

Studies of house dust mites can now fully embrace the “-omics” era

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In 1988, the first DNA sequence of a gene encoding a protein from the house dust mite *Dermatophagoides pteronyssinus* was described.¹ Now, 26 years later, the first genome of the related house dust mite *Dermatophagoides farinae* has been reported in this edition of the *Journal*,² as has its transcriptome and microbiome. The data generated by Chan et al² from the Chinese University of Hong Kong and Shenzhen University represents a vast resource that will help acarologists to understand mite biology *per se* and allergists to further their knowledge of mite allergens and allergenicity. The latter will be of particular interest to readers of this *Journal*.

In this regard the authors have not only confirmed the structures of 25 of the previously described *D farinae* allergens curated in the International Union of Immunological Societies Allergen Nomenclature Sub-Committee database but also described their gene structure. In addition, they have been able to determine the sequence of the *D farinae* amylase allergen (Der f 4), which, until this report, had not been determined, although it had been known to be present in *D farinae* extracts.³ Furthermore, the mite genome, in combination with proteomics, has enabled the authors to extend the list of known *Dermatophagoides* species allergens.

Using a serum pool from patients with mite allergy, they identified 12 novel allergenic proteins, including ferritin, cofilin (an actin-binding protein), secreted inorganic pyrophosphatase (denominated Der f 30-32, respectively), ubiquinol-cytochrome c reductase binding protein-like protein (denominated Der f 24), myosin alkali light chain protein, and DFP2. Six of these were then examined further by using individual sera, and they showed that the ubiquinol-cytochrome c reductase binding protein-like protein was a major allergen using immunoblotting and ELISA. In contrast, Der f 31 and Der f 32 were shown to be minor allergens, and the remainder were shown to be nonallergenic.

The finding that Der f 24 is a major allergen raises the question of why it has not been previously identified. However, this

situation is not unprecedented. For example, the 8-kDa *D pteronyssinus* allergen Der p 23 was identified as a major allergen by using a cDNA expression screening approach.⁴ By definition, the population examined in the authors' study had been exposed to the allergen (unless the reactivity detected reflects cross-reactivity), and therefore there could be several reasons for the failure to identify such allergens previously. For example, it is possible that they are present at very low concentrations in extracts used for screening purposes because of the types of culture, extraction techniques, or both used. Similarly, it is possible that the geographic origins of the population used to donate screening sera can influence the spectrum of allergens recognized, as has been demonstrated for the group 4 amylases, perhaps reflecting diet or other environmental factors.⁵

It will be important to determine whether Der f 24 is allergenic in different parts of the world and whether the allergen can be detected in house dust. Similarly, because the majority of mite allergens are associated with mite feces, it would be useful to know whether the Der f 24 allergen is present in such material or whether it is a somatic allergen. Despite these caveats and as the authors indicate, the mite genome used in combination with modern proteomic techniques will likely help define the complete catalog of *Dermatophagoides* mite allergens for the first time. In addition to identifying novel allergens, a bioinformatics approach has enabled the authors to identify a number of mite proteins that in nonmite species have been shown to be allergenic, particularly in fungi (eg, enolase and aldehyde dehydrogenase). However, it remains to be determined whether these orthologues are allergenic in mites.

The authors have also interrogated the genome for the presence of allergens reported in other mite species but yet to be specifically identified in *Dermatophagoides* species. Three such allergen groups have been described thus far, including Blo t 12 and Blo t 19 from *Blomia tropicalis* and Tyr p 24 (troponin C) from *Tyrophagus putrescentiae*. Although the authors did not look for Tyr p 24, they failed to find homologues of Blo t 12, a chitin-binding allergen, and Blo t 19, a cysteine-rich antibacterial peptide homologue of a group of peptides common in helminthic parasites, such as *Ascaris* species. In this regard a Blo t 12 homologue has been identified in *Lepidoglyphus destructor* (but not yet Blo t 19), indicating the group 12 allergens are essential in at least 2 mite species. The authors indicate that the failure to detect either in the *D farinae* genome could reflect the draft nature of the library or that the *Dermatophagoides* species mites do not produce homologous proteins. However, it is possible that orthologues are present but that homology is extremely low, making them difficult to recognize. In this regard *Blomia* and *Lepidoglyphus* species belong to the Glycyphagoidae family in contrast to *Dermatophagoides* species, which belong to the Pyroglyphidae family. Several studies show that there is only modest sequence identity (about 40%) and correspondingly low

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immunologic cross-reactivity between allergen groups belonging to these families.⁶

The precise biochemical function of the Blo t 12 allergen is unknown, but it possesses a C-terminal chitin-binding domain belonging to the CBM14 superfamily (also called the peritrophin-A domain).⁷ Although not specifically detected in the genome, this *Blomia* species allergen shows some homology with the pyroglyphid mite group 15, 18, and 23 allergens, principally because of this domain.⁷ *Blomia tropicalis* possesses homologues of the first 2 groups of allergens, indicating the importance of these proteins to mite physiology, irrespective of family. A *Blomia* species group 23 allergen has yet to be described, but Blo t 12 bears some resemblance to the members of this group, although there is a significant difference in size. For example, they both possess a C-terminal CBD14 domain and an N-terminus of unknown function and are found in mite feces, suggesting the 2 groups might be functionally related.

While determining the *D farinae* genome, the authors were able to sequence the associated mite microbiome. The data obtained highlight the presence of a rich diversity of bacterial species and confirm that the mite allergens thus far described, as well as the NlpC/P60 protein, are derived from mites rather than from bacteria. The microbiome was dominated by *Enterobacter* species, *Staphylococcus* species, and *Escherichia coli*, with the former (*Enterobacter cloacae*) being detected in the mite gut by using immunohistochemistry. The majority of the species characterized were gram-negative organisms, with many being associated with soil, reflecting mite ecology in the home. Although the authors acknowledge that the data obtained might be biased toward such genera, several of the prominent species detected were gram-positive. Whether the spectrum of organisms determined in this culture of *D farinae* will be similar to that found in cultures obtained from different geographic locations or even in mites obtained from carpet dust or mattress dust in the same location remains to be determined because the microbiome will probably reflect the bacterial species in local dust. In addition, it is also possible that the microbiome detected might have been influenced by the particular mite culture techniques used.

Despite these caveats, however, species from several of the gram-negative and gram-positive genera identified by the authors have previously been isolated from mites by using conventional culture techniques, as well as 16S ribosomal DNA sequencing techniques,^{8,9} suggesting that there is a “core” microbiome. The previously reported bacteria include *Enterobacter* species, *Bacillus* species, *Staphylococcus* species, *Rhizobium* species, *Bartonella* species, *E coli*, *Pseudomonas* species, and *Acinetobacter* species. The authors suggest that some of the species identified play an important symbiotic role because mites reared in the presence of antibiotics did not survive, which is consistent with various reports showing the suppressive effects of antibiotic treatment on storage mite growth.¹⁰ In addition, certain Kyoto Encyclopedia of Genes and Genomes pathway enzymes identified in the genome were associated with the microbiome rather than with the genome.

Finally, it is also possible that the mite microbiome (as well as the associated domestic microbiome) might play some immunomodulatory role because of its ability to generate an array of microbial-associated molecular patterns that are likely to comprise any mite material entering the respiratory tract. At present, there is much interest in determining the influence of microbial-associated molecular patterns on the development of or protection against allergic diseases.^{11,12} The extensive data on the mite microbiome are likely to have a beneficial influence on further studies in this area.

In conclusion, the determination of the *D farinae* genome, transcriptome, and microbiome is a major achievement. The genome is now one of only a few arachnid genomes present in the public domain, including *Ixodes scapularis* (black-legged tick), *Rhipicephalus microplus* (cattle tick), and *Tetranychus urticae* (spider mite), and it represents the only available house dust mite transcriptome and microbiome. The authors have highlighted the utility of such data, and others will similarly be able to exploit them to help answer many important questions pertaining to acarology and allergology for many years to come. For those of us with an interest in mites, allergens, and allergy, the determination of the genomes of the other clinically important mites is eagerly awaited.

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