Dietary Treatment with Extensively Hydrolyzed Casein Formula Containing the Probiotic *Lactobacillus rhamnosus* GG Prevents the Occurrence of Functional Gastrointestinal Disorders in Children with Cow's Milk Allergy

Rita Nocerino, RN[1,2], Margherita Di Costanzo, MD[1,2], Giorgio Bedogni, MD, PhD[3], Linda Cosenza, MD[1,2], Ylenia Maddalena, MD[1,2], Carmen Di Scala, RDN[1,2], Giusy Della Gatta, RDN[1,2], Laura Carucci, MD[1,2], Luana Voto, RDN[1], Serena Coppola, RDN[1,2], Anna Maria Iannicelli, RN[1], and Roberto Berni Canani, MD, PhD[1,2,4,5]

Objective To investigate whether the addition of the probiotic *Lactobacillus rhamnosus* GG (LGG) to the extensively hydrolyzed casein formula (EHCF) for cow's milk allergy (CMA) treatment could reduce the occurrence of functional gastrointestinal disorders (FGIDs).

Study design This cohort study included children with a positive history for CMA in the first year of life who were treated with EHCF alone or in combination with LGG and had evidence of immune tolerance acquisition to cow's milk for at least 12 months. FGID was diagnosed according to the Rome III diagnostic criteria by investigators unaware of previous treatment. A cohort of consecutive healthy children was also evaluated as a control population.

Results A total of 330 subjects were included, 110 per cohort (EHCF, EHCF+LGG, and healthy controls). The rate of subjects with ≥1 FGID was significantly lower in the EHCF+LGG cohort compared with the EHCF cohort (40% vs 16.4%; $P < .05$). In the EHCF+LGG cohort, a lower incidence was observed for all components of the main study outcome. The prevalence of FGIDs in the healthy cohort was lower than that in the EHCF cohort and similar to that in the EHCF+LGG cohort. The incidence rate ratio of FGIDs for the EHCF+LGG cohort vs the EHCF cohort (0.40; 95% CI, 0.25-0.65; $P < .001$) was unmodified after correction for age at CMA diagnosis, breastfeeding, weaning time, and presence of a first-degree relative with an FGID.

Conclusions These results confirm the increased risk for developing FGIDs in children with CMA and suggest that EHCF+LGG could reduce this risk. *(J Pediatr 2019; ■:1-6)*.

Functional gastrointestinal disorders (FGIDs), defined as a variable combination of chronic or recurrent gastrointestinal symptoms that cannot be explained in terms of structural or biochemical abnormalities, are a very common problem in childhood.[1] FGIDs are the result of any combination of motility disturbance, visceral hypersensitivity, altered mucosal and immune function, altered gut microbiota, and altered central nervous system processing.[2] There is increasing evidence suggesting that FGIDs can result from immune system dysregulation and gut microbiota dysbiosis.[3-8]

Cow's milk allergy (CMA) is a common food allergy in early childhood, with an estimated prevalence of 2%-3%.[9] CMA usually occurs in the first months of life and is associated with gastrointestinal inflammation and gut dysbiosis.[10] There is an emerging evidence pointing to CMA as a predisposing condition in patients with FGIDs.[11-15] Genome-wide association studies have identified single nucleotide polymorphisms common to allergies that confer a risk for FGIDs.[16-18] This evidence indicates the importance of effective strategies to prevent FGIDs in children with CMA.

Dietary intervention with extensively hydrolyzed casein formula (EHCF) supplemented with the probiotic *Lactobacillus rhamnosus* GG (LGG) has shown benefits in decreasing inflammation and gastrointestinal symptoms in children with CMA,[19] as well as in reducing the duration of disease and the occurrence of other allergic manifestations later in life.[20-22] Multiple mechanisms might be responsible for these clinical effects, including a positive effect on gut dysbiosis and epigenetic regulation of immune and nonimmune gene expression.[23-25] *L rhamnosus* GG also has been proposed for the treatment of pediatric FGIDs.[8]

CFU	Colony-forming units
CMA	Cow's milk allergy
EHCF	Extensively hydrolyzed casein formula
FGID	Functional gastrointestinal disorder
IRR	Incidence rate ratio
LGG	*Lactobacillus rhamnosus* GG
PWRM	Poisson working regression model
QPGS-RIII	Rome III diagnostic criteria

From the [1]Department of Translational Medical Science, University of Naples Federico II, Naples, Italy; [2]CEINGE Advanced Biotechnologies; [3]Clinical Epidemiology Unit, Liver Research Center, Basovizza, Trieste, Italy; [4]European Laboratory for the Investigation of Food-Induced Diseases; and [5]Task Force on Microbiome Studies, University of Naples Federico II, Naples, Italy

Supported in part by the Italian Ministry of Health (PE-2011-02348447) and by an unrestricted grant from Mead Johnson Nutrition to the Department of Translational Medical Science of the University of Naples Federico II. The Italian Ministry of Health and Mead Johnson Nutrition had no influence on the study design; the collection, analysis, and interpretation of data; the writing of the manuscript; or the decision to submit the manuscript for publication. The authors declare no conflicts of interest.

https://doi.org/10.1016/j.jpeds.2019.06.004

The present study was designed to test whether a dietary intervention with EHCF supplemented with LGG could influence the occurrence of FGIDs later in life in children who have outgrown CMA.

Methods

This prospective, open, nonrandomized trial was conducted from February 2013 to June 2018 in a cohort of potentially eligible children (both sexes, aged 4-6 years) with a previous positive clinical history of CMA still receiving follow-up for scheduled auxologic controls at a tertiary center for pediatric allergies. At enrollment, all subjects were in stable clinical condition, with no symptoms related to CMA. The inclusion criteria were children with a diagnosis of CMA obtained in the first year of life with the double-blind placebo-controlled food-challenge reference method, treated with EHCF (Nutramigen; Mead Johnson Nutrition, Chicago, Illinois) or EHCF containing the probiotic LGG (Nutramigen LGG; Mead Johnson Nutrition); gastrointestinal symptoms at CMA diagnosis; and oral challenge–proven evidence of acquisition of oral tolerance to cow's milk proteins from at least 12 months before enrollment. According to the manufacturer, the LGG concentration in the EHCF + LGG formulation is 2.5×10^7 to 5×10^8 colony-forming units (CFU)/g, and the guaranteed LGG concentration is 1.46×10^7 CFU/100 mL (1×10^6 CFU/g).

Patients who had been treated with other prebiotic or probiotic products and/or antibiotics in the previous 3 months and those with other food allergies, other allergic diseases, eosinophilic disorders of the gastrointestinal tract, chronic systemic diseases, congenital cardiac defects, active tuberculosis, autoimmune diseases, genetic diseases and chromosomal abnormalities, primary or secondary immunodeficiencies, chronic inflammatory bowel diseases, celiac disease, lactose intolerance, obesity, autism, neuropsychiatric disorders, evidence of *Helicobacter pylori* infection, cystic fibrosis, metabolic diseases, malignancy, chronic pulmonary diseases, malformations of the gastrointestinal and/or respiratory tract, history of gastrointestinal tract surgery, insufficient reliability or presence of conditions making the patient's compliance with the protocol unlikely, and participation in other studies were excluded.

During the same study period, consecutive healthy sex and age-matched children with a negative clinical history for any allergic condition and not at risk for atopic disorders (no first-degree family member affected by allergy) who were visiting our center because of minimal surgical procedures or vaccination program were also enrolled. Only subjects who met the inclusion criteria were invited to participate in the study.

Ethics

The study protocol, subject information sheet, informed consent form, and clinical chart were reviewed and approved by the Ethics Committee of the University of Naples Federico II. The study was conducted in accordance with the Declaration of Helsinki (Tokyo revision, 2004), according to Good Clinical Practice standards (CPMP/ICH/135/95), and the current Decree Law 196/2003 regarding personal data and all the requirements set out in the European regulations on this subject. The study has been registered in the Clinical Trials Protocol Registration System (ClinicalTrials.gov NCT01901380).

Data Collection

At baseline, after obtaining informed consent from the parent/guardian of each child, the clinical status of all study subjects was carefully assessed by a multidisciplinary team comprising pediatricians, pediatric gastroenterologists, pediatric nurses, and dietitians to exclude those with concomitant comorbidities. The presence of infectious diseases or other diseases was ruled out by means of a complete physical examination, including vital signs, neurologic status, growth status, nutritional status, hydration, skin evaluation, otoscopy, evaluation of oral cavity, respiratory/abdomen/lymph node examination, and genital examination.

Anamnestic, demographic, and clinical data (including data related to CMA); information on sociodemographic factors, family, and living conditions; parental history of allergic diseases and FGIDs; maternal smoking during pregnancy and environmental tobacco smoke exposure; number of siblings; and pet ownership were obtained from the parents of each child, collected in a specific clinical chart, and entered into the study database.

Two pediatric gastroenterologists, unaware of previous treatment for CMA and blinded to the study cohorts and study aims, performed a full clinical evaluation and submitted the validated questionnaires on the Rome III diagnostic criteria (QPGS-RIII)[26] to the parents to establish a possible diagnosis of FGID. In case of discordance regarding the presence of FGID, a further evaluation was performed by a third pediatric gastroenterologist. To conduct the study, the QPGS-RIII was translated into the Italian language. Focus groups of parents attending primary care clinics confirmed their understanding of the questionnaire. Consenting parents received information on the definitions of terms and symptoms of FGIDs before completing the QPGS-RIII Italian version.

Before the start of the study, all the involved researchers attended an investigator meeting in which the study protocol was described and the procedures and definitions were shared. All study procedures and assessments were performed as shown in **Figure 1** (available at www.jpeds.com).

Data Quality Assurance

All data were recorded anonymously. At the study center, designated investigators were required to enter all collected data in a case report form. Two researchers performed separate checks of data completeness, clarity, consistency, and accuracy and instructed site personnel to make any required corrections or additions.

Using a single data-entry method, all data recorded in the case report form were entered in the study database by the same researcher. Then the study dataset was reviewed, and

Table I. Characteristics of the study population

Characteristics	EHCF	EHCF+LGG	Healthy controls
Number of subjects	110	110	110
Male sex, n (%)	65 (59.1)	65 (59.1)	66 (60)
Cesarean delivery, n (%)	65 (59.1)	75 (68.2)	70 (63.6)
Term born, n (%)	99 (90)	94 (85.5)	98 (89.1)
Breastfed for at least 2 months, n (%)	79 (71.8)	70 (63.6)	78 (70.9)
Age at weaning, mo, median (IQR)	5 (4-6)	5 (4-6)	5 (4-6)
Siblings, n, median (IQR)	1 (0-1)	1 (0-1)	1 (0-1)
Familial risk for allergy, n (%)	81 (73.6)	82 (74.5)	76 (69.1)
FGID in first-degree relatives, n (%)	41 (37.3)	42 (38.2)	31 (28.2)
Exposure to passive smoking, n (%)	34 (30.9)	31 (28.2)	30 (27.3)
Maternal smoking during pregnancy, n (%)	30 (27.3)	27 (24.5)	28 (25.5)
Exposure to pets, n (%)	21 (19.1)	12 (10.9)	15 (13.6)
Immunoglobulin E-mediated CMA mechanism, n (%)	45 (40.9)	36 (32.7)	—
Age at CMA diagnosis, mo, median (IQR)	4 (2-8)	4 (2-9)	—
Gastrointestinal symptoms at CMA onset, n (%)	110 (100.0)	110 (100)	—
Cutaneous symptoms at CMA onset, n (%)	44 (40.0)	33 (30)	—
Respiratory symptoms at CMA onset, n (%)	10 (9.1)	8 (7.3)	—
Age at acquisition of immune tolerance to CMP, mo, median (IQR)	32 (18-38)	24 (15-34)*	—
Age at completion of QPGS-RIII questionnaire, mo, median (IQR)	59 (54-67)	61 (56-66)	60 (56-64)

CMP, cow's milk proteins.
*$P < .05$, EHCF+LGG vs EHCF.

data cleaning and verification were performed according to standard procedures. Once the databases was deemed complete and accurate, it was locked, and the statistical analysis was performed.

Study Outcomes

The primary outcome was the incidence of any FGIDs, as defined by Rome III diagnostic criteria,[26] in children who had outgrown CMA.

Sample Size

We calculated that 110 subjects with a previous double-blind placebo-controlled food-challenge–confirmed CMA diagnosis per group were needed to detect a difference of 0.18 in the incidence of FGIDs in the EHCF+LGG cohort vs the EHCF cohort (Pearson χ^2 test) at an α level of 0.05 and with a power of 0.80, under the expectation of an incidence of 0.44 in the EHCF+LGG group and 0.26 in the LGG group.

Statistical Analyses

Most continuous variables were not Gaussian-distributed, and all are reported as 50th percentile (median) and 25th and 75th percentiles (IQR). Discrete variables are reported as the number and proportion of subjects with the characteristic of interest. A Poisson working regression model (PWRM) with robust 95% CIs was used to estimate the incidence of and predictors of FGIDs. (A PWRM was used because a binomial regression model failed to converge for some of the models of interest. As expected, the estimates made by the binomial regression model and by the PWRM agreed in all cases in which the 2 converged). Univariable and multivariable PWRMs were used to evaluate the associations of FGIDs (discrete; 0 = no, 1 = yes) with potential predictors in 4 prespecified models with the following predictors: (M1) the formula used to treat previous CMA (discrete; 0 = EHCF, 1 = EHCF + LGG); (M2) as in model M1 plus sex (discrete; female = 0, male = 1) and age at diagnosis of CMA (continuous, in months); (M3) as in model M2 plus breastfed for ≥2 months (discrete; 0 = no, 1 = yes); and (M4) as in model M3 plus a first-degree relative with an FGID (discrete; 0 = no, 1 = yes). Multivariable fractional polynomials were used to verify that the multivariable associations of age and weaning with FGIDs were linear.[27] Marginal probabilities of FGIDs for the EHCF and EHCF+LGG cohorts were calculated from the PWRM model M1. The same probability was calculated separately for the healthy children.[28] Exact Poisson regression was used to evaluate the associations of FGID components with formula use (discrete; 0 = EHCF, 1 = EHCF + LGG).[29] (Here exact Poisson regression was used because of the unreliability of the asymptotic approximation in the presence of a small number of events). The duration of follow-up was taken into account by all the PWRM models as "exposure time". All statistical analyses were performed using Stata 15.1 (StataCorp, College Station, Texas).

Results

The flow of the subjects during the study is reported in **Figure 2** (available at www.jpeds.com). A total of 467 consecutively potentially eligible children were contacted and invited to participate to the study, of whom 52 refused to participate. The remaining 415 subjects were examined for eligibility, and 85 were excluded because of the presence of exclusion criteria, leaving a total of 330 children, 110 each in the EHCF, EHCF+LGG, and healthy control cohorts. All the children were from families of middle socioeconomic status and lived in urban areas. Baseline demographic and anamnestic features were similar among the 3 study cohorts, with the exception of the time of immune tolerance acquisition, which was reduced in the EHCF+LGG cohort compared with the EHCF cohort (**Table I**).

Table II reports the number and proportion of incident cases of FGIDs in the 3 cohorts. **Figure 3** plots the incidence rates of FGIDs in the EHCF+LGG and EHCF cohorts as estimated by the PWRM, taking into account the follow-up time. The absolute incidence of FGIDs was 0.40 (robust 95% CI, 0.31-0.50) in the EHCF cohort and

Table II. Number and proportion of incident cases of FGIDs in the 3 cohorts

FGIDs	EHCF	EHCF+LGG	Healthy controls
Total number	110	110	110
At least 1 FGID, n (%)	44 (40.0)	18 (16.4)	23 (20.9)
Cyclic vomiting, n (%)	2 (1.8)	1 (0.9)	2 (1.8)
Functional diarrhea, n (%)	0 (0.0)	0 (0.0)	40 (0.0)
Functional dyspepsia, n (%)	14 (12.7)	4 (3.6)	4 (3.6)
Rumination syndrome, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Irritable bowel syndrome, n (%)	6 (5.5)	5 (4.5)	3 (2.7)
Constipation, n (%)	18 (16.4)	7 (6.4)	11 (10.0)
Abdominal pain, n (%)	18 (16.4)	8 (7.3)	6 (5.5)
Abdominal migraine, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Aerophagia, n (%)	8 (7.3)	7 (6.4)	6 (5.5)
Nonretentive fecal incontinence, n (%)	2 (1.8)	1 (0.9)	2 (1.8)

0.16 (robust 95% CI, 0.09-0.23) in the EHCF+LGG cohort. This corresponds to a relative risk difference of −60% (95% CI, −79% to −40%) for EHCF+LGG vs EHCF ($P < .001$). In comparison, the incidence rate of FGIDs in the healthy cohort was 0.21 (robust 95% CI, 0.12 to 0.29), lower than that seen in the EHCF group and closer to that of the EHCF+LGG group (data not plotted).

Table III displays the association between formula use and the incidence of FGIDs in the EHCF+LGG cohort vs the LGG cohort as estimated by PWRM, taking into account the duration of follow-up. The incidence rate ratio (IRR) associated with use of the EHCF+LGG formula vs the LGG formula (IRR, 0.40; 95% CI, 0.25-0.65) was virtually unmodified after correction for age at CMA diagnosis (model M2), after further correction for breastfeeding and weaning duration (model M3), and, finally, for having a first-degree relative with an FGID (model M4).

In the EHCF+LGG cohort, a lower incidence was observed for all components of the main study outcome. The IRRs of the single components of FGIDs in the EHCF+LGG cohort vs the LGG cohort (exact Poisson regression, taking into account the follow-up time) were 0.49 (exact 95% CI, 0.01-9.48; $P = .99$) for cyclic vomiting, 0.28 (exact 95% CI, 0.07-0.90; $P = .03$) for functional dyspepsia, 0.82 (exact 95% CI, 0.20-3.24; $P = .98$) for irritable bowel syndrome, 0.38 (exact 95% CI, 0.14-0.96; $P = .04$) for constipation, 0.44 (exact 95% CI, 0.17-1.06; $P = .07$) for abdominal pain, 0.86 (exact 95% CI, 0.27-2.73; $P = .98$) for aerophagia, and 0.49 (exact 95% CI, 0.01-9.48; $P = .99$) for nonretentive fecal incontinence.

Discussion

The results of the present cohort study confirm a higher incidence of FGIDs in children with a history of CMA in the first months of life compared with children without a positive history of CMA. These findings support the hypothesis that prolonged gastrointestinal inflammation during an early period of neuronal plasticity may facilitate the occurrence of altered enteric nervous system hypersensitivity and dysmotility.[14,15]

We found that dietary intervention with EHCF+LGG could influence the occurrence of FGIDs in children aged 4-6 years who had outgrown CMA. In particular, a significant difference between the EHCF and the EHCF+LGG groups

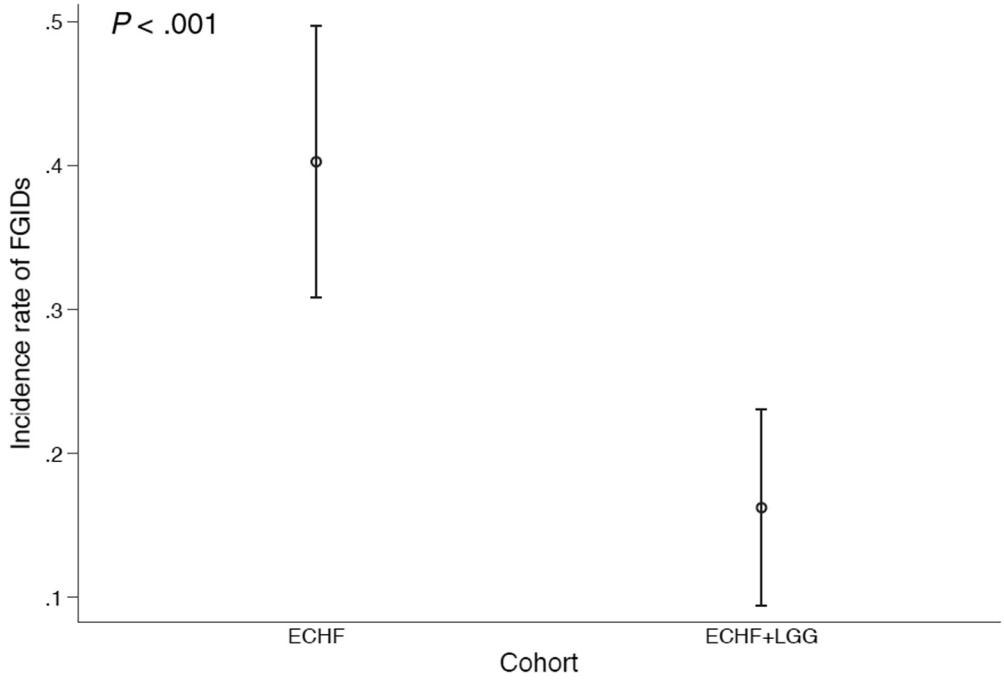

Figure 3. Incidence rates of FGIDs in the EHCF and EHCF+LGG cohorts. Values are mean and 95% CI as estimated by a robust PWRM using the duration of follow-up as the exposure time.

Table III. IRRs for the association between formula use and FGIDs

Variables	Model M1	Model M2	Model M3	Model M4
EHCF	1.00	1.00	1.00	1.00
EHCF+LGG	0.40 (0.25-0.65)*	0.40 (0.25-0.65)*	0.41 (0.25-0.65)*	0.40 (0.25-0.64)*
Age at CMA diagnosis, mo		1.09 (1.04-1.13)*	1.09 (1.04-1.14)*	1.08 (1.04-1.13)*
Male sex		0.62 (0.42-0.92)†	0.62 (0.42-0.92)†	0.62 (0.42-0.92)*
Breastfed for ≥2 mo			1.07 (0.70-1.63)	1.07 (0.72-1.61)
Weaning, mo			0.91 (0.77-1.08)	0.93 (0.79-1.11)
First-degree relative with FGIDs				1.41 (0.94-2.09)
Number of observations	220	220	220	220

Values are IRRs and 95% CIs as estimated by a robust PWRM using duration of follow-up as the exposure time.
*$P < .001$.
†$P < .05$.

was observed for the most common forms of FGIDs observed in children with CMA: functional dyspepsia, functional constipation, and functional abdominal pain.[11-15] Multiple mechanisms might be responsible for this protective effect, including a positive epigenetic regulation of immune and nonimmune gene expression and of gut microbiota structure and function (ie, increased production of the short-chain fatty acid butyrate), as was recently demonstrated in children receiving this dietary regimen.[23-25] In particular, the increased butyrate production could be relevant, considering that this gut microbiota-derived metabolite has been proposed for the management of FGIDs,[30,31] and that it is able to shape gastrointestinal tract motility, as well as visceral and central pain perception, also through epigenetic mechanisms.[32-34]

All these findings support the hypothesis of a pivotal role for gut microbiota dysbiosis and consequent alterations of intestinal immune and nonimmune functions in the pathogenesis of FGIDs,[2] and for the potential for LGG use to prevent these conditions in children with CMA.

Our study has several strengths. It included a large sample of children with a previous confirmed diagnosis of CMA followed by specialists at a tertiary pediatric allergy center with a high follow-up rate, the FGID diagnosis was performed by pediatric gastroenterologists, and the effect on the primary outcome was clinically relevant. Another strength is the availability of a cohort of healthy children, which, although the study was not designed to make formal between-group comparisons, helps put our findings into a more applied perspective by providing estimates of FGID incidence in healthy peers of children with CMA. Nonetheless, this study has some limitations. The main limitation is that it is a cohort study and not a randomized controlled trial. Another limitation is that our data cannot be generalized to children with conditions that were reasons for exclusion from the study. The effect of EHCF+LGG vs EHCF alone in these children remains to be addressed by future studies. Although our results show that EHCF+LGG reduces the occurrence of FGIDs, longer follow-up times are needed to test whether this effect persists over the long term. Finally, further studies are needed to elucidate the mechanisms of this beneficial effect.

In summary, this cohort study performed in a well-characterized population of children with previous diagnosis of CMA shows that EHCF+LGG can reduce the occurrence of FGIDs. Negative modulation of bidirectional pathways in the brain-gut-immuno-endocrine-microbiota axis could lead to an increased risk for FGIDs in children with CMA,[14] but our present results suggest that EHCF+LGG can counteract these pathways modulating the interaction among gut microbiota, epigenetic mechanisms, immune system, and gastrointestinal function. These effects lead to a positive impact on long-term protection against FGIDs in children with CMA. Future randomized controlled trials are needed to definitely ascertain the effect of EHCF+LGG in these children. ■

We thank the children and their parents for their enthusiastic participation in and dedication to the study and their visits to our department. We also thank all involved family pediatricians, nurses, technicians, and staff members for their support during this study.

Submitted for publication Mar 17, 2019; last revision received May 9, 2019; accepted Jun 4, 2019.

Reprint requests: Rita Nocerino, RN, Researcher Pediatric Nurse, University of Naples Federico II, Department of Translational Medical Science, Via S. Pansini, Naples 80131, Italy. E-mail: ritanocerino@alice.it

References

1. Hyams JS, Di Lorenzo C, Saps M, Shulman RJ, Staiano A, van Tilburg M. Childhood functional gastrointestinal disorders: child/adolescent. Gastroenterology 2016;150:1456-68.
2. Drossman DA. Functional gastrointestinal disorders: history, pathophysiology, clinical features, and Rome IV. Gastroenterology 2016;150:1262-79.
3. Liebregts T, Adam B, Bredack C, Gururatsakul M, Pilkington KR, Brierley SM, et al. Small bowel homing T cells are associated with symptoms and delayed gastric emptying in functional dyspepsia. Am J Gastroenterol 2011;106:1089-98.
4. Keely S, Walker MM, Marks E, Talley NJ. Immune dysregulation in the functional gastrointestinal disorders. Eur J Clin Invest 2015;45:1350-9.
5. Walker MM, Warwick A, Ung C, Talley NJ. The role of eosinophils and mast cells in intestinal functional disease. Curr Gastroenterol Rep 2011;13:323-30.
6. Walker MM, Talley NJ, Prabhakar M, Pennaneac'h CJ, Aro P, Ronkainen J, et al. Duodenal mastocytosis, eosinophilia and intraepithelial lymphocytosis as possible disease markers in the irritable bowel syndrome and functional dyspepsia. Aliment Pharmacol Ther 2009;29:765-73.
7. Walker MM, Aggarwal KR, Shim LS, Bassan M, Kalantar JS, Weltman MD, et al. Duodenal eosinophilia and early satiety in functional dyspepsia: confirmation of a positive association in an Australian cohort. J Gastroenterol Hepatol 2014;29:474-9.
8. Pärtty A, Rautava S, Kalliomäki M. Probiotics on pediatric functional gastrointestinal disorders. Nutrients 2018;10:E1836.

9. Sicherer SH. Epidemiology of food allergy. J Allergy Clin Immunol 2011;127:594-602.
10. Feehley T, Plunkett CH, Bao R, Choi Hong SM, Culleen E, Belda-Ferre P, et al. Healthy infants harbor intestinal bacteria that protect against food allergy. Nat Med 2019;25:448-53.
11. Saps M, Lu P, Bonilla S. Cow's milk allergy is a risk factor for the development of FGIDs in children. J Pediatr Gastroenterol Nutr 2011;52:166-9.
12. Di Nardo G, Cremon C, Frediani S, Lucarelli S, Villa MP, Stanghellini V, et al. Allergic proctocolitis is a risk factor for functional gastrointestinal disorders in children. J Pediatr 2018;195:128-33.e1.
13. Tan TK, Chen AC, Lin CL, Shen TC, Li TC, Wei CC. Preschoolers with allergic diseases have an increased risk of irritable bowel syndrome when reaching school age. J Pediatr Gastroenterol Nutr 2017;64:26-30.
14. Pensabene L, Salvatore S, D'Auria E, Parisi F, Concolino D, Borrelli O, et al. Cow's milk protein allergy in infancy: a risk factor for functional gastrointestinal disorders in children? Nutrients 2018;10:E1716.
15. Koloski N, Jones M, Walker MM, Veysey M, Zala A, Keely S, et al. Population-based study: atopy and autoimmune diseases are associated with functional dyspepsia and irritable bowel syndrome, independent of psychological distress. Aliment Pharmacol Ther 2019;49:546-55.
16. Brady RE, Glovsky MM, Opelz G, Terasaki P, Malish DM. The association of an HLA "asthma-associated" haplotype and immediate hypersensitivity in familial asthma. Int J Immunogenet 1981;8:509-17.
17. Kontakioti E, Domvri K, Papakosta D, Daniilidis M. HLA and asthma phenotypes/endotypes: a review. Hum Immunol 2014;75:930-9.
18. Vazquez-Roque MI, Camilleri M, Smyrk T, Murray JA, O'Neill J, Carlson P, et al. Association of HLA-DQ gene with bowel transit, barrier function, and inflammation in irritable bowel syndrome with diarrhea. Am J Physiol-Gastrointest Liver Physiol 2012;303:G1262-9.
19. Baldassarre ME, Laforgia N, Fanelli M, Laneve A, Grosso R, Lifschitz C. *Lactobacillus* GG improves recovery in infants with blood in the stools and presumptive allergic colitis compared with extensively hydrolyzed formula alone. J Pediatr 2010;156:397-401.
20. Berni Canani R, Nocerino R, Terrin G, Coruzzo A, Cosenza L, Leone L, et al. Effect of *Lactobacillus* GG on tolerance acquisition in infants with cow's milk allergy: a randomized trial. J Allergy Clin Immunol 2012;129:580-2.
21. Berni Canani R, Nocerino R, Terrin G, Frediani T, Lucarelli S, Cosenza L, et al. Formula selection for management of children with cow's milk allergy influences the rate of acquisition of tolerance: a prospective multicenter study. J Pediatr 2013;163:771-7.e1.
22. Berni Canani R, Di Costanzo M, Bedogni G, Amoroso A, Cosenza L, Di Scala C, et al. Extensively hydrolyzed casein formula containing *Lactobacillus rhamnosus* GG reduces the occurrence of other allergic manifestations in children with cow's milk allergy: 3-year randomized controlled trial. J Allergy Clin Immunol 2017;139:1906-13.e4.
23. Berni Canani R, Sangwan N, Stefka AT, Nocerino R, Paparo L, Aitoro R, et al. *Lactobacillus rhamnosus* GG supplemented formula expands butyrate producing bacterial strains in food allergic infants. ISME J 2016;10:742-50.
24. Berni Canani R, Paparo L, Nocerino R, Cosenza L, Pezzella V, Di Costanzo M, et al. Differences in DNA methylation profile of Th1 and Th2 cytokine genes are associated with tolerance acquisition in children with IgE-mediated cow's milk allergy. Clin Epigenetics 2015;7:38.
25. Paparo L, Nocerino R, Cosenza L, Aitoro R, D'Argenio V, Del Monaco V, et al. Epigenetic features of FoxP3 in children with cow's milk allergy. Clin Epigenetics 2016;8:86.
26. Rasquin A, Di Lorenzo C, Forbes D, Guiraldes E, Hyams JS, Staiano A, et al. Childhood functional gastrointestinal disorders: child/adolescent. Gastroenterology 2006;130:1527-37.
27. Royston P, Sauerbrei W. Multivariable model-building: A pragmatic approach to regression analysis based on fractional polynomials for modelling continuous variables. Chichester, UK: Wiley; 2008.
28. Williams R. Using the margins command to estimate and interpret adjusted predictions and marginal effects. Stata J 2012;12:308-31.
29. Mehta CR, Patel NR. Exact logistic regression: theory and examples. Stat Med 1995;14:2143-60.
30. Scarpellini E, Lauritano EC, Lupascu A, Petruzzellis C, Novi ML, Roccarina D, et al. Efficacy of butyrate in the treatment of diarrhoea-predominant irritable bowel syndrome. Dig Liver Dis 2007;(Suppl 1):19-22.
31. Załęski A, Banaszkiewicz A, Walkowiak J. Butyric acid in irritable bowel syndrome. Prz Gastroenterol 2013;8:350-3.
32. Fukui H, Xu X, Miwa H. Role of gut microbiota-gut hormone axis in the pathophysiology of functional gastrointestinal disorders. J Neurogastroenterol Motil 2018;24:367-86.
33. Russo R, De Caro C, Avagliano C, Cristiano C, La Rana G, Mattace Raso G, et al. Sodium butyrate and its synthetic amide derivative modulate nociceptive behaviors in mice. Pharmacol Res 2016;103:279-91.
34. Stilling RM, van de Wouw M, Clarke G, Stanton C, Dinan TG, Cryan JF. The neuropharmacology of butyrate: the bread and butter of the microbiota-gut-brain axis? Neurochem Int 2016;99:110-32.

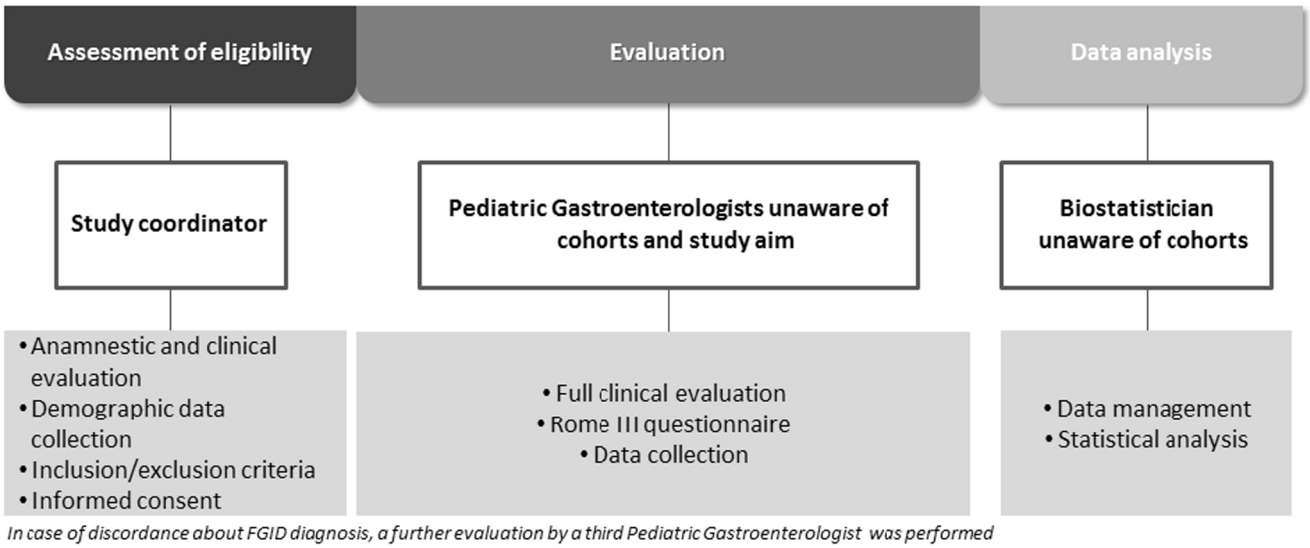

Figure 1. Study design.

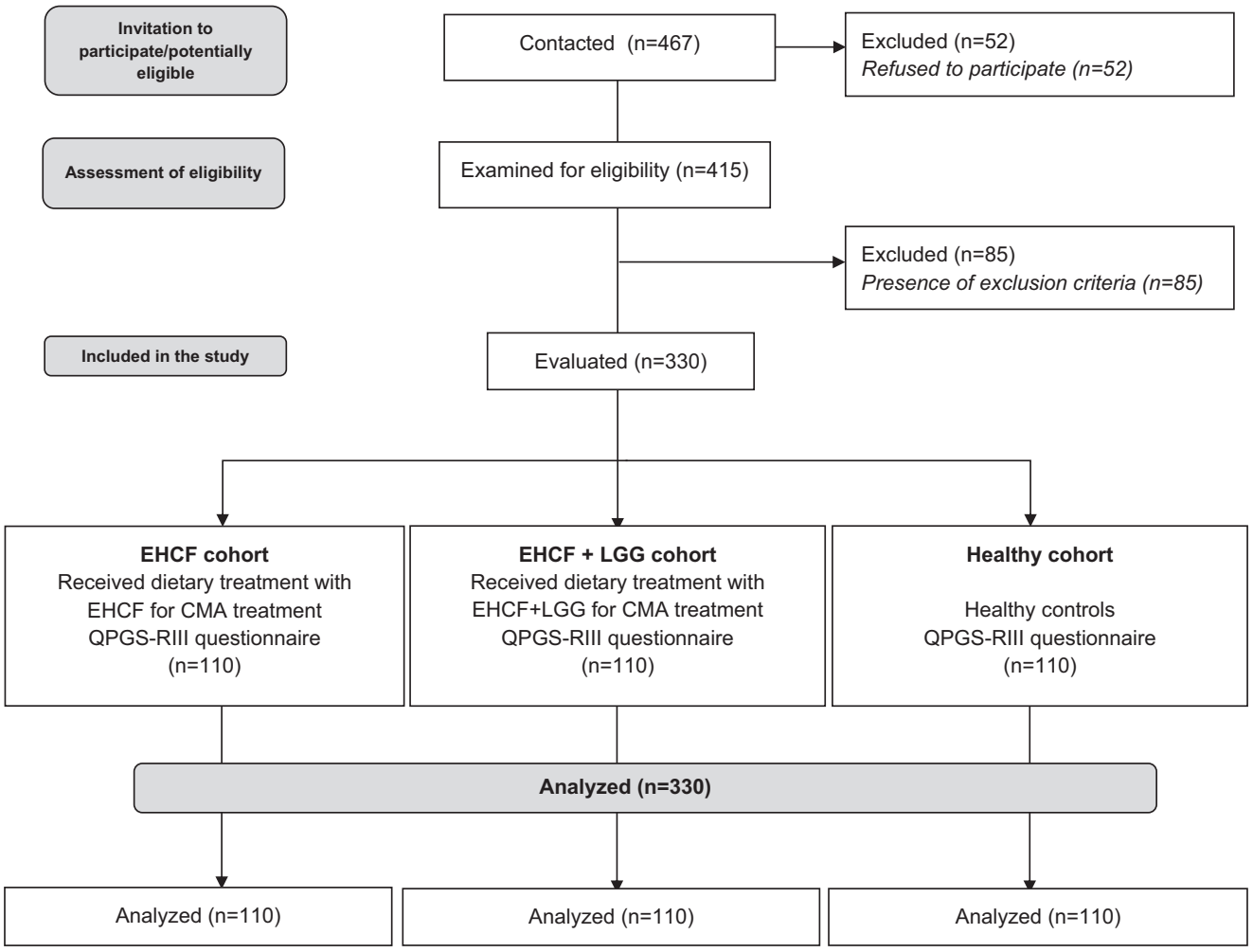

Figure 2. Flow of children through the study.