for each patient and sensitisation to pollen allergens and other foods. All 10 patients had positive specific IgE antibodies for rice, rPru p 3 and peach. Interestingly, 5 patients with rice allergies (P1, P4, P7, P8 and P9) also presented with adverse allergic reactions to maize, which was confirmed by DBPCFC (unpublished data).

All patients who underwent rice DBPCFC demonstrated reactions to the active meal and no reactions to the placebo meal. The symptoms elicited during the challenge corresponded to those reported in the clinical histories for P6, P8, P9 and P10, while patients P1, P2, P4 and

P7 had milder symptoms involving the same organ as reported in their respective clinical histories.

Four of the 8 patients challenged manifested objective symptoms (P2, P4, P6 and P8) during the challenge or immediately after test completion. Two patients (P1 and P7) with a history of asthma following rice ingestion complained of cough, but did not require albuterol because the symptoms spontaneously subsided 5–10 min after onset. Their spirometry parameters were not significantly different compared to baseline. Given that the symptoms were only subjective, we repeated the challenge three times and obtained the same results; thus, we considered the challenge results to be positive. Only patient P10 experienced oral allergy symptoms during the challenge, and P9 complained of nausea and abdominal pain.

SDS-PAGE and IgE Immunoblotting

For the IgE immunoblotting analysis, we enrolled all of the patients with a positive rice DBPCFC and the 2 pa-

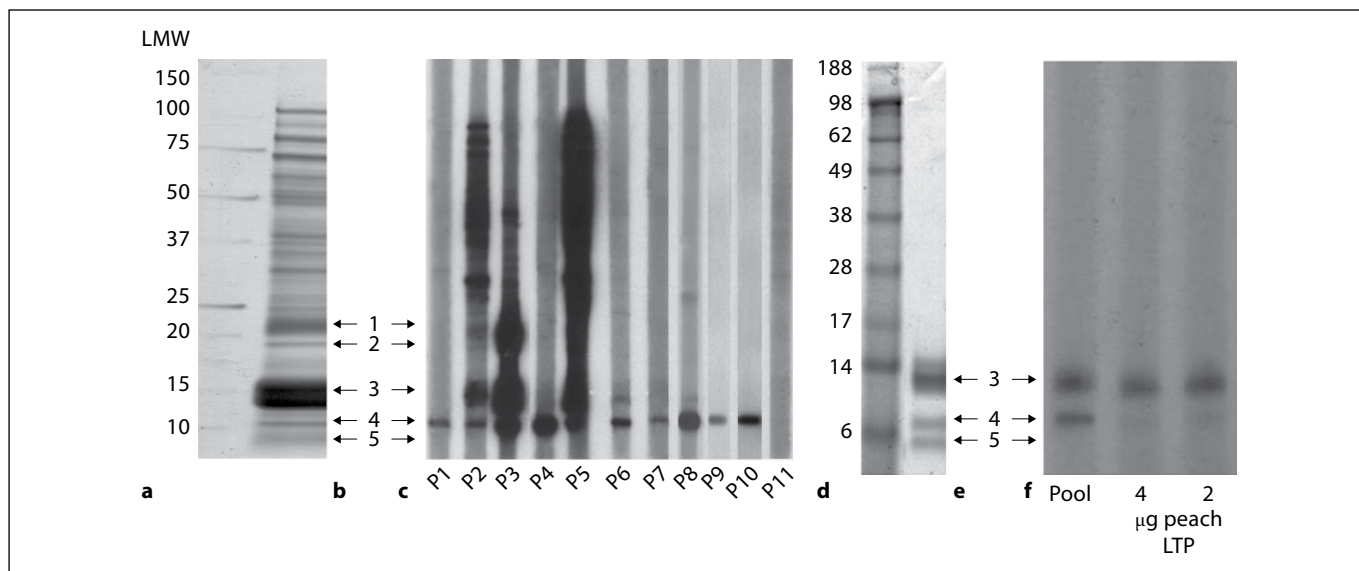


Fig. 2. Whole rice extract, SDS-PAGE (a); IgE-binding proteins excised from the gel and N-terminal amino acid sequenced (b); IgE immunoblotting results using individual serum (patients P1–10 and negative control, P11; c); partially purified rice extract,

SDSPAGE (d); identified proteins (e), and IgE immunoblotting inhibition (f) using purified peach LTP at different concentrations to inhibit the IgE activity of pooled serum of patients P4 and P6.

Table 2. Rice protein identification by N-terminal sequencing

Band	Protein name	UniProtKB entry	MW nominal/deduced from SDS-page (kDa)	N-terminal sequences ¹
1 and 2	α -amylase/subtilisin inhibitor	P29421	21,17/19	²³ APPPVYDTEGHE
3	α -amylase inhibitor/putative allergenic protein	Q8H4M4	17,27/14	²⁸ XHRGQVVYTPYQ
4	LTP	Q01QK9	11,345/11	²⁶ ITCGQVNSAV
5	LTP 2	Q10ST8	9,535/7	²⁸ AGCNAGQLTV

¹ The number indicates the position of the first sequenced amino acid as provided by the respective database sequence.

tients with rice-induced anaphylaxis. SDS-PAGE of raw rice extract showed several bands with apparent molecular weights ranging from 10 to 100 kDa (fig. 2a). Raw rice IgE immunoblotting of each single patient's serum is shown in figure 2c.

All patients (100%) showed IgE antibodies specific to a protein component of approximately 9 kDa. Other IgE-binding proteins were recognised at 14–16 kDa in 5 patients (50%) and in another 3 patients were recognized in 3 bands at 20 kDa, 33 kDa and approximately 50 kDa (37.5%).

Biochemical Characterisation of Raw Rice Allergens

Protein Identification. The IgE-reactive protein bands are shown in figure 2b and the identified rice proteins are listed in table 2. Band 1 contained a rice α -amylase/subtilisin inhibitor (P29421), whereas a rice α -amylase inhibitor (Q8H4M4), already known as a putative allergenic protein, was present in band 3. Band 4 contained a protein corresponding to an LTP (Q01QK9), and a different LTP (LTP 2, Q10ST8) was present in band 5. The protein contained in band 2 was not identified.

IgE-Immunoblotting and Immunoblotting Inhibition of Partially Purified Rice Allergens. The cationic-exchange profile of the raw rice extract showed a well-resolved gradient peak in addition to the exclusion peak. This fraction was collected, concentrated and loaded onto a gel-filtration column. As in the cation-exchange chromatography, the gel-filtration chromatographic profile also presented good resolution, and the peak containing proteins of low molecular weight was concentrated and analysed by SDS-PAGE IgE immunoblotting and immunoblotting inhibition. The electrophoretic profile of the peak of interest revealed three protein bands at approximately 6, 9 and 14 kDa (fig. 2a); only the proteins observed at 9 and 14 kDa were detected in a pooled serum sample from P4 and P6 (fig. 2c). Moreover, inhibition of IgE immunoblotting, performed by pre-incubating the pooled serum with different concentrations of purified peach LTP, showed a complete inhibition of the IgE binding of the 9 kDa protein component.

Relationship between Rice Allergy and Pru p 3 Sensitisation. A total of 68 of the 84 rice-sensitised patients were also sensitised to Pru p 3 whereas 16 were not. All of the patients presenting clinical symptoms to rice had positive anti-rPru p 3 IgE levels. Moreover, anti-rPru p 3 IgE levels in these 10 patients with rice allergies were significantly higher than in sensitised but rice-tolerant patients (Mann-Whitney test: $p = 0.0052$). Anti-rPru p 3 IgE levels were also significantly higher in patients with rice-induced symptoms than in other Pru p 3-sensitised patients (Mann-Whitney test: $p = 0.0354$). However, we did not find a significant correlation between positive IgE levels to anti-rPru p 3 and clinically evident rice allergy by Fisher's exact test.

Discussion

In this study, we described the cases of 10 patients with rice allergies who were recruited from a cohort of 148 patients with peach allergies that had previously been assembled to study the relationship between the severity of allergic reactions to peaches and the pattern of IgE reactivity to peach allergens [19]. We confirmed rice allergies in patients based on DBPCFC and identified rice allergens using the sera of allergic patients. In the peach study, patients were divided into two groups according to the clinical severity of their reaction to peaches (mild and severe). Interestingly, all of the patients with rice allergies were members of the severe

peach allergy group [19, 30]. From our study, we can confirm that rice can elicit clinical reactions in patients with peach allergies.

The 10 cases of systemic reactions to rice were demonstrated either by DBPCFC or a documented history of rice-induced anaphylaxis. All 10 patients had positive specific IgE antibodies for rice, rPru p 3 and peaches. Only 8 of the 10 patients underwent rice DBPCFC because 2 patients had experienced anaphylactic reactions to rice and were thus not eligible for challenge [30].

All 8 patients who underwent rice DBPCFC reacted to the active meal and not to the placebo meal. The symptoms reported during the rice challenge were nearly all consistent with the clinical histories and were in keeping with typical IgE-mediated food allergies. In some cases symptoms were milder than those reported in the clinical histories, probably because the more gradual ingestion of rice during the challenge compared to consumption at home or challenge interruption after symptom manifestation at a low dose of rice, as observed in P1.

The majority of patients manifested objective symptoms during the challenge or immediately after test completion. The majority of patients reporting a history of acute asthma after rice ingestion complained only of cough during challenge without any modification of spirometry values. Two patients also reported histories of respiratory reactions to vapours produced by boiling rice. However, we did not attempt to confirm this by an inhalation challenge because this procedure has not been standardised. It is noteworthy that Enrique et al. [7] reported 3 subjects who experienced asthma following the inhalation of raw rice (rice thrown during a wedding) but tolerated ingested rice. In our cases, the patients complained of symptoms upon vapour inhalation and after ingestion. Thus, it is possible that the cough that developed after rice ingestion could be considered a respiratory reaction, similar in pathogenesis to that provoked by acid reflux due to incomplete gastric digestion. Unlike the symptoms of rice allergies reported by other investigators, the symptoms reported herein were confirmed by oral provocation, making these rice-induced symptoms evidence-based and not simply reports of clinical histories.

Considering the cumulative provocation doses, 1 patient (P1) reacted to low dose and another to an intermediate dose, while the majority completed the test and reacted to the maximum cumulative provocation dose. In this study, one of the challenge-positive patients (P8) reported symptoms that involved two or more organ systems, thus fulfilling the criteria for rice-induced anaphy-

laxis. However, this patient did not require emergency treatment with epinephrine. One of the patients challenged (P10) reported only oral allergy syndrome as a challenge symptom.

The fact that patients with severe food allergies can exhibit reactions to small amounts of hidden food has prompted many studies to evaluate clinical reactivity using a standardised challenge. In fact, the lowest provoking dose has been reported for some of the more commonly allergenic foods, such as wheat, peanut and soy. However, there are no reports in the literature that verify the lowest provoking dose for rice under standardised DBPCFC conditions. Our study shows that DBPCFC symptoms can be induced at a cumulative dose of 3.1 g of rice flour. Importantly, the provoking doses were observed in patients who did not present with a history of very severe reactions. Present guidelines discourage the use of food challenges in patients with severe food allergies, and therefore the threshold dose for an incriminated food in these exquisitely allergic patients cannot be directly identified [30].

A major finding of our study was the clinical association of rice allergy with peach hypersensitivity. Our result confirms the finding of Enrique et al. [7]; however, in that study, subjects with peach allergies developed respiratory symptoms after the inhalation of raw rice dust, whereas our patients developed symptoms upon ingestion of the rice meal and only in a few cases also upon exposure to cooked rice vapours. This relationship can be explained by the IgE reactivity pattern of our patients that, as in the Spanish study [7], was characterised by a positive reaction to a 9 kDa LTP1 protein (fig. 2b) that shares a 53% amino acid sequence homology with peach LTP. This homology may account for the high rate of in vivo cross-reactivity between peach and rice LTPs. However, the per cent homology is not sufficiently high to induce a high rate of rice hypersensitivity in LTP-positive patients with peach allergies, as we observed only 10 patients with clinical manifestations of rice allergies out of 104 peach-LTP-positive patients [30]. This finding could have two potential explanations. First, it is possible that a rice allergy only develops in patients with elevated levels of anti-Pru p 3 IgE, as suggested by the significant difference in anti-Pru p 3 IgE levels between Pru p 3-sensitised rice-symptomatic and non-symptomatic patients (Mann-Whitney test: $p = 0.00354$). Although our study does not provide irrefutable laboratory evidence for the clinical relevance of LTP in rice allergy, the fact that all of the patients were previously skin tested with purified peach LTP [19] and the fact that there is a high degree of

homology between rice and peach LTP, made skin-tests with purified rice LTP redundant. An alternative explanation for the low incidence of rice allergy in the study population could be that the simultaneous sensitisation to other allergens plays a role in inducing rice symptoms. For example, we observed that 5 out of 10 patients also reacted to a 16-kDa allergen (P2, P3, P5, P6 and P8) belonging to the α -amylase inhibitor family and 3 of these 5 patients (P2, P3 and P5) also reacted to a 20-kDa allergen identified as an α -amylase subtilisin inhibitor. The 16-kDa allergen belongs to a family of serine-type endopeptidase inhibitors closely related to the rice seed allergenic protein (RA) allergens, encompassing proteins with α -amylase inhibitor activities. These RA allergens were characterised approximately 20 years ago by Adachi et al. [31], and more recently, Satoh et al. [32] confirmed their allergenic relevance in subjects with rice allergies by means of two-dimensional SDS-PAGE. Using a proteomics approach, these two studies demonstrated that at least 3 allergenic proteins belong to the RA family, encoded by a multigene family consisting of at least 4 genes. The authors showed that these were the most important rice allergens in Asia. In fact, in these countries, rice is the only relevant allergenic source and α -amylase inhibitors are the major allergens recognised. Indeed, Ikezawa [33] obtained hypoallergenic rice by hydrolysis of the α -amylase inhibitor.

Although in our study the α -amylase inhibitor behaved as a major allergen, reactivity was not observed for all patients, and no patient manifested reactivity exclusively to this allergen. Therefore, the clinical role of α -amylase inhibitors in our patients still needs to be determined [14]. In our model, namely a highly selected population of peach-allergic patients, LTP appeared to be the most relevant allergen because all of the patients were LTP reactive and 5 out of 10 patients (P1, P4, P7, P9 and P10) reacted exclusively to this protein. These findings demonstrate the role of LTP in rice food allergies confirmed by DBPCFC or documented anaphylaxis. Lastly, the association of rice allergies and maize hypersensitivity deserves mention, as this correlation can also be ascribed to sensitisation to LTP in both rice and maize. It is worth noting that 5 of the 8 challenged rice-allergic patients (P1, P4, P7, P8 and P9) also had histories of allergic reactions to maize and were positive to maize DBPCFC, whereas wheat was well tolerated. Thus, it seems striking that 50% of the patients with rice allergies in this study were also allergic to maize and LTP sensitised. The relationship observed between peach, rice and maize LTP was not detected between peach and wheat

LTP, suggesting that there is higher cross-reactivity in the former group, as previously reported by Pastorello et al. [34].

All of these observations demonstrate that the clinical features of rice allergies are related to the structural homology of LTP, allowing rice to be classified as a food involved in an 'LTP syndrome'. Furthermore, the LTP 2 protein was also identified in rice, but it did not appear to be allergenic, which is analogous to the finding observed in other allergenic foods containing LTP 2 [35, 36]. In conclusion, this study clearly demonstrates that in the

Mediterranean region, rice can elicit clinical reactions in patients who are allergic to peaches, and very often this reactivity is due to a structural protein homology between rice and peach LTP. Furthermore, we found that rice allergy symptoms are closely related to anti-rPru p 3 IgE levels, as all of the subjects with challenge-proven rice allergies were sensitised to rPru p 3; the patients with the highest anti-rPru p 3 titres were all allergic to rice, whereas rice allergy was not present in all anti-rPru p 3-positive patients.

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