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The emerging pathogen *Paecilomyces variotii* - a novel and important fungal allergen source

To the Editor,

Fungal allergy represents a worldwide public health burden.[1] Owing to their ability to colonize and germinate in the respiratory mucosa, fungi can have a far greater impact on the patients' immune system than other respiratory allergen sources.[1] However, the lack of knowledge about allergenic fungi is one of the factors that contribute to a general underdiagnosis of fungal allergy.[2] In the present study, we investigated the allergenic potential of *Paecilomyces variotii*, a heat-resistant mold ubiquitously found in soil, decomposing organic material, food products, and clinical samples, that is regarded as an emerging cause of serious, sometimes life-threatening, infections, and is often detected in the indoor environment.[3–6] Even though *P. variotii* has already been suggested as an aeroallergen source and occupational hazard, no allergens have yet been identified or characterized by this species.[7]

IgE immunoblots, performed with four different pools of sera from mold-sensitized patients (Table S1), showed the presence of several IgE-reactive proteins in *P. variotii* extract (Figure 1A), suggesting the species' high allergenic potential (no reaction was observed in immunoblots incubated with the detection antibody only—data not shown). Mass spectrometry analysis led to the identification of the first three *P. variotii* allergens, which interestingly all represent enzymes. Protein A (~36 kDa) was identified as a transaldolase, an enzyme that is already known as an allergen in different fungal species.[8] Protein B (~37 kDa) was identified as a glyceraldehyde 3-phosphate dehydrogenase, which represents an important allergen in various sources,[9] and Protein C (~48 kDa) was identified as an enolase. Enolases have also already been identified as highly conserved and cross-reactive allergens in various allergen sources including different fungi.[10,11] The proteins were submitted to the WHO/IUIS Allergen Nomenclature Subcommittee and were tentatively assigned the allergen names Pae v 1[a], Pae v 2[b], and Pae v 6[c]. The IgE-reactive protein bands marked D to H still have to be identified by peptide mass fingerprinting.

Full-length cDNAs coding for the *P. variotii* proteins Pae v 1, Pae v 2, and Pae v 6 were generated, cloned into a bacterial expression vector, and recombinant proteins were produced in *Escherichia coli* with a C-terminal hexa-histidine-tag. Reducing and non-reducing SDS-PAGEs (Figure S1) and immunoblots, performed with an anti-histidine-tag antibody, verified the purity of the recombinant proteins and showed that all proteins possess the capability to form disulfide bridges and to oligomerize under non-reducing conditions (Figure S1). Moreover, circular dichroism spectroscopy analysis of the recombinant allergens showed far-UV spectra with broad minima between 210 and 220 nm, which suggested the presence of both, alpha-helical structures, and beta-sheets, and indicated that the proteins represent properly folded molecules (Figure S2).

ELISAs performed with sera from mold-allergic patients showed the IgE-binding capacity of the three recombinant proteins. With OD values of more than 3.0 (Figure 1B), the enolase rPae v 6 showed the highest levels of IgE reactivity, whereas the IgE reactivities of rPae v 1 (maximum OD value of 0.8) and rPae v 2 (maximum OD value of 0.390) were considerably lower. Furthermore, IgE inhibition immunoblots, where nitrocellulose-blotted protein extracts from *P. variotii* were exposed to serum pools that had been pre-incubated with either one of the recombinant proteins or, for control purposes, with buffer only, showed that the recombinant allergens rPae v 1, rPae v 2, and rPae v 6 were able to significantly reduce patients' IgE-binding to their natural counterparts (Figure 1C). This indicates that the recombinant molecules contain the IgE-binding epitopes of their natural counterparts and suggests that rPae v 1, rPae v 2, and rPae v 6 could be used as tools for *in vitro* diagnosis of *P. variotii* sensitization. Pre-incubation with rPae v 2, a GAPDH, caused the reduction of IgE-binding to several proteins with molecular weights of 25–45 kDa. This suggests the presence of different Pae v 2 variants with different posttranslational modification and Pae v 2 degradation products in the natural fungal extract. The presence of GAPDH variants, which can also influence the molecule's different functions, has already been suggested in the literature.[12]

Analysis of the prevalence of IgE recognition in a cohort of patients, sensitized to different mold species, by ELISA revealed different frequencies of IgE reactivity. In Figure S3, the presence (in green) or the absence (in red) of IgE-binding to the recombinant allergens is displayed for each patient. rPae v 1 was recognized by 22% (11 out of 49) of the tested patients, rPae v 2 by 6% (3 out of 50), and 68% of the tested patients (39 out of 57) reacted with rPae v 6. Among the patients tested with all three recombinant allergens, rPae v 1 was recognized by 22% (9 out of 41), rPae v 2 by 5% (3 out of 41), and rPae v 6 by 61% (25 out of 41) of the patients. Based on these results, rPae v 1 and rPae v 2 can be regarded as minor allergens of *P. variotii*, whereas

[a]NCBI Nucleotide Sequence Accession Number: MN953053

[b]NCBI Nucleotide Sequence Accession Number: MN953054

[c]NCBI Nucleotide Sequence Accession Number: MN93795

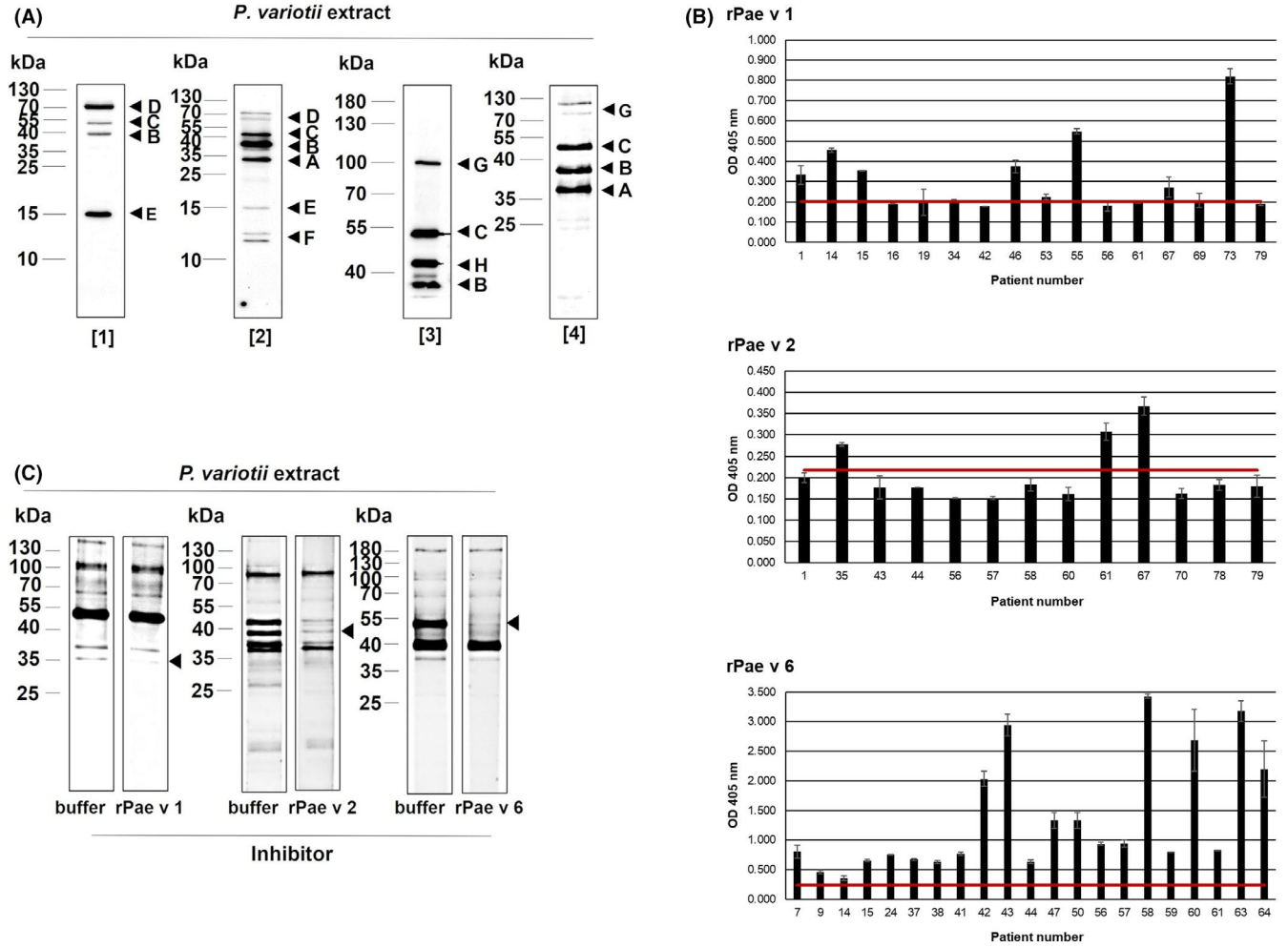

FIGURE 1 Identification and characterization of IgE-reactive proteins from *Paecilomyces variotii*. (A) Nitrocellulose-blotted proteins from *P. variotii* were exposed to four different pools of sera (pool 1: sera 1, 2, 26–28, and 65; pool 2: sera 3, 29–31, 40, 66, and 72; pool 3: sera 4–6, 48, 52, and 54; pool 4: sera 53, 56–61, 67, 77, and 78) from mold-allergic patients. IgE-reactive proteins identified by mass spectrometry are marked with A, B, and C; not yet identified bands are marked with D, E, F, G, and H. Molecular weight markers are indicated in the left margins. (B) Determination of IgE reactivity of rPae v 1, rPae v 2, and rPae v 6 by ELISA using sera from mold-sensitized patients. Results are displayed as mean OD values, and standard deviations from two technical replicates are indicated as error bars in the bar charts. The red line represents the assay's cutoff value, which was calculated from the mean OD values plus two times the standard deviation of sera from three non-allergic individuals. C, Inhibition immunoblots: blotted proteins in *P. variotii* extracts were exposed to different pools of sera (rPae v 1: sera 55 and 73; rPae v 2: sera 35, 39, and 61; and rPae v 6: sera 25, 57, 71, and 74–76), which had been pre-incubated with rPae v 1, rPae v 2, or rPae v 6, or, for control purposes, with buffer only. Molecular weight markers are indicated in the left margins. Arrows mark the molecular weights of the natural allergens Pae v 1, Pae v 2, and Pae v 6 in the fungal extract

rPae v 6 can be classified as a major mold allergen. Furthermore, evaluation of the allergenic activity of the recombinant major allergen rPae v 6 in basophil activation tests (BATs) showed a dose-dependent expression of CD63 in basophils from patient 4, suggesting the molecule's biological activity (Figure S4 in the supporting information).

The availability of an anti-enolase antibody allowed us to further investigate the release kinetics of the enolase Pae v 6 from *P. variotii* spores. The experiments showed that the spores from *P. variotii* immediately released this major allergen upon exposure to a humid milieu (Figure S5 in the supporting information). It can thus be expected that Pae v 6 would be rapidly secreted when the spores get in direct contact with the mucosa, leading to immediate allergic symptoms on the accessible mucosa surfaces such as the conjunctiva and the nose.

In conclusion, during this study the strong IgE reactivity of *Paecilomyces variotii* was demonstrated. Furthermore, the species first three allergens, tentatively designated Pae v 1, Pae v 2, and Pae v 6, were identified, recombinantly produced, and characterized regarding their biochemical and immunological features. It was shown that the three recombinant allergens represent IgE-reactive molecules, with Pae v 1 and Pae v 2 representing minor fungal allergens, whereas Pae v 6 can be classified as a major fungal allergen. We are aware that the use of sera from patients allergic to known fungal allergen sources is a limitation of our study, because, in this way, it was much more likely to identify highly conserved, potentially

cross-reactive allergens rather than species-specific allergenic molecules. Indeed, one of the three identified allergens is the highly conserved fungal pan-allergen enolase. However, since *P. variotii* has not yet been thoroughly investigated as an allergen source, it was not possible to overcome this limitation. Nevertheless, the obtained data together with the facts that exposure to *P. variotii* represents an important component of the indoor mycobiota[5] and is increasingly recognized as an important cause of infections[13] suggest that this species should be considered as a potential allergen source and should be included in routine fungal allergy diagnosis.

The description of the used methods can be found in the supporting information.

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CONFLICT OF INTEREST

All authors declared no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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COVID-19 vaccines tolerated in patients with paclitaxel and docetaxel allergy

To the Editor,

After initial reports of anaphylaxis to the messenger RNA (mRNA) COVID-19 vaccines, the Centers for Disease Control and Prevention (CDC) put forth guidance stating that patients with a history of anaphylaxis to vaccine components like polyethylene glycol (PEG) should not receive the mRNA COVID-19 vaccines.[1] To address this clinical challenge and decrease vaccine hesitancy, we published an approach to guide COVID-19 vaccination in high-risk allergy individuals.[2–4] While the etiology of anaphylaxis to mRNA COVID-19 vaccines remains unclear, PEG continues to be an important focus.[5,6] Paclitaxel contains polyoxyl-35 castor oil—a PEG derivative and structurally similar to the excipient in Pfizer-BioNTech and Moderna COVID-19 vaccines—and docetaxel contains polysorbate 80—the excipient in Janssen COVID-19 vaccine. Given this, we sought to assess the utility of pre-vaccine excipient skin testing (ST), risk stratification, and COVID-19 vaccine tolerability in oncology patients with a history of paclitaxel or docetaxel hypersensitivity reaction (HSR).

We included consecutive paclitaxel or docetaxel HSR patients referred to Mass General Brigham allergy/immunology for evaluation prior to COVID-19 vaccination. Evaluation included clinical risk assessment and excipient ST as previously described.[2,4] Clinical details were obtained by electronic health record (EHR) review. COVID-19 vaccine tolerance was determined by an allergy/immunology physician (AB) using EHR review, phone call, and/or e-mail. This study was approved by the Massachusetts General Brigham Institutional Review Board and deemed minimal risk.

Between December 30, 2020, and April 2, 2021, 21 patients with paclitaxel (n = 17) or docetaxel (n = 4) HSR were referred (Table 1). Most were female (n = 20, 95%) and white (n = 20, 95%). Approximately half of HSRs occurred in the past 4 years (n = 11, 52%), and 14 (67%) HSRs were severe grade 3.[7] Twenty patients (95%) had negative excipient ST. One paclitaxel HSR patient had positive ST (methyl-prednisolone acetate PEG3350, 0.4 mg/ml intradermal) and tolerated the Janssen COVID-19 vaccine (Table 2). All patients completed COVID-19 vaccination with no reaction (n = 19, 90%) or with mild symptoms treated with antihistamines alone (n = 2, 10%). Of 17 patients with history of paclitaxel HSR, 12 (71%) received an mRNA COVID-19 vaccine and none had a reaction.

This case series of 21 patients suggests that patients with paclitaxel (containing the excipient polyoxyl-35 castor oil/PEG derivative) or docetaxel (containing the excipient polysorbate 80) HSRs tolerate COVID-19 vaccination. While 2 patients (10%) developed reactions after COVID-19 vaccination, symptoms resolved with antihistamines alone. The reactions do not appear related to the patient's specific excipient allergy history (e.g., docetaxel HSR patient developed reaction with mRNA vaccine), and in fact, no reactions occurred in paclitaxel HSR patients that received an mRNA COVID-19 vaccine. It remains unclear if the paclitaxel HSR patient with positive intradermal PEG ST would have tolerated mRNA COVID-19 vaccination, but experience to date indicates that positive PEG intradermal ST does not predict reactions to mRNA COVID-19 vaccines.[4]

CDC guidance advises patients with PEG allergy to proceed with Janssen COVID-19 vaccination while patients with polysorbate 80 allergy can proceed with mRNA COVID-19 vaccines.[1] However, if both COVID-19 vaccine platforms are not routinely accessible, allergy/immunology consultation may be useful for allergy risk assessment and vaccine guidance. Furthermore, if mRNA COVID-19 vaccines are proven optimal for oncology patients, allergy assessment and limited excipient ST (e.g., Miralax, PEG-3350)[2,4] can provide reassurance prior to mRNA COVID-19 vaccination in patients with HSR to chemotherapeutics containing PEG. We continue to advise 30-minute monitoring post–COVID-19 vaccination for all patients with any history of anaphylaxis per CDC guidance.[1]

This study was limited by its small sample from a single institution and retrospective study design. Additionally, the study included patients with a history of hypersensitivity to drugs (i.e., paclitaxel) that do not contain the specific form of PEG contained in the vaccines, which is a PEGylated form of PEG forming PEGylated lipids. It has been frequently reported that hypersensitivity to drugs containing PEG (in its native form) or its derivatives (and not containing the