Secretor Status Strongly Influences the Incidence of Symptomatic Norovirus Infection in a Genotype-Dependent Manner in a Nicaraguan Birth Cohort

Yaoska Reyes,[1,2] Fredman González,[1] Lester Gutiérrez,[1] Patricia Blandón,[1] Edwing Centeno,[1] Omar Zepeda,[1] Christian Toval-Ruíz,[1] Lisa C. Lindesmith,[3] Ralph S. Baric,[3] Nadja Vielot,[4] Marta Diez-Valcarce,[5,6] Jan Vinjé,[5] Lennart Svensson,[2,7] Sylvia Becker-Dreps,[3,4] Johan Nordgren,[2,a] and Filemón Bucardo[1,a]

[1]Department of Microbiology and Parasitology, National Autonomous University of Nicaragua–León, León, Nicaragua, [2]Division of Molecular Medicine and Virology, Linköping University, Linköping, Sweden, [3]Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA, [4]Department of Family Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA, [5]Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA, [6]Rollins School of Public Health, Emory University, Atlanta, Georgia, USA, and [7]Division of Medicine, Karolinska Institute, Solna, Sweden

Background. The role of histo-blood group on the burden and severity of norovirus gastroenteritis in young infants has not been well documented.

Methods. Norovirus gastroenteritis was assessed in 443 Nicaraguan children followed from birth until 3 years of age. Stool samples were tested for norovirus by reverse-transcription quantitative polymerase chain reaction (RT-qPCR), and histo-blood group antigens (HBGAs) were determined by phenotyping of saliva and blood. Hazard ratios and predictors of norovirus acute gastroenteritis (AGE) outcome stratified by HBGA were estimated using Cox proportional hazards models.

Results. Of 1353 AGE episodes experienced by children, 229 (17%) tested positive for norovirus with an overall incidence of 21.9/100 child-years. Secretor children were infected as early as 2 months of age and had a higher incidence of norovirus GII compared to nonsecretor children (15.4 vs 4.1/100 child-years, $P = .006$). Furthermore, all GII.4 AGE episodes occurred in secretor children. Children infected with GI (adjusted odds ratio [aOR], 0.09 [95% confidence interval {CI}, .02–.33]) or non-GII.4 viruses (aOR, 0.2 [95% CI, .07–.6]) were less likely to have severe AGE compared to GII.4-infected children.

Conclusions. Secretor status in children strongly influences the incidence of symptomatic norovirus infection in a genogroup or genotype-dependent manner and provides evidence that clinical severity in children depends on norovirus genotypes.

Keywords. birth cohort; histo-blood group antigens; incidence; Nicaragua; norovirus; secretor.

Noroviruses are a major cause of sporadic and epidemic acute gastroenteritis (AGE) in people of all ages worldwide. They are associated with an estimated 685 million cases of diarrhea annually and are responsible for approximately 200 000 deaths including 71 000 deaths among children in developing countries [1]. Noroviruses are a genetically diverse group of single-stranded RNA viruses in the family Caliciviridae with a high mutation rate (1.21×10^{-2} to 1.41×10^{-2} substitutions per site per year) [2, 3]. Viruses belonging to genogroup (G) I and II are associated with most infections in humans. They can be further classified in 9 GI genotypes and at least 23 GII genotypes [4]. Since the mid-1990s, GII.4 viruses are the most prevalent strains with new GII.4 strains, called variants, emerging every 2–7 years and replacing previous dominant variants [5, 6]. Both in adults and children, GII.4 viruses are associated with more severe disease compared to other genotypes [7, 8].

Several in vitro and in vivo studies have suggested a role of human histo-blood group antigens (HBGAs) in the susceptibility to norovirus infections [9–13]. The synthesis of these HBGAs is mediated by fucosyltransferases and glycosyltransferases under the genetic control of the FUT2 (secretor), FUT3 (Lewis), and ABO(H) genes. The so-called "nonsecretors" (inactivated FUT2 enzyme) do not express HBGAs in mucosa and secretions and have been found to be resistant to several noroviruses, including the predominant GII.4 genotype, with only a few reported exceptions [14–16]. In contrast, some genotypes such as GII.1, GII.2, GII.3, GII.7, and GI.3 have been observed in both secretor and nonsecretor individuals [17]. Secretor status can also modify susceptibility in different degrees; for example, GII.6 viruses have been reported to preferentially, but not exclusively, infect secretors [18]. The Lewis-positive phenotype has been associated with increased susceptibility to GI viruses in some studies, whereas GII viruses generally are observed in both Lewis-negative and Lewis-positive individuals [19, 20]. However, more studies are needed to elucidate the role of Lewis

Received 22 March 2021; editorial decision 8 June 2021; accepted 10 June 2021; published online June 15, 2021.

[a]J. N. and F. B. contributed equally to this work as co–senior authors.

Correspondence: Yaoska Reyes, MSc, Campus Medico, 2nd floor, Department of Microbiology and Parasitology, National Autonomous University of Nicaragua, León, León 00068, Nicaragua (yaoska.reyes@liu.se).

antigens in norovirus susceptibility. A recent meta-analysis suggested that the blood types A, B, and AB do not affect overall susceptibility to norovirus infection, whereas blood type O was associated with higher susceptibility [21].

The majority of the above-described findings have been reported as results from challenge studies, outbreaks, or active/passive surveillance. Population-based birth cohorts have provided valuable data on norovirus infection, immunity, and risk factors, as they represent the entire population and follow risk over time [22]. In an Ecuadorian birth cohort, secretor status or blood group was not associated with risk of norovirus infection; 88% of the children in this study were secretors [23]. In an Indian birth cohort, however, secretor status was associated with risk of GI norovirus diarrhea; 61% of the children in this study were secretors [24]. Data are still limited on the role of host genetic factors at the genotype level.

The aim of this study was to describe the incidence of norovirus AGE in children in a birth cohort in Nicaragua and to determine the role of HBGAs in susceptibility and disease severity in a genogroup- and genotype-dependent manner. The results are discussed in the context of vaccine composition and clinical trials evaluating the efficacy of pediatric norovirus vaccines.

MATERIALS AND METHODS

Ethical Considerations

The protocol and questionnaire used in this study were reviewed and approved by the Ethical Committee for Biomedical Research of Universidad Nacional Autónoma de Nicaragua–León (Acta number 2-2017) and the University of North Carolina at Chapel Hill (study number 16-2079). All participants' families have provided informed consent for study participation, including biobanking of samples for future unspecified research.

Subjects and Data Collection

A total of 444 Nicaraguan infants were enrolled in a population-based birth cohort study investigating childhood gastroenteritis (Sapovirus-Associated Gastro-Enteritis [SAGE] study) between June 2017 and July 2018. In this birth cohort, field workers made weekly household visits with active surveillance for gastroenteritis up to 3 years. Furthermore, clinical characteristics were collected for each episode of AGE from the child's caregiver. A detailed description of the cohort has been described elsewhere [25]. One child was excluded due to early dropout (≤5 weeks).

Definition of Acute Gastroenteritis and Norovirus Episodes

AGE was defined as per the SAGE study protocol, described elsewhere [25]. A norovirus AGE episode was defined based on positive results by reverse-transcription quantitative polymerase chain reaction (RT-qPCR) in a sample taken during or within 14 days of the beginning of the gastroenteritis episode. A time period of ≥30 days was required to separate 2 different episodes of norovirus AGE [26] if caused by the same genotype (or genogroup, if genotype information was not available). Infection with a different genogroup or genotype at any time was considered a new episode. The clinical severity score of norovirus AGE episodes was assigned as described by Vielot and co-workers [25].

Stool and Saliva Sampling

Stool samples were collected as described elsewhere [25]. Saliva samples for HBGA phenotyping were collected by pipette and stored at –20°C.

RNA Purification for Viral Screening

The RNA extraction was made from a mix of 135 µL of 10% stool suspensions plus 5 µL of coliphage MS2 used as internal extraction control to validate the results of the assay [27]. Signal amplification of the internal control ensured no inhibitors in the sample. The QIAamp Viral RNA Mini Kit (Qiagen, Valencia, California) was used following the manufacturer's instructions. A total of 60 µL of RNA was collected and stored at –80°C until RT-qPCR.

RT-qPCR Assay for Norovirus Detection

Viral RNA was tested by real-time PCR for GI and GII noroviruses in a duplex format by using the AgPath-ID One-Step RT-PCR Kit (Applied Biosystems, Foster City, California). In brief, 5 µL of RNA was added to a reaction mixture consisting of 12.5 µL of 2× RT-PCR Buffer mix, 1 µL of 25× RT-PCR Enzyme Mix, 1.7 µL of Detection Enhancer, 1 µL of a primer/probe mix (400 nmol/L of each oligonucleotide primer Cog1F, Cog1R, Cog2F, and Cog2R, and 200 nmol/L of each TaqMan Probe Ring 1E and Ring 2) [28, 29], as well as 100 nM of each primer and probe (MS2.F/R and MS2.P) for a MS2 bacteriophage internal amplification, and 3.75 µL of RNAse free water, to final volume of 20 µL. The real-time PCR reactions were performed in a 96-well reaction plate using the Roche LightCycler 96 Instrument. PCR was performed under the following conditions: 45°C for 10 minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds. Cycle threshold (Ct) values of <37 and <35 were considered positive for norovirus GII and GI, respectively [30].

Nucleotide Sequencing

All norovirus-positive samples were shipped to the Centers for Disease Control and Prevention (Atlanta, Georgia) for conventional RT-PCR followed by Sanger sequencing of the RT-PCR products [30]. Sequences were genotyped using online norovirus typing tools (https://norovirus.ng.philab.cdc.gov/). Samples with Ct values ≤33 were submitted for sequencing [31].

RT-qPCR Assay for Viral Coinfections

All samples were tested by qPCR for other enteric viruses: sapovirus, astrovirus, and rotavirus. RT-qPCR for sapovirus

was described elsewhere [32]. Astrovirus and rotavirus detection were performed as described by Liu et al [33].

Secretor, Lewis, and ABO Phenotyping

Secretor and Lewis phenotyping on saliva collected from children at 6 months of age was performed by an in-house enzyme-linked immunosorbent assay described by Nordgren and co-workers [34]. The Ulex europaeus lectin peroxidase (UEA-I, Sigma-Aldrich, Sweden) was used for secretor phenotyping and the monoclonal anti–Lewis-a and anti–Lewis-b from Seraclone and Diaclone (Bio-Rad, Uppsala, Sweden), respectively, were used for Lewis phenotyping. Children were classified as follows: secretor/Lewis-b (Se^+Le^+), secretor/Lewis-negative (Se^+Le^-), nonsecretor/Lewis-a (Se^-Le^+) and nonsecretor/Lewis-negative (Se^-Le^-). ABO phenotyping was performed by hemagglutination test. Twenty-eight children did not provide a blood sample; therefore, ABH phenotyping was made in saliva as described elsewhere [34]. In saliva from 5 children collected at 6 months, Lewis and/or secretor phenotypes showed discrepancies—that is, negative secretor phenotype using the UEA-I assay but Lewis-b phenotype. In these children, saliva samples from 12, 18, and 24 months were examined to define Lewis and secretor phenotypes.

Statistical Analysis

We analyzed data between 12 June 2017 and 19 June 2020. Incidence (episodes/100 child-years) of norovirus gastroenteritis and differences in median time to first norovirus episode were calculated for norovirus genogroups and genotypes. The Cox proportional hazards model was used to obtain hazard ratios (HRs) and their 95% confidence intervals (CIs) and corresponding cumulative hazard curves, to compare the relative hazard of norovirus infection by secretor status, Lewis antigens, and ABO blood type. The reference groups were defined as secretor, Se^+Le^+, and type O, respectively. For ABO incidence and HR analyses, only secretor children were included, as nonsecretors do not express the ABO antigens on mucosal surfaces. Children with AB phenotype were excluded due to low frequency (n = 5). The severity of the first norovirus AGE episode was only explored in children with known infecting genotype. Severity score was arbitrarily dichotomized as either above or below the median value (not severe, ≤6; severe, >7). Multivariate logistic regression was subsequently performed to compare the odds of severe infection by norovirus genotype (GII.4 vs GI genotype and non-GII.4 genotypes). This model was adjusted for age of the child during the first episode and coinfections with rotavirus, astrovirus, or sapovirus. Statistical analyses were performed in SPSS 21.0 software (IBM, Armonk, New York). A P value <.05 was considered statistically significant.

RESULTS

Distribution of HBGA Phenotypes in Children Included in the Cohort

Most of the children in the cohort were Se^+Le^+ (76%) followed by Se^+Le^- (13%) and, in lesser proportions, Se^-Le^+ (8%) and Se^-Le^- (3%) (Table 1). In secretor children, the ABO blood group distribution was the following: O (72.5%), A (18.7%), B (7.6%), and AB (1%).

Norovirus Incidence in Association With Secretor Status

Between 12 June 2017 and 19 June 2020, the 443 children in the birth cohort experienced 1497 episodes of diarrhea and/or vomiting over 759 child-years, of which 1347 were sampled for norovirus screening. Of these, 229 (17%) tested norovirus positive, of which 165 (72%) were documented as primary symptomatic infection and occurred between 2 and 33 months of age (median, 14 months [interquartile range {IQR}, 9–19 months]). Sixty-six (40%) of the primary episodes occurred in children of ≤12 months of age. One hundred eighteen of the 165 norovirus-positive children (72%) experienced only 1 symptomatic norovirus episode during the entire study, whereas 47 children (28%) had recurrent norovirus AGE episodes. Of the 229 norovirus-positive episodes, 94 (41%) were norovirus GI, 135 (58%) were norovirus GII, and 2 (1%) were coinfections with both genogroups.

The overall incidence of norovirus was 21.9 cases per 100 child-years, being higher in secretor children than in nonsecretors (23.3 vs 12.3/100 child-years, P = .019). Further stratification by genogroup shows that the incidence of norovirus GII was approximately 4-fold higher in secretors than in nonsecretors (15.4 vs 4.1/100 child-years, P = .006), whereas the incidence of norovirus GI was similar between the secretors and nonsecretors (7.8 vs 8.2/100 child-years, P = .648) (Table 1). On average, AGE with norovirus GI occurred significantly later in life (median, 16 months [IQR, 12–19 months]) compared with norovirus GII (median, 13 months [IQR, 7–19 months]) (P = .020; Figure 1D and 1G).

Norovirus Genotypes Observed in the First and Second Norovirus AGE Episodes

A total of 114 (50%) of the norovirus-positive samples (n = 229) were genotyped; low viral load (Ct value >33) precluded successful genotyping in the remaining samples. In total, 11 different capsid genotypes were observed (4 GI and 9 GII) with genotype GII.4 being the most predominant (39%), followed by GI.3 (22%), GI.5 (13%), and GII.12 (9%) (Table 2). GII.4 was the most predominant genotype (42%) in primary norovirus infections, followed by GI.3 (18%), GII.12 (9%), GII.17 (9%), and GI.5 (9%). In contrast, GI.3 (34%) was the most common genotype in secondary symptomatic infection, followed by GII.4 (26%) and GI.5 (22%). [P16] and [P3] were the most common P-types observed. The most common combination of genotypes

Table 1. Incidence Rate (per 100 Child-Years) of the First Norovirus Gastroenteritis Episode by Histo-Blood Group Antigen Status in Children Followed in the Birth Cohort in Nicaragua, 2017–2020

	Incidence Rate									
	Overall		Genogroup				Genotype[a]			
HBGA Profile	No.	(%)	No.	GI	No.	GII	No.	GII.4	No.	GII Non-GII.4
Secretor phenotype										
Secretor (n = 396 [89%])	153	23.3[b]	50	7.8	103	15.4[b]	37	5.7[c]	24	3.6
Nonsecretor (n = 47 [11%])	12	12.3	8	8.2	4	4.1	0	0.0	2	2.1
Secretor/Lewis phenotype										
Se^+Le^+ (n = 338 [76%])	123	22.9	43	7.6	86	15.2	30	5.2	21	3.7
Se^+Le^- (n = 58 [13%])	30	26.3	8	9.2	15	17.1	7	9.2	3	3.4
Se^-Le^+ (n = 36 [8%])	11	14.8	8	10.7	3	4.0	0	0.0	1	1.3
Se^-Le^- (n = 11 [3%])	1	4.3	0	0.0	1	4.3	0	0.0	1	4.2
ABO blood type[d]										
A (n = 74 [18.7%])	26	21.0	8	6.4	18	14.5	6	6.1	2	2.0
B (n = 30 [7.6%])	12	23.4	4	7.8	8	15.6	2	3.9	1	1.9
O (n = 287 [72.5%])	113	23.8	38	8.0	75	15.6	28	5.9	21	4.4
Total (N = 443)	165	21.9	58	7.8	107	14.1	37	5.0	26	3.4

Abbreviations: HBGA, histo-blood group antigen; Le^-, Lewis negative; Le^+, Lewis positive; Se^-, nonsecretor; Se^+, secretor.

[a]These rates are based on 61 norovirus-positive samples that were successfully genotyped and could be classified into GII.4 or non-GII.4, respectively.

[b]$P < .05$.

[c]$P < .01$.

[d]n = 396; only analyzed for secretor children. AB group (n = 5) was excluded from analysis.

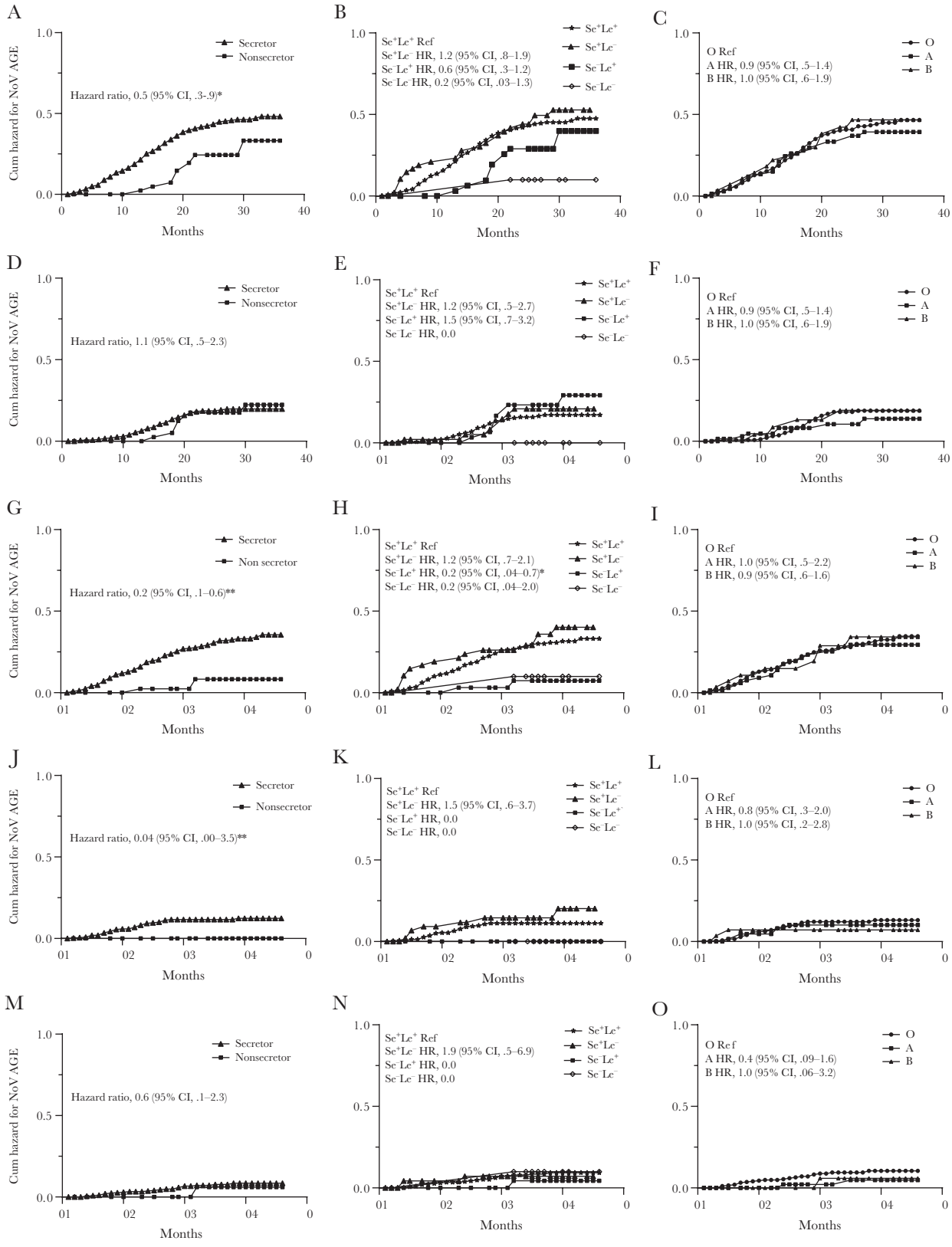

Figure 1. Cumulative hazard curves to estimate time to norovirus infection, stratified by histo-blood group antigens. *A–C*, All norovirus. *D–F*, Norovirus genogroup I. *G–I*, Norovirus genogroup II. *J–L*, Norovirus GII.4. *M–O*, Norovirus GII, non-GII.4. **P* <.05; ***P* <.01. Abbreviations: AGE,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

Table 2. Distribution of Genotype-Specific Norovirus Gastroenteritis Episodes by Histo-Blood Group Antigen Phenotype

Phenotype	Norovirus Genotype											
	GII.4 (n = 44)	GII.12 (n = 10)	GII.17 (n = 8)	GII.14 (n = 5)	GII.21 (n = 2)	GII.1 (n = 1)	GII.5 (n = 1)	GII.6 (n = 1)	GII.15 (n = 1)	GI.3 (n = 25)	GI.4 (n = 1)	GI.5 (n = 14)	GI.7 (n = 1)
Secretor phenotype													
Secretor	44 (100)	10 (100)	8 (100)	5 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	22 (88)	1 (100)	13 (93)	1 (100)
Nonsecretor	0 (0)	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	3 (12)	0 (0)	1 (2.1)	0 (0)
Secretor/Lewis phenotype													
Se^+Le^+	34 (77)	9 (90)	8 (100)	3 (60)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	18 (72)	1 (100)	10 (71)	1 (100)
Se^+Le^-	10 (23)	1 (10)	0 (0)	2 (40)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (16)	0 (0)	3 (21)	0 (0)
Se^-Le^+	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	3 (12)	0 (0)	1 (7)	0 (0)
Se^-Le^-	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
ABO blood type (secretor)													
O	34 (77)	9 (90)	6 (75)	4 (80)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	17 (68)	1 (100)	10 (71)	1 (100)
A	6 (14)	0 (0)	2 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (16)	0 (0)	3 (21)	0 (0)
B	2 (4.5)	1 (10)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4)	0 (0)	0 (0)	0 (0)
AB	2 (4.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Data are presented as No. (%).

Abbreviations: Le^-, Lewis negative; Le^+, Lewis positive; Se^-, nonsecretor; Se^+, secretor.

was GII.4[P16] and GI.3[P3]. Only GII.4 Sydney and GII.17 were detected with 2 different P-types (Supplementary Table 1).

Nonsecretor Status and its Effect on Norovirus Acute Gastroenteritis Episodes

The first observed norovirus AGE occurred at 2 months of age in secretor children; in contrast, the first norovirus AGE in nonsecretor children occurred at 13 months of age (Figure 1A). Nonsecretor children were 50% less likely to have norovirus AGE as compared to secretors at any time point during the surveillance period (HR, 0.5 [95% CI, .3–.9]; $P = .024$) (Figure 1A). No strong effect regarding Lewis phenotype was observed on norovirus incidence, although Se^-Le^- was the phenotype with the lowest risk for norovirus AGE (HR, 0.2 [95% CI, .03–1.3]) compared to other groups (Se^-Le^+, Se^+Le^+, Se^-Le^+) (Figure 1B), but the numbers were low, warranting careful interpretation. A similar analysis to estimate the risk of norovirus was performed with ABO blood groups for secretor children, and no difference in the overall risk for norovirus AGE was observed (Figure 1C).

Correlation Between Norovirus Genotype Infection and HBGA Phenotypes

Norovirus GII.4, GII.12, GII.17, and GII.14 caused disease in Se^+Le^+ and Se^+Le^- children. In contrast, GI.3 and GI.5 viruses were found in both Se^+Le^+ and Se^-Le^+ (Table 2). Interestingly, the 2 GII.21 infections were found in nonsecretor (Se^-Le^+ and Se^-Le^-) children. Children with O blood type were infected by all norovirus genotypes observed in this study, and the GII.4 genotype infected children of any blood type (Table 2).

Norovirus Genotypes and severity

Multivariate logistic regression analysis showed that children with a first AGE episode caused by GII.4 were more likely to experience severe gastroenteritis as compared with children infected with GI (adjusted odds ratio [aOR], 0.09 [95% CI, .02–.33]; $P < .001$) and GII of non-GII.4 genotype (aOR, 0.2 [95% CI, .07–.6]; $P = .006$) (Table 3).

Effect of GII.4 First Symptomatic Infection on Reinfections

None of the 37 children experiencing first AGE episodes due to GII.4 were reinfected with the same genotype, with the exception of 1 child who experienced a secondary episode of GII.4 Sydney infection 41 days after the first episode. However, 9 children with prior GII.4 gastroenteritis had GI norovirus (GI.3 and GI.5) during the second norovirus AGE episode (Table 4). A total of 6 of 17 (35%) genotyped reinfections were defined as severe (AGE score, 8–11); all severe secondary infections occurred in secretor children.

DISCUSSION

Only a limited number of birth cohort studies have investigated the role of genetic factors in burden of disease and norovirus [11, 23, 35]. This study provides data on the association between

HBGAs and the incidence of genogroup or genotype-specific norovirus gastroenteritis in children enrolled in a longitudinal birth-cohort study conducted in Nicaragua.

The overall incidence of norovirus (21.9/100 child-years) is in accordance to what has been found in other studies in low- and middle-income settings such as Ecuador, Chile, India, Brazil, and Peru [11, 23, 24, 36, 37], demonstrating that norovirus is a major cause of AGE in young children. There was a marked difference in the incidence rate of overall norovirus AGE between secretor and nonsecretor children (23.3 vs 12.3/100 child-years, $P = .019$), in contrast to what was reported in an Ecuadorian cohort where rates of infection were similar (52.8 vs 45.9/100 person-years) [23]. This difference could be due to the circulation of non-GII.4 norovirus genotypes infecting nonsecretors in Ecuador. In the current study, the incidence of GII norovirus was significantly higher in secretor than in nonsecretor children (15.4 vs 4.1, $P = .006$), but this association was not observed for GI infections, as previously shown in an Indian birth cohort [24]. Similar to the observations in Ecuador, all symptomatic GII.4 infections were found among secretor children. These findings are in line with previous reports showing that nonsecretor individuals are generally protected against GII.4 viruses as well as several other genotypes [18, 38].

We further analyzed genotype-specific susceptibility patterns of the genotypes in our cohort. Interestingly, the only GII non-GII.4 genotype found in nonsecretors was GII.21 (n = 2). To date, no specific norovirus genotype has been described to exclusively infect nonsecretor individuals. GII.21 and GII.13 viruses were previously reported as a unique genetic lineage showing a novel glycan binding site distinct from other GII noroviruses, recognizing Lewis-a antigens [39] present in Lewis-positive nonsecretors. However, we found GII.21 in both Lewis-positive and Lewis-negative nonsecretors. GII.21 viruses are rare and have sporadically been reported in the United States and several Asian countries but more often in wastewater [40–42]. As nonsecretor individuals are globally more rare than secretors, more studies are needed to explore if nonsecretor individuals are more susceptible to GII.21 infection.

No notable difference was found between ABO and the overall risk of norovirus symptomatic infection (Figure 1C). Norovirus GII.4 AGE was observed in secretor children of all blood types, in agreement with previous observations that GII.4 viruses interact with all ABO antigens [21]. Importantly, the HBGA distribution of the children in this cohort was dominated by Se^+ and O blood type, which is different from European and African populations and could thus result in a different norovirus molecular epidemiology at the population level, such as higher frequency of secretor-dependent genotypes.

No studies have investigated how HBGAs mediate susceptibility over time. Our results show that the first norovirus AGE episode occurred later in life in nonsecretor vs secretor children (13 vs 2 months). Moreover, norovirus GII episodes appear earlier in life compared to GI episodes. Similarly, a cohort from Peru noted a tendency toward earlier age for symptomatic GII cases in comparison with GI cases [26]. In our cohort, the globally dominant GII.4 genotype infected children with a median age of 9 months (IQR, 2–29 months). This finding is in agreement with a previous report from Nicaragua showing that the highest detection rate of symptomatic GII.4 infections occurred in children between 7 and 12 months of age [7]. In addition, secretor children with primary GII.4 gastroenteritis experienced more severe symptoms than children with primary GI and GII non-GII.4 episodes and also with those experiencing secondary episodes. The mechanism behind severe GII.4 episodes might involve immunological factors, microbiome composition and intestinal glycobiology of the host, or increased efficiency of infectivity or replication of the virus. Additional functional studies are needed to better elucidate the underlying mechanisms. This study also suggest that future vaccines against norovirus need to be offered during the first months of life especially in countries where natural exposure to these viruses occurs early in life. GII.4 vaccines would be most beneficial for secretors, but a multivalent vaccine would also benefit nonsecretor children who are susceptible to other genotypes and also for populations with a higher prevalence of nonsecretors such as Southeast and East Asian countries [43, 44]. For instance, the rotavirus vaccines Rotarix and RotaTeq are more immunogenic in secretor children and both vaccines contain a secretor-dependent rotavirus strain [45, 46].

Interestingly, children who had their first symptomatic episode associated with GII.4 infections had fewer secondary GII episodes as compared with secondary GI episodes (4 vs 9), with only 1 child having a secondary GII.4 episode. However, since this secondary episode occurred 41 days after the first episode, and with the same GII.4 Sydney P[16] variant, prolonged shedding is suggested. This correlates well with the findings in a

Table 3. Association Between Severity of Acute Gastroenteritis in the First Norovirus Episode and Infecting Norovirus Genotype

Variable	Severe	Not Severe	Crude OR (95% CI)	*P* Value	Adjusted OR (95% CI)[a]	*P* Value
GII.4 (n = 37)	26	11	Ref		Ref	
GI genotyped (n = 26)	5	21	0.20 (.06–.66)	.002	0.09 (.02–.33)	<.001
GII non-GII.4 (n = 25)	8	17	0.5 (.2–1.5)	.197	0.2 (.07–.6)	.006

Abbreviations: CI, confidence interval; OR, odds ratio.

[a]Model adjusted for age and coinfection with other viruses (sapovirus, astrovirus, and rotavirus).

Table 4. Genotypes Found During the First Norovirus Episode and Second Norovirus Episode in the Birth Cohort

Genotype in the First Episode	No. (Age, mo, at AGE, Median [IQR])	Genotype and No. of Children With Secondary Infection (Median Time Frame Between Infections, mo [IQR])[a]						
		GII.4	GII.12	GII.14	GI.5	GI.3	GI NT	GII NT
GII.4	37 (9 [7–14])	1 (1.4)[b]	1 (7)	1 (10)	4 (6 [6–12.5])	5 (14 [7–16])	…	2 (7.5 [7–8])
GII.12	8 (7 [5.2–8.7])	3 (5 [4–4.5])	…	…	…	…	…	…
GII.17	8 (19.5 [14.5–23])	…	…	…	…	…	…	1 (2)
GII.6	1 (4)	…	…	…	…	1 (12)	…	…
GII.14	4 (7 [5.2–8.5])	…	…	…	…	…	…	…
GII.21	2 (22)	…	…	…	…	…	…	…
GII.5	1 (16)	…	…	…	…	…	…	…
GII.15	1 (17)	…	…	…	…	…	…	…
GII NT	45 (15 [10–23])	…	…	…	1 (7)	1 (7)	2 (6 [2–10])	2 (4.5 [2–7])
All GII	107 (13 [7–19])	4	1	1	5	7	2	5
GI.3	16 (18 [14.5–20.5])	…	…	…	…	…	1 (6)	6 (4 [3–5])
GI.5	8 (14.5 [9–18])	…	…	…	…	…	1 (1)	…
GI.4	1 (20)	…	1 (3)	…	…	…	…	…
GI.7	1 (15)	…	…	…	…	…	…	…
GI NT	32 (16 [11–19])	1 (5)	…	…	…	1 (6)	1 (2)	2 (3)
All GI	58 (16 [12–19])	1	1	…	…	1	2	8
All norovirus	165 (14 [9–19])	5	2	1	5	8	4	13

Abbreviations: AGE, acute gastroenteritis; IQR, interquartile range; NT, not typed.

[a]Six of 17 (35%) genotyped reinfections were defined as severe (AGE score, 8–11).

[b]GII.4 Sydney P[16] was detected in both the primary and secondary infection.

Peruvian birth cohort where children had >60% homotypic protection against norovirus GII.4 and 48% heterotypic protection against GI.3 [26]. However, our results need to be interpreted with caution due to the low number of samples genotyped, and serological studies should be performed for confirmation.

This study has several limitations. Only AGE cases were included and no asymptomatic cases were analyzed; thus, the study cannot account for all norovirus infections during the 3 years the children were followed. The common observation of asymptomatic norovirus infections (6%–9%) warrants further study [47]. Second, self-reporting of AGE and missing stool collections may have resulted in a lower number of reported cases and therefore the incidence of norovirus may be underestimated. Third, the secretor status and O phenotypes predominated in this cohort, which has an unusually low prevalence of nonsecretor compared to global levels; thus, risk estimates for the lower prevalence phenotypes in our study setting may be less precise. Fourth, only 50% of norovirus-positive samples could be genotyped, which might have influenced the severity analyses in the context of genotypes. Successful genotyping is directly associated with high viral load. A further limitation is that we performed only phenotyping and not genotyping as a complementary test for secretor status. Hence, we cannot exclude that some children who were phenotyped as nonsecretors are instead weak secretors, that is, having reduced but not completely inactivated FUT2 enzyme activity. However, because weak secretors are rare in Nicaragua [45] and have a similar susceptibility pattern to norovirus as nonsecretor individuals, [48], this likely did not have a major impact on the results.

In conclusion, the results from this cohort study show that secretor status in children strongly correlates with the incidence and time point of symptomatic norovirus infection in a genogroup- or genotype-dependent manner. The results further provide evidence that clinical severity in children depends on the infecting norovirus genotype. These results are important for understanding the natural symptomatic infections in the context of developing pediatric norovirus vaccines.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. The authors thank all mothers and infants who participated in the study for their collaboration. We also appreciate all of the members of the SAGE team, especially the field workers: Merling Balmaceda, Vanessa Bolaños, Nancy Corea, Jhosselyng Delgado, Marvel Fuentes, Yadira Hernandez, Llurvin Madriz, Patricia Mendez, Yuvielka Martinez, Maria Mendoza, Ruth Neira, Xiomara Obando, Veronica Pravia, Yorling Picado, Aura Scott, and Mileydis Soto .

Financial support. This work was supported by the National Institute of Allergy and Infectious Diseases at the National Institute of Health (R01AI127845 and K24AI141744). Y. R., F. G., L. G., and O. Z. are supported by an international research capacity building award from the Fogarty International Center at the National Institute of Health (D43TW010923). This work was supported by the Swedish Research Council (2014-02827 and 2018-02862 to L. S.) and the Mucosa Infection and Inflammation Center at Linköping University (to L. S.).

Potential conflicts of interest. All authors: No reported conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Lopman BA, Steele D, Kirkwood CD, Parashar UD. The vast and varied global burden of norovirus: prospects for prevention and control. PLoS Med **2016**; 13:e1001999.
2. Boon D, Mahar JE, Abente EJ, et al. Comparative evolution of GII.3 and GII.4 norovirus over a 31-year period. J Virol **2011**; 85:8656–66.
3. Victoria M, Miagostovich MP, Ferreira MS, et al. Bayesian coalescent inference reveals high evolutionary rates and expansion of norovirus populations. Infect Genet Evol **2009**; 9:927–32.
4. Chhabra P, de Graaf M, Parra GI, et al. Updated classification of norovirus genogroups and genotypes. J Gen Virol **2019**; 100:1393–406.
5. Hoa Tran TN, Trainor E, Nakagomi T, Cunliffe NA, Nakagomi O. Molecular epidemiology of noroviruses associated with acute sporadic gastroenteritis in children: global distribution of genogroups, genotypes and GII.4 variants. J Clin Virol **2013**; 56:185–93.
6. Parra GI, Squires RB, Karangwa CK, et al. Static and evolving norovirus genotypes: implications for epidemiology and immunity. PLoS Pathog **2017**; 13:e1006136.
7. Bucardo F, Reyes Y, Becker-Dreps S, Bowman N, Gruber JF, Vinjé J, et al. Pediatric norovirus GII.4 infections in Nicaragua, 1999–2015. Infect Genet Evol **2017**; 55:305–12.
8. Burke RM, Shah MP, Wikswo ME, et al. The norovirus epidemiologic triad: predictors of severe outcomes in US norovirus outbreaks, 2009-2016. J Infect Dis **2019**; 219:1364–72.
9. Bucardo F, Kindberg E, Paniagua M, Grahn A, Larson G, Vildevall M, et al. Association of histo-blood group

antigens with susceptibility to norovirus infection may be strain-specific rather than genogroup dependent. Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis **2013**; 198:104280.
10. Nordgren J, Sharma S, Kambhampati A, Lopman B, Svensson L. Innate resistance and susceptibility to norovirus infection. PLoS Pathog **2016**; 12:e1005385.
11. Cantelli CP, Fumian TM, Malta FC, et al. Norovirus infection and HBGA host genetic susceptibility in a birth community-cohort, Rio de Janeiro, Brazil. Infect Genet Evol **2020**; 82:104280.
12. Lindesmith L, Moe C, Marionneau S, et al. Human susceptibility and resistance to Norwalk virus infection. Nat Med **2003**; 9:548–53.
13. Ruvoën N, Le Pendu J. Genetic susceptibility to norovirus infection [in French]. Pathol Biol (Paris) **2013**; 61:28–35.
14. Carlsson B, Kindberg E, Buesa J, et al. The G428A nonsense mutation in FUT2 provides strong but not absolute protection against symptomatic GII.4 norovirus infection. PLoS One **2009**; 4:e5593.
15. Costantini VP, Cooper EM, Hardaker HL, et al. Epidemiologic, virologic, and host genetic factors of norovirus outbreaks in long-term care facilities. Clin Infect Dis **2016**; 62:1–10.
16. Jin M, He Y, Li H, et al. Two gastroenteritis outbreaks caused by GII Noroviruses: host susceptibility and HBGA phenotypes. PLoS One **2013**; 8:e58605.
17. Nordgren J, Svensson L. Genetic susceptibility to human norovirus infection: an update. Viruses **2019**; 11:226.
18. Currier RL, Payne DC, Staat MA, et al. Innate susceptibility to norovirus infections influenced by FUT2 genotype in a United States pediatric population. Clin Infect Dis **2015**; 60:1631–8.
19. Nordgren J, Nitiema LW, Ouermi D, Simpore J, Svensson L. Host genetic factors affect susceptibility to norovirus infections in Burkina Faso. PLoS One **2013**; 8:e69557.
20. Kubota T, Kumagai A, Ito H, et al. Structural basis for the recognition of Lewis antigens by genogroup I norovirus. J Virol **2012**; 86:11138–50.
21. Liao Y, Xue L, Gao J, Wu A, Kou X. ABO blood group-associated susceptibility to norovirus infection: a systematic review and meta-analysis. Infect Genet Evol **2020**; 81:104245.
22. Cannon JL, Lopman BA, Payne DC, Vinjé J. Birth cohort studies assessing norovirus infection and immunity in young children: a review. Clin Infect Dis **2019**; 69:357–65.
23. Lopman BA, Trivedi T, Vicuña Y, et al. Norovirus infection and disease in an Ecuadorian birth cohort: association of certain norovirus genotypes with host FUT2 secretor status. J Infect Dis **2015**; 211:1813–21.
24. Menon VK, George S, Sarkar R, et al. Norovirus gastroenteritis in a birth cohort in Southern India. PLoS One **2016**; 11:e0157007.
25. Vielot NA, González F, Reyes Y, et al. Risk factors and clinical profile of sapovirus-associated acute gastroenteritis in early childhood: a Nicaraguan birth cohort study. Pediatr Infect Dis J **2021**; 40:220–6.
26. Chhabra P, Rouhani S, Browne H, et al. Homotypic and heterotypic protection and risk of reinfection following natural norovirus infection in a highly endemic setting. Clin Infect Dis **2021**; 72:222–9.
27. Rolfe KJ, Parmar S, Mururi D, et al. An internally controlled, one-step, real-time RT-PCR assay for norovirus detection and genogrouping. J Clin Virol **2007**; 39:318–21.
28. Kageyama T, Kojima S, Shinohara M, et al. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. J Clin Microbiol **2003**; 41:1548–57.
29. Trujillo AA, McCaustland KA, Zheng DP, et al. Use of TaqMan real-time reverse transcription-PCR for rapid detection, quantification, and typing of norovirus. J Clin Microbiol **2006**; 44:1405–12.
30. Cannon JL, Barclay L, Collins NR, et al. Genetic and epidemiologic trends of norovirus outbreaks in the United States from 2013 to 2016 demonstrated emergence of novel GII.4 recombinant viruses. J Clin Microbiol **2017**; 55:2208–21.
31. Chhabra P, Browne H, Huynh T, et al. Single-step RT-PCR assay for dual genotyping of GI and GII norovirus strains. J Clin Virol **2021**; 134:104689.
32. Oka T, Katayama K, Hansman GS, et al. Detection of human sapovirus by real-time reverse transcription-polymerase chain reaction. J Med Virol **2006**; 78:1347–53.
33. Liu J, Kabir F, Manneh J, et al. Development and assessment of molecular diagnostic tests for 15 enteropathogens causing childhood diarrhoea: a multicentre study. Lancet Infect Dis **2014**; 14:716–24.
34. Nordgren J, Sharma S, Bucardo F, et al. Both Lewis and secretor status mediate susceptibility to rotavirus infections in a rotavirus genotype-dependent manner. Clin Infect Dis **2014**; 59:1567–73.
35. Colston JM, Francois R, Pisanic N, et al. Effects of child and maternal histo-blood group antigen status on symptomatic and asymptomatic enteric infections in early childhood. J Infect Dis **2019**; 220:151–62.
36. O'Ryan ML, Lucero Y, Prado V, et al. Symptomatic and asymptomatic rotavirus and norovirus infections during infancy in a Chilean birth cohort. Pediatr Infect Dis J **2009**; 28:879–84.
37. Saito M, Goel-Apaza S, Espetia S, et al; Norovirus Working Group in Peru. Multiple norovirus infections in a birth

cohort in a Peruvian periurban community. Clin Infect Dis **2014**; 58:483–91.
38. Frenck R, Bernstein DI, Xia M, et al. Predicting susceptibility to norovirus GII.4 by use of a challenge model involving humans. J Infect Dis **2012**; 206:1386–93.
39. Liu W, Chen Y, Jiang X, et al. A unique human norovirus lineage with a distinct HBGA binding interface. PLoS Pathog **2015**; 11:e1005025.
40. Yahiro T, Wangchuk S, Wada T, et al. Norovirus GII.21 in children with diarrhea, Bhutan. Emerg Infect Dis **2015**; 21:732–4.
41. Koo ES, Kim MS, Choi YS, Park KS, Jeong YS. Occurrence of novel GII.17 and GII.21 norovirus variants in the coastal environment of South Korea in 2015. PLoS One **2017**; 12:e0172237.
42. Vega E, Barclay L, Gregoricus N, Shirley SH, Lee D, Vinjé J. Genotypic and epidemiologic trends of norovirus outbreaks in the United States, 2009 to 2013. J Clin Microbiol **2014**; 52:147–55.
43. Koda Y, Soejima M, Liu Y, Kimura H. Molecular basis for secretor type alpha(1,2)-fucosyltransferase gene deficiency in a Japanese population: a fusion gene generated by unequal crossover responsible for the enzyme deficiency. Am J Hum Genet **1996**; 59:343–50.
44. Yu LC, Yang YH, Broadberry RE, Chen YH, Chan YS, Lin M. Correlation of a missense mutation in the human secretor alpha 1,2-fucosyltransferase gene with the Lewis(a^+b^+) phenotype: a potential molecular basis for the weak secretor allele (Sew). Biochem J **1995**; 312:329–32.
45. Bucardo F, Reyes Y, Rönnelid Y, et al. Histo-blood group antigens and rotavirus vaccine shedding in Nicaraguan infants. Sci Rep **2019**; 9:10764.
46. Lee B, Dickson DM, deCamp AC, et al. Histo-blood group antigen phenotype determines susceptibility to genotype-specific rotavirus infections and impacts measures of rotavirus vaccine efficacy. J Infect Dis **2018**; 217:1399–407.
47. Bucardo F. Understanding asymptomatic norovirus infections. EClinicalMedicine **2018**; 2–3:7–8.
48. Liu P, Wang X, Lee JC, et al. Genetic susceptibility to norovirus GII.3 and GII.4 infections in Chinese pediatric diarrheal disease. Pediatr Infect Dis J **2014**; 33:e305–9.