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# Immunoproteomics of processed beef proteins reveal novel galactose-α-1,3-galactose-containing allergens

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**Keywords**
beef processing; carbohydrate; immunoproteomics; red meat allergy; α-Gal epitope.

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## Abstract

**Background:** Red meat allergy presents a novel form of food allergy with severe delayed allergic reactions where IgE antibodies are directed against the carbohydrate α-Gal epitope. Food preparation and processing can influence the allergenicity of proteins. The aim of this study was to characterize the proteomic profile of different beef preparations and to investigate their α-Gal reactivity and potential allergenicity.

**Methods:** Extracts from raw, boiled, fried, and medium rare prepared beef were assessed by 2D PAGE for the comparison of protein profiles. IgE-binding proteins were identified using immunoblot-coupled proteomic analysis using sera from red meat-allergic patients. Presence of the α-Gal epitope was verified using anti-α-Gal antibody and IgE inhibition immunoblot with α-Gal.

**Results:** Multiple IgE-binding proteins were detected in the different beef preparations, many of which were also recognized by the anti-α-Gal antibody. Protein spots reacting with IgE in patient sera were analyzed by MS/MS, resulting in identification of 18 proteins with high identification scores. Seven of the 18 beef allergens identified using meat-allergic patient sera were also recognized by the anti-α-Gal monoclonal antibody, and four of them were stabile to thermal treatment. Furthermore, a dose-dependent inhibition of red meat-allergic patients' IgE to beef by α-Gal was demonstrated.

**Conclusions:** We show that the α-Gal epitope is commonly present in IgE-reactive beef proteins recognized by meat-allergic patients. Seven novel α-Gal-containing IgE-binding proteins were identified, of which four were stable to heat treatment. Thus, the allergenicity of red meat proteins is preserved even upon different thermal cooking.

Red meat allergy has been identified as a new syndrome of food allergy presenting with severe allergic reactions after consumption of red meat (e.g., beef, lamb, or pork). While most allergic reactions to food are directed against protein epitopes and occur rapidly after ingestion of the allergen, allergic reactions to red mammalian meat are directed against the carbohydrate galactose-α-1,3-galactose (α-Gal) epitope and occur several hours after intake.

IgE antibody responses to α-Gal, which is abundant in glycolipids and glycoproteins from nonprimate mammals, were first recognized when patients treated with the monoclonal antibody cetuximab experienced anaphylactic reactions upon first injection (1). It was revealed that the reactions were caused by pre-existing IgE antibodies directed against the α-Gal epitope on the Fab portion of cetuximab. Further studies led to the realization that IgE to α-Gal was also associated with a novel form of food allergy presenting as anaphylaxis,

**Abbreviations**
*B. taurus*, *Bos taurus*; CBB, coomassie brilliant blue; LC, liquid chromatography; MS/MS, tandem mass spectrometry; α-Gal, galactose-α-1,3-galactose.

urticaria, or angioedema occurring 3–6 h after consumption of mammalian meat (2–10).

Another cause of meat allergy is the 'pork–cat syndrome' caused by IgE antibodies toward cat serum albumin, which cross-reacts with porcine albumin and can lead to severe allergic reactions when pork is consumed (11). In addition, clinical cross-reactivity between beef and cow's milk due to the known beef allergens bovine serum albumin (BSA), bovine immunoglobulin G (IgG), and bovine actin has been reported previously in children (12–16).

It is well known that heating or other treatments of proteins can modify IgE-binding properties by changing the conformation of the epitopes. Changing the allergen conformation by thermal treatments often destroys conformational IgE-binding epitopes, but sometimes it can produce new epitopes and enhance the allergenicity (17). It has previously been reported that cooking often destroys or modifies heat-labile beef allergens, but some patients still report allergic response to boiled/fried mammalian meat (18–20).

Recently, it has been reported that laminin γ-1 and collagen α-1 (VI) chain from beef are α-Gal-containing allergens (21). Those findings were in line with previous research demonstrating cross-reactivity of red meat with gelatin, a derivative of collagen (7), and that laminins contain α-Gal at the post-translational modification level (22).

In this study, we have characterized the proteomic profile of different beef preparations (raw, medium rare, fried, and boiled) and investigated their potential allergenicity among red meat-allergic patients. Using immunoproteomics, we have identified novel α-Gal-containing proteins that bind IgE from meat-allergic patients.

## Methods

### Patients and sera

Sera from 15 meat-allergic patients reporting delayed allergic reactions following consumption of red meat attending the Allergy Unit at Södersjukhuset, Stockholm, were used in the study. All patients were examined by a physician experienced in allergic diseases and responded to a detailed questionnaire regarding clinical episodes and symptoms after consuming mammalian meat. All patients had IgE antibodies to α-Gal (range 6.6–128 $kU_A/l$) and beef (range 0.5–28 $kU_A/l$) determined by ImmunoCAP System (Phadia AB/Thermo Fisher Scientific, Uppsala, Sweden). Sera were either used individually or as pool 1 (median IgE level 31 $kU_A/l$ to α-Gal and 9.7 $kU_A/l$ to beef based on patient no. 5–9 in Table 1) in 1D immunoblotting, or pool 2 (median IgE level 49 $kU_A/l$ to α-Gal and 9.8 $kU_A/l$ to beef based on patient no. 9–14 in Table 1) in 2D immunoblotting. Serum from an atopic subject lacking IgE to α-Gal and beef (<0.1 $kU_A/l$) was used as negative control. The study was approved by the local ethics committee.

### Beef preparation and protein extraction

Meat from beef was cut in pieces (10 g each, containing 2.3 g of protein according to nutrient data service from USDA, http://ndb.nal.usda.gov/). Raw meat extract was prepared by homogenizing one piece with 30 ml of phosphate buffer saline (PBS), pH 7.4, with a POLYTRON homogenizer (PT2100, Kinematica, Luzern, Switzerland), centrifuged at 4347 ***g*** for 30 min at 4°C. Supernatant was collected and fil-

**Table 1** Characteristic of meat-allergic patients

| | | | | IgE* | | |
|---|---|---|---|---|---|---|
| Patient no. | Age /sex | Reaction | Time to reaction† | Total | α-Gal | Beef |
| 1 | 69/M | AE, GI, U | 6 | 260 | 54 | 5 |
| 2 | 71/M | AE, GI, U | 4 | 280 | 23 | 11 |
| 3 | 35/F | ANA, AE, GI, U | 6–7 | 270 | 46 | 7.2 |
| 4 | 71/M | ANA, AE, GI, U | 6 | 48 | 6.6 | 0.5 |
| 5 | 72/M | AE, U | 6 | 420 | 128 | 16 |
| 6 | 46/M | ANA, AE, GI, U | 4–7 | 1800 | 24 | 4.5 |
| 7 | 67/F | ANA, AE, U | 6 | 550 | 31 | 22 |
| 8 | 57/M | GI, U | 4–12 | ND | 22 | 1.9 |
| 9 | 46/F | U | 2–7 | 240 | 110 | 9.7 |
| 10 | 43/F | GI, U | 3 | 360 | 61 | 12 |
| 11 | 54/F | ANA, AE, U | 1–2 | 87 | 12 | 5.4 |
| 12 | 75/M | GI | ND | 340 | 37 | 6 |
| 13 | 38/F | AE, GI, U | 4–5 | 120 | 37 | 9.9 |
| 14 | 42/M | GI, U | 6 | 270 | 80 | 28 |
| 15 | 64/M | GI U | 5 | 705 | >100 | 60.5 |

M, male; F, female; AE, angioedema; ANA, anaphylaxis; GI, gastrointestinal symptoms; U, urticaria; ND, not determined; Age = years.
*ImmunoCAP IgE results: Total IgE levels are expressed in kilounits per liter, and allergen-specific IgE levels are expressed in kilounits of allergen per liter.
†Reported time to reaction expressed in hours.

tered through 0.8-μm filter, and protein content was measured with BCA™ assay (Pierce, Rockford, IL, USA). To obtain fried, boiled, and medium rare meat for protein extraction, heating procedure was performed as previously described with some differences (12). Briefly, boiled meat was prepared by boiling the meat in water for 20 min at 80°C. Fried and medium rare meat was prepared in a frying pan for 20 min at 80°C and for 5 min at 50°C, respectively. Extract preparation was carried out as described above.

### SDS-PAGE and immunoblot analysis

Proteins from the differently processed meat extract were separated by SDS-PAGE and stained with Coomassie Brilliant Blue (CBB) or analyzed by immunoblotting under reducing conditions. Ten micrograms of proteins per lane was analyzed using serum from five meat-allergic patients (no. 1–5 in Table 1). Immunoblot inhibition was performed in the presence of 100 μg/ml α-Gal (thyroglobulin, Sigma-Aldrich Inc., St. Louis, MO, USA) using patient serum pool 1. Detection of α-Gal-containing proteins was performed using mouse monoclonal anti-α-Gal antibody (M86, Enzo Life Science Inc., Farmingdale, NY, USA). For details, see Supporting information.

### Deglycosylation assay

Enzymatic removal of glycoprotein was performed in 1 mg/ml raw beef extract with PNGase F kit (New England BioLabs, Ipswich, MA, USA) according to manufacturer's instruction. The deglycosylated product was tested on immunoblot developed with monoclonal anti-α-Gal antibody.

## 2D PAGE

Proteins from beef extracts (75 μg) were separated by 2D PAGE and detected using patient serum pool 2 and mouse anti-α-Gal monoclonal antibody. For details, see Supporting information.

### Protein identification by mass spectrometry

Protein spots of interest from 2D gel were manually excised and subjected to in-gel digestion with trypsin from porcine pancreas (Proteomic Grade, BioReagent, Dimethylated; Sigma-Aldrich) as previously described (23). Obtained peptides were analyzed by nano-liquid chromatography tandem mass spectrometry (LC-MS/MS) as described previously (24). For details, see Supporting information.

### Sequence analysis

A homology search of proteins identified from the amino acid sequences was performed with BLASTp on the NCBI nonredundant protein sequence database. For prediction of N-glycosylation sites, identified proteins were analyzed using the online software Structure Feature Analysis Tool (http://hive.biochemistry.gwu.edu) (25).

### Absorption experiment

The capacity of α-Gal and raw beef extract to inhibit IgE binding to solid-phase-bound bovine thyroglobulin, as a marker of α-Gal sensitization for research purposes, was measured using the ImmunoCAP System. Serum from one meat-allergic patient (#15) was preincubated with twofold dilutions (0.125–4000 μg/ml) of either thyroglobulin or raw beef extract.

## Results

### Meat-allergic patients recognize different processed beef proteins

IgE binding to proteins in different processed beef extracts was investigated with sera from five meat-allergic patients (no. 1–5 in Table 1). Patterns of IgE binding to several soluble proteins of beef (25–250 kDa) were detected in patients, but not in the negative control (Fig. 1A). For comparison of IgE-binding properties between differently processed beef, all extracts were analyzed with serum pool 1 from meat-allergic patients. Multiple reactive bands were detected for all the different beef preparations from 25 to 150 kDa (Fig. 1B). In raw and medium rare meat, stronger reactive band was seen between 30 and 100 kDa. As the α-Gal epitope is often attached to larger structural glycoproteins, we also investigated the high molecular weight area (>150 kDa) and found some IgE-binding proteins in this range. Furthermore, boiled and fried meat showed similar reactive protein bands, from 37 to 75 kDa, although with weaker expression compared with raw and medium rare beef extracts, which could be an indication of reduced ability to bind IgE (Fig. 1B). Preincubation of patient serum pool 1 with 100 μg/ml of α-Gal resulted in the disappearance of several IgE-binding protein bands (Fig. 1B). In addition, a separate immunoblot was developed with anti-α-Gal antibody (Fig. 1C), showing similar binding patterns to the different processed beef extracts as the patient serum pool.

To verify that the detected IgE-binding beef proteins indeed contained α-Gal, the N-linked glycoproteins were removed from the raw meat extract by deglycosylation. An immunoblot developed with anti-α-Gal antibody (Fig. 1D) displayed removal of all protein bands besides one band around 37 kDa.

### Protein profiles of different processed beef extracts

The differently cooked beef preparations separated on SDS-PAGE (Fig. 2A) showed overall similar profiles when comparing raw and medium rare beef extracts, as well as when comparing boiled and fried beef. However, some differences were noted. Boiled beef extract contained bands with higher molecular weight, ≥250 kDa, probably originating from thermally induced aggregation of proteins that were not observed in raw and medium rare meat extracts. When comparing the 2D PAGE protein profiles of raw and medium rare beef extracts (Fig. 2B, C), we were able to identify 53 matched protein spots. Boiled and fried beef extracts also showed similar profiles on 2D PAGE (Fig. 2D, E), and 35 protein

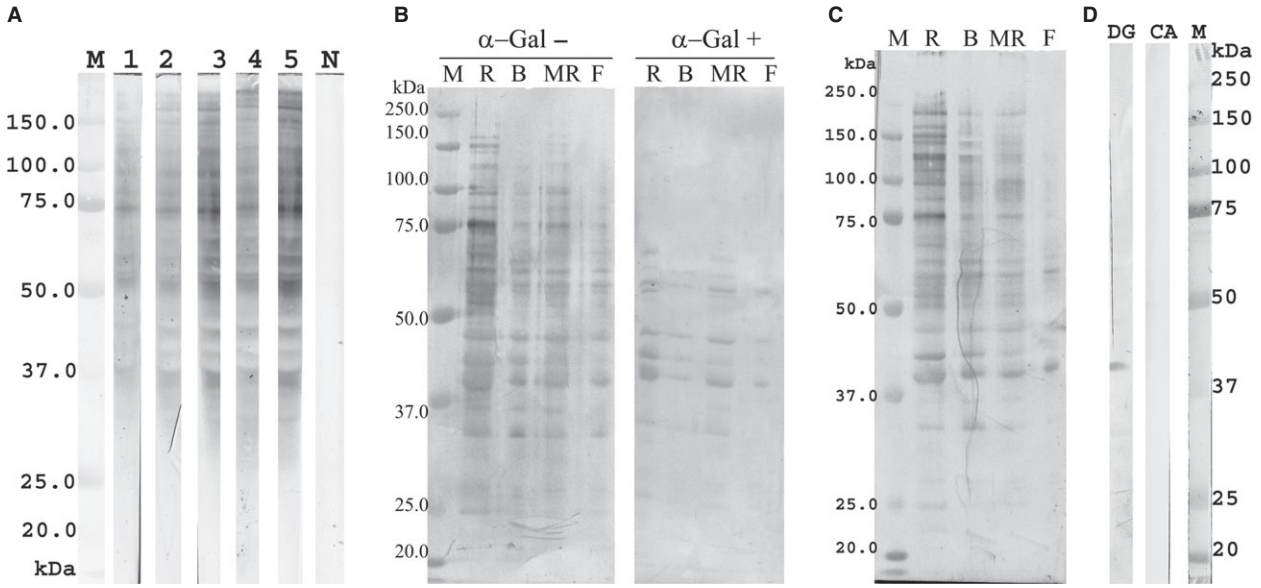


**Figure 1** Immunoblot reactivity to meat proteins. (A) IgE binding to raw beef extract in serum from five meat-allergic patients (no. 1-5) and a negative control (N). (B) IgE-binding profile of different processed beef extracts in serum pool 1 (from meat-allergic patients) without or with preincubation of α-Gal (100 μg/ml). (C) α-Gal-binding profiles of different processed beef extract using monoclonal anti-α-Gal antibody. (D) Deglycosylated raw beef extract with monoclonal anti-α-Gal antibody. M, molecular weight marker; N, negative control; R, raw; B, boiled; MR, medium rare; F, fried; DG, deglycosylated; CA, control antibody.

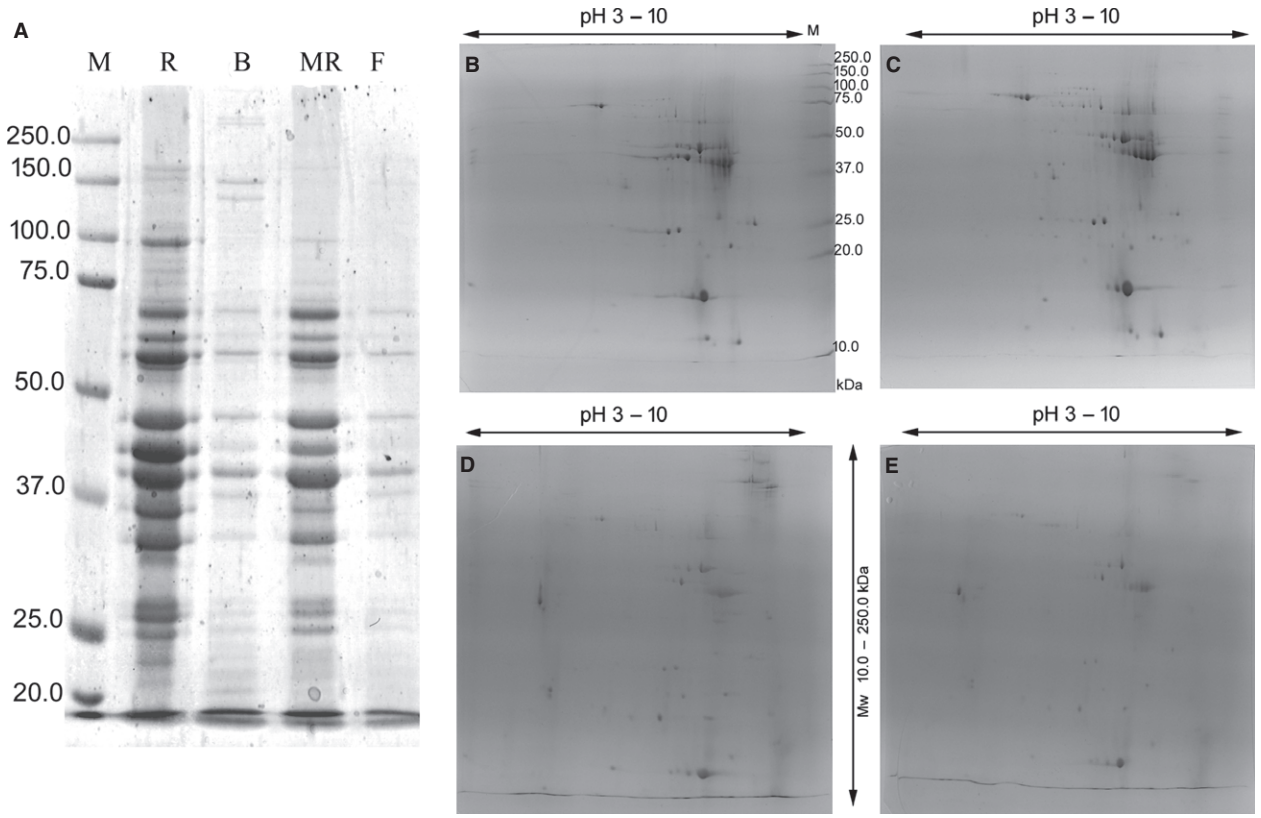


**Figure 2** 1D PAGE and 2D PAGE protein profile of different processed beef extracts. (A) 1D PAGE and 2D PAGE of raw beef extract (B), medium rare beef extract (C), boiled beef extract (D), fried beef extract (E). M, molecular weight marker; R, raw; B, boiled; MR; medium rare; F, fried.

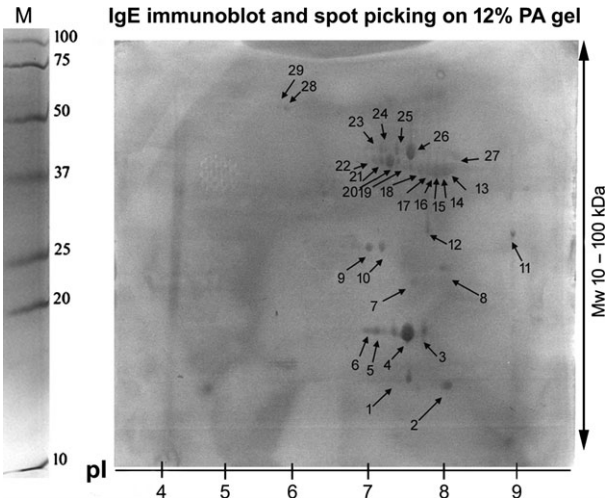


**Figure 3** 2D immunoblot analysis of raw beef extract developed with serum pool 2 (meat-allergic patients). Protein spots labeled 1–29 were used for peptide mass print analysis and protein identification (Table S1). M, molecular weight marker.

matches were identified. Some of the proteins, mostly around 17 and 50 kDa, were still present after the thermal treatment.

### Identification of IgE-binding water-soluble beef allergens

To identify proteins with IgE-binding capacity, a 2D immunoblot was developed using serum pool 2. Twenty-nine IgE-binding protein spots around 15–100 kDa were observed (Fig. 3) and analyzed by peptide mass fingerprint. MS/MS spectra of these proteins gave high identification scores for 18 proteins from the *Bos taurus (B. taurus)* database (Table S1). The well-known beef allergens myoglobin and BSA were also among the identified proteins.

Sequence analysis showed that the proteins identified from *B. taurus* have high homology with the corresponding proteins from *Homo sapiens* (Table S2), with ≥85% similarities for all proteins. Prediction of N-glycosylation sites for the identified proteins revealed sites in 10 of the 18 proteins and some of them contained more than one glycosylation site (Table S2).

### Identification of α-Gal-containing proteins

To identify α-Gal-containing proteins, a 2D immunoblot was developed with the anti-α-Gal antibody, this time with separation on 10% PA gel to investigate a wider molecular mass range (Fig. 4). The α-Gal-containing proteins appeared to have mainly basic pI values (pI ≥7), which was also seen for the IgE-binding proteins detected with the serum pool. When comparing the immunoblots developed with either serum pool or the anti-α-Gal antibody, many IgE-binding proteins that also contain the α-Gal epitope were identified (Figs 3 and 4). According to pI and Mw values on 2D PAGE and immunoblots as well as prediction of N-glycosylation sites, we identified seven proteins (triosephosphate isomerase, carbonic anhydrase 3, lactate dehydrogenase A, creatine kinase M-type, aspartate aminotransferase, β-enolase, and α-enolase) as IgE-reactive α-Gal-containing proteins (Table 2). Comparison of protein profiles between raw and cooked beef extracts revealed that four of the identified α-Gal-containing allergens (creatine kinase M-type, aspartate aminotransferase, β-enolase, and α-enolase) were stable to heat treatment (bold-faced, Table 2).

### α-Gal epitope inhibition

A meat-allergic patient serum (#15) was preincubated with either α-Gal or raw beef extract (0.125–4.0 mg/ml) prior to measurement of α-Gal-specific IgE. The dose-dependent inhibition of IgE to α-Gal with raw meat extract and α-Gal showed a maximum inhibition of 44% obtained using 4.0 mg/ml raw meat extract compared with 86% with α-Gal (Figure S1).

## Discussion

In this study, we used an immunoproteomic approach to identify α-Gal-containing proteins in differently processed

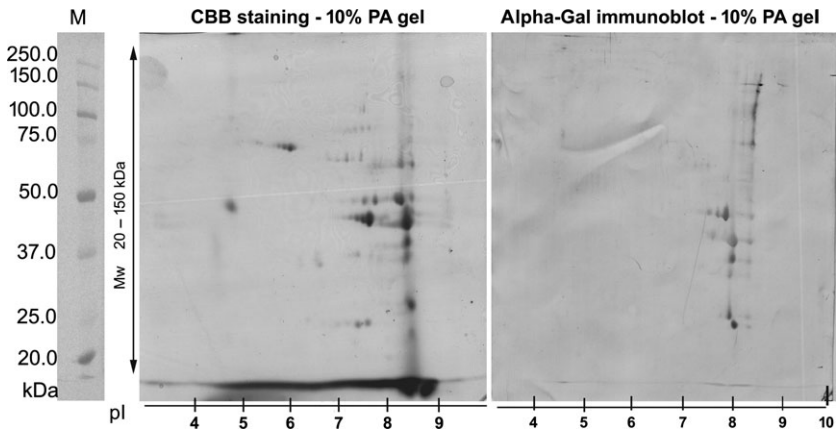


**Figure 4** 2D immunoblot analysis of raw beef extract developed with monoclonal anti-α-Gal antibody. In addition, CBB staining of 10% PA gel. M, molecular weight marker.

**Table 2** α-Gal-containing proteins identified by nano-LC-MS/MS and *Bos taurus* FASTA database search after in-gel tryptic digestion of proteins in raw beef extract

| Protein Name | Accession No.* | Mw (kDa); pI | Identified spot† |
|---|---|---|---|
| Triosephosphate isomerase | NP_001013607.1 | 26.7; 6.92 | 9, 10 |
| Carbonic anhydrase 3 | NP_001029609.1 | 29.4; 7.84 | 12, 16 |
| Lactate dehydrogenase A | BAA14170.1 | 35.6; 8.00 | 15, 16 |
| **Creatine kinase M-type** | AAD30974.10 | 43.0; 7.12 | 17–18, 20–26 |
| **Aspartate aminotrasferase** | P33097.3 | 46.4; 7.49 | 17–18, 21–26 |
| **Beta-enolase** | NP_001029874.1 | 47.1; 7.72 | 19, 24–27 |
| **Alpha-enolase** | DAA21263.1 | 47.3; 6.80 | 24–27 |

Bold-faced protein contains α-Gal and survived heat treatments.
*Database NCBIInr July 2013.
†SEQUEST algorithm.

beef extracts. Seven proteins were identified as novel α-Gal-containing IgE-binding proteins recognized by meat-allergic patients, and of these, four proteins were stable to heat treatment. Most of the meat-allergic patients recognized similar IgE-binding protein bands in beef extracts, with a wide size range of 25–250 kDa, in line with previous research (12). We noted that many of the IgE-binding proteins from the different processed meat extracts had preserved allergenicity even after thermal treatment, and preincubation of the sera with α-Gal removed most of this reactivity. The heat-stable proteins were also recognized by the anti-α-Gal antibody. The patient sera (pool) and the anti-α-Gal antibody showed a similar binding pattern to the different thermally processed beef extracts, indicating that most of the proteins still preserve their α-Gal reactivity after heat treatment. This is in line with data from our red meat-allergic patients who often report allergic reactions after ingestion of cooked meat. The reactions are delayed, which has recently been confirmed in food challenges by Commins and colleagues who also reported that basophil activation occurred in the same time frame as the clinical symptoms (10).

To identify IgE-binding proteins carrying the α-Gal epitope and whether they survive thermal processing, all meat extracts were analyzed by powerful immunoproteomic tools: high-resolution 2D PAGE, 2D immunoblot, and MS/MS analysis. Takahashi et al. (21) recently demonstrated α-Gal epitopes on two IgE-binding proteins from beef in the high molecular weight area (laminin γ-1 and collagen α-1(VI) chain). Due to unclear separation of low molecular weight proteins, they reported that other proteins could not be analyzed. Our results show not only clearly separated beef proteins in the lower molecular weight area (20–100 kDa), but also high similarity between raw and medium rare meat (53 matched protein spots), as well as high similarity between boiled and fried meat (35 matched protein spots). Furthermore, multiple protein spots reacted in the same extent with patient sera as with the anti-α-Gal monoclonal antibody.

Using high-resolution MS/MS analysis, we identified 18 IgE-binding proteins. Some of the identified proteins are well-known beef allergens (myoglobin and BSA) (15), but the majority has not previously been reported as beef allergens or α-Gal-containing proteins. Seven of the 18 IgE-binding proteins are novel α-Gal-containing proteins, and four of these survived heat treatment. The new identified proteins are cytoplasmic proteins, playing essential roles in the metabolic pathways (26, 27). Some of them have various functions; for example, enolase is included in processes such as growth control, hypoxia tolerance, and allergic responses and may also stimulate immunoglobulin production (28).

We noticed that the α-Gal-containing high molecular weight IgE-binding proteins were in low abundance compared with lower molecular mass α-Gal proteins recognized by the monoclonal anti-α-Gal antibody, which is specific for the Galα1→3Gal-R epitope (29). Other proteins known to carry α-Gal are thyroglobulin, fibrinogen, IgG, fibronectin, and integrin (β1 subunit) (30, 31). These proteins were not detected in this study, presumably due to their low solubility and very high molecular size. Denatured type I bovine collagen has been identified as the major allergenic component of gelatin (32), and meat-allergic patients have been reported to react to gelatin (7). Sequence analysis revealed that the identified *B. taurus* proteins and corresponding human proteins are highly homologous (≥85%).

This suggests that α-Gal, a post-translational modification of nonprimate mammals, is an important epitope of these proteins. However, highly homologous proteins without α-Gal were also identified.

To explore whether IgE responses to α-Gal can be inhibited with raw meat extract, competitive inhibition assay was performed. We found that the raw meat extract was able to inhibit 44% of the IgE response to α-Gal compared with an 86% inhibition using α-Gal at the highest concentration of respective inhibitor (4 mg/ml). The results indicate that IgE binding to α-Gal is restricted to exposed α-Gal moieties as previously reported (33) and that red meat-allergic patients also have α-Gal-independent IgE response to beef.

In conclusion, we have shown that the α-Gal epitope is commonly present in beef proteins recognized by red meat-allergic patients' IgE. We have identified 18 IgE-binding proteins with high identification score from *B. taurus*. By immunoproteomics and MS/MS analysis, seven novel α-Gal-containing beef allergens were described, of which four were stable to heat treatment: creatine kinase M-type, aspartate aminotransferase, β-enolase, and α-enolase. Thus, the allergenicity of red meat proteins is preserved even upon thermal processing. Furthermore, we demonstrate that red meat-allergic patients also have IgE responses to non-α-Gal-containing proteins. Red meat allergy is a novel form of food allergy, and the process of digestion, absorption, and delivery of α-Gal-containing molecules to the circulation is an important but complex issue, which remains to be elucidated.

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## Author contributions

DA and TAT participated in all stages of the project, did the experiments, and are the main authors of the manuscript. DA, TAT, CH, TCV, and MvH designed the project and contributed to interpretation of the data. All authors provided critical review of the manuscript.

## Conflicts of interest

The authors declare that they have no conflicts of interest.

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Inhibition assay demonstrating dose-dependent inhibition of serum IgE to α-Gal using two-fold dilutions of raw beef extract and thyroglobulin (0.125–4 mg/ml)

**Data S1.** Material and Methods.

**Table S1.** Protein identification by nanoLC-MS/MS and *Bos taurus* FASTA database search after in-gel tryptic digestion of proteins in raw beef extract.

**Table S2.** Homology between *Homo sapiens* and *Bos taurus* and prediction of N-glycosylation sites.

## References

1. Chung CH, Mirakhur B, Chan E, Le QT, Berlin J, Morse M et al. Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. *N Engl J Med* 2008;**358**:1109–1117.
2. Commins SP, Satinover SM, Hosen J, Mozena J, Borish L, Lewis BD et al. Delayed anaphylaxis, angioedema, or urticaria after consumption of red meat in patients with IgE antibodies specific for galactose-alpha-1,3-galactose. *J Allergy Clin Immunol* 2009;**123**:426–433.
3. Jacquenet S, Moneret-Vautrin DA, Bihain BE. Mammalian meat-induced anaphylaxis: clinical relevance of anti-galactose-alpha-1,3-galactose IgE confirmed by means of skin tests to cetuximab. *J Allergy Clin Immunol* 2009;**124**:603–605.
4. Nunez R, Carballada F, Gonzalez-Quintela A, Gomez-Rial J, Boquete M, Vidal C. Delayed mammalian meat-induced anaphylaxis due to galactose-alpha-1,3-galactose in 5 European patients. *J Allergy Clin Immunol* 2011;**128**:1122–1124.
5. Commins SP, James HR, Kelly LA, Pochan SL, Workman LJ, Perzanowski MS et al. The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose-alpha-1,3-galactose. *J Allergy Clin Immunol* 2011;**127**:1286–1293.
6. Hamsten C, Starkhammar M, Tran TA, Johansson M, Bengtsson U, Ahlen G et al. Identification of galactose-alpha-1,3-galactose in the gastrointestinal tract of the tick Ixodes ricinus; possible relationship with red meat allergy. *Allergy* 2013;**68**:549–552.
7. Mullins RJ, James H, Platts-Mills TA, Commins S. Relationship between red meat allergy and sensitization to gelatin and galactose-alpha-1,3-galactose. *J Allergy Clin Immunol* 2012;**129**:1334–1342.
8. Morisset M, Richard C, Astier C, Jacquenet S, Croizier A, Beaudouin E et al. Anaphylaxis to pork kidney is related to IgE antibodies specific for galactose-alpha-1,3-galactose. *Allergy* 2012;**67**:699–704.
9. Hamsten C, Tran TA, Starkhammar M, Brauner A, Commins SP, Platts-Mills TA et al. Red meat allergy in Sweden: association with tick sensitization and B-negative blood groups. *J Allergy Clin Immunol* 2013;**132**:1431–1434.
10. Commins SP, James HR, Stevens W, Pochan SL, Land MH, King C et al. Delayed clinical and ex vivo response to mammalian meat in patients with IgE to galactose-alpha-1,3-galactose. *J Allergy Clin Immunol* 2014;**134**:108–115.
11. Drouet M, Sabbah A. The pork/cat syndrome or crossed reactivity between cat epithelia and pork meat. *Monogr Allergy* 1996;**32**:164–173.
12. Werfel SJ, Cooke SK, Sampson HA. Clinical reactivity to beef in children allergic to cow's milk. *J Allergy Clin Immunol* 1997;**99**:293–300.
13. Ayuso R, Lehrer SB, Lopez M, Reese G, Ibanez MD, Esteban MM et al. Identification of bovine IgG as a major cross-reactive vertebrate meat allergen. *Allergy* 2000;**55**:348–354.
14. Restani P, Ballabio C, Tripodi S, Fiocchi A. Meat allergy. *Curr Opin Allergy Clin Immunol* 2009;**9**:265–269.
15. Fiocchi A, Restani P, Riva E, Qualizza R, Bruni P, Restelli AR et al. Meat allergy: I-Specific IgE to BSA and OSA in atopic, beef sensitive children. *J Am Coll Nutr* 1995;**14**:239–244.
16. Kanny G, de Hauteclocque C, Moneret-Vautrin DA. Food anaphylaxis to bovine serum albumin. *J Allergy Clin Immunol* 1998;**101**:137–139.
17. Davis PJ, Williams SC. Protein modification by thermal processing. *Allergy* 1998;**53**(Suppl 46):102–105.
18. Fiocchi A, Bouygue GR, Sarratud T, Terracciano L, Martelli A, Restani P. Clinical tolerance of processed foods. *Ann Allergy Asthma Immunol* 2004;**93**(5 Suppl 3):S38–S46.
19. Fiocchi A, Restani P, Riva E. Beef allergy in children. *Nutrition* 2000;**16**:454–457.
20. Fiocchi A, Restani P, Riva E, Mirri GP, Santini I, Bernardo L et al. Heat treatment modifies the allergenicity of beef and bovine serum albumin. *Allergy* 1998;**53**:798–802.
21. Takahashi H, Chinuki Y, Tanaka A, Morita E. Laminin gamma-1 and collagen alpha-1 (VI) chain are galactose-alpha-1,3-galactose-bound allergens in beef. *Allergy* 2014;**69**:199–207.
22. Arumugham RG, Hsieh TC, Tanzer ML, Laine RA. Structures of the asparagine-

linked sugar chains of laminin. *Biochim Biophys Acta* 1986;**883**:112–126.
23. Shevchenko A, Tomas H, Havlis J, Olsen JV, Mann M. In-gel digestion for mass spectrometric characterization of proteins and proteomes. *Nat Protoc* 2006;**1**:2856–2860.
24. Apostolovic D, Luykx D, Warmenhoven H, Verbart D, Stanic-Vucinic D, de Jong GA et al. Reduction and alkylation of peanut allergen isoforms Ara h 2 and Ara h 6; characterization of intermediate- and end products. *Biochim Biophys Acta* 2013;**1834**:2832–2842.
25. Lam PV, Goldman R, Karagiannis K, Narsule T, Simonyan V, Soika V et al. Structure-based comparative analysis and prediction of N-linked glycosylation sites in evolutionarily distant eukaryotes. *Genomics Proteomics Bioinformatics* 2013;**11**:96–104.
26. Joseph P, Suman SP, Rentfrow G, Li S, Beach CM. Proteomics of muscle-specific beef color stability. *J Agric Food Chem* 2012;**60**:3196–3203.
27. Bax ML, Sayd T, Aubry L, Ferreira C, Viala D, Chambon C et al. Muscle composition slightly affects in vitro digestion of aged and cooked meat: identification of associated proteomic markers. *Food Chem* 2013;**136**:1249–1262.
28. Aaronson RM, Graven KK, Tucci M, McDonald RJ, Farber HW. Non-neuronal enolase is an endothelial hypoxic stress protein. *J Biol Chem* 1995;**270**:27752–27757.
29. Thall AD, Maly P, Lowe JB. Oocyte Gal alpha 1,3Gal epitopes implicated in sperm adhesion to the zona pellucida glycoprotein ZP3 are not required for fertilization in the mouse. *J Biol Chem* 1995;**270**:21437–21440.
30. Everett ML, Lin SS, Worrell SS, Platt JL, Parker W. The footprint of antibody bound to pig cells: evidence of complex surface topology. *Biochem Biophys Res Commun* 2003;**301**:751–757.
31. Thall A, Galili U. Distribution of Gal alpha 1—3Gal beta 1—4GlcNAc residues on secreted mammalian glycoproteins (thyroglobulin, fibrinogen, and immunoglobulin G) as measured by a sensitive solid-phase radioimmunoassay. *Biochemistry* 1990;**29**:3959–3965.
32. Wahl R, Kleinhans D. IgE-mediated allergic reactions to fruit gums and investigation of cross-reactivity between gelatine and modified gelatine-containing products. *Clin Exp Allergy* 1989;**19**:77–80.
33. Lammerts van Bueren JJ, Rispens T, Verploegen S, van der Palen-Merkus T, Stapel S, Workman LJ et al. Anti-galactose-alpha-1,3-galactose IgE from allergic patients does not bind alpha-galactosylated glycans on intact therapeutic antibody Fc domains. *Nat Biotechnol* 2011;**29**:574–576.