CASE REPORT
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Identification of New Potential Allergens from Green-lipped Mussel (*Perna Canaliculus*)

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ABSTRACT

The green-lipped mussel (*Perna canaliculus*) originates from New Zealand. To preserve the health benefits of green-lipped mussel meat, it is freeze-dried to make a long-lasting powder. The powder is used to treat arthritis because of its potential anti-inflammatory properties. The report describes a 54-year-old woman who developed immediate rhinoconjunctival and respiratory symptoms after inhaling green-lipped mussel powder she gave to her dog for arthritis.

A skin prick test with green-lipped mussel powder was performed. Protein extracts from *P canaliculus* were separated by sodium dodecyl–sulfate polyacrylamide (SDS) gel electrophoresis and probed with serum from patients and serum preincubated with green-lipped mussel extract. Bound immunoglobulin E (IgE) was detected by specific anti-human-IgE antibodies, and IgE-binding proteins were subsequently identified by liquid chromatography and mass spectrometry.

The skin prick test was positive for green-lipped mussel. Specific IgE against green-lipped mussel extract was detected using Western immunoblotting. These potential allergenic proteins were identified by mass spectrometry as actin, tropomyosin, and paramyosin.

All three allergens are reported for the first time for *P canaliculus*. Actin is a major allergen in *Paphia textile*, paramyosin in *Sarcoptes scarbiei,* and tropomyosin in *Haliotis discus*. For all IgE-binding proteins, the software AllCatPro predicted high allergenicity, supporting our conclusion that these proteins from *P canaliculus* may also be allergenic. The identification of allergens from *P canaliculus* provides the opportunity for specific tests to assess the frequency of allergic reactions to *P canaliculus*.

Keywords: Anaphylaxis; Electrophoresis; IgE; Immunoblotting; Mussel

INTRODUCTION

Green-lipped mussel (*Perna canaliculus)* is a bivalve mollusk endemic to New Zealand. Extracts from this mussel are supposed to have antioxidant, angiotensin-converting enzyme (ACE) inhibitory, and antimicrobial properties. They are marketed as dietary supplements, nutritional, and therapeutic products to treat osteoarthritis.[1-5] Occupational asthma has been reported in workers harvesting green-lipped mussels indicating an allergic potential.[6] However, so far, no specific allergens have been identified. This is a new aspect of green-lipped mussel powder, which has been studied intensively before, especially for its anti-inflammatory properties.[2,7,8]

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We report on a 54-year-old atopic woman who started feeding her dog green-lipped mussel powder to improve her arthritis. Upon handling the powder, she experienced symptoms of an allergic reaction.

MATERIALS AND METHODS

The patient experienced symptoms of increasing severity with an itchy, runny nose, and shortness of breath each time she handled the dust from the powdered extract of green-lipped mussel. The symptoms required emergency treatment with inhaled adrenaline and oxygen supply.

When presenting to our Comprehensive Allergy Center about three months later, a skin prick test was performed with inhalation and food allergens (ALK-Abello, Germany) as well as with green-lipped mussel powder (Kräuterland Natur-Ölmühle: 100% pure New Zealand green-lipped mussel powder concentrate) undiluted and in the dilution levels 1:10 and 1:100 in sodium chloride (NaCl) 0.9%.

In addition, fluorescence enzyme immunoassay (FEIA) (ImmunoCAP™, Thermo Fischer Scientific) was used to investigate specific serum IgE of rPen a1 (tropomyosin from shrimp), mussel, and tuna.

Furthermore, a serum blood sample was obtained from the patient for Western blot analysis.

Green-lipped mussel powder was incubated with phosphate-buffered saline (PBS) at 4°C under constant rotation overnight, the suspension was centrifuged, and the supernatant was filter sterilized. Protein concentrations were determined by Bradford assay kit. Protein extracts (20 µg) from *P canaliculus* were separated using 12% SDS-gel electrophoresis. Gels were either Coomassie-stained for identification of bands and further processed for mass spectrometry or blotted onto a nitrocellulose membrane using a Western blot chamber (XCell II Blot Module, Invitrogen) and 10 mM of N-cyclohexyl-3-aminopropanesulfonic acid (CAPS) buffer. Successful blotting was checked using Ponceau S staining. Membranes were calibrated in Tris-buffered saline Tween (TBST) and were blocked with TBST + 5% milk powder overnight. The membranes were then incubated with unprocessed serum or serum preincubated with green-lipped mussel extract. As controls, the secondary antibody alone and serum from a non-atopic individual with no history of allergic reactions to *P canaliculus* were analyzed (Figure 1). The bound IgE was detected by specific anti-human-IgE antibodies (goat antihuman IgE antibody (Sigma-Aldrich). IgE-binding proteins were cut out from Coomassie-stained gels, and digested overnight using trypsin, and resulting peptides were analyzed with mass spectrometry (QExactive HF, Thermo Scientific) equipped with a chip-based electrospray ion source (Nanomate, Advion) coupled to a nano-high-performance[9] liquid chromatography system (Ultimate 3000, Dionex) and subsequently identified as described previously.[10] For the identification of the peptides, MaxQuant software was used. To increase the stringency of the evaluation, the identification score was normalized to the molecular weight of the protein. A cut-off value of <50% of the intensity of the most intense signal of the proteins identified in the corresponding band was used. This is necessary since mass spectrometry nowadays is highly sensitive, always leading to more than one identification per band and has been used in the way described here for the identification of allergens before.[9,10] However, even by employing additional criteria, it cannot be entirely excluded that a less abundant protein possesses the allergenic potential.[10]

To determine the potential allergenicity of the proteins identified, we used Allergome, UniProt and AllCatPro databases.

RESULTS

The skin prick test was positive for green-lipped mussel powder, birch, and hazel. Enzyme-linked immunosorbent assay (ELISA) detected normal ranges of serum total IgE and tryptase. No specific IgE rPen a1 (tropomyosin from shrimp), mussels, and tuna were detected.

Three IgE-reactive protein bands were detected using Western blotting, followed by identification of these proteins of interest (Figure 1; bands 1-8) by mass spectrometry (Table 1) according to established procedures.[9] Strong IgE reactivity was found with actin (Figure 1; bands 1, 4, 5, 7, and 8), paramyosin (Figure 1; bands 2 and 3), and tropomyosin (Figure 1; band 6). The appearance of one protein in more than one band can occur due to polymerization (an inherent feature of these proteins) or degradation. To control the specificity of IgE binding, the serum of the allergic patient was preincubated with green-lipped mussel extract before Western blot detection. This procedure reduced unspecific signals, and the same holds true for the control

with the serum of a non-atopic individual not allergic to *P. canaliculus* or secondary antibody only. After these procedures, only unspecific background bands of 70 and 46 kDa remained. These controls underscore the specificity of the detection of proteins by IgE binding.

Table 1. Proteins identified by MS/MS analysis followed by MaxQuant identification of the bands of interest are shown. Listed are the band numbers (see Figure 1), proteins identified, their molecular weight, Allergome codes, UniProt accession numbers, mean intensities per dalton (mean Intens/[Da]), mean peptides identified, and the AllCatPro results.

Band	kDa	Protein ID	mean Intens/[Da]	mean pept ident	Name	AllCatPro result
1	>200	Q26065	45100378	23	Actin, adductor muscle	strong evidence
2	~200	Q26065	13832288	19	Actin, adductor muscle	strong evidence
		O96064	7966658	28	Paramyosin	strong evidence
3	100	O96064	36505880	33	Paramyosin	strong evidence
4	55	Q26065	7370241	15	Actin, adductor muscle	strong evidence
5	47	Q26065	2154427470	45	Actin, adductor muscle	strong evidence
6	38	Q9GZ70	35811842	26	Tropomyosin	strong evidence
7	27	Q26065	6412384	13	Actin, adductor muscle	strong evidence
8	25	Q26065	1440689	8	Actin, adductor muscle	strong evidence

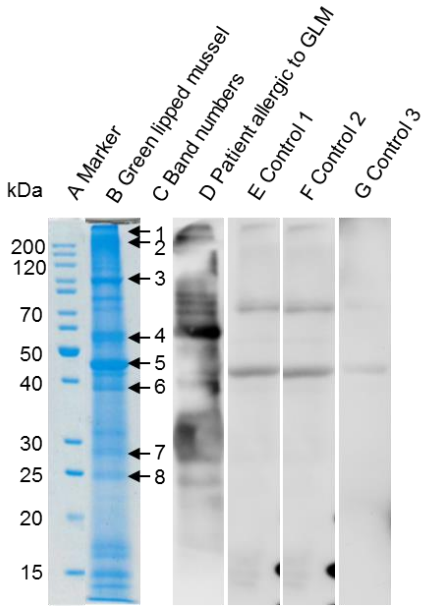

Figure 1. Identification of allergenic proteins. Protein lysate from *Perna canaliculus* was separated in an SDS-gel and either Coomassie-stained (A/ marker; B/ green-lipped mussel) or Western blotted (D/ serum from the allergic patient; E/ validation of the serum result; F/ unspecific secondary antibody binding; G/ serum of a non-allergic volunteer). Arrows and numbers mark the bands corresponding to the protein identification (C).

DISCUSSION

It has been shown that alive green-lipped mussel can decrease lung function in mussel openers.[6] So far, no specific allergens have been annotated in the Allergome Database. To our knowledge, we are the first to report an allergic reaction to green-lipped mussel powder, and this is the first description of allergens in *P canaliculus*, namely actin, tropomyosin, and paramyosin.

Actin is a highly conserved protein involved in cell motility in all eukaryotic cells. Even so, there are no entries in the AllFam Database. The in silico-prediction using AllCatPro reveals strong evidence for allergenicity. Furthermore, actin has been described previously as a major allergen in carpet clam (*Paphia textile*)[11] and as an allergen in the common periwinkle sea snail (Littorina littorea),[12] but according to the Allergome Database, it is not known as an allergen in pollen or food allergies.

The second protein identified as a potential allergen is paramyosin, a primary structural compound from invertebrate muscles. It belongs to the AllFam group AF100, which contains allergens from 6 different origins. Furthermore, AllCatPro predicts high allergenicity with paramyosin, which has been described as an allergen in other species previously.[13]

The third allergen in *P canaliculus*, tropomyosin, is a long-known allergen in seafood. It plays a central role in the calcium-dependent regulation of muscle contraction. It has been identified as an allergen for many species, as the AllFam group AF054 contains 64 entries for troponin. The allergenicity was furthermore confirmed in the carpet clam *(P textile)*,[11] in the disc abalone *(H discus)*,[14] and in silico by AllCatPro.[15]

The identification of specific allergens from *P canaliculus* in one patient opens the path for further work for a better understanding of type-1 allergy to this mussel.

STATEMENT OF ETHICS

The patient gave written consent to all clinical and in-vitro investigations.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Emelyanov A, Fedoseev G, Krasnoschekova O, Abulimity A,Trendeleva T, Barnes P J. Treatment of asthma with lipid extract of New Zealand green-lipped mussel: a randomised clinical trial. Eur Respir J. 2002;20;(3):596-600.
2. Coulson S, Palacios T, Vitetta L. Perna canaliculus (Green-Lipped Mussel): Bioactive Components and Therapeutic Evaluation for Chronic Health Conditions. Prog Drug Res. 2015; 70:91-132.
3. Jayaprakash R, Perera CO. Partial Purification and Characterization of Bioactive Peptides from Cooked New Zealand Green-Lipped Mussel (Perna canaliculus) Protein Hydrolyzates. Foods. 2020;9(7):879.
4. Grienke U, Silke J, Tasdemir D. Bioactive compounds from marine mussels and their effects on human health. Food Chem. 2014;142:48-60.
5. Scientific Opinion on the substantiation of health claims related to green lipped mussel extract and maintenance of joints, bone and muscles (ID 1571, 1813) pursuant to Article 13(1) of Regulation (EC) No 1924/2006, EFSA 2009; Journal 9, 1265. It is mandatory for all references to be indexed in PubMed. However you can put the link in a parenthesis in an appropriate place in the text. It is mandatory for all references to be indexed in PubMed. However you can put the link in a parenthesis in an appropriate place in the text.
6. Glass W I, Power P, Burt R, Fishwick D, Bradshaw L M. Work-related respiratory symptoms and lung function in New Zealand mussel openers. Am J Ind Med. 1998;34(2):163-8.
7. Coulson S, Vecchio P, Gramotnev H, Vitetta L. Green-lipped mussel (Perna canaliculus) extract efficacy in knee

osteoarthritis and improvement in gastrointestinal dysfunction: a pilot study. Inflammopharmacology. 2012.;12(2):71-6.

8. Ulbricht C, Chao W, Costa D, Nguyen Y, Seamon E, Weissner W. An evidence-based systematic review of green-lipped mussel (Perna canaliculus) by the Natural Standard Research Collaboration. J Diet Suppl. 2009;6:54-90.
9. Kespohl S, Kotschy-Lang N, Tomm J M, von Bergen M. Occupational IgE-mediated softwood allergy: characterization of the causative allergen. Int Arch Allergy Immunol. 2012;157:202-8.
10. Tomm JM, van Do T, Jende C, Simon J C, Treudler R, von Bergen M, Averbeck M. Identification of new potential allergens from Nile perch (Lates niloticus) and cod (Gadus morhua). J Investig Allergol Clin Immunol. 2013;23(5):159-67.
11. Misnan R, Bakhtiar F, Abdullah N, Murad S. Tropomyosin and Actin Identified as Major Allergens of the Carpet Clam (Paphia textile) and the Effect of Cooking on Their Allergenicity. Biomed Res Int. 2015:254152.
12. Gonzalez-Mancebo E, Trujillo-Trujillo M J, Gandolfo-Cano M, Mohedano-Vicente E, Cuesta-Herranz J, Bartolome B, et al. Actin Allergen of Common Periwinkle Sea Snail (Littorina littorea). J Investig Allergol Clin Immunol. 2019;29(11):150-2.
13. Naz S, Desclozeaux M, Mounsey K E, Chaudhry F R, WaltonSF. Characterization of Sarcoptes scabiei Tropomyosin and Paramyosin: Immunoreactive Allergens in Scabies. Am J Trop Med Hyg. 2017;97:851-60.
14. Suzuki M, Kobayashi Y, HIraki Y, Kakata H, Shiomi K. Paramyosin of the disc abalone Haliotis discus discus: Identification as a new allergen and cross-reactivity with tropomyosin. Food Chemistry. 2013;124(12):921-6.
15. Maurer-Stroh S, Krutz NL, Kern P S, Gunalan V, Nguyen MN, Limviphuvadh V, et al. AllerCatPro-prediction of protein allergenicity potential from the protein sequence. Bioinformatics. 2019;35(8):3020-7.