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## Ole e 1, Ole e 7, and Ole e 9: Identifying distinct clinical subsets of olive tree–allergic patients

*To the Editor:*

Allergy to olive pollen (*Olea europaea*) is caused in most cases by Ole e 1, but other allergens, for example, profilin (Ole e 2), polcalcin (Ole e 3 and Ole e 8), glucanase (Ole e 4 and Ole e 9), and lipid transfer protein (LTP) (Ole e 7)[1,2] may also be involved (World Health Organization/International Union of Immunological Societies database: www.allergen.org) (see Table E1 in this article's Online Repository at www.jacionline.org). Genuine molecules such as Ole e 1, Ole e 7, and Ole e 9 and panallergens such as profilin (Bet v 2.0101, Hev b 8.0204, Mer a 1.0101, and Phl p 12.0101) or polcalcin (Bet v 4.0101 and Phl p 7.0101)[3] can nowadays be detected by ImmunoCAP-ISAC.

**TABLE I.** Clinical and molecular characteristics of participants

| Characteristic | N (%) | Age (y) | Female (age) | Male (age) |
|---|---|---|---|---|
| All patients | 839 | 27 ± 16 | 435 (30 ± 15) | 404 (24 ± 15) |
| | **N (%)** | **Age (y)** | **Female (%)** | **Male (%)** |
| Ole e 1 | 708 (84.4) | 27 ± 16 | 360 (50.8) | 348 (49.2) |
| Ole e 7 | 180 (21.5) | 27 ± 13 | 90 (50) | 90 (50) |
| Ole e 9 | 85 (10.1) | 24 ± 14 | 35 (41.2) | 50 (58.8) |
| 1. RS | 674 (80.3) | 28 ± 16 | 354 (52.5) | 320 (47.5) |
| 2. AD | 189 (22.5) | 23 ± 14 | 99 (52.4) | 90 (47.6) |
| 3. OAS | 327 (39) | 27 ± 15 | 168 (51.4) | 159 (48.6) |
| 4. SR | 365 (43.5) | 28 ± 16 | 186 (51) | 179 (49) |

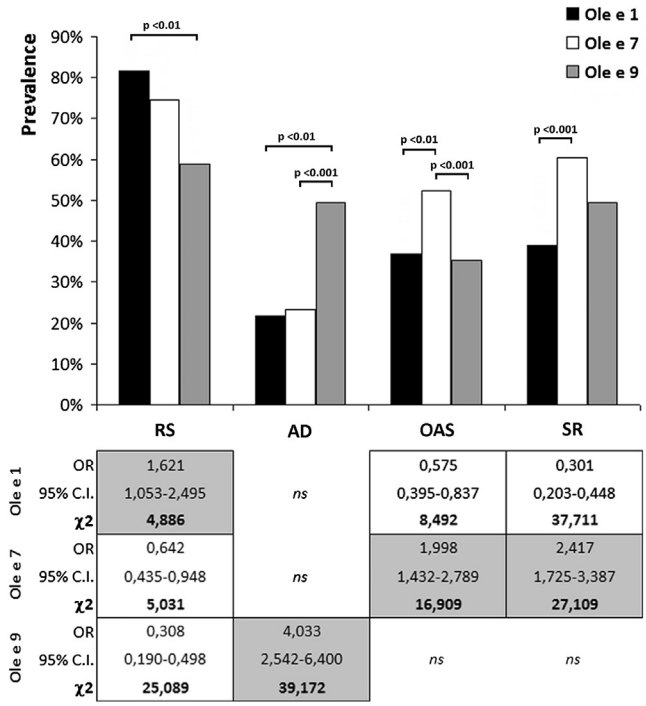
Nine hundred ninety-five consecutive Italian outpatient subjects of the Allergy Unit of IDI-IRCCS, Rome, with positive results on skin prick test with olive tree pollen extract (Stallergenes S.A., Antony, France) were studied between May 2013 and December 2014, after approval by the IDI-IRCCS Ethical Committee as well as written informed consent from the participants. At baseline, using a standardized questionnaire and before allergen testing, all patients were grouped into 4 overlapping categories: (1) asthma, allergic rhinitis, allergic conjunctivitis ("Respiratory symptoms" [RS]); (2) atopic dermatitis (AD); (3) localized oral mucosal symptoms ("Oral allergic syndrome" [OAS]); and (4) symptoms suggestive of systemic reactions to food, including urticaria, angioedema, and anaphylaxis according to Sampson et al[4] ("Severe Reactions" [SR]). Sera were collected during the first visit, and IgE reactivity was analyzed using the 112 ImmunoCAP-ISAC platform (ThermoFisher Scientific, Uppsala, Sweden).

A genuine sensitization to olive tree pollen molecules was observed in 839 of the 995 subjects (84.3%, 435 females and 404 males; mean age, 27.5 ± 16.2 years; range, 3-76 years). The remaining 156 (15.7%) reacted to profilin (n = 117, 75%), polcalcin (n = 33, 2.1%), or both (n = 6, 3.8%) and were excluded from the study. No differences in the prevalence of IgE recognition were noted between male and female patients, but males were younger (24 ± 15 years vs 30 ± 15 years; $P < .01$). The demographic characteristics and the distribution among clinical categories are summarized in Table I.

Ole e 1 reactivity was detected in 84.4% of the patients, followed by Ole e 7 (21.5%), a higher prevalence than in another Mediterranean population,[5] and Ole e 9 (10.1%). Ole e 9 reactivity was higher among males than among females (12.4% vs 8.0%; $P = .038$). Ole e 7 IgE reactivity was significantly less frequent in children younger than 15 years (12.6% vs 24.1%; $P < .001$).

The association between reactivity to olive tree molecules and the self-reported clinical profiles at baseline is shown in Fig 1.

Ole e 1 reactivity was associated with RS (81.6%; $P < .03$), but inversely related to the occurrence of local symptoms or systemic reactions to food (36.9% for OAS, $P < .005$, and 39% for SR, $P < .001$). In contrast, Ole e 7 reactors showed a clinical history suggestive of both local (52.2%; $P < .001$) and/or systemic reactions to food (60.6%; $P < .001$), and a lower prevalence of RS ($P < .03$). Accordingly, a slight inverse relationship was observed between




| | | RS | AD | OAS | SR |
|---|---|---|---|---|---|
| Ole e 1 | OR | 1,621 | ns | 0,575 | 0,301 |
| | 95% C.I. | 1,053-2,495 | | 0,395-0,837 | 0,203-0,448 |
| | χ2 | 4,886 | | 8,492 | 37,711 |
| Ole e 7 | OR | 0,642 | ns | 1,998 | 2,417 |
| | 95% C.I. | 0,435-0,948 | | 1,432-2,789 | 1,725-3,387 |
| | χ2 | 5,031 | | 16,909 | 27,109 |
| Ole e 9 | OR | 0,308 | 4,033 | ns | ns |
| | 95% C.I. | 0,190-0,498 | 2,542-6,400 | | |
| | χ2 | 25,089 | 39,172 | | |

**FIG 1.** Reactivity prevalence of olive tree molecules in 4 clinical subset of allergic patients (RS, AD, OAS, and SR). The association between olive tree pollen molecules' IgE reactivity and self-reported clinical symptoms was studied in univariate and multivariate analysis. In univariate analysis, the Mann-Whitney *U* test (2 groups) was first used to compare continuous IgE values in subjects with or without a given clinical involvement. In addition, each variable of interest was dichotomized (as negative or reactive) to study the proportion of subjects with organ involvement in the 2 groups thus obtained. Categorical variables were analyzed using the Pearson $\chi^2$. In the table, a direct *(light gray)* or inverse *(white)* association is indicated for Ole e 1 *(black columns)*, Ole e 7 *(white columns)*, and Ole e 9 *(gray columns)* reactors. *ns*, Not significant.

Ole e 1 and Ole e 7 IgE recognition ($P < .001$; $\rho = -0.218$) (see Fig E1 in this article's Online Repository at www.jacionline.org). Multiple logistic regression analysis, considering all the molecules studied as a whole, including also age and sex, showed a significant relationship between Ole e 7 reactivity and a history of OAS ($P < .01$; adjusted odds ratio [aOR], 1.730; 95% CI, 1.156-2.590) or SR ($P = 0.03$; aOR, 1.586; 95% CI, 1.060-2.371). Ole e 1 reactivity remained inversely associated with SR even after multiple adjustment ($P < .001$; aOR, 0.393; 95% CI, 0.241-0.639).

Ole e 9–positive subjects presented a positive association with AD (49.4% vs 19.5% among Ole e 9–negative subjects; $P < .001$) and a lower rate of RS ($P < .001$). Multiple regression also confirmed the finding that Ole e 9 reactivity identifies a subset of patients with a history of AD ($P < .001$; aOR, 4.189; 95% CI, 2.540-6.919) and a lower risk of RS ($P < .001$; aOR, 0.319; 95% CI, 0.193-0.527).

On the basis of these observations, Ole e 7 and Ole e 9 seem to be markers of sensitization to other panallergens, rather than specific olive pollen molecules. In effect, it has been suggested that Ole e 7 or Ole e 9 reactors might show a lower tolerance to immunotherapy at the recommended doses.[5]

Unlike mugwort and plane tree LTPs (Art v 3 and Pla a 3, respectively), which show approximately 50% sequence identity with the peach LTP Pru p 3,[6] Ole e 7 shares less than 20% of amino acid sequence with Pru p 3, and therefore seems to be neither structurally nor immunologically related to food LTPs.[7] However, our study demonstrates that Ole e 7 reactivity is in strict relationship with adverse reactions to plant-derived food (mainly peach, walnut, and peanut), thus suggesting an intriguing involvement of Ole e 7 in the LTP panallergen family, despite the lack of structural homology.[7] Moreover, about 80% of Ole e 7 reactors were also positive to at least 1 of the plant food LTP tested on the microarray versus only 30% of Ole e 1 reactors.

To our knowledge, the association between Ole e 9 reactivity and the higher prevalence of AD has never been described before. Ole e 9 contains 2 immunologically and structurally independent domains[8]: the N-terminal domain has a 1,3-β-glucanase activity, thus belonging to the pathogenesis-related protein family-2, which is implicated in latex-pollen-vegetable food allergy syndrome.[8] Accordingly, we observed a higher occurrence of latex reactivity among Ole e 9 reactors (15.3% vs 3.3% among nonreactors; $P < .001$; $\chi^2 = 25.348$; OR, 5.265; 95% CI, 2.582-10.737). Ole e 9 reactivity was never associated with a higher incidence of food reactions (OAS or SR).

In conclusion, our study shows that distinct subsets can be recognized among olive tree reactors on the basis of molecular reactivity. Ole e 1 positivity identifies the specific olive tree pollen–allergic population with RS, possibly deserving specific immunotherapy, whereas Ole e 7 and Ole e 9 IgE recognition is associated with local or systemic reactions to food and AD, respectively. This suggests that testing for olive components may be useful for a better characterization of patients with atopy, as is the case for peanut components.[9] It is likely, in the near future, that this concept could be extended to other allergens as well.

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## Proton pump inhibitor–responsive esophageal eosinophilia does not preclude food-responsive eosinophilic esophagitis

*To the Editor:*

Over the past 2 decades, eosinophilic esophagitis (EoE) has emerged as an important and increasingly prevalent antigen-mediated immunologic disorder. Current consensus recommendations advocate a therapeutic trial of proton pump inhibitor (PPI) before establishing the diagnosis of EoE.[1] Patients with EoE symptoms with resolution of eosinophilic inflammation on PPI therapy are designated as having PPI-responsive esophageal eosinophilia (PPIREE), and not EoE. PPI responsiveness is attributed to gastroesophageal reflux disease (GERD). Response to corticosteroids or elimination of dietary antigens, however, is often viewed as an inherent characteristic of EoE. Recent studies have identified that 25% to 50% of the patients with esophageal eosinophilia and suspected EoE demonstrate a histologic response to PPI.[2]

We describe 5 adults with characteristic EoE presentations who exhibited symptomatic, endoscopic, and histologic improvement when treated independently with either PPI therapy or an empiric elimination diet at 2 distinct time points (ie, patients were not on a PPI at the time of diet and not on diet at the time of PPI). The data are summarized in Table I. Each of the patients had an atopic history and presented with dysphagia and food impaction. There were 2 men and 3 women aged 24 to 72 years. Two of the 5 patients underwent the six-food elimination diet (elimination of milk, soy, egg, wheat, nuts, and seafood) therapy, with an established protocol involving a dietician.[3] The other 3 patients underwent empiric elimination with wheat (1 patient) and milk (2 patients) based on patient preference. Endoscopic features assessed using a validated classification and grading system showed reduction in inflammatory features after each therapy (Fig 1).[4] Moreover, histology demonstrated a marked reduction in esophageal eosinophilia, with normalization in all cases.

This small case series calls into question the premise that PPI responsiveness precludes the diagnosis of EoE. EoE, PPIREE, and GERD are increasingly less distinct than suggested in the current clinical approach.[5] This supposition is supported by several recent studies. Two prospective studies in adults found that neither clinical nor endoscopic features distinguished PPIREE from EoE.[6,7] Therapeutically, 2 randomized controlled trials comparing topical steroids to PPI therapy for patients with esophageal eosinophilia failed to demonstrate a statistically significant difference in histologic response between the 2 treatments.[8,9] Mechanistically, a recent study found no significant difference between EoE and PPIREE in terms of the expression of eotaxin-3 and $T_H2$ cytokines (IL-5 and IL-13).[10] Furthermore, recent translational studies have provided evidence that the PPI response in esophageal eosinophilia may be related to PPI effects on improving the mucosal integrity of the esophageal epithelial barrier as well as acid-independent PPI effects on eosinophilic inflammation.[11,12]

Several limitations to the present case series exist. The study was retrospective with a small cohort. Our findings could have been influenced by seasonal fluctuations in disease activity, although such a temporal effect has been described only in isolated case reports.[3] Spontaneous or sustained remission in EoE could have occurred although this has been infrequently described in prospective studies. Finally, esophageal eosinophilia in EoE is patchy, leading to potential for sampling error on endoscopic biopsy. This possibility was mitigated by the use of a standardized protocol of 8 esophageal biopsies (4 proximal and 4 distal esophagus) during endoscopy based on a protocol developed from our institution.[13]

Defining EoE on the basis of the absence of a therapeutic response to PPI has practical and conceptual problems.[9] Practically, the appropriate dose, duration, and durability of the PPI therapy has not been defined. Conceptually, defining a disease on the basis of treatment response is problematic. Mounting evidence refutes the notion that GERD and EoE are readily differentiated by PPI response. If acid refluxate weakens the esophageal epithelial barrier, thereby allowing access of swallowed antigens to antigen-presenting cells, is the disease process due to GERD, allergic pathogenesis, or the combination? Acknowledging this growing ambiguity is important for improving our understanding of the pathogenesis of EoE as well as for avoiding inappropriate limitations in therapeutic options for afflicted patients.

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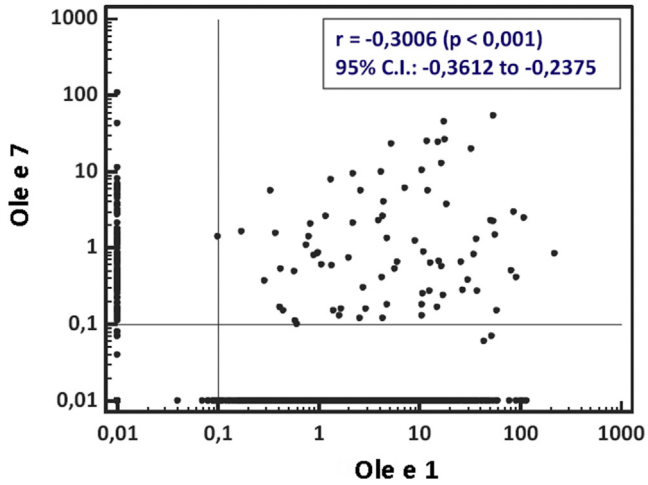


**FIG E1.** The degree of relationship between Ole e 7 and Ole e 1 IgE reactivity was analyzed using Spearman correlation ($r$) test, given the skewed distribution of the observed values. A slight significant inverse relationship was observed, with $P < .001$ and $r^2 = -0.3006$.

**TABLE E1.** Olive tree pollen allergens identified to date according to the WHO/IUIS database (www.allergen.org)

| Allergen | Biochemical name | Biological function | MW | IgE prevalence[E1,E2] |
|---|---|---|---|---|
| Ole e 1 | Common olive group 1 | Trypsin inhibitors | 16 | >70 |
| Ole e 2 | Profilin | Actin-binding proteins | 15 | >40 |
| Ole e 3 | Polcalcin | Calcium-binding proteins | 9 | 20 |
| Ole e 4* | | Glucanases | 32 | 80 |
| Ole e 5 | Superoxide dismutase | Superoxide Dismutases | 16 | 35 |
| Ole e 6 | Cysteine-enriched allergen | Unknown | 10 | 15-55 |
| Ole e 7 | Putative nonspecific LTP | LTPs | 9-10 | 47 |
| Ole e 8 | Polcalcin-like protein (4 EF-hands) | Calcium-binding proteins | 21 | 5 |
| Ole e 9 | 1,3-Beta glucanase | Glucanases | 46 | 50 |
| Ole e 10 | X8 domain–containing protein | Glycosyl hydrolase | 11 | 54 |
| Ole e 11 | Pectin methylesterase | Pectinesterases | 39.4 | |

*EF*, Calcium-binding motifs composed of 2 helixes (E and F) joined by a loop; *IUIS*, International Union of Immunological Societies; *MF*, molecular weight; *WHO*, World Health Organization.

*Ole e 4 could be a proteolytic degradation product of Ole e 9 and not a genuine allergen.