



Universiteit
Leiden
The Netherlands

Metabolomics insight into the gut microbiome of infants with cow's milk allergy

Zhu, P.

Citation

Zhu, P. (2026, January 13). *Metabolomics insight into the gut microbiome of infants with cow's milk allergy*. Retrieved from <https://hdl.handle.net/1887/4286434>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/4286434>

Note: To cite this publication please use the final published version (if applicable).

Chapter IV

Current insights into cow's milk allergy in children: microbiome, metabolome and immune response – a systematic review

Based on:

Mariyana V Savova*, Pingping Zhu*, Amy C Harms, Renate G van der Molen, Clara Belzer, Diana M Hendrickx

Current insights into cow's milk allergy in children: microbiome, metabolome and immune response – a systematic review

Pediatric Allergy and Immunology

DOI: 10.1111/pai.14084

*Authors contributed equally

Abstract

The increasing prevalence of IgE-mediated cow's milk allergy (CMA) in childhood is a worldwide health concern. There is a growing awareness that the gut microbiome (GM) might play an important role in CMA development. Therefore, treatment with probiotics and prebiotics has gained popularity. This systematic review provides an overview on the alterations of the GM, metabolome and immune response in CMA-children and animal models, including post-treatment modifications. MEDLINE, PubMed, Scopus and Web of Science were searched for studies on the GM in CMA-diagnosed children, published before March 1, 2023. A total of 21 articles (13 on children, 8 on animal models) were included. The studies suggest that the GM, characterized by an enrichment of the Clostridia class and reductions in the Lactobacillales order and *Bifidobacterium* genus, is associated with CMA in early life. Additionally, reduced levels of short chain fatty acids (SCFAs) and altered amino acid metabolism were reported in CMA-children. Commonly used probiotic strains belong to the *Bifidobacterium* and *Lactobacillus* genera. However, only *Bifidobacterium* levels were consistently upregulated after intervention, while alterations of other bacteria taxa remain inconclusive. These interventions appear to contribute to the restoration of SCFAs and amino acid metabolism balance. Mouse models indicate that these interventions tend to restore the T_{h2}/T_{h1} balance, increase the T_{reg} response, and/or silence the overall pro- and anti-inflammatory cytokine response. Overall, this systematic review highlights the need for multi-omics related research in CMA-children to gain a mechanistic understanding of this disease and to develop effective treatments and preventive strategies.

Introduction

One of the most common food allergies in early childhood is cow's milk allergy (CMA).^{1,2} Allergic reactions can be IgE-mediated, non-IgE-mediated, or a mix of both.³ Multiple studies have shown that among the children diagnosed with CMA those with IgE-mediated reactions to CM tend to have persistent symptoms and acquire tolerance slower than those with non-IgE-mediated reactions.⁴⁻⁷ At present, infants diagnosed with CMA are placed on an elimination diet consisting of an extensively hydrolyzed formula (EHF) or, if symptoms persist, an amino-acid formula (AAF).⁸ Because of the increasing evidence linking food allergies with alterations in gut microbial composition,^{9,10} modifying the gut microbiome (GM) with probiotics, prebiotics or synbiotics has emerged as a promising way to prevent and treat allergies.¹¹ However, there is still little mechanistic understanding on how the GM influences host immune health, leading to allergies, including CMA.¹² Recent technological innovations in the field of microbiome, proteomics and metabolomics have opened new doors for research and provided opportunities to address the gap in understanding the role of GM in CMA. The objective of this systematic review is to further the understanding of the relationship between the GM and CMA, by reviewing existing studies examining microbiome, metabolome, proteome, and immune response data on IgE-mediated CMA in children and animal models.

1. Methods

This systematic review is registered in PROSPERO (CRD42021290177).

2.1 Search strategy

A search in MEDLINE, PubMed, Scopus and Web of Science was performed using the queries in Table S1. The search was limited to research articles published in English before March 1, 2023.

2.2 Inclusion and exclusion criteria

Human case, case-control, and intervention studies were included only if they examined children with IgE-mediated CMA aged 0-12 years. The allergy had to be medically

diagnosed by either a skin prick test (SPT) or an IgE-specific test combined with a cow's milk food challenge. In studies with fecal transplantation (FT), the IgE-mediated CMA status of the donor must be confirmed by the diagnosis criteria used for human studies. For studies reporting data on groups of subjects diagnosed with different types of CMA, only the group with IgE-mediated CMA was reviewed. For animal studies, only case-control and intervention studies on models that included both sensitization and challenge steps were included. The studies were included only if they contained analytical data that examined the GM or metabolome and were excluded when they failed to meet the inclusion criteria, had unclear diagnosis, or involved antibiotic treatment.

2.3 Study selection

Titles, abstracts, and methods were screened independently by two of the authors MVS, PZ, DMH, and by a third author in case of disagreement. Subsequently, the full text of the studies marked as potentially eligible was retrieved and independently checked for eligibility by at least two of the authors MVS, PZ, DMH, and by a third author in case of disagreement or doubts.

4

2.4 Data extraction

For human studies, the extracted data included general study details (author, year), participant information (age, sample size), CMA diagnosis, analytical data types, data acquisition techniques, measured analytical parameters and significant results. For intervention studies, the intervention details were also extracted. If available, the age range for each group in the study was reported. When only the mean and standard deviation (SD) were available, the age was reported as mean \pm sd. The results were split in two: increased and decreased variables between the compared groups. For animal intervention studies, the extracted data included general study details, model information, challenge information, intervention details, data acquisition techniques, measured analytical parameters and significant results.

3 Results

3.1 Search strategy

Our search yielded 733, 479, 512, 897 articles in respectively Scopus, PubMed, MEDLINE and Web of Science. Forty-nine studies were eligible for inclusion. Figure 1 shows the PRISMA¹³ flow diagram. Of the 49 papers, 28 were excluded after careful consideration (Table S2).

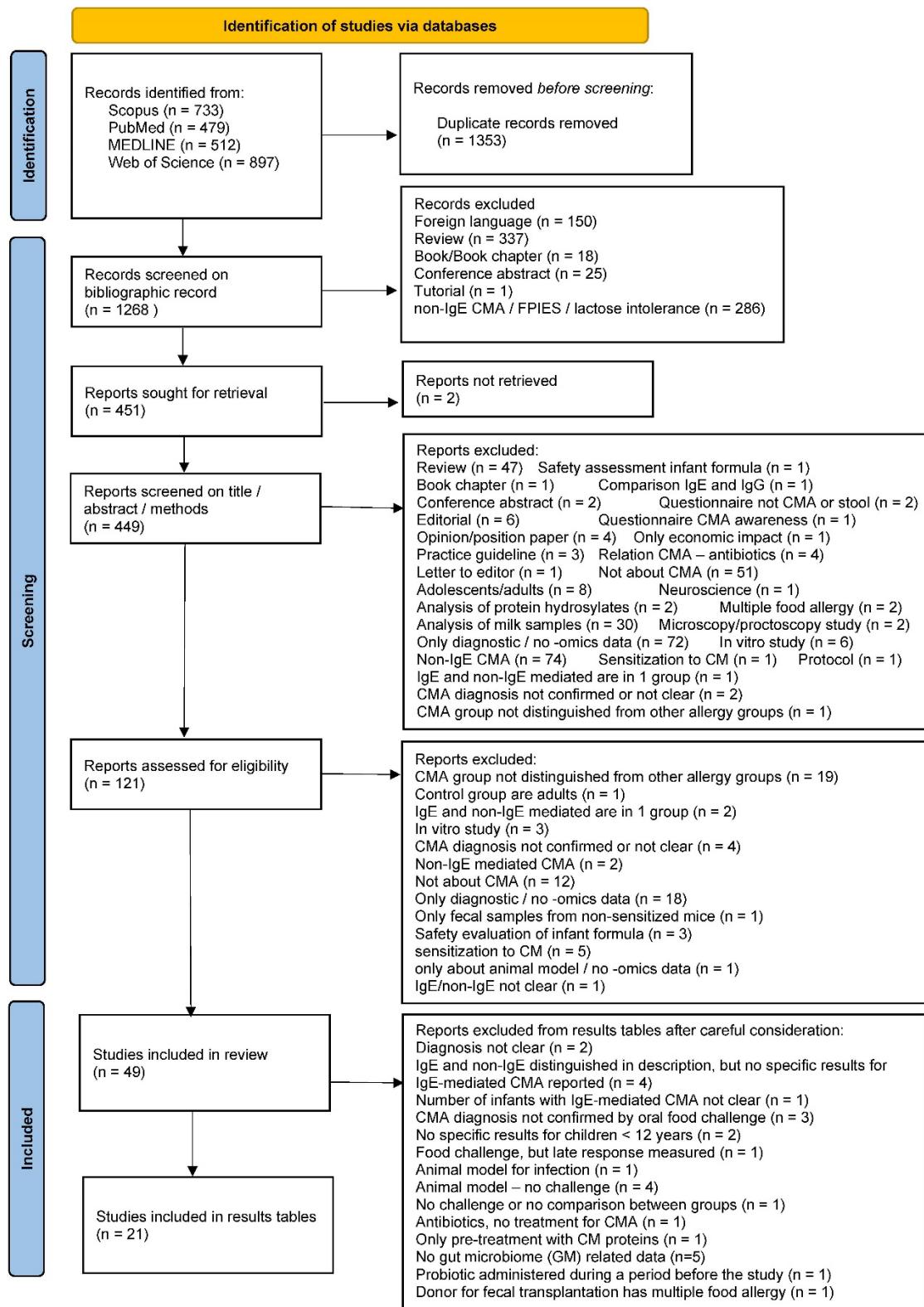


Figure 1. PRISMA flow chart for this systematic review.

3.2 Study findings

3.2.1 Human studies

CMA diagnosis criteria and measured parameters in human studies are summarized in Table S3.

3.2.1.1 Case and case-control studies

Human studies include one case and nine case-control studies (Table 1), among which four examined both the microbiome and metabolome,¹⁴⁻¹⁷ five the microbiome,¹⁸⁻²² and one the metabolome.²³ For all case-control studies, healthy controls (HC) were used except for one study²³ that considered atopic eczema/dermatitis syndrome infants as controls.

GM modifications

The GM-related studies include four case-control reports,^{15,19,17,20} four case-control findings in intervention studies,^{14,16,18,21} and one case study.²² Techniques applied for GM profile identification included bacteria culture¹⁸ and 16S rRNA gene-based approaches (DGGE,¹⁹ FISH^{14,15} and gene sequencing^{16,17,21,20,22}). Two studies applied specific probes to target certain bacteria groups,^{14,15} and six used universal probes or primers to target the V3 region,¹⁹ V4 region^{16,22} or both.^{17,20,21}

Six studies compared α - and β -diversity between CMA-group and HC, three of them noted increased^{16,19} or decreased²⁰ Shannon α -diversity difference in the CMA-groups, and one reported β -diversity (unweighted UniFrac) difference between CMA-group and HC.²¹ A single study reported a higher total bacteria count in the CMA-group.¹⁸

Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Verrucomicrobia were the primary reported GM phyla. Elevated abundances of the Firmicutes phylum were consistently observed in the CMA-groups.^{14-19,21} These included: total Firmicutes;^{17,21} the class Clostridia;¹⁷ the families *Lachnospiraceae*¹⁶ and *Ruminococcaceae*^{16,17}; the genera *Clostridium*,^{14,19} *Faecalibacterium*¹⁶, *Lactobacillus*,¹⁸ *Ruminococcus*¹⁶ and *Subdoligranulum*¹⁹ and the species *Clostridium coccoides*¹⁵ and *Clostridium celerecrescens*.¹⁹ Conversely, certain Firmicutes phylum, including the genus

*Granulicatella*²¹ and the families *Streptococcaceae*,¹⁶ *Enterococcaceae*,¹⁶ and *Acidaminococcaceae*,²⁰ decreased in the CMA-groups. Additionally, enriched bacteria of the Firmicutes phylum, including the class Clostridia, were also observed in the infants who outgrew CMA.²²

Bacteroidetes phylum members also showed varying changes in the CMA-groups.^{14,17,19–21} These included increased levels of the *Flavobacteriaceae* family,¹⁷ the *Bacteroides*^{14,19} and *Prevotella*²¹ genera, along with reduced abundance of the *Prevotellaceae* family²⁰ and the *Parabacteroides* genus.²¹ Furthermore, several bacteria from the Proteobacteria phylum, including the *Haemophilus*, *Actinobacillus* and *Klebsiella* genera,²¹ and the *Escherichia coli* species,¹⁹ increased in the CMA-groups. In contrast, total Proteobacteria,¹⁷ the *Enterobacteriaceae* family,^{16,18} and the *Escherichia* genus¹⁶ decreased. In the Actinobacteria phylum, one study reported increased *Atopobium* cluster (genus) levels,¹⁵ while *Bifidobacteriaceae* family members, including *Bifidobacterium spp.*, consistently exhibited decreased abundance in the CMA-groups.^{14,16,18,19} Additionally, the Verrucomicrobia phylum dropped in the CMA-group.²¹

Two studies reported certain bacteria only in the CMA group or the HC. The *Clostridium celerecrescens* species,¹⁹ and the *Burkholderiaceae*, *Nannocystaceae*, *Shewanellaceae*, *Thermomonosporaceae* and *Flavobacteriaceae* families were reported only in the CMA group.¹⁷ In contrast, the *Bifidobacterium bifidum* species¹⁹ and the *Methylophilaceae* and *Dietziaceae* families were exclusively detected in the HC.¹⁷

Metabolome modifications

Decreased total short chain fatty acid (SCFAs),^{14,17} along with increased butyrate and total branched-chain short fatty acids (BCSFAs),¹⁵ were reported in CMA-groups. Besides, lower pyruvate, lactate, threonine and proline, along with higher total esters, ketones, alcohol aldehydes, uridine, histidine, tyrosine, trimethylamine-N-oxide (TMAO) and arginine/histidine,¹⁴ and elevated organic acids were reported in CMA-groups.²³

Metabolome-microbiome associations

Two studies examined the association between the GM and the metabolome.^{15,17} Positive correlations were found between the *Clostridium* genus and butyrate, the *Clostridium coccoides* species and BCSFAs, and the *Bacteroides* genus and propionate.¹⁵ Isocaproate and BCSFAs were negatively related with the *Bifidobacterium* genus.¹⁵ Additionally, lactate was found to be negatively correlated with *Bacteroides* genus¹⁷ and *Clostridium coccoides* species,¹⁵ but positively correlated with *Bifidobacterium* genus.¹⁵

Chapter IV

Table1. Human case and case-control studies in infants/children. Abbreviations: see Supplementary Excel file.

Age years (y); months (m)	Analytical techniques	Analytical data	Sample size (CMA/control)	Results:		Reference
				Increase	Decrease	
2-12 m	Bacterial culture (CFU)	Microbiome	46/46	Baseline: Total bacteria count, Anaerobic bacteria After 6 months: Anaerobes count, Lactobacilli count and proportion	Baseline: Yeast count After 6 months: Bifidobacteria count and proportion, Enterobacteria proportion, Yeast proportion	Thompson-Chagoyan <i>et al.</i> ¹⁸
0.55 ± 0.20 y	GC-MS	Metabolomics	16/16	beta-hydroxybutyrate, adipate, isocitrate, homovanillate, suberate, tartarate, 3-indoleacetate, 5-hydroxyindoleacetate	Not reported	#Salmi <i>et al.</i> ²³
2-12 m	FISH-FC (16S rRNA gene specific probes); GC-FID	Microbiome, Metabolomics	46/46	<i>Clostridium coccoides</i> group, <i>Atopobium</i> cluster, butyrate, BCSFA	Not reported	Thompson-Chagoyan <i>et al.</i> ¹⁵
6.5-10.4 m	FISH (16S rRNA gene specific probes); GC-MS; NMR;	Microbiome, Metabolomics	18/18	<i>Bacteroides</i> , <i>Clostridium</i> , Total esters, ketones, alcohols, aldehydes; Uridine, histidine, tyrosine, TMAO, arginine/histidine	Bifidobacteria, Total SCFAs (major difference: acetate and butyrate), Pyruvate, Lactic acid, threonine, proline	Francavilla <i>et al.</i> ¹⁴
5-8 y	PCR-DGGE (V3 regions + 16S rRNA gene- specific primers)	Microbiome	12/12	GM α -diversity (Shannon diversity), <i>C. coccoides</i> diversity (Shannon diversity), <i>Bacteroides</i> , <i>Clostridium</i> , <i>Escherichia coli</i> ; only detected in CMA group: <i>C. celerecrescens</i>	<i>Bifidobacterium (B.)</i> diversity (Shannon diversity), <i>B. adolescent</i> , <i>B. longum</i> , <i>B. catenulatum</i> , and <i>B. breve</i> Only detected in control group: <i>B. bifidum</i>	Guo <i>et al.</i> ¹⁹

Table 1. Human case and case-control studies in infants/children. Abbreviations: see Supplementary Excel file.(Continued)

Age years (y), months (m)	Analytical techniques	Analytical data	Sample size (CMA/control)	Results: modifications in case versus control (case-control study) modifications in allergic versus tolerant (case study)		Reference
				Increase	Decrease	
1-12 m	qPCR- 16S rRNA (V4 re- gion), GC-FID	Microbiome	19/20	GM α -diversity (Shannon diversity), Gut microbiota evenness (Pielou's evenness), <i>Ruminococcaceae</i> , <i>Lachnospiraceae</i> , <i>Ruminococcus</i> , <i>Faecalibacterium</i>	<i>Bifidobacteriaceae</i> , <i>Streptococcaceae</i> , <i>Enterobacteriaceae</i> , <i>Enterococcaceae</i> , <i>Bifidobacterium</i> , <i>Escherichia</i>	Canani <i>et al.</i> ¹⁶
5-8 y	PCR-16s rRNA (V3-V4 regions), HPLC-UV	Microbiome, Metabolomics	6/8	Firmicutes, Clostridia, <i>Ruminococcaceae</i> , <i>Subdoligranulum</i> only detected in CMA group: <i>Burkholderiaceae</i> , <i>Nannocystaceae</i> , <i>She- wanellaceae</i> , <i>Thermomonosporaceae</i> , <i>Fla- vobacteriaceae</i>	Proteobacteria only detected in control group: <i>Methylophilaceae</i> , <i>Dietziaceae</i> , Total SCFAs	Dong <i>et al.</i> ¹⁷
10-15 m	PCR- 16S-rRNA (V3-V4 regions), qRT-PCR	Microbiome	14/14	Firmicutes, <i>Haemophilus</i> , <i>Actinobacillus</i> , <i>Prevotella</i> , <i>Klebsiella</i>	Verrucomicrobia, <i>Parabacteroides</i> , <i>Granulicatella</i>	Mennini <i>et al.</i> ²¹
4-6 m	16S-rRNA (V3-V4 regions)	Microbiome	16/34	Not reported	GM α -diversity (Shannon diversity), <i>Acidaminococcaceae</i> , <i>Prevotellaceae</i>	Mera-Berriatua <i>et al.</i> ²⁰
3-16 m	16S-rRNA (V4 re- gion)	Microbiome	226/- (3-6m: 29/-)	Fecal microbiome at 3-6 month: <i>Bacteroidetes</i> , <i>Enterobacter</i> Metagenome functional enrichment of fatty acid metabolism.	Fecal microbiome at 3-6 month: Clostridia, Firmicutes.	Bunyavanich <i>et al.</i> ²²

#AEDS as basic disease for subjects in both case and control group, and the age is calculated by the pooled mean and sd from the age groups provided in the article

3.2.1.2 Intervention studies

Eight intervention studies for CMA treatment were included (Table2).^{14,16,18,21,23–26} Two examined the GM and metabolome,^{14,16} one the GM and immune response,²⁶ four the GM,^{18,21,24,25} and one the metabolome.²³ The interventions varied across studies, including synbiotics,²⁵ prebiotics,²⁴ probiotics (species of the genus *Bifidobacterium*,^{21,26} *Lactobacillus rhamnosus* GG (LGG) species^{16,23}) and different formula types.^{14,18}

GM modifications

The GM profile was identified with bacteria culture,¹⁸ FISH,²⁵ 16S rRNA gene sequencing with specific primers/probes^{14,24,26} or targeting the V4¹⁶ or V3-V4 regions.²¹ Alterations of the phylum Firmicutes in CMA-patients were described in five intervention studies, involving treatment with EHF,¹⁸ lactose-supplemented EHF,¹⁴ LGG,¹⁶ species and strains from the *Bifidobacterium* genus.^{21,26} These interventions raised Firmicutes phylum members, including the Turicibacterales order,⁴⁸ the *Lactobacillaceae* and *Lachnospiraceae* families⁴⁸ and the genera like *Lactobacillus*,^{18,48} *Blautia*,^{16,21} *Roseburia*,¹⁶ *Coprococcus*,¹⁶ *Anaerofustis*,¹⁶ *Ruminococcus*,^{21,26} *Turicibacter*²⁶ and *Oscillospira*.²⁶ Conversely, some Firmicutes phylum members, including the Clostridia class,¹⁴ *Christensenellaceae* family⁴⁸ and genera like *Enterococcus*, *Streptococcus*,²¹ *Anaerovibrio*, *Oscillibacter*, *Bilophila*, *Dorea* and *Roseburia*²⁶ decreased under treatments.

The interventions also affected the Proteobacteria phylum²¹ and its members. The Betaproteobacteria class, the Burkholderiales order, the *Alcalligenaceae* family and the *Sutterella* genus increased in the treated group,²⁶ while some studies reported decreased levels of the Deltaproteobacteria class,²⁶ the *Enterobacteriaceae* family¹⁸ and the *Sutterella* genus.²¹ In the Bacteroidetes phylum, studies reported the interventions increased levels of the *Porphyromondaceae* family²⁶ and the *Prevotella* genus,^{21,26} and reduced levels of the *Bacteroides* and *Prevotella* genera.¹⁴ Additionally, the Actinobacteria phylum also underwent changes with interventions.^{14,18,21,25,26} The use of probiotic *Bifidobacterium* strains consistently elevated the *Bifidobacterium* genus.^{21,25,26} Increased *Bifidobacterium* were also noticed after lactose-supplemented EHF diet.¹⁴ In

contrast, the Actinobacteria phylum²¹ and its members, the genera *Bifidobacterium*,¹⁸ *Atopobium*,²¹ and *Actinomyces*,^{21,26} were decreased by the treatments. The Verrucomicrobia phylum and its *Akkermansia* genus were found increased in the treatment group.²¹

In addition to the taxonomy changes, enhanced α -diversity (chao1, observed species),²⁶ reduced total bacteria²⁴ and a decreased ratio of the *Eubacterium rectale/Clostridium coccoides* species²⁵ were reported after probiotics, pectin-based thickened AAF and synbiotics treatments, respectively.

Metabolome modifications

After the LGG-supplemented hydrolyzed whey formula (HWF) diet, CMA-patients showed increased kynurename and decreased 3-indoleacetate.²³ Additionally, butyrate increased in LGG-supplemented extensively hydrolyzed casein (EHC) formula treated CMA-patients.¹⁶ Meanwhile, lactose-supplemented EHF raised SCFAs, lactate, threonine, uridine, histidine, tyrosine, methionine, TMAO, phenylalanine, arginine/histidine and gamma–amino–butyrate/lysine, and lowered the total esters, ketones, alcohols, aldehydes and valine/isoleucine in CMA-patients.¹⁴

Immune response

The single intervention study reporting findings on the immune response showed that *Bifidobacterium bifidum* reduced allergy symptoms, lowered serum IgE and raised IgG₂ levels in CMA-patients.²⁶ The IgG₂ and IgE were respectively positively and negatively correlated with GM α -diversity (Chao1 index, observed species, community diversity index, Shannon index). The intervention decreased the pro-inflammatory cytokines TNF α , IL-1 β and IL-6 and increased the anti-inflammatory cytokine IL-10 as well.²⁶

CMA outcome

Four out of eight intervention studies discussed CMA tolerance or allergic symptoms improvement between treatment and control.^{16,24–26} Two studies noted significant improvement in allergic symptoms after treatment,^{24,26} and one reported five out of 12 infants in the treated group outgrew CMA after six months, compared to none in the control group.¹⁶

Chapter IV

Table 2. Characteristics of studies that compare CMA infants/children before and after intervention (intervention study). Abbreviations: see Supplementary Excel file.

Age years (y); months (m)	Analytical techniques	Analytical data	sample size (treatment/ control)	Intervention detail				Results: modifications in treatment versus control		Reference
				Duration (months)	Comparison groups	Control diet (Basic for- mula (BF))	Treatment diet (BF + interven- tion)	Increase	Decrease	
0.55 ± 0.20 y	GC-MS	Metabolomics	9/5	1	Treatment vs control	HWF	HWF with LGG	Kynureneate	3-indoleacetate	Salmi <i>et al.</i> ²³
2-12 m	Bacteria cul- ture (CFU)	Microbiome	46/46	6	CMA sub- jects before inter- vention	-	EHF	<i>Lactobacilli</i>	Enterobacteria Bifidobacteria	Thompson- Chagoyan <i>et al.</i> ¹⁸
6.5-10.4 m	FISH (16S rRNA-spe- cific probes), GC-MS, NMR;	Microbiome, Metabolomics	16/16	2	CMA sub- jects before inter- vention	-	EHF with 3.8% lactose	<i>Bifidobacteria</i> . LAB, SCFAs, lactate, threonine, uridine, histidine, tyrosine, methionine, TMAO, Phenylalanine, arginine/his- tidine, c-amino-butrate/lysine,	<i>Atopobium</i> , <i>Bac- teroides/Prevotella</i> , clos- tridia and sulfate-reduc- ing bacteria, Total esters, ketones, alcohols, aldehydes, Valine/isoleucine	Francavilla <i>et al.</i> ¹⁴
6.2 ± 4.3 m	qPCR (16S rRNA- spe- cific primers and probes)	Microbiome	23/17	3	Treatment vs control	RAAF	TAAF	Not reported	Total bacteria count	Dupont <i>et al.</i> ²⁴
1-12 m	qPCR- 16S rRNA (V4 region), GC-FID	Microbiome; Metabolomics	12/7	6	Treatment vs control, CMA sub- jects before intervention	EHC formula	EHC formula with LGG	After vs before intervention: <i>Blautia</i> , <i>Roseburia</i> , <i>Coprococcus</i> , Compared to control group: <i>Roseburia</i> , <i>An- aerofustis</i> . Butyrate	Not observed	Canani <i>et al.</i> ¹⁶

Table 2. Characteristics of studies that compare CMA infants/children before and after intervention (intervention study). Abbreviations: see Supplementary Excel file. (Continued)

Age years (y); months (m)	Analytical techniques	Analytical data	sample size (treatment/ control)	Intervention detail				Results: modifications in treatment versus control		Reference
				Duration (months)	Comparison groups	Control diet (Basic for- mula(BF))	Treatment diet (BF + interven- tion)	Increase	Decrease	
0.5-12 m	ELISA qPCR (16S rRNA- specific primers)	Microbiome, Immune response	123/121	6	Treatment vs control	-	<i>Bifidobacterium bifidum</i> TMC3115	After 6 months: IL-10, total IgG ₂ , GM α -diversity (chaol in- dex, observed species), <i>Bifidobacteriales</i> , <i>Bifidobacterium</i> , <i>Lactobacillaceae</i> <i>Lactobaci- llus</i> , <i>Turicibacter</i> , <i>Turicibacterales</i> , <i>Betaproteobacteria</i> , <i>Suttere- lla</i> , <i>Burkholderiales</i> , <i>Alcalligenaceae</i> , <i>Porphyromonadaceae</i> , <i>Parabacteroides</i> , <i>Ruminococcus</i> , <i>Oscillospira</i> , <i>Lachnospira</i>	After 6 months: TNF α , IL-1, IL-6, IL-10, total IgE, <i>Anaerovibrio</i> , <i>Christen- senelaceae</i> , <i>Oscillibacter</i> , <i>Bilophila</i> , <i>Dorea</i> <i>Rosebu- ria</i>) Desulfovibrionales, Deltaproteobacteria, Proteobacteria, <i>Actino- myces</i>)	Jing <i>et al.</i> ²⁶
10-15 m	PCR- 16S rRNA (V3-V4 re- gions), qRT-PCR	Microbiome	14/14	1	CMA subjects before inter- vention	-	probiotic mix: <i>Bifidobacterium breve</i> M-16V, <i>Bifidobacterium longum</i> subsp. <i>longum</i> BB536, <i>Bifidobacterium longum</i> subsp. <i>Infantis</i> M-63	<i>Verrucomicrobia</i> , Proteobacteria, <i>Akkermansia</i> , <i>Prevotella</i> , <i>Ruminococcus</i> , <i>Blautia</i> , <i>Bifidobacterium longum</i> sub- species <i>infantis</i>	Actinobacteria, <i>Actinomyces</i> , <i>Enterococcus</i> , <i>Streptococcus</i> , <i>Sutterella</i>	Mennini <i>et al.</i> ²¹
<13 m	FISH (16S rRNA s-spe- cific probes)	Microbiome	80/89	12	Treatment VS control	AAF	synbiotics: oligosaccharides (oligofructose, in- ulin), <i>Bifidobacte- rium breve</i> M- 16V	After 6 and 12 month: bifidobacteria	After 6 month: ER/CC	Chatchatee <i>et al.</i> ²⁵

3.2.2 Animal studies

The animal studies include two studies on the GM, metabolome and immune response,^{27,28} four on the GM and immune response²⁹⁻³² and two on the metabolome and immune response^{33,34} (Table 3). All animal models were on mice, details are provided in Tables S4 and S5.

GM modifications

Three interventions,^{28,31,32} two case-controls^{27,30} and one FT²⁹ study reported GM modifications. Bacteria were identified using 16S rRNA gene-targeted primers, which targeted group/species-specific bacteria³¹ or certain hypervariable regions (V3-V4,^{27,28,32} V4²⁹ and eight other regions³⁰).

In two studies comparing GM changes between CMA- and sham mice,^{27,30} one observed increased Simpson α -diversity in CMA-male-C57BL/6J mice but decreased Simpson and Shannon α -diversity in CMA-female-BALB/cJ mice.³⁰ Regardless of the strain and gender, the β -diversity (Bray-Curtis) was significant different between the two groups.³⁰ Apart from the gender and strain-specific α -diversity difference, CMA-mice showed enrichment in the phyla Bacteroidetes and Patescibacteria (female-C57BL/6J) but reduction in the phyla Verrucomicrobia, Proteobacteria (male-C57BL/6J) and Actinobacteria (female-C57BL/6J).³⁰ Compared to mice colonized with feces from healthy children (healthy-colonized mice), a FT study reported that mice with feces from CMA children (CMA-colonized mice) had higher abundances of the Clostridiales order and the *Clostridiaceae*, *Ruminococcaaceae* and *Barnesiellaceae* families, along with lower levels of the *Lachnospiraceae*, *Erysipelotrichaceae* and *Enterobacteriaceae* families.²⁹ At the genus level, the CMA-mice exhibited higher *Barnesiella* and *Clostridium_XIVa*,²⁷ and CMA-colonized mice had enhanced *Enterococcus*, *Ruminococcus*, *Coprobacillus*, *Blautia* and *Parabacteroides*.²⁹ In contrast, the *Lactobacillus*, *Parvibacter*,²⁷ *Streptococcus*, and *Salmonella*²⁹ genera, as well as *Anaerostipes caccae* species²⁹ decreased in CMA and CMA-colonized mice. Additionally, the *Bosea* genus was absent in CMA-mice.²⁷

Species and strains of the *Lactobacillus* and *Bifidobacterium* genera were used as probiotic in CMA-mouse models.^{28,31} One study reported that five out of six probiotic strains reduced the total bacteria.³¹ Another found significant differences in GM β -diversity (Bray-Curtis, UniFrac) between control and treated groups but only the *Lactobacillus rhamnosus* species increased GM richness.²⁸ At the family level, it was reported that *Prevotellaceae* and *Marinifilaceae* increased, whereas *Helicobacteraceae*, *Lachnospiraceae*, *Deferrribacteraceae*, *Clostridiaceae*, *Peptococcaceae* and *Burkholderiaceae* decreased after taking at least one probiotic.²⁸ Interestingly, the *Ruminococcaceae* family increased with *Lactobacillus rhamnosus* treatment but decreased with *Bifidobacterium longum* subsp. *infantis* treatment.²⁸ Furthermore, one study found that probiotic treatments with *Lactobacillus rhamnosus* and *Bifidobacterium animalis* subspecies *lactis* increased the *Clostridium* cluster IVa genus and the *Clostridium leptum* species.³¹ Conversely, more than three probiotic strains decreased the *Lactobacillus*, *Clostridium* cluster I/II, *Clostridium* cluster XI, *Enterococcus* and *Prevotella* genera, as well as the *Clostridium Coccoides* and *Clostridium Leptum* species.³¹ Additionally, it was reported that prebiotic administration with partially hydrolyzed whey reduced the *Lactobacillus* genus and increased the *Prevotella* genus.³²

Metabolome modifications

Two studies examined fecal SCFAs in CMA-mice with and without synbiotic intervention.^{33,34} They reported enhanced acetate³³, butyrate³³ and propionate³⁴ with synbiotic diet. However, one study only observed reduced kynurenine and N-acetylkynurenine in probiotic-treated mice.²⁸ Additionally, a FT study compared ileal transcription signatures between CMA and healthy-colonized mice.²⁹ They found upregulated metabolism of monocarboxylic acid, arachidonic acid, linoleic acid and pyruvate in CMA-colonized mice, while increased carbohydrate metabolic process in healthy-colonized mice.²⁹

CMA outcome and immune response

Among all animal studies only Feehley *et al.*²⁹ and Kostadinova *et al.*³⁴ correlated the immune response to the GM. Feehley *et al.*²⁹ reported that growth factor TGF- β receptor

and ROR2 genes in CMA-colonized mice was positively correlated with *Lachnospiraceae* family.²⁹ Meanwhile, Kostadinova *et al.*³⁴ showed that propionate was positively correlated with FOXP3+ cell frequency in the colon.³⁴

All intervention studies reported immune response data which relates to the treatment outcome.^{28,31-34} Unlike post-sensitization,²⁸ pre-sensitization³¹ intake of *Lactobacillus salivarius*, *Lactobacillus rhamnosus* and *Bifidobacterium longum subspecies infantis* successfully lowered the mast cells degranulation marker mucosal mast cell protease-1 (mMCP-1)³⁵ and BLG-specific IgE.³¹ All strains lowered the IL-4 secretion and the BLG-specific sIgG₁-to-sIgG_{2a} ratio³¹ which indicates the overall T_h2-to-T_h1 response.³⁶ The rest of the responses were strain-dependent. *Lactobacillus rhamnosus* and *Bifidobacterium longum subspecies infantis* increased T_h1 IFN- γ and T_{reg} IL-10 secretion in stimulated splenocytes, whereas *Lactobacillus salivarius* declined IFN- γ secretion.³¹ Post-challenge administration of those probiotic strains predominantly induced regulatory response.²⁸ All strains significantly increased TGF- β expression, while *Lactobacillus rhamnosus* and *Lactobacillus salivarius* interventions also increased FOXP3 and IL-10 expression. The post-sensitization intake resulted in overall cytokine suppression as well. The reduction in granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN- γ , IL-2, and IL-4 was common among the strains, while IL12p70, IL-10, IL-5 and IL-17A was strain-dependent.²⁸

Kostadinova *et al.*^{33,34} reported that synbiotic intake alone did not alleviate the acute allergic skin response but its combination with T cell-epitope-containing BLG peptides (PepMix) did.^{33,34} Notably, the combined diet reestablished the lost T_h1/T_h2 balance as evidenced by the lymphocyte distribution in the small intestine lamina propria³³ as well as the increased transcription factor (Tbet/GATA3) and cytokine (IFN- γ /IL-13) gene expression in the Peyer's Patches (PP).³⁴ Right after the intervention the immune response was predominantly regulatory. It was characterized by an increase in the mRNA expression of FOXP3 over the GATA3 and ROR γ T in the PP, as well as higher FOXP3+ over GATA3+ and T_{reg} over T_h cell frequencies in mesenteric lymph node.³⁴ Synbiotic addition had a site-dependent effect on IL-22 mRNA expression and also silenced the whey-stimulated splenocyte secretion of cytokines (IL-10, IL-5, IL-13, IL-

17A, IFN- γ) which were induced by the PepMix intake.³³ Kleinjans *et al* showed that the effect of prebiotics on allergic symptoms varied with the composition and treatment duration.³²

Table 3. CMA intervention studies with animal models. Abbreviations: see Supplementary Excel file.

Groups		Platforms	Results: [‡]		Reference
Case/Intervention	Control		Microbiome/Metabolome	CMA outcome & Immune response	
G1: <i>L. rhamnosus</i> G2: <i>B. longum</i> subsp. <i>Infantis</i> G3: <i>L. salivarius</i> G4: <i>B. bifidum</i> G5: <i>L. gasseri</i> G6: <i>B. animalis</i> subsp. <i>lactis</i>		AC: PBS	Microbiome Total bacteria ↓ G1, G2, G3, G4, G5 <i>Clostridium</i> cluster IVa ↑ G1, G6 <i>Staphylococci</i> abundance ↑ G1 <i>C. leptum</i> ↑ G1, G6 <i>Prevotella</i> ↑ G6 <i>C. leptum</i> ↓ G2, G3, G4, G5 <i>Prevotella</i> ↓ G2, G3, G4, G5 <i>Lactobacillus</i> ↓ G2, G3, G4, G5 <i>Clostridium</i> cluster I/II ↓ G2, G3, G5 <i>Clostridium</i> cluster XI ↓ G2, G3, G4 <i>C. coccoides</i> ↓ G2, G3, G4, G5 <i>Enterococcus</i> ↓ G2, G3, G4, G5 <i>Enterococcus</i> ↓ G1	Allergy markers mMCP-1 ↓ G1, G2, G3 Immunoglobulins BLG-sIgE ↓ G1, G2, G3 BLG-sIgG ₁ /sIgG _{2a} ↑ G1, G2, G3, G4, G6 Cytokines IL-4 ↓ G1, G2, G3, G4 (spleen, MLN) IFN-γ ↑ G1, G2, G6 (spleen) IFN-γ ↓ G3, G4 (spleen) IFN-γ ↑ G6 (MLN) IL-10 ↑ G1, G2, G6 (spleen) IL-10 ↑ G1, G5, G6 (MLN) mRNA expression IL-4 ↓ G2 IL-10, GATA3, ROR γ T ↓ G2, G3 FOXP3 ↑ G2, G3 IL-17a ↑ G1, G2, G3	Neau <i>et al.</i> ³¹
G1: <i>L. rhamnosus</i> G2: <i>B. longum</i> subsp. <i>Infantis</i> G3: <i>L. salivarius</i>		AC: PBS	Microbiome PCR - 16S rRNA (V3-V4 regions) Kynurenone, N-acetylkynurenone ↓ G1, G2, G3 Microbiome Richness (OTU number) ↑ G1 Beta diversity ↑ G1, G2, G3 <i>Prevotellaceae</i> ↑ G1, G2, G3 <i>Marinililaceae</i> ↑ G1, G2 <i>Ruminococcaceae</i> ↑ G1 <i>Helicobacteraceae</i> ↓ G1 <i>Ruminococcaceae</i> ↓ G2 <i>Lachnospiraceae</i> ↓ G1, G2, G3 <i>Deferrribacteraceae</i> ↓ G1, G2 <i>Clostridiaceae</i> ↓ G1 <i>Peptococcaceae</i> ↓ G1, G3 <i>Burkholderiaceae</i> ↓ G1 <i>Anaeroplasmataceae</i> ↓ G2	Cytokines GM-CSF, IL-2, IFN-γ, IL-4 ↓ G1, G2, G3 IL12p70 and IL10 ↓ G1 IL-5 ↓ G2, G3 IL17A ↓ G1, G3 mRNA expression FOXP3, IL-10 ↑ for G1 and G3 TGFβ ↑ G1, G2, G3	Esber <i>et al.</i> ²⁸
G1: pWH G2/G3: pWH + short(G2)/long (G3) scGOS/IcFOS (9:1) G4/G5: pWH + short (G4)/long (G5) scGOS/IcFOS (9:1) + pAOS	TC: W AC: PBS	Microbiota PCR (16S rRNA V3-V4 regions) Immunoglobulins ELISA	Microbiome <i>Prevotella</i> ↑ G3, G4, G5 vs G1 <i>Lactobacillus</i> ↓ G5 vs G1	Allergy markers mMCP-1 ↓ G1, G5 vs AC TSLP ↓ G1 vs AC AASR ↓ TC, G1, G2, G4, G5 vs AC SAS & body-T ↓ TC, G2 vs AC	Kleinjans <i>et al.</i> ³²
G1: mix of W peptides (PepMix) G2: scFOS and IcFOS (9:1) + <i>B. breve</i> M-16V (FF/Bb) G3: PepMix + FF/Bb	TC: W AC: PBS	Immunoglobulins ELISA Metabolites GC-FID Lymphocytes FC Cytokines IA (ex-W)	Metabolites acetate, butyrate ↑ G2 butyrate ↑ G2 vs G3, TC vs AC	Allergy markers AASR ↓ G3, TC vs AC SAS ↓ TC vs AC Lymphocytes (SI-LP) T _h 1/T _h 2 ↑ G3, TC T _{reg} , T _h 17 ↑ AC vs TC Cytokines (spleen) IFN-γ, IL-17A, IL-13, IL-5, IL-10 ↓ G3 vs G1 & TC vs AC IL-10 ↑ G3	Kostadinova <i>et al.</i> ³³

Fecal metabolome exploration in infants with CMA

Table 3. CMA intervention studies with animal models. Abbreviations: see Supplementary Excel file.(Continued)

Groups		Platforms	Microbiome/Metabolome	Results‡	Reference
Case/Intervention	Cotrol			CMA outcome & Immune response	
G1: mix of W peptides (PepMix) G2: scFOS and lcFOS (9:1) + <i>B. breve</i> M-16V (FF/Bb) G3: PepMix + FF/Bb	TC: W AC: PBS	Metabolites GC-FID Lymphocytes FC mRNA expression qPCR Immunohisto-chemistry	<u>Part 1: Post-oral tolerance</u> Metabolites butyrate ↑ G3 vs G1 propionate ↑ TC, G2, G3 vs AC Positive correlation: propionate and FOXP3+ (colon)	Allergy markers AASR ↓ G3, TC vs AC AASR ↑ G1, G2 vs G3 SAS ↓ TC vs AC <u>Part 1: Post-oral tolerance</u> Lymphocytes FOXP3+/GATA3+, T_{reg}/T_{effs} ↑ G3 vs AC, G3 vs G2, G3 vs G1 (MLN) T_{reg} ↓ G3 vs AC, G3 vs G2, TC vs AC (spleen) CD25+ ↓ G3 vs G2 DC (SI-LP) CD8α-CD11b+/CD8α+CD11b-, CD11b+CD103+ ↑ G3 CD8α+CD11b- ↓ G1 mRNA expression FOXP3/GATA3 ↑ G3 (PP) FOXP3/ROR γ T ↑ G3 vs AC, G3 vs G2, G3 vs G1 (PP) TGF- β ↑ G3 vs G2 (proximal SI) TGF- β ↓ G1 (colon) IL-22 ↑ G3 vs AC, G3 vs G1 (PP) IL-22 ↑ for G3 vs G1 (middle SI) IL-22 ↑ G2 vs AC & G2 vs G3 (colon) Galectin 9 ↓ TC Tbet/GATA3 ↓ G1 vs AC, G1 vs G3 (colon)	Kostadina <i>et al.</i> ³⁴

Table 3. CMA intervention studies with animal models. Abbreviations: see Supplementary Excel file.(Continued)

Groups		Platforms	Results‡		Reference
Case/Intervention	Control		Microbiome/Metabolome	CMA outcome & Immune response	
G1: M-C57BL/6J G2: M BALB/cJ G3: F-C57BL/6J G4: F-BALB/cJ	S: sham control (sex and strain matched to G1, G2, G3, G4 separately)	Immunoglobulins ELISA Cytokines, chemokines, and acute phase proteins: IA Microbiota 16S rRNA sequencing (8 regions)	Microbiome α-diversity ↑ G4 (Simpson and Shannon indices) α-diversity ↓ G1 (Simpson index) Immunoglobulins sIgE ↑ G2 vs S, G4 vs S, G4 vs G3 sIgG ₁ ↑ G2 vs S, G2 vs G1, G4 vs S, G4 vs G3 sIgG _{2a} ↑ G2 vs S, G2 vs G1, G4 vs S, G4 vs G3 Cytokines, chemokines, and acute phase proteins: G1 vs S: ↑ in CCL1, CSF1, IL-13, CCL17, IL-21, FGF2, CCL12, IL-10, CCL9 G2 vs S: ↓ IL-1β, IL-13, CSF2, TNFRSF1A G4 vs S: ↑ IL-15, TNFRSF1B, ICAM-1	Allergy markers Body-T ↓ G2 vs S, G4 vs S, G4 vs G3 SAS ↑ G2 vs S, G4 vs S, G4 vs G3 Immunoglobulins sIgE ↑ G2 vs S, G1 vs S, G4 vs S, G4 vs G3 sIgG ₁ ↑ G2 vs S, G2 vs G1, G4 vs S, G4 vs G3 sIgG _{2a} ↑ G2 vs S, G2 vs G1, G4 vs S, G4 vs G3 Cytokines, chemokines, and acute phase proteins: G1 vs S: ↑ in CCL1, CSF1, IL-13, CCL17, IL-21, FGF2, CCL12, IL-10, CCL9 G2 vs S: ↓ IL-1β, IL-13, CSF2, TNFRSF1A G4 vs S: ↑ IL-15, TNFRSF1B, ICAM-1	Smith <i>et al.</i> ³⁰
G1: CMA	S: Sham control	Microbiome PCR-16S rRNA (V3-V4 regions) Immunoglobulins ELISA Cytokines ELISA mRNA expression qPCR Metabolome GC-FID, RP, HILIC-MS/MS	Microbiome <i>Barnesiella</i> ↑ <i>Clostridium_XIVa</i> ↑ <i>Lactobacillus</i> ↓ <i>Parvibacter</i> ↓ Only observed in sham mice: <i>Bosea</i>	Allergy markers Body-T ↓ G1 vs S SAS ↑ G1 vs S Histamine ↑ G1 vs S mMCP-1 ↑ G1 vs S Immunoglobulins whey-sIgE, sIgG ₁ , sIgG _{2a} ↑ G1 vs S Cytokines IL-6, IL-10 ↑ G1 vs S mRNA expression IL-8, IL-33, mTOR mRNA ↑ G1 vs S	Cao <i>et al.</i> ²⁷
G1: CMA-FT G2: <i>Anaerostipes caccae</i>-FT	B-HC: breast-fed HC-FT F-HC: formula-fed HC-FT	Microbiome PCR - 16S rRNA (V4 region) Immunoglobulins ELISA Transcriptome RNA-seq, qPCR	After fecal colonization before sensitization: Microbiome G1 vs F-HC: <i>Enterococcus</i> ↑ <i>Barnesiellaceae</i> ↑ <i>Ruminococcus</i> ↑ <i>Ruminococcaceae</i> ↑ <i>Coprobacillus</i> ↑	Allergy markers mMCP-1 ↑ G1, G4 vs HC mMCP-1 ↓ G2 vs G1 Immunoglobulins BLG-specific IgE, IgG1 ↑ G1 vs HC Cytokines IL-13, IL-4 ↑ G1 vs G2	Feehley <i>et al.</i> ²⁹

Fecal metabolome exploration in infants with CMA

			<p><i>Clostridiaceae</i> ↑</p> <p><i>Clostridiales</i> ↑</p> <p><i>Blautia</i> ↑</p> <p><i>Parabacteroides</i> ↑</p> <p><i>Lachnospiraceae</i> ↓</p> <p><i>Erysipelotrichaceae</i> ↓</p> <p><i>Enterobacteriaceae</i> ↓</p> <p><i>Streptococcus</i> ↓ <i>Enterobacteriaceae</i> ↓</p> <p><i>Salmonella</i> ↓ <i>Anaerostipes caccae</i> ↓</p>	<p>Transcriptome</p> <p><i>Tgfbr3</i> ↓ G1 vs G2, G1 vs HC</p> <p><i>Ror2</i> ↓ G1, G2 vs HC</p> <p><i>Ror2</i>, <i>Tgfbr3</i> positively correlated to <i>Lachnospiraceae</i></p>	
--	--	--	-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	--

‡All results are vs AC or C or S unless state otherwise

4 Discussion and Conclusion

In general, no clear conclusion can be drawn about the GM diversity modification in CMA children, because of limited data on β -diversity^{21,30} and discordant results regarding to α -diversity in both human^{16,19,20} and animal³⁰ studies.

Taxonomic findings showed that the *Bifidobacteriaceae* family, including *Bifidobacterium* spp., were consistently reported lower in CMA-children.^{14,16,18,19} This result aligns with the consensus on the protective function of *Bifidobacterium* spp. in early life.^{37,38} Another noteworthy observation concerning GM in CMA-children is the consistent increase of the Firmicutes phylum,^{14–19,21} primarily associated with the Clostridia class. Conversely, decreased levels of bacteria of the Lactobacillales order were observed.^{16,21} The trends of Firmicutes alterations align with the findings of an animal study which reported higher *Clostridium* cluster XIVa and lower *Lactobacillus* genus in CMA-mice.²⁷ However, CMA and healthy-colonized mice were both characterized with bacteria from the Clostridia class, with *Anaerostipes caccae*, a clostridial species, showing protective effects against CMA.²⁹ Additionally, infants who resolved CMA were reported to have enriched Clostridia class at 3–6 months.²² Discordant results have also been reported regarding the protective or detrimental effect of the Clostridia class in food allergy.^{39,40} Therefore, despite the conflicting findings of the Clostridia class in this review, we lean towards suggesting that GM with enriched Clostridia class, reduced Lactobacillales order and reduced *Bifidobacterium* genus is associated with CMA in early-life.

Various intervention approaches, including probiotics, prebiotics and synbiotics, were applied to restore the balance of GM and the metabolome in CMA-children. Elevated *Bifidobacterium* genus was consistently observed post-treatment with *Bifidobacterium* strains as probiotics^{21,25,26} or after lactose-supplemented EHF treatment.¹⁴ However, the impact on the Lactobacillales order in both CMA-children and CMA-mice was less clear. Increased levels of the *Lactobacillaceae* family were reported with *Bifidobacterium*-specific probiotics²⁶ and EHF in CMA-children,¹⁸ while decreased *Enterococcus* and *Streptococcus* genera were noted in *Bifidobacterium*-treated CMA-

children.²¹ Additionally, decreased levels of *Lactobacillus* genus were reported in CMA-mice treated with *Bifidobacterium* and *Lactobacillus*-specific probiotics.^{31,32} Similarly, the effect on the Clostridia class varied. Higher levels of its members were reported in CMA-children and mice treated with probiotics.^{16,21,26,28,31} Meanwhile, reduced Clostridia class members also noted in CMA-children treated with lactose-supplemented EHF or probiotics,^{14,26} and in CMA-mice treated with probiotics.^{28,31} Therefore, it is clear that the enhancement of *Bifidobacterium* after *Bifidobacterium*-specific treatment was commonly reported, however the treatment effect on other bacteria remain inconclusive. Despite the uncertainty of most GM profile modifications, there are studies which reported improved allergic symptoms or a high resolution rate in CMA-children treated with probiotics or prebiotics.^{16,24,26}

In addition to GM modifications, CMA-children were reported to have decreased total SCFAs^{14,16} and altered amino acids and nucleotides levels.^{14,23} These findings are consistent with a recent review on the metabolic changes in children with IgE-mediated food allergies,⁴¹ and these metabolome changes appear to be restored with interventions. Increased SCFAs and balanced amino acids were reported after treatment with LGG or lactose-supplemented EHF.^{14,23} Enhanced levels of acetate,³³ butyrate,^{33,34} and propionate³⁴ were also reported in synbiotic-treated CMA-mice.

This systematic review provides an overview of the modifications of the GM, metabolome, and immune response in IgE-mediated CMA-children and CMA animal models. Comparing microbiome data between studies is challenging due to methodological variations, diverse intervention approaches, and the reporting of different taxonomic levels. Consequently, only general conclusions can be drawn based on family or higher taxonomic levels. Meanwhile, insights into metabolomics are restricted by limited scope of studied metabolites. Thus, future work should examine broader range of metabolites known to be crucial in the crosstalk between the GM and host's immune system^{41,42} and use untargeted metabolomics as hypothesis-generating strategy. Only a single human study reported microbiome and immune response data and their relationship.²⁶ Similarly, only a single animal study correlated transcriptomics and GM data,²⁹ including genes related to the immune response. Therefore, there is a

need for both human and animal studies on the correlation of the GM to the immune response. Future animal studies can build on the general treatment outcome findings in the review, namely overall cytokine silencing,^{28,33} restoration of the T_h2/T_h1 balance,^{31,33,34} and induction of regulatory response.^{28,31,34} Moreover, future work can focus on parameters already connected to allergic tolerance acquisition in human, such as induction of T_{reg} response, the production of TGF- β , IgG4, IgA.⁴³ No proteomics studies met our inclusion criteria, but a study on the fecal microbiome and metaproteome relationships in CMA-children has been published after our inclusion date.⁴⁴ Overall, discussions on multi-omics connections are rare in the reviewed studies, and none of the studies reported shotgun meta-genomics, meta-transcriptomics, or meta-proteomics for microbiome function information. Therefore, there is a clear need for more comprehensive multi-omics studies to gain a better mechanistic understanding of CMA in early life. These efforts would eventually lead to the development of better and effective treatment and preventive strategies.

Acknowledgements

We thank Ria Derkx (Wageningen University library) for her advice on the search strategy. FINANCIAL SUPPORT: This study was part of the EARLYFIT project (Partnership programme NWO Domain AES-Danone Nutricia Research), funded by the Dutch Research Council (NWO) and Danone Nutricia Research (project number: 16490).

Reference:

1. Flom, J. D. & Sicherer, S. H. Epidemiology of Cow's Milk Allergy. *Nutrients* **11**, 1051 (2019).
2. Savage, J. & Johns, C. B. Food Allergy: Epidemiology and Natural History. *Immunol Allergy Clin North Am* **35**, 45–59 (2015).
3. Guidelines for the Diagnosis and Management of Food Allergy in the United States: Report of the NIAID-Sponsored Expert Panel. *Journal of Allergy and Clinical Immunology* **126**, S1–S58 (2010).
4. Høst, A. *et al.* Clinical course of cow's milk protein allergy/intolerance and atopic diseases in childhood. *Pediatric Allergy and Immunology* **13**, 23–28 (2002).
5. Vanto, T. *et al.* Prediction of the development of tolerance to milk in children with cow's milk hypersensitivity. *The Journal of Pediatrics* **144**, 218–222 (2004).
6. Saarinen, K. M., Pelkonen, A. S., Mäkelä, M. J. & Savilahti, E. Clinical course and prognosis of cow's milk allergy are dependent on milk-specific IgE status. *Journal of Allergy and Clinical Immunology* **116**, 869–875 (2005).
7. Schoemaker, A. A. *et al.* Incidence and natural history of challenge-proven cow's milk allergy in European children – EuroPrevall birth cohort. *Allergy* **70**, 963–972 (2015).
8. Hochwallner, H., Schulmeister, U., Swoboda, I., Spitzauer, S. & Valenta, R. Cow's milk allergy: From allergens to new forms of diagnosis, therapy and prevention. *Methods* **66**, 22–33 (2014).
9. Zhao, W., Ho, H. & Bunyavanich, S. The gut microbiome in food allergy. *Annals of Allergy, Asthma & Immunology* **122**, 276–282 (2019).
10. Peroni, D. G., Nuzzi, G., Trambusti, I., Di Cicco, M. E. & Comberiati, P. Microbiome Composition and Its Impact on the Development of Allergic Diseases. *Frontiers in Immunology* **11**, 700 (2020).
11. Shi, J., Wang, Y., Cheng, L., Wang, J. & Raghavan, V. Gut microbiome modulation by probiotics, prebiotics, synbiotics and postbiotics: a novel strategy in food allergy prevention and treatment. *Critical Reviews in Food Science and Nutrition* **1–17** (2022) doi:10.1080/10408398.2022.2160962.
12. Bunyavanich, S. & Berin, M. C. Food allergy and the microbiome: Current understandings and future directions. *Journal of Allergy and Clinical Immunology* **144**, 1468–1477 (2019).
13. Page, M. J. *et al.* The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* **372**, n71 (2021).
14. Francavilla, R. *et al.* Effect of lactose on gut microbiota and metabolome of infants with cow's milk allergy. *Pediatric Allergy and Immunology* **23**, 420–427 (2012).
15. Thompson-Chagoyan, O. C. *et al.* Faecal microbiota and short-chain fatty acid levels in faeces from infants with cow's milk protein allergy. *International Archives of Allergy and Immunology* **156**, 325–332 (2011).
16. Canani, R. B. *et al.* Lactobacillus rhamnosus GG-supplemented formula expands butyrate-producing bacterial strains in food allergic infants. *The ISME Journal* **10**, 742–750 (2016).
17. Dong, Y., Fei, P., Han, Y. & Guo, L. Characterization of Fecal Microbiota, Short-Chain Fatty Acids and Lactic Acid Concentrations in 5-8-Year-Old Children with Cow Milk Protein Allergy. *28*, 64638 (2018).
18. Thompson-Chagoyan, O. C., Vieites, J. M., Maldonado, J., Edwards, C. & Gil, A. Changes in faecal microbiota of infants with cow's milk protein allergy - A Spanish prospective case-control 6-month follow-up study. *Pediatric Allergy and Immunology* **21**, e394–e400 (2010).
19. Guo, L. *et al.* Comparative Analysis of Fecal Microbiota in 5-8-Year-old Children with and without Cow Milk Protein Allergy. *26*, 6397 (2016).
20. Mera-Berriatua, L. *et al.* Unravelling the Gut Microbiota of Cow's Milk–Allergic Infants, Their Mothers, and Their Grandmothers. *Journal of Investigational Allergology and Clinical Immunology* **32**, 395–398 (2022).

Chapter IV

21. Mennini, M. *et al.* Gut Microbiota Profile in Children with IgE-Mediated Cow's Milk Allergy and Cow's Milk Sensitization and Probiotic Intestinal Persistence Evaluation. *International Journal of Molecular Sciences Article* (2021) doi:10.3390/ijms22041649.
22. Bunyavanich, S. *et al.* Early-life gut microbiome composition and milk allergy resolution. *Journal of Allergy and Clinical Immunology* **138**, 1122–1130 (2016).
23. Salmi, H., Kuitunen, M., Viljanen, M. & Lapatto, R. Cow's milk allergy is associated with changes in urinary organic acid concentrations. *Pediatric Allergy and Immunology* **21**, e401–e406 (2010).
24. Dupont, C. *et al.* Safety of a New Amino Acid Formula in Infants Allergic to Cow's Milk and Intolerant to Hydrolysates. *Journal of Pediatric Gastroenterology & Nutrition* **61**, 456–463 (2015).
25. Chatchatee, P. *et al.* Tolerance development in cow's milk-allergic infants receiving amino acid-based formula: A randomized controlled trial. *Journal of Allergy and Clinical Immunology* **149**, 650–658.e5 (2022).
26. Jing, W., Liu, Q. & Wang, W. Bifidobacterium bifidum TMC3115 ameliorates milk protein allergy in by affecting gut microbiota: A randomized double-blind control trial. *Journal of Food Biochemistry* **44**, e13489 (2020).
27. Cao, L.-H. *et al.* Food Allergy-Induced Autism-Like Behavior is Associated with Gut Microbiota and Brain mTOR Signaling. *J Asthma Allergy* **15**, 645–664 (2022).
28. Esber, N. *et al.* Three Candidate Probiotic Strains Impact Gut Microbiota and Induce Anergy in Mice with Cow's Milk Allergy. *Appl Environ Microbiol* **86**, e01203-20 (2020).
29. Feehley, T. *et al.* Healthy infants harbor intestinal bacteria that protect against food allergy. *Nat Med* **25**, 448–453 (2019).
30. Smith, N. A. *et al.* Anxiety-like behavior and intestinal microbiota changes as strain-and sex-dependent sequelae of mild food allergy in mouse models of cow's milk allergy. *Brain Behav Immun* **95**, 122–141 (2021).
31. Neau, E. *et al.* Three Novel Candidate Probiotic Strains with Prophylactic Properties in a Murine Model of Cow's Milk Allergy. *Applied and Environmental Microbiology* **82**, 1722–1733 (2016).
32. Kleijnjans, L. *et al.* Mice co-administrated with partially hydrolysed whey proteins and prebiotic fibre mixtures show allergen-specific tolerance and a modulated gut microbiota. *Beneficial Microbes* **10**, 165–178 (2019).
33. Kostadinova, A. I. *et al.* A Specific Mixture of Fructo-Oligosaccharides and Bifidobacterium breve M-16V Facilitates Partial Non-Responsiveness to Whey Protein in Mice Orally Exposed to β -Lactoglobulin-Derived Peptides. *Frontiers in Immunology* **7**, 673 (2017).
34. Kostadinova, A. I. *et al.* Dietary Intervention with β -Lactoglobulin-Derived Peptides and a Specific Mixture of Fructo-Oligosaccharides and Bifidobacterium breve M-16V Facilitates the Prevention of Whey-Induced Allergy in Mice by Supporting a Tolerance-Prone Immune Environment. *Front Immunol* **8**, 1303 (2017).
35. Vaali, K., Puimalainen, T., Wolff, H., Alenius, H. & Palosuo, T. Mucosal mast cell protease-1 (MMCP-1) as a marker of intestinal immunopathology in food allergy model. *Journal of Allergy and Clinical Immunology* **115**, S240 (2005).
36. Stevens, T. L. *et al.* Regulation of antibody isotype secretion by subsets of antigen-specific helper T cells. *Nature* **334**, 255–258 (1988).
37. Di Gioia, D., Aloisio, I., Mazzola, G. & Biavati, B. Bifidobacteria: their impact on gut microbiota composition and their applications as probiotics in infants. *Appl Microbiol Biotechnol* **98**, 563–577 (2014).
38. Saturio, S. *et al.* Role of Bifidobacteria on Infant Health. *Microorganisms* **9**, 2415 (2021).
39. Lee, K. H., Song, Y., Wu, W., Yu, K. & Zhang, G. The gut microbiota, environmental factors, and links to the development of food allergy. *Clin Mol Allergy* **18**, 5 (2020).
40. Huang, Y. J. *et al.* The microbiome in allergic disease: Current understanding and future opportunities—2017 PRACTALL document of the American Academy of Allergy, Asthma & Immunology and the European Academy of Allergy and Clinical Immunology. *Journal of Allergy and Clinical Immunology* **139**, 1099–1110 (2017).
41. De Paepe, E., Van Gijseghem, L., De Spiegeleer, M., Cox, E. & Vanhaecke, L. A Systematic Review of Metabolic Alterations Underlying IgE-Mediated Food Allergy in Children. *Molecular Nutrition & Food Research* **65**, 2100536 (2021).
42. Spertini, F. Metabolomics and allergy: Opening Pandora's box. *Journal of Allergy and Clinical Immunology* **145**, 782–784 (2020).
43. Sampson, H. A. *et al.* Mechanisms of food allergy. *Journal of Allergy and Clinical Immunology* **141**, 11–19 (2018).
44. Hendrickx, D. M. *et al.* Assessment of infant outgrowth of cow's milk allergy in relation to the faecal microbiome and metaproteome. *Sci Rep* **13**, 12029 (2023).

Supplementary Material

Table S1. Search queries

1. MEDLINE	((cow*.ti. OR cow*.ab. OR cow*.kw. OR cow*.kf.) AND (milk.ti. OR milk.ab. OR milk.kw. OR milk.kf.)) AND ((allerg*.ti. OR allerg*.ab. OR allerg*.kw. OR allerg*.kf.) OR (hypersensitiv*.ti. OR hypersensitiv*.ab. OR hypersensitiv*.kw. OR hypersensitiv*.kf.)) OR (milk hypersensitivity.sh.) AND ((microb*.ti. OR microb*.ab. OR microb*.kw. OR microb*.kf.) OR (microflora.ti. OR microflora.ab. OR microflora.kw. OR microflora.kf.) OR (16S*.ti. OR 16S*.ab. OR 16S*.kw. OR 16S*.kf.) OR (bifido*.ti. OR bifido*.ab. OR bifido*.kw. OR bifido*.kf.) OR (bacter*.ti. OR bacter*.ab. OR bacter*.kw. OR bacter*.kf.) OR (lachno*.ti. OR lachno*.ab. OR lachno*.kw. OR lachno*.kf.) OR (rumino*.ti. OR rumino*.ab. OR rumino*.kw. OR rumino*.kf.) OR (veillo*.ti. OR veillo*.ab. OR veillo*.kw. OR veillo*.kf.) OR (entero*.ti. OR entero*.ab. OR entero*.kw. OR entero*.kf.) OR (microbiota.sh. OR bifidobacterium.sh. OR bacteroidaceae.sh. OR bacteroides.sh. OR ruminococcus.sh. OR veillonellaceae.sh. OR veillonella.sh. OR enterobacteriaceae.sh.) AND ((child*.ti. OR child*.ab. OR child*.kw. OR child*.kf.) OR (infant*.ti. OR infant*.ab. OR infant*.kw. OR infant*.kf.) OR (baby.ti. OR baby.ab. OR baby.kw. OR baby.kf.) OR (babies.ti. OR babies.ab. OR babies.kw. OR babies.kf.) OR (toddler*.ti. OR toddler*.ab. OR toddler*.kw. OR toddler*.kf.) OR (newborn*.ti. OR newborn*.ab. OR newborn*.kw. OR newborn*.kf.) OR (infant.sh. OR child.sh. OR child. preschool.sh. OR infant. newborn.sh.))
2. PubMed	((cow[Title/Abstract] OR cow's[Title/Abstract]) AND milk[Title/Abstract]) AND (allerg*[Title/Abstract] OR hypersensitiv*[Title/Abstract]) OR ((milk hypersensitivity[MeSH Terms])) AND (((microb*[Title/Abstract]) OR (microflora[Title/Abstract]) OR (16S[Title/Abstract]) OR (bifido*[Title/Abstract]) OR (bacter*[Title/Abstract]) OR (lachno*[Title/Abstract]) OR (rumino*[Title/Abstract]) OR (veillo*[Title/Abstract]) OR (entero*[Title/Abstract])) OR ((microbiota[MeSH Terms]) OR (microbiotas[MeSH Terms]) OR (human microbiome[MeSH Terms]) OR (human microbiomes[MeSH Terms]) OR (microbiome[MeSH Terms]) OR (microbiome, human[MeSH Terms]) OR (microbiomes[MeSH Terms]) OR (16s ribosomal rna[MeSH Terms]) OR (ribosomal rna, 16s[MeSH Terms]) OR (rna, 16s ribosomal[MeSH Terms]) OR (bifidobacterium[MeSH Terms]) OR (bacteroidaceae[MeSH Terms]) OR (bacteroides[MeSH Terms]) OR (ruminococcus[MeSH Terms]) OR (veillonellaceae[MeSH Terms]) OR (veillonella[MeSH Terms]) OR (enterobacteriaceae[MeSH Terms])))) AND (((child*[Title/Abstract]) OR (infant*[Title/Abstract]) OR (baby[Title/Abstract]) OR (babies[Title/Abstract]) OR (toddler*[Title/Abstract]) OR (newborn*[Title/Abstract])) OR ((infant[MeSH Terms]) OR (child[MeSH Terms]) OR (child, preschool[MeSH Terms]) OR (infant, newborn[MeSH Terms])))
3. Scopus	(TITLE-ABS-KEY (cow* W/6 milk)) AND ((TITLE-ABS-KEY (allergy) OR (TITLE-ABS-KEY (hypersensitiv*)) AND ((TITLE-ABS-KEY (microb*) OR (TITLE-ABS-KEY (microflora) OR ((TITLE-ABS-KEY (16s*) OR ((TITLE-ABS-KEY (bifido*) OR ((TITLE-ABS-KEY (bacter*) OR ((TITLE-ABS-KEY (lachno*) OR ((TITLE-ABS-KEY (rumino*) OR ((TITLE-ABS-KEY (veillo*) OR ((TITLE-ABS-KEY (entero*)) AND (((TITLE-ABS-KEY (child) OR ((TITLE-ABS-KEY (infant) OR ((TITLE-ABS-KEY (baby) OR ((TITLE-ABS-KEY (toddler) OR ((TITLE-ABS-KEY (newborn))
4. Web of Science	(TI=(cow* AND milk) OR AB=(cow* AND milk) OR AK=(cow* AND milk) OR KP=(cow* AND milk)) AND ((TI=(allergy) OR AB=(allergy) OR AK=(allergy) OR KP=(allergy)) OR (TI=(hypersensitiv*)) OR AB=(hypersensitiv*) OR AK=(hypersensitiv*) OR KP=(hypersensitiv*)) AND ((TI=(microb*) OR AB=(microb*) OR AK=(microb*) OR KP=(microb*)) OR (TI=(microflora) OR AB=(microflora) OR AK=(microflora) OR KP=(microflora)) OR (TI=(16s*) OR AB=(16s*) OR AK=(16s*) OR KP=(16s*)) OR (TI=(bifido*) OR AB=(bifido*) OR AK=(bifido*) OR KP=(bifido*)) OR (TI=(bacter*) OR AB=(bacter*) OR AK=(bacter*) OR KP=(bacter*)) OR (TI=(lachno*) OR AB=(lachno*) OR AK=(lachno*) OR KP=(lachno*)) OR (TI=(rumino*) OR AB=(rumino*) OR AK=(rumino*) OR KP=(rumino*)) OR (TI=(veillo*) OR AB=(veillo*) OR AK=(veillo*) OR KP=(veillo*)) OR (TI=(entero*) OR AB=(entero*) OR AK=(entero*) OR KP=(entero*)) AND ((TI=(child) OR AB=(child) OR AK=(child) OR KP=(child)) OR (TI=(infant) OR AB=(infant) OR AK=(infant) OR KP=(infant)) OR (TI=(baby) OR AB=(baby) OR AK=(baby) OR KP=(baby)) OR (TI=(toddler) OR AB=(toddler) OR AK=(toddler) OR KP=(toddler)) OR (TI=(newborn) OR AB=(newborn) OR AK=(newborn) OR KP=(newborn)))

Table S2. Information and reasons for the 28 papers excluded after careful consideration

Index	Author and year	Exclusion reason
1	Pohjavuori <i>et al.</i> , 2004 ¹	Diagnosed IgE-mediated CMA based on a CM challenge and skin prick tests or antigen-specific IgE of any antigen tested (including also egg-white, cat, dog and birch).
2	Viljanen <i>et al.</i> , 2005a ²	
3	Barros <i>et al.</i> , 2017 ³	
4	Viljanen <i>et al.</i> , 2005b ⁴	Distinguished between IgE-mediated and non-IgE mediated CMA in the description of the allergic subjects but did not report any specific results for IgE-mediated CMA.
5	Burks <i>et al.</i> , 2015 ⁵	
6	Dong <i>et al.</i> , 2018 ⁶	
7	Jarvinen <i>et al.</i> , 2014 ⁷	Reported 29 infants with IgE-mediated CMA in their table with clinical characteristics. However, elevated levels of cow's milk specific IgE were reported in only 13 infants. The corresponding author was contacted by email, but was unable to supply additional data because the research was done in a previous institution
8	Mercer <i>et al.</i> , 2009 ⁸	CMA was diagnosed based on total and CM specific IgE levels and CMA-related symptoms, but no oral food challenge was used to confirm CMA.
9	Taniuchi <i>et al.</i> , 2005 ⁹	Included several subjects whose diagnosis was not confirmed by an oral food challenge, but by a cow's milk elimination diet
10	Kendler <i>et al.</i> , 2006 ¹⁰	Did not confirm CMA by oral food challenge
11	Hol <i>et al.</i> , 2008 ¹¹	Used a food challenge, but diagnosed children based on their late response, which does not point to IgE-mediated CMA
12	Shek <i>et al.</i> , 2005 ¹²	
13	Yamamoto-Hanada <i>et al.</i> , 2023 ¹³	Included both children below 12 years old as well as adolescents and/or adults, but results for children were not reported separately
14	Hill <i>et al.</i> , 1989 ¹⁴	
15	Hauer <i>et al.</i> , 1997 ¹⁵	Did not include any gut microbiome data or intervention targeting the gut microbiome
16	Szabó and Eigenmann, 2000 ¹⁶	
17	Paparo <i>et al.</i> , 2016 ¹⁷	Studied infants that received probiotics in the past, before entering the study, and therefore could not be compared to the probiotic intervention studies discussed in this review
18	Gotteland <i>et al.</i> , 1992 ¹⁸	Studied CM protein absorption after <i>E. coli</i> infection
19	Morin <i>et al.</i> , 2012 ¹⁹	
20	Shandilya <i>et al.</i> , 2016 ²⁰	Animals models were sensitized to CM, but did not receive a food challenge, thus focus on CM sensitization rather than CMA
21	Wróblewska <i>et al.</i> , 2020 ²¹	
22	Maiga <i>et al.</i> , 2017 ²²	
23	Pescuma <i>et al.</i> , 2019 ²³	Two of the three experiments had no challenge, while in the third one there was no comparison between (allergy or treatment) groups
24	Graversen <i>et al.</i> , 2021 ²⁴	Focused on antibiotics instead of treatment for CMA.
25	Liu <i>et al.</i> , 2023 ²⁵	Studied the effect of pre-treatment with whey or beta-lactoglobulin (BLG) before sensitization
26	Mauras <i>et al.</i> , 2019 ²⁶	The CMA donor used for fecal transplantation had multiple food allergy
27	Schouten <i>et al.</i> , 2009 ²⁷	
28	Adel-Patient <i>et al.</i> , 2020 ²⁸	No GM-related data, do not mention how the treatment changed the GM

Fecal metabolome exploration in infants with CMA

Table S3. CMA diagnosis and measured variables for all human studies.

Abbreviations: see Supplementary Excel file.

Author and year	CMA diag-nosis	Measured variables		
		Microbiome	Metabolomics	Immune response
Thompson-Chagoyan <i>et al.</i> , 2010 ²⁹	CM-specific IgE, SPT, DBPCFC	Aerobes, Anaerobes, Enterobacteria, Bifidobacteria, <i>Lactobacilli</i> , Clostridia	-	-
Salmi <i>et al.</i> , 2010 ³⁰	CM-specific IgE, SPT, DBPCFC	-	Urine: 37 organic acids, Creatinine	-
Thompson-Chagoyan <i>et al.</i> , 2011 ³¹	CM-specific IgE, SPT, DBPCFC	10 targeted probes: <i>Bifidobacterium</i> , <i>Bacteroides</i> , Enterobacteria, <i>Streptococcus</i> , <i>Lactobacillus</i> , <i>Atopobium</i> , <i>Clostridium coccoides</i> , <i>Clostridium leptum</i> , <i>Clostridium perfringens</i> sps., <i>Clostridium difficile</i> sps.	Feces: Lactate, SCFA (acetate, propionate, butyrate, isocaproic acid), Branched-chain short fatty acids (BCSFA).	-
Francavilla <i>et al.</i> , 2012 ³²	CM-specific IgE, SPT, DBPCFC	13 targeted probes: Domain bacteria, negative control, <i>Bifidobacterium</i> , <i>Bacteroides/Prevotella</i> , Eubacterium rectale/Clostridium coccoides, <i>Lactobacillus/Enterococcus</i> , <i>Streptococcus/Lactococcus group</i> , <i>Escherichia coli</i> , Sulfate-reducing bacteria (SRB), <i>Atopobium</i> group, <i>Corynebacterium</i> group, <i>Clostridium histolyticum</i> , <i>Clostridium lituseburense</i>	GC-MS (feces): 15 organic metabolites (esters, ketones, Alcohols, sulfur compounds, hydrocarbons, SCFA); NMR (feces): pyruvic acid, lactic acid, uridine, histidine, tyrosine, threonine, methionine, proline, TMAO, arginine/histidine, valine / isoleucine, phenylalanine, gamma-amino-butyric acid/lysine	-
Guo <i>et al.</i> , 2016 ³³	Analysis of serum samples, SPT, DBPCFC	Dominant bacteria, <i>Bifidobacterium</i> , <i>Lactobacillus</i> , <i>C. coccoides</i> , Microbiota diversity (Shannon-Weaver index, dice similarity coefficient)	-	-
Canani <i>et al.</i> , 2016 ³⁴	Clinical history, CM-specific IgE, DBPCFC	Dominant bacteria, Microbiota Alpha diversity (Shannon index) and Evenness (Pielou's evenness index)	Feces: butyrate	-
Dong <i>et al.</i> , 2018 ³⁵	CM-specific IgE, SPT, DBPCFC	Dominant bacteria, Microbiota Alpha diversity (Chao1, ACE, Simpson, Shannon, and coverage indices)	Feces: SCFAs (acetate, butyrate, propionate, isobutyrate), lactate	-
Mennini <i>et al.</i> , 2021 ³⁶	CM-specific IgE, SPT, DBPCFC	PCR : Dominant bacteria; qRT-PCR; <i>B. breve</i> , <i>B. longum</i> subsp. <i>longum</i> , <i>B. longum</i> subsp. <i>infantis</i> Microbiota Alpha diversity (Observed, Chao1 and Shannon indices) and beta diversity(unweighted UniFrac)	-	-

Chapter IV

Mera-Berriatua et al., 2022 ³⁷	Clinical history of IgE-mediated food allergy, SPT	Dominant bacteria Microbiota Alpha diversity (Shannon index) and beta diversity (Bray-Curtis distance)	--	-
Bunyavanich et al., 2016 ³⁸	CM-specific IgE, SPT, CM challenge or AD with CM-specific IgE	Microbiome (feces): Dominant bacteria; Microbiota Alpha diversity (Faith's phylogenetic diversity) and beta diversity (unweighted UniFrac)	-	-
Dupont et al., 2015 ³⁹	CM-specific IgE, SPT, or both positive cutaneous tests and IgE, DBPCFC	Total bacteria, <i>Clostridium</i> cluster IV, <i>Bacteroides/ Prevotella</i> group, <i>Bifidobacterium</i> , <i>Lactobacillus/ Leuconostoc/Pediococcus</i> group, <i>Clostridium</i> cluster XIVa, <i>Clostridium</i> cluster XI, <i>Clostridium</i> cluster I/II, <i>Staphylococcus</i> , <i>Enterococcus</i> , <i>Escherichia coli</i>	Plasma: Amino acids (cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine, valine) Feces: butyrate	-
Chatchatee et al., 2022 ⁴⁰	CM-specific IgE, SPT, DBPCFC	bifidobacteria and ER/CC group		
Jing et al. 2020 ⁴¹	SPT, IgE, DBPCFC	dominant bacteria microbiota Alpha diversity (number of OTUs, Chao1, Shannon, Simpson index) and beta diversity (weighted and unweighted UniFrac)	-	Immuno-globulins Total IgE, IgG ₂ (serum) Cytokines TNF α , IL-1 β , IL-6, IL-10 (serum)

Table S4. Model information for all animal studies. Abbreviations: see Supplementary Excel file.

Sensitization					Challenge		Intervention details		n size/group ††	Author and year
					Intradermal	Intragastric				
Animal/Strain (gender)	Allergen: Dose(mg)	Adjuvant: Dose (µg)	Period† (wk)	Administration	Allergen: Dose (ug)	Allergen: Dose(mg)	Introduction	Duration		
C3H/HeOuJ mice (F)	W:20	CT:10	5	i.g.	W:20	W:50	Pre-S		6-8	Kostadinova <i>et al.</i> 2017a ⁴²
C3H/HeOuJ mice (F)	W:20	CT:10	5	i.g.	W:20	W:50	Pre-S	6-9d‡	6-8	Kostadinova <i>et al.</i> 2017b. ⁴³
C3H/HeOuJ mice (F)	W:20	CT:10	5	i.g.	W:6	W:50	Long WS Short Pre-S	Long 7.5wk Short 5d	7-10	Kleinjans <i>et al.</i> 2019 ⁴⁴
BALB/cByJ mice (F)	W:15	CT:10	5	i.g.	-	BLG:60	WS	6wk	30	Neau <i>et al.</i> , 2016 ⁴⁵
BALB/cByJ mice (F)	W:15	CT:10	5	i.g.	-	BLG:60	Post-S	20d	10 -12	Esber <i>et al.</i> 2020 ⁴⁶
Germ-free C3H/HeN (M and F)	BLG:20	CT:10	5	i.g.	-	BLG:2*100	-	-	6-42	Feehley <i>et al.</i> 2019 ⁴⁷
C3H/HeN mice (M)	W/W/W: 10/100/0.5	CT/CT/Alum: 10/10/2	5/2/2	i.g./i.g./i.p.	-	W:50	-	-	3-7	Cao <i>et al.</i> 2022 ⁴⁸
C57BL/6J and BALB/cJ (M and F)	BLG:1	CT:10	5	i.g.	-	W:50	-	-	5-10	Smith <i>et al.</i> 2021 ⁴⁹

†All administrations are performed weekly

†† Intervention group sizes (not control group)

‡ Synbiotic diet for 9 days, peptide mix intake for 6 days

Table S5. Measured variables for all animal studies. Abbreviations: see Supplementary Excel file.

Author and year	Measured variables		
	Microbiome	Metabolomics	Immune response
Neau <i>et al.</i> ⁴⁵	11 bacteria primers, all bacteria	-	Igs: Total and BLG-s IgE, IgG ₁ , IgG _{2a} (plasma) Cytokines: IFN- γ , IL-12p70, IL-4, IL-5, and IL-10 (spleen, MLN) mRNA expression: ifn-g, il-4, il-10, tgf-b, il-17a, t-bet, gata3, roryt, foxp3 (ileum)
Esber <i>et al.</i> ⁴⁶	α (Shannon index) and β (Bray-Curtis distance, UniFrac distance) diversity	Feces: SCFA, Plasma: other metabolites	Igs: BLG- sIgE, sIgG ₁ , sIgG ₂ (plasma) Cytokines: IL-17A, IL-2, GM-CSF, IL-4, IFN- γ , IL-10, IL-5, IL-12p70 (spleen) mRNA expression: gata3, tbet, foxp3, roryt, ifny, tnfa, il4, il10, and tgf β (ileal)
Kleinjans <i>et al.</i> ⁴⁴	All bacteria	-	Igs: W- sIgE, sIgG ₁ , sIgG _{2a} (serum)
Kostadinova <i>et al.</i> ⁴²	-	Feces: acetic acid, propionic acid, butyric acid	Igs: W- and BLG- sIgE, sIgG ₁ , sIgG _{2a} (serum) Lymphocytes: T cells, DC (spleen, MLN, SI-LP) Cytokines: IL-5, IL-13, IL-10, IL-17A, IFN- γ (Spleen, MLN, SILP)
Kostadinova <i>et al.</i> ⁴³		<u>Part 1: Post-oral tolerance</u> Metabolites Feces: acetic acid, propionic acid, butyric acid, valeric acid	mRNA expression: Foxp3, Tbet, GATA3, Ror γ T, IL-10, galectin-9, TGF- β , IL-13, IFN- γ , IL-22 (PP, SI (proximal, middle), colon) Immunohistochemistry: Foxp3+ cells (colon) <u>Part 2: Post-challenge</u> mRNA expression: Foxp3, Tbet, GATA3, Ror γ T, IL-10, galectin-9, TGF- β , IL-13, IFN- γ , and IL-22 (PP, spleen) Lymphocytes: T _{reg} (LP)
Smith <i>et al.</i> ²⁴	α (Shannon, Simpson indices) and β (Bray-Curtis) diversity	-	Igs: BLG-sIgE, sIgG ₁ ; sIgG _{2a} (serum) Cytokines, chemokines, and acute phase proteins: e.g. IL-10, IL-13, IL-15, IL-1 β , IL-31, IL-21, CCL1, CCL9, CCL12, CCL17, FGF2, CDF1, CSF2, TNFSF1A, TNFRSF1B, ICAM-1 (plasma)
Cao <i>et al.</i> ²³	All bacteria, α and β diversity	-	Igs: W- sIgE, sIgG ₁ , sIgG _{2a} (serum) Cytokines: IL-6, IL-10 (serum) mRNA expression: IL-4, IL-8, IL-33, IL-1 β , TGF- β , GAPDH, mTOR mRNA
Feehley <i>et al.</i> ⁴⁷	α (Shannon index) and β (weighted UniFrac) diversity Pielous's evenness		Igs: BLG-specific IgE, IgG ₁ (serum) Cytokines: IL-13, IL-4 (spleen) ex-W Transcriptome: 32 genes (IEC)

Reference:

1. Pohjavuori, E. *et al.* Lactobacillus GG effect in increasing IFN- γ production in infants with cow's milk allergy. *Journal of Allergy and Clinical Immunology* **114**, 131–136 (2004).
2. Viljanen, M. *et al.* Probiotics in the treatment of atopic eczema/dermatitis syndrome in infants: a double-blind placebo-controlled trial. *Allergy* **60**, 494–500 (2005).
3. Barros, K. V. *et al.* Evidence for Involvement of IL-9 and IL-22 in Cows' Milk Allergy in Infants. *Nutrients* **9**, 1048 (2017).
4. Viljanen, M. *et al.* Probiotic effects on faecal inflammatory markers and on faecal IgA in food allergic atopic eczema/dermatitis syndrome infants. *Pediatr Allergy Immunol* **16**, 65–71 (2005).
5. Burks, A. W. *et al.* Synbiotics-supplemented amino acid-based formula supports adequate growth in cow's milk allergic infants. *Pediatric Allergy and Immunology* **26**, 316–322 (2015).
6. Dong, P., jing Feng, J., yong Yan, D., jing Lyu, Y. & Xu, X. Early-life gut microbiome and cow's milk allergy- a prospective case - control 6-month follow-up study. *Saudi Journal of Biological Sciences* **25**, 875–880 (2018).
7. Järvinen, K. M. *et al.* Role of maternal elimination diets and human milk IgA in the development of cow's milk allergy in the infants. *Clinical & Experimental Allergy* **44**, 69–78 (2014).
8. Mercer, N. *et al.* Duodenal Intraepithelial Lymphocytes of Children with Cow Milk Allergy Preferentially Bind the Glycan-Binding Protein Galectin-3. *Int J Immunopathol Pharmacol* **22**, 207–217 (2009).
9. Taniuchi, S. *et al.* Administration of Bifidobacterium to infants with atopic dermatitis: Changes in fecal microflora and clinical symptoms. *Journal of Applied Research* **5**, 387–396 (2005).
10. Kendler, M., Uter, W., Ruegger, A., Shimshoni, R. & Jecht, E. Comparison of fecal microflora in children with atopic eczema/dermatitis syndrome according to IgE sensitization to food. *Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology* **17**, 141–147 (2006).
11. Hol, J. *et al.* The acquisition of tolerance toward cow's milk through probiotic supplementation: A randomized, controlled trial. *Journal of Allergy and Clinical Immunology* **121**, 1448–1454 (2008).
12. Shek, L. P. C., Bardina, L., Castro, R., Sampson, H. A. & Beyer, K. Humoral and cellular responses to cow milk proteins in patients with milk-induced IgE-mediated and non-IgE-mediated disorders. *Allergy* **60**, 912–919 (2005).
13. Yamamoto-Hanada, K. *et al.* Combination of heat-killed Lactiplantibacillus plantarum YIT 0132 (LP0132) and oral immunotherapy in cow's milk allergy: a randomised controlled trial. *Beneficial Microbes* **14**, 17–29 (2023).
14. Hill, D. J., Firer, M. A., Ball, G. & Hosking, C. S. Recovery from milk allergy in early childhood: Antibody studies. *The Journal of Pediatrics* **114**, 761–766 (1989).
15. Hauer, A. C., Breese, E. J., Walker-Smith, J. A. & MacDonald, T. T. The Frequency of Cells Secreting Interferon- γ and Interleukin-4, -5, and -10 in the Blood and Duodenal Mucosa of Children with Cow's Milk Hypersensitivity. *Pediatr Res* **42**, 629–638 (1997).
16. Szabó, I. & Eigenmann, P. a. Allergenicity of major cow's milk and peanut proteins determined by IgE and IgG immunoblotting. *Allergy* **55**, 42–49 (2000).
17. Paparo, L. *et al.* Epigenetic features of FoxP3 in children with cow's milk allergy. *Clin Epigenetics* **8**, 86 (2016).
18. Gotteland, M., Crain-Denoyelle, A. M., Heyman, M. & Desjeux, J. F. Effect of Cow's Milk Protein Absorption on the Anaphylactic and Systemic Immune Responses of Young Rabbits during Bacterial Diarrhoea. *Int Arch Allergy Immunol* **97**, 78–82 (1992).
19. Morin, S. *et al.* Delayed bacterial colonization of the gut alters the host immune response to oral sensitization against cow's milk proteins. *Molecular Nutrition & Food Research* **56**, 1838–1847 (2012).
20. Shandilya, U. K., Sharma, A., Kapila, R. & Kansal, V. K. Probiotic Dahi containing Lactobacillus acidophilus and Bifidobacterium bifidum modulates immunoglobulin levels and cytokines expression in whey proteins sensitised mice. *Journal of the Science of Food and Agriculture* **96**, 3180–3187 (2016).
21. Wróblewska, B. *et al.* Effect of Low-Immunogenic Yogurt Drinks and Probiotic Bacteria on Immunoreactivity of Cow's Milk Proteins and Tolerance Induction-In Vitro and In Vivo Studies. *Nutrients* **12**, 3390 (2020).
22. Maiga, M. A. *et al.* Neonatal mono-colonization of germ-free mice with Lactobacillus casei enhances casein immunogenicity after oral sensitization to cow's milk. *Molecular Nutrition & Food Research* **61**, 1600862 (2017).
23. Pescuma, M., Hébert, E. M., Font, G., Saavedra, L. & Mozzi, F. Hydrolysate of β -lactoglobulin by Lactobacillus delbrueckii subsp. bulgaricus CRL 656 suppresses the immunoreactivity of β -lactoglobulin as revealed by in vivo assays. *International Dairy Journal* **88**, 71–78 (2019).

4

24. Graversen, K. B. *et al.* Partially Hydrolysed Whey Has Superior Allergy Preventive Capacity Compared to Intact Whey Regardless of Amoxicillin Administration in Brown Norway Rats. *Front Immunol* **12**, 705543 (2021).
25. Liu, M. *et al.* Oral pretreatment with β -lactoglobulin derived peptide and CpG co-encapsulated in PLGA nanoparticles prior to sensitizations attenuates cow's milk allergy development in mice. *Frontiers in Immunology* **13**, 1053107 (2023).
26. Mauras, A. *et al.* Gut microbiota from infant with cow's milk allergy promotes clinical and immune features of atopy in a murine model. *Allergy* **74**, 1790–1793 (2019).
27. Schouten, B. *et al.* Cow Milk Allergy Symptoms Are Reduced in Mice Fed Dietary Synbiotics during Oral Sensitization with Whey. *The Journal of Nutrition* **139**, 1398–1403 (2009).
28. Adel-Patient, K. *et al.* Administration of Extensive Hydrolysates From Caseins and Lactobacillus rhamnosus GG Probiotic Does Not Prevent Cow's Milk Proteins Allergy in a Mouse Model. *Frontiers in Immunology* **11**, 1700 (2020).
29. Thompson-Chagoyan, O. C., Vieites, J. M., Maldonado, J., Edwards, C. & Gil, A. Changes in faecal microbiota of infants with cow's milk protein allergy - A Spanish prospective case-control 6-month follow-up study. *Pediatric Allergy and Immunology* **21**, e394–e400 (2010).
30. Salmi, H., Kuitunen, M., Viljanen, M. & Lapatto, R. Cow's milk allergy is associated with changes in urinary organic acid concentrations. *Pediatric Allergy and Immunology* **21**, e401–e406 (2010).
31. Thompson-Chagoyan, O. C. *et al.* Faecal microbiota and short-chain fatty acid levels in faeces from infants with cow's milk protein allergy. *International Archives of Allergy and Immunology* **156**, 325–332 (2011).
32. Francavilla, R. *et al.* Effect of lactose on gut microbiota and metabolome of infants with cow's milk allergy. *Pediatric Allergy and Immunology* **23**, 420–427 (2012).
33. Guo, L. *et al.* Comparative Analysis of Fecal Microbiota in 5-8-Year-old Children with and without Cow Milk Protein Allergy. *26*, 6397 (2016).
34. Canani, R. B. *et al.* Lactobacillus rhamnosus GG-supplemented formula expands butyrate-producing bacterial strains in food allergic infants. *The ISME Journal* **10**, 742–750 (2016).
35. Dong, Y., Fei, P., Han, Y. & Guo, L. Characterization of Fecal Microbiota, Short-Chain Fatty Acids and Lactic Acid Concentrations in 5-8-Year-Old Children with Cow Milk Protein Allergy. *28*, 64638 (2018).
36. Mennini, M. *et al.* Gut Microbiota Profile in Children with IgE-Mediated Cow's Milk Allergy and Cow's Milk Sensitization and Probiotic Intestinal Persistence Evaluation. *International Journal of Molecular Sciences Article* (2021) doi:10.3390/ijms22041649.
37. Mera-Berriatua, L. *et al.* Unravelling the Gut Microbiota of Cow's Milk–Allergic Infants, Their Mothers, and Their Grandmothers. *Journal of Investigational Allergology and Clinical Immunology* **32**, 395–398 (2022).
38. Bunyavanich, S. *et al.* Early-life gut microbiome composition and milk allergy resolution. *Journal of Allergy and Clinical Immunology* **138**, 1122–1130 (2016).
39. Dupont, C. *et al.* Safety of a New Amino Acid Formula in Infants Allergic to Cow's Milk and Intolerant to Hydrolysates. *Journal of Pediatric Gastroenterology & Nutrition* **61**, 456–463 (2015).
40. Chatchatee, P. *et al.* Tolerance development in cow's milk–allergic infants receiving amino acid–based formula: A randomized controlled trial. *Journal of Allergy and Clinical Immunology* **149**, 650–658.e5 (2022).
41. Jing, W., Liu, Q. & Wang, W. Bifidobacterium bifidum TMC3115 ameliorates milk protein allergy in by affecting gut microbiota: A randomized double-blind control trial. *Journal of Food Biochemistry* **44**, e13489 (2020).
42. Kostadinova, A. I. *et al.* A Specific Mixture of Fructo-Oligosaccharides and Bifidobacterium breve M-16V Facilitates Partial Non-Responsiveness to Whey Protein in Mice Orally Exposed to β -Lactoglobulin-Derived Peptides. *Frontiers in Immunology* **7**, 673 (2017).
43. Kostadinova, A. I. *et al.* Dietary Intervention with β -Lactoglobulin-Derived Peptides and a Specific Mixture of Fructo-Oligosaccharides and Bifidobacterium breve M-16V Facilitates the Prevention of Whey-Induced Allergy in Mice by Supporting a Tolerance-Prone Immune Environment. *Front Immunol* **8**, 1303 (2017).
44. Kleinjans, L. *et al.* Mice co-administrated with partially hydrolysed whey proteins and prebiotic fibre mixtures show allergen-specific tolerance and a modulated gut microbiota. *Beneficial Microbes* **10**, 165–178 (2019).
45. Neau, E. *et al.* Three Novel Candidate Probiotic Strains with Prophylactic Properties in a Murine Model of Cow's Milk Allergy. *Applied and Environmental Microbiology* **82**, 1722–1733 (2016).
46. Esber, N. *et al.* Three Candidate Probiotic Strains Impact Gut Microbiota and Induce Anergy in Mice with Cow's Milk Allergy. *Appl Environ Microbiol* **86**, e01203-20 (2020).
47. Feehley, T. *et al.* Healthy infants harbor intestinal bacteria that protect against food allergy. *Nat Med* **25**, 448–453 (2019).

Fecal metabolome exploration in infants with CMA

48. Cao, L.-H. *et al.* Food Allergy-Induced Autism-Like Behavior is Associated with Gut Microbiota and Brain mTOR Signaling. *J Asthma Allergy* **15**, 645–664 (2022).
49. Smith, N. A. *et al.* Anxiety-like behavior and intestinal microbiota changes as strain-and sex-dependent sequelae of mild food allergy in mouse models of cow's milk allergy. *Brain Behav Immun* **95**, 122–141 (2021).

Table S6. Abbreviations

Abbreviation	Full name/definition
16S rRNA	16S ribosomal ribonucleic acid
AAF	amino acid formula
AASR	acute allergic skin response (ear swelling)
AC	allergic control
AEDES	atopic eczema/dermatitis syndrome
BCSFAs	branched-chain short fatty acids
BLG	beta-lactoglobulin
body-T	body temperature
CFU	colony-forming unit
CM	cow's milk
CMA	cow's milk allergy
DC	dendritic cells
DGGE	denaturing gradient gel electrophoresis
DBPCFC	Double-blind, placebo-controlled food challenge
EHF	extensively hydrolyzed formula
ELISA	Enzyme-linked immunosorbent assay
ER/CC	Eubacterium rectale/Clostridium coccoides
ex-BLG	ex-vivo res-stimulation with BLG
ex-W	ex-vivo res-stimulation with whey
F	female
FC	flow cytometry
FF/Bb	short and long chain FOS and B. breve M-16V
FISH	fluorescent in situ hybridization
FOS	fructo-oligosaccharides
FOXP3	forkhead box P3
FT	fecal transplantation
G	group
GATA3	GATA Binding Protein 3
GC-FID	GC-flame ionization detector
GC-MS	gas-chromatography-mass spectrometry
GM	gut microbiome
GM-CSF	Granulocyte macrophage colony-stimulating factor
GOS	galacto-oligosaccharides
HC	healthy controls
HILIC	Hydrophilic interaction chromatography
HPLC-UV	high-performance liquid chromatography-ultraviolet detector
HWF	hydrolysed whey formula
IA	immunoassay (other than ELISA)
i.p.	intraperitoneal
i.g.	intragastric
i.d.	intradermally
IEC	Intestinal epithelial cell(s)
IFN- γ	Interferon-gamma
Ig(s)	immunoglobulin(s)
IL	interleukin
LAB	lactic acid bacteria
lcFOS	long chain fructo-oligosaccharides
LGG	Lactobacillus rhamnosus GG
LP	lamina propria
M	male
MLN	mesenteric lymph node
mMCP-1	mucosal mast cell protease-1
MS	mass spectrometry
MS/MS	Tandem mass spectrometry
NMR	nuclear magnetic resonance
OTU	operational taxonomic unit
pAOS	pectin-derived acidic oligosaccharide

Fecal metabolome exploration in infants with CMA

PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PP	Peyer's Patches
qPCR	quantitative PCR
qRT-PCR	quantitative real-time PCR
RAAF	reference amino acid formula
Ror2	Receptor Tyrosine Kinase Like Orphan Receptor 2
ROR γ T	retinoid-Related Orphan Receptor gamma t
RP	reverse phase
SAS	systematic anaphylaxis scores
SCFAs	Short-chain fatty acids
scFOS	short chain FOS
scGOS	short chain galacto-oligosaccharides
sd	standard deviation
SI	small intestine
sIg	specific Immunoglobulin
SI-LP	small intestine lamina propria
sp.	single unnamed species (of a certain genus)
spp.	multiple species (of a certain genus)
SPT	skin prick test
TAAF	thickener amino acid formula
Tbet	T-box transcription factor
TC	tolerant control
Tgfb3	Transforming growth factor beta receptor III
TGF- β	Transforming growth factor beta
T _h	T helper cell
T _{eff}	effector T cells
TMAO	trimethylamine-N-oxide
TNF α	tumor necrosis factor alpha
T _{reg}	T regulatory cell
TSLP	thymic stromal lymphopoietin
UPLC-MS/MS	ultra-performance liquid chromatography with tandem mass spectrometry
W	whey
Pre-S	pre-sensitization
Post-S	post-sensitization
WS	whole study
wk	week(s)

