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Enrichment of formula in probiotics or prebiotics and risk of infection and allergic diseases up to age 5.5 years in the nationwide ELFE cohort

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Conflict of interest:

Amandine Divaret-Chauveau has been invited to allergology and pediatric congresses (inscription and accommodation) by Mead Johnson, Sodilac and Nutricia. Karine Adel-Patient has been invited by Stallergènes to speak at a conference, outside of the submitted work. None of the other authors declare any conflicts of interest.

Abbreviations:

FOS: fructo-oligosaccharides

GOS: galacto-oligosaccharides

GBTM: group-based trajectory modelling

LRTI: lower respiratory tract infection

PANDiet: Probability of Adequate Nutrient intake based Diet quality index

RCT: randomized controlled trials

URTI: upper respiratory tract infection

1 **ABSTRACT**

2 **Background:** An increasing number of infant and follow-on formulas are enriched with
3 probiotics and/or prebiotics; however, evidence for health effects of such enrichment in early
4 childhood remains inconclusive.

5 **Objective:** The present study aimed to assess, whether consumption of formula enriched with
6 probiotics or prebiotics was associated with the risk of infection and allergic diseases in early
7 childhood.

8 **Methods:** Analyses involved data for 8,389 formula-fed children from the *Etude*
9 *Longitudinale Française depuis l'Enfance* (ELFE) cohort. Enrichment of the formula with
10 probiotics or prebiotics that was consumed from age 2 to 10 months was identified by the
11 formula ingredient list. Lower respiratory tract infection (LRTI), upper respiratory tract
12 infection (URTI), gastrointestinal infection, wheezing, asthma, food allergy and itchy rash
13 were prospectively reported by parents up to age 5.5 years. Adjusted logistic regression
14 models were used to assess associations between consumption of enriched formula and risk of
15 infection and allergic diseases.

16 **Results:** At age 2 months, more than half of formula-fed infants consumed probiotic-enriched
17 formula and only one in 10 consumed prebiotic-enriched formula. Consumption of
18 *Bifidobacterium lactis*-enriched formula at 2 months was associated with a lower risk of LRTI
19 (OR[95% CI]=0.84[0.73-0.96]). Consumption of *Bifidobacterium breve*-enriched formula up
20 to 6 months was associated with a higher risk of LRTI (OR[95% CI]=1.75[1.29-2.38]) and
21 asthma (OR[95% CI]=1.95[1.28-2.97]), while its consumption from 6-10 months was
22 associated with a lower risk of LRTI (OR[95% CI]=0.64[0.48-0.86]) and asthma
23 (OR[95% CI]=0.59[0.40-0.88]). Moreover, consumption of *Streptococcus thermophilus* from
24 6-10 months was associated with a higher risk of asthma (OR[95% CI]=1.84[1.29-2.63]). No
25 significant association was found for gastrointestinal infection, food allergy, and itchy rash.

26 Overall, consumption of prebiotic-enriched formula was not significantly associated with
27 infection and allergy risk.

28 **Conclusions:** Associations between consumption of probiotic-enriched formula and risk of
29 respiratory symptoms differ according to the strain considered and consumption period.

30 Further well-designed studies are needed to confirm these results.

31 **Keywords:** Infancy, Enrichment, Infant formula, Prebiotic, Probiotic, Infection, Allergy,

32 Birth cohort

33 INTRODUCTION

34 There is increasing evidence that the microbiome could influence the infant's immune system
35 development and that an imbalanced microbial composition and reduced diversity (dysbiosis)
36 could promote susceptibility to metabolic and immune- related diseases (1, 2). Colonization
37 of the digestive tract and formation of the newborn's microbiota begins mainly at birth, and
38 both its composition and function are variable for the first 3 years of life, after which it
39 stabilizes (2, 3). The infant gut microbiome composition is affected by several factors such as
40 the maternal microbiota composition, mode of delivery, feeding practices, environmental
41 exposures and antibiotics use (2, 4-7).

42 Breastfeeding is the preferred nutrition option for newborns because it provides all of their
43 nutritional needs (8). Human breast milk is a complex biofluid that contains proteins, lipids,
44 carbohydrates and various minerals and vitamins (7, 9). It also contains a wide range of non-
45 nutritional bioactive components and a variety of both prebiotics and probiotics that ensure
46 neonate gut colonization by microbes beneficial for metabolism, immune development and
47 lifelong health (1, 5, 10). According to the Food and Agriculture Organization and the World
48 Health Organization, probiotics can be considered "living microorganisms, which when
49 administered in adequate amounts confer health benefits on the host", while prebiotics are
50 defined as "non-digestible food ingredients that beneficially affect the host by selectively
51 stimulating the growth and/or activity of one or a limited number of bacterial species already
52 established in the colon, and thus in effect improve host health" (11).

53 Although exclusive breastfeeding for the first 6 months of life or at least 4 months is the
54 nutritional recommendation for healthy term infants (8), several studies of infant feeding
55 practices have shown a high level of non-compliance with these recommendations (12-14).
56 When exclusive breastfeeding is not attainable, infant formula is the preferred option. To

57 reproduce some of the beneficial aspects of breastmilk, a large number of formulas are
58 enriched with probiotics, prebiotics, or both (i.e., symbiotics) (15, 16).
59 Several randomized controlled trials (RCTs) have assessed the effect of probiotic-, prebiotic-
60 or symbiotic-enriched formula on infant health. Some showed a beneficial effect of probiotics
61 and prebiotics on respiratory or gastrointestinal infections, antibiotics use, atopic dermatitis or
62 other types of allergies (17-22), but others did not report any association with incidence of
63 diarrhoea episodes, respiratory illnesses and allergic manifestations (18-24). Moreover,
64 systematic reviews concluded that evidence was not conclusive for recommending the routine
65 use of probiotic-, prebiotic- or symbiotic-enriched formula in healthy term infants (25-28).
66 The Committee on Nutrition from the European Society for Pediatric Gastroenterology,
67 Hepatology and Nutrition also concluded lack of data to recommend their routine use (29).
68 In this study, we aimed to describe the consumption of probiotic- or prebiotic-enriched
69 formula and its association with risk of infection and allergic diseases in early childhood.

70 **SUBJECTS AND METHODS**

71 **Study design**

72 This analysis was based on data from the *Etude Longitudinale Française depuis l'Enfance*
73 (ELFE) study, a multidisciplinary nationwide birth cohort including 18,329 children born in
74 2011 in 320 participating maternity units among a random sample of 349 maternities in
75 mainland France (30). Inclusion began in April 2011 and took place during 25 selected
76 recruitment days over four waves of 4 to 8 days each that covered all four seasons. Inclusion
77 criteria were singleton or twins born after 33 weeks' gestation, from mothers aged 18 years or
78 older and not planning to move outside of metropolitan France in the next 3 years. Foreign
79 families also participated in the study if mothers were able to read French, Arabic, Turkish or
80 English.

81 Participating mothers had to provide written consent for their own and their child's
82 participation. Fathers signed the consent form for the child's participation when present at
83 inclusion or were informed about their rights to oppose it. The ELFE study was approved by
84 the Advisory Committee for Treatment of Health Research Information (Comité Consultatif
85 sur le Traitement des Informations pour la Recherche en Santé), the National Data Protection
86 Authority (Commission Nationale Informatique et Libertés), and the National Statistics
87 Council.

88 **Data collection**

89 Families were followed by phone interview at age 2 months, 1 year, 2 years, 3.5 years and 5.5
90 years. They also completed a monthly internet/paper-based questionnaire on infant feeding
91 from age 3 to 10 months.

92 ***Infant milk feeding***

93 At the 2-month interview, if relevant, the age at first introduction of infant formula was
94 collected.

95 From age 2 to 10 months, the name and brand of the formula used was collected monthly for
96 formula-fed infants. Classification of all reported infant formulas was based on their
97 nutritional characteristics (ingredient list and nutritional composition). Enrichment of the
98 formula with probiotics (*Bifidobacterium breve* [BC50], *Bifidobacterium lactis* [BB12],
99 *Bifidobacterium infantis*, other *Bifidobacterium*, *Lactobacillus reuteri* [DSM 17938],
100 *Lactobacillus fermentum* [CECT5716], *Lactobacillus rhamnosus*, other non-specified
101 *Lactobacillus*, *Streptococcus thermophilus*) or with prebiotics (galacto-oligosaccharides
102 [GOSs] and fructo-oligosaccharides [FOSs]) was identified from the ingredient list.

103 Infants receiving both breast milk and formula were classified according to the type of
104 formula they received.

105 ***Infections and allergies***

106 In this study, severe/frequent infections, as well as allergies from age 2 months to 5.5 years
107 were assessed as follows:

- 108 - Ever gastrointestinal infection: parental report of at least one hospitalization for
109 gastroenteritis/dehydration or emergency consultation for diarrhea/vomiting/dehydration
- 110 - Ever upper respiratory tract infection (URTI): parental report of at least one
111 hospitalization for URTI or emergency consultation for otalgia, at least three otitis events
112 from birth, at least three laryngitis events from birth, or at least three angina events from
113 birth
- 114 - Ever lower respiratory tract infection (LRTI): parental report of at least one bronchiolitis
115 event or one hospitalization for bronchitis/bronchiolitis/pneumopathy
- 116 - Ever wheezing, asthma, itchy rash or food allergy: at least one parental report of
117 wheezing in the chest, medical diagnosis of asthma, itchy rash, or medical advice to
118 avoid certain foods due to a food allergy.

119 ***Other variables***

120 Parental socio-demographic characteristics considered in this study were: maternal age at
121 delivery (18–24, 25–29, 30–34, ≥ 35 years), number of older children in the household (no
122 sibling, one sibling, at least two siblings), maternal migration status (“majority population”
123 which included women born to two French parents [inside or outside of France]),
124 “descendants of migrants” including women born in France with at least one non-French
125 parent, “migrants” including women not born in France and without French citizenship at
126 birth), maternal education level (up to lower secondary, upper secondary, intermediate, 3-year
127 university degree, at least 5-year university degree), employment status during pregnancy
128 (employed, unemployed, not in the labour force [e.g., housewife, student, disabled, retired])

129 and monthly household income per consumption unit (<€750, €751–1,111, €1,112–1,500,
130 €1,501–1,944, €1,945–2,500, >€2,500). From the postal code of residence, we determined the
131 region of residence (Paris region, North, East, East Paris Basin, West Paris Basin, West,
132 South-West, South-East, Mediterranean) and the city size (rural area, urban area; >2000
133 inhabitants for rural area).

134 Maternal health characteristics included self-reported height and pre-pregnancy weight used
135 to calculate pre-pregnancy body mass index (BMI) (<18.5, 18.5–24.9, 25.0–29.9,
136 ≥ 30.0 kg/m²), smoking status (never smoker, smoker only before pregnancy, smoker only in
137 early pregnancy, smoker throughout pregnancy) and diet quality during pregnancy (measured
138 by using the Probability of Adequate Nutrient intake based Diet quality index [PANDiet]
139 score, which reflects nutrient-based reference guidelines adapted for pregnancy; total scores
140 range from 0 to 100) (31).

141 Newborn characteristics were collected from the medical record: child sex, gestational age,
142 birth weight and delivery mode (C-section, vaginal). Birth weight categories (small, adequate
143 or large for gestational age) were defined according to Audipog reference curves (32). The 2-
144 month questionnaire collected the type of the physician consulted for the first visit after
145 delivery (paediatrician, other child doctor, general practitioner, other including emergency).

146 Parent and sibling history of allergy (hay fever, asthma, eczema) was collected at the 2-month
147 interview. Children were considered to have a family history of allergy if at least one parent
148 or sibling had a history of allergy.

149 **Study sample**

150 Children whose parents withdrew their consent within the first year and requested deletion of
151 their data (n=57) were excluded from this study. We also randomly excluded one twin out of
152 two (n=287) to avoid family clustering.

153 For the present analysis, children not followed at the 2-month interview (n=1,696), those
154 exclusively breastfed at age 2 months (n=5,045), those without sufficient data on the infant
155 formula consumed at age 2 months (n=667), those with a medical diagnosis of cow's milk
156 protein allergy at age 2 months (n=193) or those without any data on infection and allergy
157 from age 2 months to 5.5 years (n=1,995) were excluded. The main analyses then involved
158 8,389 children (**Figure 1**).

159 **Statistical analyses**

160 *Descriptive statistics*

161 Differences between excluded children (n=9,596) and children included in the main analyses
162 (n=8,389) were assessed with Student *t* test and chi-square test for continuous and categorical
163 variables, respectively.

164 To take into account the inclusion procedure and biases related to non- consent and attrition,
165 the data were weighted (33) in sensitivity analyses. Weighting also included calibration on
166 margins from the state register's statistical data and the 2010 French National Perinatal study
167 (34) for the following variables: age, region, marital status, migration status, education level,
168 and primiparity.

169 *Main analyses*

170 Multiple imputations were performed to deal with missing data (covariates and longitudinal
171 trajectories of the type of formula consumed from age 2 to 10 months) (35, 36). We assumed
172 that data were missing at random, i.e. that missing values in the data set may depend on the
173 value of other observed variables, but cannot depend on the values of unobserved variables
174 (37). We generated five independent data sets by using the fully conditional specification
175 method (MI procedure) and the calculated estimates of pooled effects (MIANALYSE
176 procedure; SAS software). Missing data for categorical variables were imputed by using a

177 multinomial model, for ordinal or binary variables by using logistic regression and for the
178 continuous variables by using linear regression (36).

179 Adjusted associations between consumption of enriched formula and risk of infection or
180 allergic diseases were examined by logistic regression models adjusted for confounding
181 factors. Potential confounding factors were identified first from the literature, then selected by
182 using the directed acyclic graphs method (38). The main model was adjusted for maternal and
183 household characteristics (maternal age, education level, migration status, employment during
184 pregnancy, smoking status, city size, region of residence, maternal diet quality during
185 pregnancy [PANDiet score], number of older children, household income and family history
186 of allergy), child characteristics (sex, gestational age, birth weight category, type of physician
187 consulted after discharge, C-section delivery, age at infant formula introduction) and variables
188 related to study design (maternity size and recruitment wave). A second model was performed
189 to additionally account for the use of antibiotics up to age 1 year.

190 Formula enriched with probiotics or prebiotics was first examined at the 2-month follow-up
191 (enriched vs not enriched). Then, to account for the temporal evolution in the consumption of
192 prebiotic or probiotic-enriched formula between child age 2 and 10 months, we identified
193 groups of children with similar longitudinal patterns of consumption by using the Nagin's
194 method for group-based trajectory modelling (GBTM) (39) with the TRAJ procedure from
195 SAS software. These trajectories were modeled for children who consumed formula for at
196 least 2 follow-ups from age 2 to 10 months.

197 *Sensitivity analyses*

198 Sensitivity analyses were conducted for children whose parents did not report any eczema,
199 wheezing, gastrointestinal infection or respiratory tract infection at age 2 months (n=6,502), to
200 address potential reverse causation bias.

201 Additional sensitivity analyses involved exclusively formula-fed infants at age 2 months
202 (n=6,251). Moreover, to deal with the issue of change in infant formula from birth to the 2-
203 month interview, a sensitivity analysis involved children without any change in infant formula
204 up to age 2 months (n=4,399). Finally, given our previous findings on the association between
205 partially hydrolyzed formula and allergy (40), a sensitivity analysis was conducted among
206 infants consuming non-hydrolyzed formula (n=7,805).

207 To deal with selection and attrition bias, a sensitivity analysis was conducted with weighted
208 data according to the weighting described previously. Finally, as the main analyses used
209 multiple imputation to deal with missing data, the main analyses were replicated on the
210 complete-case sample.

211 All analyses were carried out with SAS 9.4 (SAS Institute, Cary, NC, USA).

212 **RESULTS**

213 As compared with excluded children, included children frequently were the first born (46.6%
214 vs 42.3%, $p < 0.0001$), were born to older mothers (mean age 31.0 ± 4.9 vs 30.6 ± 5.2 years,
215 $p = 0.00003$), mothers who were employed during pregnancy (75.2% vs 67.3%, $p < 0.0001$) and
216 had a higher education level (40.8% vs. 35.9% with at least 3-year university degree,
217 $p < 0.0001$). The characteristics of included children are described in **Table 1**.

218 **Enrichment of formulas with prebiotics and probiotics**

219 At the 2-month follow-up, more than half of formula-fed infants consumed probiotic-enriched
220 formula. The enrichment with prebiotics usually using a mixture of GOS and FOS, concerned
221 9.1% (95% confidence interval=8.4%-9.8%) of infants.

222 The weighted prevalence of formula-fed infants consuming probiotic-enriched formula at the
223 2-month follow-up is presented in **Figure 2**. The main probiotics added to infant formula

224 were *Streptococcus thermophilus*, *Lactobacillus reuteri* (DSM 17938), and *Bifidobacterium*
225 *breve* (BC50), followed by *Lactobacillus fermentum* (CECT5716) and *Bifidobacterium lactis*
226 (*BB12*). Enrichment with *S. thermophilus* was always associated with another enrichment (*B.*
227 *breve*, *B. lactis* or *L. reuteri*). The combined enrichment with *Bifidobacterium infantis* and
228 *Lactobacillus rhamnosus* was consumed by less than 3% of infants and was not further
229 examined.

230 The longitudinal trajectories of consumption of formulas enriched with probiotics or
231 prebiotics from age 2 to 10 months are presented in **Supplementary Figure 1**. For *B. breve*
232 and *S. thermophilus*-enriched formula, a 4-group solution was identified with infants
233 consuming 1) never-enriched formula, 2) enriched formula up to age 5-6 months, 3) enriched
234 formula from age 6-7 months and 4) enriched formula throughout infancy. For *L. fermentum*
235 and *L. reuteri*-enriched formula, a 3-group solution was identified with infants consuming 1)
236 never-enriched formula, 2) occasionally-enriched formula and 3) enriched formula throughout
237 infancy. For *B. lactis*-enriched formula, a 2-group solution was identified with infants
238 consuming 1) never-enriched formula and 2) enriched formula throughout infancy. For GOS-
239 enriched formulas, a 3-group solution was identified, with infants consuming 1) never-
240 enriched formula, 2) early use of enriched formula and 3) delayed use of enriched formula.

241 **Probiotic-enriched formula and risk of infection or allergic diseases up to age 5.5 years**

242 The findings of unadjusted analyses are presented in **Supplemental tables 1 and 2**.

243 ***Bifidobacterium* enrichment**

244 In the main model, compared to infants consuming non-*Bifidobacterium*-enriched formula,
245 those consuming *B. lactis*-enriched formula at age 2 months were at lower risk of LRTI and
246 wheezing from age 2 months to 5.5 years (**Table 2**). We found no significant association
247 between *Bifidobacterium* enrichment and URTI, asthma (**Table 2**), gastrointestinal infection,

248 food allergy or itchy rash up to age 5.5 years (**Table 3**). Further adjustment for antibiotic use
249 up to age 1 year did not substantially modify the risk estimates, but the observed associations
250 between consumption of *B. lactis*-enriched formula and wheezing were no longer significant
251 (**data not shown**).

252 The observed associations for the consumption of *Bifidobacterium*-enriched formula and the
253 risk of respiratory symptoms, gastrointestinal infection, food allergy and itchy rash were
254 consistent in analyses among exclusively formula-fed infants (**Supplementary tables 3 and**
255 **4**), in weighted analyses and analyses in the complete-case sample (**Supplementary tables 5-**
256 **8**). Similar findings were also observed in the other sensitivity analyses (analyses conducted
257 in subsamples of infants without any symptoms up to 2 months, infants without any change in
258 formula up to age 2 months (**Supplementary tables 9-12**), and infants consuming non-
259 hydrolyzed formula (**data not shown**).

260 According to the GBTM method, infants always consuming *B. lactis*-enriched formula from
261 age 2 to 10 months were also at lower risk of URTI and asthma up to age 5.5 years (**Table 2**).
262 Consumption from age 6 months of *B. breve*-enriched formula was related to a lower risk of
263 LRTI and asthma up to age 5.5 years, whereas consumption in the first months only was
264 related to a higher risk of LRTI and asthma up to age 5.5 years (**Table 2**). Similar findings
265 were observed after further adjustment for antibiotic use (data not shown) and in sensitivity
266 analyses (**Supplementary tables 3-12**).

267 *Lactobacillus enrichment*

268 In the main model, compared to consumption of non-*Lactobacillus*-enriched formula,
269 consumption of *L. reuteri*-enriched or *L. fermentum*-enriched formula at age 2 months was not
270 associated with occurrence of infection or allergic diseases up to age 5.5 years (**Tables 2 and**
271 **3**). After further adjustment for antibiotic use, consumption of *L. reuteri*-enriched formula at

272 age 2 months was related to a lower risk of URTI up to age 5.5 years (OR=0.83 and 95%
273 CI=0.72-0.97; data not shown).

274 According to the GBTM method, occasional, or regular consumption of *L. reuteri*-enriched
275 formula from age 2 to 10 months was not associated with occurrence of infection or allergic
276 diseases (**Tables 2 and 3**). Occasional consumption of *L. fermentum*-enriched formula from
277 age 2 to 10 months was related to a higher risk of URTI up to age 5.5 years, but not regular
278 consumption. Consumption of *L. fermentum*-enriched formula was not related to other
279 infections or allergic diseases. Similar findings were observed after further adjustment for
280 antibiotic use (data not shown) and in sensitivity analyses (**Supplementary tables 3-12**).

281 *Streptococcus thermophilus* enrichment

282 In the main model, compared to consumption of non-*S. thermophilus*-enriched formula at age
283 2 months, consumption of *S. thermophilus*-enriched formula was not related to infections or
284 allergic diseases up to age 5.5 years (**Tables 2 and 3**). Similar findings were observed after
285 further adjustment for antibiotic use (data not shown).

286 According the GBTM method, consumption of *S. thermophilus*-enriched formula from age 2
287 to 10 months was related to a higher risk of URTI up to age 5.5 years. In addition,
288 consumption of *S. thermophilus*-enriched formula up to age 6 months was related to a lower
289 risk of asthma up to age 5.5 years, whereas consumption from 6 months was related to a
290 higher risk of asthma (**Table 2**). Consumption of *S. thermophilus*-enriched formula was not
291 related to other infections or allergic diseases (**Tables 2 and 3**). Similar findings were
292 observed after further adjustment for antibiotic use (data not shown) and in sensitivity
293 analyses (**Supplementary tables 3-12**).

294 **Prebiotic-enriched formula and risk of infection or allergic diseases up to age 5.5 years**

295 Consumption of prebiotic-enriched formula at age 2 months or between 2 and 10 months was
296 not related to infection and allergic diseases, except for the association between early use of
297 GOS-enriched formula and a lower risk of URTI (**Tables 2 and 3**). However, this association
298 was no longer significant after further adjustment for antibiotic use up to age 1 year (data not
299 shown). The observed findings for the consumption of prebiotic-enriched formula and the risk
300 of infection and allergic diseases were similar in sensitivity analyses (**Supplementary tables**
301 **3-12**).

302 **DISCUSSION**

303 Among children born in 2011, more than half of formula-fed infants had consumed probiotic-
304 enriched formula at age 2 months. The infant formula consumed was often enriched with *L.*
305 *reuteri* (DSM 17938) or *B. breve* (BC50) combined with *S. thermophilus*, followed by *L.*
306 *fermentum* (CECT5716) and *B. lactis* (BB12) combined with *S. thermophilus*. Overall, the
307 consumption of probiotic-enriched formula was related to the risk of respiratory diseases
308 (respiratory tract infection, wheezing, asthma) up to age 5.5 years, but not with
309 gastrointestinal infection, food allergy, and itchy rash. However, the observed associations for
310 respiratory diseases were not consistent depending on the strains used for enrichment, as well
311 as the consumption period of the formula. No significant association was found for the
312 consumption of prebiotic-enriched formula.

313 As in our study, most RCTs highlighted null or beneficial effect of probiotic enrichment on
314 respiratory diseases or respiratory infection-related outcomes (25, 29). However, when
315 considering the strains, results are less constituent. Indeed, only one RCT including 80
316 healthy 6-month-old children in Spain showed a lower number of episodes of respiratory
317 infections with consumption of formula enriched with *L. salivarius* CECT5713, compared
318 with non-enriched formula (17). Another RCT involving 81 healthy infants in Finland who

319 consumed infant formula before age 2 months also showed a protective effect of formula
320 enriched with *B. lactis BB12* combined with *L. rhamnosus* GG ATCC53103 on the incidence
321 of recurrent respiratory infections during the first year of life (19). However, in this study,
322 probiotics were not added to the formula during manufacturing, but rather as capsules
323 (prebiotics/placebo) directly added to the formula. In addition, consumption of probiotic-
324 enriched formula was not related to the incidence of respiratory infections.

325 In the ELFE study, consumption of probiotic-enriched formula was not associated with the
326 risk of itchy rash and food allergy, which is consistent with the findings observed in other
327 studies (29). The consumption of probiotic-enriched formula was also not associated with
328 gastrointestinal infection in this study. Although some RCTs showed that consumption of
329 probiotic-enriched formula (e.g. enrichment with *B. lactis BB12* alone or in combined with *S.*
330 *thermophilus*, *L.s reuteri* ATCC 55730 or *L. salivarius* CECT5713) had a protective effect on
331 gastrointestinal infection outcomes (17, 18, 25), others examining the effect of formula
332 enriched with *B. lactis BB12* combined with *L. rhamnosus* GG ATCC53103, *B. longum*
333 *BL999* combined with *L. rhamnosus* LPR, or *B. breve* C50 combined with *S. thermophilus* or
334 with *L. johnsonii* La1 found non-significant associations (19, 24, 25, 29).

335 In the present study, the effect of probiotic enrichment was evaluated for each probiotic strain,
336 although some formulas were enriched with a combination of strains. This methodological
337 approach does not consider the synergistic effect of probiotics strains; however, the different
338 strains studied were adjusted for each other in all models. Interactions between strains were
339 also tested and were not significant in any model.

340 Overall, studies that evaluated the effect of probiotic-enriched formula on childhood health
341 showed inconsistent findings. This observation could be explained by the considerable
342 heterogeneity in the RCT designs. Indeed, the types of probiotics and strains as well as the
343 combinations used differed across studies, while the different probiotics strains have specific

344 properties. The measured outcomes and their definitions, duration and timing of intervention
345 also differ across studies. Further studies with a long follow-up, large sample size and using
346 similar methodology (same inclusion criteria, duration and timing of intervention, probiotics
347 strains, combinations and dose used) are needed to establish the effects of probiotic-enriched
348 formula on childhood health.

349 Regarding the effect of prebiotics-enriched formula consumption on infection and allergy in
350 childhood, available studies are limited and the results are inconclusive (20-23, 26, 27, 29).
351 We found no significant association between prebiotic-enriched formula consumption and the
352 risk of gastrointestinal infection, respiratory symptoms and allergic diseases, consistent with
353 the findings observed in some RCTs (20, 21, 23). In contrast, one RCT involving healthy term
354 infants in Italy, from parents with a history of allergy (atopic eczema, allergic rhinitis, or
355 asthma) found lower number of episodes of URTI during the 2-year follow-up among infants
356 consuming hypoallergenic formula enriched with a mixture of short-chain GOS and long-
357 chain FOS than those consuming the same formula without enrichment (21). In addition, one
358 RCT found a significantly lower number of children with at least 1 episode of acute diarrhoea
359 and lower number of diarrheal episodes during the 12-month follow-up in the group receiving
360 GOS/FOS-enriched formula as compared with the control group (20), and another found that
361 the cumulative incidences of allergic manifestations (atopic dermatitis, recurrent wheezing,
362 and allergic urticaria) were lower in the intervention group, than in the control group (21).

363 To our knowledge, no other longitudinal cohort has assessed the association between
364 probiotic or prebiotic formula enrichment and infection/allergic manifestations, which hinders
365 international comparison of our results.

366 The present prospective study involved children included in a large French birth cohort with
367 detailed data on infant diet, which allowed us to examine among formula-fed infants and

368 under real conditions of use, the effect of formula enrichment with probiotics or prebiotics on
369 infection and allergic diseases in childhood. In this cohort, the number of infants exposed to
370 probiotic-enriched formula was higher than that observed in the trials (mostly small sample
371 sizes). This high proportion could possibly be attributable to popular beliefs about the health
372 benefits of probiotics. However, a previous study using the ELFE cohort data showed few
373 social and health-related factors associated with the use of prebiotic- or probiotic-enriched
374 formula (41), which suggests that reverse causality bias is limited in the present study. Indeed,
375 the authors found that only family income and breastfeeding duration were related to the use
376 of prebiotic- or probiotic-enriched formula at age 2 months and the type of infant formula
377 used was fairly stable between 2 and 10 months.

378 Although a wide range of socio-demographic and economic data was considered in the
379 present study, other unmeasured factors such as dietary supplements containing probiotics or
380 prebiotics consumed by mothers or infants and intake of prebiotics from complementary
381 foods might have led to potential residual confounding. In this cohort, health data were
382 reported by parents and not validated by medical records, which could lead to a potential
383 measurement bias. However, the items used were derived from international ones (42) to limit
384 this bias. In addition, repeated data on health-related outcomes in childhood limited memory
385 bias. The various sensitivity analyses performed to examine the robustness of our results and
386 address selection and reverse causation bias showed very similar findings, which suggests that
387 if there were any potential biases, they should have a limited impact on our findings.

388 In conclusion, this observational study showed that probiotic-enriched formulas are often
389 consumed, but few show a real health benefit. The consumption of prebiotic-enriched formula
390 does not have convincing effects on health. Replication in other observational studies and
391 larger well-designed RCTs are needed to confirm these results and possibly identify the most

392 interesting strains and optimal “window of opportunity” for prebiotic and probiotic-enriched
393 formula consumption.

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Table 1 Characteristics of children and families included in the main analyses (n=8,389)

	Missing values (n)	% (n) or mean (SD)
Family characteristics		
Maternal age at delivery (years)	0	31 (4.9)
Maternal education level	294	
Up to lower secondary		3.4% (279)
Upper secondary		36% (2,911)
Intermediate		24.7% (1,999)
3-year university degree		17.4% (1,411)
At least 5-year university degree		18.5% (1,495)
Maternal employment during pregnancy	2	
Employed		75.2% (6,306)
Unemployed		11.3% (949)
Out the labor force		13.5% (1,132)
Household income (€/month/consumption unit)	247	1661.2 (974.8)
Residence in rural area	2	23.3% (1,951)
Smoking during pregnancy	100	
Never smoker		54.3% (4,503)
Smoker only before pregnancy		24.7% (2,047)
Smoker only in early pregnancy		4.1% (336)
Smoker throughout pregnancy		16.9% (1,403)
Maternal pre-pregnancy BMI (kg/m²)	127	23.7 (5)
Children in household	0	
No sibling		46.6% (3,907)
One sibling		36.2% (3,040)
At least 2 siblings		17.2% (1,442)
Maternal diet quality during pregnancy (PANDiet 0-100 score)	978	54.9 (9)
Child characteristics		
Boys	0	51% (4,282)
Gestational age (weeks)	132	39.2 (1.5)
Child birth weight	241	
Small weight for gestational age		10% (811)
Adequate weight for gestational age		80.1% (6,528)
Large weight for gestational age		9.9% (809)
Age at infant formula introduction (months)	3	0.4 (0.5)
Ever infection from age 2 months to 5.5 years		
Gastrointestinal infection	0	13.4% (1,122)
Upper respiratory tract infection	0	32.4% (2,716)
Lower respiratory tract infection	0	37.8% (3,172)
Ever allergic diseases from age 2 months to 5.5 years		
Wheezing	0	42.4% (3,556)
Medical diagnosis of asthma	0	14.1% (1,179)
Itchy rash	0	45.9% (3,849)
Food allergy	0	6.3% (525)

BMI body mass index; *PANDiet* Probability of Adequate Nutrient intake-based Diet quality index

Table 2 Adjusted associations between consumption of enriched formula at 2 months and from 2 to 10 months and the risk of respiratory diseases up to 5.5 years (n=8,389)

	<i>n</i>	Upper respiratory tract infection		Lower respiratory tract infection		Wheezing		Asthma	
		OR [95% CI]	P	OR [95% CI]	P	OR [95% CI]	P	OR [95% CI]	P
Consumption at 2 months									
Enrichment in Bifidobacterium			0.1		0.03		0.2		0.3
No Bifidobacterium	5,805	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
<i>Bifidobacterium breve</i> (BC50)	1,372	1.19 [1.00; 1.41]		1.09 [0.93; 1.29]		1.06 [0.90; 1.24]		0.97 [0.77; 1.21]	
<i>Bifidobacterium lactis</i> (BB12)	732	0.95 [0.82; 1.10]		0.84 [0.73; 0.96]		0.87 [0.75; 0.99]		0.83 [0.68; 1.01]	
Other Bifidobacterium or unspecified strain	480	0.87 [0.71; 1.08]		1.15 [0.95; 1.40]		1.10 [0.91; 1.34]		1.25 [0.96; 1.62]	
Enrichment in Lactobacillus			0.2		0.5		0.8		0.1
No Lactobacillus	6,043	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
<i>Lactobacillus reuteri</i> (DSM 17938)	1,679	0.86 [0.75; 1.00]		1.09 [0.95; 1.25]		1.00 [0.87; 1.14]		0.85 [0.70; 1.02]	
<i>Lactobacillus fermentum</i> (CECT5716)	480	1.04 [0.87; 1.24]		0.96 [0.81; 1.15]		1.08 [0.91; 1.28]		1.19 [0.95; 1.50]	
Other Lactobacillus or unspecified strain	187	1.18 [0.87; 1.60]		0.89 [0.67; 1.20]		0.95 [0.71; 1.26]		1.06 [0.72; 1.55]	
Enrichment in Streptococcus			0.5		0.6		0.3		0.3
No Streptococcus	6,295	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
<i>Streptococcus thermophilus</i>	2,094	0.96 [0.86; 1.07]		0.97 [0.87; 1.08]		1.06 [0.95; 1.17]		1.08 [0.94; 1.26]	
Enrichment in prebiotics			0.6		0.9		0.5		0.7
No prebiotics	7,456	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
FOS/GOS or GOS only	933	0.98 [0.91; 1.06]		1.00 [0.93; 1.08]		1.03 [0.96; 1.11]		0.98 [0.89; 1.09]	
Consumption between 2 and 10 months									
Enrichment in <i>B. breve</i> (BC50)			0.4		0.002		0.4		0.003
Never	6,053	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
Up to 6 months	284	1.16 [0.85; 1.60]		1.75 [1.29; 2.38]		1.28 [0.96; 1.72]		1.95 [1.28; 2.97]	
From 6 months	748	1.01 [0.74; 1.40]		0.64 [0.48; 0.86]		0.90 [0.67; 1.20]		0.59 [0.40; 0.88]	
Always	1,304	0.79 [0.62; 1.02]		0.90 [0.70; 1.15]		0.90 [0.71; 1.15]		0.74 [0.52; 1.05]	
Enrichment in <i>B. lactis</i> (BB12)			0.01		0.2		0.1		0.04
Never	7,598	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
Always	791	0.80 [0.68; 0.95]		0.89 [0.76; 1.05]		0.89 [0.76; 1.04]		0.78 [0.62; 0.99]	
Enrichment in <i>L. fermentum</i> (CECT5716)			0.05		0.3		0.03		0.3

Never	7,717	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
Sometimes	329	1.21 [1.02; 1.44]		1.13 [0.96; 1.34]		1.13 [0.95; 1.34]		1.15 [0.92; 1.44]	
Always	343	0.92 [0.78; 1.10]		0.90 [0.76; 1.06]		1.03 [0.87; 1.22]		0.95 [0.75; 1.20]	
Enrichment in <i>L. reuteri</i> (DSM 17938)			0.2		0.5		0.9		0.6
Never	6,468	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
Sometimes	590	1.00 [0.88; 1.14]		1.07 [0.94; 1.22]		1.02 [0.90; 1.15]		0.98 [0.82; 1.17]	
Always	1,331	0.94 [0.84; 1.05]		0.94 [0.85; 1.05]		1.00 [0.90; 1.10]		0.97 [0.84; 1.12]	
Enrichment in <i>S. thermophilus</i>			0.08		0.4		0.8		0.003
Never	5,218	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
Up to 6 months	471	0.97 [0.76; 1.25]		0.81 [0.64; 1.03]		0.97 [0.77; 1.22]		0.66 [0.47; 0.94]	
From 6 months	812	0.81 [0.61; 1.09]		1.13 [0.86; 1.50]		0.99 [0.75; 1.29]		1.84 [1.29; 2.63]	
Always	1,888	1.34 [1.06; 1.69]		1.10 [0.88; 1.37]		1.10 [0.89; 1.36]		1.15 [0.85; 1.57]	
Enrichment in GOS			0.1		0.5		0.2		0.3
Never	7,205	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
Early use	487	0.87 [0.76; 0.99]		0.96 [0.85; 1.10]		1.08 [0.95; 1.22]		1.06 [0.88; 1.28]	
Delayed use	697	1.13 [0.97; 1.32]		0.99 [0.84; 1.16]		0.87 [0.75; 1.01]		0.88 [0.69; 1.12]	

CI confidence interval; FOS fructo-oligosaccharides; GOS galacto-oligosaccharides; OR odd ratio

Logistic regressions adjusted for maternal and household characteristics (maternal age, education level, migration status, employment during pregnancy, smoking status, pre-pregnancy BMI, diet quality during pregnancy, city size, region of residence, household income, family history of allergy and number of older children), child characteristics (sex, gestational age, birth weight category, type of physician consulted after discharge, C-section delivery and age at infant formula introduction) and ELFE study design variables (maternity size and recruitment wave). For a given period, all enrichments were considered simultaneously.

Table 3 Associations between consumption of enriched formula at 2 months and from 2 to 10 months and the risk of gastrointestinal infections, food allergies or itchy rash up to 5.5 years (n=8,389)

	Gastrointestinal infection		Food allergy		Itchy rash	
	OR [95% CI]	P	OR [95% CI]	P	OR [95% CI]	P
Consumption at 2 months						
Enrichment in Bifidobacterium		0.3		0.4		0.7
No Bifidobacterium	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
<i>Bifidobacterium breve</i> (BC50)	0.93 [0.75; 1.17]		0.87 [0.64; 1.18]		1.07 [0.91; 1.25]	
<i>Bifidobacterium lactis</i> (BB12)	0.92 [0.76; 1.12]		0.91 [0.68; 1.21]		1.00 [0.88; 1.15]	
Other Bifidobacterium or unspecified strain	1.27 [0.98; 1.64]		1.01 [0.67; 1.52]		1.00 [0.82; 1.21]	
Enrichment in Lactobacillus		0.5		0.2		0.8
No Lactobacillus	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
<i>Lactobacillus reuteri</i> (DSM 17938)	1.15 [0.95; 1.39]		0.78 [0.59; 1.02]		0.95 [0.83; 1.08]	
<i>Lactobacillus fermentum</i> (CECT5716)	0.94 [0.72; 1.21]		0.79 [0.55; 1.12]		1.00 [0.84; 1.19]	
Other Lactobacillus or unspecified strain	0.87 [0.58; 1.29]		1.74 [1.01; 3.01]		1.11 [0.83; 1.47]	
Enrichment in Streptococcus		0.9		0.06		0.3
No Streptococcus	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
<i>Streptococcus thermophilus</i>	1.01 [0.88; 1.17]		1.21 [0.99; 1.49]		0.94 [0.85; 1.04]	
Enrichment in prebiotics		0.3		0.1		0.5
No prebiotics	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
FOS/GOS or GOS only	1.05 [0.95; 1.17]		0.89 [0.76; 1.04]		0.98 [0.91; 1.05]	
Consumption between 2 and 10 months						
Enrichment in <i>B. breve</i> (BC50)		0.6		0.7		0.2
Never	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
Up to 6 months	1.21 [0.78; 1.88]		1.20 [0.69; 2.08]		1.29 [0.96; 1.73]	
From 6 months	1.06 [0.70; 1.62]		1.02 [0.56; 1.87]		0.91 [0.69; 1.20]	
Always	0.87 [0.62; 1.24]		1.00 [0.62; 1.63]		0.84 [0.66; 1.06]	
Enrichment in <i>B. lactis</i> (BB12)		0.8		0.6		0.5
Never	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
Always	1.03 [0.82; 1.28]		1.08 [0.81; 1.44]		0.95 [0.81; 1.10]	
Enrichment in <i>L. fermentum</i> (CECT5716)		0.5		0.1		0.9

Never	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
Sometimes	1.14 [0.89; 1.46]		0.79 [0.54; 1.16]		0.98 [0.83; 1.16]	
Always	0.86 [0.67; 1.11]		1.00 [0.69; 1.44]		1.03 [0.87; 1.21]	
Enrichment in <i>L. reuteri</i> (DSM 17938)		0.7		0.1		0.2
Never	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
Sometimes	1.08 [0.90; 1.30]		1.15 [0.90; 1.47]		1.02 [0.90; 1.16]	
Always	0.96 [0.83; 1.13]		0.82 [0.66; 1.01]		0.93 [0.84; 1.03]	
Enrichment in <i>S. thermophilus</i>		0.5		0.4		0.3
Never	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
Up to 6 months	0.84 [0.59; 1.20]		1.27 [0.83; 1.96]		0.83 [0.66; 1.04]	
From 6 months	0.89 [0.60; 1.33]		0.73 [0.41; 1.31]		1.08 [0.83; 1.41]	
Always	1.17 [0.86; 1.59]		0.82 [0.54; 1.26]		1.14 [0.92; 1.40]	
Enrichment in GOS		0.1		0.2		0.6
Never	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
Early use	1.18 [0.99; 1.42]		0.83 [0.62; 1.10]		0.95 [0.83; 1.07]	
Delayed use	0.80 [0.63; 1.02]		1.03 [0.72; 1.49]		1.08 [0.93; 1.26]	

CI confidence interval; FOS fructo-oligosaccharides; GOS galacto-oligosaccharides; OR odd ratio

Logistic regressions adjusted for maternal and household characteristics (maternal age, education level, migration status, employment during pregnancy, smoking status, pre-pregnancy BMI, diet quality during pregnancy, city size, region of residence, household income, family history of allergy and number of older children), child characteristics (sex, gestational age, birth weight category, type of physician consulted after discharge, C-section delivery and age at infant formula introduction) and ELFE study design variables (maternity size and recruitment wave). For a given period, all enrichments were considered simultaneously.