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Elucidating the α -Gal syndrome at the molecular allergen level

To the editor,

The galactose- α -1,3-galactose (α -Gal) syndrome (AGS) is a novel food allergy with delayed severe allergic reactions after mammalian meat consumption, caused by IgE against the carbohydrate epitope α -Gal present in mammalian products. We have recently characterized a cohort of AGS patients, and demonstrated that atopy increased the risk of anaphylactic symptoms in the respiratory system¹. This finding prompted us to elucidate the sensitization pattern in AGS patients in more depth, by using the multiplex ImmunoCAP Immuno Solid-phase Allergen Chip (ISAC), and address the clinical relevance of sensitization to individual allergen molecules.

A total of 138 patients with IgE antibodies to α -Gal (ImmunoCAP) and a doctor's diagnosis of AGS were enrolled in the study (Table S1). Almost half of the patients reported anaphylactic symptoms (47.8%). Patients' sera were tested on ISAC¹¹². A detailed description of patient inclusion, IgE measurements and statistics can be found in the supplementary file.

Due to the role of α -Gal in AGS, we investigated the carbohydrate response more closely. We found that AGS patients were not more prone to develop IgE against carbohydrate residues other than α -Gal. IgE against α -Gal was detected in 92.8% of the patients, because of the slightly lower sensitivity of ISAC compared to ImmunoCAP. Only thirty-one patients (23%) were sensitized against other cross-reactive carbohydrate domains, mainly glycosylated grass pollen allergens (Phl p 4 15.9% and Cyn d 1 18.8%). This percentage is similar to patients with inhalant allergy (23%)².

Figure 1A shows a heat map of the IgE reactivity to the most frequently recognized allergen families. A complete heatmap and an explanatory table are available in figure S1 and table S2. After food allergens, grass pollen and tree pollen were the most common allergen sources (both 33%, dominated by Bet v 1 and Phl p1), followed by the PR-10 proteins (31%, due to cross-reactivity with Bet v 1) and the animal dander group (27%, predominantly Fel d 1) (Table S3), which is similar to the general Swedish population³.

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The analysis on a molecular level revealed furthermore that IgE analysis to domestic animals in AGS patients needs to be based on allergen molecules to be able to identify primary sensitization. We found that the majority of the AGS patients were sensitized to cat (75%) and dog (85%) dander extracts (Fig 1B), due to the presence of α -Gal in these allergen sources ⁴. When the patients' sera were analyzed for cat and dog allergen molecules, the low frequency of genuine cat (Fel d 1) and dog (Can f 1 and 5) sensitization became apparent (Fig 1B, 21.7% and 10.1% respectively).

Next, we investigated if sensitization to specific allergen molecules was associated with AGS symptoms. Anaphylactic patients showed a significantly higher frequency of IgE only against food allergens compared to non-anaphylactic patients (Fig. 2A). On a single allergen level, only patients with IgE against the milk protein lactoferrin had a higher risk of anaphylaxis compared to negative patients (Fig. 2B, OR 4.1; 95% CI 1.5-11.1; P=0.006). The observed IgE reactivity against lactoferrin is likely due to the α -Gal present on lactoferrin ⁵. We speculate that its relation with anaphylaxis is due to higher α -Gal-specific IgE levels in these patients (Fig. 2C), in combination with distinct characteristics of α -Gal-specific IgE antibodies like a higher affinity, which is linked to anaphylaxis ⁶. These data provide a lead for further investigation of lactoferrin-IgE as a potential marker of increased risk of anaphylaxis.

In conclusion, for the first time the IgE response of AGS patients has been dissected on a broad molecular allergen level. We report new insights into AGS that will help improve the clinical management of AGS patients.

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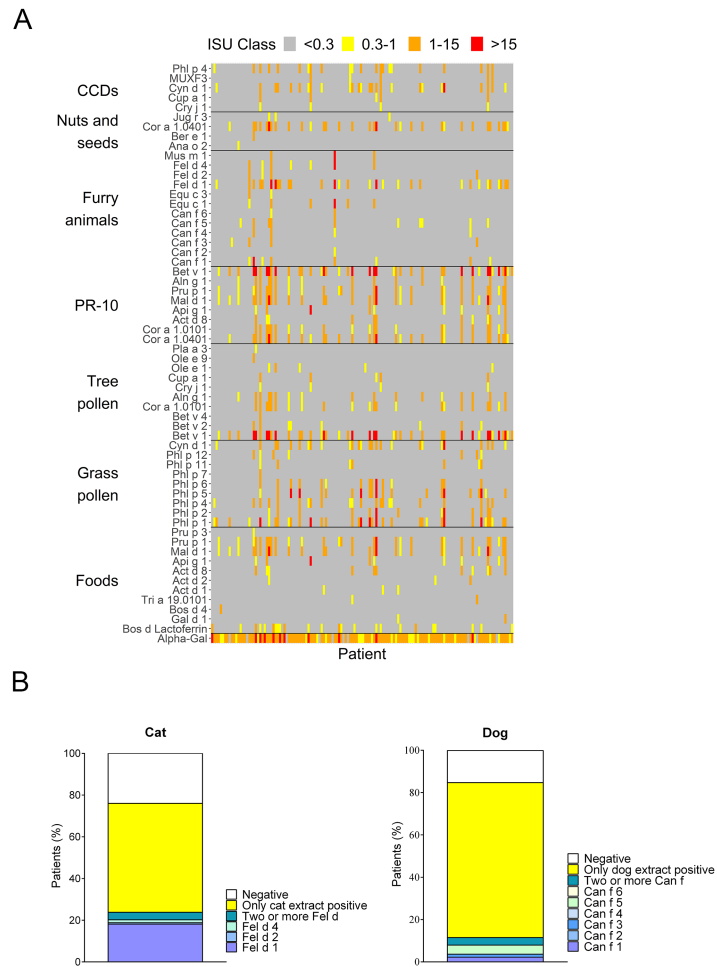
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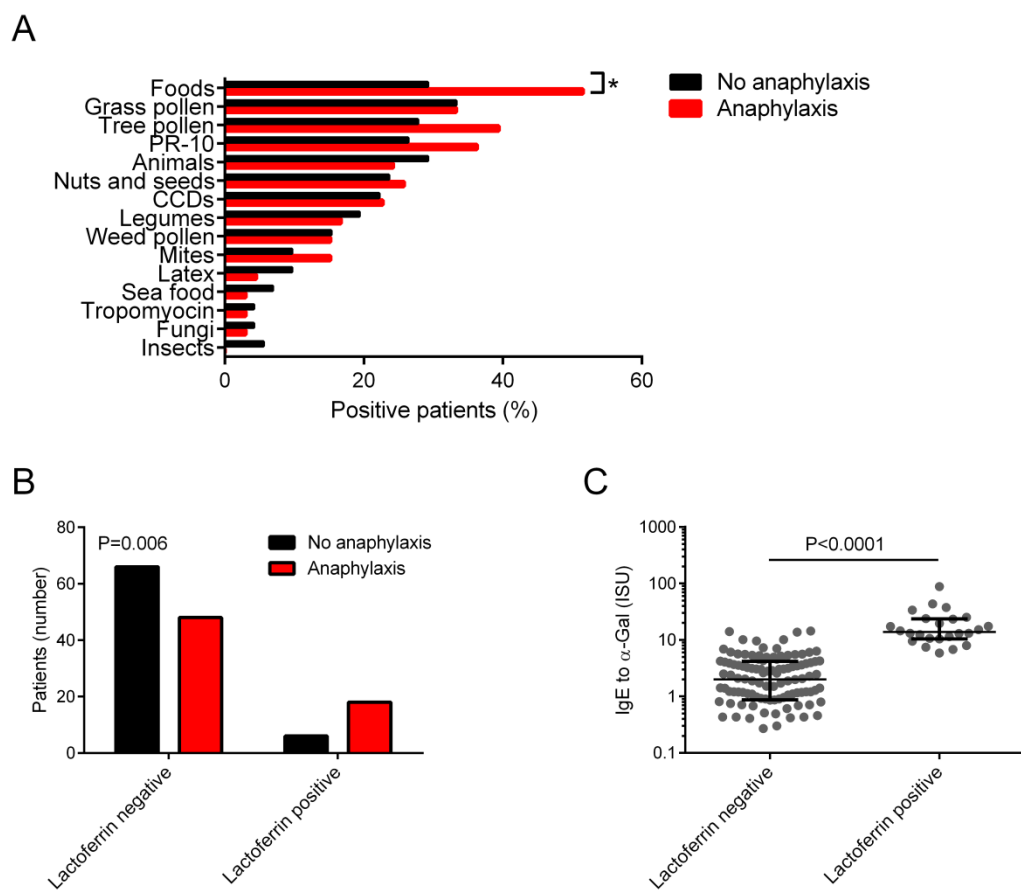
Figure legends

Figure 1. Sensitization patterns of AGS patients. Heat map representing the main allergens belonging to the most recognized allergen families (A). Only molecules with at least one subject having ISU ≥ 0.3 are shown. Frequencies of sensitization against protein extracts and individual allergens for the α -Gal sources cat and dog (B).

Figure 2. Comparison of sensitization frequencies in patients with and without anaphylaxis. Protein groups in AGS patients suffering from anaphylaxis compared to patients without anaphylaxis (A). Frequency of anaphylaxis in lactoferrin negative and positive patients (B). Levels of α -Gal specific IgE in lactoferrin negative and positive patients (C). Data is shown as median with interquartile range.



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1 **Supporting information**

2 **Elucidating the α -Gal syndrome at the molecular allergen level**

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Material and methods

Patient cohort

Patients with symptoms of delayed allergic reactions following mammalian meat consumption attended the Allergy Unit at Södersjukhuset in Stockholm. They were examined and diagnosed by the same allergologist with many years of experience in food allergy. Blood samples were drawn for analysis of allergen-specific IgE antibodies. Diagnosis of mammalian meat allergy was defined as symptoms after mammalian meat consumption and IgE to α -Gal (≥ 0.1 kU_A/L) measured by ImmunoCAP. In total, 138 patients were enrolled in the study. All patients answered a detailed questionnaire regarding the onset of symptoms. Anaphylaxis was defined as a severe, usually rapid onset and potentially life-threatening systemic hypersensitivity reaction from several organ systems, always including respiratory and / or cardiovascular manifestations. In addition, symptoms from the skin, mucous membranes, gastrointestinal tract and the central nervous system are usually seen ¹.

Atopy was defined as IgE antibodies to one or more of the inhalant allergen molecules from birch, hazelnut, timothy grass, and mugwort pollen (Bet v 1, Cor a 1.01, Phl p 1, Phl p 11, Phl p 2, Phl p 5, Phl p 6, Art v 1, Art v 3), and from cat, dog and horse (Fel d 1, Fel d 4, Can f 1, Can f 2, Can f 4, Can f 5, Can f 6, Equ c 1) and from mites and mold (Der f 1, Der f 2, Der p 1, Der p 2, Cla h 8). Patients were diagnosed with airborne allergy if they were atopic and had airway symptoms after exposure to birch, timothy or furry animals. One-hundred and twenty-five of the patients had previously been characterized based on analysis of allergen extracts by ImmunoCAP and symptoms ². The study was approved by the local ethics committee (Ethical permit No 2011/1604-31/2, 2018/2483-32),

performed in accordance with the declaration of Helsinki, and all patients gave their written informed consent.

ImmunoCAP and ISAC IgE determination

Serum samples were tested by ImmunoCAP and the updated ISAC¹¹² (Thermo Fisher AB, Uppsala, Sweden). Total IgE and allergen-specific IgE antibody reactivity to α -Gal (bovine thyroglobulin, o215), birch, timothy, cat (e1) and dog (e5) were analyzed by ImmunoCAP. The cutoff for total IgE was ≥ 2 kU/L and for allergen-specific IgE ≥ 0.1 kU_A/L. The updated ISAC¹¹² was used for determining IgE reactivity against allergen molecules. The chip contains 112 allergen molecules from 48 allergen sources, including α -Gal (the same bovine thyroglobulin molecule as on ImmunoCAP). Analysis was performed according to the manufacturer's instructions³. The results were evaluated using Phadia Microarray Image Analysis (MIA) software. The cutoff for IgE detection was set at 0.3 ISAC standardized units (ISU) with a scale ranging from Low (0.3–3 ISU-E), Moderate to High (3–15 ISU-E), and Very High (> 15 ISU-E).

Statistical analysis

Statistical analysis was performed with GraphPad Prism, version 6 (GraphPad software, La Jolla, CA, USA). Measurements of IgE antibodies were compared using Mann-Whitney U tests. Frequencies were compared using Fisher's exact test. Spearman's rank correlation was used to calculate the strength and direction of an association between two variables. A P-value of less than 0.05 was considered significant.

Results

Table S1. Patient characteristics.

Parameter	
Sex (M:F)	68:70
Age (median years, min-max)	48.5 (17-76)
α -Gal IgE (median kU _A /L, min-max)	16.0 (0.26-144.0)
Total IgE (median kU/L, min-max)	121.5 (8.6-3600.0)
Percentage α -Gal IgE of total IgE (median, min-max)	14 (0.0-66.0)
A/O blood group (percentage)	97.6%
Frequency anaphylaxis	47.8%
Frequency angioedema	60.1%
Frequency urticaria	90.5%
Frequency GI symptoms	74.6%

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85 Table S2. Explanatory list of allergen abbreviations.

Protein group	Allergen source	Allergen molecules
Foods	Cow's milk (<i>Bos domesticus</i>)	Bos d 4, Bos d lactoferrin
Foods	Chicken (<i>Gallus domesticus</i>)	Gal d 1
Foods	Wheat (<i>Triticum aestivum</i>)	Tri a 19
Foods	Kiwi (<i>Actinidia deliciosa</i>)	Act d 1, 2, 8
Foods/PR-10	Celery (<i>Apium graveolens</i>)	Api g 1
Foods/PR-10	Apple (<i>Malus domestica</i>)	Mal d 1
Foods	Peach (<i>Prunus persica</i>)	Pru p 1, 3
Grass pollen	Timothy (<i>Phleum pratense</i>)	Phl p 1, 2, 4, 5, 6, 7, 11, 12
Grass pollen/CCD	Bermuda grass (<i>Cynodon dactylon</i>)	Cyn d 1
Tree pollen	Birch (<i>Betula verrucosa</i>)	Bet v 1, 2, 4
Tree pollen/PR-10	Hazel (<i>Corylus avellana</i>)	Cor a 1.0101
Tree pollen/PR-10	Alder (<i>Alnus glutinosa</i>)	Aln g 1
Tree pollen/CCD	Japanese cedar (<i>Cryptomeria japonica</i>)	Cry j 1
Tree pollen/CCD	Cypress (<i>Cupressus arizonica</i>)	Cup a 1
Tree pollen	Olive (<i>Olea europaea</i>)	Ole e 1, 9
Tree pollen	Plane (<i>Platanus acerifolia</i>)	Pla a 3
PR-10 proteins	Peanut (<i>Arachis hypogaea</i>)	Ara h 8
PR-10 proteins	Soy bean (<i>Glycine max</i>)	Gly m 4
PR-10 proteins	Celery (<i>Apium graveolens</i>)	Api g 1
PR-10 proteins	Peach (<i>Prunus persica</i>)	Pru p 1
PR-10 proteins	Birch (<i>Betula verrucosa</i>)	Bet v 1
Furry animals	Dog (<i>Canis familiaris</i>)	Can f 1, 2, 3, 4, 5, 6b
Furry animals	Horse (<i>Equus caballus</i>)	Equ c 1, 3
Furry animals	Cat (<i>Felix domesticus</i>)	Fel d 1, 2, 4
Furry animals	Mouse (<i>Mus musculus</i>)	Mus m 1
Nuts and seeds	Cashew nut (<i>Anacardium occidentale</i>)	Ana o 2
Nuts and seeds	Brazil nut (<i>Bertholletia excelsa</i>)	Ber e 1
Nuts and seeds/PR-10	Hazelnut (<i>Corylus avellana</i>)	Cor a 1.0401
Nuts and seeds	Walnut (<i>Juglans regia</i>)	Jug r 3
CCDs	Bromelain	MUXF3
CCDs	Timothy (<i>Phleum pratense</i>)	Phl p 4
Legumes	Peanut (<i>Arachis hypogaea</i>)	Ara h 1, 2, 3, 8, 9
Legumes	Soy bean (<i>Glycine max</i>)	Gly m 4, 5, 6
Weed pollen	Mugwort (<i>Artemisia vulgaris</i>)	Art v 1, 3
Weed pollen	Goosefoot (<i>Chenopodium album</i>)	Che a 1
Weed pollen	Mercury (<i>Mercurialis annua</i>)	Mer a 1
Weed pollen	Wall pellitory (<i>Parietaria judaica</i>)	Par j 2
Weed pollen	Saltwort (<i>Salsola kali</i>)	Sal k 1
Mites	Blomia (<i>Blomia tropicalis</i>)	Blo t 5
Mites	Dermatophagoides (<i>Dermatophagoides farinae</i>)	Der f 1, 2
Mites	Dermatophagoides (<i>Dermatophagoides pteronyssinus</i>)	Der p 1, 2, 10, 23
Mites	Lepidoglyphus (<i>Lepidoglyphus destructor</i>)	Lep d 2
Latex	Latex (<i>Hevea brasiliensis</i>)	Hev b 5, 6, 8
Sea food	Cod (<i>Gadus callarias</i>)	Gad c 1

Sea food	Shrimp (<i>Penaeus monodon</i>)	Pen m 1, 2
Tropomyosin	Shrimp (<i>Penaeus monodon</i>)	Pen m 1
Tropomyosin	Dermatophagoides (<i>Dermatophagoides pteronyssinus</i>)	Der p 10
Tropomyosin	Cockroach (<i>Blattella germanica</i>)	Bla g 7
Tropomyosin	Anisakis (<i>Anisakis simplex</i>)	Ani s 3
Fungi	Alternaria (<i>Alternaria alternata</i>)	Alt a 1, 6
Fungi	Aspergillus (<i>Aspergillus fumigatus</i>)	Asp f 1, 3, 6
Fungi	Cladosporium (<i>Cladosporium herbarum</i>)	Cla h 8
Insects	Cockroach (<i>Blattella germanica</i>)	Bla g 1, 7

Table S3. Sensitization frequencies (≥ 0.3 ISU) among the different allergen sources and families.

Protein group	Included components	Nr of positive patients
Foods	Lactoferrin, Gal d 1, Bos d 4, Tri a 19, Act d 1,2,8, Api g 1, Mal d 1, Pru p 1,3	55 (40%)
Grass pollen	Phl p 1,2,4,5,6,7,11,12, Cyn d 1	46 (33%)
Tree pollen	Bet v 1,2,4, Cor a 1.0101, Aln g 1, Cry j 1, Cup a 1, Ole e 1,9, Pla a 3	46 (33%)
PR-10 proteins	Cor a 1.0401,1.0101, Ara h 8, Gly m 4, Act d 8, Api g 1, Mal d 1, Pru p 1, Aln g 1, Bet v 1	43 (31%)
Animals	Can f 1,2,3,4,5,6b, Equ c 1,3, Fel d 1,2,4, Mus m 1	37 (27%)
Nuts and seeds	Ana o 2, Ber e 1, Cor a 1.0401, Jug r 3	34 (25%)
CCDs	Cry j 1, Cup a 1, Cyn d 1, MUXF3, Phl p 4	31 (23%)
Legumes	Ara h 1,2,3,8,9, Gly m 4,5,6	25 (18%)
Weed pollen	Art v 1,3, Che a 1, Mer a 1, Par j 2, Sal k 1	21 (15%)
Mites	Blo t 5, Der f 1,2, Der p 1,2,10,23, Lep d 2	17 (12%)
Latex	Hev b 5,6,8	10 (7%)
Sea food	Gad c 1, Pen m 1,2	7 (5%)
Tropomyosin	Pen m 1, Der p 10, Bla g 7, Ani s 3	5 (4%)
Fungi	Alt a 1,6, Asp f 1,3,6, Cla h 8	5 (4%)
Insects	Bla g 1,7	4 (3%)

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