A Novel Galacto-Oligosaccharide (RP-G28) Promotes Beneficial Adaptations to the Human Gut Microbiome in Patients with Lactose Intolerance

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Summary

Non-persistence of the lactase enzyme in the small intestinal mucosa affects 65-70% percent of the population globally, commonly referred to as lactose intolerance (LI). In susceptible individuals, fermentation of lactose in the colon can produce carbon dioxide, hydrogen and methane leading to a range of abdominal and bowel-related symptoms, including abdominal pain, cramping, discomfort, bloating, distension, flatulence, and diarrhea.

Treatments that modify the gut microbiota can be used to treat disease-associated alterations in the gut microbiome (dysbiosis). Galacto-oligosaccharides (GOS) are prebiotics that are not digested in the small intestine reaching the colon where they stimulate growth of lactose-metabolizing bacteria. Elevated populations of *Lactobacillus* and *Bifidobacterium* species enhance β-galactosidase activity and GOS utilization, resulting in enhanced fermentation of lactose and reducing lactose-derived gas production in the colon, to alleviate the symptoms of LI.

Methods

Study design. A multicenter, randomized, double-blind, placebo-controlled, parallel group clinical trial was conducted to determine the efficacy, safety, and tolerability of two dose regimens of RP-G28 in subjects with moderate to severe LI. 377 LI patients received either low (n=127) or high (n=123) doses of RP-G28 or placebo (n=127). The lower dose of RP-G28 was administered as 5 grams twice daily on days 1-10 followed by 7.5 g twice daily on days 11-30. The higher dose of RP-G28 was administered as 7.5 g twice daily for days 1-10, followed by 10 g twice daily on days 11-30.

Gut microbiome analysis. 16S rRNA amplicon sequencing was performed utilizing patient fecal (stool) samples, which were obtained on day 0, day 31 and day 61. Amplification using universal primers targeting the V4 region of the bacterial 16S rRNA gene[1]. A total of 1,050 DNA samples corresponding to 345 subjects receiving placebo or RP-G28 treatments were analyzed also by high-throughput qPCR targeting bifidobacteria and lactobacilli using specific 16S rRNA gene and GroEL probes[2, 3].

Data analysis. Bioinformatics analysis of bacterial 16S rRNA amplicon sequencing data was conducted using the Quantitative Insights Into Microbial Ecology (QIIME) software[4].
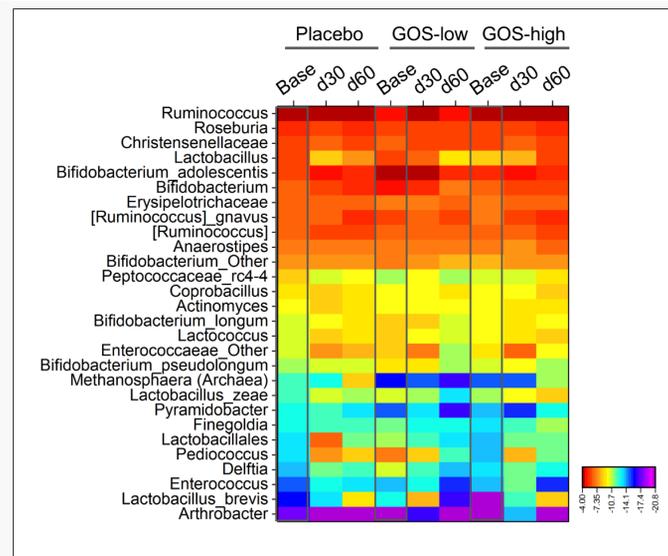
Results

RP-G28 impact on overall gut microbiome composition and diversity

➢ Analysis of similarities (ANOSIM) and permutational multivariate analysis of variance (PERMANOVA) using of unweighted Unifrac similarity matrices showed moderate but statistically significant correlations (ANOSIM $0.01 > R < 1$ and $P < 0.05$; PERMANOVA > 1 pseudo-F < 6, P = 0.05) between microbiome composition and treatment group, sex, individual patient, age, race, and BMI. Conversely, ethnicity, alcohol, and smoking had no impact on the microbiome of participants.

➢ No significant differences (One way ANOVA with Tukey pairwise comparisons P < 0.05) in Shannon diversity (H) and species richness (S) indexes were observed between treatment groups at different days (not shown).

Table 1. Strength and statistical significance of sample grouping determined by the nonparametric tests ANOSIM and PERMANOVA using a distance matrix as primary output

Significant Parameters	PERMANOVA		ANOSIM	
	Pseudo-F	P value	R²	P value
Sex	5.09	1E⁻⁰⁶	0.016	0.01
Run	3.3	1E⁻⁰⁶	0.05	1E⁻⁰⁶
Site	2.8	1E⁻⁰⁶	0.09	1E⁻⁰⁶
Patient ID	2.5	1E⁻⁰⁶	0.94	1E⁻⁰⁶
Age category	2.38	1E⁻⁰⁶	0.019	0.02
Race	1.9	1E⁻⁰⁶	0.018	0.025
BMI category	1.9	1E⁻⁰⁶	0.015	0.03
Treatment Group (TG)	1.86	1E⁻⁰⁶	0.008	1E⁻⁰⁶
Resp3_v5	1.65	0.0009	0.005	0.04
Resp_v8	1.65	0.001	0.003	0.02
Visit Number (VN)	1.34	0.0004	0.01	1E⁻⁰⁶

### Cohorts were similar at baseline with different impact by treatments on each group

➢ GOS treatment resulted in measurable changes in microbiome profiles and significant changes in relative abundance of 28 taxa (Kruskall Wallis FDR corrected p < 0.05).

RP-G28 induced significant increases in the abundance of phylum Actinobacteria, family Bifidobacteriaceae, and genus *Bifidobacterium*
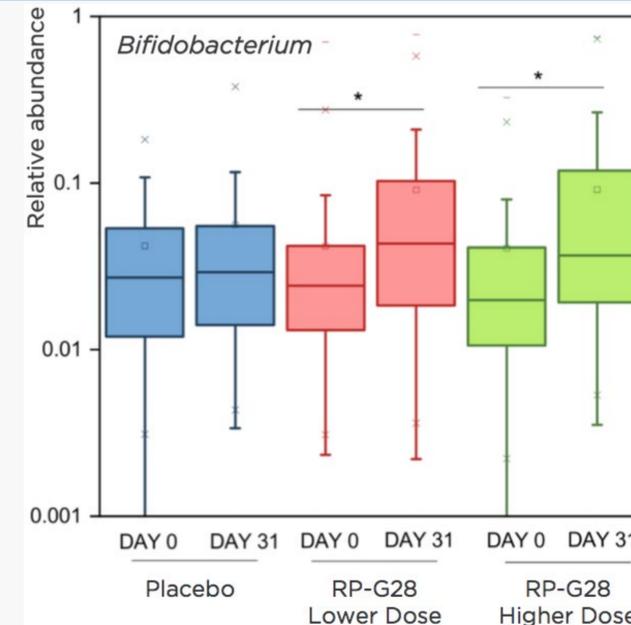
➢ *B. angulatum, B. gallicum* and *B. longum* were increased at day 31 in both treatments. *B. bifidum, B. breve,* and *B. catenulatum* were increased at days 31 and 61.

➢ Bifidobacteria increased in 77.7% (77/99) of patients in the RP-G28 groups compared to 52.1% (49/94) in the placebo group

### Top 10 taxa differentiate Placebo and RP-G28 determined by Random Forest
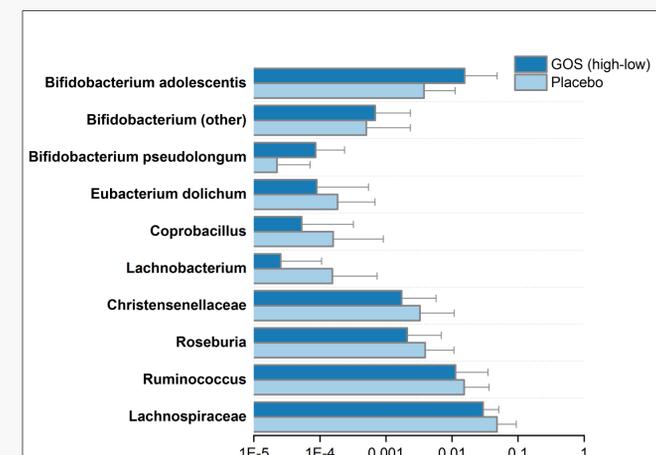
➢ Increased abundance of *Bifidobacterium* (other), *B. adolescentis* and *B. pseudolongum*, and decreased abundance of the families Lachnospiraceae and Christensenellaceae, and the genera *Roseburia, Ruminococcus, Coprobacillus,* and *Eubacterium dolichum* characterized the GOS-treated groups.

Clinical Outcomes

Patients Reporting Meaningful Improvement in Lactose Intolerance Symptoms

- RP-G28 treatment resulted in improved tolerance as compared to placebo.
- Patients consumed 2x more milk after treatment compared to the placebo group (p=0.014)
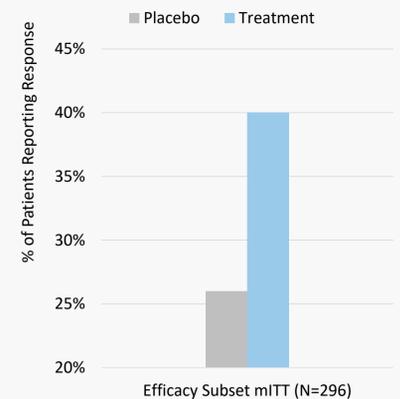
Conclusions

➢ RP-G28 had a moderate impact on overall gut microbiome composition and diversity. Sex, interindividual variation, age, race, BMI also impacted the microbiome.

➢ RP-G28 induced significant increases in the abundance of *Bifidobacterium* species providing a plausible explanation for the clinical benefits observed with this therapeutic in LI patients.

➢ RP-G28 acts on lactose intolerant individuals by increasing the abundance of gut microbes that can rapidly metabolize lactose without production of gas

References

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Acknowledgments