Cell Genomics

CellPress
OPEN ACCESS

Preview

Unlocking the genetic influence on milk variation and its potential implication for infant health

Claudia Nussbaum[1] and Sarah Kim-Hellmuth[2,3,*]
[1]Division of Neonatology, Department of Pediatrics, Dr. von Hauner Children's Hospital, University Hospital LMU Munich, Munich, Germany
[2]Department of Pediatrics, Dr. von Hauner Children's Hospital, University Hospital LMU Munich, German Center for Child and Adolescent Health (DZKJ), partner site Munich, Munich, Germany
[3]Computational Health Center, Institute of Translational Genomics, Helmholtz Munich, Neuherberg, Germany
*Correspondence: sarah.kimhellmuth@helmholtz-munich.de
https://doi.org/10.1016/j.xgen.2024.100676

Human milk has long been recognized for its critical role in infant and maternal health. In this issue of *Cell Genomics*, Johnson et al.[1] apply a human genetics and genomics approach to shed light on the complex relationship between maternal genetics, milk variation, and the infant gut microbiome.

For a long time, breast milk was merely considered a food for newborns and infants, optimally adapted to their needs by its composition of macro- and micronutrients. However, we now know that it represents much more than just a form of nutrition.[2] Breast milk has been described as highly specialized, personalized medicine that has profound impact on both child and maternal health.[3] A central aspect is the modification of the child's immune system and the prevention of infections through breast milk. The shaping of the child's microbiome plays a significant role here, facilitated by the unique composition of breast milk with human milk oligosaccharides (HMOs) among other components. While knowledge about the mechanisms of various milk components is growing, relatively little is known about the factors that modify the variability in human milk, particularly the role of maternal genetics in this process and its effect on child health.

Understanding how genetic variation contributes to phenotypic variation in humans is a major endeavor in human genetics and genomics. Large-scale expression quantitative trait locus (eQTL) studies have significantly advanced our understanding of how genetic variation influences gene expression and have built enormous resources for the research community.[4,5] These studies have revealed that eQTLs are highly context specific and vary considerably between different tissues, cell types, and cell states.[4,6,7] Furthermore, context-specific eQTLs have been shown to be particularly relevant to disease associations compared to standard eQTLs,[8] highlighting the importance of studying functional regulatory variants under physiologically relevant conditions. Although major advancements have been made, characterizing the context specificity of eQTLs is still far from complete. Molecular human genetics studies related to women's health or developmental stages, for example, have been scarce. In addition, while quantitative traits have been expanded to many other molecular phenotypes (e.g., epigenetics, splicing, protein abundance), only a few studies examine the potential downstream consequences of molecular QTLs at the tissue or organism level.

In this issue of *Cell Genomics*, Johnson et al.[1] tackled this knowledge gap by using a multi-omics approach that integrates maternal genetics, human milk transcriptomics and composition, and infant fecal metagenomics. They aimed to characterize milk eQTLs, uncover connections between milk gene expression and milk composition, and determine how these factors influence the infant gut microbiome (Figure 1). The analysis involved 146–310 mother-infant pairs from the MILK study. Bulk RNA sequencing (RNA-seq) of cell pellets from 1-month postpartum milk samples revealed that 34.5% of the milk transcriptome can be traced back to three genes that encode key proteins involved in milk production i.e., beta- and kappa-casein (CSN2 and CSN3) and lactalbumin (LALBA). Several associations between maternal and milk traits and milk genes were identified, e.g., a significant negative association of milk volume with expression of the circadian clock gene PER2, indicating a potential role of the circadian rhythm on milk production. Regarding milk IL-6 concentration, a significant correlation was seen with genes that showed enrichment for inflammatory response and other immune pathways, which was attributed to a larger estimated proportion of immune cells in the milk.

A central aspect of the work of Johnson et al. is the association mapping of milk RNA-seq profiles with maternal genetic data ($n = 230$), leading to the identification of 2,790 genes with an eQTL in *cis*, which corresponds to ~16% of all genes tested. 482 of the identified eQTLs were found to be specific for the lactating mammary gland. These milk-specific eQTLs regulate genes that are central to key biological pathways in milk production (such as the synthesis of casein or lactose), as well as to innate immune responses. Milk eQTLs showed the strongest overlap with eQTLs in secretory tissues like the salivary gland or pancreas,[4] while the overlap with non-lactating breast tissue was lower. These observations exemplify that studying non-lactating breast tissue is not suitable for making conclusions about the genetic regulation of milk production. Through colocalization analysis, the researchers were able to identify seven loci that are likely to share a causal variant affecting both milk gene expression and breast cancer risk. For example,

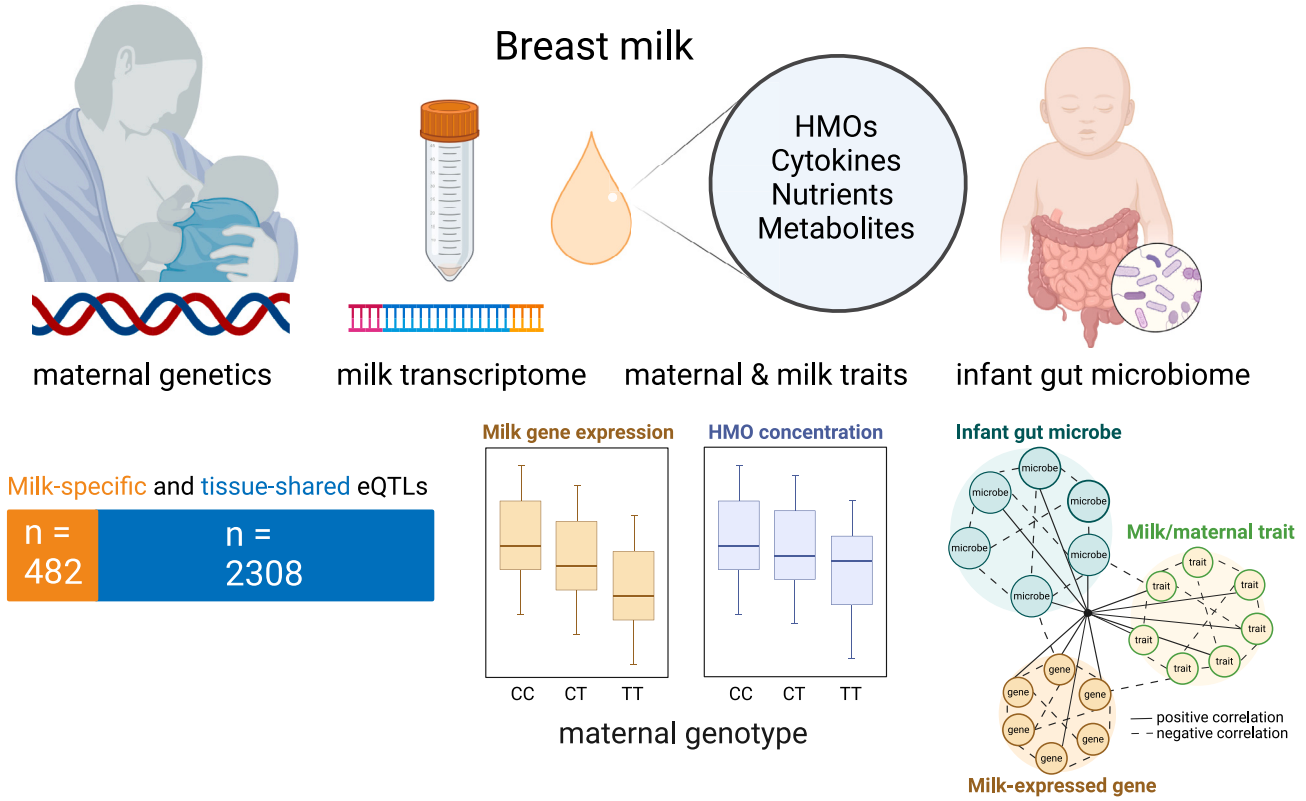

Figure 1. Linking maternal genetics with milk variation, composition, and the infant gut microbiome
Partly adapted from Johnson et al.[1]

the expression of the LMX1B gene in milk was associated with a higher risk of breast cancer, suggesting that genetic factors influencing lactation-specific gene expression may also contribute to the development of breast cancer.

The authors further analyzed the association between milk gene expression and HMO composition at 1 month (n = 310). HMOs promote the growth of beneficial bacteria like *Bifidobacteria* in the infant's gut and vary widely in composition and concentration among women. Genes associated with the expression of 22 different HMO traits showed enrichment for pathways related to protein biosynthesis, localization, and degradation, as well as immune response and regulation. The influence of maternal genetic variability on HMO concentrations was tested for seven glycosyltransferase genes with a significant milk eQTL. For three of these genes (i.e., FUT2, GCNT3, and B3GNT3), an association of maternal genotypes with HMO concentrations was demonstrated. Finally, metagenomic sequencing of fecal samples was performed at 1 and 6 months of age (n = 146) and mapped with milk gene expression, identifying nine gene sets correlated with the microbial taxa or pathways in the infant gut, e.g., genes related to the lysosome and the fatty acid metabolism. The simultaneous analysis of milk components provides possible explanations for how differences in milk gene expression influence the variability of milk composition and, in turn, affect the infant's microbiome. An example of this is the association of milk-expressed genes from the JAK/STAT pathway with the concentration of pro-inflammatory cytokines (IL-6) in milk and the reduced growth of *Bifidobacterium infantis*.

Leveraging the human genetics and genomics toolbox, this study advances our knowledge of the genetic influence on lactation biology in humans and how it is potentially connected to maternal and infant health. Although the number of detected eQTLs was significantly lower compared to other, larger eQTL studies, likely due to the limited sample size, it should still be recognized that this is the first study of its kind not only to explore the impact of maternal genetics on human milk gene expression but also to provide first insights into how this may influence infant immune functions and health. It should also be acknowledged that the collection of high-quality, sensitive human specimens, such as breast milk, along with the corresponding metadata, can be a challenge during the often-exhausting early months of a child's life for mothers, which can significantly complicate recruitment. This highlights the importance of close collaboration and exchange across clinical and basic research disciplines when expanding population-scale human genetics studies into a clinical setting.

Immunity and inflammation were a common thread across study results. Numerous inflammatory genes in milk were positively correlated with milk IL-6 and multiple HMOs while showing inverse correlation with the abundance and

growth of *Bifidobacterium* in the infant gut at 1 month and *Escherichia* at 6 months, underscoring the immunological role of lactation in shaping the infant gut microbiome. Given this immunological role, human milk has been suggested to be more about immune protection than primarily nutrition.[2] This effect extends beyond the immediate defense against pathogens provided by components like antibodies, immune cells, and enzymes. Much more, it aligns with the "Developmental Origins of Health and Disease" hypothesis, in that human milk modulates child development and long-term outcomes by influencing critical imprinting events during a sensitive developmental window.[9] The colonization of the neonatal gut by a diverse range of microbes in the first months of life is crucial to this process, with human milk being one of the factors through which maternal genetics can impact the infant's immune phenotype. Future studies could address this immune-modulatory role of human milk further in longitudinal studies—as milk composition changes dramatically over time—with dense phenotyping of maternal (microbiome, immunology), milk (antibodies, enzymes, single cells), and infant (genetics, immunology) traits in diverse populations. With their study, Johnson et al. have provided an important milestone for future work to complete our understanding of this fascinating "mother-breastmilk-infant triad."[10]

ACKNOWLEDGMENTS

This work was funded by the Deutsche Forschungsgemeinschaft (DFG) SFB/TRR359-B06 (491676693) and SFB/TRR237-B29 (369799452) and the BMBF German Center for Child and Adolescent Health (DZKJ) (01GL2406A). C.N. is further supported by the Medical and Clinician Scientist Program (MCSP) of the Medical Faculty, LMU Munich. Figure 1 was created with BioRender.com.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Johnson, K.E., Heisel, T., Allert, M., Fürst, A., Yerabandi, N., Knights, D., Jacobs, K.M., Lock, E.F., Bode, L., Fields, D.A., et al. (2024). Human milk variation is shaped by maternal genetics and impacts the infant gut microbiome. Cell Genom. *4*, 100638. https://doi.org/10.1016/j.xgen.2024.100638.
2. Gura, T. (2014). Nature's first functional food. Science *345*, 747–749. https://doi.org/10.1126/science.345.6198.747.
3. Victora, C.G., Bahl, R., Barros, A.J.D., França, G.V.A., Horton, S., Krasevec, J., Murch, S., Sankar, M.J., Walker, N., and Rollins, N.C.; The Lancet Breastfeeding Series Group (2016). Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. Lancet *387*, 475–490. https://doi.org/10.1016/s0140-6736(15)01024-7.
4. GTEx Consortium (2020). The GTEx Consortium atlas of genetic regulatory effects across human tissues. Science *369*, 1318–1330. https://doi.org/10.1126/science.aaz1776.
5. Kerimov, N., Tambets, R., Hayhurst, J.D., Rahu, I., Kolberg, P., Raudvere, U., Kuzmin, I., Chowdhary, A., Vija, A., Teras, H.J., et al. (2023). eQTL Catalogue 2023: New datasets, X chromosome QTLs, and improved detection and visualisation of transcript-level QTLs. PLoS Genet. *19*, e1010932. https://doi.org/10.1371/journal.pgen.1010932.
6. Kim-Hellmuth, S., Aguet, F., Oliva, M., Muñoz-Aguirre, M., Kasela, S., Wucher, V., Castel, S.E., Hamel, A.R., Viñuela, A., Roberts, A.L., et al. (2020). Cell type–specific genetic regulation of gene expression across human tissues. Science *369*, eaaz8528. https://doi.org/10.1126/science.aaz8528.
7. Cuomo, A.S.E., Nathan, A., Raychaudhuri, S., MacArthur, D.G., and Powell, J.E. (2023). Single-cell genomics meets human genetics. Nat. Rev. Genet. *24*, 535–549. https://doi.org/10.1038/s41576-023-00599-5.
8. Umans, B.D., Battle, A., and Gilad, Y. (2021). Where Are the Disease-Associated eQTLs? Trends Genet. *37*, 109–124. https://doi.org/10.1016/j.tig.2020.08.009.
9. Ames, S.R., Lotoski, L.C., and Azad, M.B. (2023). Comparing early life nutritional sources and human milk feeding practices: personalized and dynamic nutrition supports infant gut microbiome development and immune system maturation. Gut Microb. *15*, 2190305. https://doi.org/10.1080/19490976.2023.2190305.
10. Bode, L., Raman, A.S., Murch, S.H., Rollins, N.C., and Gordon, J.I. (2020). Understanding the mother-breastmilk-infant "triad.". Science *367*, 1070–1072. https://doi.org/10.1126/science.aaw6147.