

Human milk oligosaccharides, RV1 seroconversion, and rotavirus diarrhea risk in a vaccinated Nicaraguan birth cohort

Sylvia Becker-Dreps, MD, MPH

Departments of Family Medicine and Epidemiology

University of North Carolina at Chapel Hill

(Dissertation work of Rebecca Rubinstein, MD-PhD candidate)

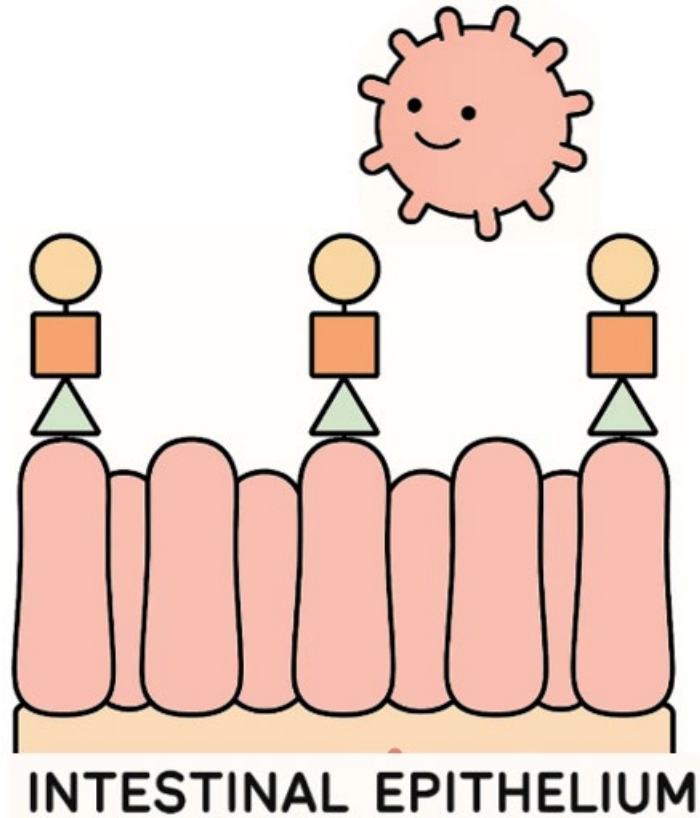
Outline

1. Background: rotavirus susceptibility and HMOs
2. Study design and methods
3. Results: HMOs and RV1 seroconversion
4. Results: HMOs and rotavirus disease risk
5. Limitations & Implications

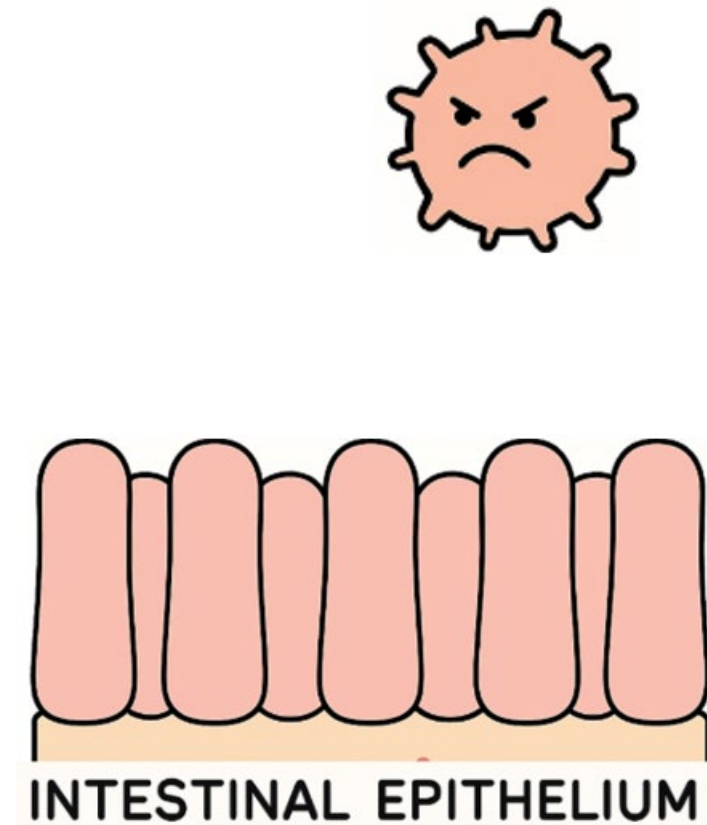


Secretor glycan expression and rotavirus susceptibility

Secretors (having functional FUT2) display fucosylated glycans



Non-secretors do not display these glycans



Secretors have a higher risk of rotavirus gastroenteritis

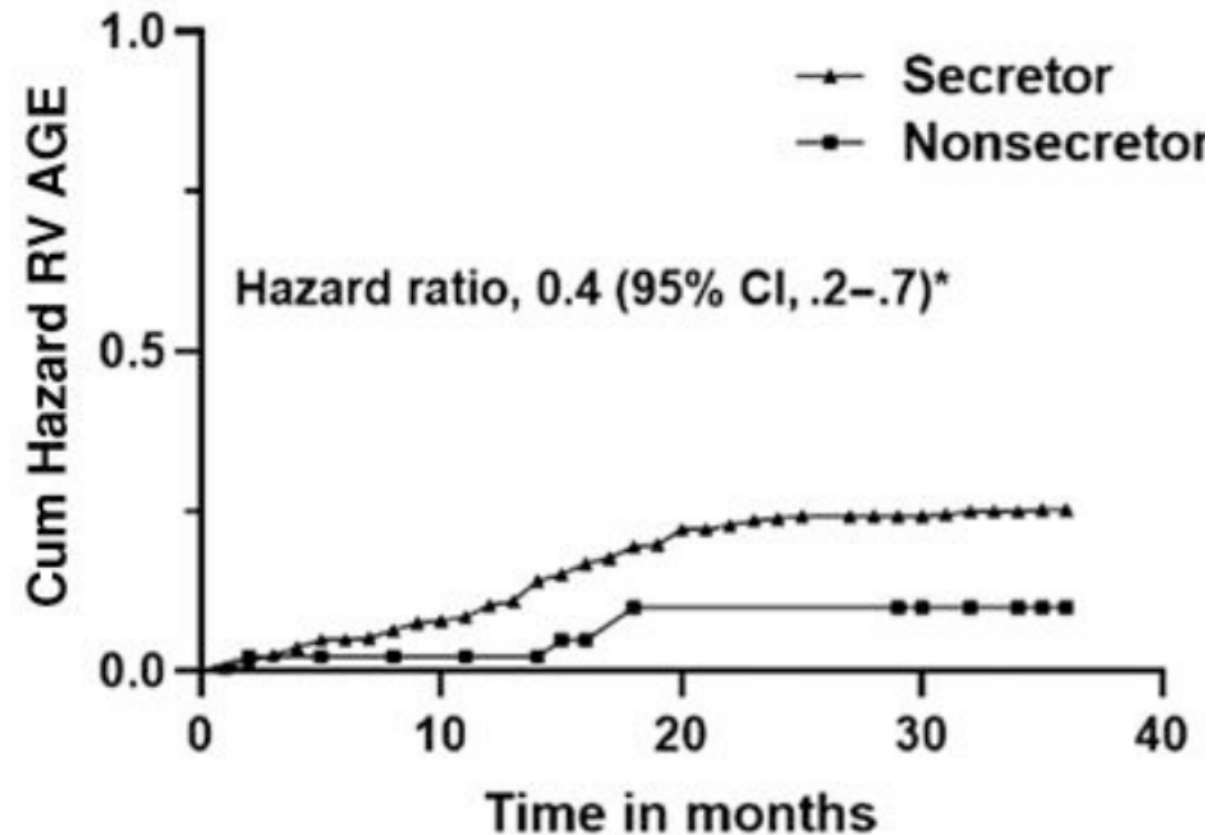
The Journal of Infectious Diseases

MAJOR ARTICLE

Nonsecretor Phenotype Is Associated With Less Risk of Rotavirus-Associated Acute Gastroenteritis in a Vaccinated Nicaraguan Birth Cohort



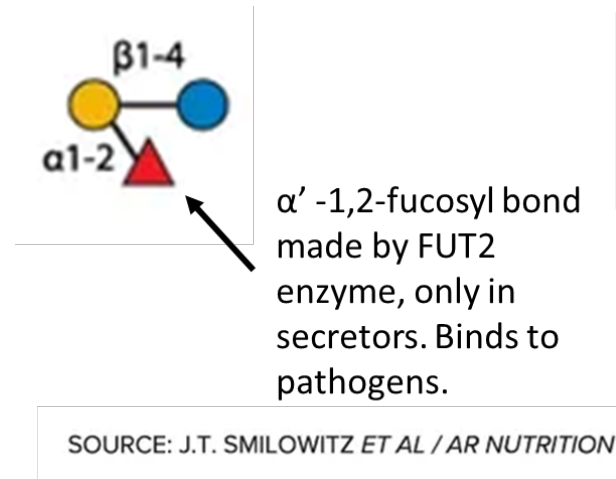
Yaoska
Reyes



FUT2 also shapes human milk composition

- Determine the types of glycans secreted into saliva and milk
- In milk, these glycans are called **human milk oligosaccharides (HMOs)**
 - Categorized as fucosylated, neutral non-fucosylated, and sialylated

**2'fucosyllactose
(2'FL) expressed
abundantly in
secretor mothers**



- **HMOs: the 3rd most abundant solid component of milk, yet infants can't digest them**
 - “Prebiotics” that shape the gut microbiome
 - May block pathogens by serving as soluble receptors (“decoy receptors”)
 - Genotype-specific interactions (Laucirica, et al. J Nutr. 2017; Ramani, et al. Nat Commun 2019)
 - Influence the developing immune system

Differences in HMO concentration in human milk by secretor status

319 Nicaraguan mothers, 1-month post-partum (medians and Q1, Q3)			
HMO ($\mu\text{g/mL}$)	Secretor mother (n=289)	Non-secretor mother (n=30)	p-value*
2'FL	2916.6 [2001.1, 4149.9]	7.8 [4.2, 13.8]	0.001
LNFP-I	1029.5 [567.2, 1604.3]	173.5 [131.6, 229.3]	0.001
DFLNT	968.6 [519.6, 1572.1]	343.9 [144.3, 457.4]	0.001
LNT	596.3 [370.9, 888.8]	1232.7 [603.2, 1828.9]	0.16
LNFP-II	562.3 [374.3, 815.3]	1159.7 [732.4, 1468.6]	0.045
6'SL	497.5 [330.0, 694.6]	626.8 [379.6, 887.1]	0.001
3'FL	375.7 [234.3, 587.0]	1119.4 [576.5, 1583.2]	0.068
FLNH	275.6 [177.9, 377.2]	347.9 [264.7, 661.3]	0.001
LSTc	285.3 [196.8, 403.5]	207.6 [152.1, 312.7]	0.001
DSLNH	224.2 [143.4, 331.7]	217.8 [162.5, 354.0]	0.62
DFLac	250.3 [163.5, 439.2]	22.9 [14.0, 28.2]	0.016
FDSLNH	193.8 [132.1, 316.7]	500.5 [280.9, 758.8]	0.002
DSLNT	174.1 [113.9, 259.4]	167.1 [112.3, 287.0]	0.001
LNnT	151.7 [99.9, 219.1]	126.8 [72.1, 189.1]	0.81
LNH	148.4 [98.9, 220.8]	136.0 [97.9, 218.6]	0.98
3'SL	113.8 [84.6, 175.4]	105.7 [76.1, 127.8]	0.002
LSTb	88.2 [57.8, 129.3]	110.2 [71.2, 173.8]	0.001
DFLNH	82.2 [46.4, 138.3]	205.9 [122.5, 293.3]	0.001
LNFP-III	46.4 [29.2, 76.5]	48.8 [20.2, 71.2]	0.72

* Group differences were tested using Kruskal-Wallis-tests.



Study aim



- Determine the association between HMO concentrations in early life and
- RV1 seroconversion
 - Rotavirus AGE risk over the first 36 months of life

Study Population

Sapovirus-Associated GastroEnteritis (SAGE) **birth cohort** in León, Nicaragua

(Vielot et al., 2021 *Ped Infect Dis J*)



1) Enrolled 409 breastfed newborns born June '17-July '18 in Perla Maria district

2) Exclusion criteria:

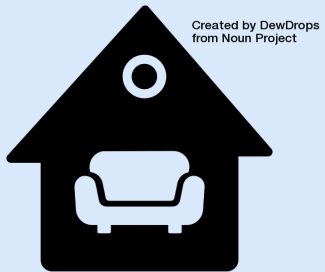
- birthweight <2000g; GA<36 weeks
- major health condition
- another child in household already enrolled

3) Limited analysis to 297 children providing complete sera set to 12 mo., were still breastfeeding and provided a milk sample 1 mo. postpartum

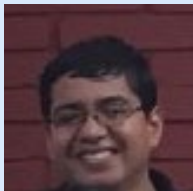
297 mother-child dyads followed weekly for 36 months

Baseline Data

Baseline household members and household characteristics



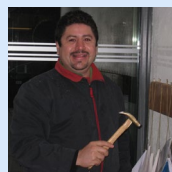
Nadja Vielot



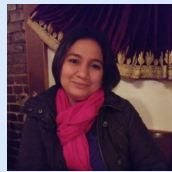
Christian Toval Ruiz



Maternal (milk) and child (saliva) HBGA phenotype



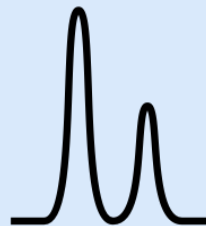
Filemón Bucardo



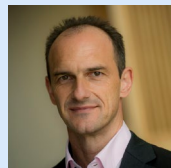
Yaoska Reyes



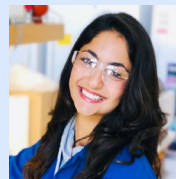
Breastmilk 1 month post-partum (1 time point)



19 HMOs measured with HPLC-FL



Lars Bode



Annalee Furst

Weekly Data



Gastroenteritis Episodes



Stool pathogen qPCR



Filemón Bucardo



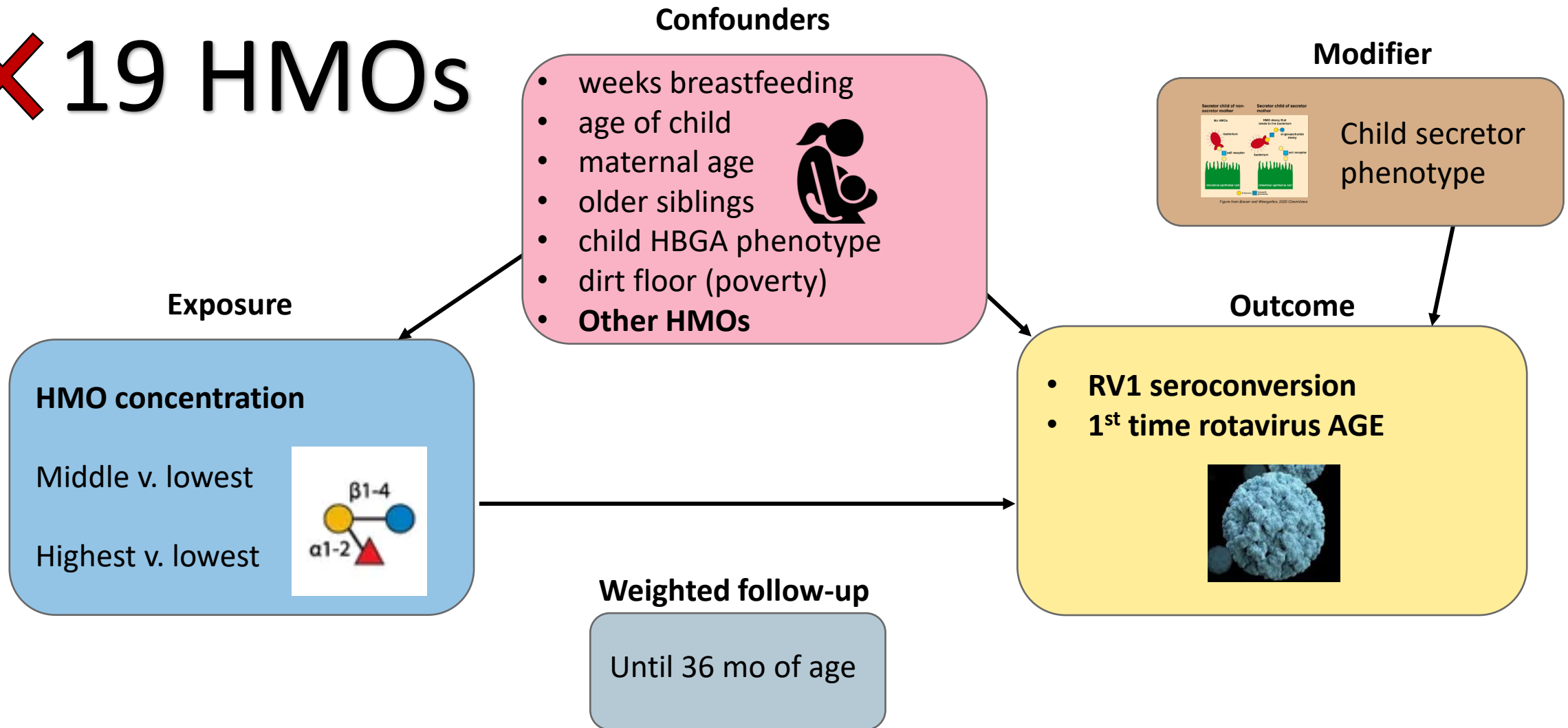
Yaoska Reyes



Breastfeeding assessed weekly

Statistical analysis

X 19 HMOs



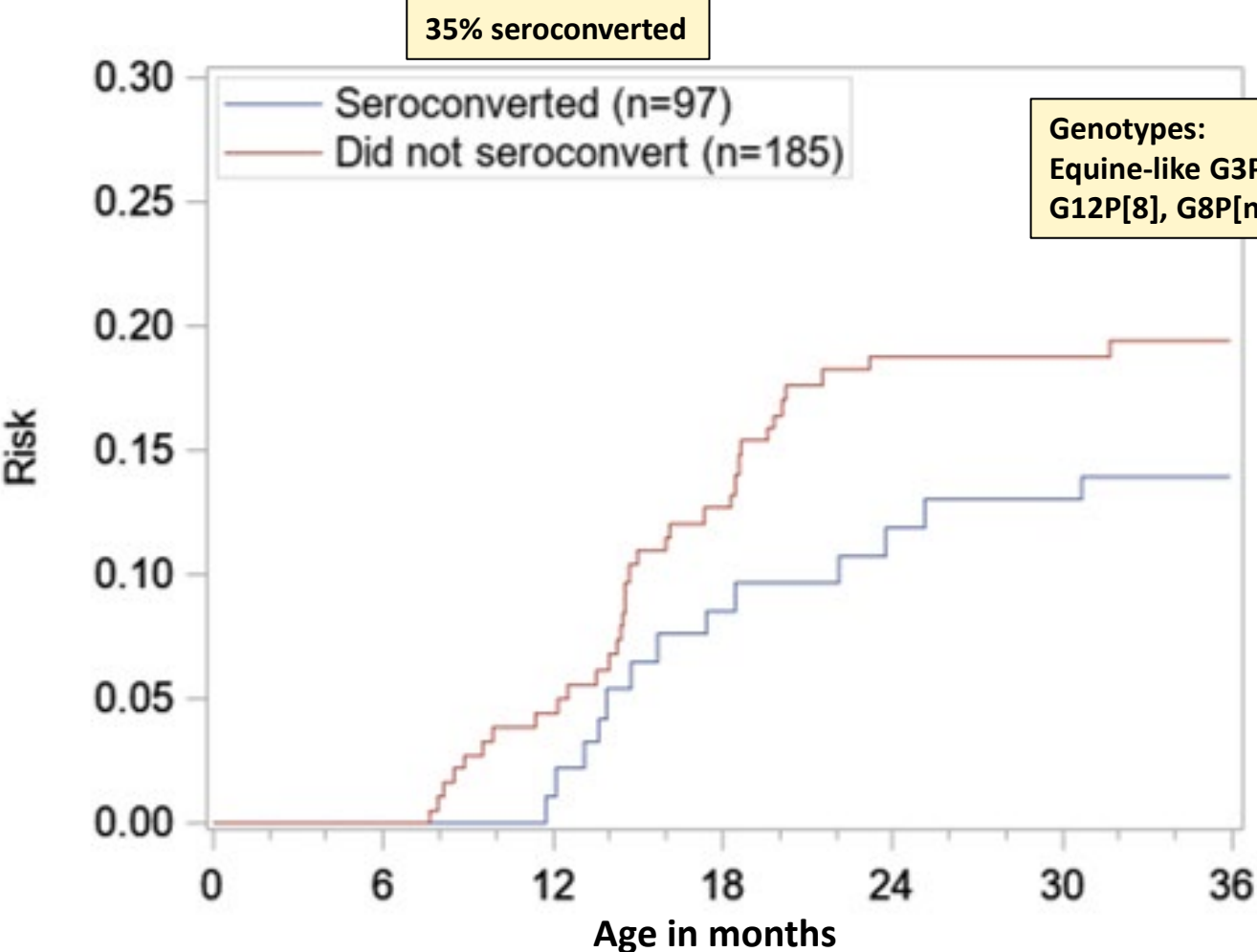
Results: Characteristics of 297 mother/child dyads

Frequency (%) or median (IQR^a)

Characteristics	Seroconverted (n= 103)	Did not seroconvert (n=194)	Not assessed for seroconversion (n=84)
Child sex, female	53 (51.4)	97 (50.0)	40 (47.6)
Mother is secretor	96 (93.2)	179 (92.3)	73 (86.9)
Child is secretor	93 (90.3)	168 (86.6)	79 (94.0)
<u>Lewis phenotype (children)</u>			
<i>Lewis A phenotype</i>	7 (6.8)	20 (10.3)	4 (4.8)
<i>Lewis B phenotype</i>	78 (75.7)	148 (76.3)	70 (83.3)
<i>Lewis negative</i>	18 (17.4)	26 (13.4)	10 (11.9)
Age of child at HMO measurement (mo.)	1.3 (1.2, 1.5)	1.3 (1.2, 1.4)	1.3 (1.2, 1.5)
Age of child at 1 st RV1 dose	2.0 (2.0, 2.1)	2.0 (2.0, 2.1)	2.0 (2.0, 2.1)
<i>Missing date of 1st dose</i>	1	2	8
Age of child at 2 nd RV1 dose	4.0 (4.0, 4.2)	4.0 (4.0, 4.1)	4.0 (4.0, 4.1)
<i>Missing recorded date of 2nd dose</i>	29	49	20
Number of household children <3 yrs			
<i>0-1</i>	61 (59.2)	124 (63.9)	49 (58.3)
<i>2</i>	42 (40.8)	70 (36.1)	35 (41.7)
Mother primary caregiver	57 (55.3)	116 (59.8)	41 (48.8)
Home floor construction			
<i>Dirt</i>	30 (29.1)	59 (30.4)	27 (32.1)
<i>Improved Floor</i>	73 (70.9)	135 (69.6)	57 (67.9)
Mother's age at birth of study child	23.7 (20.3, 28.9)	24.0 (19.8, 27.3)	23.8 (19.9, 27.0)
Mother's formal education (yrs)	13.0 (10.0, 14.0)	13.0 (10.0, 17.0)	13.0 (11.0, 16.0)

**Median duration of any breastfeeding →
18.7 months (8.3, 32.2)**

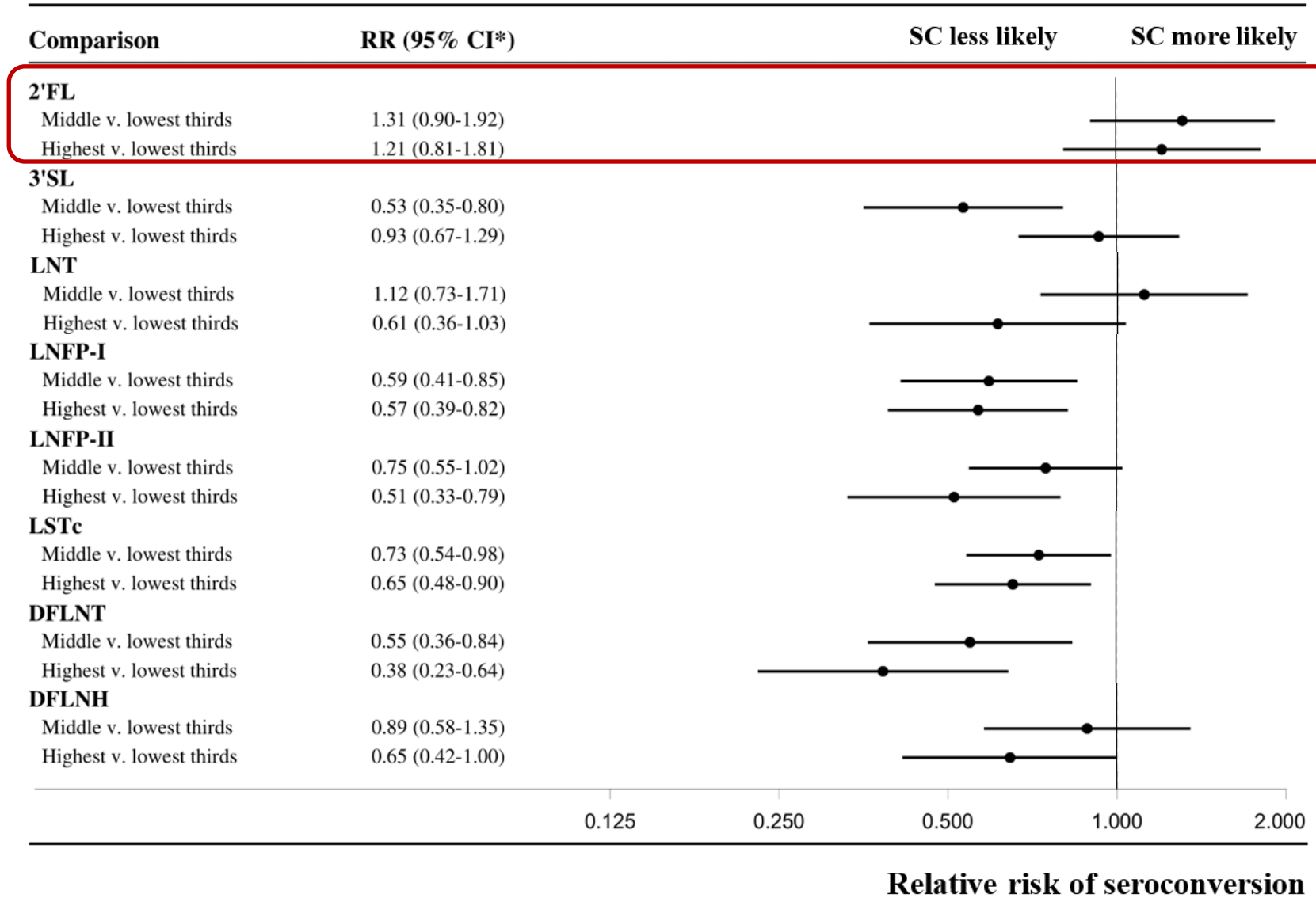
Results: rotavirus risk by seroconversion status



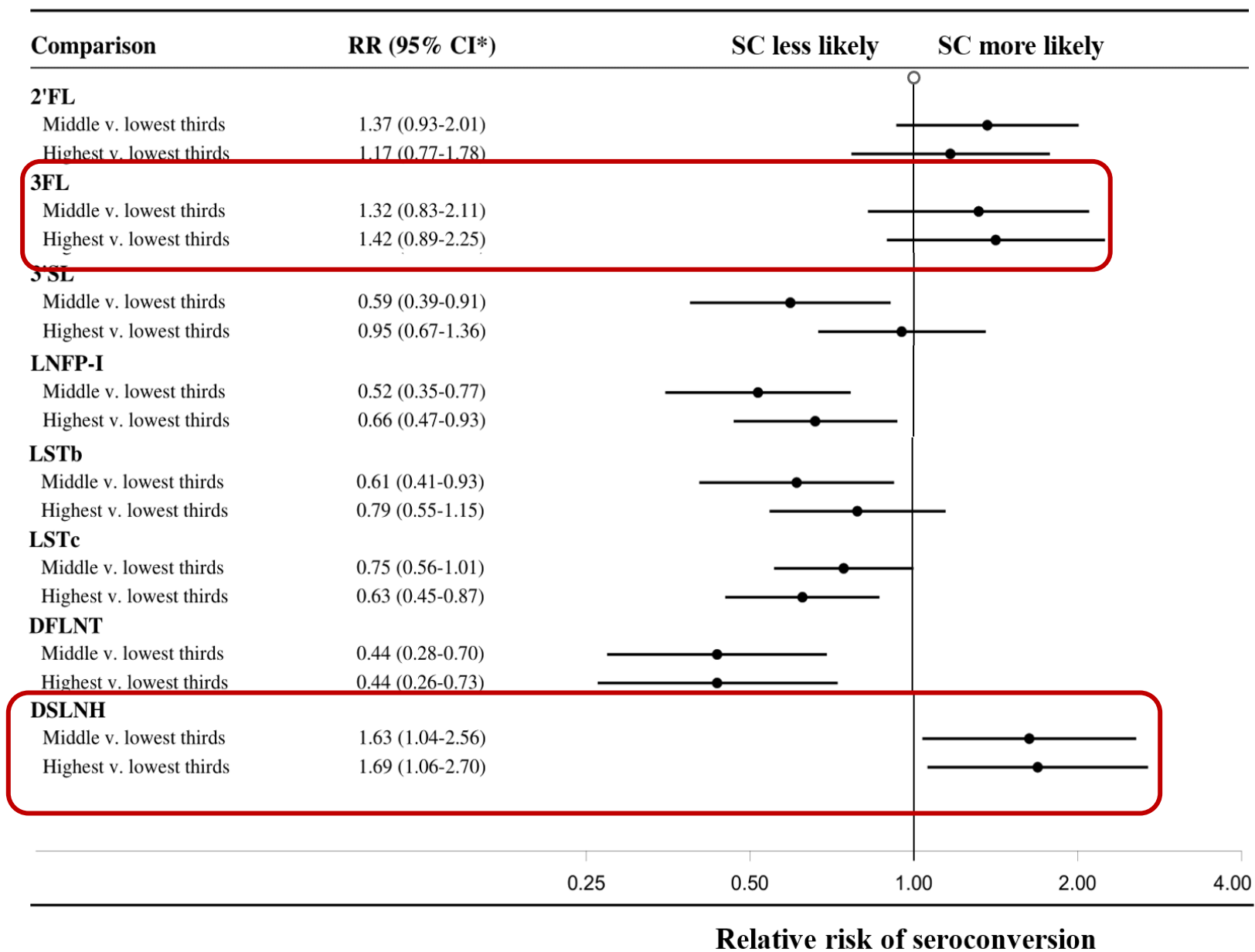
Among the 58 children IgA seropositive PRIOR to vaccination, 97% failed to seroconvert

Weighted for: weeks of exclusive breastfeeding prior to seroconversion ascertainment, currently breastfeeding at time of seroconversion ascertainment, mother's secretor status, child's secretor and Lewis status.

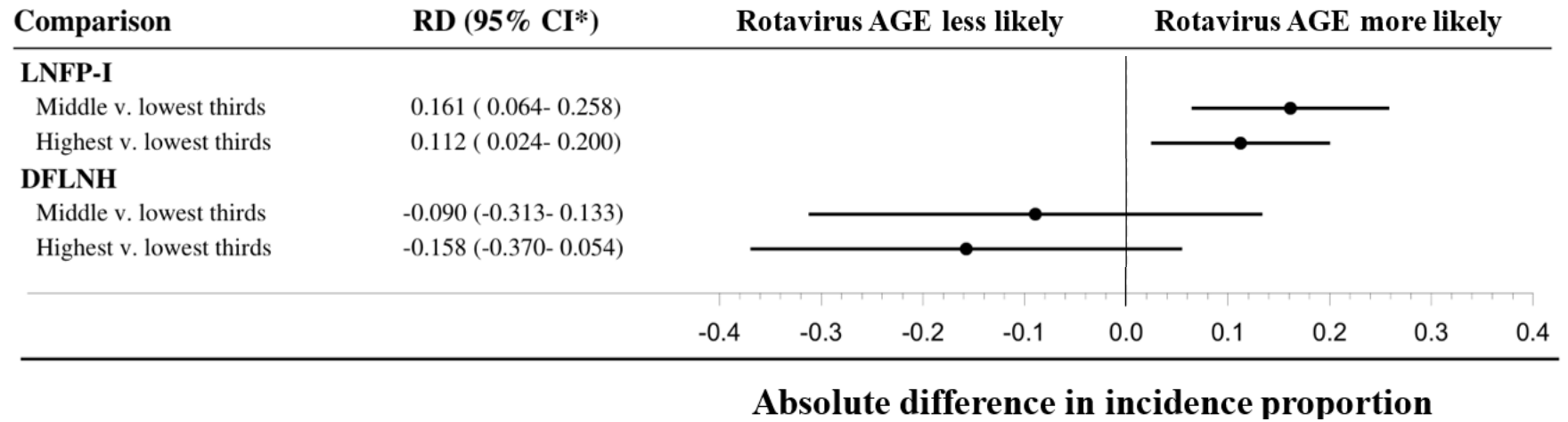
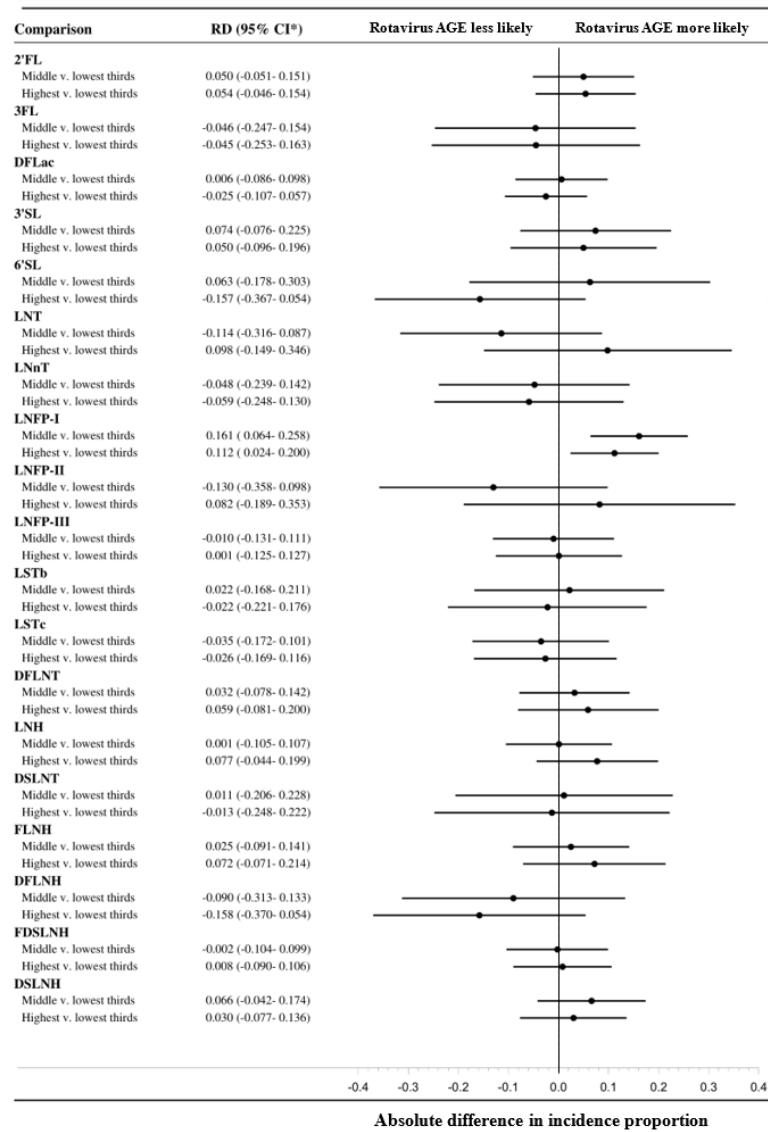
Results: RV1 seroconversion by HMO tertile (all children)



Results: RV1 seroconversion by HMO tertile (secretors)



Results: Rotavirus AGE by HMO tertile (all children)



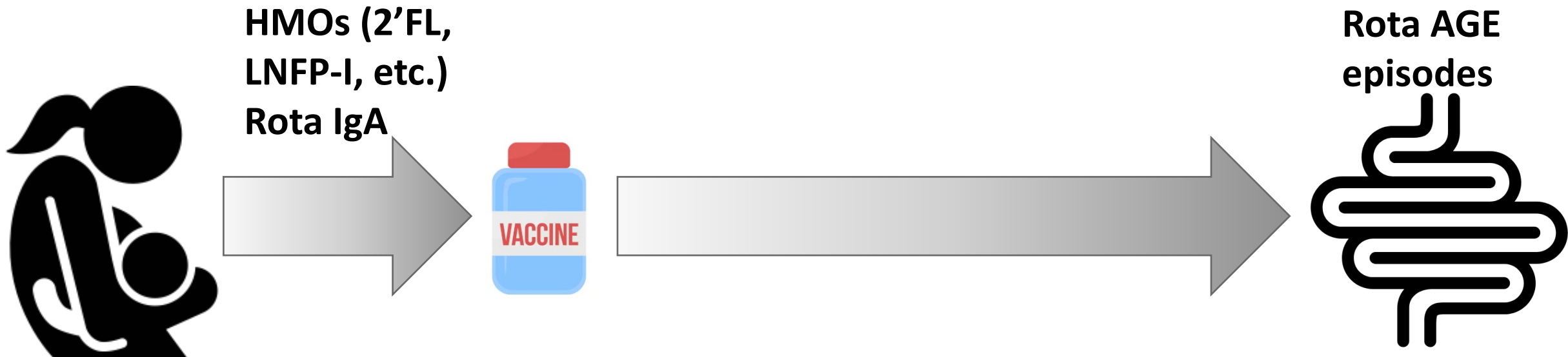
Absolute difference in incidence proportion

Limitations

- HMOs measured at one timepoint
- Could not measure exact daily volumes of milk received
- Did not measure levels of milk IgA antibodies against rotavirus
 - Secretor mothers may have higher IgA levels
- P[8] rotaviruses were in circulation at the time of this study; should be repeated in a population with more P[6] genotypes

Conclusion

- The concentrations of several fucosylated and sialylated HMOs in maternal milk were associated with RV1 seroconversion
- Less clear associations were seen with 36-month rotavirus AGE risk, although many other factors could influence risk over this time



While secretor phenotype is not modifiable, HMOs are somewhat modifiable



- **Breast is still best**
- HMO supplementation of formula should be guided by evidence
 - Several HMOs were associated with less seroconversion
 - Especially important in regions with high AGE burden
- If found efficacious in clinical trials, HMO supplements could be provided to breastfed babies
 - Vitamin D supplementation currently recommended for breastfeeding infants in the US

Agradecimientos al equipo Nica



Filemón Bucardo

Roberto Herrera

Christian Toval

Lester Gutiérrez

Yaoska Reyes

Patricia Blandón

Samuel Vilchez

Omar Zepeda

Sylvia Becker-Dreps

Fredman González

Marlen Morales

Edwing Centeno

Thank you to my collaborators and funders



**Rebecca Rubinstein,
MD-PhD candidate**



Filemón Bucardo, PhD



Lars Bode, PhD



Jessie Edwards, PhD



Nadja Vielot, PhD



National Institute of
Allergy and
Infectious Diseases

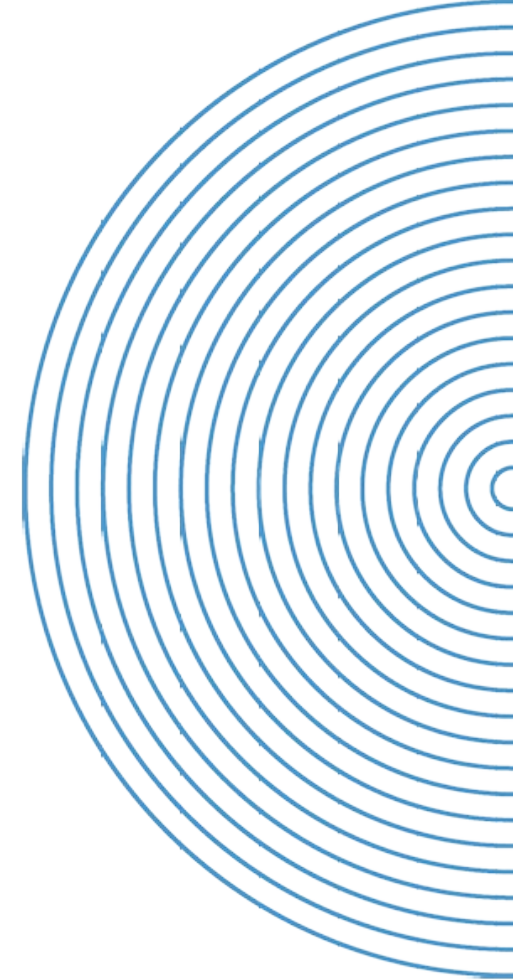


Fogarty International Center
Advancing Science for Global Health

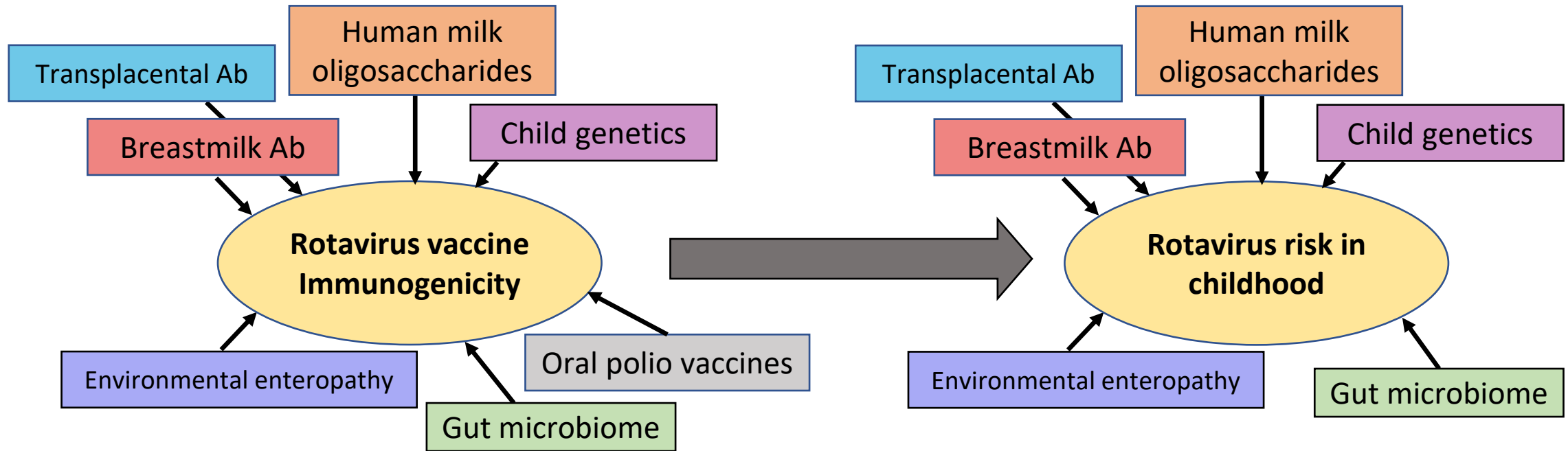


GILLINGS SCHOOL OF
GLOBAL PUBLIC HEALTH

QUESTIONS

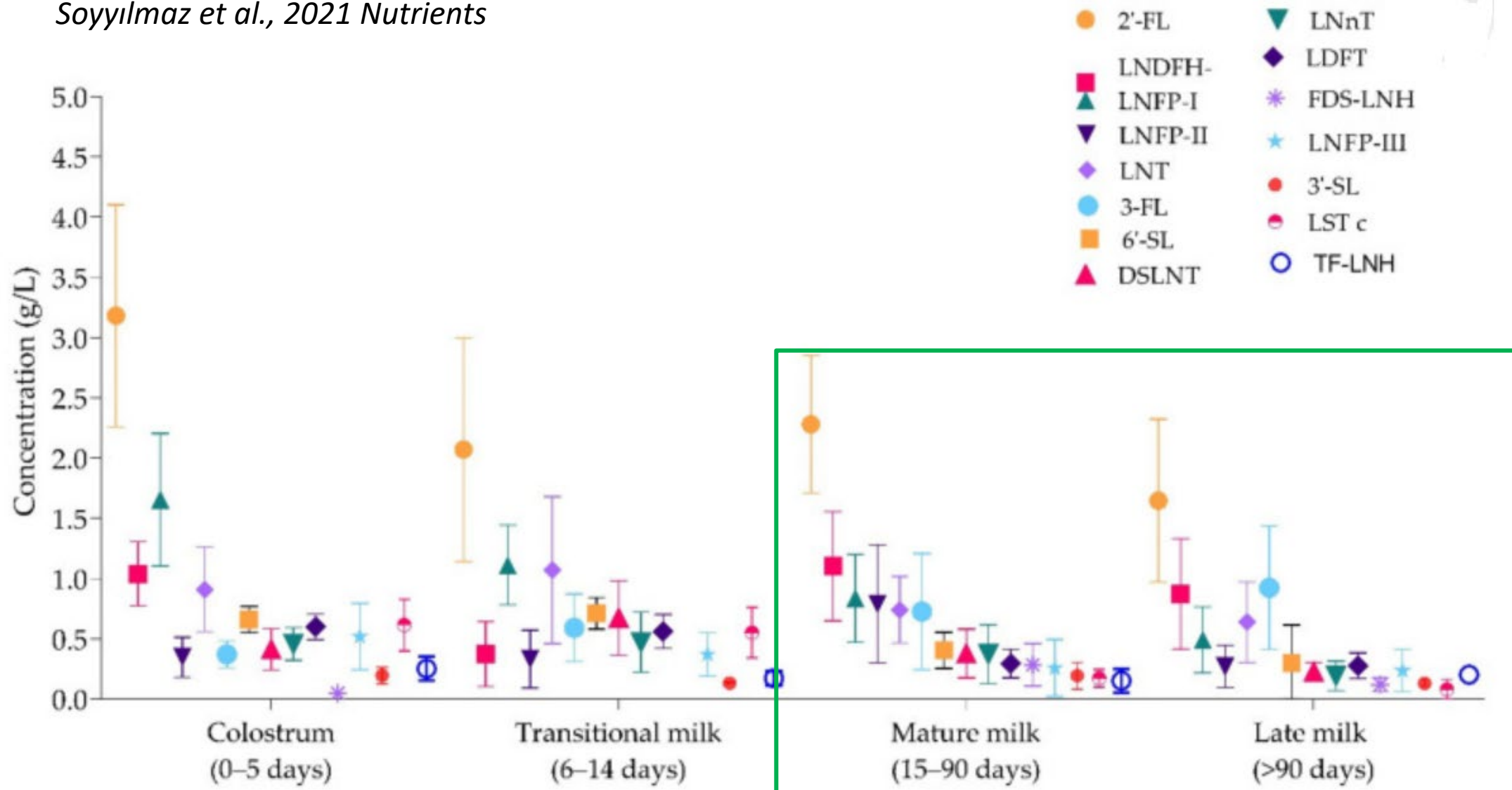


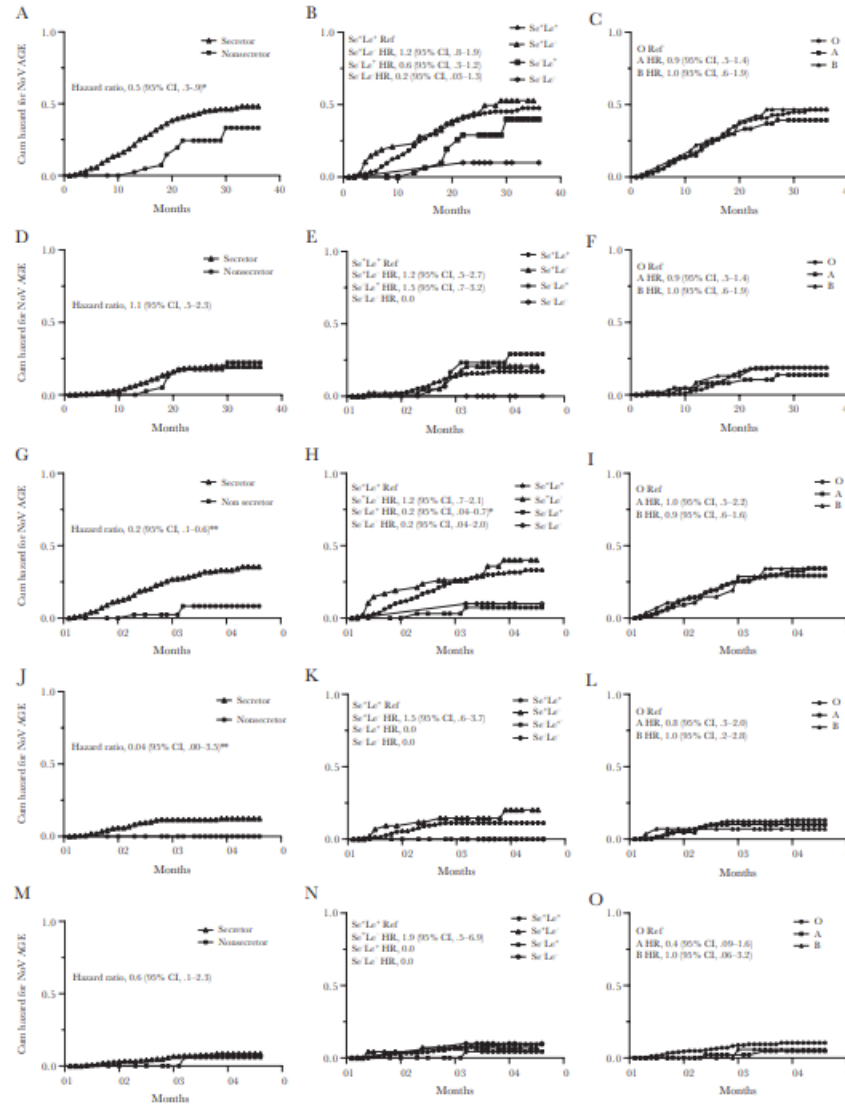
Factors influencing vaccine performance and disease risk



15 most abundant HMOs through lactation periods

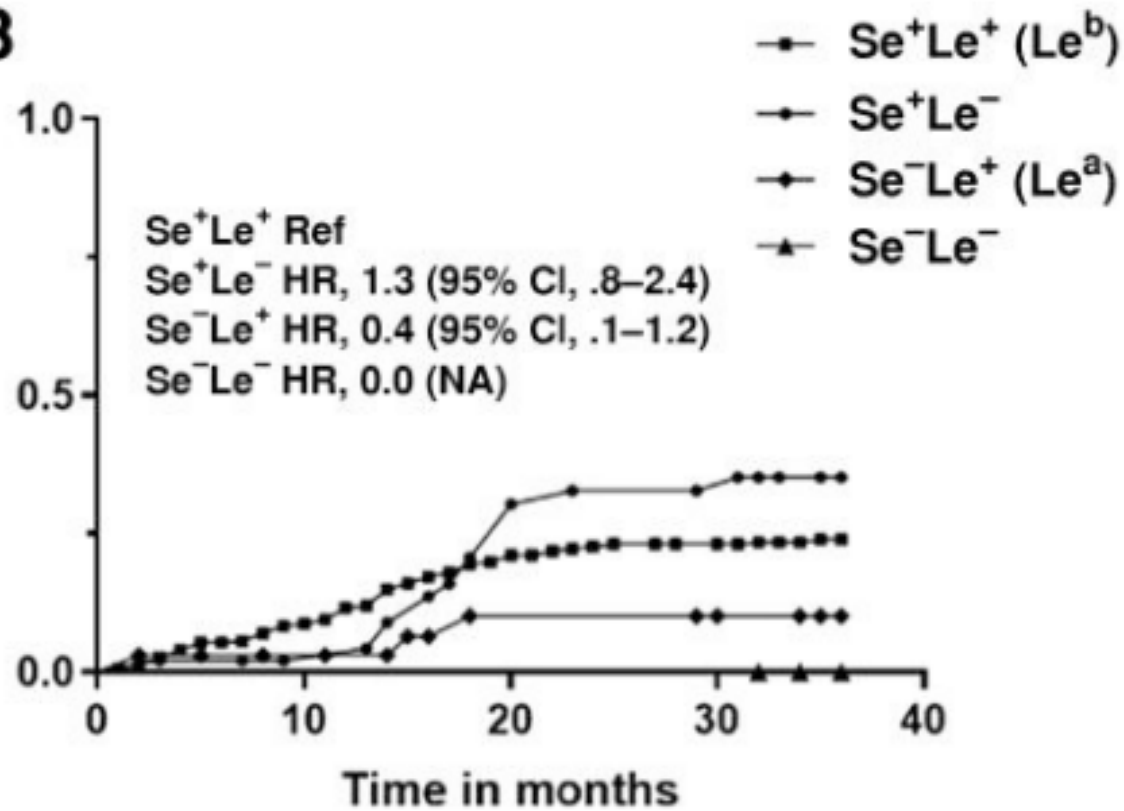
Soyyilmaz et al., 2021 Nutrients





Cumulative hazard curves to estimate time to norovirus infection, stratified by histo-blood group antigens

Figure 1. Cumulative hazard curves to estimate time to norovirus infection, stratified by histo-blood group antigens. A–C, All norovirus. D–F, Norovirus genogroup I. G–I, Norovirus genogroup II. J–L, Norovirus GII.4. M–O, Norovirus GII, non-GII.4. * $P < .05$; ** $P < .01$. Abbreviations: AGE, acute gastroenteritis; CI, confidence interval; HR, hazard ratio; Le⁻, Lewis negative; Le⁺, Lewis positive; NoV, norovirus; Se⁻, nonsecretor; Se⁺, secretor.

B**C**