

Lipid transfer proteins: the most frequent sensitizer in Italian subjects with food-dependent exercise-induced anaphylaxis

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Summary

Background Specific food-dependent exercise-induced anaphylaxis (S-FDEIAn) is a distinct form of food allergy in which symptoms are elicited by exercise performed after ingesting food to which the patient has become sensitised. Non-specific FDEIAn (NS-FDEIAn) is a syndrome provoked by exercise performed after ingesting any food.

Objective We sought to identify the culprit allergenic molecules in patients with FDEIAn, combining 'classic' allergy testing with an allergenic molecule-based microarray approach for IgE detection.

Methods All subjects were evaluated who reported at least one episode of anaphylaxis in association with physical exercise performed within 4 h after a meal. We performed skin prick tests (SPT) with commercial food extracts, prick plus prick tests (P + P) with fresh foods (P + P), and serum specific IgE assays by means of both the ImmunoCAP (CAP) and the ISAC 89 microarray system (ISAC).

Results Among our 82 FDEIAn patients, the most frequent suspected foods were tomato, cereals, and peanut. SPT, P + P, and CAP displayed different degrees of sensitivity. Each test disclosed some positivities not discovered by others. Seventy-nine subjects were positive to at least one food (49 to more than 20), whereas three were negative. All suspected foods were positive to at least one of SPT, P + P, and CAP. When tested using the ISAC, 64 (78%) subjects were positive to Pru p 3 [peach lipid transfer protein (LTP)], 13 were positive to other food allergen molecules, and five displayed negative results to all food allergenic molecules. Overall, 79 patients probably had S-FDEIAn and the other 3 NS-FDEIAn.

Conclusions Multiple food hypersensitivity represents a clinical hallmark of a large percentage of FDEIAn patients. The very high prevalence of IgE to the LTP suggests a role of this allergen group in causing S-FDEIAn.

Keywords food-dependent exercise-induced anaphylaxis, lipid transfer protein, microarray, specific IgE

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Introduction

Exercise-induced anaphylaxis (EIA) is a physical allergy elicited by mild to vigorous exercise alone [1, 2]. Food-dependent exercise-induced anaphylaxis (FDEIAn) is a distinct form of EIA, provoked by the combination of food ingestion and physical exercise [2–4]. In some cases, specific foods cause anaphylaxis when physical exercise follows their ingestion [specific FDEIAn (S-FDEIAn)]. Neither exercise nor food ingestion

alone elicits this syndrome. Other patients suffer from EIA after all meals followed by exercise, regardless of the food eaten [non-specific FDEIAn (NS-FDEIAn)] [5, 6].

The mechanism by which food ingestion could provoke an adverse reaction only when combined with physical exertion is still not fully understood. Either positive skin tests or serum specific IgE reactivity to the same foods consumed before the reaction or both suggest an IgE-mediated pathogenesis. In these subjects,

exercise might increase the absorption of recently-ingested, but partially-digested, food proteins, including allergens, into the circulation, from where they migrate into the perivascular and tissue spaces, where mast cells armed with allergen-specific IgE reside [2, 4]. Furthermore, increased histamine serum levels in some FDEIAN patients after challenges with culprit foods, followed by physical exercise, have been correlated with the clinical response [7–9].

Since the first report of S-FEDIAN in 1979 [3], several foods, such as shrimp, shellfish, and wheat-derived products, have been reported as triggers [2, 4, 6, 8–11]. In a study by Shadick et al. [12], one-third of the 279 subjects with EIAN reported one or more foods as triggers. In European countries, vegetables are the most common food allergens causing FDEIAN [6, 10, 13]. Moreover, multiple food reactivity seems to represent a clinical hallmark of some FDEIAN patients [6, 10, 13].

Because of the severity of FDEIAN, it is important to identify the causative food in order to prevent the allergic reaction. Skin prick tests (SPT) using commercial or fresh food preparations, as well as serum specific IgE assays with extracts, are routinely used. In addition, IgE testing for allergenic molecules can improve the allergist's diagnostic resources [14], especially in patients with wheat-dependent EIAN [15–19]. In a study by Matsuo et al. [16], the simultaneous measurement of specific IgE using ω -5 gliadin and high molecular weight glutenin increased the sensitivity and the specificity of wheat-dependent EIAN diagnosis.

In the present study, we combined a 'classic' diagnostic protocol – which includes both *in vivo* (skin tests) and *in vitro* (a second-generation test for detecting specific IgE) tests [6, 10] – with an allergenic-molecule-based microarray system by means of an allergenic-molecule-based microarray system to evaluate patients with FDEIAN in an attempt to identify as many positive foods as possible. This 'classic' protocol was applied in a previous study of ours [6], in which 24 of the present subjects were first evaluated.

Our diagnostic work-up also included an exercise challenge after a meal without any of the foods associated with skin test or specific IgE assay positivity.

Materials and methods

Patient selection

We studied all the patients seen in the allergy units of C.I. Columbus and Oasi Maria S.S. between January 1997 and June 2008 reporting at least one episode of anaphylaxis in association with physical exercise performed within 4 h after a meal. Anaphylaxis was diagnosed according to the clinical criteria proposed by Sampson et al. [20] and included in the recent

document by the PRACTALL study group [2]. As codified by Dohi et al. [8], urticaria was the kind typical of EIAN (wheal diameter, > 10–15 mm, unlike cholinergic urticaria, in which wheal diameter is < 5 mm), collapse was not due to a dysfunction of an organ, such as the heart, lung, or brain, and anaphylaxis was not experienced in response to warm baths, showers, fever, or other increases in core body temperature. Sixty-six of the 82 patients had been treated with epinephrine, steroids, and anti-histamines in emergency rooms (and medical records of 58 of them were available). The remaining 16 patients had been treated by general practitioners, and five of them did not receive epinephrine.

Patients followed the same study protocol.

An exhaustive interview was carried out using a detailed questionnaire. Patients were carefully asked about all foods ingested during the 24-h period preceding each episode. Foods repeatedly eaten prior to exercise without causing symptoms were also recorded.

Prior to the study, all subjects received information about the possible risks of the diagnostic work-up, and written informed consent was obtained from each patient or the parents of those under 18 years of age. The protocol was approved by the respective institutional review boards (protocol number: Oasi Maria S.S., No. 02/011/1997).

A physical examination, including electrocardiogram, echocardiogram, and pulmonary function tests, was performed to diagnose any heart or lung diseases. Allergy investigations including tests as listed below were carried out as well.

In vivo testing

Skin prick tests were performed with a panel of commercial glycerinated allergenic extracts (20 inhalant and 26 food extracts; Bayropharm, Milan, Italy) on three different days on the volar forearms. Inhalants included pollen from grasses (*Phleum pratense*, *Lolium perenne*, *Poa pratensis*), *Artemisia*, *Parietaria*, *Plantago*, a ragweed mixture, as well as olive, birch, beech, cypress, and plane trees, *Dermatophagoides pteronyssinus* and *farinae*, dog and cat dander, *Alternaria alternata*, *Cladosporium herbarum*, *Aspergillus* mixture, and *Penicillium* mixture. The panel of food allergens, all available as extracts 1 : 20 w/v, included a mixture of meats (lamb, rabbit, beef, pork, and chicken), egg white, cow's milk, cod, shrimp, wheat, rye, barley, oat, maize, rice, pea, peanut, soybean, bean, walnut, almond, tomato, potato, carrot, garlic, onion, apple, celery, banana, and peach. Because Bayropharm ceased production of the aforesaid reagents, Stallergenes extracts (Antony, France) have been used since July 2005. As the meat mixture and the oat extract were no longer available from the latter manufacturer, we replaced the

meat mixture with single meat extracts (lamb, rabbit, beef, pork, and chicken) by Stallergenes. SPT were also performed with any suspected food (e.g., hazelnut) not included in the aforesaid panel.

Fresh food prick tests using the prick plus prick (P + P) technique described by Dreborg and Foucard [21] were also performed on a different day using the last nine food sources cited above (from tomato to peach) and for kiwi, lentil, fennel, parsley, basil, and lettuce, as well as any other suspected food (e.g., grape and mulberry). All SPT were performed using sterile stainless steel standardized lancets (Stallergenes).

A SPT was considered positive when a wheal of at least 3 mm in diameter, surrounded by erythema, was observed. Positive responses were rated from 1+ to 4+ according to a grading system based on the relation between the reactions induced by the allergen and the histamine reference [22]. Positive and negative controls were applied using histamine at 10 mg/mL and a glycerinated solution, respectively.

In vitro testing

Blood samples were collected when patients were evaluated and sera were stored at -20°C until assayed. Total serum IgE levels were detected by means of the ImmunoCAP Total IgE (Phadia, Uppsala, Sweden) with a detection range of 2–5000 IU/L.

Specific IgE detection for each of the food allergens (with the exception of the meat mixture or single meats), as well as for any suspect food, was performed using the ImmunoCAP system (CAP; Phadia) at the time of inclusion of the patients. We considered a positive result to be a value of 0.35 kU/L or greater.

Since 2005, specific IgE detection has also been performed using the Immuno Solid-phase Allergen Chip microarray system (ISAC; VBC-Genomics, Vienna, Austria), according to manufacturer instructions, where 89 molecules were immobilised in triplicate. We first evaluated the sera of patients we had studied since January 1997, then those of newly enrolled FDEIAn subjects. The allergenic molecule panel represented on the ISAC included allergens from the following food sources: wheat (including the gliadin fraction), barley, peach, kiwi, carrot, apple, hazelnut, cashew nut, celery, pineapple, cow's milk, hen's egg, *Anisakis*, snail, and shrimp. The ISAC chip also bears allergenic molecules from non-food allergens from the following sources: latex, bee venom, dog, cat, mites, cockroaches, *Alternaria*, *Aspergillus*, *Cladosporium*, and pollen from grasses, *Parietaria*, mugwort, as well as hazel, birch, alder, plane, cypress, and olive trees. A number of allergenic sources, including foods, were represented by panallergens like profilin, Bet v 1-like, lipid transfer protein (LTP), and tropomyosin. Using microtechnology for

spotting allergenic molecules, this test allows multiplex testing using 20 μL of serum from each subject and obtaining results on all of them after a single run [23]. For the specific purpose of comparing allergenic molecule IgE prevalence, we used a gender/age matching control cohort of 11 109 allergic subjects (6261 female, age range between 9 and 47 years old, median 24.8) not affected by FDEIAn seen at IDI-IRCCS and tested using the same ISAC 89. Considering the absence of any background in the testing, as ascertained by using sera with total IgE values above 10 000 IU/L, a cut-off value of 0.01 kU/L in the ISAC IgE result was established for positivity.

Food and exercise challenges

In order to confirm or exclude the responsibility of a meal, regardless of the intake of a suspected food, a treadmill ergonometric stress test was performed as previously reported [6, 10]. Briefly, at gradually increasing speeds, each patient performed the test ninety minutes after a meal where any of the foods associated with a positive SPT or IgE positive assay were excluded [Food plus Exercise Challenge (FEC)] [10]. Equipment and trained personnel for emergency intervention were available during all challenges. Tests were stopped immediately if the patient began to experience symptoms like itching, flushing, urticaria, dyspnea, or abdominal cramps; otherwise the test was continued until muscle exhaustion occurred, which was always after the patients had achieved 90% or more of their predicted maximum heart rate and oxygen consumption [10]. Each challenge was performed around noon. The patients had been fasting since the evening before. Patients were asked not to take leukotriene modifiers during the week before the test, as well as long-acting β_2 -agonists, bronchial and nasal corticosteroids, antihistamines, and nedocromil sodium for at least 2 days before, and short-acting β_2 -agonists for 6 h before the challenge. In the asthmatic patients, the challenge was started if the baseline FEV_1 was at least 80% of the predicted value.

Because the patient histories clearly indicated that the suspected foods were tolerated if not followed by exercise, and considering results from our previous study where the oral provocation tests with suspected foods without exercise were negative in FDEIAn patients [10], we did not perform such tests during the present study.

Data management and statistics

All data generated during the present study, whether produced *in vivo* or *in vitro*, were saved in the InterAll allergy electronic record (version 3; Allergy Data

Laboratories sc, Latina, Italy). InterAll is a web-based application cross-linked to the Allergome platform (<http://www.allergome.org>). Allergome regularly feeds the InterAll e-record with updated and unique codes of all allergenic preparations used for testing, whether extracts or molecules, and with codes for all allergy testing methods, thus allowing fully structured data to be saved in the local database.

Data analysis was carried out using the GraphPad software (Prism 5.01; GraphPad Software, Inc., La Jolla, CA, USA). Statistical evaluation was performed using the statistical package SPSS Release Version 13.0 (SPSS Inc., Chicago, IL, USA). Differences were considered significant at P values of < 0.05 .

The degree of relationship between the quantitative variables studied was analysed using Pearson's correlation test, and the most commonly used bivariate correlation technique. The chi-square test was used to investigate whether distributions of categorical variables differ from one another. Differences between prevalence were evaluated using the non-parametric Mann-Whitney U -test. The statistical significance cut-off level was set at $P < 0.01$.

For the estimation of the huge amount of data generated by the analysis of 89 allergenic molecules in all subjects studied, we applied the same approach used for genomics data analysis [24]. The results of SPT, P + P, CAP, and ISAC were expressed as heat-map in Fig. 1. Each square in the heat-map represents the presence (red) or the absence (black) of IgE recognition of any tested allergens. Untested allergens were represented in grey. The red colour intensity of every square in the heat-map is directly associated with the intensity of responses to SPT or P + P expressed as a score ranging from 1+ to 4+ [22] or with the kU/L of measured IgE.

Results

We studied 82 patients (41 females), age range between 9 and 47 years old (mean 22.78 ± 7.32); male subjects were significantly younger (19.9 ± 5.9 vs. 25.6 ± 7.5 , Mann-Whitney Rank Sum Test $P < 0.01$). Our work-up was carried out 7 ± 23 months (range, 1–192) after the most recent anaphylactic reaction.

Thirty-one subjects had experienced only one FDEIAn episode, 35 subjects between 2 and 5, nine between 6 and 10, and seven patients more than 10. Fourteen patients had also suffered from 1 to 13 episodes of exercise-induced urticaria-angioedema.

Overall, 16 patients had negative histories of allergic or allergy-like diseases other than FDEIAn. Of the other 66 subjects, 54 suffered from an IgE-mediated allergy to either inhalants (36 subjects) or food (4), or both (14). Among the remaining 12 patients, six had urticaria or angioedema unrelated to food intake or exer-

cise (Table 1). During their FDEIAn episodes, the latter patients had suffered symptoms in addition to the urticarial eruptions: all had experienced angioedema (mainly of the face), five dyspnea (one of these also colic, one choking, and two loss of consciousness), and two hypotension (one associated with dyspnea). These additional symptoms allowed us to rule out flare-ups of their chronic urticaria.

Fifteen of the 82 FDEIAn subjects could not recall any specific suspected food.

The other 67 suspected a specific food in at least one episode. The most frequent ones were tomato (either fresh or processed), wheat, and vegetable (peanut, sunflower etc.) oils (Table 2); most patients recalled more than one of the most frequent suspected foods.

The time elapsed between meal and exercise ranged from 30 to 240 min (median 60 min), and the duration of exercise before the onset of symptoms ranged from 10 to 50 min as reported by the patients.

The activities most frequently associated with FDEIAn symptoms were playing soccer, followed by running, dancing, aerobics, and tennis (Table 3). The level of exertion ranged from walking to running.

Twenty-three subjects recalled other contributing factors on at least one FDEIAn episode: 10 had taken non-steroidal anti-inflammatory drugs (NSAIDs) before one of the attacks; five reported more frequent FDEIAn episodes during the period of their seasonal rhinitis (one of them had also taken acetylsalicylic acid before the reaction); two were menstruating; two were stressed; three were both menstruating and stressed; and one reported unfavourable climatic conditions.

The most frequent symptoms were angioedema (93.9%), followed by urticaria (91.5%), flushing (89%), dyspnea (79.2%), hypotension (34.1%), gastrointestinal symptoms (nausea, diarrhoea, colic, vomiting: 24.7%), and upper respiratory tract symptoms (choking, throat constriction, hoarseness: 7.4%). Physical examinations and instrumental studies revealed hypertrophy of the right ventricle of the heart in one patient, hypertension in another, coronary anomalies in a third one, and a prolapsed mitral valve in two. Overall such findings did not influence the clinical allergy picture and did not contraindicate the FEC.

Allergy test results

The median [25th percentile–75th percentile] serum total IgE concentration was 153.5 kU/L [71.5–303]. The allergy testing was positive for airborne allergens in 68 (82.9%) patients. Diagnostic data from the 50 symptomatic subjects, all affected by rhino-conjunctivitis, plus 18 who did not suffer from respiratory symptoms (Table 1) are summarised in Fig. 1, each panel representing one of the four methods used in the present study.

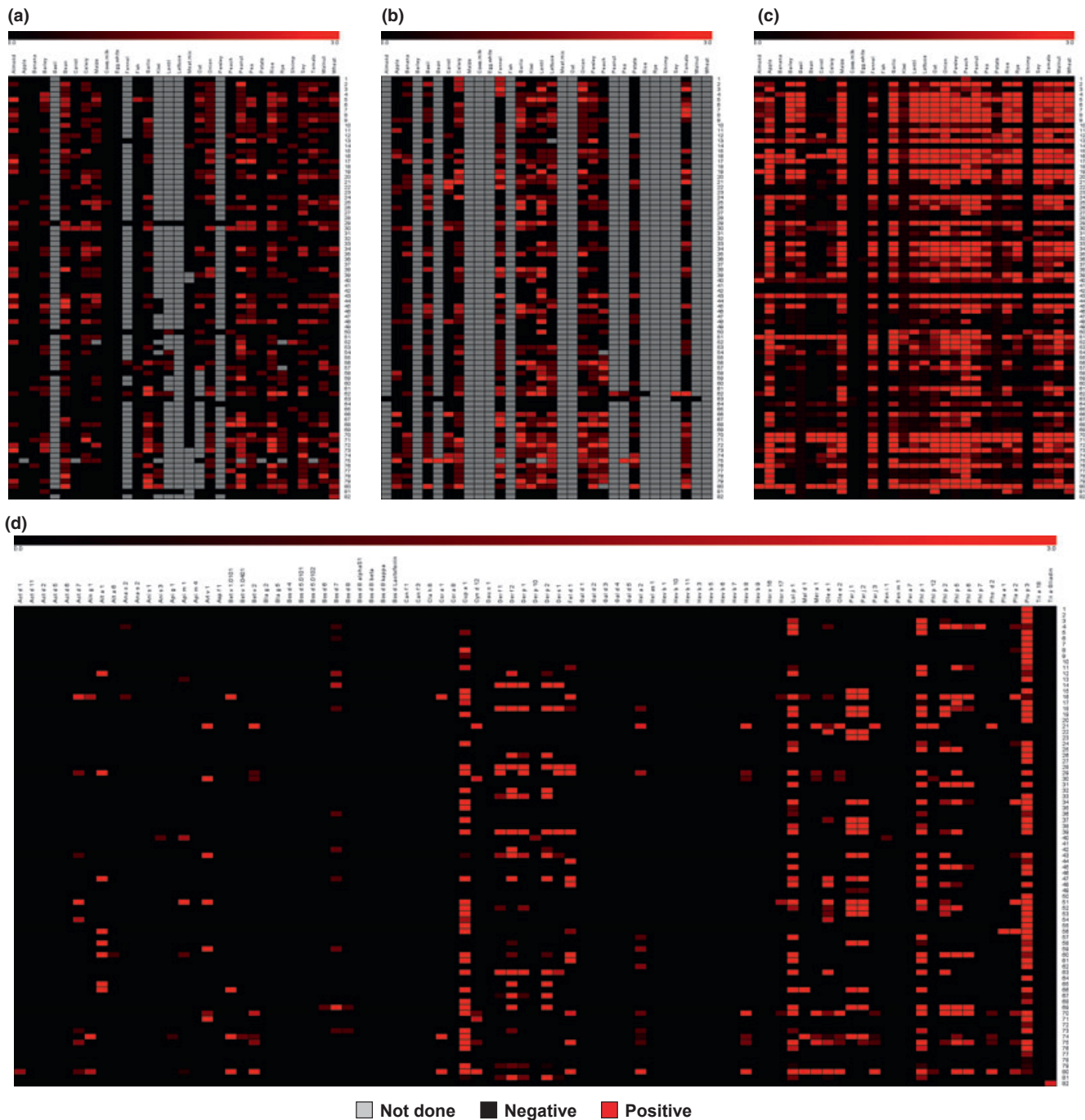


Fig. 1. Heat-map representation of results obtained by skin prick tests (SPT; a), prick + prick tests (P + P; b), IgE detection by ImmunoCAP (IgE; c) and IgE detection by ISAC microarray (d). Each line on the heat-map represents a patient, tested allergens are in columns, and results are reported as positive (red), negative (black), or untested (grey). The red colour intensity of every square on the heat-map is directly associated with the intensity of responses to SPT or P + P expressed as a score ranging from 1+ to 4+ or with the kU/L of measured IgE.

On the basis of SPT and CAP positive results to the above reported allergen panel, 79 of 82 patients were sensitised to at least one food, and 49 of them to more than 20 (up to 27) distinct food sources. Three subjects had no detectable reactivity after *in vitro* and *in vivo* tests.

Figure 1 and Table 4 illustrate the list of assessed foods, the total number of subjects positive to each food, and the number of positive results detected by SPT (Fig. 1a), P + P (Fig. 1b), or specific IgE (Fig. 1c) evaluation, respectively.

Table 1. Clinical characteristics of patients

Personal history	No. of patients	No. of positive to inhalants	No. of positive to foods	No. of positive to lipid transfer protein
Rhino-conjunctivitis	25*	25	25	20
Rhino-conjunctivitis + asthma	11	11	11	8
Rhino-conjunctivitis + oral allergy syndrome	7	7	7	7
Rhino-conjunctivitis + food hypersensitivity [†]	7	7	7	5
Urticaria-angioedema	6	2	6	6
Food hypersensitivity [†]	4	4	4	4
Cholinergic urticaria	2	2	1	1
Contact dermatitis	2	1	2	0
ASA hypersensitivity	2	0	2	1
Negative [‡]	16	9	14	12
Total	82	68	79	64

*One of them presented an IgE-mediated hypersensitivity to *Vespula* spp. venom.

[†]Systemic hypersensitivity reactions to foods unrelated to exercise.

[‡]Negative histories of allergic or allergy-like diseases other than FDEIA.

Table 2. Suspected food-dependent exercise-induced anaphylaxis-triggering food in 67 patients

Suspected food	Reactions No. (%)
Tomato	25 (30.4)
Wheat	8 (9.8)
Vegetable oils	7 (8.5)
Pea	5 (6.1)
Fennel	4 (4.9)
Hazelnut	4 (4.9)
Peanut	4 (4.9)
Maize	3 (3.7)
Garlic	3 (3.7)
Lettuce	3 (3.7)
Peach	3 (3.7)
Shrimp	3 (3.7)
Legumes	2 (2.4)
Walnut	2 (2.4)
Rice	2 (2.4)
Almond	1 (1.2)
Apple	1 (1.2)
Grape	1 (1.2)
Mulberry	1 (1.2)

As shown in Table 4, the most frequently positive foods were maize and peanut (70 patients), apple (69), fennel, lentil, onion, and peach (66 patients).

The foods suspected on the basis of history data were positive in almost all cases. Each of the three methods (SPT, P + P, CAP) disclosed some reactivity not discovered by the other two. P + P testing revealed a higher number of positive results than those obtained with SPT (Table 4). Except for almond, bean, fish, and shrimp, CAP positive results constantly outnumbered those of SPT. Regarding foods assessed with both CAP and P + P, the former method recorded more positive

Table 3. Patients' reported physical activities while experiencing anaphylaxis

Type of exercise	Reactions No. (%)
Football	29 (27.4)
Running	27 (25.5)
Dancing	13 (12.3)
Aerobics	11 (10.4)
Tennis	7 (6.6)
Gymnastic	5 (4.7)
Basket	4 (3.8)
Track	4 (3.8)
Swimming	2 (1.9)
Hockey	1 (0.9)
Karate	1 (0.9)
Riding	1 (0.9)
Cycling	1 (0.9)

results for all foods, except for celery, kiwi, and basil. Figure 1d reports ISAC results using a heat-map representation. Sixty-four (78%) out of 82 patients had IgE to the peach LTP, Pru p 3, thus representing the most common sensitizer in our FDEIA subjects, whereas profilin, PR-10, tropomyosin, and the gliadin fraction were positive in 15.8%, 4.8%, 1.2%, and 1.2%, respectively (Fig. 2). LTP prevalence in the FDEIA subjects had a strong statistically significant difference when compared with the control group, whereas the same was not true for the remaining food allergens, including wheat gliadin (Fig. 2).

Cypress pollen allergen Cup a 1 and timothy grass pollen allergen Phl p 1, and its homologous in rye grass pollen allergen Lol p 1, bound IgE from 45% of FDEIA subjects; 25.6% of patients were IgE positive to the *Parietaria* allergens (both Par j 2 and Par j 1), 50% to

Table 4. Results of allergologic evaluations of food allergens in the 82 food-dependent exercise-induced anaphylaxis patients

Food	No. Pos.	SPT	P + P	CAP
Meat mix*	1	1	n.p.	0
Egg white	1	0	n.p.	1
Cow's milk	3	1	n.p.	2
Fish	3	3	n.p.	0
Wheat	55	4	n.p.	25
Rye	54	5	n.p.	25
Barley	57	3	n.p.	28
Oats	60	0	n.p.	41
Maize	70	2	n.p.	40
Rice	61	8	n.p.	14
Peas	58	7	n.p.	26
Peanuts	70	6	n.p.	22
Soy	63	6	n.p.	21
Beans	50	33	n.p.	7
Walnut	62	3	n.p.	27
Almond	37	11	n.p.	8
Shrimp	8	3	n.p.	3
Tomato	65	0	1	23
Carrot	26	0	2	11
Garlic	63	0	2	15
Onion	66	0	1	16
Apple	69	0	1	50
Celery	59	2	10	7
Banana	38	1	11	12
Peach	66	0	0	31
Potato	45	1	5	23
Kiwi	50	n.p.	16	12
Lentils	66	n.p.	11	14
Fennel	66	n.p.	9	19
Lettuce	65	n.p.	11	21
Parsley	64	n.p.	3	15
Basil	51	n.p.	19	16

Food, food assessed by allergologic tests: skin prick tests with commercial extracts (SPT), prick plus prick tests with fresh foods (P + P), and ImmunoCAP (CAP); No. Pos. = total positivities; SPT = No. of positivities detected only by SPT; P + P = No. of positivities detected only by P + P; CAP = No. of positivities detected only by CAP; n.p. = not performed.

*See text. The difference between the total positivities and the sum of the positivities detected only by a single test (SPT, P + P, or CAP) is accounted for by those detected by more than one test.

at least one genuine grass pollen allergen, and 23.7% to genuine mite allergens (Fig. 2). In the ISAC, none of the patients were IgE reactive to latex or hen's egg allergens. The control group showed significantly higher prevalence for *Parietaria*, grass, and mite allergens (Fig. 2).

Table 5 reports IgE reactivity results to LTP, profilin and PR-10 by matching them with those obtained by testing every allergenic extract using conventional diagnostic methods. As shown in this table, the chi-square test suggests that there was a correlation

between reactivity to Pru p 3 and the vast majority of plant foods tested in the study group.

FEC results

Seventy-five subjects agreed to undergo this test. The three patients with negative results in all food allergens, one of whom suffered from cholinergic urticaria, experienced anaphylactic reactions (pruritus, flushing, urticaria, dyspnea, and/or hypotension), which promptly resolved after intramuscular epinephrine, as well as intravenous 6-methyl-prednisolone (80 mg) and chlorphenamine (10 mg). The remaining 72 FDEIAn subjects tolerated the FEC.

Overall, 79 patients probably had S-FDEIAn and the other three NS-FDEIAn.

Discussion

A large percentage of our subjects probably suffer from a specific form of FDEIAn. In the etiopathogenesis of such form, an essential role is played by the ingestion of one or more foods to which the patient is sensitive. A challenge test consisting of ingestion of the suspected food followed by physical exercise [suspected food plus exercise challenge (SFEC)] is the only reliable method to identify the causative food and to diagnose the disease. Nevertheless, although it is a good way to elicit the clinical reaction, it does not provide information about what links several foods in causing symptoms. However, the procedure is not only harmful, but may also be lengthy and costly, especially in patients positive to several foods, who constitute a large percentage of all FDEIAn patients [6, 13]. Moreover, this test failed to provoke clinical symptoms in about one-third of more than 80 subjects previously evaluated [25], including eight patients described in our previous study [10]. Therefore, the SFEC might be performed only for investigative purposes or to clarify the causative role of certain foods, for instance those important in an athlete's diet. To diagnose S-FDEIAn, a negative response to the FEC, together with the observation that avoidance of positive foods from 4 to 6 h before physical exertion prevents further EIA episodes, may be sufficient.

The aim of our diagnostic protocol is to identify any foods to which patients are sensitised in order to enable such patients to safely resume their physical activities. In this connection, we emphasise the usefulness of testing both *in vivo* and *in vitro* a great number of foods. The combined use of SPT, P + P, and CAP allowed us to identify numerous positive results. Generally, each test disclosed some positive results not discovered by others. The P + P revealed positivity (especially to fruits and vegetables) not disclosed by SPT. The negative results in some FDEIAn patients reported by others

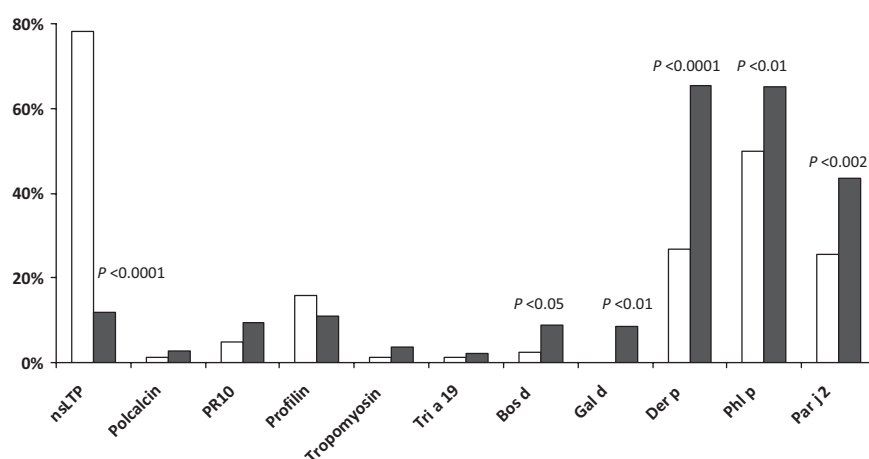


Fig. 2. Prevalence of IgE to 'genuine' food allergens, panallergens, and environmental allergens in the FDEIA allergic population (white bars) and in the 11 109 allergic controls (black bars).

Table 5. LTP, profilin, and PR-10 reactivity compared to allergenic extract results in the 82 food-dependent exercise-induced anaphylaxis patients

LTP							PR10		Profilin		LTP							PR10		Profilin		LTP							PR10		Profilin										
		N		P		N		P		N		P		N		P		N		P		N		P		N		P		N		P		N		P		N		P	
Almond	N	16	29	43	2	38	7	Lettuce	N	12	5	17	0	17	0	Almond	χ^2	10.773	p	0.001	n.s.	n.s.	Lettuce	χ^2	26.915	p	0.000	n.s.	0.044												
	P	2	35	35	2	31	6		P	6	59	61	4	52	13		P	0.000						n.s.	n.s.					P	0.000	n.s.	0.044								
Apple	N	11	2	13	0	12	1	Meat	N	0	0	0	0	0	0	Apple	χ^2	35.410	p	0.000	n.s.	n.s.	Meat	χ^2	n.s.	p	n.s.	n.s.													
	P	7	62	65	4	57	12		P	1	0	1	0	1	0		P	n.s.						n.s.	n.s.				P	n.s.	n.s.	n.s.									
Banana	N	12	32	44	0	41	3	Oat	N	11	11	22	0	20	2	Banana	χ^2	n.s.	p	4.869	0.027	5.811	Oat	χ^2	13.807	p	0.000	n.s.	n.s.												
	P	6	32	34	4	28	10		P	7	53	56	4	49	11		P	n.s.						0.016	n.s.					P	0.000	n.s.	n.s.								
Barley	N	10	15	25	0	25	0	Onion	N	11	5	16	0	14	2	Barley	χ^2	6.838	p	0.009	n.s.	6.776	Onion	χ^2	25.412	p	0.000	n.s.	n.s.												
	P	8	49	53	4	44	13		P	7	59	62	4	55	11		P	0.009						n.s.	0.009					P	0.000	n.s.	n.s.								
Basil	N	12	19	30	1	30	1	Parsley	N	12	6	18	0	17	1	Basil	χ^2	8.171	p	0.004	n.s.	5.958	Parsley	χ^2	26.915	p	0.000	n.s.	n.s.												
	P	6	45	48	3	39	12		P	6	58	60	4	52	12		P	0.004						n.s.	0.015					P	0.000	n.s.	n.s.								
Bean	N	15	17	31	1	29	3	Peach	N	15	1	16	0	15	1	Bean	χ^2	19.028	p	0.000	n.s.	n.s.	Peach	χ^2	59.814	p	0.000	n.s.	n.s.												
	P	3	47	47	3	40	10		P	3	63	62	4	54	12		P	0.000						n.s.	n.s.					P	0.000	n.s.	n.s.								
Carrot	N	11	45	56	0	51	5	Peanut	N	12	0	12	0	12	0	Carrot	χ^2	n.s.	p	n.s.	n.s.	Peanut	χ^2	49.981	p	0.000	n.s.	n.s.													
	P	7	19	22	4	18	8		P	6	64	66	4	57	13		P	n.s.					n.s.	n.s.					P	0.000	n.s.	n.s.									
Celery	N	12	11	23	0	21	2	Pea	N	13	11	24	0	22	2	Celery	χ^2	17.042	p	0.000	n.s.	n.s.	Pea	χ^2	20.554	p	0.000	n.s.	n.s.												
	P	6	53	55	4	48	11		P	5	53	54	4	47	11		P	0.000						n.s.	n.s.					P	0.000	n.s.	n.s.								
Maize	N	11	1	12	0	12	0	Potato	N	10	27	37	0	34	3	Maize	χ^2	39.878	p	0.000	n.s.	n.s.	Potato	χ^2	n.s.	p	n.s.	n.s.													
	P	7	63	66	4	57	13		P	8	37	41	4	35	10		P	0.000						n.s.	n.s.				P	n.s.	n.s.	n.s.									
Cow's milk	N	17	62	75	4	66	13	Rice	N	11	10	20	1	19	2	Cow's milk	χ^2	n.s.	p	n.s.	n.s.	Rice	χ^2	15.257	p	0.000	n.s.	n.s.													
	P	1	2	3	0	3	0		P	7	54	58	3	50	11		P	n.s.					n.s.	n.s.					P	0.000	n.s.	n.s.									
Egg white	N	18	63	77	4	68	13	Rye	N	11	17	28	0	27	1	Egg white	χ^2	n.s.	p	n.s.	n.s.	Rye	χ^2	7.457	p	0.006	n.s.	4.808	0.028												
	P	0	1	1	0	1	0		P	7	47	50	4	42	12		P	n.s.					n.s.	n.s.						P	0.006	n.s.	4.808	0.028							
Fennel	N	11	5	16	0	15	1	Shrimp	N	14	60	70	4	61	13	Fennel	χ^2	25.412	p	0.000	n.s.	n.s.	Shrimp	χ^2	n.s.	p	n.s.	n.s.													
	P	7	59	62	4	54	12		P	4	4	8	0	8	0		P	0.000						n.s.	n.s.				P	n.s.	n.s.	n.s.									
Fish	N	17	62	76	3	68	11	Soy	N	14	5	19	0	19	0	Fish	χ^2	n.s.	p	n.s.	n.s.	Soy	χ^2	36.631	p	0.000	n.s.	4.659	0.031												
	P	1	2	2	1	1	2		P	4	59	59	4	50	13		P	n.s.					n.s.	n.s.						P	0.000	n.s.	4.659	0.031							
Garlic	N	11	8	19	0	17	2	Tomato	N	10	7	17	0	16	1	Garlic	χ^2	18.648	p	0.000	n.s.	n.s.	Tomato	χ^2	17.019	p	0.000	n.s.	n.s.												
	P	7	56	59	4	52	11		P	8	57	61	4	53	12		P	0.000						n.s.	n.s.					P	0.000	n.s.	n.s.								
Kiwi	N	13	19	32	0	31	1	Walnut	N	15	5	19	1	18	2	Kiwi	χ^2	10.682	p	0.001	n.s.	6.374	Walnut	χ^2	43.449	p	0.000	n.s.	n.s.												
	P	5	45	46	4	38	12		P	3	59	59	3	51	11		P	0.001						n.s.	0.012					P	0.000	n.s.	n.s.								
Lentil	N	13	3	16	0	15	1	Wheat	N	11	16	27	0	25	2	Lentil	χ^2	40.800	p	0.000	n.s.	n.s.	Wheat	χ^2	8.295	p	0.004	n.s.	n.s.												
	P	5	61	62	4	54	12		P	7	48	51	4	44	11		P	0.000						n.s.	n.s.					P	0.004	n.s.	n.s.								

LTP, lipid transfer protein; n.s., not statistically significant.

[8, 26, 27] could thus be explained by the fact that they did not use tests with fresh foods, which are often more sensitive than those with commercial extracts [28–30], even in case of FDEIA [5, 7], or that they used a limited panel of allergens. Many uncommon food antigens

may be ingested in composite dishes like salads, sauces, pizzas, and certain ethnic foods. Stricker et al. [31], using a panel of 79 food antigens, demonstrated that 7% of cases reported as idiopathic anaphylaxis were not truly idiopathic.

The CAP disclosed numerous positive results, and in some cases proved to be more sensitive than *in vivo* tests, probably because the solid phase coupling of extracts allows greater stability of critical allergens for specific IgE binding. These features have made it useful in the diagnosis of classic food allergies, particularly in predicting symptomatic IgE-mediated food hypersensitivity [32].

In our opinion, thorough allergy testing also contributes to understanding the irregularity or unpredictability of FDEIAN, which may or may not occur even given the same level of exertion or the same amount of time elapsed after apparently identical meals. In effect, such irregularity may be partially explained by sensitisations to seasonings, such as garlic, onion, basil, and parsley, present in many dishes, but rarely recalled by patients and rarely included in allergen panels. However, the irregularity might also be explained by contributing factors we noted – and also reported by others [1, 33–36] – such as the use of NSAIDs prior to physical exertion, stress, menstruation, and seasonal or unfavourable climatic conditions.

In any case, multiple food hypersensitivity represents a hallmark of some FDEIAN patients. However, microarrays allowed us to identify a common allergenic determinant, the LTP, in 78% of the 82 patients, including most of those who recalled wheat and maize as suspected foods. In fact, even though some studies [15–19] identified molecules like ω -5 gliadin and high molecular weight glutenin as responsible sensitizers in wheat-dependent EIAN, LTP can also be the responsible molecules of severe IgE-mediated reactions to cereals such as wheat and maize. In a recent study on European patients with IgE-mediated wheat hypersensitivity [37], the most important wheat allergens were the α -amylase/trypsin inhibitor subunits, which were present in all three protein fractions of raw and cooked wheat. However, other important allergens were a 9-kDa LTP in the albumin/globulin fraction and several low molecular weight glutenin subunits in the gluten fraction. Regarding maize, a study by Pastorello et al. [38] identified a 9-kDa LTP as the major allergen of raw maize in a population of 22 patients who had suffered anaphylactic reactions to it. Moreover, a subsequent study by the same group [39] proved that maize LTP maintains its IgE-binding capability after heat treatment, thus being the most eligible candidate for a causative role in severe anaphylactic reactions to cooked maize. With regard to tomato, the most frequent suspect food in our FDEIAN patients, Pravattoni et al. [40] identified an IgE-binding LTP in fresh tomato peel, pulp, and seeds, as well as in tomato derivatives.

In subjects with multiple food hypersensitivity, probably not all the positive foods were responsible for FDE-

IAN. It would be useful to establish the causative role played by each one by performing exercise challenges after their ingestion. As previously said, however, such procedure is not only harmful, but also time-consuming, because of the numerous positive foods. Conversely, we emphasise the usefulness of the FEC in our diagnostic protocol. If negative, FEC indicates foods that can safely be consumed prior to exercise. A negative FEC also reassures patients that, when the suspect positive food is not eaten, post-prandial exercise will not cause symptoms. A positive FEC is indicative of NS-FDEIAN.

FDEIAN is, in many cases, an extremely complex form of food allergy, which appears in association with exercise and sometimes other factors. It is thus important to carefully assess hypersensitivity to foods in these patients, as also suggested by other authors [13]. In our study, we used the same extensive panel of food allergens for each patient. In clinical practice, this panel could be individualised in order to limit investigation costs. For the same reason, *in vitro* tests for foods to which they have proved to be not very sensitive could be omitted. A great advantage might come from the use of an extensive panel of food-derived allergenic molecules tested by the novel microarray technology we used. A comprehensive panel of LTP, including the already identified ones (45 according to the official WHO-IUIS web site and <http://www.allergome.org> accessed in April 2011) and others to be identified from sources described in the present study, could be very helpful in suggesting to FDEIAN patients what to include or exclude in their meals before doing exercise. In effect, our 79 S-FDEIAN patients were instructed to avoid all positive foods during the four hours preceding physical exercise, and all but one of the 67 subjects (12 subjects dropped out) followed by us for 55.8 ± 32.7 months (range 1–122 months) did not experience reactions. Only one subject experienced an anaphylactic reaction during the follow-up, when she ran one hour after a meal containing *radicchio* (variety of chicory), which was not included in the first allergy exam and was strongly positive to P + P in the second one. Therefore, avoidance of foods associated with SPT, P + P, and/or IgE positive results for at least 4 h before exercise allowed all our S-FDEIAN patients to continue their physical activity without suffering any disturbance, and actually eliminated the unpredictability of this syndrome.

In conclusion, 79 patients probably had S-FDEIAN and the other 3 NS-FDEIAN. In 64 (81%) of the 79 subjects suspected of S-FDEIAN, the histories, allergy data, and responses to elimination diets suggest a possible etiological role for specific foods containing LTP.

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