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COMPARE
Comprehensive Protein Allergen Resource

COMPARE Peer-Review Panel (PRP) Statement – 11 January 2019

Justification of removal from COMPARE database of sequence accession # BAW32535.1 of GFP-like protein (Akane) from the octocoral *Scleronephthya gracillima*

In response to questions about the validity of the inclusion of a GFP-like protein from *Scleronephthya gracillima* in the COMPARE database in 2018 (accession # BAW32535.1), the peer-review panel (PRP) of the database has re-reviewed the entry during the review process for the 2019 database version. During the review process leading to the 2018 version of the COMPARE database, the protein in question was reviewed for the first time, based on a single publication by Y. Kato et al. (Luminescence, 2017; 32: 1009-1016). The evidence for allergenicity was considered quite weak at that time as well, but a conservative approach was taken and the sequence was entered in COMPARE 2018.

In the course of 2018, PRP took the precaution to email Y. Kato to ask whether any new evidence was collected, in particular, whether IgE binding to the full-length recombinant protein was demonstrated. It was communicated that this was not done and that the research on the putative allergenicity of the protein was discontinued. Therefore, PRP reconvened for a careful and detailed re-review of the original study.

As a result of this re-review, the COMPARE PRP has decided to remove the sequence (accession # BAW32535.1) from the 2019 database. The justification for this removal can be summarized as follows:

- The pools of sera used as allergic and healthy control pool are ill-defined. The allergic pool is described as a pool originating from “fisherman catching spiny lobster ……, who exhibited two or more of the following symptoms: conjunctivitis, rhinitis, dermatitis and bronchial asthma”. It is unclear whether these symptoms occurred during their profession or not. Evaluation to include reported triggers and investigation of sensitivity to other relevant occupational allergens was not reported. The controls are described as “healthy volunteers who did not exhibit allergy from octocoral”, although symptoms to the octocoral are in fact not explicitly reported for the allergic subjects. Nothing about potential further atopic background (or absence thereof) is mentioned for the control subjects. For neither of the pools is it reported how many sera were used. Overall, therefore, the conclusion is that the pools used are very poorly defined.
- Kato et al. separated a crude extract of the octocoral *S. gracillima* by SDS-PAGE and show on immunblot that a 22kDa band is recognized by IgE from both the allergic and the control pool, and that a 27 kDa band is exclusively recognized by IgE from the allergic pool. Based on this they conclude that the 27 kDa protein is an allergen and the 22 kDa band is not. In fact, however, the binding to the 22 kDa band is much stronger, both by allergic and control pool, than the binding to the 27 kDa band.

- Kato et al. performed size-exclusion chromatography of the crude extract of the octocoral *S. gracillima*. They show IgE binding of the allergic pool by ELISA to ~20 fractions, but they do not report or show the absence of ELISA reactivity of the control pool.
- They then pooled these IgE-binding fractions and further separated them by anion exchange chromatography. A fraction named “Fra. (2.10)” was considered the fraction of interest, but the authors do not confirm IgE binding by ELISA. They do show IgE binding to the 22kDa (allergic and control) and 27 kDa (allergic only) bands on immunoblot, similar as observed for the crude extract. They go on performing an immunoblot inhibition with crude octocoral *S. gracillima* extract. The 27 kDa band is almost completely inhibited. The authors claim that the 22 kDa band is unaffected, but this is debatable since there clearly is a decrease in intensity of the 22 kDa band.
- They also perform a 2D SDS-PAGE/immunoblotting. In this case the strength of IgE recognition of the 22 kDa spots is even much stronger (by both allergic and control pool) compared to the very faint recognition of three 27 kDa spots. Moreover, one of these faint spots is also recognized by the control pool.
- The authors perform trypsin digestion of the 22 and 27 kDa bands from the 1D SDS-PAGE, followed by MS analysis of the resulting peptides. In the 27 kDa band they identify a sequence that matches amino acids 71-80 of a fluorescent protein from *Acropora aculeus*: YPADI/LPDYF. In the 22 kDa band they identify a peptide that matches amino acids 81-91 of a fluorescent protein from *Montastrea annularis*: QSFPEGFSWER. When performing a similar procedure on the two 27 kDa IgE-binding spots from the 2D SDS-PAGE, they find the QSFPEGFSWER that was found in the 22 kDa band from the 1D SDS-PAGE. In other words, both the 22 kDa and the 27 kDa band contain the QSFPEGFSWER sequence.
- Based on this sequence they used RACE protocols to clone the protein from the octocoral *S. gracillima*. The full sequence is reported and contains both peptides identified adjacent to each other at positions 71-91: YPADIPDYFQSFPEGFSWER. Strangely, the authors do not discuss that the most likely explanation is that the 22kDa is just a truncated version of the 27 kDa.
- The authors expressed the full-length protein and performed experiments to study the fluorescent properties of the protein, but did not demonstrate IgE binding of the allergic pool to the full length recombinant, and absence of binding by the control serum pool.
- The authors do not discuss that the amino acid sequence contains a consensus sequence for N-linked glycosylation at positions 41-43: NLT. We do not know whether the natural protein is glycosylated at this site, but if it is, this may explain the observation that IgE-binding to the 27 kDa band can be inhibited with crude octocoral extract, and that the 22 kDa band cannot: the 22 kDa band may be an N-terminally truncated version of the full protein, resulting in removal of the glycosylation site. In this scenario, IgE binding to the fluorescent protein could be to the carbohydrate exclusively.

In conclusion, the re-review has identified many flaws in the allergenicity claim, so it was decided to remove the sequence from the 2019 version of the COMPARE database.

The HESI COMPARE database program is committed to transparency and open dialog. Individuals or organizations are invited to submit their questions or inquiries via the “Contact us” portal in the COMPARE database website. HESI staff will respond if the information is readily available or will relay the inquiries to PRP if a more in-depth analysis is required.

Furthermore HESI, the COMPARE Steering Team, and the PRP want to uphold the high standards of quality aimed for the COMPARE Database. For that reason, two actions are being taken:

- Decisions and comments from the reviewers recorded during the PRP review process will be made available via a downloadable "Transparency Document" in the database page (under "Documentation"), starting with the release of COMPARE' 2019.
- As an extra precaution, PRP has committed to check back entries approved in past versions of COMPARE to identify eventual similar scenarios. A statement with proper justification, similar to this one, will be released in the event that other sequences are to be removed from COMPARE.

For more details about the PRP review process, please visit: http://comparedatabase.org/process-development/ and click on "Peer review of candidate entries".

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