GLOBAL CARDIOLOGY SCIENCE AND PRACTICE: COVER SHEET

# Alpha-gal syndrome: Implications for cardiovascular disease

**John Bianchi, Anneke Walters, Zachary W. Fitch*, Joseph W. Turek**

* zwfitch@gmail.com

DOI: https://doi.org/10.21542/gcsp.2019.20
VOLUME: 2019
ISSUE: 3
ARTICLE: 20

PMC PUBLICATION DATE: 31 DECEMBER 2019
PMC COLLECTION DATE: 31 DECEMBER 2019
PMC ARTICLE TYPE: review-article

ARTICLE TYPE: Review article
SUBMITTED: 22 AUGUST 2019
ACCEPTED: 28 OCTOBER 2019
RUNNING HEAD: Bianchi et al. GCSP 2019:20

CITE THIS ARTICLE AS: Bianchi J, Walters A, Fitch ZW, Turek JW. Alpha-gal syndrome: Implications for cardiovascular disease, Global Cardiology Science and Practice 2019:20 https://doi.org/10.21542/gcsp.2019.20

**Review article**

# Alpha-gal syndrome: Implications for cardiovascular disease

**John Bianchi[1], Anneke Walters[1], Zachary W. Fitch[2,*], Joseph W. Turek[2]**

*1. Revivicor, 1700 Kraft Drive, Suite 2400, Blacksburg, VA 24060, USA*
*2. Division of Thoracic and Cardiovascular Surgery, Department of Surgery, Duke University Medical Center, Durham, NC, USA*

* zwfitch@gmail.com

## Abstract

Alpha-gal syndrome (AGS) refers to a potentially life-threatening allergy to the molecule galactose-α1,3-galactose (gal), which is expressed on most mammalian tissues but, importantly, is not expressed by humans. This syndrome can manifest as an allergic reaction to mammalian meat products, but other sources of mammalian tissue can also provoke an immune response, including injectable and implantable medical products. This syndrome has been linked to coronary atherosclerosis, and medical products that express gal are routinely used in cardiology and cardiac surgery. This article seeks to discuss potential implications of alpha syndrome as it relates to cardiovascular health and to heighten awareness in the cardiovascular community about this emerging public health issue.

## Prevalence of galactose-α1,3-galactose

Most mammalian species (including New World monkeys, cows, pigs, goats, horses, sheep, rabbits and mice) express the galactose-α1,3-galactose (gal) disaccharide sugar on cells and tissue surfaces[1-4]. Gal expression results from the catalytic activity of the α1,3-galactosyltransferase enzyme encoded by the glycoprotein α1,3-galactosyltransferase gene (GGTA1)[1-3, 5]. Certain mammalian species, such as catarrhines (humans, apes, and Old World monkeys), do not have a functional GGTA1 gene[6-8] and correspondingly do not express gal[1, 3, 4]. Additionally, gal has been documented to be absent in fish, amphibians, reptiles, and birds[3, 9, 10]. The function of gal is unknown[3], but it is clearly not essential for survival[1, 3].

## Prevalence of IgM, IgG, and IgA anti-gal antibodies

Mammalian species that do not produce gal such as humans and Old World primates have been well documented to possess natural anti-gal antibodies[1-3, 11, 12]. It has been reported that these natural antibodies occur as different isotypes, including IgM, IgG, and IgA [1, 2, 13]. In humans, anti-gal antibodies are among the

most abundant immunoglobulins, with some studies reporting that 1-3% of circulating immunoglobulins are directed against gal[3, 11-15]. Anti-gal immunoglobulin titers may be attenuated or amplified by various factors; a vegetarian diet reduces titers while implantation of bioprosthetic heart valves increases titers[16, 17].

## Prevalence of IgE anti-gal antibodies

More recently, van Nunen, Commins and others [18-24] have described a unique population with high titers of anti-gal IgE. Anti-gal IgE develops in a subset of people after an index exposure to gal. On re-exposure to gal, this subset of people can develop a severe IgE-mediated hypersensitivity reaction that can manifest as anaphylaxis (including urticaria, tachycardia, angioedema, syncope, and hypotension) with many patients requiring emergency care.

The condition, termed "alpha-gal syndrome" (AGS), is incited by exposure to gal through tick bites, even in patients who previously tolerated exposure to gal through red meat consumption. Although the Lone Star tick is the culprit in the United States, bites from certain other tick species around the world cause a similar hypersensitivity to gal[23-31].

The National Institute of Health (NIH) recently highlighted AGS and noted that it is often unrecognized or misdiagnosed [32]. For AGS patients, a tick bite can lead to a hypersensitivity reaction that characteristically manifests as anaphylaxis three to six hours after consumption of mammalian meat products, even in patients who previously tolerated red meat for their entire lives [20, 22, 33].

Others who have elevated anti-gal IgE levels (allergen specific positivity to gal) due to a tick bite remain asymptomatic after red meat consumption but may manifest anaphylaxis after exposure to injected or implanted mammalian derived medical products [19, 34-37]. For this reason, allergists have described patients as allergen negative (alpha-gal-specific IgE levels below a cutoff value; typically 0.1 or 0.35 kUA/L), allergen positive (alpha-gal-specific IgE levels above the cutoff value), and patients with alpha-gal syndrome (alpha-gal-specific IgE levels above the cutoff value and a history of clinical anaphylaxis after red meat consumption).

The reported prevalence of individuals in the United States with elevated allergen-specific titers of anti-gal IgE (i.e. allergen positive) has been reported to be in the range of 8% to 46%, with highest prevalence within the geographic distribution of the Lone Star tick (Figure 1)[21, 38-41]. Similar prevalence rates have been reported in other regions around the world (Table 1) [27, 42, 43].

Children within the geographic distributions of certain ticks are projected to have allergen positive prevalence comparable to the adult population[33]. As one might expect, hunters and forest service workers have been reported to have a prevalence that is more than twice that of the general population[21, 43]. It appears that the prevalence of AGS equates to 10% of the allergen-positive population. Thus, in the southeastern United States, approximately 3% of the general population exhibits anaphylaxis after consumption of mammalian meat.

## Unique characteristics of the allergen-positive population

Allergen-positive patients have been identified to have higher titers of anti-gal IgG, with more IgG subtype 1 and less IgG subtype 2 than allergen negative populations[44]. Furthermore, allergen-positive patients manifest a significant difference in coronary artery disease when compared to an allergen-negative cohort [38]. This suggests that IgE sensitization to alpha gal may be a novel modifiable risk factor for coronary atherosclerosis, especially in patients 65-years and younger. By eating mammalian food products, patients sensitized to gal may be contributing to coronary artery disease.

*Table 1. Prevalence of α-gal allergen positivity (e.g Alpha-gal-specific IgE titers fall above the threshold for positivity)*

| Region | Location | Reference | Threshold for positivity (Anti-gal IgE allergen titer) | Prevalence of positivity |
|---|---|---|---|---|
| United states | Southeastern US | Commins[21] | >=0.35IU/ml | 20% |
| | North Carolina | Commins[21] | >=0.35IU/ml | 20% |
| | North Carolina | Burk[39] | >=0.35 kUA/L | 22% |
| | Tennessee , | Commins[21] | >=0.35IU/ml | 22% |
| | Virginia | Wilson[38] | >=0.1kU/L | 26.3% |
| | Virginia | Commins[21] | >=0.35IU/ml | 18% |
| | Boston | Commins[21] | >=0.35IU/ml | <1% |
| | Northern California | Commins[21] | >=0.35IU/ml | 2% |
| Africa | Kabati, Kenya (rural) | Commins[21] | >=0.35IU/ml | 76% |
| | Thika, Kenya (industrial town) | Commins[21] | >=0.35IU/ml | 29% |
| South America | Esmeraldas Province, Ecuador | Commins[21] | >=0.35IU/ml | 37% |
| Europe | Germany General population | Fischer[43] | >=0.1kUA/L | 15% |
| | Germany hunters and forest service workers | Fischer[43] | >=0.1kUA/L | 35% |
| | Spain | Gonzalez-Quintela[42] | >=0.1kUA/L | 5.5% |
| | Denmark | Gonzalez-Quintela[42] | >=0.1kUA/L | 8.1 |
| | Norrbotten, Sweden (age 18 y) | Commins[21] | >=0.35IU/ml | <1% |
| | Sweden (10%), | Apostolovic[27] | Not reported | 10% |

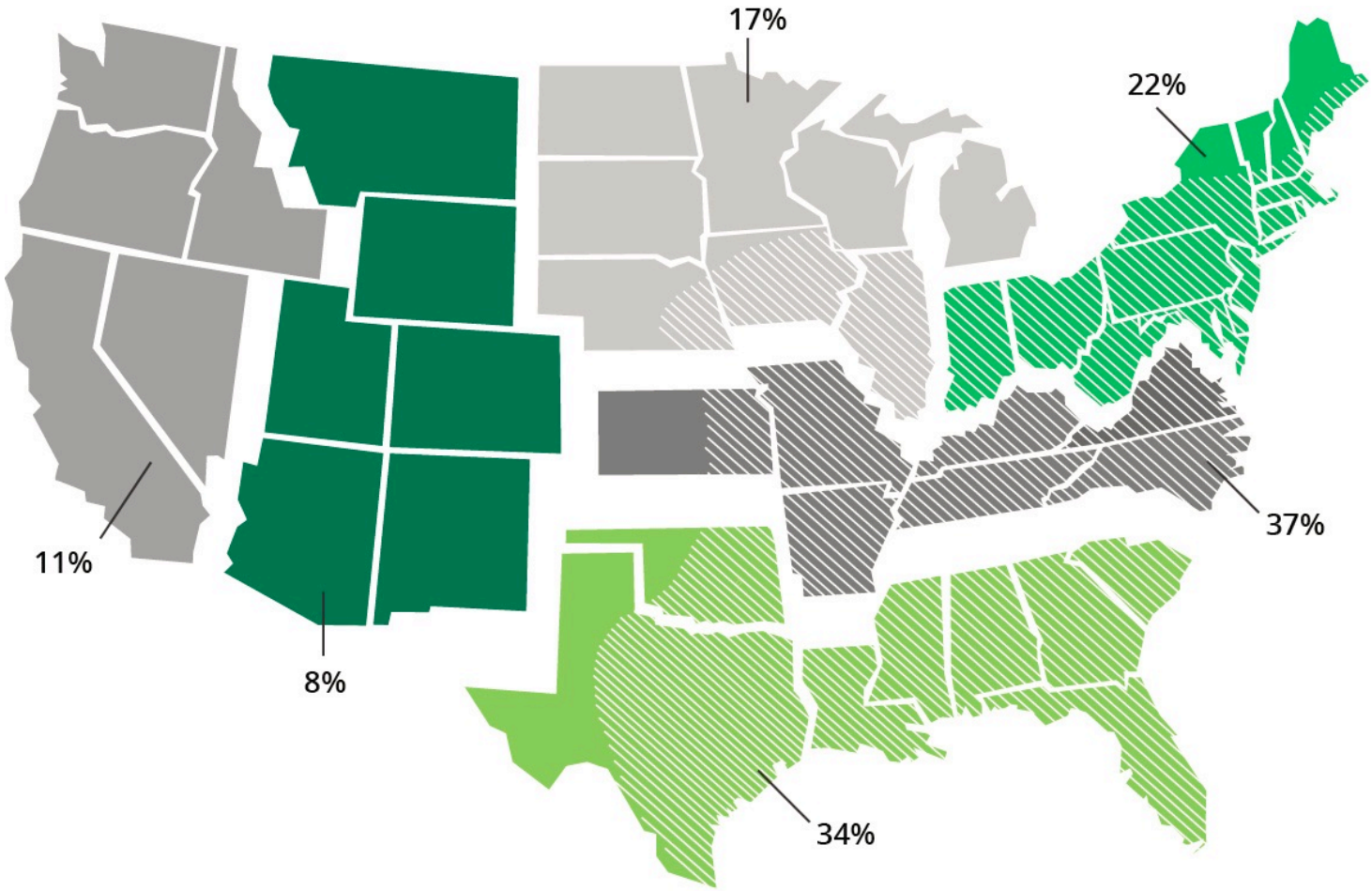


Figure 1. *Surveillance for IgE to alpha-gal. Percent positive rates are presented for IgE to alpha-gal within each of six regions in the United States, 2012-2013 (7300 samples). Percentages refer to the percentage of samples submitted for testing that tested positive. Diagonal white lines on the map represent the known geographic distribution of the Lone Star tick (from Olafson, P. Ticks and the mammalian meat allergy. USDA Beef Research, (2015)).*

## Putative mechanisms of IgE sensitization

A central question is why some individuals who tolerate gal exposure through red meat consumption for years go on to develop an allergy to gal after a tick bite. Wilson et al[45] have proposed that tick-induced α-gal sensitization is due to an activation of the innate immune system through one of at least three possible mechanisms. First, the damage and local trauma from the bite may release local damage-associated molecular patterns (DAMPs) that activate innate immune cells, leading in turn to activation of the adaptive immune system, including the formation of plasma cells that produce gal-specific IgE through T-cell-dependent germinal center reactions (or possibly through a T-cell independent process, though this is unlikely). Second, the tick bite may introduce gal while at the same time introducing tick-associated microbes that could act as pathogen-associated molecular patterns (PAMPs) and likewise cause the innate immune system to direct an adaptive immune response. According to these theories, gal is an innocent bystander that is swept up at the scene of an immune response initiated by local damage or by microbes. Third, it has been proposed that gal itself could be perceived as a PAMP and initiate a response in its own right.

## Clinical implications of gal in whole organ xenotransplantation

The fact that gal has been confirmed on mammalian cells and tissues has significant clinical implications in whole organ xenotransplantation (i.e., pig to human, pig to Old World primate)[1, 2, 4]. This is due to the fact that gal is the major antigen expressed on pig cells and tissues to which natural anti-gal antibodies bind [3, 9, 13, 46]. The binding of anti-gal antibodies to gal activates the complement system within minutes to hours of discordant tissue, cell, or organ transplantation and the host effectively rejects the transplanted material [2, 4].

## Clinical implications of gal in human therapeutic products

The health concerns for alpha-gal IgE positive patients (especially patients remaining asymptomatic after meat consumption) who may be under consideration for mammalian derived medicinal products has been well stated by Fischer et al [43]:

> *"In our opinion, clinical tolerance to mammalian meat and innards cannot be considered the same as clinical tolerance to intravenous application of alpha-gal-containing drugs. Due to this potential risk, a special warning regarding the intravenous administration of alpha gal-containing drugs may be needed in all individuals who display alpha-gal-IgE positivity."*

Others[25] concur and have identified case studies that highlight different classes of medicinal products that "may prove risky in people who are gal sensitized [allergen positive]"[25]. These include:

- Drugs including cetuximab[37], heparin[47],
- Gelatin including capsules[48], tablets[36], suppositories, colloids[49], and vaccines[50, 51]
- Collagen including corneal shields[52], hemostatic agents[47] or other scaffolds
- Magnesium stearate[36]
- Mammalian derived heart valves[34, 35, 54]

Although some health care providers observe that AGS patients may tolerate administration of gal containing therapeutics, these patients require unique and special care that places additional economic burden while exposing them to potential harm[55, 56].

## *In vitro* or *in vivo* testing in laboratory animals of medicinal products

The understanding of the health implications for the allergen-positive population is new, and emerging. For medicinal products currently in use, Muglia indicates [53]:

> *"Pharmaceutical manufacturers do not currently test products for alpha-gal content. Additionally, they are not required by the Food and Drug Administration to report changes in inactive ingredients on the package insert."*
>
> *"Manufactures do not report alpha-gal content in their package inserts or test for alpha-gal content in products. Inactive ingredient information can change at any time, and the FDA does not require manufacturers to disseminate this information."*

As one might expect, and prior to human use, many of the medical products including bioprosthetic heart valves are routinely evaluated in sheep[57], rabbits[58], etc. However, these models do not challenge the gal-mediated immune response because these animals all naturally express the gal molecule and therefore do not produce anti-gal antibodies[59]. The only appropriate model would involve animal subjects that produce anti-gal antibodies such as Old World non-human primates (NHP). Although NHPs have been well documented to have anti-gal IgG and IgM antibodies, corresponding anti-gal IgE values particularly after a challenge with the lone star tick (or comparable tick, or tick extract) were not noted in the scientific literature.

Given the growing awareness of this issue, surgeons have demanded medical manufactures eliminate gal from medicinal products[60]. Similarly, NIH-NIAID Director Anthony S. Fauci specifically identified the alpha gal allergy as "a serious and growing public health problem that urgently requires more research." [32]

## Cardiovascular medicinal products

The persistence of gal on acellular xenografts derived from bovine or porcine sources has been implicated in both acute and chronic responses. The current standard of care with bioprosthetic valves is crosslinking the collagen matrix with glutaraldehyde that reduces antigenicity by "hiding" or "masking" antigens including gal[16, 61]. Unfortunately, the glutaraldehyde treatment obliterates the natural regenerative properties of the graft and residual gal remains [17, 59, 62, 63]. The persistent exposure of the recipient's immune system to gal is implicated in failure of current heart valves by calcification via a chronic IgM/ IgG response [17, 59, 62-64].

More concerning for AGS patients receiving heart valves is that the acute IgE response has been linked to immediate post-operative anaphylaxis,[35] blood culture negative endocarditis,[54, 65] and rapid destruction of the valve [34, 54]. In addition, anaphylaxis during other cardiac procedures have been attributed to administration of heparin (derived from porcine intestines) or the use of the hemostatic agent Gelfoam (derived from porcine skin) [36 56 47]. Given the widespread use of heparin, it is of special concern and it must be noted that there have been very few documented cases of anaphylaxis due to heparin use. Although

screening patients for anti-gal IgE titers prior to cardiovascular surgery may be an effective tactic to identify the optimum surgical intervention in order to prevent operative or immediate post-operative anaphylaxis, Hawkins documented several years after implantation of a bioprosthetic valve an allergen negative patient may seroconvert to allergen positive (after a tick bite) and subsequently acutely reject the implanted valve [34].

### *Decellularization strategies to remove gal*

Some entities have tried to remove gal via decellularization[17, 57] to remove immunogenic components including gal; however, these results have not been successful [61, 66, 67]. Allergists, particularly those examining the gal epitope on food products, have identified substantial flaws in any strategy intended to remove gal from tissue matrices via fluid washes. Raw, boiled, fried, beef or pork products have been examined to understand persistence of the gal epitope to typical cooking methods and regardless of treatment, alpha gal persisted [31, 68].

Many proteins were identified as having the alpha gal epitope attached, including heat stabile proteins that were confirmed to be reactive to allergen positive serum [31, 68]. Similarly, Bovine thyroglobulin (BTG) that is well known to be decorated with gal was subjected to a simulated digestion process. Although the BTG was broken down into many different smaller proteins, gal persisted and was reactive to allergen positive patient serum[69]. Others have identified gal to be inherent or bound to the collagen matrix including collagen and laminin – the major structural components of the extracellular matrix (ECM) [70].

Of note, Mullins has described the extreme conditions employed to produce gelatin intended as a human therapeutic that "uses a combination of acid and alkaline hydrolysis, followed by heat extraction at temperatures up to 90°C, then sterilized at temperatures >100°C"[49]. Regardless, gal persisted in these gelatin-derived colloids and resulted in anaphylaxis in AGS patients after intravenous exposure [49]. Perhaps, decellularization may be effective at removing unbound or soluble proteins that have gal. However, any decellularization strategy intense enough to remove gal chemically bound to ECMs to prevent adverse reactions in the allergen positive population would need to break chemical bonds subsequently degrading the matrix to the point of obliterating its biomechanical properties and rendering any resulting ECM useless.

## Establishment of an engineered pig that does not have gal

Revivicor, Inc. (Blacksburg, VA) has utilized its expertise in somatic cell nuclear transfer (SCNT), in combination with gene targeting techniques, to establish a unique proprietary genetically engineered (GE) pig, GalSafe®, that has both alleles of GGTA1 inactive, meaning that gal is not detectable in these pigs [71, 72]. The GalSafe® pig is phenotypically normal [73, 74] to a comparable non-engineered pig except for its genetically engineered trait (Figure 2). In addition, GalSafe® pigs produce anti-gal IgM and IgG [75]. Of note, Revivicor has demonstrated safety and efficacy of the GalSafe® pig by essentially completing all necessary steps for regulatory approval of the pig [76] with the FDA-CVM (data on file) that provides a foundation for pursuing various raw materials for fabrication into acellular scaffolds (tissue grafts without viable cells) for distribution as implantable human use medical product. Any tissue derived from the GalSafe® pig

including heart valves, pericardium, vascular conduits and others may serve as materials for human use medical products.

## Implications for cardiology and cardiac surgery

Gal hypersensitivity should be recognized as a relevant issue to cardiac surgeons and cardiologists. As noted above, patients may be at increased risk for severe coronary artery disease if they are sensitized to gal. As a result, consumption of red meat may be a modifiable risk factor that could decrease morbidity and mortality in patients who are sensitized to gal.

The fact that patients can be exposed to gal through implantation of bioprosthetic heart valves and other devices presents a different set of challenges, especially for surgeons. Future studies should better characterize the relationship between elevated anti-gal antibody titers and bioprosthetic valve function. For example, patients with bioprosthetic valves known to have elevated anti-gal antibody titers should undergo echocardiography to evaluate valve function. At the same time, a non-human primate model of gal sensitization should be developed so that gal sensitization as it relates to bioprosthetic valves and other devices can be studied in a controlled manner. Non-human primate work should investigate new methods of decellularization and other processing techniques designed to mitigate the immunogenicity of gal. Importantly, non-human primate work should also directly compare the implantation of bioprosthetic heart valves from bioengineered animals such as GalSafe® pigs to the implantation of valves from wild type animals in non-human primates sensitized to gal. In the future, the standard of care may be implantation of bioprosthetic valves only if they are free of gal expression.

In the meantime, identifying patients with a gal allergy is important so that they can be properly managed in the perioperative period. Before implanting a gal-containing medical device (especially in areas endemic to pathogenic species sensitizing to gal), patients should be asked about food allergies, and especially about an allergy or intolerance to red meat or pork. They should also be asked about an allergy to the drug cetuximab, as this has been implicated in alpha gal allergy. If time permits, a referral to an allergist for specific testing may be appropriate. For patients suspected or confirmed to be sensitized to gal, the medical record should be updated to reflect this allergy. Manning and colleagues have recently written a thorough review of anesthesia considerations for patients with alpha gal syndrome, including a list of common perioperative drugs and other products that may contain alpha gal and should be avoided in patients with an alpha gal allergy [77]. While alpha gal sensitization is a relatively newly described condition, its potentially serious implications demand increased education efforts and vigilance in the perioperative period, especially in regions like the southeast United States where a high prevalence of sensitization exists.

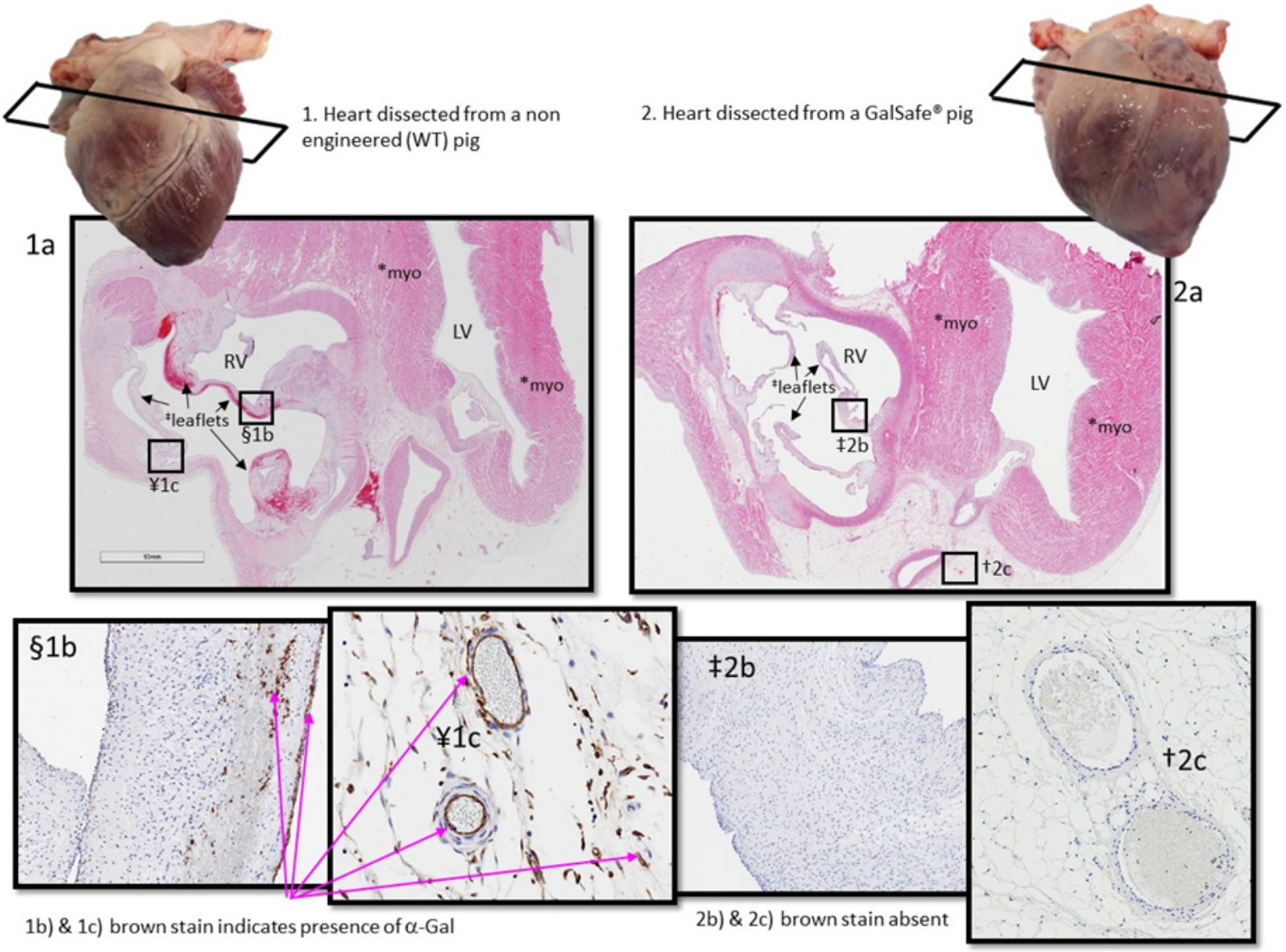


**Figure 2.** Evaluation of the H&E slides (transverse section at level of the tricuspid/atrioventriculure valve) confirmed that the gross morphological characteristics of the GalSafe® heart (2a) is indistinguishable from a heart derived from a WT pig (1a) and confirmed normal morphology for heart tissue layers and structures (e.g. *myocardium, blood vessels and valve leaflets) with no observed differences between GalSafe® and WT genotypes. The cross section of heart valves from WT and GalSafe® pigs were stained with GS-isolectin B4 (brown) to detect gal. Gal is present (+) on the WT heart valve and is indicated by the brown stain present (pink arrows) in the representative enlarged images of the (1b) valve leaflet and (1c) blood vessels, respectively. Gal was not detected (-) in comparable areas, (2b) leaflet and (2c) blood vessels from the GalSafe® pigs.

**Conflict of interest statement:** John Bianchi and Anneke Walters are employees of Revivicor Inc, which develops genetically modified pigs, including gal-safe pigs. No other author has a conflict of interest.

## References


1. Joziasse, D.H. & Oriol, R. Xenotransplantation: the importance of the Galalpha1,3Gal epitope in hyperacute vascular rejection. *Biochimica et biophysica acta* **1455**, 403-418 (1999).
2. Kobayashi, T. & Cooper, D.K.C. in [Alpha]-gal and anti-gal : [alpha] 1,3-galactosyltransferase, [alpha]-gal epitopes, and the natural anti-gal antibody subcellular biochemistry. (eds. U. Galili & J.L. Ávila) 229-257 (Kluwer Academic/Plenum, New York; 1999).
3. Macher, B.A. & Galili, U. The Galalpha1,3Galbeta1,4GlcNAc-R (alpha-Gal) epitope: a carbohydrate of unique evolution and clinical relevance. *Biochimica et biophysica acta* **1780**, 75-88 (2008).
4. Sandrin, M.S. & McKenzie, I.F. Gal alpha (1,3)Gal, the major xenoantigen(s) recognised in pigs by human natural antibodies. *Immunological reviews* **141**, 169-190 (1994).
5. Joziasse, D.H., Shaper, J.H., Van den Eijnden, D.H., Van Tunen, A.J. & Shaper, N.L. Bovine alpha 1----3-galactosyltransferase: isolation and characterization of a cDNA clone. Identification of homologous sequences in human genomic DNA. *The Journal of biological chemistry* **264**, 14290-14297 (1989).
6. Galili, U. & Swanson, K. Gene sequences suggest inactivation of alpha-1,3-galactosyltransferase in catarrhines after the divergence of apes from monkeys. *Proceedings of the National Academy of Sciences of the United States of America* **88**, 7401-7404 (1991).
7. Joziasse, D.H., Shaper, J.H., Jabs, E.W. & Shaper, N.L. Characterization of an alpha 1----3-galactosyltransferase homologue on human chromosome 12 that is organized as a processed pseudogene. *The Journal of biological chemistry* **266**, 6991-6998 (1991).
8. Larsen, R.D., Rivera-Marrero, C.A., Ernst, L.K., Cummings, R.D. & Lowe, J.B. Frameshift and nonsense mutations in a human genomic sequence homologous to a murine UDP-Gal:beta-D-Gal(1,4)-D-GlcNAc alpha(1,3)-galactosyltransferase cDNA. *The Journal of biological chemistry* **265**, 7055-7061 (1990).
9. Galili, U., Shohet, S.B., Kobrin, E., Stults, C.L. & Macher, B.A. Man, apes, and Old World monkeys differ from other mammals in the expression of alpha-galactosyl epitopes on nucleated cells. *The Journal of biological chemistry* **263**, 17755-17762 (1988).
10. Oriol, R. et al. Major carbohydrate epitopes in tissues of domestic and African wild animals of potential interest for xenotransplantation research. *Xenotransplantation* **6**, 79-89 (1999).
11. Teranishi, K., Manez, R., Awwad, M. & Cooper, D.K. Anti-Gal alpha 1-3Gal IgM and IgG antibody levels in sera of humans and old world non-human primates. *Xenotransplantation* **9**, 148-154 (2002).
12. Galili, U., Mandrell, R.E., Hamadeh, R.M., Shohet, S.B. & Griffiss, J.M. Interaction between human natural anti-alpha-galactosyl immunoglobulin G and bacteria of the human flora. *Infection and immunity* **56**, 1730-1737 (1988).
13. Cooper, D.K. et al. Identification of alpha-galactosyl and other carbohydrate epitopes that are bound by human anti-pig antibodies: relevance to discordant xenografting in man. *Transplant immunology* **1**, 198-205 (1993).
14. Galili, U., Rachmilewitz, E.A., Peleg, A. & Flechner, I. A unique natural human IgG antibody with anti-alpha-galactosyl specificity. *The Journal of experimental medicine* **160**, 1519-1531 (1984).
15. Minanov, O.P. et al. Anti-GaL IgG antibodies in sera of newborn humans and baboons and its significance in pig xenotransplantation. *Transplantation* **63**, 182-186 (1997).
16. Boer, U. et al. Antibody formation towards porcine tissue in patients implanted with crosslinked heart valves is directed to antigenic tissue proteins and alphaGal epitopes and is reduced in healthy vegetarian subjects. *Xenotransplantation* **24** (2017).
17. Bloch, O. et al. Immune response in patients receiving a bioprosthetic heart valve: lack of response with decellularized valves. *Tissue engineering. Part A* **17**, 2399-2405 (2011).
18. Platts-Mills, T.A., Schuyler, A.J., Tripathi, A. & Commins, S.P. Anaphylaxis to the carbohydrate side chain alpha-gal. *Immunology and allergy clinics of North America* **35**, 247-260 (2015).
19. Steinke, J.W., Platts-Mills, T.A. & Commins, S.P. The alpha-gal story: lessons learned from connecting the dots. *The Journal of allergy and clinical immunology* **135**, 589-596; quiz 597 (2015).
20. Commins, S.P. & Platts-Mills, T.A. Delayed anaphylaxis to red meat in patients with IgE specific for galactose alpha-1,3-galactose (alpha-gal). *Current allergy and asthma reports* **13**, 72-77 (2013).

21. Commins, S.P. et al. The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose-alpha-1,3-galactose. *The Journal of allergy and clinical immunology* **127**, 1286-1293 e1286 (2011).
22. Carter, M.C. et al. Identification of alpha-gal sensitivity in patients with a diagnosis of idiopathic anaphylaxis. *Allergy* (2017).
23. van Nunen, S. Tick-induced allergies: mammalian meat allergy, tick anaphylaxis and their significance. *Asia Pacific allergy* **5**, 3-16 (2015).
24. Van Nunen, S.A., O'Connor, K.S., Clarke, L.R., Boyle, R.X. & Fernando, S.L. An association between tick bite reactions and red meat allergy in humans. *The Medical journal of Australia* **190**, 510-511 (2009).
25. van Nunen, S.A. Tick-induced allergies: mammalian meat allergy and tick anaphylaxis. *The Medical journal of Australia* **208**, 316-321 (2018).
26. Arslan Lied, G. Red meat allergy induced by tick bites: A Norwegian case report. *European annals of allergy and clinical immunology* **49**, 186-188 (2017).
27. Apostolovic, D. et al. The red meat allergy syndrome in Sweden. *Allergo journal international* **25**, 49-54 (2016).
28. Kaloga, M. et al. Allergy to Red Meat: A Diagnosis Made by the Patient and Confirmed by an Assay for IgE Antibodies Specific for Alpha-1,3-Galactose. *Case reports in dermatology* **8**, 10-13 (2016).
29. Chinuki, Y., Ishiwata, K., Yamaji, K., Takahashi, H. & Morita, E. Haemaphysalis longicornis tick bites are a possible cause of red meat allergy in Japan. *Allergy* **71**, 421-425 (2016).
30. Jappe, U. et al. Meat allergy associated with galactosyl-alpha-(1,3)-galactose (alpha-Gal)-Closing diagnostic gaps by anti-alpha-Gal IgE immune profiling. *Allergy* **73**, 93-105 (2018).
31. Hilger, C. et al. Two galactose-alpha-1,3-galactose carrying peptidases from pork kidney mediate anaphylactogenic responses in delayed meat allergy. *Allergy* **71**, 711-719 (2016).
32. National Institute of Health. **NIAID Scientists Link Cases of Unexplained Anaphylaxis to Red Meat Allergy**. 28 November 2017, https://www.niaid.nih.gov/news-events/niaid-scientists-link-cases-unexplained-anaphylaxis-red-meat-allergy.
33. Kennedy, J.L. et al. Galactose-alpha-1,3-galactose and Delayed Anaphylaxis, Angioedema, and Urticaria in Children. *Pediatrics* **131**, e1545-1552 (2013).
34. Hawkins, R.B., Frischtak, H.L., Kron, I.L. & Ghanta, R.K. Premature Bioprosthetic Aortic Valve Degeneration Associated with Allergy to Galactose-Alpha-1,3-Galactose. *Journal of cardiac surgery* **31**, 446-448 (2016).
35. Mozzicato, S.M., Tripathi, A., Posthumus, J.B., Platts-Mills, T.A.E. & Commins, S.P. Porcine or bovine valve replacement in 3 patients with IgE antibodies to the mammalian oligosaccharide galactose-alpha-1,3-galactose. *The journal of allergy and clinical immunology. In practice* **2**, 637-638 (2014).
36. Muglia, C., Kar, I., Gong, M., Hermes-DeSantis, E.R. & Monteleone, C. Anaphylaxis to medications containing meat byproducts in an alpha-gal sensitized individual. *The journal of allergy and clinical immunology. In practice* **3**, 796-797 (2015).
37. Chung, C.H. et al. Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. *The New England journal of medicine* **358**, 1109-1117 (2008).
38. Wilson, J.M. et al. IgE to the Mammalian Oligosaccharide Galactose-alpha-1,3-Galactose Is Associated With Increased Atheroma Volume and Plaques With Unstable Characteristics-Brief Report. *Arteriosclerosis, thrombosis, and vascular biology* **38**, 1665-1669 (2018).
39. Burk, C.M., Beitia, R., Lund, P.K. & Dellon, E.S. High rate of galactose-alpha-1,3-galactose sensitization in both eosinophilic esophagitis and patients undergoing upper endoscopy. *Diseases of the esophagus : official journal of the International Society for Diseases of the Esophagus* **29**, 558-562 (2016).
40. Altrich, M.L., Blum, S.P. & Foster, S.M. Alpha-Gal IgE Sensitization in the United States; Surveillance Update. *Journal of Allergy and Clinical Immunology* **135**, AB37 (2015).
41. Olafson, P. Ticks and the mammalian meat allergy. USDA Beef Research, (2014).

42. Gonzalez-Quintela, A. et al. IgE antibodies to alpha-gal in the general adult population: relationship with tick bites, atopy, and cat ownership. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology* **44**, 1061-1068 (2014).
43. Fischer, J. et al. Prevalence of type I sensitization to alpha-gal in forest service employees and hunters. *Allergy* **72**, 1540-1547 (2017).
44. Rispens, T., Derksen, N.I., Commins, S.P., Platts-Mills, T.A. & Aalberse, R.C. IgE production to alpha-gal is accompanied by elevated levels of specific IgG1 antibodies and low amounts of IgE to blood group B. *PloS one* **8**, e55566 (2013).
45. Wilson, J.M., Schuyler, A.J., Schroeder, N. & Platts-Mills, T.A. Galactose-alpha-1,3-Galactose: Atypical Food Allergen or Model IgE Hypersensitivity? *Current allergy and asthma reports* **17**, 8 (2017).
46. Good, A.H. et al. Identification of carbohydrate structures that bind human antiporcine antibodies: implications for discordant xenografting in humans. *Transplantation proceedings* **24**, 559-562 (1992).
47. Sell-Dottin, K., Sola, M. & Caranasos, T. Impact of Newly Emerging Alpha-Gal Allergies on Cardiac Surgery: A Case Series. *Clin Surg* **2**, 1-3 (2017).
48. Vidal, C., Méndez-Brea, P., López-Freire, S. & González-Vidal, T. Vaginal Capsules: An Unsuspected Probable Source of Exposure to α-Gal. *J Investig Allergol Clin Immunol* **26**, 338-339 (2016).
49. Mullins, R.J., James, H., Platts-Mills, T.A. & Commins, S. Relationship between red meat allergy and sensitization to gelatin and galactose-alpha-1,3-galactose. *The Journal of allergy and clinical immunology* **129**, 1334-1342 e1331 (2012).
50. Stone, C.A., Jr. et al. Anaphylaxis after zoster vaccine: Implicating alpha-gal allergy as a possible mechanism. *The Journal of allergy and clinical immunology* **139**, 1710-1713 e1712 (2017).
51. Akella, K., Patel, H., Wai, J., Roppelt, H. & Capone, D. Alpha Gal-Induced Anaphylaxis to Herpes Zoster Vaccination. *Chest* **152**, a6 (2017).
52. Mullins, R.J., Richards, C. & Walker, T. Allergic reactions to oral, surgical and topical bovine collagen. Anaphylactic risk for surgeons. *Australian and New Zealand journal of ophthalmology* **24**, 257-260 (1996).
53. Indrani, K., Min, G., Christine, M., A., M.C. & R., H.-D.E. Alpha-Gal (Mammalian Meat) Allergy: Implications for Pharmacists. *Pharmacy Times* (2015).
54. Fournier, P.E. et al. A deadly aversion to pork. *Lancet* **377**, 1542 (2011).
55. Pinson, M.L. & Waibel, K.H. Safe administration of a gelatin-containing vaccine in an adult with galactose-alpha-1,3-galactose allergy. *Vaccine* **33**, 1231-1232 (2015).
56. Kleiman, A.M., Littlewood, K.E. & Groves, D.S. Delayed Anaphylaxis to Mammalian Meat Following Tick Exposure and Its Impact on Anesthetic Management for Cardiac Surgery: A Case Report. *A & A case reports* **8**, 175-177 (2017).
57. O'Brien, M.F. et al. The SynerGraft valve: a new acellular (nonglutaraldehyde-fixed) tissue heart valve for autologous recellularization first experimental studies before clinical implantation. *Seminars in thoracic and cardiovascular surgery* **11**, 194-200 (1999).
58. Lim, H.G., Kim, S.H., Choi, S.Y. & Kim, Y.J. Anticalcification effects of decellularization, solvent, and detoxification treatment for genipin and glutaraldehyde fixation of bovine pericardium. *Eur J Cardiothorac Surg* **41**, 383-390 (2012).
59. Mangold, A. & Ankersmit, H.J. Reviewing alpha-Gal in valve immunology. *Eur J Cardiothorac Surg* **41**, 1214-1215 author reply 1215-1216 (2012).
60. Ankersmit, H.J., Copic, D. & Simader, E. When meat allergy meets cardiac surgery: A driver for humanized bioprosthesis. *The Journal of thoracic and cardiovascular surgery* **154**, 1326-1327 (2017).
61. Moroni, F. & Mirabella, T. Decellularized matrices for cardiovascular tissue engineering. *American journal of stem cells* **3**, 1-20 (2014).
62. Konakci, K.Z. et al. Alpha-Gal on bioprostheses: xenograft immune response in cardiac surgery. *European journal of clinical investigation* **35**, 17-23 (2005).
63. Mangold, A. et al. Alpha-Gal specific IgG immune response after implantation of bioprostheses. *The Thoracic and cardiovascular surgeon* **57**, 191-195 (2009).
64. Kasimir, M.T. et al. Presence and elimination of the xenoantigen gal (alpha1, 3) gal in tissue-engineered heart valves. *Tissue engineering* **11**, 1274-1280 (2005).
65. Loyens, M. et al. Link between endocarditis on porcine bioprosthetic valves and allergy to pork. *International journal of cardiology* **167**, 600-602 (2013).

66. Simon, P. et al. Early failure of the tissue engineered porcine heart valve SYNERGRAFT in pediatric patients. *Eur J Cardiothorac Surg* **23**, 1002-1006; discussion 1006 (2003).
67. Perri, G. et al. Early and late failure of tissue-engineered pulmonary valve conduits used for right ventricular outflow tract reconstruction in patients with congenital heart disease. *Eur J Cardiothorac Surg* **41**, 1320-1325 (2012).
68. Apostolovic, D. et al. Immunoproteomics of processed beef proteins reveal novel galactose-alpha-1,3-galactose-containing allergens. *Allergy* **69**, 1308-1315 (2014).
69. Apostolovic, D. et al. Peptidomics of an in vitro digested alpha-Gal carrying protein revealed IgE-reactive peptides. *Scientific reports* **7**, 5201 (2017).
70. 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* **69**, 199-207 (2014).
71. Phelps, C.J. et al. Production of alpha 1,3-galactosyltransferase-deficient pigs. *Science.* **299**, 411-414 (2003).
72. Dai, Y. et al. Targeted disruption of the alpha1,3-galactosyltransferase gene in cloned pigs. *Nature biotechnology* **20**, 251-255 (2002).
73. Fisher, M.B. et al. Potential of healing a transected anterior cruciate ligament with genetically modified extracellular matrix bioscaffolds in a goat model. *Knee Surg Sports Traumatol Arthrosc* **20**, 1357-1365 (2012).
74. Liang, R., Fisher, M., Yang, G., Hall, C. & Woo, S.L. Alpha1,3-galactosyltransferase knockout does not alter the properties of porcine extracellular matrix bioscaffolds. *Acta Biomater* **7**, 1719-1727 (2011).
75. Fang, J. et al. Anti-gal antibodies in alpha1,3-galactosyltransferase gene-knockout pigs. *Xenotransplantation* **19**, 305-310 (2012).
76. CVM Guidance for Industry #187: Regulation of Genetically Engineered Animals Containing Heritable Recombinant DNA constructs. (2015).
77. Dunkman WJ, Rycek W, Manning MW. What Does a Red Meat Allergy Have to Do With Anesthesia? Perioperative Management of Alpha-Gal Syndrome. *Anesth Analg*. May 2018:1. doi:10.1213/ANE.0000000000003460