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Pla a 2 and Pla a 3-reactivity identify Plane tree-allergic patients with respiratory symptoms or food allergy.

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Abstract

Nine hundred thirty-nine rPla a 1, nPla a 2 and rPla a 3 ImmunoCAP ISAC reactors were studied. nPla a 2^{pos} MUXF3^{pos} but Pla a $1/2^{neg}$ subjects were excluded from the study because they were CCD reactors. Amongst the 764 remaining participants, 71.9% were Pla a 3^{pos}, 54.1% Pla a 2^{po}s and 10.9% Pla a 1^{pos}. Among Pla a 3 reactors, 89.6% were Pru p 3^{pos} and 86.8% Jug 3^{pos}, but the strongest IgE recognition relationship was observed between Pla a 3 and Jug r 3. Distinctive clinical subsets could be documented among plane-tree allergic patients. Pla a 3 reactors had both local and systemic food-induced reactions, but lower past respiratory symptoms occurrence. Pla a 2 reactivity was associated with respiratory symptoms but inversely related to systemic reactions to food. Co-sensitization to Pla a 2 and Pla a 3 was associated with a lower past incidence of severe food-induced reactions.

Short title: CRD unveil clinical subsets in Plane-tree allergy

Key words.

Pla a 2, Pla a 3, plane tree, ns-lipid transfer protein, Jug r 3

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The Plane-tree (*Platanus acerifolia*) family, from the order Proteales, is broadly distributed in southern Europe with 8 to 17% sensitization rates in exposed people(1). The most important allergens (*www.allergen.org*) are the 18 kDa invertase-inhibitor Pla a 1(2), the 43 kDa glycoprotein Pla a 2 displaying a polygalacturonase activity(3) and the non-specific lipid transfer protein (nsLTP) Pla a 3(4), showing 45% sequence identity with Pru p 3 from peach(5;6). Recently it has become possible to verify plane tree reactivity by simultaneously evaluating these three genuine molecules (rPla a 1, nPla a 2 and rPla a 3) with a micro-arrayed procedure allowing the immediate comparison with other nsLTPs, profilins, polcalcins or the Cross-reactive carbohydrate determinants (CCDs) molecules spotted on the array.

Nine hundred thirty-nine consecutive Italian plane-tree reactors were detected as rPla a 1, nPla a 2 or rPla a 3 reactors out of 3,385 outpatient subjects of the Allergy Unit of the dermatological institute IDI-IRCCS, Rome, between September 2013 and August 2016. Fifty-two (7.3%) patients studied were mono-sensitive to plane pollen, 90.6% were sensitized to other pollen sources, 25.3% to moulds, 48.3% to animal epithelia and 51.4% to house dust mites. At baseline, patients were categorized on the basis of clinical symptoms reported at the time of the first visit by a physician blinded to Plant tree reactivity data, as follows: (1), symptoms localised to the oral mucosa (‘*oral allergy syndrome’; OAS*); (2), systemic reactions, including urticaria, angioedema, respiratory difficulty and/or pre-syncope/syncope, due to ingestion of fruits, tree nut or peanut (‘*Systemic reaction to food’; SRtoF*); (3), symptoms of rhino-conjunctivitis and/or bronchial asthma only (‘*Respiratory symptoms’; RS*). None of the selected patients has been previously treated with allergen specific immunotherapy to plane tree due to their respiratory allergy.

IgE reactivity of sera from all participants, collected and stored at the time of the first visit, was analyzed using the latest commercially available ImmunoCAP-ISAC platform (ThermoFisher Scientific, Uppsala, Sweden).

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We observed a genuine plane tree reactivity in 764/939 patients (398 female and 366 male; mean age 29±16 years, range 6 months-82 years). One hundred seventy-five participants, both Pla a 2 mono-reactors and MUXF3-positive, were excluded from the study because CCD reactors, and non-Plane tree allergic patients as further confirmed by negative skin prick test for plane tree extracts(7). Our data demonstrate that an isolated IgE immuno-reactivity to Pla a 2 should always be interpreted cautiously, since this natural glycosylated molecule could score positive in CCDs reactors with no clinical significance (8;9). CCDs do not act as allergens *in vivo* and have no clinical role(7), but their occurrence could drive to an *in vitro* misleading positive result when natural purified glycoproteins such as nPla a 2 are tested(10).

The demographic, clinical and molecular characteristics of study patients are summarized in Table 1.

Pla a 3 was detected in 71.9% participants (549), followed by Pla a 2 (54.1%) and Pla a 1 (10.9%). Pla a 2 IgE recognition occurrence was significantly higher in males (63.7% vs 45.2% in females, $p<0.01$). Taken together, these data reveal a peculiar molecule recognition pattern in our Italian group of patients if compared to what described in literature, where the Pla a 1 and Pla a 2 sensitization frequencies ranged from 80% to 90% (2;3), and Pla a 3 reached no more than 45% in Spanish plane pollen allergic patients (4). In previous reports, Pla a 1 has been described as the major Plane tree allergen, recognized by the vast majority of patients in Spain (2;11), and recently it was suggested that Pla a 1 could be the major allergen in plane tree allergic patients without peach reactivity(12). Surprisingly, in our Italian cohort, mainly represented by Pla a 3^{pos} subjects, Pla a 1 reactivity was found in less than 11% of the total Plane tree allergic population. Differences among Italy and Spain could be the result of the levels of exposure to plane tree pollen in the two

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countries, since pollen concentrations are higher (reaching some hundreds of grains) in Spanish cities, than in central in Italy (generally <100) (19).

Four hundred ninety-two (89.6%) Pla a 3 reactors were Pru p 3^{pos} and 477 (86.8%) were Jug 3^{pos}. Three hundred twenty-one (42%) patients were Pla a 3 mono-reactors (90.6% of them Pru p 3^{pos} and 86.9% Jug r 3^{pos}), 175 (22.9%) were Pla a 2 mono-reactors and only 22 (2.9%) participants were Pla a 1^{pos} but both Pla a 2/Pla a 3^{neg}. Two hundred twenty participants (28.8%) were Pla a 2/Pla a 3^{pos}.

A moderate relationship was observed between Pla a 1 and Pla a 2 IgE recognition (Pearson Correlation (r) test: 0.538). On the other hand reactivity to the Plane tree nsLTP Pla a 3, showed a strong positive relationship with Jug r 3 (r= 0.7239), Ara h 9 (r= 0.6588), Cor a 8 (r= 0.6549) and Pru p 3 (r= 0.6436) ($p< 0.001$ in all cases), whilst no relationship was observed among Pla a 3, Par j 2, Ole e 7 or Tri a 14.

It has been suggested that Pla a 1 and Pla a 2 reactivity is the signature of a genuine plane tree pollen sensitization, whereas Pla a 3 reactivity should be considered the consequence of a primary sensitization to plant-food LTPs, mainly Pru p 3 in the Mediterranean area(4;13;14), although respiratory symptoms could be induced by the molecular components found in peach leaves (15). As a consequence, Pla a 3 reactivity, the most frequent plane tree sensitization found in our Italian cohort, could represent a marker of nsLTP reactivity associated with an higher risk of food allergy and, accordingly, cross-reactivity in subjects allergic to both peach and plane pollen (but not in peach reactors without plane pollen allergy) has been recently found (12). We recently observed a significant correlation between immuno-reactivity to Pla a 3 and nsLTPs from tree nut/peanut in a

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large population affected by nsLTP allergy(6), and similarly, we found an associations with Jug r 3, Ara h 9 and Cor a 8 reactivity, even to a higher extent than with Pru p 3 in our Italian cohort of Pla a 3^{pos} plane tree allergic people, suggesting the possibility of a primary sensitization alternative to peach.

Plane tree pollen sensitization, has been often associated with food allergy (16-19), and therefore we sought to verify if the molecular IgE recognition profile in our Italian cohort could be associated with specific clinical pictures. The association between Plane tree reactivity and the self-reported clinical profiles documented at baseline is shown in Figure 1. No age difference was recorded comparing patients with food allergy (FA) (26±15-years), RS (31±18) or both (26±15). In patients with both FA and RS, respiratory allergy always preceded FA. Patients >14-years-old showed a higher prevalence of SRtoF (60.5% vs 49.3%, $p<0.05$).

Pla a 2 reactivity was significantly linked to a history of RS but inversely associated to SRtoF, indicating that Pla a 2^{pos} subjects had a low frequency of adverse reaction to food. On the other hand, a history of both OAS or SRtoF was significantly associated with Pla a 3 IgE recognition, but such reactivity was inversely related to the presence of rhino-conjunctivitis or bronchial asthma. The simultaneous occurrence of Pru p 3 reactivity was associated with an even higher risk of severe reaction (chi-squared=6,391, $p<0.01$, OR=2.572 95% CI=1.2-5.4). Furthermore, participants with a history of SRtoF showed significantly higher Pla a 3 ($p<0.01$) and lower Pla a 2 ($p<0.05$) IgE ISU-E values (data not shown). These molecules are therefore able to identify two subgroups of patients showing different clinical behaviours, similarly to Olive tree allergen sensitization in which Ole e 1 and Ole e 7 were linked to RS and SRtoF respectively (20). Pla a 2/Pla a 3 double reactors had a history of OAS (chi-squared=14.151; OR=1.83; C.I.=1.3-2.5 $p<0.001$) but were never associated with SRtoF.

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Multiple logistic regression analysis, including all the molecules studied as well as age and sex, showed a significant association between a history of RS and Pla a 2 reactivity ($p < 0.05$; ORa= 1.48, 95% C.I.= 1.03-2.13). IgE reactivity to both Pla a 2 ($p = 0.007$; ORa= 1.59, 95% C.I.= 1.14-2.23), and Pla a 3 ($p < 0.0001$; ORa=2.93, 95% C.I.=2.0-4.29) was linked to a history of OAS. Pla a 1 ($p < 0.05$; ORa=0.61, 95% C.I.=0.37-0.99) and Pla a 2 ($p < 0.03$; ORa=0.67, 95% C.I.=0.47-0.95) reactivity remained inversely associated with SRtoF, whilst Pla a 3 ($p < 0.0001$; ORa=4.01, 95% C.I.=2.73-5.89) recognition was strongly associated to SRtoF, even after multiple adjustment.

In conclusion, different subsets can be recognized among Plane tree reactors based on molecular reactivity. Once ruled out a potential CCDs recognition, nPla a 2 positivity along with Pla a 1, identifies the genuine Plane tree allergic population affected by RS, and therefore eligible for specific immunotherapy, whilst Pla a 3 IgE recognition is associated to OAS or SRtoF. It appears that testing for plane tree components may be useful for a better characterization of atopic patients. It is likely that this concept may be expanded to other allergens as well.

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	Total	***Local Reaction***	***Systemic Reaction***	***Respiratory Symptoms***
	(n=764)	(n=356)	(n=446)	(n=536)
Sex (M|F)	366|398	167|189	196|250	261|275

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Age (y)± SD	29±16	28±14	29±15	27,4±16,6
Pla a 1	10.9%	9%	7.8%	11.9%
Pla a 2	54.1%	54.2%	43.9%	57.6%
Pla a 3	71.9%	80.9%	85.2%	69%

Table 1. Clinical, demographic and Plane tree molecules recognition profiles of participants

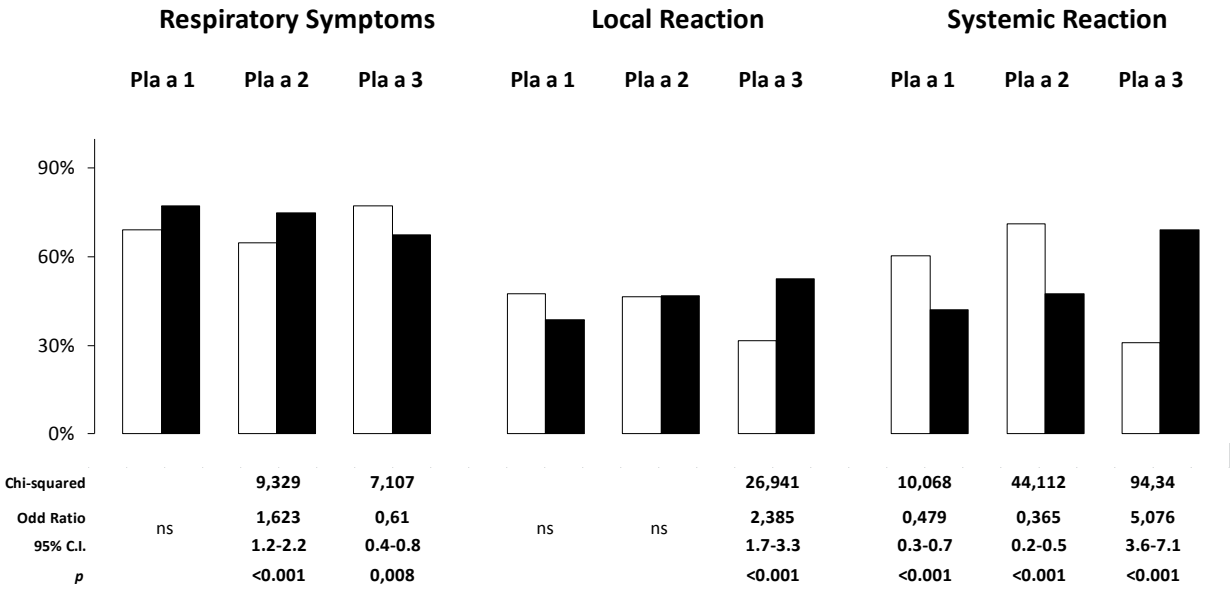

Figure 1

Figure Legend

Figure 1. Frequencies of specific symptoms (Local, Systemic Reaction to food or Respiratory Symptoms) among patients sensitized (black bars) or not sensitized (white bars) to the respective Plane tree molecule. The crude odd ratios and the 95% confidence intervals are reported where significant direct (>1 - red) or inverse (<1 - green) associations were observed.

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