

Phl p 4: An early indicator of grass pollen allergy?



Angelico Mendy, MD, PhD, and Darryl C. Zeldin, MD Research Triangle Park, NC

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Grass pollen is ubiquitous and is a major cause of respiratory allergy in temperate regions where grass pollen sensitization accounts for nearly two-thirds of all sensitizations to outdoor allergens.¹ Of the numerous grass species, the few allergenically relevant belong primarily to the *Pooideae* subfamily, which includes timothy grass (*Phleum pratense* [Phl p]).¹ To date, 13 Phl p molecules have been described and the major ones include Phl p 1, Phl p 4, and Phl p 5.^{1,2} Phl p 1 is the most prevalent Phl p molecule; it belongs to group 1 allergens that are recognized in 95% of patients with grass pollen allergy and thus can be used as a diagnostic marker.² Phl p 4 is a glycosylated protein with cross-reactive carbohydrate determinants (CCDs) that has a high frequency of IgE reactivity and low allergenicity because sensitized individuals develop IgE antibodies against the poorly antigenic CCDs.^{1,2} Contrary to Phl p 4, Phl p 5 is highly allergenic and consists of 2 active domains that result in the production of high IgE levels.^{1,2} The remaining timothy grass allergens are minor (Phl p 2, Phl p 6, and Phl p 11) or non-grass-specific cross-reacting allergens (Phl p 7 and Phl p 12).^{1,2} The chemical name, function, and IgE positivity of Phl p molecules are detailed in Table I.

In recent years, the use of allergen molecules of Phl p instead of its extract for the diagnosis and/or early prediction of allergic diseases, known as component-resolved diagnosis (CRD), has become mainstream.^{3,4} Previously, in the German Multicentre Allergy Study, Hatzler et al³ applied the CRD approach to demonstrate that weak mono- or oligomolecular sensitization to Phl p often occurs in children years before the onset of seasonal allergic rhinitis. They also showed that the IgE response to Phl p in childhood increases in molecular complexity over years through “molecular spreading” to cause a rise in the number of recognized molecules.³ In the April 2020 issue of the *Journal*, Westman et al⁵ investigated the usefulness of sensitization to Phl p molecules during early childhood in the prediction of allergic rhinitis to grass pollen in later childhood and adolescence.⁵ The study included a subset of 763 participants from the BAMSE (Barn/Children, Allergy/Asthma, Milieu, Stockholm, Epidemiology)

birth cohort who had serum collected and analyzed for IgE specific to the different Phl p allergen molecules and to timothy grass extract at ages 4, 8, and 16 years.⁵ They defined grass pollen allergic rhinitis by parental report of symptoms with exposure to grass. The serum samples were analyzed for IgE reactivity against Phl p molecules, CCD-containing molecules, and timothy extracts. In keeping with previous investigations,³ the study reported that sensitization to Phl p 1, Phl p 4, and Phl p 5 occurred early in childhood and that the prevalence of sensitization to any Phl p molecule, which increased with age, tended to be higher than sensitization to timothy extracts. In their analysis, the authors found that sensitization to Phl p 1 and Phl p 4 at age 4 years was associated with subsequent allergic rhinitis, even after adjustment for other Phl p molecules. The study concluded that in addition to Phl p 1, Phl p 4 might be an unrecognized early predictor of grass pollen allergy.⁵ These results are interesting and potentially clinically relevant; however, the study has both strengths and limitations and raises important questions that need to be discussed.

The finding of Phl p 4 as an early predictor of grass pollen is novel and surprising, especially given the low allergenicity of the molecule. Although the biological and clinical importance of Phl p 1 has been previously demonstrated, the potential clinical relevance of Phl p 4 before this report was doubtful.³ Westman et al⁵ had the advantage of using a sample from the BAMSE study, a large population-based Swedish birth cohort of 4089 children born between 1994 and 1996. Over the 16-year follow-up, the study authors reported a high response rate (78%) and performed a well-characterized exposure assessment. The IgE testing comprehensively analyzed 8 Phl p molecules and 5 CCD molecules using the MedALL chip, an assay that allows for the simultaneous determination of different allergen molecules with small amounts of serum.⁶ Using appropriate statistical analyses to determine the association of Phl p molecules with subsequent allergic rhinitis, the authors reported not only highly significant but also consistent findings. For both Phl p 1 and Phl p 4, sensitization at age 4 years was associated with increased odds of allergic rhinitis at ages 8 and 16 years.⁵ Likewise, sensitization at age 8 years was positively associated with allergic rhinitis at age 16 years, and the results remained significant even after mutually adjusting for the significant Phl p molecules in their analysis.

Although the finding on Phl p 4 is novel, a major limitation of the study is that the determination of Phl p 4 sensitization was based on the natural or native form of Phl p 4 (nPhl p 4), which produces false-positive results due to cross-reactivity of the CCDs with other plant allergens.² Indeed, in their study, more than 50% of the children aged 4 and 8 years had concomitant IgE reactivity with the tested CCDs. Therefore, the results need to be confirmed with more specific and reliable testing using recombinant Phl p 4.² Moreover, the study by Westman et al was conducted on a subset of 763 children of the 4089 BAMSE participants. Although the authors compared the subsample with the overall BAMSE population that included the study participants, they did not rule out the

From the National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park.

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Corresponding author: Darryl C. Zeldin, MD, NIEHS/NIH, 111 Alexander Dr, A214, Research Triangle Park, NC 27709. E-mail: zeldin@niehs.nih.gov.

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TABLE I. Chemical name, function, and IgE positivity of timothy grass allergens

Timothy grass	Chemical name	Function	IgE positivity*
Major specific allergens			
Phl p 1	CCD-bearing protein	Beta-expansin	95%
Phl p 4	CCD-bearing protein	Berberine bridge enzyme	70%-75%
Phl p 5	Group 5 (Ribonuclease)	Ribonuclease	50%-95%
Minor specific allergens			
Phl p 2	Group 2 (expansin-like protein)	Unknown	55%-65%
Phl p 3	Group 3 (expansin-like protein)	Unknown	60%
Phl p 6	Group 6	Unknown	44%-75%
Phl p 11	Ole e 1-like protein	Trypsin inhibitor	32%-43%
Cross-reactive allergens			
Phl p 7	Polcalcin	Calcium-binding protein	7%-10%
Phl p 12	Profilin	Actin-binding protein	15%

*Percent of IgE positivity among grass pollen-sensitized individuals.

possibility of selection bias by comparing the baseline characteristics of the 763 participants with the remaining 3326 nonparticipants. Furthermore, 1699 children from BAMSE had sera available at all 3 time points and of them, only 800 were randomly sampled for microarray testing. Whether these 800 children differ from the remaining nonselected 899 children is also unclear. Moreover, the generalizability of the findings to other populations exposed to temperate grass or to racially diverse populations needs to be firmly established. The BAMSE cohort only included white children born in the Swedish city of Stockholm; whether these findings can be replicated in multiethnic or rural populations is uncertain, especially given the high cross-reactivity of nPhl p 4.² Finally, the outcome of allergic rhinitis to grass pollen was defined by parental report, which has previously been shown to underestimate the true prevalence of the condition.⁷ In this regard, Westman et al⁵ compared parental report of grass pollen allergic rhinitis with a combination of allergic rhinitis and sensitivity to timothy extracts and found high sensitivity, specificity, and positive predictive value at age 16 years.

In summary, grass pollen allergy is common and is a major cause of allergic rhinitis. Recent molecular diagnosis testing and CRD might help in the diagnosis or early prediction of the condition. In a large population-based study, Westman et al reported that sensitization not only to Phl p 1 but also to Phl p 4, a highly cross-reactive and poorly antigenic molecule, might be an

early predictor of grass pollen allergen. The determination of Phl p 4 sensitization using a poorly sensitive test based on nPhl p 4 and the limited generalizability of the findings to diverse populations are study weaknesses that warrant further investigation before Phl p 4 can be clinically used as an early predictor of grass pollen allergic rhinitis.

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