

BRIEF COMMUNICATION

Anaphylaxis to galacto-oligosaccharides – an evaluation in an atopic population in Singapore

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Abstract

Anaphylaxis to galacto-oligosaccharides (GOS), a prebiotic, has been described in atopic patients following its supplementation in commercial milk formula in South-East Asia. The epidemiology of this usual allergy to a carbohydrate is unknown. This study evaluated the prevalence of allergy to two formulations of commercial GOS, Vivinal™ GOS (vGOS) and Oligomate™, in an atopic cohort. Atopic subjects ($n = 487$) from two specialist allergy clinics were surveyed via structured questionnaire and underwent skin prick tests to GOS. Subjects with positive skin prick tests to GOS ($n = 30$, 6.2%) underwent basophil activation tests, and a subset ($n = 13$) underwent oral challenge tests to both formulations of GOS. Six subjects had positive challenges to vGOS; and none to Oligomate. By extrapolating the BAT and oral challenge results, the prevalence of allergy to vGOS is estimated at up to 3.5% (95% CI 2.2–5.5%) of our atopic population. Our findings show that GOS allergy may be common amongst atopics in Singapore.

Recognition of the benefits of prebiotics (1) has led to the supplementation of galacto-oligosaccharides (GOS) in commercially available milk formulations and dairy products in recent years. GOS is synthesized enzymatically and consists of a mixture of sugar chains of varying lengths, up to seven units at most (2). They are nondigestible oligosaccharides that, when ingested, function as prebiotics. Although available in consumer products globally, reports of allergic reactions and anaphylaxis to GOS have been confined to the Asian region – Japan (3), Singapore (4), Malaysia and Thailand (unpublished), and likely Vietnam (5). This is an unusual allergy as the trigger is a pure carbohydrate (6). GOS, being a small molecule, is unlikely to cross-link IgE on mast cells, but it has been shown in animal studies to be immunogenic when coupled with carrier proteins (3); there is suggestion that in patients with GOS allergy, this protein component may be one found on the cell surface (4). The five

reported cases from Singapore were above 5 years old, atopic and tolerant to cow's milk. They developed anaphylaxis following the ingestion of between 40 and 250 ml of cow's milk formula containing between 0.16 and 2.0 grams of GOS (4). All patients were sensitized to GOS based on skin prick tests (SPT) and basophil activation tests (BAT) indicating an IgE-mediated allergy.

In Singapore, the market for GOS is restricted as an ingredient in several brands of commercially available powdered milk formulae for infants, children and pregnant/lactating mothers. The exposure to GOS in the general population is therefore limited. We sought to ascertain whether GOS allergy in our population is a rare phenomenon or that its prevalence is limited by the lack of exposure. In addition, we sought to establish the utility of the BAT in predicting GOS allergy. Hence, we evaluated the prevalence of allergy to two formulations of commercial GOS in an atopic cohort. The commercial formulations were Vivinal GOS (vGOS) (FrieslandCampina Domo, Amersfoort, the Netherlands) (2), which is the only preparation that is marketed in Singapore, and Oligomate 55N GOS (Yakult, Tokyo, Japan) (7), which is not marketed in Singapore. They differ in their bacterial

Abbreviations

BAT, basophil activation test; GOS, galacto-oligosaccharides; SPT, skin prick test; vGOS, Vivinal galacto-oligosaccharides.

source of the β -galactosidase used in the manufacturing process – *Bacillus circulans* in vGOS and *Sporobolomyces singularis* and *Kluyveromyces lactis* in Oligomate. As a consequence, vGOS and Oligomate differ in their galacto-oligosaccharide composition.

The inclusion criteria were based on the clinical features of our five cases reported previously (4): age 5 years and above with the history of atopic disease (eczema, allergic rhinitis and/or asthma), sensitized to dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and/or *Blomia tropicalis*) which is the major allergen in our population, as an indicator of atopy (8); and no history of cow's milk or GOS allergy. Subjects were recruited from the Allergy Clinics of two tertiary hospitals in Singapore. Written informed consent was obtained from all participants, and this study was approved by ethics review boards of both institutions. A structured questionnaire and SPT to two concentrations of vGOS (7.2 and 14.4 mg/ml of vGOS) were administered. All subjects with positive SPT (≥ 3 mm wheal) to either concentration of vGOS were defined as sensitized to GOS; they then had blood drawn for BAT to vGOS products. Adult (≥ 21 years) participants who had a positive SPT to vGOS were invited to undergo an oral challenge to both commercial GOS products on two separate occasions. SPT and BAT to Oligomate 55N GOS were performed for those who underwent challenge.

A total of 487 participants (64.7% female) with ages ranging from 5 to 60 years were surveyed. Their atopic conditions were eczema (43.1%), asthma (47.2%), allergic rhinitis (84.2%) and food allergies (24.6%), which included shellfish, peanuts, fish, tree nuts, various legumes and fruits. There were 30 participants (6.2%) sensitized to vGOS on SPT. On multivariate analysis, there was no significant association between vGOS sensitization and sensitization to any of the dust mites, type of atopic disorder or demographic factors.

Thirteen of the 30 participants consented to open challenge to vGOS and Oligomate (on separate days) in increasing doses up to a cumulative dose of 4 g. Six subjects had a positive response and one equivocal response to vGOS upon ingestion of doses of between 0.5 and 2 g; none reacted to Oligomate challenge. The positive reactions occurred 15–70 min after ingestion of vGOS, four of whom had anaphylaxis as per the criteria set out by the World Allergy Organisation (9); the details are in Table 1. Another subject not included as challenge positive had sneezing and chest tightness 4 h after ingestion which resolved spontaneously and was classified as 'equivocal'; however, no other likely explanation for the symptoms in this subject could be found (summarized in Fig. 1).

We then plotted receiver operating characteristic curves for the BAT results for all subjects who underwent challenge, at each concentration of vGOS that their blood was stimulated with, up to 1000 μ g/ml of vGOS. For CD203c, the optimal concentration was 100 μ g/ml of vGOS, with a cut-off stimulation index (stimulation index = stimulation by allergen/basal stimulation) of 2.43 showing a sensitivity of 66.7% and specificity of 100%. For CD63, the optimal concentration was 1 μ g/ml of vGOS, with a cut-off of acti-

Table 1 Allergic reactions to vGOS challenge

Subject number	Cumulative dose of vGOS ingested (grams)	Time to onset of symptoms after ingestion (minutes)	Specific symptoms/signs
1	2	45	Sneeze, itchy and running nose, itchy eyes, cough
2	2	30	Sneeze, urticaria, laryngeal tightness
3	2	30	Sneeze, laryngeal tightness
4	0.6	15	Sneeze, blocked nose, wheezing, laryngeal tightness
5	2	30	Chest tightness, cough
6	4	70	Sneeze, itch, diarrhoea

vated basophils of 1.65% showing a sensitivity of 66.7% and specificity of 100%. Taking a positive result to be either CD63 or CD203c positive using these cut-offs, five of the six challenge-positive patients were vGOS BAT positive, and all of the challenge-negative and equivocal subjects were vGOS BAT negative. Excluding the equivocal case, vGOS BAT had a sensitivity of 83% (95% CI 36–99%) and specificity of 100% (95% CI 52–100%).

Further, subjects with positive vGOS challenge were more likely to have larger vGOS SPT wheals than those with negative vGOS challenge (median wheal size [range]: vGOS challenge positive = 6 mm [range 3–8] for both vGOS 7.2 and 14.4 mg/ml; vGOS challenge negative = 4 mm [range 0–4] for vGOS 7.2 mg/ml; and 3 mm [range 0–5] for vGOS 14.4 mg/ml; $P < 0.019$).

Following descriptions of GOS allergy in Japan (3), Yakult Japan modified their manufacturing process of GOS by changing the source of β -galactosidase from *Bacillus circulans* to *Sporobolomyces singularis* and *Kluyveromyces lactis*. This resulted in lower concentration of the branched chain sugar Gal β 1-4(Gal β 1-4Gal β 1-6)Glc, which they suspect to be the allergenic epitope (3). The modified GOS, with commercial name Oligomate 55N, was the other preparation tested in this study. All thirteen of our subjects passed the oral challenge to Oligomate. All were also SPT and BAT negative to Oligomate.

To estimate the prevalence of vGOS allergy in our atopic population, two approaches were used. One, where the prevalence is based on the proportion of positive challenges in those sensitized to GOS, gives an estimated prevalence rate of 2.8%. Given that most of those who truly had GOS allergy also were positive to BAT, the second method based on this observation is as follows. We used our derived vGOS BAT positivity criteria and determined that from the atopic population survey, 15 of 30 subjects with positive SPT were vGOS BAT positive. Given that five of six challenge-positive

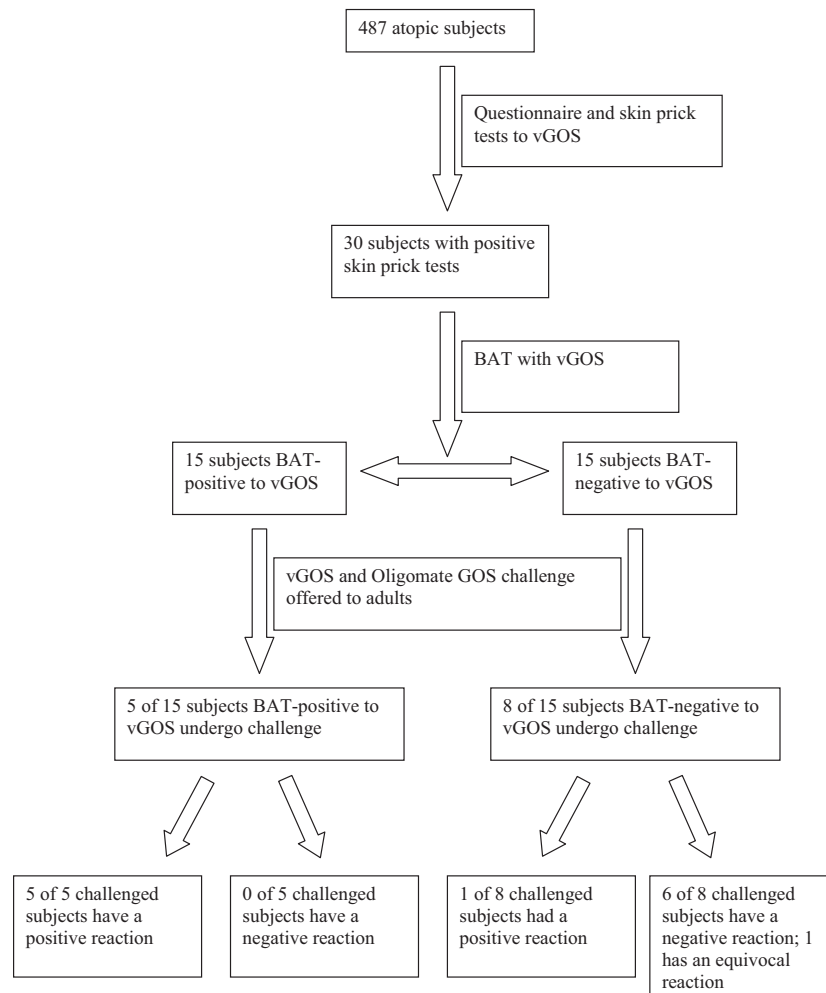


Figure 1 Chart depicting workflow and outcomes of survey on GOS allergy in an atopic population. BAT, basophil activation test; vGOS, Vivinal® galacto-oligosaccharide.

subjects were BAT positive, and none of seven challenge-negative/equivocal subjects were BAT positive, this yielded a true-positive rate of 100% and false-negative rate of 14.3%. Extrapolating these results, the prevalence of allergy to GOS is estimated at up to 3.5% (95% CI 2.2–5.5%) of our atopic population (see Table 2 for formula used). This estimate excludes the atopic people who had a negative SPT to vGOS. However, this estimate may not be entirely accurate as subjects were drawn from patients attending a specialized clinic in a tertiary hospital. Furthermore, due to the small number of subjects from which the ROC curves were derived, this limits the accuracy of the BAT and, thus, the precision of this estimate.

In conclusion, the estimated prevalence of true vGOS allergy may be up to 3.5% in the Singapore atopic population aged 5–60, which is significant given that the atopic population constitutes about one-fifth of the entire local population. The risk of allergy to different GOS formulations is variable, and this is likely related to structural differences. The relatively few cases

Table 2 Mathematical formulae for estimating prevalence of vGOS allergy in the atopic population

1. Estimated prevalence in cohort, using only challenge results = (Number of positive challenges/total number of subjects challenged) × (Number of subjects with GOS SPT positive/total number of subjects in cohort)
Hence $(6/13) \times (30/487) = 2.8\%$
 2. Estimated prevalence (%) in cohort, using BAT positivity criteria and challenge results = [(True positive rate of BAT × number of BAT positive) + (False negative rate of BAT × number of BAT negative)] × 100/Total number of subjects in cohort
Hence,
 $[(1.00 \times 15) + (0.143 \times 15)] \times 100/487 = 3.5\%$
- Assumptions:
1. All vGOS skin prick test-negative subjects are not GOS-allergic.
 2. No difference in likelihood of GOS allergy between those who consented to challenge, compared to those who refused or not eligible.

reported probably reflect limited exposure. The specific geographical restriction of GOS allergy cases to Japan and South-East Asian region implies a primary sensitizer confined to these regions, but this requires further investigation.

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Author contributions

Jian Yi Soh supervised the challenges, drafted the manuscript and provided final approval for its publication. Chiung-Hui Huang performed the basophil activation tests, contributed to the manuscript draft and provided final approval for its publication. Wen Chin Chiang substantially contributed to the acquisition of data, contributed to the manuscript draft and provided final approval for its publication. Genevieve V Llanora supervised the challenges, contributed to the manuscript draft and provided final approval for its publica-

tion. Alison Joanne Lee supervised the challenges, contributed to the manuscript draft and provided final approval for its publication. Wenyin Loh substantially contributed to the acquisition of data, contributed to the manuscript draft and provided final approval for its publication. Yue Lin Cherlyn Chin assisted in the conception of the study, contributed to the manuscript draft and provided final approval for its publication. Victoria Yu Jia Tay assisted in the conception of the study, contributed to the manuscript draft and provided final approval for its publication. Yiong Huak Chan supervised the data interpretation and statistical analysis, contributed to the manuscript draft and provided final approval for its publication. Dianne Delsing provided the extracts required for the study, revised the manuscript critically for intellectual content and provided final approval for its publication. Bee Wah Lee conceived the study and study protocol, participated in manuscript writing and provided final approval for its publication.

Conflicts of interest

Jian Yi Soh, Chiung-Hui Huang, Wen Chin Chiang, Genevieve V Llanora, Alison Joanne Lee, Wenyin Loh, Yue Lin Cherlyn Chin, Victoria Yu Jia Tay and Yiong Huak Chan declare there is no conflict of interest for the conception, performing, and writing nor for the decision to submit the article for publication. Dianne Delsing is an employee of FrieslandCampina, the company which markets Vivinal GOS. Bee Wah Lee receives honoraria supported by Danone Nutricia, Nestle Nutrition and Abbott Nutrition for lectures at conferences.

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