



## Letter

## Wheat-dependent exercise-induced anaphylaxis caused by a lipid transfer protein and not by $\omega$ -5 gliadin

Wheat-dependent exercise-induced anaphylaxis (WDEIA) is characterized by severe allergic symptoms from physical effort performed after wheat ingestion in patients allergic to wheat. The most important allergen in WDEIA is represented by  $\omega$ -5 gliadin,<sup>1</sup> although low- and high-molecular-weight glutenins have been described as causative allergens.<sup>2</sup> This report describes 3 patients with WDEIA, in whom the causative allergen was a 9-kDa wheat lipid transfer protein (LTP). Each of these patients had a history of severe allergy to peach and was re-evaluated for several episodes of severe systemic allergic reactions of unknown origin. Patient P1, a 17-year-old boy, had a history of numerous emergency visits for anaphylaxis since infancy, without a clear association to any foods or exercise. Patient P2, a 15-year-old boy with a history of atopic dermatitis, allergic asthma, and oral allergy syndrome to Prunoidae fruits presented with frequent episodes of severe anaphylaxis in the previous 2 years, apparently after eating any kind of cereal. Patient P3, a 21-year-old woman, known at the authors' center for severe oral allergy syndrome to peach and anaphylaxis with maize, was re-evaluated for the occurrence of 4 anaphylactic reactions after eating pizza and dancing and after eating pizza and performing cardiopulmonary resuscitation at work (Table 1 presents clinical details).

Patients P1 and P2 underwent a double-blinded, placebo-controlled food challenge (DBPCFC) at rest with wheat, rice, and maize; patient P3, who had a known allergy to maize, did not undergo the maize challenge. Challenges for wheat, rice, and maize were performed as previously described.<sup>3,4</sup> For a negative DBPCFC result at rest, the patients underwent an open exercise food challenge (OEFC): outdoor running was performed for 20 minutes, 1 hour after having ingested 100 g of wheat, maize, or rice; cereal OEFCs were separated by 1-week intervals. For details of the OEFC, see the "eMethods" section. The exercise reported in the history was not repeated for the challenge to better monitor heart rate.

Specific serum IgE antibodies for wheat, maize, rice,  $\omega$ -5 gliadin, peach, rPru p 3, and *Phleum pratense* were determined by the ImmunoCAP system (ThermoFisher Scientific, Uppsala, Sweden). IgE immunoblotting was performed with wheat, maize, and rice extracts and with purified wheat LTP according to methods previously described.<sup>5</sup> Extraction of Osborne fractions of wheat proteins was performed as published previously.<sup>5</sup> Maize and rice proteins were extracted as previously described.<sup>6</sup> An IgE immunoblotting inhibition of a wheat albumin–globulin fraction was performed after incubating the serum of patient P2 with peach- and maize-purified LTPs. Wheat LTP purification was performed using a method described by Capocchi et al<sup>7</sup> and LTP1 identity was

confirmed by mass spectrometry/mass spectrometry as previously described.<sup>7</sup> Table 1 presents demographic data, clinical histories, specific IgE, challenge results, observed clinical symptoms, and management of anaphylactic reactions for the 3 patients. Cereal DBPCFC results at rest were negative for all 3 patients except for the rice DBPCFC, which showed a positive result in patient P2. All patients showed a positive wheat OEFC reaction; the maize OEFC reaction was positive only in patient P2. The rice OEFC was performed in patients P1 and P3 and yielded negative results (Table 1 presents the clinical results of the wheat OEFC).

The IgE immunoblotting of the wheat albumin–globulin fraction showed reactivity toward a 9-kDa protein for all 3 patients. IgE immunoblotting of the wheat-purified 9-kDa protein confirmed the presence of a pure IgE-reactive 9-kDa component (Figure E1A).

The IgE immunoblotting for maize and rice with sera from the 3 patients showed a strong reactivity toward an IgE-binding component at 9 kDa; immunoblotting inhibition of the wheat albumin–globulin fraction by peach- and maize-purified LTPs showed the total disappearance of the 9-kDa band (Figure E1B).

In conclusion, this report has described 3 patients with peach allergy, positivity to Pru p 3, and WDEIA. In each case, the only IgE-binding protein observed at the wheat immunoblotting was the 9-kDa LTP, and no reactivity was observed to other proteins of the gliadin and/or glutenin fractions. These results suggest that in patients with WDEIA, wheat LTP (Tri a 14) can play an important allergenic role, at least in Italy. However, regarding the clinical significance of this finding, the cross-reactivity between peach and wheat LTPs was not great and only a small percentage of patients allergic to peach LTP have wheat allergy. Thus, detection of a positive Tri a 14 titer should always be verified by an OEFC before confirming the diagnosis of WDEIA.

Interestingly, all 3 patients were sensitized to maize and rice LTPs, but each presented with a different pattern of clinical expression: patient P2 showed positivity to rice DBPCFC at rest and manifested food-dependent exercise-induced anaphylaxis with maize, whereas patient P3 exhibited maize anaphylaxis. This observation confirmed the findings of a recent Italian study demonstrating that LTP is the most frequent sensitizer in patients with food-dependent exercise-induced anaphylaxis.<sup>8</sup>

Thus, it is clear that in Italy patients with WDEIA should undergo an initial investigation for wheat LTP sensitization; however, the authors believe that wheat LTP sensitization should be investigated outside Italy in patients with WDEIA and negativity to  $\omega$ -5 gliadin. Furthermore, patients with LTP sensitivity and WDEIA should be investigated for possible reactions to maize and rice because variable reactivities were observed to these cereals.

**Table 1**Demographic data, clinical history, specific IgE, challenge results, observed clinical symptoms during wheat challenge and exercise, and management of anaphylactic reactions in 3 patients<sup>a</sup>

| Patient number | Sex    | Reported food allergies | Age at FDEIA diagnosis (y) | Trigger food | Type of exercise                                       | Symptoms (ED visits)   | Specific IgE (kU/L) |      |      | DBPCFC |              |                 | OEFC |   |    | Wheat OEFC HR 170–190 beats/min | Wheat-related anaphylactic manifestation                          | Anaphylaxis Management                                               |
|----------------|--------|-------------------------|----------------------------|--------------|--------------------------------------------------------|------------------------|---------------------|------|------|--------|--------------|-----------------|------|---|----|---------------------------------|-------------------------------------------------------------------|----------------------------------------------------------------------|
|                |        |                         |                            |              |                                                        |                        | rPru p 3            | W    | R    | M      | ro-5 gliadin | Phleum pratense | W    | R | M  |                                 |                                                                   |                                                                      |
| P1             | male   | stone fruits            | 17                         | wheat        | soccer                                                 | U, AE, Rh, D (4)       | 34.7                | 1.56 | 1.46 | 4.92   | <0.35        | 0.81            | –    | – | –  | –                               | urticaria, angioedema, intense lachrymation, nasal obstruction    | epinephrine IM, hydrocortisone IV, chlorphenamine IM                 |
| P2             | male   | stone fruits, maize     | 15                         | wheat, maize | soccer                                                 | E, U, AW, C, GI, H (3) | 6.94                | 5.70 | 8.81 | 9.86   | <0.35        | 85.6            | –    | + | NP | +                               | urticaria, dyspnea, ear edema, nausea, abdominal cramps, PA 90/50 | epinephrine IM, methylprednisone IV, salbutamol INH, cetirizine PO   |
| P3             | female | stone fruits, maize     | 21                         | wheat        | brisk walk, work-related cardiopulmonary resuscitation | U, D, H, GI            | 2.42                | 0.23 | 0.2  | 0.97   | <0.35        | 1.92            | –    | – | NP | +                               | urticaria, dyspnea, wheezing, rhinitis                            | epinephrine IM, hydrocortisone IV, chlorphenamine IM, salbutamol INH |

Abbreviations: AE, angioedema; AW, wheezing; C, cough; D, dyspnea; DBPCFC, double-blinded, placebo-controlled food challenge; E, erythema; ED, emergency department; FDEIA, food-dependent exercise-induced anaphylaxis; GI, nausea, abdominal pain; H, arterial hypotension 90/50; HR, heart rate; IM, intramuscular; INH, inhaled; IV, intravenous; M, maize; NP, not performed; OEFC, open exercise food challenge; PA, arterial blood pressure; PO, per oral; R, rice; Rh, rhinitis; U, urticaria; W, wheat.

<sup>a</sup>Clinical details of the OEFC with rice and maize are not presented.

## Supplementary Data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.anai.2014.01.012>.

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## eMethods

The challenge was supervised by a physician and immediate access to the emergency department was available for severe reactions; the challenge was interrupted for symptoms and/or objective signs. Forced expiration volume in 1 second was measured before and during the challenge; a positive reaction was

defined as a 15% decrease in forced expiration volume in 1 second. For the OEFC, the open oral cereal challenge with wheat and maize and/or rice was performed after a 48-hour cereal-free diet and repeated every 3 days in case of negative results. The outdoor running was continued until a maximum heart rate of 170 to 190 beats/min was reached for a maximum of 10 minutes.

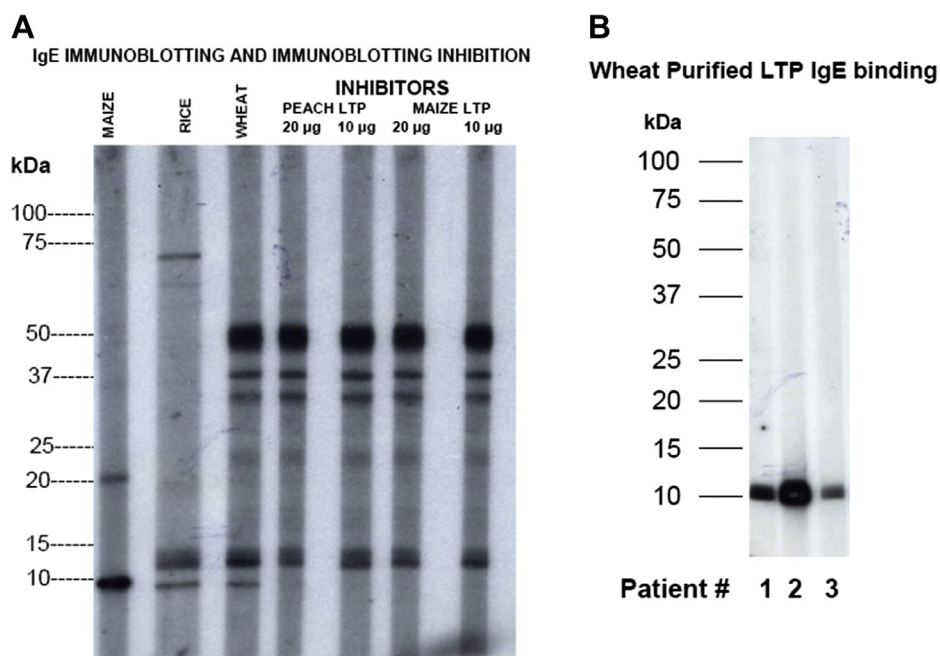


**Figure E1.** (A) Maize, rice, and wheat IgE immunoblots with serum from patient P2 (lanes 1–3) and wheat IgE immunoblot inhibition by peach and maize lipid transfer proteins (LTPs) at 20 and 10  $\mu$ g (lanes 3–7). (B) IgE immunoblotting of the 9-kDa wheat-purified LTP with sera from 3 patients.