MAJOR ARTICLE

Predicting Susceptibility to Norovirus GII.4 by Use of a Challenge Model Involving Humans

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Background. GII.4 is the predominant norovirus genotype worldwide. Challenge models involving humans have shown the association of human histo-blood group antigens (HBGAs) and susceptibility to infection with Norwalk virus (GI.1 norovirus), but the association of HBGAs and infection with other noroviruses is based on results of epidemiological studies. We performed the first GII.4 challenge study involving humans and prospectively evaluated the relationship between HBGAs and norovirus infection and associated illness.

Methods. Forty healthy adults (23 secretors and 17 nonsecretors of HBGAs) were challenged with 5×10^4 reverse-transcription polymerase chain reaction (RT-PCR) units of GII.4 norovirus. Subjects were assessed daily for clinical illness, and stool specimens were evaluated for norovirus by RT-PCR. Infection was defined by detection of norovirus and/or seroconversion to GII.4 antibody.

Results. Of the 23 secretors, 16 (70%) were infected with norovirus, 13 (57%) became ill (characterized by vomiting and/or diarrhea), and 12 (52%) developed norovirus-associated illness. In contrast, only 1 nonsecretor (5.9%) became ill, and another nonsecretor shed virus for a single day ($P < .001$ for each variable, compared with secretors). Infection occurred in secretors regardless of ABO blood group. Illness was mild to moderate in severity and lasted 1–3 days.

Conclusions. Secretor status determined the susceptibility to norovirus GII.4 challenge. This human challenge model should be useful for evaluating norovirus vaccines and antiviral agents.

Clinical trials registration. NCT01322503.

Norovirus, a genus of the Caliciviridae family, is divided into 5 genogroups (GI-GV), of which GI and GII are the major causes of nonbacterial epidemics of acute gastroenteritis in humans [1, 2]. Each genogroup can be further divided into genotypes, and >30 genotypes of GI and GII human noroviruses have been identified [1]. While most genotypes circulate in limited geographical locations, the GII.4 genotype has been identified worldwide since the mid-1990s [3–6]. Additionally, epidemiological studies from many countries have found GII.4 to be responsible for up to 70%–80% of all outbreaks and sporadic gastroenteritis related to norovirus [6–8]

Both GI and GII noroviruses have been found to recognize the human histo-blood group antigens (HBGAs) [9–12]. However, different genogroups and genotypes of noroviruses recognize different HBGAs, and up to 8 receptor-binding patterns have been described [11–13]. Challenge studies involving humans have demonstrated a relationship between HBGAs and infection due to Norwalk virus, the prototypical norovirus [9, 10, 14]. But such evidence for other genotypes, including GII.4 noroviruses, is based on epidemiological data [15–18].

In this study, we performed the first challenge study in humans that involved a GII.4 norovirus strain and prospectively evaluated the relationship between susceptibility and the presence of specific HBGAs. This study has led to the development of a human challenge model that uses a GII strain with genotype 4, the most common norovirus genotype. This model will be useful for future vaccine and antiviral evaluations.

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METHODS

Subjects

Men and women aged 18–49 years and in good general health were recruited from the community. Subjects were excluded if they worked in health care, food service, or day care; were pregnant or nursing; or had close contact with persons at risk for complications from norovirus infection.

Potentially eligible subjects were screened by enzyme-linked immunosorbent assay (ELISA) for antibody to the challenge strain, and those with a titer of <1:1600 remained eligible. Subjects were required to have normal results of physical and laboratory evaluations, including liver function tests, renal function tests, and complete blood count; negative results of tests for human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and pregnancy; negative results of tests for bacterial and parasitic infections in stool; and normal findings of electrocardiography. Finally, subjects were evaluated for phenotypic evidence of the presence of a FUT-2 gene, as determined by identification of HBGAs in the saliva, as described below [11, 19].

The study was approved by the institutional review boards of the Cincinnati Children's Hospital Medical Center, as well as by the US Army Medical Research and Materiel Command's Human Research Protection Office. Written informed consent was obtained from all participants prior to enrollment.

Challenge Strain

The challenge strain (Cin-1) was isolated in 2003 from a stool sample collected from a man who developed a 3-day illness characterized by diarrhea, vomiting, nausea, abdominal cramps, and fever. Reverse-transcription polymerase chain reaction (RT-PCR) analysis of the stool identified a GII.4 strain of norovirus that bound to the A, B, and H HBGAs. The ORF-2 encoding capsid protein was sequenced and submitted to the National Center for Biotechnology Information (GenBank accession number JQ965810). Phylogenetic analysis indicated that the strain belonged to the 2002 cluster within norovirus GII.4 [20]. Before production of the challenge strain began, the donor underwent extensive screening for several pathogens, including *Treponema pallidum*, *Mycobacterium tuberculosis*, hepatitis A virus (HAV), HBV, HCV, HIV types 1 and 2, and human T-lymphotropic virus types 1 and 2. The donor stool sample was processed in a College of American Pathologist–certified facility to produce the viral challenge pool. Donor stool was also tested and found to be negative for rotavirus, enteric adenovirus, astrovirus, picornavirus (including [HAV]), enterotoxin, *Mycoplasma* species, and *Mycobacteria* species. At the end of the production process, the stool was tested for *Mycoplasma* species, bacterial endotoxin, and retroviruses, as well as for adventitious virus that might have been introduced during the purification process. Testing for rotavirus and astrovirus was performed at CCHMC using the Rotaclone kit (Meridian Diagnostics, Cincinnati, OH) and RIDASCREEN kit (R-Biopharm, Darmstadt, Germany), respectively. Real-time PCR for detection of adenovirus was perfomed in the laboratory of Dr N. Jothikumar (Centers for Disease Control and Prevention, Atlanta, GA). All other stool testing was performed by AppTec Laboratory Services (St. Paul, MN). The norovirus concentration of the final product (ie, the challenge pool) was quantified and found to contain approximately 5×10^4 RT-PCR units per milliliter. Donor evaluation and stool processing and evaluation were reviewed by the Food and Drug Administration, and an Investigational New Drug application to administer the challenge norovirus strain was submitted and approved.

Study Design

The study was conducted in 2 groups of 20 subjects. Approximately equal numbers of secretors and nonsecretors were selected for participation in each of the 2 study cohorts. Study staff and subjects were blinded to the secretor status of the subject. An unblinded study member reviewed the screening data and provided the study coordinator with the list of identification numbers of eligible subjects.

On the day of challenge, subjects refrained from eating and drinking for 90 minutes, and then they ingested 60 mL of 2% sodium bicarbonate solution. Approximately 2 minutes later, subjects were asked to drink approximately 5×10^4 RT-PCR detection units of the challenge virus diluted in 80 mL of sterile water. The dose of virus administered was based on a previous study demonstrating that at least 50% of susceptible volunteers became ill after ingestion of a GI.1 norovirus at doses ranging from 10^4 to 10^8 PCR detectable units [9]. Because all doses in the previous study produced high rates of infection, in the current study, we elected to administer a dose that approximated the lowest dose used by Lindesmith et al [9]. Five minutes after administration of the inoculum, subjects ingested another 60 mL of 2% sodium bicarbonate solution and then refrained from eating and drinking for at least 90 minutes.

Subjects remained in the inpatient facility for a minimum of 4 days following challenge or until they were asymptomatic, whichever was longer. During the inpatient stay, subjects were evaluated twice daily by an investigator. All stool specimens were collected, graded, and weighed. All emesis was also measured. Daily, at least 1 aliquot of stool was saved from all subjects and tested for norovirus shedding. Additionally, during each 8-hour shift among inpatient healthcare staff, subjects with diarrhea had up to 1 sample collected during the period of diarrhea, to assess for norovirus shedding.

Oral rehydration solution was provided to all subjects who developed vomiting or diarrhea. Subjects unable to maintain

hydration status with oral rehydration solution were to be provided intravenous fluids. Prior to discharge, the safety clinical laboratory analysis was repeated, and if findings were abnormal, they were followed to resolution or stability.

For the 6 days following discharge from the inpatient unit, subjects kept a memory aid to record gastrointestinal and systemic reactions or fever. Subjects returned to the outpatient clinic 1 day and 30 days after discharge from the inpatient unit, to evaluate their health. At the 30-day postchallenge visit, blood was collected to measure antibody titers to the challenge virus, and a stool sample was collected to evaluate for the presence of norovirus.

Before enrollment of the second cohort, the study safety monitoring committee reviewed the safety data from the first cohort and approved continuation of the study.

Study Definitions

Secretors were defined as subjects with H1, Le^b, or Le^y detected in their saliva. Nonsecretors were defined as subjects without H1, Le^b, and Le^y but with Le^a or Le^x detected in their saliva. Norovirus infection was defined as RT-PCR detection of norovirus in the stool any time during the inpatient challenge stay and/or as seroconversion (defined as a 4-fold rise in ELISA antibody titer between days 0 and 30). Illness was defined as the presence of diarrhea (defined as ≥3 loose or liquid stools in a 24-hour period) and/or vomiting during the observation period. Subjects were classified as having norovirus-associated illness if they had norovirus infection and diarrhea and/or vomiting during the observation period.

Laboratory Evaluations

HBGA Typing

The saliva-based binding assay described in our previous studies was used [11, 19]. In brief, saliva samples collected from each subject during screening were diluted to a 1:1000 ratio and coated onto microtiter plates for detection of salivary HBGAs by monoclonal antibodies specific to Le^a, Le^b, Le^x, Le^y, and H type 1 (Covance, Princeton, NJ). The bound antibodies were then detected by horseradish peroxidase–conjugated goat anti-mouse immunoglobulin G (IgG) or immunoglobulin M antibodies (Millipore, Billerica, MA) and goat anti-mouse IgG3 (ICL, Portland, OR), followed by addition of substrate solution (Kirkegard & Perry Laboratories, Gaithersburg, MD). A positive and negative control were included in each plate. Each sample, in addition to the positive and negative controls, was tested in duplicate, and the values of the replicate wells were compared. If either the ratio of the maximum optical density (OD) to the minimum OD was >1.5 or if the absolute difference in the ODs between the replicate wells was >0.15, the sample was retested. Samples with an OD of ≥0.20 were considered positive for the specific HBGA.

Norovirus ELISA

Serum antibody titers to norovirus were determined using an antigen-capture enzyme immunoassay [11, 20]. In brief, rabbit serum anti-norovirus was used to coat microtiter plates, followed by addition of purified recombinant P particles made from the challenge GII.4 strain. After washing, 2-fold dilutions of serum samples, beginning at a 1:100 dilution, were added to wells. The bound antibodies were detected by horseradish peroxidase–conjugated rabbit anti-human IgG antiserum (MP Biomedicals, Solon, OH). All assays included positive and negative control serum. An OD of >0.20 was considered to indicate a positive reaction, and the norovirus antibody titer was expressed as the highest dilution that had an OD of ≥0.2.

Receptor-Blocking Assay for Norovirus Antibody

A saliva-based assay [21, 22] was used to measure serum antibodies that block norovirus binding to HBGAs. Briefly, a boiled saliva sample from a secretor was used to coat microtiter plates. Serial dilutions of serum samples from individual subjects were incubated with the recombinant P particles of GII.4 norovirus (VA-387) before being added to the microtiter plates. The P particles bound to the saliva were then detected by an anti-norovirus antibody, followed by a horseradish peroxidase–conjugated second antibody, as described above. The receptor-blocking titers were determined on the basis of a 50% reduction in the OD for each serum sample, compared with wells without serum. The OD range of unblocked control was 0.9–1.1. The 50% blocking titer was defined as the highest dilution at which the OD (after subtraction of the blank) was ≤50% of the OD of the P particle alone (no serum).

Binding to the Challenge Norovirus in Saliva Specimens

Saliva samples collected from subjects were tested for their ability to bind P particles of the challenge GII.4 norovirus, using a previously described method [21] Briefly, saliva specimens were diluted with phosphate-buffered saline (ratio, 1:1000) and coated on microtiter plates. Following addition of P particles and incubation, the bound P particles were detected by a norovirus-specific antibody, followed by a secondary antibody, as described above. An OD of >0.20 for a saliva specimen was considered positive for a binding reaction.

Detection of Norovirus in Stool Specimens

The presence of norovirus in stools was determined using RT-PCR analysis. RNA was extracted from 120 μL of a 10% stool specimen, using the Trizol reagent (Gibco, Gaithersburg, MD) according to the manufacturer's instructions. The previously described primer set p289H,I/p290H,I,J,K, which targets the RNA polymerase gene of norovirus, was used [23, 24]. RT-PCR was performed for 30 cycles, with denaturation at 94°C for 30 seconds, reannealing at 49°C for 1 minute, and extension at 72°C for 1.5 minutes [25]. The resulting RT-PCR

Table 1. Demographic Characteristics of Adults Who Underwent Voluntary Challenge With Norovirus GII.4

Characteristic	Overall (n = 40)	Secretors (n = 23)	Nonsecretors (n = 17)
Age, y, mean (range)	28.2 (19–48)	30.8 (19–48)	24.7 (19–33)
Sex			
Female	21	11	10
Male	19	12	7
Race			
African American	31	18	13
White	9	5	4

Secretors were defined as subjects with H1, Le^b, or Le^y detected in their saliva, and nonsecretors were defined as subjects without H1, Le^b, and Le^y but with Le^a or Le^x detected in their saliva.

products were detected by agarose gel electrophoresis. For samples with weak signals or questionable sizes of RT-PCR products, RT-PCR was repeated, followed by sequencing of the RT-PCR amplicon.

Statistical Analysis

All comparisons were made using a 2-tailed Fisher exact test. *P* values of $\leq .05$ were considered statistically significant.

RESULTS

To identify subjects for the challenge study, 171 individuals were screened, and 128 (75%) were found to be secretors. Of the secretors, 59 (46%) had an anti-norovirus antibody titer of ≤1600, compared with 34 of 43 nonsecretors (79%) ($P < .001$). Following physical examination, safety laboratory evaluations, and electrocardiography, 60 subjects remained eligible. Of these, 23 secretors and 17 nonsecretors were enrolled in 2 cohorts of 20 subjects per cohort and received the challenge virus. Demographic characteristics of the 40 subjects are listed in Table 1.

Infection with norovirus occurred in 16 secretors (70%) following challenge, compared with 1 nonsecretor (6%) ($P < .001$; Table 2). Additionally, although infection with norovirus in the secretor group typically was associated with repeated identification of norovirus in the stool, the single nonsecretor who was infected had norovirus identified from a single stool sample and did not develop illness. Of the 23 secretors, 13 (57%) developed illness, of whom 12 met the definition of norovirus-associated illness (ie, vomiting and/or diarrhea plus RT-PCR detection of norovirus in the stool and/or seroconversion). The remaining subject experienced nausea and vomiting for 1 day but did not have virus detected or undergo seroconversion. In comparison, 1 nonsecretor became ill but, on the basis of study definitions, did not have norovirus infection ($P < .001$ for differences between secretors and nonsecretors in the incidence of any illness and the incidence of norovirus-associated illness). Diarrhea occurred in 9 secretors (39%) and 0 nonsecretors, whereas vomiting occurred in 7 secretors (30%) and 1 nonsecretor (6%). Four secretors developed both vomiting and diarrhea. Infection was seen in secretors from all ABO blood groups, including all 5 secretors with blood group B, 4 of whom became ill.

Table 2. Effects of Secretor Status on Norovirus Infection, Any Illness, and Norovirus-Associated Illness Among Adults After Norovirus GII.4 Challenge

Group	Norovirus Infection, No. (%)	Any Illness, No. (%)	Norovirus-Associated Illness, No. (%)
Secretor (n = 23)	16[a] (70)	13[a] (57)	12[a] (52)
Shed norovirus	14 (61)	10 (44)	10 (44)
Seroconverted to anti-norovirus Ab	12 (52)	10 (44)	10 (44)
Shed and seroconverted	10 (44)	8 (35)	8 (35)
Nonsecretor (n = 17)	1 (6)	1 (6)	0
Shed norovirus	1 (6)	0	0
Seroconverted to anti-norovirus Ab	0	0	0
Shed and seroconverted	0	0	0

Secretors were defined as subjects with H1, Le^b, or Le^y detected in their saliva, and nonsecretors were defined as subjects without H1, Le^b, and Le^y but with Le^a or Le^x detected in their saliva. See Methods for definitions of norovirus infection, any illness, and norovirus-associated illness.

Abbreviation: Ab, antibody.

[a] $P < .001$, compared with nonsecretors overall.

Vomiting and diarrhea typically had an onset of 48–72 hours after administration of the challenge virus. Symptoms were mild to moderate and were relatively short lived, with resolution within 48 hours. All ill subjects were successfully managed with oral rehydration fluids. The first symptoms associated with illness were malaise, nausea, and abdominal gurgling, which typically began 12–24 hours before the onset of vomiting or diarrhea. We found no association between preexisting antibody titers (detected by ELISA or receptor blocking) and infection or illness within the limited range of the subjects' titers.

Virus was first detected by RT-PCR the day following challenge (in 5 of 20 secretors) and was detected for a mean of 5.2 days (range, 2–30 days; Table 3). The peak of norovirus shedding occurred 3 days after challenge, when 11 secretors (47.8%) had the virus detected in their stool. Three secretors (13.4%) were shedding virus 8 days after challenge, but only 1 secretor (4.4%) had the virus detected 30 days after challenge.

Saliva specimens from all secretors but no nonsecretors bound the recombinant P particles of the challenge strain

Table 3. Daily Prevalence of Norovirus Shedding Among Adults After Norovirus GII.4 Challenge

	Day After Challenge						
Group	1	2	3	4	5	8	30
Secretor	5	10	11	8	5	3	1
Nonsecretor	0	1	0	0	0	0	0

Secretors were defined as subjects with H1, Le^b, or Le^y detected in their saliva, and nonsecretors were defined as subjects without H1, Le^b, and Le^y but with Le^a or Le^x detected in their saliva.

(data not shown). Before challenge, receptor-blocking antibody was detected in 17 secretors and 0 nonsecretors ($P < .001$; Tables 4, 5). Thirty days after challenge, 13 secretors had a 4-fold increase from prechallenge titers of receptor-blocking antibody, compared with 0 nonsecretors ($P < .001$).

Although solicited adverse events were fairly common in both study groups, many symptoms, including abdominal gurgling, abdominal pain, nausea, anorexia, malaise, and headache, were more frequent among secretors, compared with nonsecretors, Table 6.

DISCUSSION

To our knowledge, the current study is the first to prospectively randomize subjects by secretor status to test the association of HBGA type and the susceptibility of humans to infection with norovirus. Additionally, this is the first report of subjects being challenged with a GII.4 norovirus, the genotype most commonly associated with current gastrointestinal outbreaks [6–8]. Compared with previous norovirus challenges, in the present study, approximately 50% of the study group was predicted to be susceptible to the challenge strain on the basis of secretor status. Because some previous studies suggested that preexisting antibodies may be associated with immunity to norovirus infection [26, 27], subjects were required to have a low level of GII.4 antibodies, in an attempt to minimize the effect of antibodies on infection.

Previous challenge and epidemiological studies of Norwalk virus performed in the 1970–1980s demonstrated a variable susceptibility to infection, even after multiple exposures to the virus [26, 28–30]. The retrospective analysis of samples collected during previous challenge studies with Norwalk virus (a GI.1 norovirus), performed by Lindesmith et al [9] and later confirmed by Hutson et al [14], showed that the risk of infection was related to the FUT-2 gene. Approximately 80% of people have an active FUT-2 allele, leading to expression of the H type 1 HBGAs on mucosal surfaces and to their detection in saliva and other body fluids [31]. Presence of the HBGAs on the surfaces of the intestinal epithelial cells is postulated to influence host susceptibility by allowing attachment of the virus. Lindesmith et al [9] analyzed saliva samples collected during previous Norwalk virus challenge studies and found that, among 55 secretors, 34 (62%) were infected following challenge with the virus, compared with 0 of 22 nonsecretors.

Table 4. Norovirus-Specific Laboratory Results Among Adult Nonsecretors After Norovirus GII.4 Challenge

		Serum IgG Titer		BT50	
Subject	RT-PCR Finding After Challenge	Screening	Day 30 After Challenge	Day 0	Day 30 After Challenge
FRN313	Negative	800	1600	<50	<50
FRN319	Negative	1600	3200	<50	<50
FRN321	Negative	100	100	<50	<50
FRN344	Negative	800	800	<50	<50
FRN353	Negative	800	100	<50	<50
FRN365	Negative	800	1600	<50	<50
FRN372	Negative	200	200	<50	<50
FRN378	Negative	1600	1600	<50	<50
FRN421	Negative	1600	1600	<50	<50
FRN423	Negative	400	800	<50	<50
FRN432	Negative	800	1600	<50	<50
FRN436	Negative	800	800	<50	<50
FRN437	Negative	1600	1600	<50	<50
FRN444	Negative	400	800	<50	<50
FRN469	Positive	800	800	<50	<50
FRN472	Negative	1600	1600	<50	<50
FRN474	Negative	400	400	<50	<50

Nonsecretors were defined as subjects without H1, Le^b, and Le^y but with Le^a or Le^x detected in their saliva. No saliva samples bound to the GII.4 challenge strain.

Abbreviations: BT50, blocking titer, 50%; IgG, immunoglobulin G; RT-PCR, reverse-transcription polymerase chain reaction.

Table 5. Norovirus-Specific Laboratory Results Among Adult Secretors After Norovirus GII.4 Challenge

Subject	RT-PCR Finding After Challenge	Serum IgG Titer		BT50	
		Screening	Day 30 After Challenge	Day 0	Day 30 After Challenge
FRN311	Negative	800	800	100	100
FRN327	Positive	1600	6400	100	200
FRN335	Negative	800	25 600	<50	400
FRN339	Negative	800	3200	50	100
FRN350	Positive	1600	25 600	200	800
FRN352	Negative	1600	3200	<50	50
FRN354	Positive	1600	25 600	100	800
FRN359	Positive	800	6400	100	800
FRN366	Negative	800	6400	100	400
FRN371	Negative	1600	3200	200	200
FRN384	Negative	1600	1600	100	100
FRN385	Positive	1600	6400	100	200
FRN403	Positive	1600	6400	<50	100
FRN406	Positive	1600	25 600	<50	200
FRN409	Positive	200	25 600	<50	800
FRN420	Positive	1600	6400	50	100
FRN429	Negative	400	1600	<50	<50
FRN434	Positive	1600	25 600	100	400
FRN439	Positive	1600	12 800	50	800
FRN460	Positive	1600	12 800	50	400
FRN466	Positive	1600	3200	100	800
FRN470	Negative	1600	6400	200	400
FRN478	Positive	800	6400	100	400

Secretors were defined as subjects with H1, Le^b, or Le^y detected in their saliva. Saliva samples from all secretors showed binding to the GII.4 challenge strain.
Abbreviations: BT50, blocking titer, 50%; IgG, immunoglobulin G; RT-PCR, reverse-transcription polymerase chain reaction.

In the current study, a strong association of secretor status with the clinical outcome of the subjects was also observed for GII.4 norovirus. Of the 23 subjects with an active FUT-2 allele (ie, secretors) and who were thus predicted to be at increased susceptibility to infection, 16 (70%) were infected, compared with only 1 of 17 subjects (6%) without an active FUT-2 allele (ie, nonsecretors) ($P < .001$). Similarly, of the 23 secretors, 12 (52%) developed symptomatic illness, compared with only 1 (6%) of the 17 nonsecretors ($P < .001$). Thus, we were able to prospectively confirm the previously assumed association between an active FUT-2 allele and norovirus infection and development of disease and to extend this observation to GII.4 noroviruses.

Table 6. Solicited Adverse Events Among Adults After Norovirus GII.4 Challenge

Adverse Event	Secretors, No.	Nonsecretors, No.
Fever	0	0
Abdominal gurgling	18	8
Malaise	15	2
Abdominal pain	14	3
Nausea	13	3
Headache	11	6
Anorexia	8	1
Chills	5	2
Muscle aches	4	1

Data denote adverse events of any severity or grade. No subject developed a grade III adverse event. Secretors were defined as subjects with H1, Le^b, or Le^y detected in their saliva, and nonsecretors were defined as subjects without H1, Le^b, and Le^y but with Le^a or Le^x detected in their saliva.

Another important finding of the study was that the GII.4 strain used was able to infect people of all ABO blood groups. Of the 5 subjects who were secretors and had blood group B, all were infected, and illness developed in 4. This contrasts with findings involving the prototype Norwalk virus, in which type B secretors had decreased in vitro binding of the virus and a decreased risk of infection [10–12, 14]. The susceptibility of people to GII.4 norovirus regardless of ABO blood group [15–18] perhaps explains why this genotype has become the most commonly circulating genotype worldwide.

The duration of shedding following infection with the GII.4 virus also differed slightly from studies involving the prototype Norwalk virus. In a study by Atmar et al, adult subjects

challenged with Norwalk virus shed virus for a median of 28 days (range, 13–56 days) [32]. In contrast, in the present study, although all 14 secretors who shed did so for 3 days, by day 5, only 5 were shedding, dropping to 3 by day 8 and to only 1 by day 30. The study by Atmar et al used quantitative RT-PCR or immunomagnetic capture RT-PCR, which may be more sensitive than the regular RT-PCR used in our study. Additionally, it is possible that the shedding characteristics of GI norovirus differs from that of GII strains. Further studies are needed to clarify the differences in shedding between the study by Atmar et al and the current study.

Future evaluations will look to improve our GII.4 norovirus challenge model by determining the minimal dose that can reliably induce illness and/or infection. In the current study, on the basis of previous work by Lindesmith and colleagues, we selected 5×10^4 RT-PCR units of virus as the challenge dose [12]. While the 70% infection rate among susceptible hosts (ie, secretors) was comparable to results reported by Lindesmith et al [9], a recent publication suggested that a dose as low as 48 RT-PCR units may be equally effective in inducing infection [32, 33]. For this study, we excluded subjects with an antibody titer of >1:1600, to maximize infection rates. However, this impaired our ability to evaluate the relationship between levels of preexisting antibody and protection. Future studies should evaluate the protective role of preexisting immunity by challenging subjects with a wide range of levels of preexisting antibody. Finally, more work is needed to explore other potential factors that help determine susceptibility to noroviruses. It is unclear why 30% of the subjects for whom a high risk of infection was predicted (ie, secretors with low levels of preexisting antibody) did not become infected following challenge.

In summary, the current study is the first to prospectively evaluate and demonstrate the relationship between susceptibility to a specific norovirus and the presence of specific HBGAs. These major secretor antigens likely act as attachment factors for norovirus attachment. Thus, people lacking these antigens (ie, nonsecretors) are naturally resistant to infection with norovirus. Additionally, we have established a new challenge model, using a GII.4 norovirus that can be used to evaluate prophylactic and therapeutic interventions for norovirus infection.

Notes

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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.

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