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SYSTEMATIC REVIEW

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Current insights into cow's milk allergy in children: Microbiome, metabolome, and immune response—A systematic review

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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 of 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 GM in CMA-diagnosed children, published before 1 March 2023. A total of 21 articles (13 on children and 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 the 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.

KEYWORDS
cow's milk allergy, gut microbiota, immune response, infant, metabolomics, mouse model, synbiotics

Mariyana V. Savova and Pingping Zhu shared first authorship.

1 | BACKGROUND

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 cow's milk tend to have persistent symptoms more often 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 of 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 GM and CMA, by reviewing existing studies examining microbiome, metabolome, proteome, and immune response data on IgE-mediated CMA in children and animal models.

Key message

The gut microbiome (GM) may play an important role in the development of cow's milk allergy (CMA). Treatments targeting the GM, such as prebiotics, probiotics, and synbiotics, may therefore be key prevention and treatment strategies. This systematic review reports on 21 studies, including 13 human studies and 8 animal studies studying GM's relation to CMA. Our findings suggest that GM characterized by an enriched Clostridia class, reduced Lactobacillales order, and reduced *Bifidobacterium* genus is typical in CMA children. Our results highlight that mechanistic insights, which can be obtained by means of multi-omics approaches, are required to study CMA and develop effective preventive and treatment strategies.

2 | 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, and 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.

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 ± 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, and 897 articles in, respectively, Scopus, PubMed, MEDLINE and Web of Science. Forty-nine studies were eligible for inclusion. Figure 1 shows the PRISMA[13] flow diagram. Of the 49 papers, 28 were excluded after careful consideration Table S2.

3.2 | Study findings

3.2.1 | Human studies

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

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 study[23] that considered atopic eczema/dermatitis syndrome infants as controls.

GM modifications. The GM-related studies include four case–control reports,[15,17,19,20] four case–control findings in intervention studies,[14,16,18,21] and one case study.[22] Techniques applied for GM profile identification included bacteria culture[18] and 16S rRNA gene-based approaches (DGGE,[19] FISH[14,15] and gene sequencing[16,17,20–22]). 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 decreased[20] 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* family[20] 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* genus[16] 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* species[19] 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 to the *Bifidobacterium* genus.[15] Additionally, lactate was found to be negatively correlated with *Bacteroides* genus[17] and *Clostridium coccoides* species,[15] but positively correlated with *Bifidobacterium* genus.[15]

Intervention studies

Eight intervention studies for CMA treatment were included (Table 2).[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]

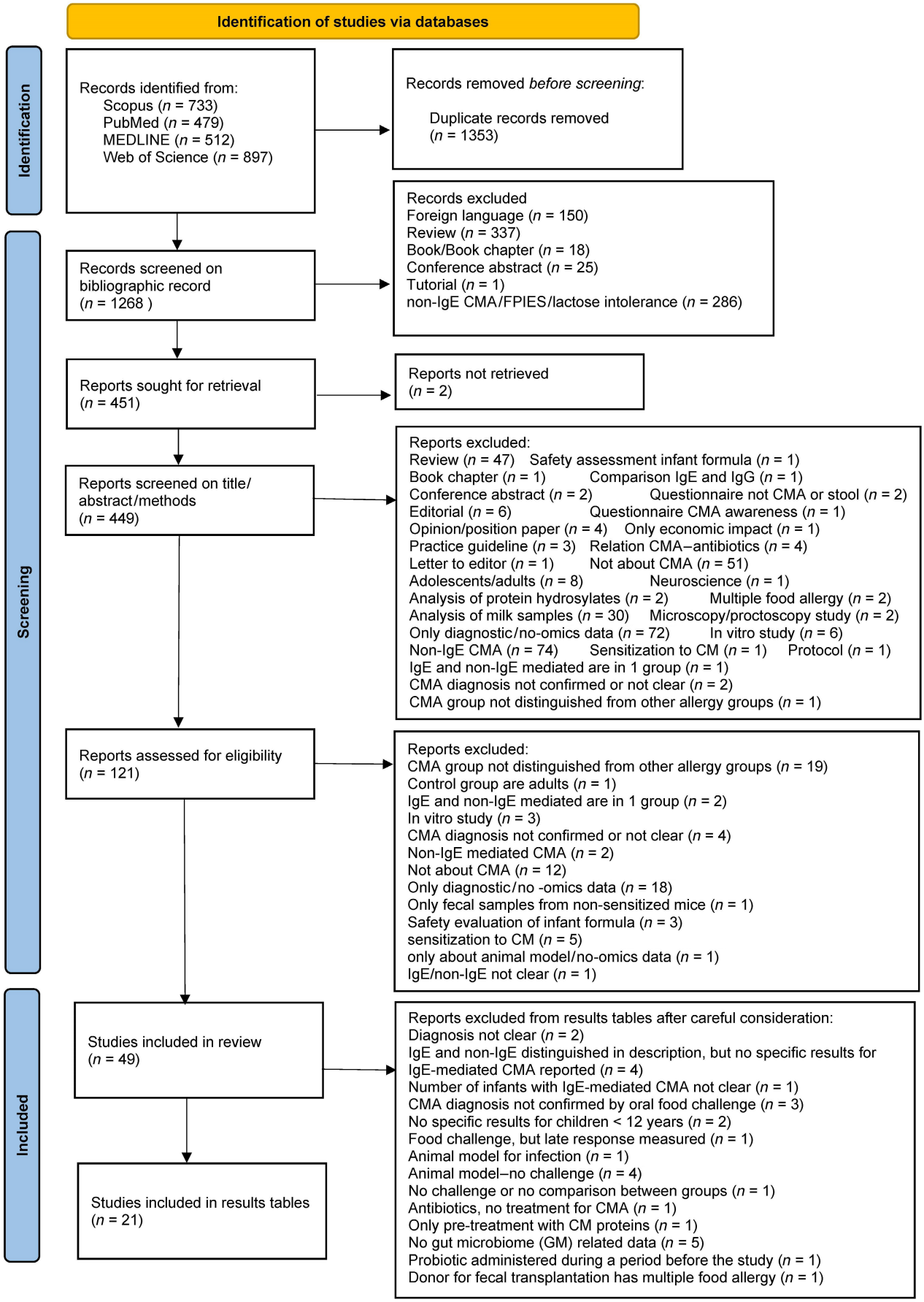

FIGURE 1 PRISMA flowchart for this systematic review.

TABLE 1 Human case and case–control studies in infants/children.

Age				Results: modifications in case vs. control (case–control study), modifications in allergic vs. tolerant (case study)		
years (y); months (m)	Analytical techniques	Type of analytical data	Sample size (CMA/control)	Increase	Decrease	Reference
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 et al.[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 et al.[23,a]
2–12 m	FISH-FC (16S rRNA gene-specific probes); GC-FID	Microbiome, Metabolomics	46/46	*Clostridium coccoides* group, *Atopobium* cluster, butyrate, BCSFA	Not reported	Thompson-Chagoyan et al.[15]
6.5–10.4 m	FISH (16S rRNA gene-specific probes); GC–MS; NMR	Microbiome, Metabolomics	18/18	*Bacteroides*, *Clostridium*, 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 et al.[14]
5–8 y	PCR-DGGE (V3 regions +16S rRNA gene-specific primers)	Microbiome	12/12	GM α-diversity (Shannon diversity), *C. coccoides* diversity (Shannon diversity), *Bacteroides*, *Clostridium*, *Escherichia coli*; only detected in the CMA group: *C. celerecrescens*	*Bifidobacterium* (*B.*) diversity (Shannon diversity), *B. adolescent*, *B. longum*, *B. catenulatum*, and *B. breve* Only detected in the control group: *B. bifidum*	Guo et al.[19]
1–12 m	qPCR-16S rRNA (V4 region), GC-FID	Microbiome	19/20	GM α-diversity (Shannon diversity), Gut microbiota evenness (Pielou's evenness), *Ruminococcaceae*, *Lachnospiraceae*, *Ruminococcus*, *Faecalibacterium*	*Bifidobacteriaceae*, *Streptococcaceae*, *Enterobacteriaceae*, *Enterococcaceae*, *Bifidobacterium*, *Escherichia*	Canani et al.[16]

(Continues)

TABLE 1 (Continued)

Age				Results: modifications in case vs. control (case–control study), modifications in allergic vs. tolerant (case study)		
years (y); months (m)	Analytical techniques	Type of analytical data	Sample size (CMA/control)	Increase	Decrease	Reference
5–8 y	PCR-16 s rRNA (V3-V4 regions), HPLC-UV	Microbiome, Metabolomics	6/8	Firmicutes, Clostridia, *Ruminococcaceae*, *Subdoligranulum* Only detected in the CMA group: *Burkholderiaceae*, *Nannocystaceae*, *Shewanellaceae*, *Thermomonosporaceae*, *Flavobacteriaceae*	Proteobacteria only detected in the control group: *Methylophilaceae*, *Dietziaceae*, Total SCFAs	Dong et al.[17]
10–15 m	PCR-16S-rRNA (V3-V4 regions), qRT-PCR	Microbiome	14/14	Firmicutes, *Haemophilus*, *Actinobacillus*, *Prevotella*, *Klebsiella*	Verrucomicrobia, *Parabacteroides*, *Granulicatella*	Mennini et al.[21]
4–6 m	16S-rRNA (V3-V4 regions)	Microbiome	16/34	Not reported	GM α-diversity (Shannon diversity), *Acidaminococcaceae*, *Prevotellaceae*	Mera-Berriatua et al.[20]
3–16 m	16S-rRNA (V4 region)	Microbiome	226/– (3–6 m: 29/–)	Fecal microbiome at 3–6 months: *Bacteroidetes*, *Enterobacter* Metagenome functional enrichment of fatty acid metabolism	Fecal microbiome at 3–6 months: Clostridia, Firmicutes	Bunyavanich et al.[22]

Note: For abbreviations, see Appendix S1.

[a]AEDS as basic disease for subjects in both case and control groups, and the age is calculated by the pooled mean and SD from the age groups provided in the article.

TABLE 2 Characteristics of studies that compare CMA infants/children before and after intervention (intervention study).

Age				Intervention detail				Results: modifications in treatment vs. control		
years (y); months (m)	Analytical techniques	Type of analytical data	Sample size (treatment/control)	Duration (months)	Comparison groups	Control diet (Basic formula (BF))	Treatment diet (BF + intervention)	Increase	Decrease	Reference
0.55 ± 0.20 y	GC–MS	Metabolomics	9/5	1	Treatment vs. control	HWF	HWF with LGG	Kynurenate	3-indoleacetate	Salmi et al.[23]
2–12 m	Bacteria culture (CFU)	Microbiome	46/46	6	CMA subjects before intervention	–	EHF	*Lactobacilli*	Enterobacteria Bifidobacteria	Thompson-Chagoyan et al.[18]
6.5–10.4 m	FISH (16S rRNA-specific probes), GC–MS, NMR;	Microbiome, Metabolomics	16/16	2	CMA subjects b before intervention	–	EHF with 3.8% lactose	*Bifidobacteria*, LAB, SCFAs, lactate, threonine, uridine, histidine, tyrosine, methionine, TMAO, Phenylalanine, arginine/histidine, c–amino–butyrate/lysine	*Atopobium*, *Bacteroides/Prevotella*, clostridia, and sulfate-reducing bacteria, Total esters, ketones, alcohols, aldehydes, Valine/isoleucine	Francavilla et al.[14]
6.2 ± 4.3 m	qPCR (16S rRNA-specific primers and probes)	Microbiome	23/17	3	Treatment vs. control	RAAF	TAAF	Not reported	Total bacteria count	Dupont et al.[24]
1–12 m	qPCR-16S rRNA (V4 region), GC-FID	Microbiome; Metabolomics	12/7	6	Treatment vs. control, CMA subjects before intervention	EHC formula	EHC formula with LGG	After vs. before intervention: *Blautia*, *Roseburia*, *Coprococcus*, Compared to the control group: *Roseburia*, *Anaerofustis*. Butyrate	Not observed	Canani et al.[16]

(Continues)

TABLE 2 (Continued)

Age				Intervention detail				Results: modifications in treatment vs. control		
years (y); months (m)	Analytical techniques	Type of analytical data	Sample size (treatment/control)	Duration (months)	Comparison groups	Control diet (Basic formula (BF))	Treatment diet (BF + intervention)	Increase	Decrease	Reference
0.5–12 m	ELISA qPCR (16S rRNA-specific primers)	Microbiome, Immune response	123/121	6	Treatment vs. control	–	*Bifidobacterium bifidum* TMC3115	After 6 months: IL-10, total IgG_2, GM α-diversity (chao1 index, observed species), Bifidobacteriales, *Bifidobacterium*, *Lactobacillaceae*, *Lactobacillus*, *Turicibacter*, *Turicibacterales*, *Betaproteobacteria*, *Sutterella*, *Burkholderiales*, *Alcalligenaceae*, *Porphyromondaceae*, *Parabacteroides*, *Ruminococcus*, *Oscillospira*, *Lachnospira*	After 6 months: TNFα, IL-1, IL-6, IL-10, total IgE, *Anaerovibrio*, *Christensenelaceae*, *Oscillibacter*, *Bilophila*, *Dorea*, *Roseburia* Desulfovibrionales, Deltaproteobacteria, *Proteobacteria*, *Actinomyces*	Jing et al.[26]
10–15 m	PCR-16S rRNA (V3-V4 regions), qRT-PCR	Microbiome	14/14	1	CMA subjects before intervention	–	Probiotic mix: *Bifidobacterium breve* M-16 V, *Bifidobacterium longum* subsp. *longum* BB536, *Bifidobacterium longum* subsp. infantis M-63	Verrucomicrobia, Proteobacteria, *Akkermansia*, *Prevotella*, *Ruminococcus*, *Blautia*, *Bifidobacterium longum subspecies infantis*	Actinobacteria, *Actinomyces*, *Enterococcus*, *Streptococcus*, *Sutterella*	Mennini et al.[21]
<13 m	FISH (16S rRNA s-specific probes)	Microbiome	80/89	12	Treatment vs. control	AAF	Synbiotics: oligosaccharides (oligofructose, inulin), *Bifidobacterium breve* M-16 V	After 6 and 12 months: bifidobacteria	After 6 months: ER/CC	Chatchatee et al.[25]

Note: For abbreviations, see Appendix S1.

TABLE 3 CMA intervention studies with animal models.

Groups			Results[a]		
Case/Intervention	**Control**	**Platforms**	**Microbiome/Metabolome**	**CMA outcome and immune response**	**Reference**
G1: *L. rhamnosus* *G2*: *B. longum* subsp. *infantis* *G3*: *L. salivarius* *G4*: *B. bifidum* *G5*: *L. gasseri* *G6*: *B. animalis* subsp. *lactis*	AC: PBS	*Immunoglobulins* ELISA *Cytokines* IA (ex-BLG) *mRNA expression* q-PCR *Microbiome* qPCR (16 s rRNA-specific primers); bacteria culture	*Microbiome* Total bacteria ↓ G1, G2, G3, G4, G5 *Clostridium* cluster IVa ↑ G1, G6 *Staphylococci* abundance ↑ G1 *C. leptum*↑ G1, G6 *Prevotella*↑ G6 *C. leptum* ↓ G2, G3, G4, G5 *Prevotella*↓ G2, G3, G4, *Lactobacillus* ↓ G2, G3, G4, G5 *Clostridium* cluster I/II↓ G2, G3, G5 Clostridium cluster XI ↓ G2, G3, G4 *C. coccoides*↓ G2, G3, G4, G5 *Enterococcus* ↓ G2, G3, G4, G5 *Enterococcus* ↓ 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, RORγT ↓ G2, G3 FOXP3 ↑ G2, G3 IL-17a ↑ G1, G2, G3	Neau et al.[31]
G1: *L. rhamnosus* *G2*: *B. longum* subsp. *infantis* *G3*: *L. salivarius*	*AC*: PBS	*Microbiome* PCR -16S rRNA (V3-V4 regions) *Metabolome* GC-FID, UPLC-MS/MS *Immunoglobulins* ELISA *Cytokines* IA (ex-BLG) *mRNA expression* qPCR	*Metabolome* Kynurenine, N-acetylkunurenine ↓ G1, G2, G3 *Microbiome* Richness (OTU number) ↑ G1 Beta diversity ↑ G1, G2, G3 *Prevotellaceae* ↑ G1, G2, G3 *Marinifilaceae* ↑ G1, G2 *Ruminococcaceae* ↑ G1 *Helicobacteraceae* ↓ G1 *Ruminococcaceae* ↓ G2 *Lachnospiraceae* ↓ G1, G2, G3 *Deferribacteraceae* ↓ G1, G2 *Clostridiaceae* ↓ G1 *Peptococcaceae* ↓ G1, G3 *Burkholderiaceae* ↓ G1 *Anaeroplasmataceae*↓ 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 et al.[28]
G1 pWH *G2/G3*: pWH + short(G2)/long (G3) scGOS/lcFOS (9:1) *G4/G5*: pWH + short (G4)/long (G5) scGOS/lcFOS (9:1) + pAOS	*TC*: W *AC*: PBS	*Microbiota* PCR (16S rRNA V3-V4 regions) *Immunoglobulins* ELISA	*Microbiome* *Prevotella* ↑ G3, G4, G5 vs. G1 *Lactobacillus* ↓ G5 vs. G1	*Allergy markers* mMCP-1 ↓ G1, G5 vs. AC TSLP ↓ G1 vs. AC AASR ↓ TC, G1, G2, G4, G5 vs. AC SAS and body-T ↓ TC, G2 vs. AC	Kleinjans et al.[32]

(Continues)

TABLE 3 (Continued)

Groups			Results[a]		
Case/Intervention	**Control**	**Platforms**	**Microbiome/Metabolome**	**CMA outcome and immune response**	**Reference**
G1: mix of W peptides (PepMix) *G2*: scFOS and lcFOS (9:1)+ *B. breve* M-16 V (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_h1/T_h2 ↑ G3, TC T_{reg}, T_h17 ↑ AC vs. TC *Cytokines* (spleen) IFN-γ, IL-17A, IL-13, IL-5, IL-10 ↓ G3 vs. G1 and TC vs. AC IL-10 ↑ G3	Kostadinova et al.[33]
G1: mix of W peptides (*PepMix)* *G2*: scFOS and lcFOS (9:1)+ *B. breve* M-16 V (*FF/Bb)* *G3*: PepMix + FF/Bb	*TC*: W *AC*: PBS	*Metabolites* GC-FID *Lymphocytes* FC *mRNA expression* qPCR *Immunohistochemistry*	<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_{regs}/T_{effs} ↑ G3 vs. AC, G3 vs. G2, G3 vs. G1 (MLN) T_{regs} ↓ G3 vs. AC, G3 vs. G2, TC vs. AC (spleen) CD25+ ↓ G3 vs. G2 *DC* (SI-LP) $CD8\alpha^-CD11b^+/CD8\alpha^+CD11b^-$, $CD11b^+CD103^-$ ↑ G3 $CD8\alpha^+CD11b^-$↓ G1 *mRNA expression* FOXP3/GATA3 ↑ G3 (PP) FOXP3/RORγT ↑ 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 and G2 vs. G3 (colon) Galectin 9 ↓ TC Tbet/GATA3 ↓ G1 vs. AC, G1 vs. G3 (colon) <u>Part 2: Post-challenge</u> *Lymphocytes* (SI-LP) CD25+ Tcells ↑ G3 CD25+ Tcells ↑ G3 vs. G2 T_{reg} ↑ G1 *mRNA expression* (PP) Tbet/GATA3 ↑ G3 IFN-γ/IL-13 ↑ G3 vs. AC and G3 vs. G2	Kostadinova et al.[34]

TABLE 3 (Continued)

Groups			Results[a]		
Case/Intervention	**Control**	**Platforms**	**Microbiome/Metabolome**	**CMA outcome and immune response**	**Reference**
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) Bacteroidetes↑G3 Patescibacteria↑G3 Verrucomicrobia↓ G1 Proteobacteria↓ G1 Actinobacteria↓G3	*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 et al.[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* *Barnesiella*↑ *Clostridium*_XIVa↑ *Lactobacillus*↓ *Parvibacter*↓ Only observed in sham mice: *Bosea*	*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 et al.[27]

(Continues)

TABLE 3 (Continued)

Groups			Results[a]		
Case/Intervention	Control	Platforms	Microbiome/Metabolome	CMA outcome and immune response	Reference
G1: CMA FT *G2*: *Anaerostipes caccae*-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: *Enterococcus*↑ *Barnesiellaceae*↑ *Ruminococcus*↑*Ruminococcaceae*↑ *Coprobacillus* ↑ *Clostridiaceae* ↑ *Clostridiales* ↑ *Blautia* ↑ *Parabacteroides*↑ *Lachnospiraceae*↓ *Erysipelotrichaceae*↓ *Enterobacteriaceae*↓ *Streptococcus*↓*Enterobacteriaceae*↓ *Salmonella*↓*Anaerostipes caccae* ↓ *Transcriptome* *G1* vs. *F-HC*: (Mroh7, Cntn1, Slc9b2, Letm2, Acot12, Abcc2, Cyp3a59, Cyp2b10, Lrrn1, Me1, Akr1c19, Gstm1, Ces1f) ↑ (Tgfbr3, Acta1, Ror2, Slc22a13, Fbp1, Apcdd1) ↓	*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 *Transcriptome* Tgfbr3 ↓ G1 vs. G2, G1 vs. HC Ror2 ↓ G1, G2 vs. HC Ror2, Tgfbr3 positively correlated to *Lachnospiraceae*	Feehley et al.[29]

Note: For abbreviations, see Appendix S1.

[a]All results are vs. AC or C or S unless stated otherwise.

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 V4[16] 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,[26] the Lactobacillaceae and Lachnospiraceae families[26] and the genera like *Lactobacillus*,[18,26] *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* family[26] and genera like *Enterococcus*, *Streptococcus*,[21] *Anaerovibrio*, *Oscillibacter*, *Bilophila*, *Dorea*, and *Roseburia*[26] decreased under treatments.

The interventions also affected the Proteobacteria phylum[21] 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* family[18] and the *Sutterella* genus.[21] In the Bacteroidetes phylum, studies reported the interventions increased levels of the *Porphyromondaceae* family[26] 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 phylum[21] 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 to increase in the treatment group.[21]

In addition to the taxonomy changes, enhanced α-diversity (chao1, observed species),[26] reduced total bacteria,[24] and a decreased ratio of the *Eubacterium rectale*/*Clostridium coccoides* species[25] 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 kynurenate 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 IgG_2 levels in CMA patients.[26] The IgG_2 and IgE were respectively positively and negatively correlated with GM α-diversity (Chao1 index, observed species, community diversity index, and Shannon index). The intervention decreased the pro-inflammatory cytokines TNFα, IL-1β, 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 5 out of 12 infants in the treated group outgrew CMA after 6 months, compared to none in the control group.[16]

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 response[29–32] 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 FT[29] study reported GM modifications. Bacteria were identified using 16S rRNA gene-targeted primers, which targeted group/species-specific bacteria[31] or certain hypervariable regions (V3-V4,[27,28,32] V4[29] and eight other regions[30]).

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 significantly 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), an 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* species[29] 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 the 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*, Deferribacteraceae, 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 acetate,[33] butyrate,[33] and propionate[34] with a synbiotic diet. However, one study only observed reduced kynurenine and N-acetylkunurenine in probiotic-treated mice.[28] Additionally, an 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 were positively correlated with the *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-sensitization[31] 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 $sIgG_1$-to-$sIgG_{2a}$ ratio[31] 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 propria[33] 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 RORγT 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, and IFN-γ) which were induced by the PepMix intake.[33] Kleinjans et al.[32] showed that the effect of prebiotics on allergic symptoms varied with the composition and treatment duration.

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 α-diversity in both human[16,19,20] and animal[30] 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 probiotics[26] 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 were 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 that 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 nucleotide 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 propionate[34] 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, IgG_4, and 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 more effective treatment and preventive strategies.

AUTHOR CONTRIBUTIONS

Diana M. Hendrickx: Formal analysis; investigation; writing – review and editing; supervision. **Mariyana V. Savova:** Formal analysis; investigation; writing – original draft. **Pingping Zhu:** Investigation; writing – original draft; formal analysis. **Amy C. Harms:** Supervision; writing – review and editing. **Renate G. van der Molen:** Investigation; writing – review and editing. **Clara Belzer:** Conceptualization; funding acquisition; investigation; supervision; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known conflicts of interest.

PEER REVIEW

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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