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Perspective

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Gut microbiome immaturity and childhood acute lymphoblastic leukaemia

In the format provided by the authors and unedited

SUPPLEMENTARY DATA

SUPPLEMENTARY METHODS

SUPPLEMENTARY TABLES

SUPPLEMENTARY FIGURES

SUPPLEMENTARY METHODS:

Types of evidence sources considered for main data analysis: All types of primary clinical studies were considered in the present perspective article. Grey literature with sufficient information to assess eligibility was also considered. Data from narrative reviews, opinion articles and animal studies were excluded. Only studies with titles and abstracts in English were considered.

Participants: In the present perspective article, we only included studies involving participants who were diagnosed with acute leukaemia up to the age of 19 years. Studies including participants with an additional diagnosis of any chronic medical condition were not considered in order to exclude potential confounding effects on the gut microbiome. Prior antibiotic administration was not deemed a feasible exclusion criterion, given the very high frequency of antibiotic administration around the time of leukemia diagnosis and during treatment. This information, however, was sought during the data extraction process to assist with the interpretation of findings (Figure 2).

Concept: The concept of interest in this perspective article was gut microbiome diversity and composition measured by sequencing techniques, with no restrictions on the measures reported. Studies relying solely on culture-based techniques for examining the gut microbiome were not included due to the poor sensitivity of such methods and their inability to provide information about the overall microbiome composition. Studies investigating the oral microbiome were also excluded, as the oral and stool microbiomes of cancer patients have been shown to have different relative co-abundance patterns and different sensitivity to prior antibiotic treatment[1,2]. Studies examining the microbiome of other body sites were not considered.

Context: The present perspective article aimed to explore clinical evidence on gut microbiome diversity and composition the context of a new diagnosis of childhood acute leukaemia prior to administration of any chemotherapy.

Study Selection & Data Charting: The records identified from all databases were imported to the commercially available reference management software Endone (Clarivate Analytics, PA, USA). After manual duplicate removal, the title and abstract of each record was screened according to the Participant-Concept-Context Framework. The reference lists of all studies selected for inclusion in the present review were also screened for potentially eligible records. The final selection of studies was confirmed by all authors.

Analysis of published results: The direction of differences in gut microbiome diversity and composition (relative abundance) between healthy children and children with ALL, together with statistical significance levels, were directly extracted from the published results of selected studies. For the study of Liu et al.[3], which reported differences in relative abundance only at the level of species, individual participant data were used to conduct a linear discriminant analysis of effect size (LEfSE) at other taxonomic levels using an LDA score >2.0 and $p<0.05$[4].

SUPPLEMENTARY TABLES

Supplementary Table 1: Trends of α-diversity, β-diversity & relative abundance of major phyla during early childhood based on the results of selected studies of healthy children.

α-diversity	**Shannon Diversity Index**	Odamaki, 2020[6]
	4 months	1.9*
	10 months	2.8*
	28 months	4.1*
	73 months	4.3*

β-diversity	Roswall, 2021[7]	**Weighted Unifrac distance**
	Birth	0.92*
	4 months	0.82*
	12 months	0.85*
	36 months	0.72*
	60 months	0.70*

Changes in Relative abundance of major phyla over time	**Stewart, 2021[8]**	**Proteobacteria**	**Actinobacteria**	**Firmicutes**	**Bacteroides**
	3 months	15%	53%	26%	<1%
	12 months	6%	27%	55%	-
	20 months	4%	21%	61%	-
	40 months	4%	21%	61%	10%
	Odamaki, 2020[6]	**Proteobacteria**	**Actinobacteria**	**Firmicutes**	**Bacteroides**
	4 months	4%*	50%*	25%*	<1%*
	10 months	3%*	43%*	38%*	<1%*
	28 months	<1%*	13%*	76%*	2%*
	73 months	<1%*	11%*	78%*	4%*

*median values estimated from boxplots of published studies

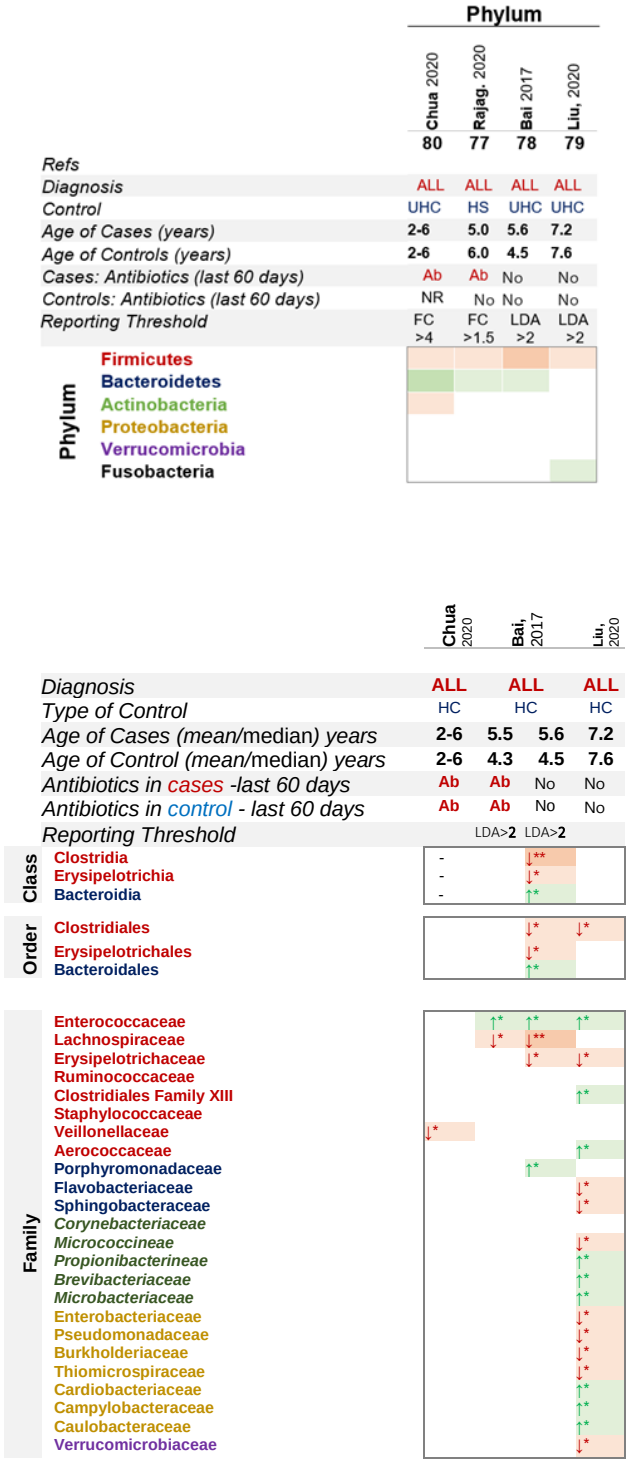
Supplementary Table S2: Search strategy (A) and results of database search.

A. Search Strategy for MEDLINE (Pubmed), Embase & Embase Classic (Ovid) and Cochrane Database

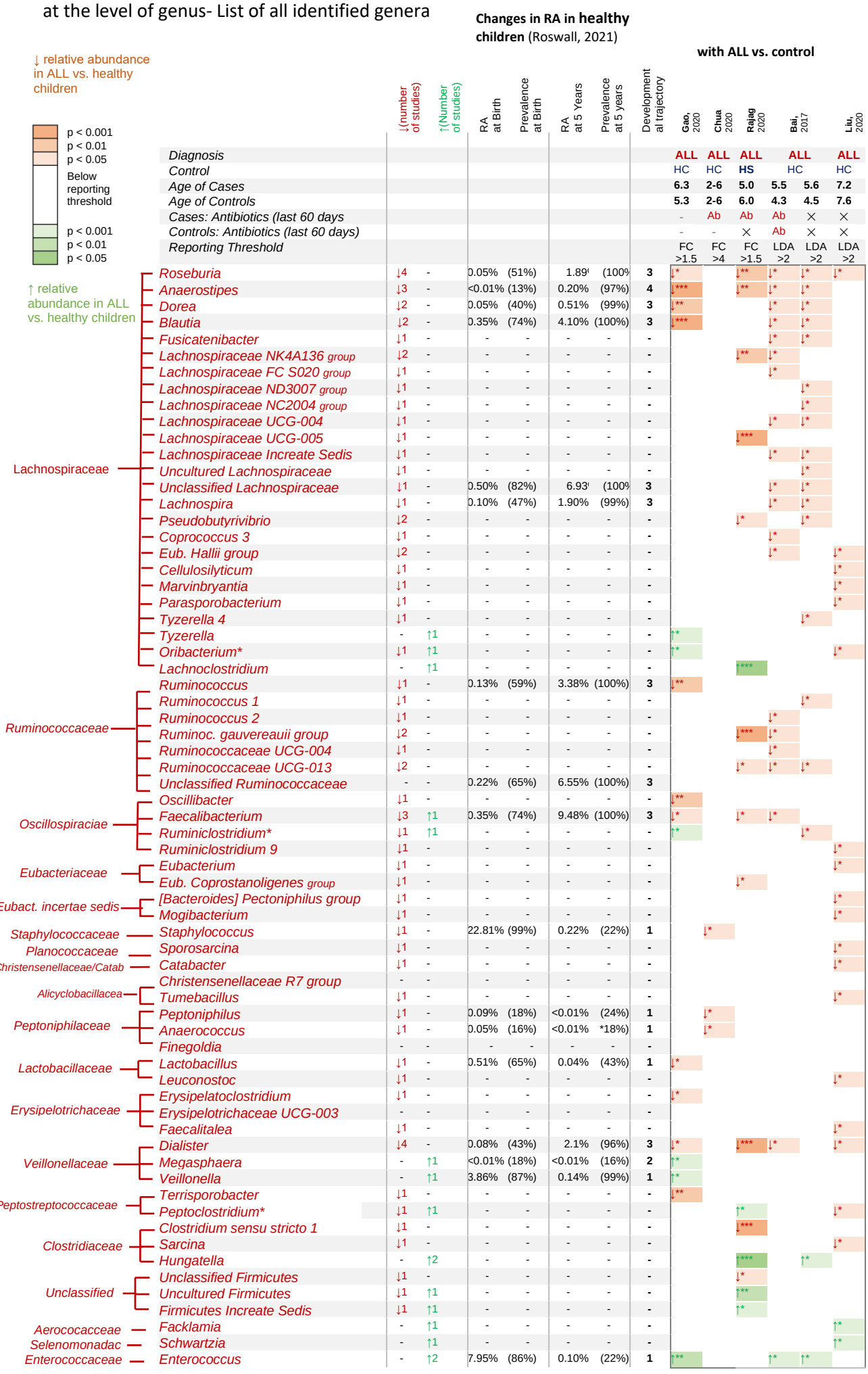
Database	**MEDLINE (Pubmed)**
Publication period	From inception until [20/10/2022]
Search strategy	((microbiome or microbiota) AND (Leukemia or Leukaemia))—all fields
Articles retrieved	n= 261
Database	**Embase & Embase Classic (Ovid)**
Publication period	From inception until [20/10/2022]
Search strategy	1. exp microbiome/ or microbiome.mp. or exp bacterial microbiome/ 2. microbiota.mp. or exp microflora/ 3. 1 or 2 4. exp leukemia/ or leukemia.mp. 5. leukaemia.mp. or exp leukemia/ 6. 4 or 5 7. 3 and 6
Articles retrieved	n=1305

SUPPLEMENTARY FIGURES

Supplementary Figure 1: Differences in gut microbiome composition at the level of class, order and family between newly diagnosed children with ALL and healthy children.

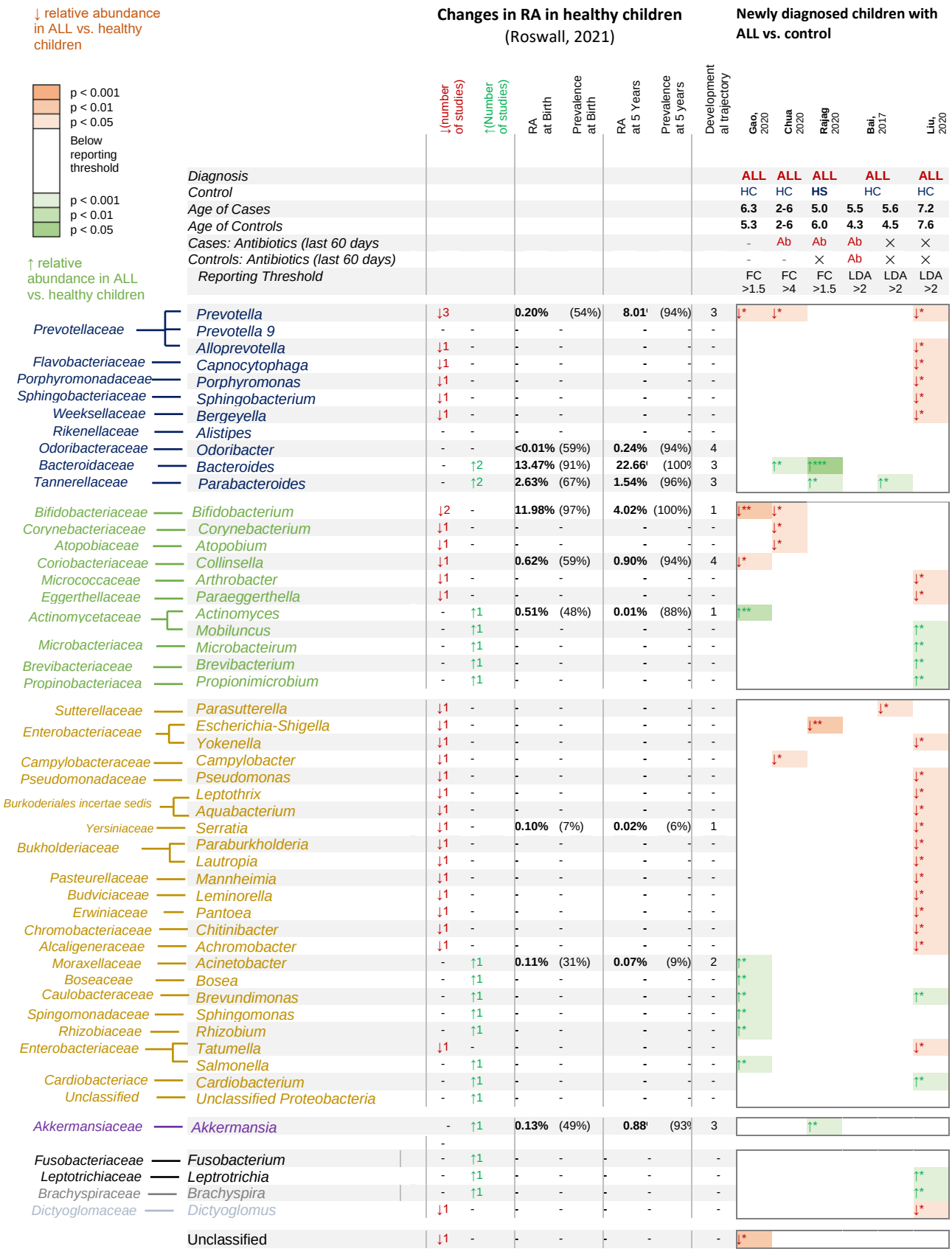


Supplementary Figure 2: Differences in gut microbiome composition in children with ALL vs. healthy children at the level of genus- List of all identified genera



	↓(number of studies)	↑(Number of studies)	RA at Birth	Prevalence at Birth	RA at 5 Years	Prevalence at 5 years	Development al trajectory	Gao, 2020	Chua 2020	Rajag 2020	Bai, 2017		Liu, 2020
Diagnosis								ALL	ALL	ALL	ALL		ALL
Control								HC	HC	HS	HC		HC
Age of Cases								6.3	2-6	5.0	5.5	5.6	7.2
Age of Controls								5.3	2-6	6.0	4.3	4.5	7.6
Cases: Antibiotics (last 60 days								-	Ab	Ab	Ab	×	×
Controls: Antibiotics (last 60 days)								-	-	×	Ab	×	×
Reporting Threshold								FC >1.5	FC >4	FC >1.5	LDA >2	LDA >2	LDA >2
Roseburia	↓4	-	0.05%	(51%)	1.89	(100%	3	↓*		↓**	↓*	↓*	↓*
Anaerostipes	↓3	-	<0.01%	(13%)	0.20%	(97%)	4	↓***		↓**	↓*	↓*	
Dorea	↓2	-	0.05%	(40%)	0.51%	(99%)	3	↓**			↓*	↓*	
Blautia	↓2	-	0.35%	(74%)	4.10%	(100%)	3	↓***			↓*	↓*	
Fusicatenibacter	↓1	-	-	-	-	-	-				↓*	↓*	
Lachnospiraceae NK4A136 group	↓2	-	-	-	-	-	-			↓**	↓*		
Lachnospiraceae FC S020 group	↓1	-	-	-	-	-	-				↓*		
Lachnospiraceae ND3007 group	↓1	-	-	-	-	-	-					↓*	
Lachnospiraceae NC2004 group	↓1	-	-	-	-	-	-					↓*	
Lachnospiraceae UCG-004	↓1	-	-	-	-	-	-				↓*	↓*	
Lachnospiraceae UCG-005	↓1	-	-	-	-	-	-			↓***			
Lachnospiraceae Increate Sedis	↓1	-	-	-	-	-	-				↓*	↓*	
Uncultured Lachnospiraceae	↓1	-	-	-	-	-	-					↓*	
Unclassified Lachnospiraceae	↓1	-	0.50%	(82%)	6.93	(100%	3				↓*	↓*	
Lachnospira	↓1	-	0.10%	(47%)	1.90%	(99%)	3				↓*	↓*	
Pseudobutyrivibrio	↓2	-	-	-	-	-	-			↓*		↓*	
Coprococcus 3	↓1	-	-	-	-	-	-				↓*		
Eub. Hallii group	↓2	-	-	-	-	-	-				↓*		↓*
Cellulosilyticum	↓1	-	-	-	-	-	-						↓*
Marvinbryantia	↓1	-	-	-	-	-	-						↓*
Parasporobacterium	↓1	-	-	-	-	-	-						↓*
Tyzerella 4	↓1	-	-	-	-	-	-					↓*	
Tyzerella	-	↑1	-	-	-	-	-	↑*					
*Oribacterium**	↓1	↑1	-	-	-	-	-	↑*					↓*
Lachnoclostridium	-	↑1	-	-	-	-	-			↑***			
Ruminococcus	↓1	-	0.13%	(59%)	3.38%	(100%)	3	↓**					
Ruminococcus 1	↓1	-	-	-	-	-	-					↓*	
Ruminococcus 2	↓1	-	-	-	-	-	-				↓*		
Ruminoc. gauvereauii group	↓2	-	-	-	-	-	-			↓***	↓*		
Ruminococcaceae UCG-004	↓1	-	-	-	-	-	-				↓*		
Ruminococcaceae UCG-013	↓2	-	-	-	-	-	-			↓*	↓*	↓*	
Unclassified Ruminococcaceae	-	-	0.22%	(65%)	6.55%	(100%)	3						
Oscillibacter	↓1	-	-	-	-	-	-	↓**					
Faecalibacterium	↓3	↑1	0.35%	(74%)	9.48%	(100%)	3	↓*		↓*	↓*		
*Ruminiclostridium**	↓1	↑1	-	-	-	-	-	↑*				↓*	
Ruminiclostridium 9	↓1	-	-	-	-	-	-						↓*
Eubacterium	↓1	-	-	-	-	-	-						↓*
Eub. Coprostanoligenes group	↓1	-	-	-	-	-	-			↓*			
[Bacteroides] Pectoniphilus group	↓1	-	-	-	-	-	-						↓*
Mogibacterium	↓1	-	-	-	-	-	-						↓*
Staphylococcus	↓1	-	22.81%	(99%)	0.22%	(22%)	1		↓*				
Sporosarcina	↓1	-	-	-	-	-	-						↓*
Catabacter	↓1	-	-	-	-	-	-						↓*
Christensenellaceae R7 group	-	-	-	-	-	-	-						
Tumebacillus	↓1	-	-	-	-	-	-						↓*
Peptoniphilus	↓1	-	0.09%	(18%)	<0.01%	(24%)	1		↓*				
Anaerococcus	↓1	-	0.05%	(16%)	<0.01%	*18%)	1		↓*				
Finegoldia	-	-	-	-	-	-	-						
Lactobacillus	↓1	-	0.51%	(65%)	0.04%	(43%)	1	↓*					
Leuconostoc	↓1	-	-	-	-	-	-						↓*
Erysipelatoclostridium	↓1	-	-	-	-	-	-	↓*					
Erysipelotrichaceae UCG-003	-	-	-	-	-	-	-						
Faecalitalea	↓1	-	-	-	-	-	-						↓*
Dialister	↓4	-	0.08%	(43%)	2.1%	(96%)	3	↓*		↓***	↓*		↓*
Megasphaera	-	↑1	<0.01%	(18%)	<0.01%	(16%)	2	↑*					
Veillonella	-	↑1	3.86%	(87%)	0.14%	(99%)	1	↑*					
Terrisporobacter	↓1	-	-	-	-	-	-	↓**					
*Peptoclostridium**	↓1	↑1	-	-	-	-	-			↑*			↓*
Clostridium sensu stricto 1	↓1	-	-	-	-	-	-			↓***			
Sarcina	↓1	-	-	-	-	-	-						↓*
Hungatella	-	↑2	-	-	-	-	-			↑***		↑*	
Unclassified Firmicutes	↓1	-	-	-	-	-	-			↓*			
Uncultured Firmicutes	↓1	↑1	-	-	-	-	-			↑**			
Firmicutes Increate Sedis	↓1	↑1	-	-	-	-	-			↑*			
Facklamia	-	↑1	-	-	-	-	-						↑*
Schwartzia	-	↑1	-	-	-	-	-						↑*
Enterococcus	-	↑2	7.95%	(86%)	0.10%	(22%)	1	↑**			↑*	↑*	

Supplementary Figure 2 (continued): Difference in gut microbiome composition (genus level) in children with ALL vs. healthy children- List of all identified taxa

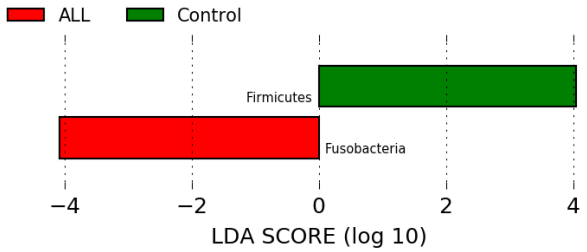


	↓(number of studies)	↑(Number of studies)	RA at Birth	Prevalence at Birth	RA at 5 Years	Prevalence at 5 years	Developmental trajectory	Gao, 2020	Chua 2020	Rajag 2020	Bai, 2017		Liu, 2020
Diagnosis								ALL	ALL	ALL	ALL		ALL
Control								HC	HC	HS	HC		HC
Age of Cases								6.3	2-6	5.0	5.5	5.6	7.2
Age of Controls								5.3	2-6	6.0	4.3	4.5	7.6
Cases: Antibiotics (last 60 days								-	Ab	Ab	Ab	×	×
Controls: Antibiotics (last 60 days)								-	-	×	Ab	×	×
Reporting Threshold								FC >1.5	FC >4	FC >1.5	LDA >2	LDA >2	LDA >2
Prevotella	↓3		**0.20%**	(54%)	**8.01**	(94%)	3	↓*	↓*				↓*
Prevotella 9	-	-	-	-	-	-	-						
Alloprevotella	↓1	-	-	-	-	-	-						↓*
Capnocytophaga	↓1	-	-	-	-	-	-						↓*
Porphyromonas	↓1	-	-	-	-	-	-						↓*
Sphingobacterium	↓1	-	-	-	-	-	-						↓*
Bergeyella	↓1	-	-	-	-	-	-						↓*
Alistipes	-	-	-	-	-	-	-						
Odoribacter	-	-	**<0.01%**	(59%)	**0.24%**	(94%)	4						
Bacteroides	-	↑2	**13.47%**	(91%)	**22.66**	(100	3		↑*	↑***			
Parabacteroides	-	↑2	**2.63%**	(67%)	**1.54%**	(96%)	3			↑*		↑*	
Bifidobacterium	↓2	-	**11.98%**	(97%)	**4.02%**	(100%)	1	↓**	↓*				
Corynebacterium	↓1	-	-	-	-	-	-		↓*				
Atopobium	↓1	-	-	-	-	-	-		↓*				
Collinsella	↓1		**0.62%**	(59%)	**0.90%**	(94%)	4	↓*					
Arthrobacter	↓1	-	-	-	-	-	-						↓*
Paraeggerthella	↓1	-	-	-	-	-	-						↓*
Actinomyces	-	↑1	**0.51%**	(48%)	**0.01%**	(88%)	1	↑**					
Mobiluncus	-	↑1	-	-	-	-	-						↑*
Microbacteirum	-	↑1	-	-	-	-	-						↑*
Brevibacterium	-	↑1	-	-	-	-	-						↑*
Propionimicrobium	-	↑1	-	-	-	-	-						↑*
Parasutterella	↓1	-	-	-	-	-	-				↓*		
Escherichia-Shigella	↓1	-	-	-	-	-	-			↓**			
Yokenella	↓1	-	-	-	-	-	-						↓*
Campylobacter	↓1	-	-	-	-	-	-		↓*				
Pseudomonas	↓1	-	-	-	-	-	-						↓*
Leptothrix	↓1	-	-	-	-	-	-						↓*
Aquabacterium	↓1	-	-	-	-	-	-						↓*
Serratia	↓1	-	**0.10%**	(7%)	**0.02%**	(6%)	1						↓*
Paraburkholderia	↓1	-	-	-	-	-	-						↓*
Lautropia	↓1	-	-	-	-	-	-						↓*
Mannheimia	↓1	-	-	-	-	-	-						↓*
Leminorella	↓1	-	-	-	-	-	-						↓*
Pantoea	↓1	-	-	-	-	-	-						↓*
Chitinibacter	↓1	-	-	-	-	-	-						↓*
Achromobacter	↓1	-	-	-	-	-	-						↓*
Acinetobacter	-	↑1	**0.11%**	(31%)	**0.07%**	(9%)	2	↑*					
Bosea	-	↑1	-	-	-	-	-	↑*					
Brevundimonas	-	↑1	-	-	-	-	-	↑*					↑*
Sphingomonas	-	↑1	-	-	-	-	-	↑*					
Rhizobium	-	↑1	-	-	-	-	-	↑*					
Tatumella	↓1	-	-	-	-	-	-						↓*
Salmonella	-	↑1	-	-	-	-	-	↑*					
Cardiobacterium	-	↑1	-	-	-	-	-						↑*
Unclassified Proteobacteria	-	↑1	-	-	-	-	-						
Akkermansia	-	↑1	**0.13%**	(49%)	**0.88**	(93%	3			↑*			
Fusobacterium	-	↑1	-	-	-	-	-						
Leptotrichia	-	↑1	-	-	-	-	-						↑*
Brachyspira	-	↑1	-	-	-	-	-						↑*
Dictyoglomus	↓1	-	-	-	-	-	-						↓*
Unclassified	↓1	-	-	-	-	-	-	↓*					

Supplementary Figure 3: Differences in gut microbiome composition at the level of species as reported by the study of Liu et al. 2020

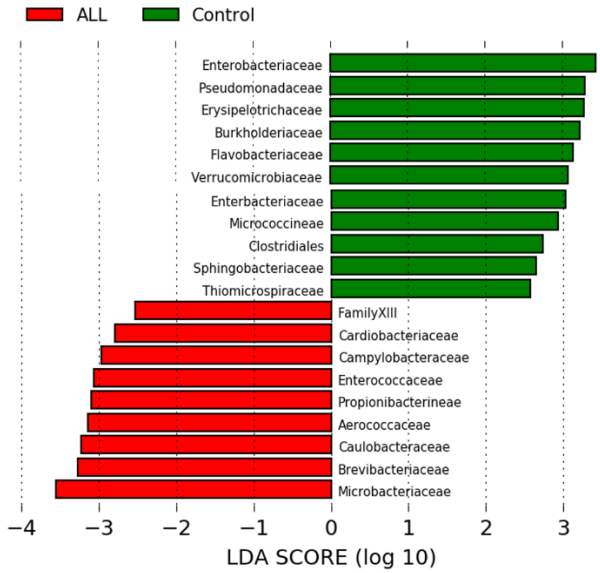
Supplementary Figure 4: LefSE re-analysis of individual participant data from the study of Liu et al (2020) at the level of (a) Phylum, (b) Family, (c) Genus using an LDA score >2.0 and p<0.05.

(a) Phylum

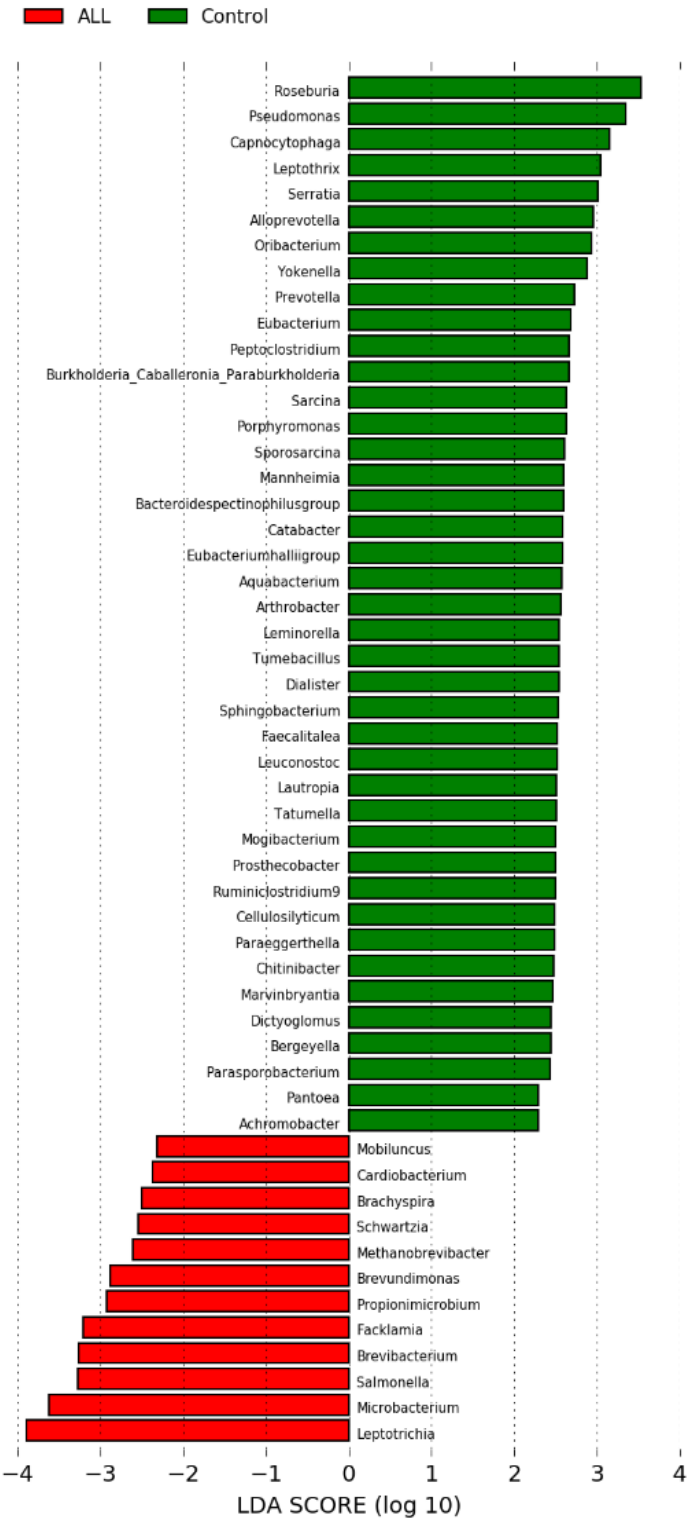


(b) Family

(b) Family



(c) Genus



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