Total transcriptome, proteome, and allergome of Johnson grass pollen, which is important for allergic rhinitis in subtropical regions

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Background: Genomic data are lacking for many allergen sources. To circumvent this limitation, we implemented a strategy to reveal the repertoire of pollen allergens of a grass with clinical importance in subtropical regions, where an increasing proportion of the world's population resides.
Objective: We sought to identify and immunologically characterize the allergenic components of the Panicoideae Johnson grass pollen (JGP; *Sorghum halepense*).
Methods: The total pollen transcriptome, proteome, and allergome of JGP were documented. Serum IgE reactivities with pollen and purified allergens were assessed in 64 patients with grass pollen allergy from a subtropical region.
Results: Purified Sor h 1 and Sor h 13 were identified as clinically important allergen components of JGP with serum IgE reactivity in 49 (76%) and 28 (43.8%), respectively, of patients with grass pollen allergy. Within whole JGP, multiple cDNA transcripts and peptide spectra belonging to grass pollen allergen families 1, 2, 4, 7, 11, 12, 13, and 25 were identified. Pollen allergens restricted to subtropical grasses (groups 22-24) were also present within the JGP transcriptome and proteome. Mass spectrometry confirmed the IgE-reactive components of JGP included isoforms of Sor h 1, Sor h 2, Sor h 13, and Sor h 23.
Conclusion: Our integrated molecular approach revealed qualitative differences between the allergenic components of JGP and temperate grass pollens. Knowledge of these newly identified allergens has the potential to improve specific diagnosis and allergen immunotherapy treatment for patients with grass pollen allergy in subtropical regions and reduce the burden of allergic respiratory disease globally. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *Allergome, allergen components, allergic rhinitis, IgE, Johnson grass pollen, pollen allergy, proteome, transcriptome*

The incidences of allergic rhinitis (AR) and asthma have reached a plateau in developed nations, but the prevalence of allergic respiratory diseases shows greater variability in countries with emerging economies.[1] AR afflicts 500 million persons worldwide, adversely effecting patient health and quality of life.[2-5] The disease carries a substantial financial burden through lost productivity and increased health care costs.[6,7]

Grass pollen allergen sources vary according to climatic region, with subtropical grass pollens being more abundant in the subtropics.[8-10] Current demographic data indicate that the population of subtropical climates is increasing[11] and that tropical zones are widening polewards.[12] For example, the population in subtropical states of the United States is estimated at approximately 52.3 million, having increased by 18.3% between 2000 and 2010 (US Census Bureau for Florida, Louisiana, Mississippi, and Texas).[13] The contribution of subtropical grasses to allergic respiratory diseases, such as AR and asthma, is predicted to increase with global warming because of a competitive advantage for subtropical grass species over temperate grasses and a widening of their distribution.[14-16]

Airborne grass pollen levels affect hospital admissions for asthma.[17-19] However, most research has concentrated on the allergenicity of pollens from temperate grass species, such as timothy grass *(Phleum pratense)*.[20,21] Emerging evidence from subtropical regions indicates that subtropical pollen allergens show distinct immunologic reactivity from temperate grass pollens.[22-28] The allergenic molecules derived from the subtropical grass pollens characterized thus far differ considerably from homologous temperate grass pollen allergens in terms of amino acid sequence.[29,30] Johnson grass *(Sorghum halepense)*

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Supported by the University of Queensland Collaboration Industry Engagement Fund and a cosponsorship from Stallergenes Pty Ltd. J.M.D. is supported by a National Health and Medical Research Council of Australia Development Grant (1017441). The University of Queensland Diamantina Institute proteomics facility was supported by the National Health and Medical Research Council of Australia Major Equipment and Infrastructure grant and a Ramaciotti Foundations Equipment Gift. M.M.H. is supported by Australian Research Council Future Fellowship (FT120100251).

Disclosure of potential conflict of interest: E. K. Gilding has received salary and research support from the Australian Research Council (LP0883808) and has received payment for lectures from The University of Queensland. J. M. Davies has received research support from The University of Queensland Collaborative Industry Engagement Fund, the National Health and Medical Research Council, the Asthma Foundation of Queensland, and the Australian Society for Clinical Immunology and Allergy; has consultant arrangements with Stallergenes Australia; is employed by The University of Queensland; has received payment from Stallergenes Pty Ltd and GlaxoSmithKline for lectures delivered by J. M. Davies; the university has received travel support from Thermo Fisher; is the named inventor on patent granted in Australia and applied for in the United States for Novel immunogenic molecules and uses thereof: Immunogenic protein Pas n 1 from Bahia grass pollen; receives in-kind support for development of her research from Thermo Fisher by way of provision of materials; and receives in-kind support for development of her research from Sullivan Nicolaides Pathology by way of provision of pathology services. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication December 5, 2013; revised May 18, 2014; accepted for publication June 13, 2014.

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0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2014.06.034

Abbreviations used
AR: Allergic rhinitis
2D: Two-dimensional
JGP: Johnson grass pollen
MS: Mass spectrometry
pI: Isoelectric point
SPT: Skin prick test

is a perennial weed of the Panicoideae subfamily of grasses that is distributed globally throughout the subtropics.[10,31,32] Although the 2 dominant pollen allergen components of Bahia grass *(Paspalum notatum)* have been determined[29,33] and allergen components of Bermuda grass *(Cynodon dactylon)* pollen have been extensively characterized, relatively little is known of Johnson grass pollen (JGP) allergens. We previously showed that 77% of patients with AR from a subtropical region of Queensland, Australia, were sensitized to JGP.[10] The sequence of the group 1 allergen of JGP, Sor h 1, has been described, and recombinant Sor h 1 reacted with a group 1–specific mAb.[34] However, allergic sensitivity of patients with grass pollen allergy to JGP has not been described. Here we investigate serum IgE reactivity of patients from a subtropical region with the dominant allergens purified from JGP based on the amount within the total pollen protein and observed IgE reactivity by using immunoblotting.

As with many clinically important allergen sources, the full genomic sequence of JGP is unknown. This can hamper implementation of in-depth molecular approaches to allergen discovery. We comprehensively analyze the proteome, transcriptome, and allergome of JGP using advanced molecular and bioinformatic techniques that do not rely on genomic data. This detailed approach provides a powerful and versatile data set of the clinically relevant allergen components within JGP and supports the future development of targeted immunodiagnosis and immunotherapies tailored to better meet the needs of patients with subtropical grass pollen allergy.

METHODS

Study participants

Participants were recruited consecutively from immunology or respiratory clinics at the Princess Alexandra Hospital, Brisbane, Australia, and regional parts of Queensland, Australia, with informed consent as approved by the Metro South Human Research Ethics Committee. Subjects were tested for allergic sensitivity to a panel of 10 common aeroallergen extracts, including Johnson, Bahia *(Paspalum notatum)*, Bermuda *(Cynodon dactylon)*, or Ryegrass *(Lolium perenne)* pollen extracts by using skin prick tests (SPTs; Hollister-Stier, Spokane, Wash), according to guidelines of the Australian Society for Clinical Immunology and Allergy.[10] The inclusion criteria for the group of patients with grass pollen allergy was a history of AR consistent with pollen allergy and an SPT response (>3 mm in diameter) to the pollen extract of at least 1 grass species (n = 64). Sera of 5 additional patients with AR from the temperate region of Melbourne were included in one assay. Nonatopic subjects with no history of allergic disease and no positive SPT response (n = 19) and subjects with histories of AR and asthma with SPT responses to allergens other than grass pollens, frequently house dust mite, cat dander, or *Alternaria* species, were included as control subjects (n = 23). Subjects less than 18 years of age and those receiving immunotherapy were excluded. Sera were obtained from participants by means of venipuncture.

One- and 2-dimensional gel electrophoresis and immunoblotting

JGP (Greer, Lenoir, NC) extracted in PBS was separated by means of 14% SDS-PAGE (10 μg per lane) and immunoblotted for mAb or serum IgE reactivity with the following modifications to published methods.[30] Immunoblots were incubated overnight with patient sera diluted 1:50 before incubation with rabbit anti-human IgE (Dako, Glostrup, Denmark) diluted 1:10,000 for 2 hours and subsequently goat anti-rabbit IgG–horseradish peroxidase conjugate (Promega, Madison, Wis) at 1:10,000 for 2 hours. IgE immunoblots were developed for 5 minutes by means of chemiluminescence (SuperSignal WestPico; Thermo Scientific, Rockford, Ill). Immunoblots probed with the mAbs 6C6[30] and AF6[35] were visualized by using standard 1,4-dichloronapthol development.[30]

JGP (50 μg per DryGel strip, pH 3-11; GE Healthcare, Uppsala, Sweden) was separated by charge and size by means of 2-dimensional (2D) gel electrophoresis and stained with Coomassie Brilliant Blue.[30] 2D gels of JGP were immunoblotted, as described above. 2D gels of JGP spiked with isoelectric focusing standard proteins were examined to determine the observed molecular weights and isoelectric focusing points of IgE reactive components.

Serum IgE reactivity with purified Sor h 1 and Sor h 13

The 2 dominant allergenic components of JGP were purified from an aqueous extract of JGP by means of ammonium sulfate precipitation, hydrophobic interaction, and size exclusion chromatography, as previously described.[33,36] Sera were tested for IgE reactivity with whole JGP extract (5 μg/mL) and purified allergens (1 μg/mL) by means of ELISA.[30]

Statistical analysis

Data were assessed for normality by using the Kolmogorov-Smirnov test. Differences between groups were assessed by using the Mann-Whitney *U* test for nonparametrically distributed data. Within-group differences were assessed by using the Wilcoxon signed-rank test for paired data. Correlations were determined with the Spearman rank test for paired data. *P* values of less than .05 were considered significant.

Transcriptome sequencing

Total RNA was extracted from pollen and treated with DNAse.[37] mRNA sequencing was performed with the Illumina RNA-seq pipeline by the Beijing Genomics Institute (Shenzhen, China). The total JGP transcriptome sequence assembly and analysis methods are detailed in the Methods section in this article's Online Repository at www.jacionline.org.

JGP allergens were deduced by means of comparison of translated JGP transcripts to the comprehensive allergen database Allergome.org (January 2014; www.allergome.org[38]) through BLASTP software.[39]

Mass spectrometry

Thirty micrograms of whole JGP extract was separated on SDS-PAGE into 8 × 1–mm bands. Liquid handler–assisted in-gel digest and shotgun proteomics analysis was performed by using an Agilent 6510 QTOF coupled with an HPLC-ChipCUBE.[40] Protein spots cut from Coomassie-stained 2D gels were processed similarly. The resulting mass spectrometry (MS)/MS spectra were searched against specified databases by using Spectrum Mill (Agilent B.04.00.127; Agilent, Technologies, Santa Clara, Calif). Cysteine carbamidomethylation and methionine oxidation were selected as fixed and variable modifications, respectively. Precursor and product mass tolerance were set to ±20 ppm and ±50 ppm, respectively. The peptide cutoff score was set to 9.

Proteome and allergome analysis

In the absence of knowledge of the Johnson grass genome, the mass spectra of tryptic digest peptide fragments observed in whole JGP and excised