DE GRUYTER
OPEN
Romanian Journal of Rhinology, Vol. 6, No. 21, January - March 2016
DOI: 10.1515/rjr-2016-0002

LITERATURE REVIEW

Polcalcins as pollen panallergens in allergic rhinitis

Florin-Dan Popescu, Mariana Vieru
Department of Allergology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

ABSTRACT

Polcalcins are highly cross-reactive calcium-binding allergen components specifically expressed in pollen from trees, grasses and weeds. The grass allergen component rPhl p 7, a recombinant non-glycosylated calcium-binding protein of 2-EF-hand type, is the most cross-reactive polcalcin and may be used as a polcalcin biomarker of IgE-mediated hypersensitivity. Polcalcin sensitization, which appears to be linked to geographical factors, level and time of pollen exposure, has to be assessed in allergic rhinitis patients with multiple pollen sensitizations and may be useful for a better targeted prescription of allergen immunotherapy.

KEYWORDS: polcalcin, pollen, allergic rhinitis

INTRODUCTION

Pollen grains of wind-pollinated plants, named anemophilous plants, are important outdoor sources of **aeroallergens**, and exposure to such type of pollen depends on the plant species, wild spread or cultivation, geographic area, altitude, air currents, temperature, precipitation and other weather events. Pollen extracts for European and North American skin testing panels include native extracts of pollen from trees/shrubs from *Betulaceae*, *Oleaceae*, *Platanaceae* and *Cupressaceae* families, grass pollen from *Poaceae* family, and pollen from weeds belonging to *Asteraceae* and *Urticaceae* families[1-7].

Pollen IgE sensitization in patients with allergic rhinitis is conventionally revealed by skin prick testing with natural allergen extracts and/or native aeroallergen-specific serum IgE antibody tests, and it is relevant only in case of corresponding suggestive symptoms. Serum specific IgE to allergen components, detected using singleplex or multiplex assays, have proven utility as component-resolved diagnosis biomarkers in the assessment of sensitization to pollen allergens, allowing the clinician to confirm genuine sensitization to a specific allergen plant source and guide an accurate prescription of allergen immunotherapy, important in many regions of the world with great plant biodiversity and/or where pollen seasons may overlap. Component-resolved specific diagnosis is also important due to the high heterogeneity of geographically-related sensitization profiles. Moreover, molecular-based diagnosis improves the understanding of clinically relevant IgE sensitization to cross-reactive allergen components from aeroallergen sources and foods[8-12].

Candidates biomarkers for **molecular allergy diagnostics** to identify specific allergen sensitivities to major pollen allergen markers are Bet v 1 (*Fagales* trees from the *Betulaceae* and *Fagaceae* families, such as birch, hazel, alder, beech and oak), Ole e 1 (*Oleaceae* family plants, such as olive and ash), Jun a 1 (cedar, cypress), Phl p 1/5 (*Pooideae* subfamily grasses), Amb a 1 (ragweed), Art v 1 (mugwort) and Par j 2 (pellitory)[9,12,13]. Some opinions consider that the added value of component-resolved diagnosis mainly lies in the correct identification of allergic rhinitis patients

Corresponding author: Florin-Dan Popescu, MD, PhD, Associate Professor, Department of Allergology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania, Address: "Nicolae Malaxa" Clinical Hospital, 12 Sos. Vergului, Sector 2, Bucharest, Romania
e-mail: florindanpopescu@allergist.com

who are not sensitized to major pollen allergens and in whom prescription of allergen immunotherapy is anticipated, as patients sensitized to minor allergens alone are unlikely to receive sufficient amounts of responsible allergen to achieve a successful outcome [10]. The application of *in vitro* component-resolved approach to the selection of patients more likely to better respond to allergen immunotherapy has been suggested, with the hypothesis that multisensitized patients with broad patterns of IgE reactivity to multiple minor allergens, or cross-reactive panallergens such as profilins or polcalcins, may show poor responses to allergen immunotherapy[9,13]. In some authors experience, at least for grass pollen sublingual immunotherapy, this type of therapy was found to be efficacious irrespective of the patients' baseline IgE sensitization profiles[14,15]. Molecular allergy diagnosis can change allergen immunotherapy prescription in complex pollen areas[16]; therefore, the hypothesis that component-resolved diagnosis-guided prescription improves specific immunotherapy efficacy deserves to be tested[17]. It is important to mention that the molecular-based allergy diagnosis introduced the possibility of better targeted prescription of allergen immunotherapy[18].

PANALLERGEN FAMILIES

Members of the **panallergen** families include profilins, ubiquitous proteins in all pollens and numerous plant foods, and polcalcins, Ca^{2+}-binding proteins present in all pollens. In the case of IgE sensitizations to plant panallergens, minor allergens profilin and/or polcalcin, diagnostic pollen extracts lack analytical specificity and show all-over positive inducing misleading results. Some pollen-allergic individuals produce positive sensitization tests (skin prick tests, IgE serology) to numerous or occasionally many pollen types, independent of their plant taxonomic relationship. Approximately 15% to 30% of pollen-allergic individuals are sensitized to profilin, and about 5%, and according to some authors up to 20%, are sensitized to polcalcins. In less than 2%, sensitization occurs to both panallergens in the same individual[12,19].

IgE sensitization to polcalcin and profilin usually occurs in regions with high grass pollen exposure, and this leads to the induction of a broad IgE antibody repertoire specific for grass pollen with numerous molecular allergen components[12,20,21]. Sensitization to profilin seems to occur sooner than sensitization to polcalcin, thus sensitization to this calcium-binding allergen may serve as a marker in the long-term development of allergic rhinitis[22]. In addition, profilin-sensitized allergic individuals can develop oropharyngeal and, rarely, systemic symptoms after the ingestion of various plant foods[11,12,23].

If we consider the panallergen families, only profilins are distributed ubiquitously throughout the plant kingdom. This class is responsible for allergic reactions to a multitude of evolutionary unrelated pollen and food allergen sources. Being present exclusively in pollen grains of plants, polcalcins are not involved in pollinosis-associated plant food allergies, but may be discussed in allergic reactions to ingestion of edible products containing pollen grains of specific plants[9,11,24]. In a case of urticaria to *Stigma maydis* infusion, high level of specific IgE against polcalcin rPhl p 7, calcium-binding protein likely to cross-react with Zea m 7 from maize pollen, was reported[24]. Moreover, cross-reactivity between the pollen of wind-pollinated weeds and other *Asteraceae* insect-pollinated plants is a major mechanism of bee pollen supplements-induced anaphylaxis, likely attributable to pollen allergen components, such as polcalcins, profilins and lipid-transfer proteins[11,25].

POLCALCINS AND ALLERGIC RHINITIS

Polcalcins belong to a Ca^{2+}-binding protein (CBP) family sharing common domains termed EF-hands (helix-loop-helix motifs, which consist of two perpendicularly placed alpha-helices and an inter-helical loop forming a single calcium-binding site). Due to their ability to bind and transport calcium as well as to interact with a variety of ligands in a calcium-dependent manner, CBPs have important biological functions in eukaryotic cells. After parvalbumin, an important fish allergen, calcium-binding allergens were discovered in pollen grains of trees, grasses and weeds and, more recently, as autoallergens in humans[23,26].

The **polcalcin** family is one of the most relevant families of calcium-binding allergens, and their allergenicity is primarily associated with the Ca^{2+}-bound form of the protein. The biologic function of polcalcins is still unclear. However, due to their pollen-specific localization and their ability to bind calcium, it has been proposed that they are involved in the control of intracellular calcium levels during pollen germination and in the signalling processes in the pollen tube growth. These cross-reactive molecular allergen components have been described as panallergens in various pollen sources (grass, tree and weed pollen), not in plant foods or latex (polcalcin expression seems to be restricted to pollen), are highly conserved among species and their amino acid sequences share a high degree of sequence identity across species, of about 60%-90%[23,27,28]. Rarely occurring as sole sensitizers in patients with clinical symptoms, polcalcins are minor allergens, only about 5-20% of pollen sensitized individuals having IgE responses to these cross-reactive allergenic molecules[12,19]. The clinical relevance of

polcalcin sensitization is linked to geographical factors and to the level of exposure to different allergenic sources. It has been shown that among weed pollen-allergic patients, IgE reactivity to polcalcins from mugwort and ragweed, is much higher in Italians (21 to 28%) when compared to Austrians (10%)[29]. Monitoring of polcalcin-sensitized patients should be performed as these individuals are at risk of developing multiple pollen sensitizations[23].

In agreement with the number of calcium-binding EF-hand motifs, at least three types of polcalcins were described in pollen: polcalcins displaying two (birch Bet v 4, alder Aln g 4, olive tree Ole e 3, ash Fra e 3, lilac Syr v 3, Timothy grass Phl p 7, Bermuda grass Cyn d 7, ragweed Amb a 9, mugwort Art v 5, goosefoot Che a 3), three (birch Bet v 3, ragweed Amb a 10), and four (juniper Jun o 4, olive tree Ole e 8) calcium-binding domains. The three-dimensional structure of polcalcin is characterized by alpha-helices. The monomer with a molecular weight of 8 to 9 kDa, reveals the typical polcalcin structural domain. The monomeric Bet v 4 from birch is composed of two symmetrically arranged EF-hands bringing the two bound calcium ions into spatial proximity. Dimeric Timothy grass Phl p 7 contains two of these basic structural domains, while four of these domains were observed in the tetrameric goosefoot Che a 3[23]. It seems noteworthy that the calcium-binding property of polcalcins affects both the profilin IgE-reactivity and thermostability. Calcium association induces conformational changes in the three-dimensional structure. Two conformational states of calcium-binding proteins can be distinguished, the closed calcium-free "apo", and the open calcium-associated "holo" forms. It was suggested that the apo-forms are less stable to thermal denaturation and display decreased IgE-reactivity when compared to their calcium-bound counterparts[19, 23, 26]. Moreover, changes in polcalcin quaternary structure, termed molecular metamorphosis, are induced and controlled by a combination of EF-hand rearrangements and domain swapping[30].

The hierarchy of IgE cross-reactivity was established for important polcalcins (rPhl p7 > rAln g 4 > rJun o 4 > rBet v 3), and identified rPhl p 7 as the EF-hand allergen component containing most IgE epitopes[31]. From the pollen polcalcins available for routine use, recombinant non-glycosylated calcium-binding protein of 2-EF-hand type, the grass allergen component rPhl p 7, is the most cross-reactive polcalcin and it may serve as efficient biomarker of IgE-mediated hypersensitivity to this entire group of homologous proteins[9, 23]. Recently, *Sorghum bicolor* polcalcin (Sor b 7) was found to be closely related with polcalcins from *Cynodon dactylon*, *Phleum pratense* and *Oryza sativa* grass species[32].

CONCLUSIONS

In conclusion, allergists and ENT specialists must know that polcalcins are highly cross-reactive calcium-binding allergens specifically expressed in pollen tissues. Polcalcin sensitization, which seems to be linked to geographical factors, level and time of pollen exposure, has to be assessed in patients with multiple pollen sensitizations[9,23]. The question of whether sensitization to this minor allergen should preclude subsequent allergen immunotherapy has never been evaluated prospectively. Retrospectively, it was suggested in some publications that less successful courses of allergen immunotherapy may be associated with panallergen sensitization to profilin and/or polcalcin[9,12,33]. Monosensitization to these pollen panallergens is considered to be uncommon, and is presumed to be less suitable for allergen immunotherapy, because the content of profilin and polcalcin in pollen extracts and their subsequent therapeutic effect is not so clear[12].

Conflict of interest: The authors have no conflict of interest.

Contribution of authors: All authors have equally contributed to this work.

REFERENCES

1. D'Amato G., Cecchi L., Bonini S., Nunes C., Annesi-Maesano I., Behrendt H., Liccardi G., Popov T., van Cauwenberge P. - Allergenic pollen and pollen allergy in Europe. Allergy., 2007;62(9):976-990.
2. Bernstein I.L., Li J.T., Bernstein D.I., Hamilton R., Spector S.L., Tan R., Sicherer S., Golden D.B., Khan D.A., Nicklas R.A., Portnoy J.M., Blessing-Moore J., Cox L., Lang D.M., Oppenheimer J., Randolph C.C., Schuller D.E., Tilles S.A., Wallace D.V., Levetin E., Weber R.; American Academy of Allergy, Asthma and Immunology; American College of Allergy, Asthma and Immunology. - Allergy diagnostic testing: an updated practice parameter. Ann. Allergy. Asthma Immunol., 2008;100(3 Suppl 3):S1-148.
3. Bousquet J., Heinzerling L., Bachert C., Papadopoulos N.G., Bousquet P.J., Burney P.G., Canonica G.W., Carlsen K.H., Cox L., Haahtela T., Lodrup Carlsen K.C., Price D., Samolinski B., Simons F.E., Wickman M., Annesi-Maesano I., Baena-Cagnani C.E., Bergmann K.C., Bindslev-Jensen C., Casale T.B., Chiriac A., Cruz A.A., Dubakiene R., Durham S.R., Fokkens W.J., Gerth-van-Wijk R., Kalayci O., Kowalski M.L., Mari A., Mullol J., Nazamova-Baranova L., O'Hehir R.E., Ohta K., Panzner P., Passalacqua G., Ring J., Rogala B., Romano A., Ryan D., Schmid-Grendelmeier P., Todo-Bom A., Valenta R., Woehrl S., Yusuf O.M., Zuberbier T., Demoly P.; Global Allergy and Asthma European Network; Allergic Rhinitis and its Impact on Asthma. - Practical guide to skin prick tests in allergy to aeroallergens. Allergy., 2012;67(1):18-24.
4. Heinzerling L., Mari A., Bergmann K.C., Bresciani M., Burbach G., Darsow U., Durham S., Fokkens W., Gjomarkaj M., Haahtela T., Bom A.T., Wöhrl S., Maibach H., Lockey R. - The skin prick test - European standards. Clin. Transl. Allergy., 2013;3(1):3.
5. Passali D., Bellusi L.M., Kern E.B., Sarafoleanu C., Popescu F.D. -

Rhinosinusal inflammation and infections: modern thinking and current treatment. Bucharest: Editura Academiei Romane, 2013;p.101-167.

6. Popescu F.D., Tamiian M., Albert K. - Multilingual approach to the panel of aeroallergens suggested for skin prick testing in European Union patients. Romanian Journal of Rhinology., 2014;14(4):93-98.
7. Popescu F.D. - European standards and North American practice parameters for skin prick testing panels in allergic rhinitis and asthma. Romanian Journal of Rhinology., 2015;17(5):19-23.
8. Canonica G.W., Ansotegui I.J., Pawankar R., Schmid-Grendelmeier P., van Hage M., Baena-Cagnani C.E., Melioli G., Nunes C., Passalacqua G., Rosenwasser L., Sampson H., Sastre J., Bousquet J., Zuberbier T.; WAO-ARIA-GA2LEN Task Force: Allen K.,. Asero R, Bohle B.,. Cox L, de Blay F., Ebisawa M., Maximiliano-Gomez R., Gonzalez-Diaz S., Haahtela T., Holgate S., Jakob T., Larche M., Matricardi P.M., Oppenheimer J., Poulsen L.K., Renz H.E., Rosario N., Rothenberg M., Sanchez-Borges M., Scala E., Valenta R. - A WAO - ARIA - GA²LEN consensus document on molecular-based allergy diagnostics. World Allergy Organ. J., 2013;6(1):17.
9. Popescu F.D. - Molecular biomarkers for grass pollen immunotherapy. World J. Methodol., 2014;4(1):26-45.
10. Van Gasse A.L., Mangodt E.A., Faber M., Sabato V., Bridts C.H., Ebo D.G. - Molecular allergy diagnosis: status anno 2015. Clin. Chim. Acta., 2015;444:54-61.
11. Popescu F.D. - Cross-reactivity between aeroallergens and food allergens. World J. Methodol., 2015;5(2):31-50.
12. Kleine-Tebbe J., Matricardi P.M., Hamilton R.G. - Allergy work-up including component-resolved diagnosis: how to make allergen-specific immunotherapy more specific. Immunol. Allergy Clin. North. Am., 2016;36(1):191-203.
13. Douladiris N., Savvatianos S., Roumpedaki I., Skevaki C., Mitsias D., Papadopoulos N.G. - A molecular diagnostic algorithm to guide pollen immunotherapy in southern Europe: towards component-resolved management of allergic diseases. Int Arch Allergy Immunol., 2013;162:163-172.
14. Baron-Bodo V., Horiot S., Lautrette A., Chabre H., Drucbert A.S., Danzé P.M., Sénéchal H., Peltre G., Galvain S., Zeldin R.K., Horak F., Moingeon P. - Heterogeneity of antibody responses among clinical responders during grass pollen sublingual immunotherapy. Clin. Exp. Allergy., 2013;43(12):1362-1373.
15. Moingeon P. - Biomarkers for Allergen Immunotherapy: A "Panoromic" View. Immunol. Allergy Clin. North. Am., 2016;36(1):161-79.
16. Sastre J., Landivar M.E., Ruiz-García M., Andregnette-Rosigno M.V., Mahillo I. - How molecular diagnosis can change allergen-specific immunotherapy prescription in a complex pollen area. Allergy., 2012;67(5):709-711.
17. Stringari G., Tripodi S., Caffarelli C., Dondi A., Asero R., Di Rienzo Businco A., Bianchi A., Candelotti P., Ricci G., Bellini F., Maiello N., Miraglia del Giudice M., Frediani T., Sodano S., Dello Iacono I., Macrì F., Peparini I., Povesi Dascola C., Patria M.F., Varin E., Peroni D., Comberiati P., Chini L., Moschese V., Lucarelli S., Bernardini R., Pingitore G., Pelosi U., Tosca M., Cirisano A., Faggian D., Travaglini A., Plebani M., Matricardi P.M.; Italian Pediatric Allergy Network (I-PAN). - The effect of component-resolved diagnosis on specific immunotherapy prescription in children with hay fever. J. Allergy Clin. Immunol., 2014;134(1):75-81.
18. Passalacqua G., Canonica G.W. - Allergen immunotherapy: history and future developments. Immunol. Allergy Clin. North Am., 2016;36(1):1-12.
19. Neudecker P., Nerkamp J., Eisenmann A., Nourse A., Lauber T., Schweimer K., Lehmann K., Schwarzinger S., Ferreira F., Rösch P. - Solution structure, dynamics, and hydrodynamics of the calcium-bound cross-reactive birch pollen allergen Bet v 4 reveal a canonical monomeric two EF-hand assembly with a regulatory function. J. Mol. Biol., 2004;336(5):1141-57.
20. Hatzler L., Panetta V., Lau S., Wagner P., Bergmann R.L., Illi S., Bergmann K.E., Keil T., Hofmaier S., Rohrbach A., Bauer C.P., Hoffman U., Forster J., Zepp F., Schuster A., Wahn U., Matricardi P.M. - Molecular spreading and predictive value of preclinical IgE response to Phleum pratense in children with hay fever. J. Allergy Clin. Immunol., 2012;130(4):894-901.
21. Tripodi S., Frediani T., Lucarelli S., Macrì F., Pingitore G., Di Rienzo Businco A., Dondi A., Pansa P., Ragusa G., Asero R., Faggian D., Plebani M., Matricardi P.M. - Molecular profiles of IgE to Phleum pratense in children with grass pollen allergy: implications for specific immunotherapy. J. Allergy Clin. Immunol., 2012;129(3):834-839.
22. Orovitg A., Guardia P., Barber D., de la Torre F., Rodríguez R., Villalba M., Salcedo G., Monteseirìn J., Conde J. - Enhanced diagnosis of pollen allergy using specific immunoglobulin E determination to detect major allergens and panallergens. J. Investig. Allergol. Clin. Immunol., 2011;21(4):253-9.
23. Hauser M., Roulias A., Ferreira F., Egger M. - Panallergens and their impact on the allergic patient. Allergy Asthma Clin. Immunol., 2010;6(1):1.
24. Popescu F.D., Vieru M. - Pooideae molecular sensitization profile in selected rhinitis patients with allergy to grass pollen and maize-derived edible products. Clin. Transl. Allergy., 2015;5(Suppl 4):P21.
25. Choi J.H., Jang Y.S., Oh J.W., Kim C.H., Hyun I.G. - Bee pollen-induced anaphylaxis: a case report and literature review. Allergy Asthma Immunol. Res., 2015;7(5):513-517.
26. Valenta R., Hayek B., Seiberler S., Bugajska-Schretter A., Niederberger V., Twardosz A., Natter S., Vangelista L., Pastore A., Spitzauer S., Kraft D. - Calcium-binding allergens: from plants to man. Int. Arch. Allergy Immunol., 1998;117(3):160-166.
27. Parody N., Fuertes M.A., Alonso C., Pico de Coaña Y. - Analysis of calcium-induced conformational changes in calcium-binding allergens and quantitative determination of their IgE binding properties. Methods Mol. Biol., 2013;963:115-125.
28. Mas S., Garrido-Arandia M., Batanero E., Purohit A., Pauli G., Rodríguez R., Barderas R., Villalba M. - Characterization of profilin and polcalcin panallergens from ash pollen. J. Investig. Allergol. Clin. Immunol., 2014;24(4):257-266.
29. Wopfner N., Gruber P., Wallner M., Briza P., Ebner C., Mari A., Richter K., Vogel L., Ferreira F. - Molecular and immunological characterization of novel weed pollen pan-allergens. Allergy., 2008;63(7):872-881.
30. Magler I., Nüss D., Hauser M., Ferreira F., Brandstetter H. - Molecular metamorphosis in polcalcin allergens by EF-hand rearrangements and domain swapping. FEBS J., 2010;277(12):2598-2610.
31. Tinghino R., Twardosz A., Barletta B., Puggioni E.M., Iacovacci P., Butteroni C., Afferni C., Mari A., Hayek B., Di Felice G., Focke M., Westritschnig K., Valenta R., Pini C. - Molecular, structural, and immunologic relationships between different families of recombinant calcium-binding pollen allergens. J. Allergy Clin. Immunol., 2002;109(2):314-320.
32. Sekhar B.C., Sachin Ch, Raman B.V., Bondili J.S. - Molecular characterization and in silico analysis of Sorghum Panallergens: Profilin and Polcalin. Indian J. Exp. Biol., 2015;53(11):726-731.
33. Schmid-Grendelmeier P. - Rekombinante Allergene – Routinediagnostik oder Wissenschaft? Hautarzt., 2010;61:946-953.