Author Response Letter to Reviewers' Comments

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Science Editor; Peer Reviewer; Company Editor-in-Chief: Lian-Sheng Ma

Science Editor: Jin-Lei Wang

Reviewer Code: 03852107

Dear Editors,

We, the authors, appreciate your care and valuable suggestions for our manuscript mentioned above, which is an invited review.

I have attached the manuscript revised according to the reviewer and editor comments, questions and suggestions. The references were major revised according to Science Editor comments and requests. Point-by-point responses are listed down in this letter. We hope these changes meet your expectations and the manuscript will be accepted for publication in the world's leading journal WJM.

Best regards,

Florin-Dan Popescu

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Authors' Response to the Reviewer's Comments

Reviewer's code: 03852107
Reviewer's specific comments to authors:
Although the review is interesting, but still contains some issues, belowing (1) All Fel d 1-8 should b introduced together, with a summary of molecular mechanisms and mapping. Finally, it explains which molecules are the best as commercial IgE immunoassays. (2) The introduction of the Cat Niemann-Pick type C2 protein and Kallikrein allergens is too little, and I do not understand the author's intention to introduce the Cat Niemann-Pick type C2 protein and Kallikrein allergens. (3) Why can't IgA Fel d 5, IgM Fel d 6 and so on be available as allergen components in the current commercial IgE immunoassays? Please explain the reason and indicate whether it has potential as the commercial IgE immunoassays.

Authors' Response:

Dear reviewer,
Thank you for your valuable time spent on our manuscript, for considering it interesting, for your overall evaluation as having a good scientific quality, and especially for your valuable comments, questions and suggestions, which generated some responses and actions from our side with a major revision as requested, which we consider that improved the quality and clarity of our manuscript.

Point-by-point responses:

 Comment 1: All Fel d 1-8 should be introduced together, with a summary of molecular mechanisms and mapping. Finally, it explains which molecules are the best as commercial IgE immunoassays.

o **Response:** Thank you very much for suggesting to introduce together the molecular allergens Fel d 1 to Fel d 8, recognized by the World Health Organization/ International Union of Immunological Societies database, with a summary of molecular mechanisms, presentation of the IgE epitope mapping according to AllerBase, a great allergen knowledge database, and a closure presentation in the introduction of the best characterized and available cat allergen molecules used at this stage in commercial IgE immunoassays, according to the updated World Allergy Organization consensus document on molecular-based allergy diagnosis and mentioned also by the European *Academy* of Allergy and Clinical Immunology Molecular Allergology User's Guide and a recent Consensus document on the dog and cat allergy. Therefore, we decided to introduce a paragraph in the *Introduction* part of our manuscript (pages 5-6) to satisfy your important suggestions from Comment 1, as follows:

"To date, eight *Felis domesticus* molecular allergens have been recognized as Fel d 1 to Fel d 8 by the WHO/IUIS (World Health Organization/International Union of Immunological Societies)[4]: uteroglobin-like protein Fel d 1, serum albumin Fel d 2, cystatin Fel d 3, lipocalins Fel d 4 and Fel d 7, immunoglobulins Fel d 5 and Fel d 6, and latherin-like protein Fel d 8. Cat allergens are involved in the molecular mechanisms underlying IgE-mediated allergic sensitization and different cross-reactivities. Representative isoforms are described for these allergens: Fel d 1.0101, Fel d 2.0101, Fel d 3.0101, Fel d 4.0101, Fel d 5.0101, Fel d 6.0101, Fel d 7.0101, Fel d 8.0101, but none is mentioned as such in the commercial IgE immunoassays. Data on the IgE binding epitopes are scarce, with sequence positions mentioned only for Fel d 1. IgE epitope mapping of this dominant cat allergen revealed five sequential/linear epitopes on chain 1/Fel d 1-A and two on chain 2/Fel d 1-B, in addition to a discontinuous/conformational epitope on chain 1[5], the last one being located on the four helices of the Fel d 1 chain 1 spatially juxtaposed upon protein folding.
Currently, the best characterized and available cat allergenic molecules for commercial IgE assays are Fel d 1, Fel d 2, Fel d 4 and Fel d 7. The two types of such allergen components used in singleplex and multiplex immunoassays are recombinant (r) allergens (produced by recombinant DNA technology) and highly purified natural (n) allergens (purified from natural sources)[6]. All are included in the list of cat allergens presented in the European *Academy* of Allergy and Clinical Immunology (EAACI) Molecular Allergology User's Guide[7] and in a recent Consensus document on dog and cat allergy[8]. The characteristics of these cat allergens[7-11] are presented in Table 1 together with all other allergenic molecules recognized by the WHO/IUIS database[4]."

An additional paragraph was introduced on page 19 to mention the key molecular mechanisms of IgE sensitization and type 2 allergic inflammation:
"Allergenic molecules induce specific IgE sensitization of mast cells and trigger type 2 allergic inflammation upon re-exposure. The availability of natural purified or recombinant allergens improved the understanding of the molecular mechanisms leading to these immune responses, which vary depending on several structural and biological characteristics of these allergens. In addition, other pro-inflammatory properties of allergens must be mentioned, including late-phase allergic inflammation induced by non-IgE reactive peptides of Fel d 1 via major histocompatibility complex (MHC)-restricted T cell activation[99-101]."

 Comment 2: The introduction of the Cat Niemann-Pick type C2 protein and Kallikrein allergens is too little, and I do not understand the author's intention to introduce the Cat Niemann-Pick type C2 protein and Kallikrein allergens.

o **Response:** First, we detailed the quantity of information on the topic, considered too small, and in order to clarify our intention, we changed the structure of some paragraphs and grouped other cat allergenic molecules, different from Fel d 1 to Fel d 8, in a subchapter named *Other cat allergens* (pages 15-17). In this, we also included discussions on additional molecular explanations for cat and dog cross-sensitivity and cross-reactivity. In short, we thank you for generating these changes that clarify the structure of the manuscript part of molecular allergens presentation and for the request to justify the reason to present and discuss other molecular allergens involved in allergic cross-reactivity between cat and dog which are present or not in natural cat allergen extracts, such as the very recent discovered cat NPC2 and the recently discussed dog kallikrein allergens, even if cross-reactivity between cat and dog allergens is usually explained by structural similarities between lipocalins and albumins. This whole discussion is needed because a frequent association between cat and dog sensitization is known for several decades, and a common question is whether this is due to co-sensitization to different allergen components or cross-reactivity between cat and dog allergenic molecules. Minor language polishing was needed due to structural changes in this section.

 Comment 3: Why can't IgA Fel d 5, IgM Fel d 6 and so on be available as allergen components in the current commercial IgE immunoassays? Please explain the reason and indicate whether it has potential as the commercial IgE immunoassays.

o **Response:** Thank you for considering it necessary that we must clarify the reasons why some molecular allergens are included in commercial IgE immunoassays and some are not, either due to aspects related to production availability or possible analytical errors. This made us create a distinct paragraph in the Conclusions part (page 20) as follows:

„Precision allergy molecular diagnostic applications (PAMD@) in cat allergy involve several molecular allergens used in commercial singleplex and multiplex IgE immunoassays, Fel d 1, Fel d 2, Fel d 4 and Fel d 7, these being the allergenic components currently available on the market[100]. For other native or recombinant allergenic components to be included in such immunoassays used in clinical practice, they must not only be well characterized and experimentally validated, but must also be clinically validated and available from their production point of view. Moreover, the characteristics of the solid-phase of the immunoassay and the manner by which allergenic molecules are coupled are important to reflect their biochemical properties

and specific requirements for stability, preserving epitope complexity. Regarding native IgA Fel d 5 and IgM Fel d 6 allergen components with α-Gal IgE-binding epitopes, their use may be associated with analytical errors and impaired *in vitro* diagnostics in some patients, in such cases bovine thyroglobulin being a good molecular biomarker for α-Gal IgE sensitization[5,15,28,29,86]. Although α-Gal is present on cat Igs, cross-sensitization between cat allergens and the oligosaccharide antigen is not considered clinically relevant[100]."

Science Editor Comments:

1. Scientific quality: The manuscript describes a review of the molecular diagnosis in cat allergy. The topic is within the scope of the WJM.
(1) Classification: Grade C;
(2) Summary of the Peer-Review Report: Although the review is interesting, but still contains some issues to be addressed. The questions raised by the reviewers should be answered; and
(3) Format: There are 3 tables. A total of 104 references are cited, including 29 references published in the last 3 years. There are no self-citations (Ref. 3, 5, 25, 40, 67, 77). The topics of the self-citations are related to this study.
2. Language evaluation: Classification: Grade C. A language editing certificate issued by AJE was provided.
3. Academic norms and rules: No academic misconduct was found in the Bing search.
4. Supplementary comments: This is an invited manuscript. No financial support was obtained for the study. The topic has not previously been published in the WJM.
5. Issues raised: (1) Authors should always cite references that are relevant to their study. Please check and remove any references that not relevant to this study.
6. Recommendation: Conditional acceptance.

Authors' Response point-by-point and actions:

Dear Editor,
1. Thank you for your valuable time spent on our manuscript for the WJM.
(1) Thank you for classifying it as having good scientific quality.
(2) The questions raised by the reviewers were answered, actions and changes were made according to the previous detailed point-by-point response.
(3) Regarding the Format:
The three tables references were clarified, citing more than five references related to each table in a single citation were avoided, very small changes in the content of table 3 and its footnotes were made according to updated citations.

The total number of references was reduced to 103, several citations were removed and a few were replaced with newer ones more relevant. One self-citation (Ref. 3) was also replaced with a more relevant one represented by an American practice parameter document.

2. Regarding language evaluation, according to the provided Certificate Service Confirmation, our manuscript achieved Grade A after editing.

3. Thank you for mentioning that no academic misconduct was found.

4. This invited manuscript with an interesting and challenging topic has no financial support obtained, as you mentioned.

5. Issues raised were taken into consideration and solved.

(1) After a careful check of all references, a major revision of the References list was made, the total number of references was slightly reduced, several citations not so relevant (including one self-citation, although related to the topic) were removed and few were replaced with newer ones more important and/or relevant, as mentioned above. Some new information was added according to the references changes. The succession of the references in some parts of the list was also changed in order to always cite references that are relevant and to avoid citing more than five references in a single citation, even when separated by a hyphen.

6. We, the authors, hope that, after we resolved all issues in the manuscript based on the peer review report and made changes according to the point-by-point response to the issues raised, and after we revised the manuscript according to the Editorial Office's comments and suggestions, the final decision will be of acceptance of our manuscript for publishing in WJM.