## Role of Creatine Kinase as an Allergen in Immediate Selective Allergy to Pork Meat

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Allergic reactions can occur after ingestion of pork products [1]. Allergy to pork meat is very uncommon in isolation [2]. We present a protein identified in a series of patients with selective allergy to pork-derived foods.

Six patients were recruited for an allergy work-up between January 2014 and February 2016. They all tolerated other mammalian meats, poultry, fish, and condiments/spices. Only patient #4 reported a tick bite. No patient had received cetuximab or a gelatin-derived colloid.

Skin prick testing was performed using a subset of indoor and outdoor aeroallergens (pollens, molds, mites, dog, cat, hamster, rabbit, horse, and cow), along with a food battery (*Anisakis*, profilin, lipid transfer protein, latex, and spices) and a meat battery (pork, beef, lamb, rabbit, and chicken) (ALK-Abelló). Prick-by-prick tests were performed for raw and boiled meats (pork, beef, lamb, rabbit, chicken, and turkey), ham (cooked and dry-cured), and other pork-derived foods (chorizo, sausage, salami, and loin), with negative controls (50% glycerinated saline) and positive controls (histamine hydrochloride, 10 mg/mL). Prick-by-prick testing with bovine gelatin-derived colloid (Aventis Pharma) and additional intradermal testing with a 1:100 dilution of bovine gelatin-derived colloid were performed in order to rule out possible asymptomatic gelatin sensitization. Serum total IgE and specific IgE against meats (pork, beef, lamb, and chicken), $\alpha$-gal, gelatin, and bovine serum albumin, were measured using ImmunoCAP (Thermo Fisher Scientific) following the manufacturer's instructions. All clinical data are shown in the Supplementary Table. All patients had negative results in skin tests with bovine gelatin-derived colloid. Five nonatopic and 5 atopic individuals served as controls, and they all had negative results to skin tests with meat, including pork.

In order to identify the culprit protein, proteins from dry-cured ham were separated by SDS-PAGE according to the method of Laemmli [3] (Figure) and electrotransferred onto polyvinylidene fluoride membranes as previously described by

Towbin et al [4] before being incubated with the patients' sera. Western blot performed with the patients' sera revealed a band of 43 kDa in all cases (Figure). Mass spectrometry sequencing revealed the identity of this protein, a creatine kinase (CK) M-type, with a molecular mass of 43 260 Da and a peptide sequence of 382 amino acids (Swissprot, NCBI database).

We present a series of 6 patients diagnosed with allergy exclusively to pork-derived products in which a specific protein was recognized by all sera. These immediate allergic reactions occurred predominantly in women. Gastrointestinal tract symptoms were observed in half of the cases. However, the diagnosis was difficult to make using conventional diagnostic methods.

The role of CK in allergic diseases has been described in a patient with occupational asthma and urticaria caused by thermolabile CK in fish [5]. However, to the best of our knowledge, CK has not been previously associated with selective pork allergy. Diverse clinical pictures have been described after eating pork products [6,7], and various allergens from pork-derived products have been reported (28-30, 40, 51, and 65 kDa in meat) [6,7]. However, none of them has been associated with an immediate, isolated allergic reaction after eating raw and cooked pork-derived foods. Because all patients had symptoms immediately after ingestion of dry-cured ham and other pork-derived products, it is reasonable to think that this CK could be a thermostable allergen, unlike fish CK [5], owing to its capacity to induce allergy either via dry-cured (ham, chorizo, loin, and salami) and cooked pork-derived foods (sausages, chops, and steaks). We thought that the allergenic capacity of these dry-cured foods was similar to that of processed fish (salted, smoked, or fermented) [8], that is, not dependent on industrial processing.

CK is a marker of heat stress in pig muscle and, therefore, an indicator of the quality of the meat [9]. During transportation and lairage before slaughter, especially under warm Mediterranean climatic conditions, hyperthermia can induce diverse metabolic reactions, including anaerobic glycolysis, glycogenolysis, production of hydrogen ions (low pH), and dehydration [9]. Furthermore, factors such as female sex, specific pig race, and extended time to slaughter affect the quantity of CK in porcine muscle [9,10]. All these factors lead to pale, soft, exudative meats, with lower levels of CK than dark, firm, dry pork meats [10].

Dry-cured ham, loin, salami, and chorizo are dark, firm, dry meats that do not receive heat during home or industrial processing. In fact, they are cured by cooling and drying. Therefore, it is possible that the pork meat portions used to perform prick-by-prick testing did not contain the necessary quantity of CK to elicit a positive response in most of the patients, since this protein was the only one detected and the responsible allergen in all 6 cases. An additional explanation would be that a significant number of the patients experienced predominantly gastrointestinal tract symptoms, and in cases where the skin was not the target organ, these were the only symptoms recorded.

As for the negative CAP results, we think that if IgE against pork CK had been available for skin and serology testing, a positive result would have been identified in all 6 patients. The main problem is that there are no purified CK proteins in pork meat, and neither serology nor skin testing can be performed, even though the immunodetection studies suggest that CK could be responsible for the clinical symptoms. Therefore, the negative responses in the skin tests and immunodetection in most patients could be explained by the quantity of CK in the meat, the different target organ, and the absence of IgE against pork CK.

In conclusion, we report a series of 6 patients diagnosed with an immediate allergy exclusively to pork-derived foods that developed in adulthood (mainly women) and was induced by specific culinary products (both dry-cured and cooked). The reactions manifested as various symptoms, affecting mainly the gastrointestinal tract. We identified a CK M-type from pork meat as the associated protein in all 6 cases.

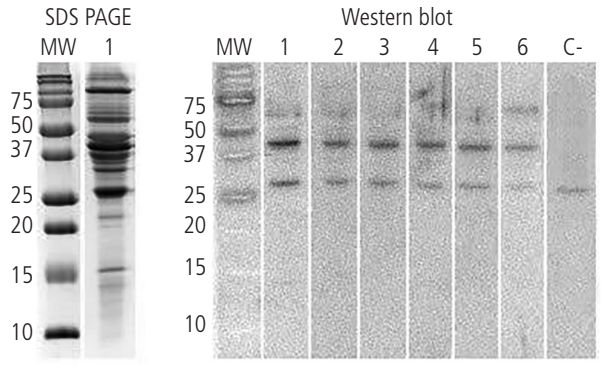


Figure. SDS PAGE with dry-cured ham. Lane 1, detected protein bands. Western blot from the 6 patients' sera. MW, molecular weight markers. Lane 1, patient #1; Lane 2, patient #2; Lane 3, patient #3; Lane 4, patient #4; Lane 5, patient #5; Lane 6, patient #6; C–, negative control serum.

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*Conflicts of Interest*

The authors declare that they have no conflicts of interest.

## References

1. Carrapatoso I, Bartolomé Zavala B, Ribeiro F, Martínez Quesada J, Segorbe Luís A. Allergy to red meat in adulthood: a case report. J Investig Allergol Clin Immunol. 2014;24(3):206-8.
2. Dominguez-Ortega J, Rodriguez-Jiménez B, Ledesma A, Kindelan C, González JM, Jiménez F. Selective allergy to raw pork. J Investig Allergol Clin Immunol. 2009;19(3):244-5.
3. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970;227(5259):680-5.
4. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets:

 

procedure and some applications. Proc Natl Acad Sci USA. 1979;76(9):4350-4.
5. Larco-Rojas X, González-Gutiérrez ML, Vázquez-Cortés S, Bartolomé B, Pastor-Vargas C, Fernández-Rivas M. Occupational asthma and urticaria in a fishmonger due to creatine kinase, a cross-reactive fish allergen. J Investig Allergol Clin Immunol. 2017;27(6):378-96.
6. Llátser R, Polo F, De La Hoz F, Guillaumet B. Alimentary allergy to pork. Crossreactivity among pork kidney and pork and lamb gut. Clin Exp Allergy. 1998;28(8):1021-5.
7. Asero R, Mistrello G, Falagiani P. Oral allergy syndrome from pork. Allergy. 1997;52(6):684-6.
8. Sletten G, Van Do T, Lindvik H, Egaas E, Florvaag E. Effects of industrial processing on the immunogenicity of commonly ingested fish species. Int Arch Allergy Immunol. 2010;151(3):223-36.
9. Jama N, Maphosa V, Hoffman LC, Muchenje V. Effect of sex and time to slaughter (transportation and lairage duration) on the levels of cortisol, creatine kinase and subsequent relationship with pork quality. Meat Science. 2016;116:43-9.
10. Bonelli AM, Schifferli R. Porcine stress syndrome. Arch Med Vet. 2001;33:2.

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