Enhanced Protection Against Diarrhea Among Breastfed Infants of Nonsecretor Mothers

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Abstract: Diarrhea is a major cause of infant mortality. Being a "nonsecretor" (having an inactive fucosylase-2 gene) protects against diarrhea by inhibiting enteric infections. Breastfeeding also protects against diarrhea; however, the impact of maternal secretor status is unknown. In the ALSPAC cohort (N = 4971), we found that breastfeeding by nonsecretor mothers was especially protective against diarrhea, which could inform new prevention strategies.

Key Words: fucosyltransferase-2, secretor status, breast milk, diarrhea, pediatrics

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D iarrhea is a major cause of infant morbidity and mortality worldwide.[1] Susceptibility is partially determined by histo-blood group antigens that serve as receptors for enteric pathogens on the intestinal epithelium.[2,3] Histo-blood group antigens secretion and expression are determined by the fucosyltransferase-2 (*FUT2*) gene. About 20% of Caucasians are homozygous "nonsecretors" for the nonsense mutation W143X (rs601338G>A),[4] while other *FUT2* single nucleotide polymorphisms (SNPs) confer nonsecretor status in other ethnic groups. Nonsecretors are resistant to pathogens that use *FUT2* antigens to infect cells, including norovirus[5] and rotavirus.[6] For example, in a US multicenter case-control study of severe rotavirus gastroenteritis,[6] 23% of control children were nonsecretors compared with 0.5% of cases, indicating nearly complete resistance among nonsecretor children. Similarly, nonsecretor status was shown to confer complete resistance against nosocomial and sporadic outbreaks of acute norovirus gastroenteritis in Sweden.[5]

Breastfeeding is another factor that modifies susceptibility to gastrointestinal infections and diarrheal disease. In a recent systematic review, breastfeeding was associated with a 63% reduced risk of diarrhea during the first 6 months of life and a 41%–88% lower odds of mortality from infectious disease during this period, depending on the exclusivity of breastfeeding.[7] This striking protection is partially attributed to the anti-infective properties of breast milk, including maternal antibodies and antimicrobial proteins such as lactoferrin.[8] In addition, breast milk contains a diverse array of human milk oligosaccharides (HMOs) that act as decoy receptors for enteric pathogens in the infant gut and serve as selective substrates for commensal gut microbes that stimulate immune development.[9] These functions are structure-specific and depend on HMO fucosylation, which is determined by the mother's *FUT2* secretor status.[10]

Thus, current evidence indicates that both infant secretor status (a fixed genetic trait) and breastfeeding (a modifiable exposure during early life) influence susceptibility to diarrheal disease. However, few studies have addressed these factors simultaneously and none, to our knowledge, have assessed their potential interaction with maternal secretor status, despite clear evidence that maternal *FUT2* activity modifies the HMO profile of breast milk, which in turn regulates microbial activity in the infant gut.[10] Understanding these relationships could inform new strategies for diarrhea prevention, with potentially life-saving implications—especially in low- and middle-income settings, where diarrhea is a leading cause of infant mortality. We studied these associations and potential interactions in the population-based Avon Longitudinal Study of Parents and Children (ALSPAC) cohort.[11]

MATERIALS AND METHODS

This study was embedded in the ALSPAC cohort, a population-based pregnancy cohort of N = 14,541 families resident in the former county of Avon, United Kingdom with expected dates of delivery between April 1, 1991 and December 31, 1992.[11] We analyzed 4971 mother-infant dyads for whom genetic data and information on infant diarrhea were available. Analyses were limited to Caucasian dyads because the cohort was primarily (>98%) Caucasian and the SNP responsible for *FUT2* secretor status is known to vary by ethnicity.[11] Ethical approval was obtained from

the ALSPAC Ethics and Law Committee and the Local Research Ethics Committee.

As described previously,[3,12] *FUT2* secretor status was defined based on the rs601338 SNP,[3,4] where G is the wild-type "secretor" allele and A is the recessive "nonsecretor" allele. To confirm recessivity, we conducted a sensitivity analysis separating the GG homozygous secretors from the GA heterozygous secretors but did not find any differences between these groups (not shown). We therefore dichotomized secretor status, combining the GA and GG genotypes as secretors and comparing them to the homozygous AA nonsecretors. Information on breastfeeding status (breastfeeding at 6 months, yes vs. no) and infant diarrhea (defined as one or more episodes) was obtained from maternal reports at 6 and 18 months. Mothers were asked if their child "had diarrhea or gastroenteritis?" and "how many times?" they had diarrhea or gastroenteritis. We conducted a sensitivity analysis where diarrhea was defined as 2 or more episodes during the time period of interest.

We used logistic regression to determine associations between infant *FUT2* secretor status and diarrhea incidence. The impact of maternal secretor status was assessed by evaluating diarrhea incidence across the 4 possible categories of maternal and infant secretor status (ie, −/−, −/+, +/−, +/+). To assess the impact of breastfeeding, we further stratified these 4 groups based on breastfeeding status at 6 months. We also formally tested for interactions between maternal secretor status, infant secretor status and breastfeeding by including interaction terms in our regression models,[13] and performed a stratified analysis to explore these potential interactions.[13] In addition, we conducted a sensitivity analysis, where diarrhea was defined as 2 or more episodes in the 0–6-month or 6–18-month timeframes. Analyses were conducted using R studio software version 3.3.2 (R Foundation for Statistical Computing) and SAS version 9.4 (SAS Institute Inc. 2013).

RESULTS

Overall, 24% of infants and 25% of mothers were nonsecretors, and 33% breastfed for at least 6 months (Fig. 1). The incidence of diarrhea among infants was 27% from 0 to 6 months and 51% from 6 to 18 months. Compared with secretor infants, nonsecretors had a 15% lower risk of diarrhea before 6 months [odds ratio (OR): 0.85; 95% confidence interval: 0.72–0.99] and 30% lower risk between 6 and 18 months (OR: 0.70; 0.61–0.81) (Fig. 1).

Independent of infant secretor status, breastfeeding was strongly protective against diarrhea during both time periods, and this effect appeared to be modified by maternal secretor status (Fig. 1 and Table 1, Supplemental Digital Content 1, http://links.lww.com/INF/E250). For example, at 0–6 months, breastfeeding tended to be more protective among infants of nonsecretor mothers (67% reduced risk; OR: 0.33; 0.24–0.44) than secretor mothers (59% reduced risk; OR: 0.41; 0.35–0.49) (*P* for interaction = 0.18). By 6–18 months, the effect of breastfeeding was lower in magnitude, but the evidence for protection remained strong and the modification by maternal secretor status was more evident (32% reduced risk from nonsecretor mothers; OR 0.68; 0.53–0.86, compared with 14% reduced risk from secretor mothers; OR 0.86; 0.75–0.99) (*P* for interaction = 0.09) (Fig. 1 and Table 1, Supplemental Digital Content 1, http://links.lww.com/INF/E250). Similar results were observed when we defined diarrhea as 2 or more episodes (Table 2, Supplemental Digital Content 2, http://links.lww.com/INF/E251). There was no evidence of interaction between breastfeeding and infant secretor status, or maternal and infant secretor status (not shown).

DISCUSSION

To our knowledge, this is the largest population-based study to assess the interaction role of breastfeeding, maternal and infant *FUT2* secretor status in infant diarrhea. Our results confirm previous findings that nonsecretor[2,3] and breastfed[9] infants are protected from diarrhea, and provide new evidence that maternal secretor status modifies the effect of breastfeeding in this context. Our findings suggest that while breastfeeding was protective in all dyads, nonsecretor milk may confer greater protection than secretor milk.[9]

Our results confirm and extend recent findings from the Southampton Women's Survey (N = 1831 UK mother-infant dyads), where Barton et al[14] reported a decreased risk of enteric illness among *FUT2* nonsecretor infants, and found that longer breastfeeding duration was associated with lower risk of diarrhea in the first 2 years of life. They found no independent effect of maternal secretor status, but they did not test the potential interaction between maternal secretor status and breastfeeding.[14] Consistent with Barton et al, our findings suggest strong protective effects from infant nonsecretor status and breastfeeding, with no independent effect of maternal secretor status; however, our interaction analysis revealed a role for maternal secretor status that is more evident among breastfeeding dyads. This novel finding supports the hypothesis that maternal secretor status influences infant susceptibility to diarrhea through its impact on breast milk composition.

Surprisingly, the apparent impact of maternal secretor status in breastfed infants was greatest in the 6–18 month time period (Fig. 1 and Table 1, Supplemental Digital Content 1, http://links.lww.com/INF/E250), after most infants had ceased breastfeeding. We hypothesize that passive immunity conferred through maternal antibodies and antimicrobial proteins in breast milk[8] is most important at earlier ages when the infant immune system is still maturing, whereas the comparably modest (yet long-lasting) effect of maternal secretor status becomes more important later in infancy, and persists even after weaning. This persistent effect could reflect a "programming" mechanism whereby HMOs in nonsecretor breastmilk shape the infant gut microbiome in a way that permanently impacts immune development and affords long-term protection against diarrhea. Further research is warranted to investigate this hypothesis and explore other possible mechanisms.

Interestingly, and contrary to the hypothesis that *FUT2*-dependent oligosaccharides (ie, those produced by secretor mothers) somehow inhibit enteric pathogens, our findings suggest that nonsecretor milk may confer greater protection than secretor milk.[9] Seemingly in contrast to our findings, a study of 93 Mexican infants found lower rates of diarrhea in those receiving milk with higher concentrations of *FUT2*-dependent oligosaccharides; however, there were no nonsecretor mothers in this study for comparison.[9] Consistent with our results, a study of 520 infants in Bangladesh, Peru and Tanzania found a lower incidence of infection by specific enteric pathogens and all-cause diarrhea among infants of nonsecretor mothers, although interactions with breastfeeding were not considered.[15] We uniquely assessed this interaction in 4971 UK infants, finding evidence that breastfeeding by nonsecretor mothers is especially protective against diarrhea, although we could not assess milk composition or identify specific pathogens in our study.

Further investigation is needed to understand how nonsecretor milk may directly or indirectly inhibit enteric infections or symptomatic disease, perhaps due to enrichment of *FUT2*-independent HMOs and/or microbiome modification. We have previously shown that, while nonsecretor milk lacks 2′-fucosyllactose and has relatively low concentrations of several other fucosylated HMOs, it is enriched in HMOs including lacto-N-fucopentaose II and sialyl-lacto-N-tetraose b.[16] Understanding how these HMOs or
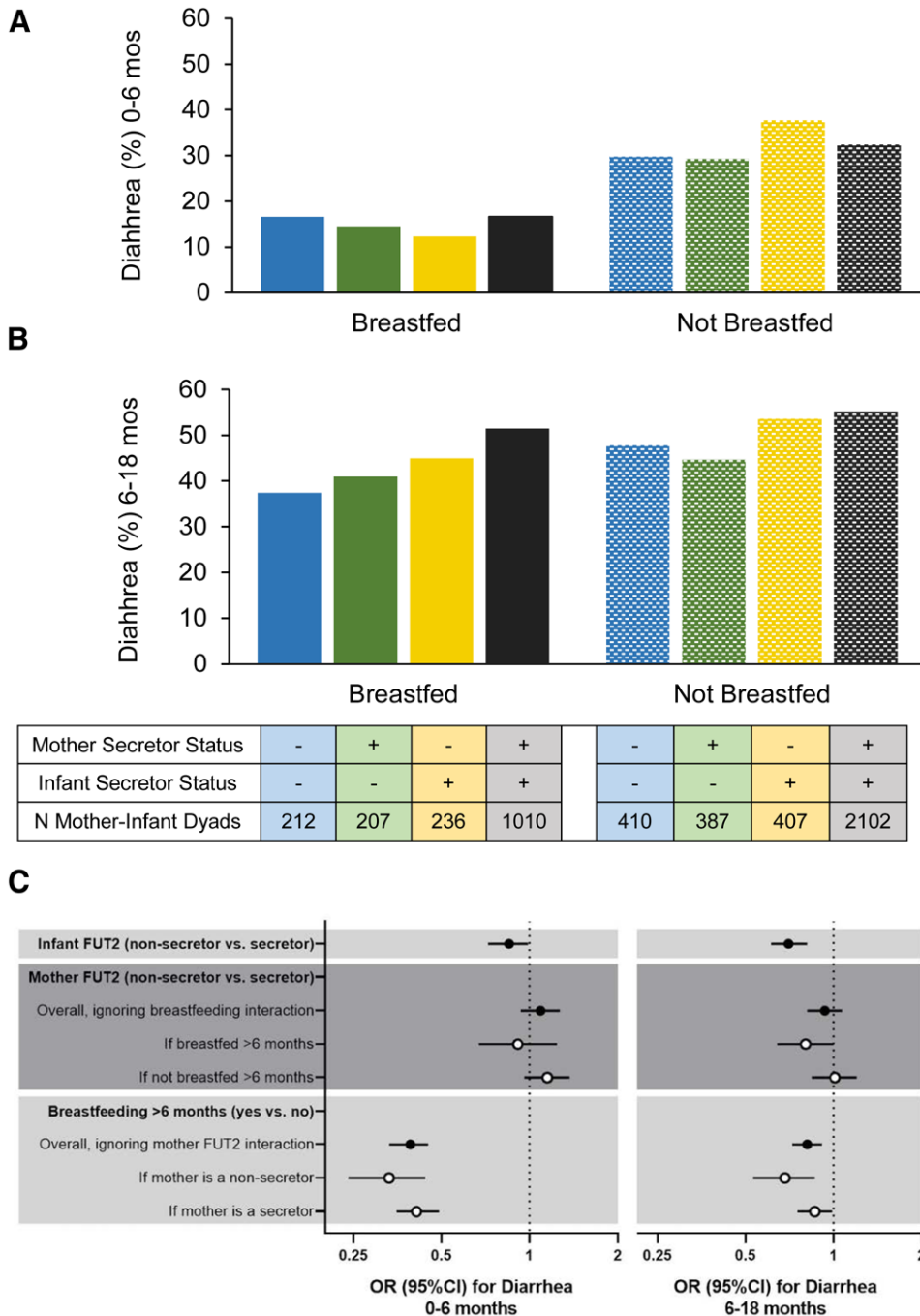
FIGURE 1. Maternal and infant secretor status, breastfeeding and infant diarrhea in the ALSPAC cohort at (A) 0–6 months (B) 6–18 months. Mother-infant dyads are classified according to *FUT2* secretor status based on their rs601338 genotype (– indicates homozygous AA nonsecretor; + indicates GA or GG secretor). Breastfeeding refers to any breastfeeding at 6 months. Estimates are from logistic regression models (C) mutually adjusted for infant *FUT2*, mother *FUT2* and breastfeeding. No interactions were detected for infant *FUT2* and maternal *FUT2*, or for infant *FUT2* and breastfeeding (interaction *P* values >0.20; not shown). CI indicates confidence interval; *FUT2*, fucosyltransferase-2.

other properties of nonsecretor milk limit enteric infections could have implications for optimizing nutrition in non-breastfed infants because synthetic HMOs (most commonly *FUT2*-dependent 2′-fucosyllactose) are now being added to infant formulas.[17] While these new formulations appear to be well tolerated by healthy infants, studies have been limited to high-income settings and have not evaluated diarrhea or tested other (*FUT2*-independent) oligosaccharides for comparison. This research could also inform strategies for the collection and provision of human donor milk, which does not currently consider secretor status.

Strengths of our study include the population-based design and the relatively large sample size, which permitted our novel assessment of the interaction between maternal secretor status and breastfeeding. Our study was limited by its reliance on parent report for diarrheal illness without specific information on the number of stools in 24 hours or deviation from usual bowel movements, which are recommended metrics for optimally defining diarrhea. Relying on parental report may have introduced recall bias and decreased the accuracy of the diarrhea variable used in the study and did not allow investigation of specific enteric pathogens. Parental reports also make it difficult to distinguish infectious diarrhea from other watery stools resulting from general gut dysbiosis or food allergy. Thus, we cannot conclude that the protective effect of breastfeeding from nonsecretor mothers against diarrhea is specific to enteric infections. In addition, there is potential for misclassification bias because healthy breastfed infants can have watery stools that can be mistaken for diarrhea, which may have led to an underestimation of the protective effect of breastfeeding. In addition, breast milk and stool were not collected, so we could not assess HMO composition or the infant microbiome. Finally, it must be noted that our results from the Caucasian UK ALSPAC cohort may not be generalizable to non-Caucasian populations in other settings.

Our results confirm previous findings that infant nonsecretor status and breastfeeding are protective against diarrhea during infancy. In addition, we provide novel evidence that breastfeeding by nonsecretor mothers may be especially protective against diarrhea in UK infants. Further studies are needed to replicate our findings in other settings, elucidate the biologic mechanisms, and determine clinical implications. Ultimately this research will inform efforts to optimize infant nutrition and understand the pathogenesis and prevention of diarrheal disease.

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