Letter to the Editor

Human milk oligosaccharides and development of cow's milk allergy in infants

To the Editor:

Human milk oligosaccharides (HMOs) provide a main substrate for infant's gut microbiota, not only to bifidobacteria but also some Bacteroides.[1] HMOs are complex glycans and the third largest solid component in human milk. HMO composition varies between women, which partially depends on genetics. For example, HMO fucosylation is mediated by the 2 fucosyltransferases FUT2 (secretor gene) and FUT3 (Lewis gene), which also determine the mother's Secretor and Lewis blood group status. Nonsecretor mothers, who lack the functional FUT2 enzyme, also lack most alpha1-2-fucosylated oligosaccharides such as 2′fucosyllactose (2′FL) and lacto-*N*-fucopentaose (LNFP I). Infants fed by nonsecretor mothers are delayed in establishment of bifidobacteria-laden microbiota.[2] Previous studies have linked

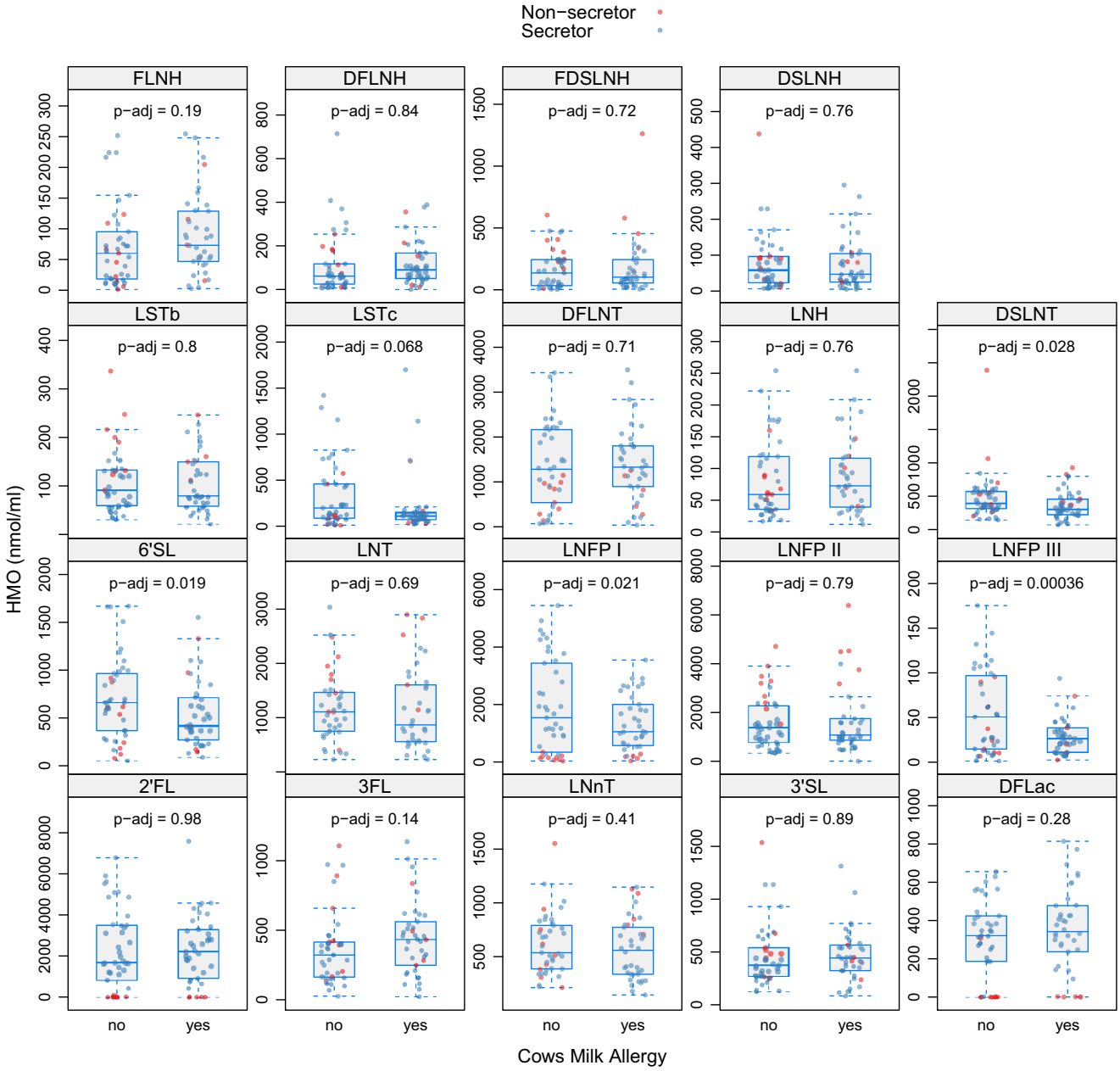

FIG 1. Human milk oligosaccharide levels in mothers who have an infant with CMA and in those with a child without CMA. Nonparametric *P* values (Wilcoxon) for the difference in distribution between CMA and non-CMA samples are indicated in each panel. For HMO abbreviations, see Table E2.

individual HMO with reduced risk of mother-to-child HIV transmission[3] and FUT2-dependent HMO with lower risk to manifest IgE-associated eczema in infants born via C-section.[4] Because there is emerging evidence in humans to support the concept that the infant gut microbiome plays a role in food sensitization/allergy,[5] we sought to compare the HMO composition in breast milk received by infants who develop cow's milk allergy (CMA) with that in infants without CMA.

We used stored human milk, foremilk collected in the morning, from a prospective birth cohort designed to assess immunologic factors in human milk, development of CMA within the first 18 months of life, and oversampled for newborns at high risk for food allergies. The results for human milk cytokines in this cohort have been previously published.[6] Clinical characteristics are presented in Table E1 in this article's Online Repository at www.jacionline.org. The earliest available milk sample was assessed from each mother: at median 1.0 month in 41 mothers of infants without CMA and at median 1.4 months in 39 mothers of infants with CMA. CMA was verified by oral food challenges at median age 6 months. HMO composition was measured by HPLC after 2-aminobenzamide labeling (see Table E2 in this article's Online Repository at www.jacionline.org). Raffinose was added to milk samples as internal standard to allow for absolute quantification (for details of Methods, see this article's Online Repository at www.jacionline.org). Freezing does not impact HMO levels, which are very stable in term milk day-to-day and diurnally.[7] The study was approved by the institutional review boards of the Helsinki University Central Hospital, the City of Helsinki, and the University of Rochester Medical Center, Rochester, NY.

In our cohort, FUT2 Secretor status did not significantly correlate with CMA ($P = .38$, Fisher test). Duration of lactation in months (ie, age of the infant) significantly correlated with levels of several HMOs (see Fig E1 in this article's Online Repository at www.jacionline.org), whereas maternal atopy only marginally associated with 1 HMO (disialyllacto-*N*-tetraose [DSLNT]; $P = .046$) and maternal age did not significantly correlate with any HMO levels. After adjusting for babies' age and maternal covariates, including atopic diseases, duration of lactation, and Secretor status, milk of mothers with an infant with CMA contained lower levels of 6′-sialyllactose (6′SL, $P = .019$), DSLNT ($P = .028$), LNFP I ($P = .021$), and LNFP III ($P = .00036$) than did milk of mothers with a non-CMA infant, and there was a trend for lower levels of LS-tetrasaccharide c (LSTc, $P = .068$) (Fig 1). After correction for multiple comparisons, the level of LNFP III remained significantly lower in mothers with a infant with CMA (29 μM vs 57 μM; 95% CI, 11-43; adjusted $P = .0069$). Infants who received low (<60 μM) LNFP III–containing milk were more likely to become affected with CMA when compared with infants who received high LNFP III–containing milk (odds ratio, 6.7; 95% CI, 2.0-22). When further classifying infants into types of CMA, all mothers with an infant with delayed-onset CMA were Secretors (active FUT2, milk containing 2′FL and LNFP I), whereas those with an infant with immediate-type (IgE-mediated) CMA were not; otherwise, HMOs were comparable between these groups (see Fig E2 in this article's Online Repository at www.jacionline.org). Levels of LSTc, DSLNT, and 6′SL ($P = .019$, .028, and .044, respectively) were lower in the milk of mothers with an infant with atopic dermatitis when compared with those with an infant without atopic dermatitis.

Cluster analysis that groups HMOs on the basis of similarity of expression patterns and baby's age (Fig 2) shows that HMOs that directly depend on FUT2 expression (2′FL and LNFP I, group B) were coexpressed, and correlate with FUT2 status, as expected. Also, noteworthy that 3 seemingly unrelated HMOs, 6′SL, LSTc, and LNFP III (group A), formed a coexpressed cluster that together significantly correlated with CMA status (multivariate analysis of variance, $P = .015$). It was not immediately obvious from the cluster analysis what regulates this expression pattern, because these 3 HMOs do not share a known biosynthetic pathway that would easily explain this pattern. It is noteworthy, however, that a third group C with FDSLNH, LNFP II, 3FL, and 3′SL negatively correlated with groups A and B, suggesting a common regulatory mechanism that diverts HMO composition between 2 different "glycotypes."

One previous study assessed the association of HMO with infant allergic status,[4] indicating that infants born by C-section at a high hereditary risk for allergic diseases might have a lower risk to manifest IgE-associated eczema at 2 years when fed breast milk with FUT2-dependent HMO. Food allergy or individual HMO components were not separately assessed. Although we have no data on C-section births in our cohort, our data suggest that it is the Lewis X antigen that is present in LNFP III, and not the FUT2 (Secretor status) that is associated with protection against CMA. Lewis X antigen is recognized by some C-type lectins. In fact, HMOs have recently been suggested to bind dendritic cell–specific intercellular adhesion molecule-3-grabbing nonintegrin, mediated by Lewis X structure.[8] It remains to be seen whether Lewis X or other HMO structures in breast milk could compete with binding of glycoproteins to dendritic cell–specific intercellular adhesion molecule-3-grabbing nonintegrin or other C-type lectins in the gastrointestinal tract, thereby modulating infant's tolerance to foods.

We cannot exclude the possibility that other substances, such as cytokines, antibodies, or exosomes, in breast milk could have contributed to the development of CMA. In addition, although our data suggest that higher LNFP III concentrations are associated with the lack of development of CMA, they are not required to prevent CMA. Therefore, other mechanisms must be in play. Findings of our small cohort need to be validated in a larger sample.

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