ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Original Article

Molecular sensitization profile of patients with lipid transfer protein syndrome and associated clinical characteristics

Perfil de sensibilização molecular de doentes com síndrome de proteínas de transferência lipídica e associação com características clínicas

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ABSTRACT

Introduction: Lipid transfer proteins (LTPs) can cause a diversity of food allergy phenotypes, broadly defined as LTP syndrome. **Objective:** The aims of this study were to characterize the molecular profile of patients with this syndrome and to evaluate any possible association with clinical phenotypes. **Methods:** Retrospective study of patients followed up from April 2011 to April 2019. Patients with LTP syndrome and sensitization to Pru p 3, diagnosed by ImmunoCAP ISAC® (Phadia, Thermo Fisher Scientific, Sweden), were selected. Statistical analysis was conducted in IBM SPSS® v20. **Results:** One hundred patients were assessed, 64% of which were females, with a mean age 27.2±11.8 years (15% pediatric). Mean age at first reaction was 19.9±10 years. According to clinical presentation, two groups were created: local reaction (LR) (n=28) and systemic reaction (SR) (n=72). The following parameters were analyzed in association with the SR group: LTP sensitization profile, co-sensitization to profilins or PR-10 proteins, presence of atopy, and gender. In univariate analysis, a positive association was found between the SR group, female sex (odds ratio [OR] 2.8, p=0.02), and presence of Jug r 3 (OR 2.6, p=0.03). There was a negative association between the SR group, the presence of Par j 2 (OR 0.16, p < 0.01), and co-sensitization to profilins (OR 0.11, p < 0.01). In multivariate analysis, only the presence of Par j 2 kept statistical significance (OR 0.023, p < 0.01). **Conclusions:** Molecular profile characterization may be useful as a predictor of disease expression in an individual, making a relevant contribution to improved follow-up of these patients. Sensitization to Par j 2 seems to provide protection for the occurrence of SR.

Keywords: Food hypersensitivity, rosacea, allergens.

RESUMO

Introdução: As proteínas de transferência lipídicas (LTP) são causa de uma variedade de fenótipos de alergia alimentar globalmente definidos como síndrome LTP. **Objetivo:** O nosso objetivo é caracterizar o perfil molecular destes doentes e avaliar associação com os fenótipos clínicos. **Metodologia:** Estudo retrospectivo em que foram selecionados doentes com síndrome de LTP e sensibilização ao alergênio molecular pru p 3 em ImmunoCAP ISAC® (Phadia, Thermo Fisher Scientific, Suécia) realizados de abril de 2011 a abril de 2019. A análise estatística foi realizada através do *software* IBM SPSS® v20. **Resultados:** Cem doentes, 64% do sexo feminino, com média de idades à data do exame de 27,2±11,8 anos (idade pediátrica - 15%). A média de idades da primeira reação foi de 19,9±10 anos. Foram constituídos dois grupos com base na apresentação clínica à data da realização do exame: local (LR) n = 28; sistêmica (SR) n = 72. Os seguintes parâmetros foram avaliados em relação ao grupo SR: perfil de sensibilização a LTP, co-sensibilização com profilinas ou PR-10, presença de atopia e gênero. Na análise univariada foi encontrada associação positiva com grupo SR para sexo feminino (*Odds ratio* (OR) 2,8, p = 0,02) e presença de Jug r 3 (OR 2,60, p = 0,03). Associaram-se negativamente à doença sistêmica a presença de Par j 2 (OR 0,16, p < 0,01) e de profilinas (OR 0,11, p < 0,01). Na análise multivariada apenas manteve significado estatístico a presença de par j 2 (OR 0,023, p < 0,01). **Conclusões:** A caracterização do perfil molecular pode ser útil como preditos da expressão da doença, sendo uma importante ferramenta no seguimento destes doentes. A presença de Par j 2 parece ser fator protetor de reação grave.

Descritores: Hipersensibilidade alimentar, *rosaceae*, alérgenos.

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Introduction

Lipid transfer proteins (LTPs) belong to the prolamin protein superfamily and are known to play a role in the defense against bacteria and fungi in plants.[1-4] Furthermore, they are resistant to heat and proteolytic enzymes and are widely distributed throughout the plant kingdom.[1,2,5] LTPs from distinct botanical sources show moderate-to-high degree of homology (35-95%) causing LTP-sensitized patients to have multiple sensitizations and clinically relevant allergies to botanically unrelated plant-derived foods, a condition universally known as LTP syndrome.[3,6]

In the Mediterranean area, LTPs are major panallergens responsible for food allergy in adults.[2,7-9] LTPs are found mainly in epidermal tissues of fruits and are a major allergen in the Rosaceae family. In addition, LTPs are present in nuts, seeds, vegetables, *Hevea brasiliensis* latex, and pollens such as mugwort (Art v 3), plane tree (Pla a 3), *Parietaria judaica* (Par j 2), and olive tree (Ole e 7).[3,5] However, peach LTP, Pru p 3, seems to be the molecule dominating the immune response to LTPs and is considered the marker for severe systemic reaction.[1,10]

LTP syndrome is extremely varied in its clinical expression, varying from asymptomatic presentation to anaphylaxis to one or multiple fruits and vegetables.[9,11] The presence of cofactors, such as exercise, fasting, and non-steroidal anti-inflammatory drugs (NSAIDs) can amplify the clinical relevance of LTP sensitization.[8,9] Component-resolved diagnosis (CRD) is invaluable to address complex syndromes such as LTP syndrome and has been used to try to identify severity markers.[5,12] Studies propose sensitization to more than five LTPs and high levels of ISAC standardized units for IgE (ISU-E) as severity factors,[4,13] and co-sensitization to birch pollen, Bet v 1, profilin and Par j 2 as an indicator of milder disease.[4,14]

Cross-reactivity occurs when a patient sensitized to a particular allergen exhibits an allergic response to a homologous allergen from different species with shared epitopes.[5,10,15] CRD involves the use of defined allergen molecules to determine the individual patient's reactivity profile in order to identify the allergens that are causing disease.[12,16] The use of microarray techniques allows us to better understand cross-reactivity syndromes.[5] ImmunoCAP™ ISAC assay is a multiplex specific IgE (sIgE) test with 112 allergen components from 48 different allergen sources, and its results are analyzed with microarray image analysis software and reported in ISU-E. The LTP family has been widely studied regarding plant-food cross-reactivity.[10]

As sensitization to LTP varies geographically,[9] the aim of this study was to characterize the Portuguese population according to their molecular sensitization profile and its association with clinical allergic phenotypes.

Materials and methods

Study design and patient selection

This is a retrospective study of 100 patients with LTP syndrome who were followed up from April 2011 to April 2019. Patients were selected according to two criteria: 1 - food-allergic reactions to peach and to at least one different plant or food not taxonomically related, 2 - in vitro sensitization to Pru p 3. Food allergy diagnosis was based on clinical symptoms and confirmed by skin prick tests and oral food challenge; these data were collected from clinical records. Sensitization to Pru p 3 (> 0.3 ISU-E) was diagnosed by ImmunoCAP ISAC® (Phadia, Thermo Fisher Scientific, Sweden).

According to clinical presentation, two groups were created: local reaction (LR), which included patients who developed symptoms localized to the oral mucosa; and systemic reaction (SR), including those who developed cutaneous (urticaria, angioedema), respiratory, gastrointestinal, or cardiovascular symptoms or anaphylaxis. The following variables were analyzed in association with the SR group: LTP sensitization profile, co-sensitization to profilins or PR-10 proteins, presence of atopy, and gender.

Statistical analysis

Statistical analysis was performed using IBM SPSS® v20. Continuous variables were expressed as mean and standard deviation (SD) for those with normal distribution and as median and interquartile range (IQR) for those with non-normal distribution. Categorical variables were expressed in absolute frequency and percentages. LTP values were also dichotomized (presence/absence of sensitization, cut-off value > 0.3 ISU-E) and reanalyzed. Univariate analysis was performed using the chi-square test for categorical variables and Mann-Whitney-U test for continuous variables. Multiple logistic regression was performed for the clinical variables to investigate

whether associations with clinical symptoms were present after simultaneously adjusting for other variables of interest. A p-value < 0.05 was considered statistically significant.

Results

Demographic and clinical findings

One hundred patients were included in the study, with a mean age of 27.2±13.9 years and a female predominance (64%). Most patients (73%) had a history of atopic diseases, with allergic rhinitis being the most significant one (72%), followed by asthma (18%), atopic dermatitis (15%), and other food allergies (10%). The mean age at first reaction of LTP syndrome was 19.9±10 years (Table 1).

Rosaceae fruits were the main trigger for first reaction (73%), followed by tree nuts (17%) and peanuts (7%). Regarding clinical expression, there were 28 patients with oral allergy syndrome, 27 with urticaria and/or angioedema, 44 with anaphylaxis, and one with respiratory symptoms. Twenty-eight patients were classified into the LR group, and 72 into the SR group.

Cofactors for reaction were identified in 21 patients: exercise (n=15), NSAID (n=4), or both (n=2).

When comparing LR with SR groups, women showed increased odds of having systemic symptoms, contrary to patients with a history of atopy, who showed decreased odds of having systemic symptoms (Figure 1). There was no significant age difference between the groups.

Molecular sensitization profile

Peach LTP (Pru p 3) had a median value of 2.2 (IQR 3.52) ISU-E, being significantly higher in patients from the SR group. Hazelnut LTP (Cor a 8) also had a significantly higher value for those in the SR group. No other LTP values were related to clinical characteristics. The association between ISU-E values for LTPs and symptoms is summarized in Table 2.

After dichotomization of ISU-E values for LTPs, the most frequently found ones were Jug r 3 (n=66), Pla a 3 (n=63) and Ara h 9 (n=61). The presence of Jug r 3 was positively correlated with the SR group, and presence of Par j 2 negatively correlated with SR. LTPs sensitization profile is summarized in Table 3.

Profilins identified were: Pru p 4, Phl p 12, Bet v 2, and Mer a 1. PR-10 proteins identified were: Bet v 1, Cor a, and Mal d 1. Presence of profilins or PR-10 proteins was considered when at least one of

Table 1
Demographic and clinical data

	Total n=100	Local reaction n=28	Systemic reaction n=72	p-value
Female gender	64 (64%)	13 (46.4%)	51 (70.8%)	0.02
Current age (years), mean±SD	27.2±13.9	27.9±13.9	25±11	0.33
Age at first reaction (years), mean±SD	19.9±10	21.6±11.8	19.3±9.3	0.52
Atopic disease	73 (73%)	25 (89.2%)	48 (67.6%)	0.01
Rhinitis	72 (72%)	24 (85.7%)	48 (67.6%)	0.07
Asthma	18 (18%)	6 (21.4%)	12 (16.7%)	0.66
Atopic dermatitis	15 (15%)	9 (32%)	6 (8.3%)	0.03
Other food allergies	10 (10%)	7 (25%)	3 (4%)	0.04

SD = standard deviation.

them was positive. Profilin sensitization negatively correlated with the SR group.

Associations between the SR group and LTP sensitization profile, co-sensitization to profilins and/or PR-10 proteins, clinical variables, atopic disease, and female gender were analyzed and are represented in Figure 1.

Multivariate analysis

Multiple logistic regression analysis was performed considering molecular sensitization profile, together with gender and history of atopy. Only the presence of Par j 2 sensitization showed a significant relationship with the SR group, regardless of other variables (odds ratio 0.023, $p < 0.01$).

Table 2
Reactivity of LTPs

	Total n=100	Local reaction n=28	Systemic reaction n=72	p-value
Pru p 3	2.2 (IQR 3.52)	1.6 (IQR 3.48)	2.5 (IQR 3.6)	0.03
Cor a 8	1 (IQR 1.42)	0.5 (IQR 1.10)	1.1 (IQR 2.35)	0.02
Jug r 3	1.75 (IQR 2.18)	1.2 (IQR 2.85)	1.9 (IQR 2.02)	0.65
Ara h 9	1.1 (IQR 1.73)	1 (IQR 1.95)	1.1 (IQR 1.69)	0.74
Tri a 14	1.6 (IQR 2.03)	1.9 (IQR 2.28)	1 (IQR 1.8)	0.73
Ole e 7	0.6 (IQR 3.50)	48 (IQR 89.9)	0.5 (IQR 2.7)	0.09
Pla a 3	1.2 (IQR 1.80)	0.85 (IQR 1.30)	1.4 (IQR 1.8)	0.12
Art v 3	1 (IQR 1.15)	0.7 (IQR 1.25)	1.1 (IQR 1.2)	0.78
Par j 2	7.3 (IQR 33.95)	12 (IQR 38.4)	4.5 (IQR 21.2)	0.36

Units in ISU-E.
LTP = lipid transfer protein; IQR = interquartile range.

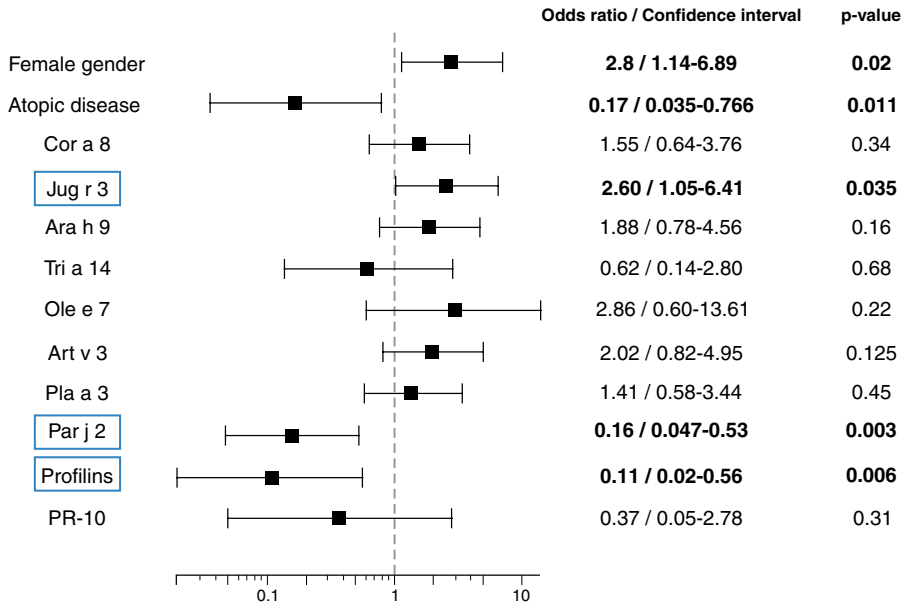

Figure 1
Graphic representation of bivariate analysis between variables of interest and systemic reaction (SR) group

Table 3
LTP sensitization profile (dichotomized)

	Total n=100	Local reaction n=28	Systemic reaction n=72	p-value
Cor a 8	47	11	36	0.34
Jug r 3	66	14	52	0.04
Ara h 9	61	14	47	0.16
Par j 2	14	9	5	< 0.01
Tri a 14	8	3	5	0.68
Ole e 7	15	2	13	0.22
Pla a 3	63	16	47	0.13
Art v 3	48	10	38	0.45
Co-sensitization				
* Profilins	8	6	2	< 0.01
** PR-10	4	2	2	0.31

LTP = Lipid transfer proteins
* At least one: Pru p 4, Phl p 12, Bet v 2, Mer a 1.
** At least one: Bet v 1, Cor a, Mal d 1.

Discussion

Our data showed that there was a significant negative association between sensitization to Par j 2 and SR in multivariate analysis. Furthermore, in bivariate analysis, history of atopy and sensitization to profilins was negatively associated with SR, and presence of Jug r 3 and female gender were positively associated with SR.

Previous studies failed to find differences between genders; hence, this finding requires validation in larger prospective studies.

The independent association of sensitization to Par j 2 with milder clinical presentation has previously been described by Scala et al.[4] Although sensitization to Jug r 3 has not been associated with SR, the study also reported it reached comparable levels of reactivity comparable to those of Pru p 3, potentially suggesting walnut as an alternative source of sensitization to LTPs.

Consistent with findings by Basagaña et al.[17] and Scala et al.[4], sensitization to profilins is apparently related to milder symptoms. Since we found a low reactivity to PR-10 proteins (probably due to geographical reasons), the same relationship could not be proved for these proteins. Both studies also report higher levels of sIgE/ISU-E to LTPs in patients with SR. We found that ISU-E levels of Pru p 3 and Cor a 8 were significantly higher in those with SR; however, that was not true for other LTPs.

Although olive and wall pellitory, along with grass pollen, are the main plants responsible for pollinosis in Portugal,[8] plane tree and mugwort were the most frequent pollens identified in our population. Art v 3 and Pla a 3 share a 41% and 46% sequence identity, respectively, with Pru p 3.[18] Previous studies found an association between Pru p 3 and sensitization to both Art v 3 and Pla a 3, further hypothesizing their role as primary sensitizers in patients with LTP syndrome, or even acting as triggers or enhancers of the disease.[4,12,19] Despite that, we found no association between sensitization to either Art v 3 or Pla a 3 and occurrence of systemic disease.

History of atopy showed association with milder symptoms. Previous studies showed similar results, implying that respiratory allergy can be an indicator of milder disease.[20]

As a limitation of our study, we identify its retrospective nature and a possible selection bias, since asymptomatic or milder symptoms patients were not indicated to perform microarray analysis. Larger prospective studies are necessary to better characterize patients with LTP syndrome.

LTP syndrome has a complex clinical pattern with several poorly defined aspects. Overcoming difficulty to predict which patients with more severe symptoms would benefit from immunotherapy is the focus of most researchers. CRD can be an important tool in addressing this problem, since specific patterns seem to relate to clinical phenotypes.

As it appears to suffer from geographical variations, it is important to characterize different populations across the globe. We hope that our paper, as well as other studies, can contribute to a widespread knowledge on recombinant allergens, since this could have potential implications for the diagnosis and for therapeutic options for allergic patients.

Conclusion

In our study, we demonstrate that the analysis of sensitization profile using molecular components increases the diagnosis accuracy in patients with LTP syndrome and sensitization to Pru p 3, allowing a possible correlation with severity reaction.

The sensitization to Par j 2 seems to provide protection for the occurrence of SR, while presence sensitization to Jug r 3 is associated with SR. We emphasize that ISU-E levels of Pru p 3 and Cor a 8 were also significantly higher in patients with SR, although the same association has not been found for other LTPs.

Further studies are needed to compare the different sensitization profiles and severity reaction in these patients.

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