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Assessing the Histo-Blood Group Antigen Blocking Antibody Response against GII.2 Norovirus (Snow Mountain Virus)

By

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Abstract

Assessing the Histo-Blood Group Antigen Blocking Antibody Response against GII.2 Norovirus (Snow Mountain Virus)

By Makoto Ibaraki

Noroviruses are one of the most common viral causes of acute gastroenteritis (AGE) globally, and each year, an estimated 684 million infections occur among both children and adults. Noroviruses can be classified into 48 genotypes and while the GII.4 has predominantly been the genotype associated with outbreaks worldwide, reports of outbreaks associated with the GII.2 genotype has recently been increasing. To determine the histo-blood group antigen (HBGA) blocking antibody response following GII.2 norovirus (Snow Mountain Virus, SMV) exposure, results from a recently conducted randomized, double blind, placebo-controlled SMV human challenge study were utilized. For the trial, SMV-specific serum immunoglobulin (Ig)A and IgG concentrations were measured through enzyme-linked immunosorbent assays (ELISA) while HBGA-blocking antibody titers were determined through a SMV-specific blockade assay. Serum antibody responses of SMV-specific IgA, IgG, and HBGA-blocking antibodies were all significantly different between SMV-infected and uninfected subjects following SMV challenge. Among the infected individuals, significant increases in HBGA-blocking antibodies were observed after receiving SMV, with the greatest increase observed on day 15 post-challenge when compared to the pre-challenge titer. The significant increases only observed among the infected subjects suggested that SMV exposure alone would not be enough to activate HBGA-blocking antibody response and that this HBGA-blocking antibody response is a reaction to SMV infection. While further research is needed in order to determine the other factors associated with SMV infection and illness, the results can be applied for the global population at risk. Findings from this study should be used to guide vaccine and screening tool development for SMV with the goal to reduce the infection and illness associated with SMV throughout the world.

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CHAPTER I: BACKGROUND

Noroviruses are a major public health concern throughout the world. Their genetic diversity, persistency in the environment, and high infectivity and pathogenicity have been documented over the last several decades. Although there are still much to learn about noroviruses and their impact on the human population, the epidemiological studies, outbreak investigations, and human challenge studies that have used various norovirus strains have allowed the scientific community to get a better understanding of noroviruses.

To interpret the findings of the GII.2 (Snow Mountain Virus) challenge study, it is important to establish a strong foundation on noroviruses. In this literature review, the structure, clinical symptoms, epidemiology, and molecular and serological diagnostic methods for noroviruses are described along with the concepts of norovirus human challenge studies and vaccine studies.

A. HISTORY OF SNOW MOUNTAIN VIRUS

Snow Mountain virus (GII.2.1976) is the prototype strain of GII.2 noroviruses which was first reported in a gastroenteritis outbreak that occurred at a resort camp in Colorado in December 1976. At least 418 individuals were involved in the outbreak and various combinations of symptoms such as vomiting (81%), diarrhea (65%), and fever (49%) were reported (1). The outbreak investigation identified that this outbreak was caused by the camp supply water that was inadequately chlorinated and contaminated from a leaking septic tank (1). A high attack

rate of 56% was reported in this outbreak. Furthermore, person-to-person transmission was observed where household contacts of the infected resort visitors also became ill (1). Since the 1976 outbreak, the Snow Mountain virus (SMV) has been detected in numerous foodborne outbreaks in the US (2-5). For example, in 1988, a foodborne outbreak associated with shrimp meals served at a retirement facility dining hall occurred in the San Francisco Bay area (3). Attack rates for residents and employees were 46% and 37%, respectively, and secondary person-to-person transmission was also observed (3).

Unlike the GII.4 genotype where new variants appear frequently, GII.2 has a lower mutation rate. In a study examining nonrecombinant GII.2 strains over a 12-year period, only 2.6% amino acid diversity was observed across the complete capsid gene of the nonrecombinant strains (6). Genetic drift, however, was observed among strains. Substitutions were found at five informative sites and amino acid position 345 consistently changed between genetic groups within the GII.2 genotype (6). Although GII.2 mutations at nucleic acid level have been observed in various studies, GII.2 mutations at amino acid level have not been changed significantly. In a longitudinal analysis of blockade antibody of GII.2 strains over 34 years, none of the polyclonal serum from GII.2.1976-infected volunteers and mouse monoclonal antibodies lost reactivity to any of the time-ordered GII.2 virus-like particles (VLPs) (7). While none of the residue changes were significant enough to ablate antibody binding, some did

moderately impact the blockade potential of antibodies (7).

B. STRUCTURE OF NOROVIRUSES AND CLASSIFICATION

Noroviruses have a non-segmented positive-strand RNA genome which is approximately 7.5 kb and contains three open reading frames (ORF). ORF1 encodes the viral RNA-dependent RNA polymerase (RdRp), ORF2 encodes the viral protein (VP1), and ORF3 encodes the minor capsid protein (VP2) (8) (**Figure 1.1**). ORF2 is predicted to start between nucleotide (nt) positions 5200 and 5225, and the estimated length is between 1716 and 1749 nts (572 – 583 aa) (9). ORF3 is hypothesized to start between nt positions 6959 and 6961, and the estimated length is between 765 and 840 nts (255 – 280 aa) (9). VP1 forms an icosahedral particle with two principal domains, the N-terminal shell (S) and C-terminal protruding (P) domains (10). The S domain surrounds the viral RNA and the P domain, which can be further categorized into the P1 and P2 subdomains (11). The P2 subdomain, which is exposed on the surface and is linked to the S domain through a flexible hinge, is hypervariable, contains the main neutralization epitopes, and binds with histo-blood group antigens (HBGA) (10, 12). Among the subdomains, the P2 subdomain is the least conserved, is often associated with strain diversity, binds to different HBGAs, and demonstrates different antigenicity (13). In contrast to VP1, VP2 is located inside the virus particle and has been identified to be involved in capsid assembly and genome encapsidation (14).

Currently, complete VP1 amino acid sequences and the ORF1 NS7 region (which specifically encodes the RdRp) nucleotide sequences are utilized to genetically classify noroviruses. The need for classifying strains became apparent in the mid-1990s. When noroviruses strains were initially clustered, strains were categorized based on partial RdRp sequences (15-17). When more sequence data became available, classification of genogroup and genotype shifted towards the usage of complete VP1 amino acid sequences where 20% sequence difference was initially used as a cut-off threshold for new genotypes and was later adjusted to 15% (11). Until recently, noroviruses were classified into six genogroups (GI – GVI), but only three, GI, GII, and GIV, have been observed in humans (18). The nomenclature criteria, however, could not keep up with the new variants that suddenly appeared and circulated around the world. Over the past two decades, a number of variants, especially GII.4 variants, have started circulating and have replaced previously dominant variants. Furthermore, an increase in norovirus diversity is anticipated through recombination that frequently occurs in the ORF1-ORF2 junction region (18). Thus, a dual nomenclature system with both RdRp (ORF1) and VP1 (ORF2) sequence data began to be used routinely in many laboratories around the world to classify noroviruses (11, 19).

In 2019, a new genotype and genogroup classification method was proposed and has started to replace the previous classification method. Based on the VP1 amino acid sequence diversity,

the genus norovirus can now be categorized into ten (GI – GX) genogroups and two tentative genogroups that will need to be confirmed when more VP1 or RdRp sequence data becomes available (11). With the new classification system, genogroups that have been observed infecting humans became GI, GII, GIV, GVIII, and GIX (11) (**Figures 1.2 and 1.3**). Strains in the ten genogroups can be further subclassified into 48 confirmed capsid genotypes (9 GI, 26 GII, 3 GIII, 2 GIV, 2 GV, 2 GVI, and 1 genotype each for GVII, GVIII, GIX, and GX) based on complete VP1 amino acid sequences and 60 confirmed P-types based on the partial nucleotide sequences of the RdRp region (11). Until recently, noroviruses were classified to specific P-types depending on the VP1 genotype of the strain. If the genotype was not known, orphan P-types or P-types with alphabet letters were assigned (20). However, the norovirus classification system has now changed where P-types and VP1 genotypes are assigned independently and dual types are designated with the capsid genotype listed first followed by P-type such as with GIX.1[GII.P15] (11).

In recent years, 50 to 70% of the annually reported global human norovirus outbreaks have been associated with infection of GII.4 strains. Some studies suggest that new GII.4 strains will emerge approximately every 2 to 7 years due to the rapid evolution and frequent recombination and predict that these new strains will cause epidemics throughout the world (21). GII.4 dominance, however, has occasionally been subverted by other GII strains. For example, during

the 2016-2017 winter season, global outbreaks of GII.2 strains exponentially increased from approximately 1% to 20% of the total human norovirus outbreaks (22). The GII.2 dominance did not last longer, where the number of cases associated with it declined the following season.

C. CLINICAL FEATURES OF NOROVIRUS INFECTIONS

Human noroviruses belong to the family *Caliciviridae* and are one of the most common viral causes of acute gastroenteritis (AGE) across the globe, where approximately 685 million illnesses due to noroviruses occur every year (21). It is estimated that over 210,000 deaths occur annually, primarily due to complications from noroviruses such as dehydration and malnutrition(23). While all individuals are at risk for norovirus infection, the very young, elderly, immunocompromised, and those with underlying medical conditions are affected by human noroviruses more severely.

Human norovirus infections are associated with a range of clinical symptoms. The incubation period is generally between 12 to 48 hours, and the classical symptoms of norovirus include sudden onset of watery diarrhea, vomiting, and abdominal pain (24). While most cases display both diarrhea and vomiting, in some cases, one of the symptoms can be presented alone (25). In a study examining all norovirus outbreaks that occurred in Catalonia between October 2004 and October 2005, diarrhea coexisted in 45.2% of the cases, and there was vomiting without diarrhea in 19.9% and diarrhea without vomiting in 34.9% (26). In the same study, the first

symptom to appear was diarrhea for 23.1% of cases and vomiting in 19.6% (26). Among the 1544 cases that were examined, the three most commonly observed symptoms were diarrhea, vomiting, and abdominal pain, and their percentages were 78.4%, 65.1%, and 67.2%, respectively (**Table 1.1**). Constitutional symptoms such as low-grade fever, malaise, myalgia, headache, and chills, frequently are observed along with the gastroenteritis (27). For most cases, symptoms resolve within 2 to 3 days. However, the clinical spectrum of illness varies greatly depending on various host factors. While about one-third of those infected are asymptomatic, vulnerable populations, such as the very young, elderly, immunocompromised, and those with underlying medical conditions, are at a greater risk for severe symptoms and complications (28). For example, there have been reports of acute renal failure leading to hemodialysis, cardiac complication from arrhythmia, and in most severe cases, even death (28). Observational studies have also indicated that frequency of symptoms varies by age. In the Catalonia epidemiological study, diarrhea (87.9%) and vomiting (52.9%) were the two predominant symptoms among individuals ≥ 65 years, and the risk of diarrhea was greater among those > 65 than in people aged < 65 years (OR: 2.61; 95% CI 1.93-3.55) (26) (**Table 1.2**). While vomiting (74.6%) and abdominal pain (91.5%) were observed more frequently in children aged < 5 years compared to those aged ≥ 5 years, and diarrhea was reported equally in both groups (26) (**Table 1.2**).

D. NOROVIRUS TRANSMISSION

Norovirus is highly infectious and known to have a low infectious dose (18-2800 viral particles) (27, 29). Norovirus is commonly transmitted from person to person and it can occur directly through the fecal-oral route, or indirectly through exposure of contaminated environmental surfaces, fomites, food, and water (30). Some recent studies also suggest that norovirus may be transmitted through air. They hypothesize that aerosolized viral particles in vomitus can be inhaled and if enough is inhaled, it can cause infection (30, 31). Foodborne transmission of norovirus can occur through contamination by infected food handlers during preparation or serving or from food items that become contaminated during agricultural production or harvesting. Leafy greens, fresh fruits, and shellfish like oysters are commonly associated with norovirus outbreaks, but any ready-to-eat food or any food handled after being cooked are potentially at risk for contamination (32). Waterborne transmission of norovirus has also been reported where in many cases, drinking or recreational water were not correctly treated or became contaminated after treatment. Outbreaks have been linked to municipal water systems, recreational water exposure during activities such as canoeing and rafting, and portable water sources at camps (30). The multiple possible transmission routes and persistence of noroviruses in the environment can lead to rapid and widespread transmission, and these factors may be some of the reasons why norovirus is commonly observed in closed settings such as cruise ships, hospital wards, and institutional residential settings.

Norovirus shedding plays a critical role in the transmission dynamics, but there are still gaps in knowledge about this topic. Until recently, the infectivity of the virus beyond the symptomatic period was not well known (33). However, with novel *in vitro* assays using human intestinal enteroids such as HEK293T, it is now becoming apparent that viruses detected during chronic phase of infection are still infectious (34). Shedding typically occurs in stool, but it can also be detected in vomitus. While the peak for viral shedding is between 2 to 5 days after infection, norovirus RNA has been detected in stool samples for up to 4 to 8 weeks in healthy individuals (33). On the other hand, in a study of immunocompromised pediatric patients, viral shedding was detected up to 418 days (34). Viral shedding is observed among both symptomatic and asymptomatic cases; however, numerous studies indicate that norovirus shedding duration is significantly longer in symptomatic cases than asymptomatic cases (35). Moreover, some studies also indicate that symptomatic patients shed higher viral loads compared to asymptomatic patients (27). Other studies suggest that timing of onset, peak, and resolution of shedding is similar among infected individuals with clinical gastroenteritis and those that do not develop symptom.

E. NOROVIRUS EPIDEMIOLOGY

Noroviruses are highly transmissible, infect people of all ages, and are often associated with winter outbreaks in institutional settings such as long-term care facilities and schools as well as closed communities like cruise ships. In the United States, norovirus infections are

responsible for over 14,000 hospitalizations, 281,000 emergency room visits, and 627,000 outpatient medical care visits annually just among children under 5 years of age (36). Globally, norovirus infections account for over 684 million episodes (95% uncertainty interval of 491 to 1,112 million) of diarrheal disease and approximately 210,000 deaths, each year, for all ages and from all possible modes of transmission (21, 37). The number of norovirus infections continue to increase rapidly each year. While noroviruses were once known to be a "cruise ship virus" or associated with an occasional foodborne outbreak during the winter season, this characterization is now outdated.

There is some heterogeneity in when norovirus outbreaks occur depending on the genotype of the norovirus. However, most norovirus associated outbreaks occur during the winter season (November to March) (38, 39). Transmission modes and outbreak settings differ among and within genogroups. For example, while GII.4 viruses have been associated with person-to-person transmission, non-GII.4 viruses such as GI.3, GII.3, and GII.12, are more frequently associated with foodborne transmission (40). Some studies also suggest that the GI genogroup more often cause waterborne transmission than the GII genogroup (41). While norovirus can be transmitted through various routes of transmission such as through food, water, and environment, the majority of the outbreaks that occur worldwide are associated with person-to-person transmission (42). In Asian countries, such as China, Japan, and South Korea, most

outbreaks have been reported in childcare centers and schools (42). In contrast, healthcare facilities, such as long-term care facilities and hospitals, are the most commonly reported settings for norovirus outbreaks in the United States and Europe (42, 43).

Among 10 birth cohort studies conducted in low- and middle-income countries (LMIC) where at least 1000 stool samples were tested routinely (i.e. Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED)) from 194 to 291 children, norovirus incidence ranged from 51 to 157 infections per 100 child-years of observation (44). These studies have also indicated that approximately 66 to 90% of children had experienced at least one norovirus infection (either symptomatic or asymptomatic) in their early childhood, which is between 1 to 5 years of age (44). Among the longitudinal studies where age stratification was conducted, more children were infected or experienced norovirus-associated diarrhea after 6 months of age as compared to those less than 6 months old (44). Older infants (6 – 11 months of age) experienced the highest incidence rate among examined age groups where the rate was 176 – 221 infections per 100 child-years.

F. GLOBAL PREVALENCE AND BURDEN OF NOROVIRUS

Despite the public health impact that noroviruses have, there is still a lack of data on the global norovirus prevalence. A study conducted in 2014 estimated the norovirus prevalence to be 18%

(95% CI: 17% – 19%) based on 175 studies spanning from 2008 to 2014 (45). More recently, a study specifically focusing on LMICs using studies from 1997 to 2018 concluded that, across all age groups in LMICs, norovirus was detected in 14% (95% CI: 14% – 15%) of symptomatic individuals and 8% (95% CI: 7% – 9%) of asymptomatic controls (46). When the same study examined norovirus prevalence specifically among children <5 years of age, it determined that norovirus detection in inpatients <5 years of age (17%; 95% CI: 17% – 18%) was larger than what was observed among outpatients of the same age (12%; 95% CI: 10% – 14%) (46). The findings from this study were similar to a meta-analysis reviewing the prevalence of norovirus among pediatric patients with gastroenteritis. In this study, the pooled prevalence of norovirus infection among children with gastroenteritis from 45 countries was 17.7% (95 CI: 16.3% – 19.2%) (47). The pooled odds ratio for the association of norovirus infection and gastroenteritis among pediatric patients was 2.7 (95% CI: 2.2 – 3.4) (47).

Other studies have specifically examined the prevalence of asymptomatic norovirus infections. Many agree with one another and estimate the current prevalence of asymptomatic norovirus infection to be approximately 7% (48, 49). When a meta-analysis stratified the prevalence by geographic regions, Africa, Meso America, and South America had higher prevalence of 15%, 14%, and 11% respectively, while Europe and North America had lower prevalence (4%) (49). It was also determined in the same study that the prevalence in communities and hospitals were

approximately the same (9%) , higher in children (8%) than adults (4%), and low among food handlers (3%) (49).

A recent study estimated the global economic burden on norovirus to be a total of $4.2 billion in direct health system costs and $60.3 billion in societal costs per year (50). The cost to society due to illness among individuals <5 years of age was $39.8 billion while it was $20.4 billion for all other age groups combined (50). The study also determined that, while norovirus incidence is similar between LMICs and high income countries, high income countries generated 62% of global health system costs. More specifically, in LMICs and high income countries, the cost to society was $45 and $274 per illness respectively. Among the various reasons mentioned in the study for the differences, productivity losses were thought to be driving much of the cost in high income countries.

G. MOLECULAR AND SEROLOGICAL DIAGNOSTIC METHODS

Rapid identification of an outbreak becomes critical when trying to control the spread. Accurate, but also prompt laboratory diagnosis becomes essential because results are needed to assist implementation of appropriate control measures in order to reduce the spread of the virus and the magnitude of the outbreaks. While molecular diagnostic tests, such as real-time PCR, have been considered as the gold standard test by multiple studies, these diagnostic tests may not be available in various clinical settings due to resource limitations. Interpretation of norovirus

diagnostic testing relies heavily on the quality of the specimen and currently, the optimal specimen for diagnosis is stool. Whole stool samples should ideally be collected during the acute phase of the illness because compared to other types of samples, whole stool samples contain a higher quantity of the virus (27). Vomitus and rectal swabs are alternative specimen types that can be used in supplement to the stool sample during outbreak investigation (30). In contrast, serum specimens are not recommended for routine diagnosis (33). While still not recommended for routine diagnosis, in recent years, serologic markers such as norovirus-specific serum and salivary IgA and IgG, HBGA-blocking antibodies, and fecal IgA are being researched in the context of human challenge studies and vaccine trials. More research, however, is still required to utilize serum specimens and other non-stool samples in clinical and laboratory settings.

Conventional RT-PCR. The first conventional reverse transcription (RT)-PCR assays were developed in the mid-1990s (19). The first-generation conventional RT-PCRs utilized various primers based solely on the first described Norwalk virus genome where it targeted a relatively conserved region of the RNA polymerase gene located in ORF1 (51). Since it underestimated the norovirus genetic diversity, it did not perform well when applied in clinical settings. The second-generation assays were developed using the sequences of additional norovirus strains, many focusing on conserved regions of the viral polymerase (15). The second-generation

conventional RT-PCR required post-PCR analysis via hybridization probes or sequencing in order to improve the sensitivity and specificity (52). Although multiple changes were made to the second-generation assays to improve detection, assay complexity and the post-amplification sample handling were one of the many challenges that a large number of researchers constantly faced.

Real-time Quantitative RT-PCR (RT-qPCR). Real-time quantitative PCR (RT-qPCR) has become the gold standard for rapid norovirus detection due to its high sensitivity and specificity along with other various advantages over other methods like conventional RT-PCR. Depending on the primers and probes used, these assays can detect numerous genogroups concurrently in various types of specimen such as stool, vomitus, and environmental, and through internal extraction and PCR controls, they can reduce false negative results. In addition, real-time RT-qPCR assays can be utilized to determine the amount of nucleic acid in the sample through a semiquantitative method as a proxy to estimate the viral load (19). With the development of real-time RT-qPCR, many of the limitations once faced with conventional RT-PCR were addressed. For example, one-step RT-qPCR assays, which perform both reverse transcription and cDNA amplification in a single reaction, require less sample handing, thus reducing the risk of cross-contamination, and have become the preferred method for clinical laboratory testing for norovirus (19).

While many initial real-time RT-qPCRs focused on the viral polymerase gene, more recent real-time RT-qPCRs have started to focus on other targets such as the conserved region at the ORF1-ORF2 polymerase-capsid junction (53). Several changes have been made to primer-probes as well. In order to further minimize the set-up time and reduce the possibility of carryover contamination, GI and GII ORF1-ORF2 primer-probe sets were optimized to be utilized in a single, multiplex TaqMan reaction (54). Through the improvement of different fluorescent labels on the probes, simultaneous detection and genogrouping became possible (54). Over the years, numerous studies have been conducted to determine the relative performance characteristics of the developed assays. Compared to the conventional assays, sensitivity of real-time RT-qPCR has been determined to be much higher. For example, a study using a multiplex real-time RT-qPCR had a clinical sensitivity of 97% for GI strains and 99% sensitivity for a TaqMan real-time RT-qPCR that targeted the ORF1-ORF2 junction (53, 54).

Enzyme Immunoassay (EIA) for antigen detection. There are a several commercially available enzyme immunoassays (EIAs) that can be used to detect norovirus GI and GII antigens in stool samples. EIAs can be applied in numerous situations ranging from large-scale clinical studies to epidemiological studies. Developing a broadly reactive EIA has been a challenge due to the many genetically distinct human norovirus genotypes and the frequent

antigenic drift among strains such as with GII.4. Thus most commercial kits available today, including the IDEIA Norovirus EIA (Oxoid, Hampshire, United Kingdom), RIDASCREEN (r-Biopharm AG, Darmstadt, Germany), and SRSV (II)-AD (Denka Seiken CO. Ltd., Tokyo, Japan), use combinations of several cross-reactive monoclonal and polyclonal antibodies that react to various VLPs (19). Previous studies have pointed out that these solid-phase sandwich-type immunoassays demonstrate a wide range of sensitivities and specificities. For example, the sensitivity and specificity of the IDEIA Norovirus EIA have ranged between 38.0 to 78.9% and 85.0 to 100%, respectively (30). Studies have shown similar results for RIDASCREEN, demonstrating a slightly lower sensitivity and specificity that ranged from 31.6 to 92.0% and 65.3 to 100%, respectively (30). Multiple studies that have evaluated the performance of these EIAs in outbreak investigations and sporadic gastroenteritis cases have demonstrated that these immunoassays are more sensitive in outbreak situations, especially when multiple samples are collected. In a large European multicenter study, the IDEIA assay resulted in a statistically significant increase in the number of norovirus outbreaks detected when six stool samples per outbreak were examined rather than three (55). Likewise, the RIDASCREEN assay was able to identify a significantly larger number of norovirus outbreaks when seven stool samples per outbreak were tested rather than three samples (55). In a study specifically examining the IDEIA assay, the sensitivity of the assay to confirm a norovirus outbreak was 44.1% when three stool samples were examined and increased to 76.9% when five samples were tested (56).

Similar to the outbreak investigations, statistical modeling suggests that a minimum of six stool samples must be tested with EIA to achieve a 90% probability of correctly detecting a norovirus outbreak (57).

The low sensitivity of commercially available EIAs observed in multiple studies can be attributed to numerous reasons. Collection of samples >72 hours after symptom onset has been directly correlated with lack of detecting norovirus by EIA (58). Moreover, storage of the fecal samples for long times before testing has also been mentioned to compromise the viral level in samples since longer waiting times would translate to higher risk of proteolytic degradation occurring (58). The genetic and antigenic diversity of norovirus strains can also explain the discrepancy in the sensitivity observed among different studies using the same kit and among different EIA kits (55). While these kits use a cocktail of monoclonal and polyclonal antibodies to detect VLPs of certain GI and GII genotypes, certain genotypes could be missed and would result in false negative results. The EIA kits currently available, although there are slight differences in their sensitivities and specificities, all have good specificities and positive predictive values (PPV), but these kits simultaneously have lower sensitivities and negative predicative values (NPV) for both outbreak investigations and sporadic gastroenteritis cases. Low NPVs suggest that EIA-negative should be confirmed with RT-qPCR results while positive EIA results strongly indicate the presence of norovirus. EIAs are useful for rapid

screening of multiple fecal samples during outbreaks since they do not require special molecular diagnostic laboratory facilities and typically have a short turn-around time. Nevertheless, because of the low sensitivity, the general scientific consensus is that caution should be exercised when interpreting EIA results.

EIA for antigen immunoglobulin detection. While EIAs are used for detection of norovirus antigens, EIAs can also be used to study immune response to norovirus infection. Most human challenge studies and outbreak investigations that have studied the immune responses associated with norovirus infection have focused on serum immunoglobulin M (IgM), IgA, and IgG (59, 60). IgM has been frequently studied because it has been shown to be a biological marker for recent norovirus infection (61, 62). Furthermore, many of these studies have all used a 4-fold increase in norovirus serum IgM, IgA, and IgG as an indication of norovirus infection (63). Studies have also used EIAs to detect norovirus-related IgG and IgA in fecal specimens. These EIAs, however, are not used frequently as EIAs that use serum specimens because less is known about fecal IgG and IgA response. Fecal IgA and IgG from norovirus infections are thought to provide protection from infection or reduce symptoms; however, studies have not been able to show any significant differences between fecal IgA levels among symptomatic and asymptomatic individuals (64-66).

Norovirus-specific salivary antibody detection with EIA. When EIAs started to receive more attention, initial focus was placed on developing sensitive EIAs to detect virus-specific IgA, IgG, and IgM in serum and IgA in stool (60). However, in the last few decades, the usage of saliva as a noninvasive alternative for serum to detect norovirus-specific antibodies has started to receive more attention because saliva collection is easy and rapid, eliminates needlestick injury risks, can be used for both children and adults, and requires little training. In a human challenge trial where 38 adults were challenged with the 8FIIa norovirus inoculum, there was good agreement between norovirus-specific salivary IgA and IgG response in the adult participants (60). When ≥4-fold increases in salivary norovirus-specific IgA or IgG were considered as an indication of norovirus infection, 12 infected participants demonstrated salivary conversions in both IgA and IgG assays and 19 uninfected subjects did not demonstrate conversion in either assay, indicating that 82% of the volunteers had concordant results. There was also an overall agreement between results from serum and salivary antibody based EIAs where agreement was seen between serum and salivary IgG responses for 89% of the volunteers and between serum IgG and salivary IgA responses for 92% of the volunteers (60). In the same human challenge study, the EIA used for norovirus-specific salivary IgA and IgG had a sensitivity and specificity of 83% and 95 to 100%, respectively (60). Similar sensitivities and specificities have been observed in community surveillance studies where in a study measuring salivary IgG responses to five common norovirus genotypes among Peruvian

children <5 years of age, the sensitivity was 71% while the specificity was 96% across the evaluated five genotypes when compared to norovirus infection diagnosed by the gold standard real time RT-qPCR (67).

When using EIAs that detect norovirus-specific salivary antibodies, how samples are collected becomes critical for accurate diagnosis. The average norovirus-specific salivary IgA levels peak around 14 days after infection and decrease by three weeks after infection (60). In contrast, norovirus-specific salivary and serum IgG levels continue to increase past three weeks after infection (60). These peak estimations are only the averages from human challenge studies, indicating that the magnitude of the norovirus-specific salivary antibodies increase and peak at different timings for infected individuals in real world situations. Thus, in order to diagnose norovirus infections, it is critical to compare anti-norovirus salivary antibody titers collected soon after infection to those of convalescent-phase saliva samples collected around 14 days post-exposure (67). If saliva specimens can be collected at 2-week intervals, immunoassays for salivary antibodies would be able to capture the rise and fall of salivary antibody titers and could potentially be applied to estimate the incidence of norovirus infections in longitudinal studies. Furthermore, these assays would also be useful for population-based prospective studies in countries where norovirus is an endemic disease.

HBGA-blocking human antibody detection with EIA. Histo-blood group antigens (HBGAs) are a family of complex glycans that can be found on the surfaces of red blood cells, on epithelial and endothelial cells throughout the body, and as soluble oligosaccharides in most bodily fluids such as saliva (68-71). HBGA expression is regulated by three genes: the FUT1, FUT2, and FUT3 genes. These genes determine the biosynthetic pathway that the HBGA will go through and this variation leads to the polymorphic ABO, Lewis, and secretor phenotypes observed among HBGAs (68). Synthesis of HBGAs starts with a disaccharide precursor and monosaccharides that are subsequently added by different of glycosyltransferases. The secretor phenotype, which is recognized by certain norovirus strains and used for binding, requires the expression of the FUT2 gene. The FUT2 gene encodes a fucosyltransferase that adds monosaccharides to the precursor (Galβ1-3GlcNAc), creating the H type 1 antigen. FUT3 or the A and B enzymes can further alter the trisaccharide where tetrasaccharides Lewis B (Le^b), A type 1, and B type 1, respectively, can be synthesized (72, 73). On the other hand, the FUT3 gene also encodes a fucosyltransferase that adds fucose residues to the precursor, leading to the synthesis of the Le^a phenotype, or the non-secretor phenotype (68). The Type 2 pathway begins with a slightly different precursor (Galβ1-4GlcNAc) and is acted on by FUT1, FUT3, A and B enzymes simultaneously, resulting in the creation of H type 2, Le^x, Le^y, A type 2, and B type 2, respectively. The Type 3 pathway precursor Galβ1-4GalNAc is catalyzed by the FUT2 gene and the resulting H type 3 product can be further modified by the A and B enzymes to generate

A or B antigens (73). Thus, the presence or absence of the FUT2 and FUT3 alleles in an individual can determine whether the individual is susceptible to certain strains of norovirus.

Outbreak investigations and human challenge studies have provided strong evidence that individuals with a non-secretor phenotype are resistant to certain norovirus strains. While non-secretor status has been repeatedly reported to be protected from infection, studies report conflicting conclusions regarding ABO HBGA status. In a Norwalk virus (GI.1) human challenge, individuals with O phenotype had a higher odds of getting infected (OR = 11.8) whereas those with B phenotype had decreased risk of infection (OR = 0.096) and symptomatic disease (74). Similar results were observed among norovirus outbreaks that occurred among British troops (75). In contrast, a GII.4 outbreak investigation in China reported that type O individuals had a reduced risk of infection and type A individuals had increased risk (76). In a waterborne outbreak investigation among Dutch children, children with HBGA type B were protected against GI infections but not against GII infections (77).

Studies have revealed that blood type and secretor status do not correlate with susceptibility to SMV infection. When saliva from subjects in a human challenge study were allowed to react with SMV VLPs, the VLPs bound to saliva from secretor positive blood type B and AB individuals (78). On the other hand, in the same study, SMV VLPs did not bind to H type 1 and

type 2 carbohydrates (78). Other studies have reported that SMV binds to H type III carbohydrates (68, 79). Recovery by H type III ligands, however, was subject-specific and weakly associated with type of stool (79). It is still unclear which particular component of the stool might be enhancing SMV attachment to HBGAs, but study results suggest that antibody, enzymes, and other protein molecules are probably not playing any role in attachment (80).

While HBGA blood type B and H type III have frequently been reported to bind with SMV VLPs, there are no reports on the binding between HBGA in porcine gastric mucin (PGM) and SMV (22, 81). PGM contain mixed type A, H1, and Lewis b HBGA and has been reported to bind with various recombinant genotypes I and II noroviruses (81). In a study examining the binding between PGM and VLPs, heated PGM also bound to VLPs (82). Since oxidation abolishes binding, the finding indicated that the protein portion of PGM was not directly associated with the binding to VLPs. In the same study, synthetic HBGA-related oligosaccharides competitively inhibited the binding of VLPs to PGM, thus suggesting that there are structural similarities between PGM receptors for VLPs and HBGA (82).

Noroviruses recognize HBGAs as receptors/coreceptors for cell entry and bind to them by using their major capsid protein, VP1. More specifically, the HBGA binding sites localized on the outermost hypervariable P2 subdomain of VP1 are used by human noroviruses to enter cells

(83). Single-crystal X-ray structures of GI and GII have shown that there are at least two binding sites for each P dimer (84). For GI, the binding sites are located within a P subunit, while for GII, the two binding sites are located at the interface between the two dimers (84). It is predicted that both strain-specific and non-specific interactions occur between the norovirus and HGBG to stabilize the binding (68). Recent studies indicate that in addition to the two reported interaction sites at the receptor-binding domain, long distance interactions between non-specific residues are being utilized to further stabilize the virus-receptor interactions (85). The antigenic variations within these binding sites on the P2 subdomain are also the reason why there are many genogroups and genotypes and why individuals are not protected when infected with a different genogroup norovirus.

In the human body, there are antibodies that block the human noroviruses from binding to HBGAs. Numerous human challenge studies suggest that the circulating serum antibodies that block HBGA binding correlate with protection from both clinical illness and infection. Thus, these antibodies have become known as surrogate neutralizing antibodies (86). Antibodies can block HBGA binding in numerous ways, including directly competing for the HBGA binding site with noroviruses, allosterically disrupting the HBGA binding site by inducing conformational changes in P domain of the noroviruses, or through steric hinderance which masks the HBGA binding site. In the case of IgA 5I2 monoclonal antibody, an antibody that

has been detected in GI.1 human challenge study volunteers, HBGA blockade is principally through steric hinderance (83). The Fab fragment of IgA 5I2, or the antigen-binding site on the antibody, recognizes and binds to a conformational epitope composed of residues from the surface-exposed loop clusters located in distal parts of the P2 subdomain. Since the HBGA binding site in GI.1 is located in a shallow depression on the distal surface of the P2 subdomain and the Fab fragment is relatively larger compared to HBGA, it is hypothesized that direct access to the HBGA binding site for optimal interaction is restricted (83). While IgA 5I2 is highly specific for GI.1, the steric hinderance mechanism is hypothesized to be a common mechanism used by other blockade antibodies. One reason is because the HBGA binding sites in GI and GII are surrounded by loop regions, which are recognized by Fab fragments as epitopes. Another reason is because the majority of the residues critical for blockade antibody binding have been observed outside of the primary HBGA binding sites. Lastly, HBGA-blocking antibodies that have been characterized in previous studies have thus far been genotype-specific and in some cases, do not cross-react even within the same genogroups; this finding suggests that these antibodies only interact with genotype-specific loop regions (83).

Some norovirus genotypes do not bind to the HBGAs; therefore, for those noroviruses, it would not be possible to measure the HBGA-blocking antibody levels against those strains (87). Although there are some limitations with using EIAs to measure HBGA-blocking antibody

levels, antibody levels that show evidence of blocking norovirus from binding to HBGAs have been proposed as a surrogate for neutralization and potentially a correlate of protection. Thus, it is important for future studies to continue focusing on HBGA-blocking antibodies (88).

H. NOROVIRUS HUMAN CHALLENGE STUDIES

In human challenge studies, virulent organisms are administered to healthy and consenting individuals under carefully controlled conditions in order to intentionally induce infection. Challenge studies have been used for various reasons. They have been applied to prove microbial pathogenicity, define host factors that contribute to acquisition of infection, identify microbial virulence factors, and determine if potential vaccine candidates are able to induce protective immunity (89). While human challenge studies have advantages, such as being able to directly apply results to the human condition and to easily collect clinical specimens, there are also notable disadvantages like difficulty with obtaining adequate sample sizes and not being able to control pre-challenge exposures that could affect responses during the challenge study. From the 1970s to the present, there have been more than a dozen norovirus human challenge studies (90) (**Table 1.3**). The majority of the challenge studies since the 1970s have focused on five genotypes: Norwalk virus (GI.1), Montgomery County (GI.5), Snow Mountain virus (GII.2), Farmington Hills (GII.4), and Hawaii virus (GII.1) (90).

Human challenges using various genotypes of norovirus inocula have been conducted over the

last several decades for research purposes, addressing questions difficult to answer through outbreak investigations, such as determining magnitude and duration of virus shedding in feces, the 50% human infectious dose for norovirus, and the cellular and humoral immune response following challenge (91-93). In a human challenge study in which 15 volunteers were challenged with SMV, significant increases in serum gamma interferon (IFN-γ) and IL-2 were observed two days after challenge (93). The amount of IFN-γ secreted depended on $CD4^+$ cells and less on $CD8^+$ cells. Furthermore, the response to SMV, which included significant increases in secretion of IFN-γ, IL-2, and serum IgG1 (subset of IgG), was dependent on a T helper 1 (Th1) cellular immune response (93). This human challenge study also observed that although serum IgG elicited by SMV was cross-reactive with Hawaii virus (HV), another GII norovirus, salivary IgA was less cross-reactive. In contrast, SMV-elicited serum IgG and salivary IgA showed no cross-reaction with Norwalk virus, a genogroup 1 norovirus (93). The cellular response observed in the human challenge study suggested that the antigenic relatedness observed in the study mirrored the genetic relatedness.

While human challenge studies have had several different specific initial objectives, they also have resulted in new findings about norovirus and immune responses. In a phase 1/2 double-blind placebo-controlled trial to determine the immunogenicity of a two dose of bivalent GI.1/GII.4 norovirus vaccine, placebo subjects that were challenged with a GII.4 inoculum

demonstrated that high levels of serum HBGA-blocking or IgA antibody to GII.4 were associated with a lower frequency of infection and illness (94). The same correlation, however, could not be determined in the study for vaccinated subjects, where the relevance of the blocking antibodies to provide protection was less apparent, suggesting that different threshold levels of serum antibody titers may be applicable for vaccine protection (94).The study also found that some placebo subjects who were not infected did not have measurable pre-challenge serum HBGA-blocking antibody (94). This finding suggested that there may be immunological mechanisms other than HBGA-blocking antibodies that are associated with norovirus protection. In fact, different human challenge studies have proposed that levels of pre-challenge Norwalk virus specific salivary IgA and Norwalk virus specific IgG memory B cells may be correlates of protection against Norwalk virus associated gastroenteritis (95, 96). In these studies, Norwalk virus specific salivary IgA levels prior to challenge correlated with reduced severity of gastroenteritis (95, 96). Furthermore, although not Norwalk virus specific, total IgA memory B cells in one of these studies also correlated with protection from Norwalk virus-associated infection and gastroenteritis (96).

Norovirus human challenge studies over the last several decades differ from one another in terms of sample size, follow-up time, and type of recruited volunteers according to the research purposes. Table 1.3 summarizes the key features of published human challenge studies. While

there are some variations, common characteristics that can be observed from these studies are that they have relatively small sample sizes and that the genotype GI.1 has been predominately used for human challenge studies compared to other genotypes. Furthermore, the studies all indicate that there is variability in the risk of infection, and more specifically, the risk of acute symptoms after norovirus infection (97).

I. NOROVIRUS VACCINE STUDIES

Currently, there are no treatments available to treat norovirus infection (98). Since noroviruses are highly antigenically diverse and infectious, stable in the environment, and pre-, post-, and asymptomatic viral shedding can occur, controlling norovirus outbreaks are challenging (99, 100). Thus, preventing norovirus infections through vaccination is an appealing public health measure. There are currently several vaccines under development. There are four vaccine candidates that are in their preclinical trials, two in phase 1, one in phase 1b, and one in phase 2b trial (101).

Among the vaccine candidates in their clinical phases, the one currently closest to getting approval to be sold on the market is a bivalent GI.1/GII.4 vaccine being developed by Takeda Pharmaceutical Company Limited (101). This vaccine was first developed as a monovalent GI.1 VLP vaccine (102). A human challenge study demonstrated that this vaccine was well tolerated and could reduce norovirus-associated AGE incidence among those challenged with

the GI.1 strain (102). Takeda then reformulated the vaccine into a bivalent GI.1/GII.4 VLP vaccine (TAK-214). In a more recent human challenge study, it was determined that this new vaccine was immunogenic and decreased norovirus associated clinical severity, but not the incidence of norovirus-associated AGE (103). In its phase 2b field efficacy study, the vaccine efficacy for moderate/severe norovirus-associated AGE from any strain was determined to be 61.8% (104). Furthermore, in a post hoc efficacy analysis, the vaccine efficacy specifically against GII.2 was determined to be 57.4% (104). The results indicated that TAK-214 may provide cross-protection against GII.2, a non-vaccine group, if an individual has had prior exposure to norovirus. The data collected in the study highlighted that vaccine efficacy may differ depending on prior norovirus exposure and that serological response is dominated by preexisting immunity (105).

Although there are currently no licensed norovirus vaccines, there are several in clinical trials. Over the last decade, clinical trials using different formulations have been conducted, and some have also examined the vaccine efficacy (**Table 1.4**). Results from these trials show promising results, and if these vaccines become licensed products, they will have a tremendous impact on reducing global norovirus morbidity. As the clinical trials progress, it will be equally critical to evaluate whether the vaccines under development can provide cross-protection across norovirus genogroups and genotypes. While including GII.4 VLPs in the vaccines are

important since GII.4 strains account for at least half of all norovirus illnesses annually, it would be important to determine if these vaccines will provide cross-protection against non-GII.4 noroviruses since non-GII.4 outbreaks also occur each year. More specifically, future human challenge studies will need to assess whether these vaccines can provide cross-protection against different variants within genotypes and between numerus genotypes circulating around the world.

CHAPTER II: MANUSCRIPT

A. TITLE, AUTHORS, ABSTRACT

Assessing the Histo-Blood Group Antigen Blocking Antibody Response against GII.2 Norovirus (Snow Mountain Virus)

By Makoto Ibaraki

Noroviruses are one of the most common viral causes of acute gastroenteritis (AGE) globally, and each year, an estimated 684 million infections occur among both children and adults. Noroviruses can be classified into 48 genotypes and while the GII.4 has predominantly been the genotype associated with outbreaks worldwide, reports of outbreaks associated with the GII.2 genotype has recently been increasing. To determine the histo-blood group antigen (HBGA) blocking antibody response following GII.2 norovirus (Snow Mountain Virus, SMV) exposure, results from a recently conducted randomized, double blind, placebo-controlled SMV human challenge study were utilized. For the trial, SMV-specific serum immunoglobulin (Ig)A and IgG concentrations were measured through enzyme-linked immunosorbent assays (ELISA) while HBGA-blocking antibody titers were determined through a SMV-specific blockade assay. Serum antibody responses of SMV-specific IgA, IgG, and HBGA-blocking antibodies were all significantly different between SMV-infected and uninfected subjects following SMV challenge. Among the infected individuals, significant increases in HBGA-blocking antibodies were observed after receiving SMV, with the greatest increase observed on day 15 post-challenge when compared to the pre-challenge titer. The significant increases only observed among the infected subjects suggested that SMV exposure alone would not be enough to activate HBGA-blocking antibody response and that this HBGA-blocking antibody response is a reaction to SMV infection. While further research is needed in order to determine the other factors associated with SMV infection and illness, the results can be applied for the global population at risk. Findings from this study should be used to guide vaccine and screening tool development for SMV with the goal to reduce the infection and illness associated with SMV throughout the world.

B. INTRODUCTION

Human norovirus was first reported in 1968 when an outbreak occurred at an elementary school in Norwalk, Ohio (106). In this outbreak, the virus affected approximately 50% of the students,

many experiencing symptoms such as nausea, vomiting, and abdominal cramps (107). Over 50 years have passed since this outbreak, but noroviruses are still one of the most common enteric pathogens and cause high morbidity throughout communities and remain a major public health concern worldwide.

Human noroviruses belong to the family *Caliciviridae* and are known to be one of the most common viral causes of acute gastroenteritis (AGE) worldwide. In the United States alone, norovirus infections are responsible for over 14,000 hospitalizations and 627,000 outpatient medical care visits just among children under 5 years of age (36). Globally, noroviruses account for over 684 million episodes of diarrheal disease (95% uncertainty interval of 491 - 1,112 million) and approximately 210,000 deaths each year (21, 37). While everyone is at risk, and infection is often characterized as being acute, norovirus infections can become life-threatening for the young, elderly, and immunocompromised. For example, a meta-analysis conducted on global norovirus-associated gastroenteritis cases among children less than 5 years found that approximately 70% of the cases occurred between the 6 to 23-month age range (108). The analysis further concluded that the proportion of cases among children less than 12 months increased from community to outpatient to inpatient settings. This finding suggests that infants more frequently experience severe norovirus illness or are more likely to seek medical care.

Noroviruses are associated with a wide range of transmission modes. While majority of the outbreaks that occur globally are associated with person-to-person transmission, foodborne and waterborne norovirus transmission can also occur (40-42). Outbreak settings are also diverse, where childcare centers, schools, long-term care facilities, and hospitals are all commonly reported settings throughout the world (42, 43).

One of the critical factors that enhances transmissibility of noroviruses is the antigenic diversity. Currently, noroviruses are categorized into ten genogroups (GI – GX) based on phylogenetic analysis of the RNA polymerase (RdRp) and the major capsid protein VP1 (9, 11). Strains in the ten genogroups can be further subclassified into 48 confirmed genotypes (11). Among them, strains within the genogroups GI, GII, GIV, GVIII, and GIX all have been reported to infect humans, with GII being the most commonly reported genogroup for human infections (11, 20, 23). It is estimated that 50 to 70% of all human norovirus outbreaks in recent years are associated with GII.4 strains. While less common, the GII.4-dominance has been occasionally subverted by other GII strains such as the GII.2 strains. During the 2016-2017 winter season, global outbreaks of GII.2 strains exponentially increased, from approximately 1% to 20% of the total human norovirus outbreaks (22).

Snow Mountain virus (GII.2.1976) is the prototype strain of GII.2 noroviruses which was first

reported in a gastroenteritis outbreak that occurred at a resort camp in Colorado in December 1976 (1). Since the initial reported outbreak, Snow Mountain virus (SMV) has been detected in numerous foodborne outbreaks in the US (2-5). While SMV has been used in previous human challenge studies, knowledge about SMV is still limited when compared to other well studied genotypes such as Norwalk virus (GI.1) (72, 92, 93, 109, 110). In particular, there is a gap in understanding the immune response to SMV. Prior studies have reported the human body uses various mechanisms of the adaptive immune system to clear up norovirus infections (94, 111). However, little is known about the pathogenesis of SMV infection and about the specific immune response that the virus elicits.

Among the various immune response mechanisms for human noroviruses, in recent years, there has been growing attention towards antibodies called histo-blood group antigen (HBGA) blocking antibodies. HBGAs are a family of complex glycans that can be found on the surfaces of red blood cells as well as epithelial and endothelial cells (68-71). Although there are some exceptions, most noroviruses bind to the HBGAs expressed on the cells and most likely use them for cell entry. High levels of HBGA-blocking antibodies that prevent noroviruses from binding to HBGAs are associated with protection from both clinical illness and infection (86). These antibodies can block HBGA binding in several ways, including directly competing for the HBGA binding site, allosterically disrupting the HBGA binding site, or through steric

hinderance which masks the HBGA binding site (83, 84). Thus, these antibodies are often referred to as correlates of protection.

To further investigate the association between SMV infection and HBGA-blocking antibody, results from a recently conducted randomized, double blind, placebo-controlled human challenge study were utilized. This report has two research goals. The first goal is to describe the levels of HBGA-blocking antibody following SMV challenge, including temporal trends in HBGA-blocking antibody titers throughout the post-challenge follow-up period. The second goal is to determine whether pre-challenge HBGA-blocking antibody concentration is a predictor for post-challenge infection and illness. The results from this study will contribute towards determining future directions for HBGA-blocking antibody research.

C. MATERIALS AND METHOD

i. CLINICAL STUDY DESIGN

The details of the challenge study have been described previously (112). To summarize, this SMV challenge study (registered at ClinicalTrial.gov under the registration no. NCT02473224) was a randomized, double blind, placebo-controlled trial for studying the optimal inoculation dosage, safety, illness, and infection of a new SMV inoculum. The study was conducted between October 2015 and November 2018 at Emory University's Hope Clinic. The primary objectives were to (1) evaluate the safety and reactogenicity of the SMV challenge stock and (2) determine a safe and optimal challenge dose of SMV to achieve illness in a high proportion

of individuals. The Emory University Institutional Review Board approved the study. Healthy adults of 18 to 49 years of age were selected for the study and challenged with either live SMV or placebo by oral administration once admitted to the inpatient hospital unit and followed for at least 45 days.

In cohorts 1 through 3, nine individuals per cohort were challenged with SMV and the remaining two received the placebo. Cohorts of subjects were sequentially challenged with one of the three doses of the SMV challenge stock beginning with 1.2×10^4 genome equivalent copies (GEC) with the objective to determine the optimal challenge dose. Once illness and safety data were available for a cohort, real-time review of the data was undertaken by the protocol team to determine the appropriate dose for the subsequent cohort. For example, if 75% or fewer subjects in Cohort 1 (challenged with a dose of 1.2×10^4 GEC of SMV) became ill, Cohort 2 received a dose that was two logs higher (1.2×10^6 GEC). If greater than 75% of subjects in Cohort 1 became ill, Cohort 2 would have received a dose that was two logs lower (1.2×10^2 GEC). Once the optimal challenge dose was determined through Cohorts 1 through 3, an additional cohort (Cohort 4), composed of eight secretor-negative subjects and three secretor-positive subjects was enrolled in the study to determine the safety, illness, and infection with the SMV challenge stock in secretor-negative individuals. All individuals in this cohort were challenged with a dose of 1.2×10^7 GEC of SMV.

ii. SEROLOGY

SMV-specific serum immunoglobulin A (IgA) and IgG titers were measured by an enzyme-linked immunosorbent assay (ELISA) for samples collected Day 1 pre-challenge and Days 6, 15, 30, and 45 post-challenge. The titer of HBGA-blocking antibodies in serum samples collected Day 1 pre-challenge and Days 6, 15, 30, and 45 post-challenge was also measured through the usage of a SMV carbohydrate-binding blockade assay developed for the study.

The SMV carbohydrate-binding blockade assay was developed based on previously reported GI.1 and GII.4 blockade assays (92, 94). Briefly, SMV virus-like particles (VLP), expressed in a baculovirus expression system, were incubated with an equal volume of 2-fold serially-diluted serum from the starting dilution (1:25) at 37°C for 1 hour. Simultaneously, a neutravidin-coated microplate (Piece Thermo Fisher Scientific, Rockford, IL) was coated with 2.5 µg/mL of blood type B-PAA-biotin (GlycoTech, Gaithersburg, MD) and incubated for 1 hour at room temperature. After the plates were washed, the sera-VLP mixture was added to the blood type B coated plate and incubated at 4°C for 2 hours. Plates were washed again and incubated at 4°C for 1 hour after the addition of SMV-specific polyclonal antibody (1:2000 dilution). Horseradish Peroxidase-conjugated goat anti-rabbit IgG (1:2000) (Sigma-Aldrich, ST. Louis, MO) was then added, and the plates were incubated for another 1 hour at 4°C. The color was developed by adding 3,3',5,5'-Tetramethylbenzidine liquid substrate (Sigma-Aldrich,

ST. Louis, MO) and stopped after 10 minutes of incubation at room temperature by adding 1M phosphoric acid. OD (optical density) was measured at 450 nm wavelength using a plate reader spectrophotometer. Each dilution of the sample was tested in duplicate, and duplicate blocking antibody titers needed to be within 2-fold of each other in order to meet QA/QC criteria. Blank wells were incubated with buffer, without VLP, but with the primary and secondary antibodies. VLP-only wells were incubated with buffer instead of serum, and these wells served as a positive control showing 100% binding of VLP and carbohydrate in the absence of serum. The BT50 (the 50% blocking titer), defined as the reciprocal of the last dilution with OD readings less than or equal to 50% of OD of the VLP only wells, was determined for each sample. Samples with a BT50 less than 25 were assigned a value of 12.5.

iii. STATISTICAL METHODS

The data were analyzed using SAS version 9.4 (SAS Institute, Cary, NC, USA). Geometric mean antibody titers (GMT) and geometric mean fold rises (GMFR) were determined for samples collected on both pre-challenge and post-challenge days. To characterize the immune response to SMV infection, the Kruskal-Wallis test was used to compare GMT and GMFR HBGA-blocking antibody and immunoglobulin differences between the infected and uninfected subjects included in the analysis.

Conditional logistic regression was applied to evaluate the relationship between pre-challenge

antibody levels and the probability of illness while controlling for several covariates. More specifically, since the sample size was small, an exact conditional logistic regression was utilized to avoid bias that could arise from sparse data. The regression model was used to assess whether pre-challenge HBGA-blocking antibody concentration could be utilized as a predictor for post-challenge illness. While there was strong interest in examining the probability of infection, this analysis was not possible since all infected subjects could not be pair-matched by age (±3 years of age) to study subjects who remained uninfected following SMV challenge.

Fisher's exact test was also utilized to evaluate the relationship between pre-challenge HBGA-blocking antibody titers and illness. This test was used to compare the presence of measurable HBGA-blocking antibody titer at pre-challenge among those who were infected with illness to those who were infected without illness. The goal for this test was to determine whether the absence of measurable HBGA-blocking antibody titers (< 25) prior to challenge was associated with the development of illness during the post-challenge follow up.

To summarize the differences between infected and uninfected subject's HBGA-blocking antibody response over time while accounting for correlation between time-points, two linear mixed models were utilized. The models were used to test the association between natural log-transformed HBGA-blocking antibody concentration and day post-challenge, stratified by

infection status accounting for random effects by subject. Unstructured correlation between different time points were taken for each individual, and the fixed effects of age, race, inoculum, and SMV-specific serum IgA were taken into consideration when modeling. While one linear mixed model considered the four post-challenge days as an ordinal variable, the other model considered these post-challenge days as a continuous variable. The outcome from the first model allowed one to see whether there was a significant increase in HBGA-blocking antibody titer between pre-challenge and specific post-challenge days. On the other hand, the results from the second model determined the average increase in HBGA-blocking antibody titer with one unit of increase in time.

D. RESULTS

i. PARTICIPANT CHARACTERISTICS

Among the 44 study participants, 38 were challenged with one of the three doses of SMV challenge inoculum, and 6 were administered a placebo. For data analysis, however, individuals with no recorded HBGA-blocking antibody data were excluded since the focus of this analysis was to determine the HBGA-blocking antibody response to SMV infection. Thus, the results from 34 individuals in the human challenge trial were used in these analyses. The study population included in the analysis was relatively young where the mean age was 33.53 years (SD = 9.51 years) and the median age was 33 years. The distribution of sex and race among infected and uninfected individuals was similar (**Table 2.1**). Secretor status was similarly distributed between the infected and uninfected groups, where the majority were secretor-

positive individuals. While episodes of AGE symptoms were frequently observed among the infected subjects, several uninfected individuals also exhibited symptoms, such as vomiting, which was consistent with past norovirus human challenge studies (113, 114).

ii. SMV INFECTION

For this study, infection was defined as SMV excretion in stool detected through RT-qPCR at any time after challenge through Day 30 or showing at least a 4-fold rise from pre-challenge SMV-specific serum IgG titer at any time after challenge through Day 30. The data analysis indicated that the observed infection rate increased as the inoculum dose increased, 0% (0/0) in Cohort 1 (1.2×10^4 GEC), 55.6% (5/9) in Cohort 2 (1.2×10^6 GEC), and 91.7% (11/12) in secretor-positive individuals that received 1.2×10^7 GEC. Among secretor-negative individuals in Cohort 4 (1.2×10^7 GEC), 87.5% (7/8) were infected. Similar to previous studies, SMV infection was not significantly associated with secretor phenotype (p-value 0.306) (93).

iii. SMV ILLNESS

Illness was defined as vomiting and/or diarrhea during the inpatient period (Day 1 through Day 5) with evidence of SMV infection. Similar to what was observed for the infection rate, illness rate increased as the inoculum dose increased. Overall, 0% (0/0) in Cohort 1, 33.3% (3/9) in Cohort 2, and 41.7% (5/12) of secretor-positive individuals that received 1.2×10^7 GEC became ill, and 0% (0/0) of the secretor-negative individuals in Cohort 4 became ill. Among those

infected, 32% (8/25) were also classified as being ill, while the remaining 68% (17/25) were infected but showed no symptoms.

iv. IMMUNE RESPONSE AGAINST SMV

To examine whether the immune response following SMV challenge differed between infected and uninfected subjects, the Kruskal-Wallis test was used. For both Day 1 and Day 6, the GMT and GMFR of HBGA-blocking antibody was not significantly different between infected and uninfected. However, both the GMT and GMFR of HBGA-blocking antibodies were significantly different between infected and uninfected individuals for Days 15, 30, and 45 (**Table 2.2**). At day 45 post-challenge, 80% (20/25) of the infected individuals still had ≥4-fold rise in HBGA-blocking antibody and 92% (23/25) of the infected subjects still had detectable HBGA-blocking antibody titers at day 45 (**Figure 2.1**).

A similar trend was observed for both SMV-specific serum IgG and IgA (**Tables 2.3 and 2.4**). For both Days 1 and 6, the GMT and GMFR of IgA and IgG were not significantly different between infected and uninfected individuals. However, GMT and GMFR were significantly different between infected and uninfected individuals for Days 15, 30, and 45 post-challenge. The trend in concentrations slightly differed from the HBGA-blocking antibodies for the later post-challenge days. While both IgG and IgA levels sharply increased from Day 6 to Day 15 post-challenge, both IgG and IgA levels did not peak at Day 15 like HBGA-blocking antibodies

and instead, continued to increase throughout the follow-up period (**Figure 2.1**).

The correlation between HBGA-blocking antibody titers and SMV-specific serum IgG and IgA titers were also examined after stratifying the population by infection status. No significant correlations between HBGA-blocking antibody and either immunoglobulin concentration were observed among the uninfected individuals. However, significant correlation was observed between both immunoglobulin levels and HBGA-blocking antibody concentrations as early as Day 6 post-challenge for subjects who were infected. The coefficient of determination was highest at Day 15 post-challenge for both immunoglobulins and was followed by a slight decline in correlation (**Table 2.5**).

v. PRE-CHALLENGE PREDICTORS OF ILLNESS

To determine whether pre-challenge HBGA-blocking antibody concentration could predict illness status following challenge, the association between the two were examined using logistic regression analysis. Since immune response may differ with age, cases with and without illness were matched for age. In addition, the natural log of HBGA-blocking antibody concentration was used as the exposure of interest. Multiple conditional logistic regression models were tested: each model controlled for a set of baseline covariates such as inoculum dose and subject race as well as chemokines such as CCL5 (RANTES) and Eotaxin. These covariates were specifically selected because of results from prior studies as well as the results

obtained through initial analysis of HBGA-blocking antibody levels. All tested conditional logistic regression models, however, concluded that the titer of pre-challenge HBGA-blocking antibody was not a statistically significant predictor of illness.

A similar hypothesis was tested using Fisher's exact test. For this test, the goal was to determine whether the absence of measurable HBGA-blocking antibody titer prior to challenge was associated with the development of illness during the post-challenge follow up. For this analysis, only those classified as infected (25/34) were included. Similar to findings of the conditional logistic regression models, this test also could not find an association between pre-challenge HBGA-blocking antibody titer and the development of illness during post-challenge.

vi. DIFFERENCES BETWEEN INFECTED AND UNINFECTED SUBJECTS OVER TIME

The trend in HBGA-blocking antibody concentrations over time was analyzed through two linear mixed models while accounting for correlation of repeated within subject measurements over time. The first linear mixed model interpreted post-challenge days as an ordinal variable while the second model interpreted post-challenge days as a continuous variable. Both models concluded that infected individuals, when compared to uninfected individuals, had statistically significant changes in HBGA-blocking antibody levels across the post-challenge days with a p-value of <0.001 and 0.006, respectively, for overall post-challenge day effect (**Figure 2.1**).

The first model indicated that, among the post-challenge days, the titers on Days 15, 30, and 45 post-challenge were significantly higher, with p-values of <0.001, <0.001, and 0.001, respectively (**Table 2.6**). In addition, the model concluded that Day 15 post-challenge had the largest average change in HBGA-blocking antibody concentration when compared to the Day 1 (pre-challenge day) titer. The expected percent increase in geometric mean from Day 1 to Day 15 was approximately 421.61% when other variables were held constant. The outcome for the second model in which time was treated as a continuous variable could be interpreted that for every one-day increase in time post-challenge, there was an expected average increase of 1.68% in HBGA-blocking antibody titer (**Table 2.6**).

One of the key reasons why linear mixed models were selected was to not only examine the relationship between HBGA-blocking antibody level and post-challenge day, but also to answer the question of how much variation in the HBGA-blocking antibody concentration was attributable to between-subject variations after controlling for baseline covariates. The intracluster correlation coefficient (ICC) was 45% (95% CI: 29.42%, 58.25%) , suggesting that 45% of the total variation among individual observations throughout the study was due to the differences at the subject or individual level. Thus, it could be assumed that for the 34 subjects included in the study, each with five repeated measures, there was notable similarity of observations within subjects and less variation was observed between subjects.

E. DISCUSSION

HBGAs are a family of complex glycans that are frequently found on the surfaces of erythrocytes, epithelial and endothelial cells, and as soluble oligosaccharides in many bodily fluids such as saliva (68, 69). Noroviruses recognize HBGAs as receptors/coreceptors for cell entry and bind to them by using their major capsid protein, VP1. Thus, HBGAs have been identified as one of the key elements required in the establishment of infection for several different genotypes of norovirus (115-117). If an individual is infected with norovirus, the body may develop antibodies that specifically block noroviruses from binding to HBGAs. These antibodies, called HBGA-blocking antibodies, can block HBGA binding in several ways, such as through directly competing for the HBGA binding site, allosterically disrupting the HBGA binding site by inducing conformational changes in the P domain of the noroviruses, or through steric hinderance, which covers up the HBGA binding site (83).

The analysis concluded that SMV infection was not dependent on secretor status, which aligned with results from previous studies (7, 93). The results from this study, along with previous studies, suggest that secretor status does not increase susceptibility to the GII.2 genotype and that both populations must be considered when developing intervention and prevention strategies against SMV. Furthermore, in this study, it was determined that HBGA-blocking antibody titer was not dependent on secretor status following SMV inoculation. The non-

significant differences in HBGA-blocking antibody response between infected secretors and non-secretors for all post-challenge days were expected because SMV infection was not dependent on secretor status and instead, was associated with HBGA-blocking antibody titers.

i. PRE-CHALLENGE HBGA-BLOCKING ANTIBODY TITER AS CORRELATE OF PROTECTION

It is still difficult to serially propagate human noroviruses using standard *in vitro* cultivation methods (57, 118, 119), thus limiting the ability to test the functions of antibodies that may neutralize noroviruses. Furthermore, some norovirus genotypes only weakly bind to HBGAs, which make measuring HBGA-blocking antibody levels challenging (120, 121). While there are several limitations, numerous human challenge studies suggest that HBGA-blocking antibodies to be a surrogate for neutralization and potentially correlate with protection from both clinical illness and infection (88, 102, 122). In contrast to these studies, the results from this study showed that pre-challenge HBGA-blocking antibody titer was not a predictor for SMV illness among infected subjects. Similar conclusions were made when both conditional logistic regression and Fisher's exact test were applied to analyze the data. The reasons for this conclusion are yet to be determined, but there are several possible explanations. One possibility is inadequate sensitivity of the serum HBGA-blocking antibody assay, resulting in misclassification of individuals. If the quantitative sensitivity of the assay was not optimal, detection of low levels of HBGA-blocking antibodies may have not been possible even if the

HBGA-blocking antibody was present. Another possibility is that the HBGA-blocking antibodies are not a functional correlate of protection, but instead are associated with other cell-mediated and/or humoral immune responses. While multiple studies have suggested that the lack of detectable blocking antibody titers correlate with a lack of protection from illness, some, similar to this study, were not able to find evidence to support this hypothesis (93, 94, 122). The third possibility is the existence of additional unrecognized mechanisms of innate immunity that prevent infection and illness. Prior studies have demonstrated a Th1- and Th2-type response to GI.1 norovirus infection, where chemokines interleukin (IL)-8, monocyte chemoattractant protein (MCP-1), and IL-10 were seen to elevate in response to GI.1 norovirus infection (123). While HBGA-blocking antibodies have been hypothesized to prevent infection, the hypothesis could be ignoring other immune response possibilities. The fourth possibility is the small study population. When the sample size is small, variability increases, making it challenging to find differences between two comparison groups. Among the 44 individuals included in the original challenge study, only results from 34 subjects could be used for these analyses. This further decreased for the conditional logistic regression model where only a total of 8 matched pairs or 16 individuals could be included in the analysis.

ii. TEMPORAL CHANGES IN SMV-SPECIFIC IMMUNOGLOBULINS AND HBGA-BLOCKING ANTIBODIES

All infected subjects, and none of the uninfected subjects, had a statistically significant increase

in SMV-specific serum IgG and IgA titers. This indicated that SMV infection, not just exposure to the virus, was essential to elicit activation of a SMV-specific humoral immune response, a similar finding to what was observed in previous Norwalk virus challenge studies (72). Studies in the past have used the presence of a norovirus VLP-reactive IgG titer as an indicator of strain exposure. However, cross-strain reactivity of anti-norovirus IgG has also been observed, suggesting that strain identification by serum IgG titer alone would lead to misclassification (72, 93, 124). While it is tempting to use this approach for outbreak investigations, a comprehensive panel of VLPs and highly sensitive serological tests would be necessary in order to use serum IgG measurements to determine current infections with noroviruses.

In this study, it was determined that, even while accounting for random variation in responses between and within subjects, post-challenge HBGA-blocking antibody titers increased significantly from pre-challenge titers for infected individuals but not for uninfected individuals. Among the infected subjects, HBGA-blocking antibody levels increased substantially following virus challenge (starting on Day 15 post-challenge). This exponential increase in HBGA-blocking antibody levels was not observed among the uninfected individuals. Similar to the serum IgG and IgA responses, this finding indicated that SMV exposure alone would not be enough to activate HBGA-blocking antibody response and that this is a reaction to SMV infection. Furthermore, this observation from the study provides

additional evidence towards the concept that HBGA-blocking antibody would be a surrogate for neutralizing antibodies.

Some studies have also demonstrated that virus-specific serum IgA as a functional correlate of protection (94). In a study examining GII.4 strains, higher GII.4-specific serum IgA levels were associated with a decreased frequency of both infection and illness. However, other studies have rejected the hypothesis of serum IgA being a correlate of protection (96). In a study examining GI.1, virus-specific serum IgA levels were not associated with protection from symptomatic illness, but instead, higher concentrations of pre-challenge GI.1-specific salivary IgA were associated with a decreased risk of norovirus gastroenteritis (96). This study also did not demonstrate that SMV-specific serum IgA was a functional correlate of protection. Among the infected subjects, higher SMV-specific serum IgA prior to challenge was not associated with protection from illness. While association between SMV-specific serum IgA titer and protection from illness could not be determined in this study, it would be important to continue investigating this relationship, along with HBGA-blocking antibody response, in future studies to identify effective vaccines that can elicit various immune responses simultaneously.

iii. STRENGTHS AND LIMITATIONS

This study is currently the largest SMV human challenge study that has been conducted to date. In addition, it is also one of the few SMV human challenge studies to assess HBGA-blocking

antibody response after an experimental viral challenge. The strengths of this analysis were the repeated measures design and the utilization of serum samples collected at pre-challenge and four post-challenge days. The usage of three different inoculum doses was another strength since immune response intensity may differ depending on the dose of the virus.

A limitation of this study is the lack of transportability to other age groups. The population at highest risk for norovirus infection are children under 5 and the elderly. However, the challenge study was conducted with healthy adults. Immune system maturation likely affects the immune response to norovirus exposure. Thus, patterns observed among adults may have not been reflective of what will be seen among children. Some other limitations of the study were the exposure history of subjects prior to the study being unknown, and possible misclassification bias due to measurement tools. Although RT-qPCR is the current gold standard for norovirus diagnosis, there could have been individuals that were misclassified as uninfected due to the fact that they shed the virus at levels below the limit of detection. From an analysis standpoint, a limitation was the small sample size. Out of the original sample of 44 subjects, only the results from 34 subjects could be used since the remaining 10 did not have HBGA-blocking antibody titers measured. Since the study population was small, the obtained results could have been merely due to chance. Thus, the results obtained from this analysis may lack internal validity.

F. REFERENCES

1. Morens DM, Zweighaft RM, Vernon TM, et al. A waterborne outbreak of gastroenteritis with secondary person-to-person spread. Association with a viral agent. *Lancet* 1979;1(8123):964-966.
2. Brondum J, Spitalny KC, Vogt RL, et al. Snow Mountain agent associated with an outbreak of gastroenteritis in Vermont. *J Infect Dis* 1985;152(4):834-837.
3. Gordon SM, Oshiro LS, Jarvis WR, et al. Foodborne Snow Mountain agent gastroenteritis with secondary person-to-person spread in a retirement community. *Am J Epidemiol* 1990;131(4):702-710.
4. Guest C, Spitalny KC, Madore HP, et al. Foodborne Snow Mountain agent gastroenteritis in a school cafeteria. *Pediatrics* 1987;79(4):559-563.
5. Truman BI, Madore HP, Menegus MA, et al. Snow Mountain agent gastroenteritis from clams. *Am J Epidemiol* 1987;126(3):516-525.
6. Iritani N, Vennema H, Siebenga JJ, et al. Genetic analysis of the capsid gene of genotype GII.2 noroviruses. *J Virol* 2008;82(15):7336-7345.
7. Swanstrom J, Lindesmith LC, Donaldson EF, et al. Characterization of blockade antibody responses in GII.2.1976 Snow Mountain virus-infected subjects. *J Virol* 2014;88(2):829-837.
8. Matthew S, Alansari K, Smatti MK, et al. Epidemiological, Molecular, and Clinical Features of Norovirus Infections among Pediatric Patients in Qatar. *Viruses* 2019;11(5).
9. Ford-Siltz LA, Mullis L, Sanad YM, et al. Genomics Analyses of GIV and GVI Noroviruses Reveal the Distinct Clustering of Human and Animal Viruses. *Viruses* 2019;11(3).
10. Miyazaki N, Taylor DW, Hansman GS, et al. Antigenic and Cryo-Electron Microscopy Structure Analysis of a Chimeric Sapovirus Capsid. *J Virol* 2015;90(5):2664-2675.
11. Chhabra P, de Graaf M, Parra GI, et al. Updated classification of norovirus genogroups and genotypes. *J Gen Virol* 2019;100(10):1393-1406.
12. Banyai K, Estes MK, Martella V, et al. Viral gastroenteritis. *Lancet* 2018;392(10142):175-186.
13. Singh BK, Leuthold MM, Hansman GS. Structural Constraints on Human Norovirus Binding to Histo-Blood Group Antigens. *mSphere* 2016;1(2).
14. Vongpunsawad S, Prasad BVV, Estes MK. Norwalk Virus Minor Capsid Protein VP2 Associates within the VP1 Shell Domain. *Journal of Virology* 2013;87(9):4818-4825.
15. Ando T, Monroe SS, Gentsch JR, et al. Detection and Differentiation of Antigenically Distinct Small Round-Structured Viruses (Norwalk-Like Viruses) by Reverse Transcription-PCR and Southern Hybridization. *Journal of Clinical Microbiology* 1995;33(1):64-71.
16. Green SM, Lambden PR, Caul EO, et al. Capsid diversity in small round-structured viruses: molecular characterization of an antigenically distinct human enteric calicivirus. *Virus*

Res 1995;37(3):271-283.

17. Vinje J, Koopmans MP. Molecular detection and epidemiology of small round-structured viruses in outbreaks of gastroenteritis in the Netherlands. *J Infect Dis* 1996;174(3):610-615.
18. Tohma K, Lepore CJ, Ford-Siltz LA, et al. Phylogenetic Analyses Suggest that Factors Other Than the Capsid Protein Play a Role in the Epidemic Potential of GII.2 Norovirus. *mSphere* 2017;2(3).
19. Vinje J. Advances in laboratory methods for detection and typing of norovirus. *J Clin Microbiol* 2015;53(2):373-381.
20. Kroneman A, Vega E, Vennema H, et al. Proposal for a unified norovirus nomenclature and genotyping. *Arch Virol* 2013;158(10):2059-2068.
21. Lopman BA, Steele D, Kirkwood CD, et al. The Vast and Varied Global Burden of Norovirus: Prospects for Prevention and Control. *PLoS Med* 2016;13(4):e1001999.
22. Mallory ML, Lindesmith LC, Brewer-Jensen PD, et al. Bile Facilitates Human Norovirus Interactions with Diverse Histoblood Group Antigens, Compensating for Capsid Microvariation Observed in 2016-2017 GII.2 Strains. *Viruses* 2020;12(9).
23. Nguyen GT, Phan K, Teng I, et al. A systematic review and meta-analysis of the prevalence of norovirus in cases of gastroenteritis in developing countries. *Medicine (Baltimore)* 2017;96(40).
24. Atmar RL, Estes MK. The epidemiologic and clinical importance of norovirus infection. *Gastroenterol Clin North Am* 2006;35(2):275-290, viii.
25. Nateghian A, Moshfegh F, Shoja Z. Study of Clinical Characteristics and Clinical Complications of Norovirus gastroenteritis in Admitted Children to the Hospital. *Journal of Pharmaceutical Research International* 2019;30(4):1-10.
26. Arias C, Sala MR, Dominguez A, et al. Epidemiological and clinical features of norovirus gastroenteritis in outbreaks: a population-based study. *Clin Microbiol Infect* 2010;16(1):39-44.
27. Cardemil CV, Parashar UD, Hall AJ. Norovirus Infection in Older Adults: Epidemiology, Risk Factors, and Opportunities for Prevention and Control. *Infect Dis Clin North Am* 2017;31(4):839-870.
28. Mattner F, Sohr D, Heim A, et al. Risk groups for clinical complications of norovirus infections: an outbreak investigation. *Clin Microbiol Infect* 2006;12(1):69-74.
29. Teunis PF, Moe CL, Liu P, et al. Norwalk virus: how infectious is it? *J Med Virol* 2008;80(8):1468-1476.
30. Robilotti E, Deresinski S, Pinsky BA. Norovirus. *Clin Microbiol Rev* 2015;28(1):134-164.
31. Alsved M, Fraenkel CJ, Bohgard M, et al. Sources of Airborne Norovirus in Hospital Outbreaks. *Clin Infect Dis* 2020;70(10):2023-2028.
32. Morton V, Jean J, Farber J, et al. Detection of noroviruses in ready-to-eat foods by using

carbohydrate-coated magnetic beads. *Appl Environ Microbiol* 2009;75(13):4641-4643.
33. Hall AJ, Vinje J, Lopman B, et al. Updated Norovirus Outbreak Management and Disease Prevention Guidelines. In: Centers for Disease Control and Prevention, ed. *Morbidity and Mortality Weekly Report*, 2011.
34. Davis A, Cortez V, Grodzki M, et al. Infectious Norovirus Is Chronically Shed by Immunocompromised Pediatric Hosts. *Viruses* 2020;12(6).
35. Wu QS, Xuan ZL, Liu JY, et al. Norovirus shedding among symptomatic and asymptomatic employees in outbreak settings in Shanghai, China. *BMC Infect Dis* 2019;19(1):592.
36. Payne DC, Vinje J, Szilagyi PG, et al. Norovirus and medically attended gastroenteritis in U.S. children. *N Engl J Med* 2013;368(12):1121-1130.
37. Weinberg GA. Outbreak Epidemiology: One of Many New Frontiers of Norovirus Biology. *J Infect Dis* 2019;219(9):1349-1352.
38. Ao Y, Wang J, Ling H, et al. Norovirus GII.P16/GII.2-Associated Gastroenteritis, China, 2016. *Emerg Infect Dis* 2017;23(7):1172-1175.
39. Iritani N, Kaida A, Abe N, et al. Increase of GII.2 norovirus infections during the 2009-2010 season in Osaka City, Japan. *J Med Virol* 2012;84(3):517-525.
40. Verhoef L, Hewitt J, Barclay L, et al. Norovirus genotype profiles associated with foodborne transmission, 1999-2012. *Emerg Infect Dis* 2015;21(4):592-599.
41. Lysen M, Thorhagen M, Brytting M, et al. Genetic diversity among food-borne and waterborne norovirus strains causing outbreaks in Sweden. *J Clin Microbiol* 2009;47(8):2411-2418.
42. Jin M, Wu S, Kong X, et al. Norovirus Outbreak Surveillance, China, 2016-2018. *Emerg Infect Dis* 2020;26(3):437-445.
43. Vega E, Barclay L, Gregoricus N, et al. Novel surveillance network for norovirus gastroenteritis outbreaks, United States. *Emerg Infect Dis* 2011;17(8):1389-1395.
44. Cannon JL, Lopman BA, Payne DC, et al. Birth Cohort Studies Assessing Norovirus Infection and Immunity in Young Children: A Review. *Clin Infect Dis* 2019;69(2):357-365.
45. Ahmed SM, Hall AJ, Robinson AE, et al. Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. *Lancet Infect Dis* 2014;14(8):725-730.
46. Mans J. Norovirus Infections and Disease in Lower-MiddleandLow-Income Countries, 1997(-)2018. *Viruses* 2019;11(4).
47. Farahmand M, Moghoofei M, Dorost A, et al. Global prevalence and genotype distribution of norovirus infection in children with gastroenteritis: A meta-analysis on 6 years of research from 2015 to 2020. *Rev Med Virol* 2021:e2237.
48. Lopman BA, Trivedi T, Vicuna 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(11):1813-1821.

49. Qi R, Huang YT, Liu JW, et al. Global Prevalence of Asymptomatic Norovirus Infection: A Meta-analysis. *EClinicalMedicine* 2018;2-3:50-58.
50. Bartsch SM, Lopman BA, Ozawa S, et al. Global Economic Burden of Norovirus Gastroenteritis. *PLoS One* 2016;11(4):e0151219.
51. Green J, Norcott JP, Lewis D, et al. Norwalk-like viruses: demonstration of genomic diversity by polymerase chain reaction. *J Clin Microbiol* 1993;31(11):3007-3012.
52. Vinje J, Vennema H, Maunula L, et al. International collaborative study to compare reverse transcriptase PCR assays for detection and genotyping of noroviruses. *J Clin Microbiol* 2003;41(4):1423-1433.
53. 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(4):1548-1557.
54. Hoehne M, Schreier E. Detection of Norovirus genogroup I and II by multiplex real-time RT- PCR using a 3'-minor groove binder-DNA probe. *BMC Infect Dis* 2006;6:69.
55. Gray JJ, Kohli E, Ruggeri FM, et al. European multicenter evaluation of commercial enzyme immunoassays for detecting norovirus antigen in fecal samples. *Clin Vaccine Immunol* 2007;14(10):1349-1355.
56. Costantini V, Grenz L, Fritzinger A, et al. Diagnostic accuracy and analytical sensitivity of IDEIA Norovirus assay for routine screening of human norovirus. *J Clin Microbiol* 2010;48(8):2770-2778.
57. Duizer E, Schwab KJ, Neill FH, et al. Laboratory efforts to cultivate noroviruses. *J Gen Virol* 2004;85(Pt 1):79-87.
58. Dimitriadis A, Bruggink LD, Marshall JA. Evaluation of the Dako IDEIA norovirus EIA assay for detection of norovirus using faecal specimens from Australian gastroenteritis outbreaks. *Pathology* 2006;38(2):157-165.
59. Matsui SM, Greenberg HB. Immunity to calicivirus infection. *J Infect Dis* 2000;181 Suppl 2:S331-335.
60. Moe CL, Sair A, Lindesmith L, et al. Diagnosis of norwalk virus infection by indirect enzyme immunoassay detection of salivary antibodies to recombinant norwalk virus antigen. *Clin Diagn Lab Immunol* 2004;11(6):1028-1034.
61. Brinker JP, Blacklow NR, Estes MK, et al. Detection of Norwalk virus and other genogroup 1 human caliciviruses by a monoclonal antibody, recombinant-antigen-based immunoglobulin M capture enzyme immunoassay. *J Clin Microbiol* 1998;36(4):1064-1069.
62. Brinker JP, Blacklow NR, Jiang X, et al. Immunoglobulin M antibody test to detect genogroup II Norwalk-like virus infection. *J Clin Microbiol* 1999;37(9):2983-2986.
63. Graham DY, Jiang X, Tanaka T, et al. Norwalk virus infection of volunteers: new insights

based on improved assays. *J Infect Dis* 1994;170(1):34-43.

64. Iritani N, Seto T, Hattori H, et al. Humoral immune responses against norovirus infections of children. *J Med Virol* 2007;79(8):1187-1193.
65. Mori K, Konishi N, Suzuki Y, et al. Comparison between patients with norovirus-related gastroenteritis and asymptomatic carriers with respect to distribution of antibody-complexed viral particles and intestinal flora. *J Med Virol* 2018;90(12):1882-1887.
66. Okhuysen PC, Jiang X, Ye L, et al. Viral shedding and fecal IgA response after Norwalk virus infection. *J Infect Dis* 1995;171(3):566-569.
67. Pisanic N, Ballard SB, Colquechagua FD, et al. Minimally Invasive Saliva Testing to Monitor Norovirus Infection in Community Settings. *J Infect Dis* 2019;219(8):1234-1242.
68. Donaldson EF, Lindesmith LC, Lobue AD, et al. Norovirus pathogenesis: mechanisms of persistence and immune evasion in human populations. *Immunol Rev* 2008;225:190-211.
69. Heggelund JE, Varrot A, Imberty A, et al. Histo-blood group antigens as mediators of infections. *Curr Opin Struct Biol* 2017;44:190-200.
70. Almand EA, Moore MD, Jaykus LA. Norovirus Binding to Ligands Beyond Histo-Blood Group Antigens. *Front Microbiol* 2017;8:2549.
71. Marionneau S, Ruvoen N, Le Moullac-Vaidye B, et al. Norwalk virus binds to histo-blood group antigens present on gastroduodenal epithelial cells of secretor individuals. *Gastroenterology* 2002;122(7):1967-1977.
72. Lindesmith L, Moe C, Marionneau S, et al. Human susceptibility and resistance to Norwalk virus infection. *Nat Med* 2003;9(5):548-553.
73. Marionneau S, Cailleau-Thomas A, Rocher J, et al. ABH and Lewis histo-blood group antigens, a model for the meaning of oligosaccharide diversity in the face of a changing world. *Biochimie* 2001;83(7):565-573.
74. Hutson AM, Atmar RL, Graham DY, et al. Norwalk virus infection and disease is associated with ABO histo-blood group type. *J Infect Dis* 2002;185(9):1335-1337.
75. Hennessy EP, Green AD, Connor MP, et al. Norwalk virus infection and disease is associated with ABO histo-blood group type. *J Infect Dis* 2003;188(1):176-177.
76. Tan M, Jin M, Xie H, et al. Outbreak studies of a GII-3 and a GII-4 norovirus revealed an association between HBGA phenotypes and viral infection. *J Med Virol* 2008;80(7):1296-1301.
77. Rockx BH, Vennema H, Hoebe CJ, et al. Association of histo-blood group antigens and susceptibility to norovirus infections. *J Infect Dis* 2005;191(5):749-754.
78. Harrington PR, Lindesmith L, Yount B, et al. Binding of Norwalk virus-like particles to ABH histo-blood group antigens is blocked by antisera from infected human volunteers or experimentally vaccinated mice. *J Virol* 2002;76(23):12335-12343.

79. Kirby AE, Kienast Y, Aldeco M, et al. Snow Mountain Virus recovery by synthetic human histo-blood group antigens is heavily influenced by matrix effects. *Sci Rep* 2020;10(1):4661.
80. Harrington PR, Vinje J, Moe CL, et al. Norovirus capture with histo-blood group antigens reveals novel virus-ligand interactions. *J Virol* 2004;78(6):3035-3045.
81. Tian P, Engelbrektson A, Mandrell R. Two-log increase in sensitivity for detection of norovirus in complex samples by concentration with porcine gastric mucin conjugated to magnetic beads. *Appl Environ Microbiol* 2008;74(14):4271-4276.
82. Tian P, Brandl M, Mandrell R. Porcine gastric mucin binds to recombinant norovirus particles and competitively inhibits their binding to histo-blood group antigens and Caco-2 cells. *Lett Appl Microbiol* 2005;41(4):315-320.
83. Shanker S, Czako R, Sapparapu G, et al. Structural basis for norovirus neutralization by an HBGA blocking human IgA antibody. *Proc Natl Acad Sci U S A* 2016;113(40):E5830-E5837.
84. Dhawane AN, Diez-Valcarce M, Gurale BP, et al. Synthesis and Evaluation of Biotinylated Bivalent HistoBlood Group Antigens for Capturing Human Noroviruses. *Bioconjug Chem* 2016;27(8):1822-1829.
85. Cao S, Lou Z, Tan M, et al. Structural basis for the recognition of blood group trisaccharides by norovirus. *J Virol* 2007;81(11):5949-5957.
86. Atmar RL, Estes MK. Norovirus vaccine development: next steps. *Expert Rev Vaccines* 2012;11(9):1023-1025.
87. Shirato H. Norovirus recognition sites on histo-blood group antigens. *Front Microbiol* 2012;3:177.
88. LoBue AD, Lindesmith L, Yount B, et al. Multivalent norovirus vaccines induce strong mucosal and systemic blocking antibodies against multiple strains. *Vaccine* 2006;24(24):5220-5234.
89. Shirley DA, McArthur MA. The utility of human challenge studies in vaccine development: lessons learned from cholera. *Vaccine (Auckl)* 2011;2011(1):3-13.
90. Leon JS, Souza M, Wang Q, et al. Immunology of Norovirus Infection. *Immunity Against Mucosal Pathogens* 2008:219-262.
91. Atmar RL, Opekun AR, Gilger MA, et al. Norwalk virus shedding after experimental human infection. *Emerg Infect Dis* 2008;14(10):1553-1557.
92. Atmar RL, Opekun AR, Gilger MA, et al. Determination of the 50% human infectious dose for Norwalk virus. *J Infect Dis* 2014;209(7):1016-1022.
93. Lindesmith L, Moe C, Lependu J, et al. Cellular and humoral immunity following Snow Mountain virus challenge. *J Virol* 2005;79(5):2900-2909.
94. Atmar RL, Bernstein DI, Lyon GM, et al. Serological Correlates of Protection against a GII.4 Norovirus. *Clin Vaccine Immunol* 2015;22(8):923-929.

95. Lindesmith LC, Beltramello M, Swanstrom J, et al. Serum Immunoglobulin A Cross-Strain Blockade of Human Noroviruses. *Open Forum Infect Dis* 2015;2(3):ofv084.
96. Ramani S, Neill FH, Opekun AR, et al. Mucosal and Cellular Immune Responses to Norwalk Virus. *J Infect Dis* 2015;212(3):397-405.
97. Teunis PFM, Le Guyader FS, Liu P, et al. Noroviruses are highly infectious but there is strong variation in host susceptibility and virus pathogenicity. *Epidemics* 2020;32:100401.
98. Mattison CP, Cardemil CV, Hall AJ. Progress on norovirus vaccine research: public health considerations and future directions. *Expert Rev Vaccines* 2018;17(9):773-784.
99. Hallowell BD, Parashar UD, Hall AJ. Epidemiologic challenges in norovirus vaccine development. *Hum Vaccin Immunother* 2019;15(6):1279-1283.
100. Pringle K, Lopman B, Vega E, et al. Noroviruses: epidemiology, immunity and prospects for prevention. *Future Microbiol* 2015;10(1):53-67.
101. Cates JE, Vinje J, Parashar U, et al. Recent advances in human norovirus research and implications for candidate vaccines. *Expert Rev Vaccines* 2020;19(6):539-548.
102. Atmar RL, Bernstein DI, Harro CD, et al. Norovirus vaccine against experimental human Norwalk Virus illness. *N Engl J Med* 2011;365(23):2178-2187.
103. Bernstein DI, Atmar RL, Lyon GM, et al. Norovirus vaccine against experimental human GII.4 virus illness: a challenge study in healthy adults. *J Infect Dis* 2015;211(6):870-878.
104. Sherwood J, Mendelman PM, Lloyd E, et al. Efficacy of an intramuscular bivalent norovirus GI.1/GII.4 virus-like particle vaccine candidate in healthy US adults. *Vaccine* 2020;38(41):6442-6449.
105. Lindesmith LC, McDaniel JR, Changela A, et al. Sera Antibody Repertoire Analyses Reveal Mechanisms of Broad and Pandemic Strain Neutralizing Responses after Human Norovirus Vaccination. *Immunity* 2019;50(6):1530-1541 e1538.
106. Kapikian AZ, Wyatt RG, Dolin R, et al. Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis. *J Virol* 1972;10(5):1075-1081.
107. Adler JL, Zickl R. Winter vomiting disease. *J Infect Dis* 1969;119(6):668-673.
108. Shioda K, Kambhampati A, Hall AJ, et al. Global age distribution of pediatric norovirus cases. *Vaccine* 2015;33(33):4065-4068.
109. Dolin R, Reichman RC, Roessner KD, et al. Detection by immune electron microscopy of the Snow Mountain agent of acute viral gastroenteritis. *J Infect Dis* 1982;146(2):184-189.
110. Steinhoff MC, Douglas RG, Jr., Greenberg HB, et al. Bismuth subsalicylate therapy of viral gastroenteritis. *Gastroenterology* 1980;78(6):1495-1499.
111. Chachu KA, Strong DW, LoBue AD, et al. Antibody is critical for the clearance of murine

norovirus infection. *J Virol* 2008;82(13):6610-6617.

112. Mulligan MJ, Rouphael N, Elsafy M. Phase I Study to Determine Optimal Human Dose for GII.2 Norovirus (Snow Mountain) Challenge Studies Final Integrated Clinical/Statistical Report. 2020:1-1492.
113. Leon JS, Kingsley DH, Montes JS, et al. Randomized, double-blinded clinical trial for human norovirus inactivation in oysters by high hydrostatic pressure processing. *Appl Environ Microbiol* 2011;77(15):5476-5482.
114. Williams AM, Ladva CN, Leon JS, et al. Changes in micronutrient and inflammation serum biomarker concentrations after a norovirus human challenge. *Am J Clin Nutr* 2019;110(6):1456-1464.
115. Hutson AM, Atmar RL, Estes MK. Norovirus disease: changing epidemiology and host susceptibility factors. *Trends Microbiol* 2004;12(6):279-287.
116. Ruvoen-Clouet N, Belliot G, Le Pendu J. Noroviruses and histo-blood groups: the impact of common host genetic polymorphisms on virus transmission and evolution. *Rev Med Virol* 2013;23(6):355-366.
117. Tan M, Jiang X. Norovirus gastroenteritis, carbohydrate receptors, and animal models. *PLoS Pathog* 2010;6(8):e1000983.
118. Herbst-Kralovetz MM, Radtke AL, Lay MK, et al. Lack of norovirus replication and histo-blood group antigen expression in 3-dimensional intestinal epithelial cells. *Emerg Infect Dis* 2013;19(3):431-438.
119. Lay MK, Atmar RL, Guix S, et al. Norwalk virus does not replicate in human macrophages or dendritic cells derived from the peripheral blood of susceptible humans. *Virology* 2010;406(1):1-11.
120. Huang P, Farkas T, Zhong W, et al. Norovirus and histo-blood group antigens: demonstration of a wide spectrum of strain specificities and classification of two major binding groups among multiple binding patterns. *J Virol* 2005;79(11):6714-6722.
121. Shirato H, Ogawa S, Ito H, et al. Noroviruses distinguish between type 1 and type 2 histo-blood group antigens for binding. *J Virol* 2008;82(21):10756-10767.
122. Reeck A, Kavanagh O, Estes MK, et al. Serological correlate of protection against norovirus-induced gastroenteritis. *J Infect Dis* 2010;202(8):1212-1218.
123. Newman KL, Moe CL, Kirby AE, et al. Human norovirus infection and the acute serum cytokine response. *Clin Exp Immunol* 2015;182(2):195-203.
124. Fankhauser RL, Monroe SS, Noel JS, et al. Epidemiologic and molecular trends of "Norwalk-like viruses" associated with outbreaks of gastroenteritis in the United States. *J Infect Dis* 2002;186(1):1-7.
125. Dolin R, Blacklow NR, DuPont H, et al. Transmission of acute infectious nonbacterial gastroenteritis to volunteers by oral administration of stool filtrates. *J Infect Dis* 1971;123(3):307-312.

126. Dolin R, Blacklow NR, DuPont H, et al. Biological properties of Norwalk agent of acute infectious nonbacterial gastroenteritis. *Proc Soc Exp Biol Med* 1972;140(2):578-583.
127. Agus SG, Dolin R, Wyatt RG, et al. Acute infectious nonbacterial gastroenteritis: intestinal histopathology. Histologic and enzymatic alterations during illness produced by the Norwalk agent in man. *Ann Intern Med* 1973;79(1):18-25.
128. Schreiber DS, Blacklow NR, Trier JS. The mucosal lesion of the proximal small intestine in acute infectious nonbacterial gastroenteritis. *N Engl J Med* 1973;288(25):1318-1323.
129. Wyatt RG, Dolin R, Blacklow NR, et al. Comparison of three agents of acute infectious nonbacterial gastroenteritis by cross-challenge in volunteers. *J Infect Dis* 1974;129(6):709-714.
130. Widerlite L, Trier JS, Blacklow NR, et al. Structure of the gastric mucosa in acute infectious bacterial gastroenteritis. *Gastroenterology* 1975;68(3):425-430.
131. Levy AG, Widerlite L, Schwartz CJ, et al. Jejunal adenylate cyclase activity in human subjects during viral gastroenteritis. *Gastroenterology* 1976;70(3):321-325.
132. Parrino TA, Schreiber DS, Trier JS, et al. Clinical immunity in acute gastroenteritis caused by Norwalk agent. *N Engl J Med* 1977;297(2):86-89.
133. Meeroff JC, Schreiber DS, Trier JS, et al. Abnormal gastric motor function in viral gastroenteritis. *Ann Intern Med* 1980;92(3):370-373.
134. Keswick BH, Satterwhite TK, Johnson PC, et al. Inactivation of Norwalk virus in drinking water by chlorine. *Appl Environ Microbiol* 1985;50(2):261-264.
135. Johnson PC, Mathewson JJ, DuPont HL, et al. Multiple-challenge study of host susceptibility to Norwalk gastroenteritis in US adults. *J Infect Dis* 1990;161(1):18-21.
136. Seitz SR, Leon JS, Schwab KJ, et al. Norovirus infectivity in humans and persistence in water. *Appl Environ Microbiol* 2011;77(19):6884-6888.
137. Mateo R, Lindesmith LC, Garg SJ, et al. Production and Clinical Evaluation of Norwalk GI.1 Virus Lot 001-09NV in Norovirus Vaccine Development. *J Infect Dis* 2020;221(6):919-926.
138. Dolin R, Levy AG, Wyatt RG, et al. Viral gastroenteritis induced by the Hawaii agent. Jejunal histopathology and serologic response. *Am J Med* 1975;59(6):761-768.
139. Treanor JJ, Madore HP, Dolin R. Development of an enzyme immunoassay for the Hawaii agent of viral gastroenteritis. *J Virol Methods* 1988;22(2-3):207-214.
140. 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(9):1386-1393.
141. El-Kamary SS, Pasetti MF, Mendelman PM, et al. Adjuvanted intranasal Norwalk virus-like particle vaccine elicits antibodies and antibody-secreting cells that express homing receptors for mucosal and peripheral lymphoid tissues. *J Infect Dis* 2010;202(11):1649-1658.
142. Kim L, Liebowitz D, Lin K, et al. Safety and immunogenicity of an oral tablet norovirus

vaccine, a phase I randomized, placebo-controlled trial. *JCI Insight* 2018;3(13).

143. Treanor JJ, Atmar RL, Frey SE, et al. A novel intramuscular bivalent norovirus virus-like particle vaccine candidate--reactogenicity, safety, and immunogenicity in a phase 1 trial in healthy adults. *J Infect Dis* 2014;210(11):1763-1771.
144. Atmar RL, Baehner F, Cramer JP, et al. Rapid Responses to 2 Virus-Like Particle Norovirus Vaccine Candidate Formulations in Healthy Adults: A Randomized Controlled Trial. *J Infect Dis* 2016;214(6):845-853.
145. Leroux-Roels G, Cramer JP, Mendelman PM, et al. Safety and Immunogenicity of Different Formulations of Norovirus Vaccine Candidate in Healthy Adults: A Randomized, Controlled, Double-Blind Clinical Trial. *J Infect Dis* 2018;217(4):597-607.
146. Chang KO, George DW, Patton JB, et al. Leader of the capsid protein in feline calicivirus promotes replication of Norwalk virus in cell culture. *J Virol* 2008;82(19):9306-9317.
147. Ghosh S, Malik YS, Kobayashi N. Animal Caliciviruses. In: Malik YS, Singh RK, Dhama K, eds. *Animal-Origin Viral Zoonoses*. Singapore: Springer, 2020:81-109.
148. Niendorf S, Jacobsen S, Faber M, et al. Steep rise in norovirus cases and emergence of a new recombinant strain GII.P16-GII.2, Germany, winter 2016. *Euro Surveill* 2017;22(4).
149. Parra GI, Squires RB, Karangwa CK, et al. Static and Evolving Norovirus Genotypes: Implications for Epidemiology and Immunity. *PLoS Pathog* 2017;13(1):e1006136.
150. Sakon N, Yamazaki K, Nakata K, et al. Impact of genotype-specific herd immunity on the circulatory dynamism of norovirus: a 10-year longitudinal study of viral acute gastroenteritis. *J Infect Dis* 2015;211(6):879-888.
151. Borrego B, Camarero JA, Mateu MG, et al. A highly divergent antigenic site of foot-and-mouth disease virus retains its immunodominance. *Viral Immunol* 1995;8(1):11-18.
152. Lindesmith LC, Donaldson EF, Lobue AD, et al. Mechanisms of GII.4 norovirus persistence in human populations. *PLoS Med* 2008;5(2):e31.
153. Lochridge VP, Hardy ME. A single-amino-acid substitution in the P2 domain of VP1 of murine norovirus is sufficient for escape from antibody neutralization. *J Virol* 2007;81(22):12316-12322.

G. TABLES

Table 1.1. Distribution of norovirus associated symptoms among cases in Catalonia study

	Age group					
	All cases[a]		1-4 years		≥ 65 years	
Symptoms	Cases	%	Cases	%	Cases	%
Diarrhea	1150	78.4	44	77.2	437	87.9
Vomiting	940	65.1	44	74.6	256	52.9
Abdominal pain	922	67.2	54	91.5	199	43.5
Nausea	712	51.9	17	30.4	157	34.1
Fever	448	31.7	19	32.2	86	17.8
Headache	417	31.3	11	20.4	55	12.0
Myalgia	303	24.1	10	18.5	60	14.2
Chills	181	15.7	3	7.7	30	7.1
General malaise	117	7.8	10	16.7	13	2.6

[a] age was unknown in 40/1544 cases (26)

Table 1.2. Norovirus associated symptoms presented by two different age groups in Catalonia study

	Children (< 5 years)		Elderly (≥ 65 years)	
Symptoms	5 - 100 years (%)	< 5 years (%)	≥ 65 years (%)	1 - 64 years (%)
Diarrhea	78.5	77.2	87.9	73.6
Vomiting	64.6	74.6	52.9	71.2
Abdominal pain	66.1	91.5	43.5	79.0
Nausea	52.8	30.4	34.1	60.9
Fever	31.7	32.2	17.8	38.9
Headache	31.7	20.4	12.0	41.4
Myalgia	24.3	18.5	14.2	29.1
Chills	15.9	7.7	7.1	20.5
General malaise	7.4	16.7	2.6	10.3

Study results from Catalonia study (26)

Table 1.3. Norovirus human challenge studies conducted over the last 50 years

Genotype	Inoculum	Exposed	Infected	Ill	Reference
GI.1	NA	9	NA	7	Dolin et al. (1971)(125)
	8fIIa	19	NA	7	Dolin et al. (1972)(126)
	8fIIa[a]	7	NA	4	Agus et al. (1973)(127)
	8fIIa	15	NA	12	Schreiber et al. (1973)(128)
	8fIIa	52	NA	30	Wyatt et al. (1974)(129)
	8fIIa	15	NA	9	Widerlite et al. (1975)(130)
	8fIIa[a]	16	NA	11	Levy et al. (1976)(131)
	8fIIa	12	NA	6	Parrino et al. (1977)(132)
	8fIIa[a]	7	NA	4	Meeroff et al. (1980)(133)
	8fIIa	59	40	32	Steinhoff et al. (1980)(110)
	8fIIa	16	14	11	Keswick et al. (1985)(134)
	8fIIa	42	29	25	Johnson et al. (1990)(135)
	8fIIa	50	41	28	Graham et al. (1994)(63)
	8fIIa & 8fIIb	77	40	23	Lindesmith et al. (2003)(72)
	8fIIb	13	10	6	Seitz et al. (2011)(136)
	8fIIb	15	7		Leon et al. (2011)(113)
	42399	49	21	20	Atmar et al. (2014)(92)
	001-09NV	16	11	10	Mateo et al. (2020)(137)
GII.1	Hawaii	23	NA	11	Wyatt et al. (1974)(129)
	Hawaii	7	NA	4	Dolin et al. (1975)(138)
	Hawaii	3	NA	1	Meeroff et al. (1980)(133)
	Hawaii	10	5	8	Treanor et al. (1988)(139)
GII.2	SMV	12	3	9	Dolin et al. (1982)(109)
	SMV	15	9	7	Lindesmith et al. (2005)(93)
NA	Montgomery	18	NA	5	Wyatt et al. (1974)(129)
GII.4	Farmington Hill	40	17	12	Frenck et al. (2012)(140)
	Farmington Hill	48	30	16	Bernstein et al. (2015)(103)

[a] studies with uncertainty about the aggregation state of the 8fIIa inoculum while it was aging

Table 1.4. Norovirus vaccine studies reported during the 21st Century

Vaccine type	Route	Vaccinated	Placebo	VE[a] (%)	IgG (%)	IgA (%)	HBGA blocking (%)	Reference
GI.1 adjuvanted monovalent	Intran[c]	40	21	NA	59.5	75.7	NA	El-Kamary et al. (2010)(141)
GI.1 monovalent	Intran	43	41	74	49	70	32	Atmar et al. (2011)(102)
GI.1 monovalent	Orally	46	20	NA	70	82.5	69.5	Kim et al. (2018)(142)
GI.1/GII.4 adjuvanted bivalent[b]	Intram[d]	76	36	NA	NA	NA	75	Treanor et al. (2014)(143)
					NA	NA	78	
GI.1/GII.4 bivalent[b]	Intram	67	65	86.4		100		Bernstein et al. (2015)(103)
						89.8		
GI.1/GII.4 adjuvanted bivalent[b]	Intram	301	153	NA	NA	94.3	79.6	Atmar et al. (2016)(144)
					NA	81.2	73.8	
GI.1/GII.4c bivalent[b]	Intram	420		NA		90.6		Leroux-Roels et al. (2017)(145)
						56.5		
GI.1/GII.4 adjuvanted bivalent[b]	Intram	2355	2357	61.8			91.3	Sherwood et al. (2020)(104)
							84.8	

[a] VE vaccine efficacy; [b] GI.1 seroresponse rate on top row and GII.4 seroresponse rate on bottom row

[c] intra denotes intranasally; [d] intram denotes intramuscularly

Table 2.1. Characteristics of SMV-challenged subjects, stratified by infection status

	Total (n=34)	Infected (n=25)	Uninfected (n=9)
Age (mean, SD)	33.53 (9.51)	33.04 (9.41)	34.89 (10.24)
Female	14 (41.2%)	11 (44.0%)	3 (33.3%)
Race			
White	10 (29.4%)	8 (32.0%)	2 (22.2%)
Black	22 (64.7%)	15 (60.0%)	7 (77.8%)
Multiple	2 (5.9%)	2 (8.0%)	0
Hispanic	2 (5.9%)	0	2 (22.2%)
AGE symptoms			
Vomit	12 (35.3%)	11 (44.0%)	1 (11.1%)
Diarrhea	8 (23.5%)	8 (32.0%)	0
Number of symptoms			
Inpatient days (mean, SD)	4.71 (4.40)	5.92 (4.41)	1.33 (2.06)
Outpatient days (mean, SD)	0.81 (1.18)	1.04 (1.24)	0
Secretor Status			
Positive	26 (76.5%)	18 (72.0%)	8 (88.9%)
Negative	8 (23.5%)	7 (28.0%)	1 (11.1%)

Infection defined as SMV excretion in stool detected through RT-qPCR at any time after challenge through Day 30 or showing at least a 4-fold rise from pre-challenge SMV-specific serum IgG at any time after challenge through Day 30

Table 2.2. HBGA-blocking antibody response to SMV antigen pre- and post-challenge

	Infection Status[a]		
	Infected (n=25)	Uninfected (n=9)	p value[b]
Day 1[c]			
GMT[d] (95% CI)	20.59 (15.96, 26.57)	25.00 (17.15, 36.44)	0.257
GMFR[e] (95% CI)	0	0	
N	25	9	
Day 6			
GMT (95% CI)	31.82 (21.11, 47.95)	50.00 (21.86, 114.39)	0.201
GMFR (95% CI)	1.57 (1.22, 2.02)	1.64 (0.89, 3.02)	0.935
N	23	7	
Day 15			
GMT (95% CI)	295.92 (141.36, 619.48)	67.30 (22.37, 202.47)	0.031
GMFR (95% CI)	14.62 (6.96, 30.68)	2.21 (0.86, 5.64)	0.018
N	23	7	
Day 30			
GMT (95% CI)	246.97 (121.62, 501.52)	50.00 (13.61, 183.69)	0.034
GMFR (95% CI)	12.20 (5.96, 24.96)	1.59 (0.48, 5.21)	0.011
N	23	6	
Day 45			
GMT (95% CI)	170.85 (93.83, 311.10)	50.00 (17.87, 139.87)	0.037
GMFR (95% CI)	8.52 (4.65, 15.63)	1.59 (0.66, 3.83)	0.010
N	22	6	

[a] Infection defined as SMV excretion in stool detected through RT-qPCR at any time after challenge through Day 30 or showing at least a 4-fold rise from pre-challenge SMV-specific serum IgG at any time after challenge through Day 30
[b] p value obtained from Kruskal-Wallis test
[c] Day 1 denotes pre-challenge
[d] GMT denotes geometric mean titer
[e] GMFR denotes geometric mean fold rise

Table 2.3. SMV-specific serum IgG response to SMV antigen pre- and post-challenge

	Infection Status[a]		
	Infected (n=25)	Uninfected (n=9)	p value[b]
Day 1[c]			
GMT[d] (95% CI)	149.69 (105.07, 213.28)	115.04 (50.94, 259.82)	0.301
GMFR[e] (95% CI)	0	0	
N	25	8	
Day 6			
GMT (95% CI)	190.17 (129.28, 279.73)	175.04 (77.49, 395.43)	0.364
GMFR (95% CI)	1.28 (1.07, 1.53)	1.15 (0.84, 1.58)	0.333
N	23	6	
Day 15			
GMT (95% CI)	812.32 (454.63, 1451.43)	154.43 (69.54, 342.96)	0.001
GMFR (95% CI)	5.47 (3.03, 9.88)	1.01 (0.82, 1.25)	0.008
N	23	6	
Day 30			
GMT (95% CI)	895.48 (537.68, 1491.39)	135.20 (56.59, 323.01)	0.001
GMFR (95% CI)	6.03 (3.38, 10.75)	0.95 (0.82, 1.09)	0.006
N	23	5	
Day 45			
GMT (95% CI)	912.26 (554.28, 1501.43)	133.72 (62.24, 287.31)	0.001
GMFR (95% CI)	6.09 (3.45, 10.74)	0.94 (0.62, 1.41)	0.004
N	22	5	

[a] Infection defined as SMV excretion in stool detected through RT-qPCR at any time after challenge through Day 30 or showing at least a 4-fold rise from pre-challenge SMV-specific serum IgG at any time after challenge through Day 30

[b] p value obtained from Kruskal-Wallis test

[c] Day 1 denotes pre-challenge

[d] GMT denotes geometric mean titer

[e] GMFR denotes geometric mean fold rise

Table 2.4. SMV-specific serum IgA response to SMV antigen pre- and post-challenge

	Infection Status[a]		
	Infected (n=25)	Uninfected (n=9)	p value[b]
Day 1[c]			
GMT[d] (95% CI)	8.13 (4.92, 13.44)	13.17 (2.44, 71.07)	0.234
GMFR[e] (95% CI)	0	0	
N	25	9	
Day 6			
GMT (95% CI)	12.39 (6.10, 25.17)	32.12 (13.84, 74.54)	0.264
GMFR (95% CI)	1.52 (0.80, 2.89)	1.10 (0.80, 1.50)	0.116
N	25	7	
Day 15			
GMT (95% CI)	54.23 (25.21, 116.69)	24.71 (10.52, 58.01)	0.072
GMFR (95% CI)	6.67 (2.70, 16.47)	0.85 (0.61, 1.18)	0.013
N	25	7	
Day 30			
GMT (95% CI)	39.89 (18.43, 86.34)	23.94 (8.35, 68.69)	0.110
GMFR (95% CI)	4.90 (2.02, 11.92)	0.86 (0.61, 1.22)	0.009
N	25	6	
Day 45			
GMT (95% CI)	50.42 (34.44, 73.82)	21.42 (6.90, 66.48)	0.033
GMFR (95% CI)	5.52 (3.32, 9.18)	0.77 (0.44, 1.34)	0.001
N	22	6	

[a] Infection defined as SMV excretion in stool detected through RT-qPCR at any time after challenge through Day 30 or showing at least a 4-fold rise from pre-challenge SMV-specific serum IgG at any time after challenge through Day 30

[b] p value obtained from Kruskal-Wallis test

[c] Day 1 denotes pre-challenge

[d] GMT denotes geometric mean titer

[e] GMFR denotes geometric mean fold rise

Table 2.5. Correlation between natural log-transformed immunoglobulins and HBGA-blocking antibody titers

	SMV-specific serum IgG		SMV-specific serum IgA	
	Infected (n=25)	Uninfected (n=9)	Infected (n=25)	Uninfected (n=9)
Day 1				
R^2	0.19	0.39	0.3	0.24
p value	0.028	0.097	0.005	0.178
N	25	8	25	9
Day 6				
R^2	0.48	0.02	0.4	0.17
p value	0.0002	0.808	0.001	0.358
N	23	6	23	7
Day 15				
R^2	0.75	0.01	0.77	0.03
p value	< 0.0001	0.873	<0.0001	0.716
N	23	6	23	7
Day 30				
R^2	0.71	0.10	0.68	0.01
p value	< 0.0001	0.61	<0.0001	0.892
N	23	5	23	6
Day 45				
R^2	0.57	0.04	0.61	0.02
p value	< 0.0001	0.749	<0.0001	0.766
N	22	5	22	6

Table 2.6. Association between covariates and natural log-transformed HBGA-blocking antibody titer among infected subjects

	Model 1[a]			Model 2[b]		
Variables	β estimates[d]	95% CI[c]	p-value	β estimates	95% CI	p-value
Visit Days (vs Day 1)[e]				0.02	0.005, 0.03	0.006
Day 6	-0.004	-0.48, 0.47	0.986	-	-	-
Day 15	1.44	0.78, 2.10	<0.001	-	-	-
Day 30	1.34	0.74, 1.94	<0.001	-	-	-
Day 45	1.05	0.48, 1.63	0.001	-	-	-
Inoculum dose	0.001	-0.0002, 0.001	0.113	0.001	-0.0001, 0.001	0.128
Age	0.008	-0.03, 0.04	0.643	0.001	-0.03, 0.03	0.953
Race (vs White)						
Black	-0.88	-1.47, -0.29	0.006	-0.80	-1.34, -0.26	0.006
Multiple	-0.35	-1.57, 0.88	0.565	-0.28	-1.44, 0.88	0.617
ln transformed IgA	0.6	0.41, 0.79	<0.001	0.88	0.73, 1.04	<0.001

Outcome is natural log transformed HBGA-blocking antibody titer
Infection defined as SMV excretion in stool detected through RT-qPCR at any time after challenge through Day 30 or showing at least a 4-fold rise from pre-challenge
[a] Model 1:linear mixed model treating post-challenge days as ordinal variable
[b] Model 2:linear mixed model treating post-challenge days as continuous variable
[c] CI is abbreviation for confidence interval
[d] β denotes coefficient
[e] reference of Day 1 only applied to Model 1

H. FIGURES

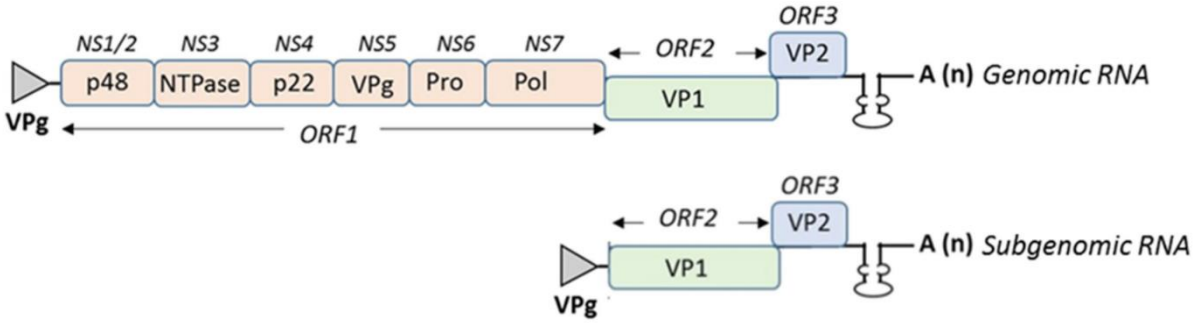

Figure 1.1. Genome map of human norovirus (genomic RNA and subgenomic RNA). The 5' end is predicted to be capped with the VPg protein (encoded by NS5). The 3' end has a short 3' untranslated region (3'-UTR) and a poly-A tail. The 66 bases of 3'-UTR and the VP2 are important for viral replication for human norovirus (146, 147).

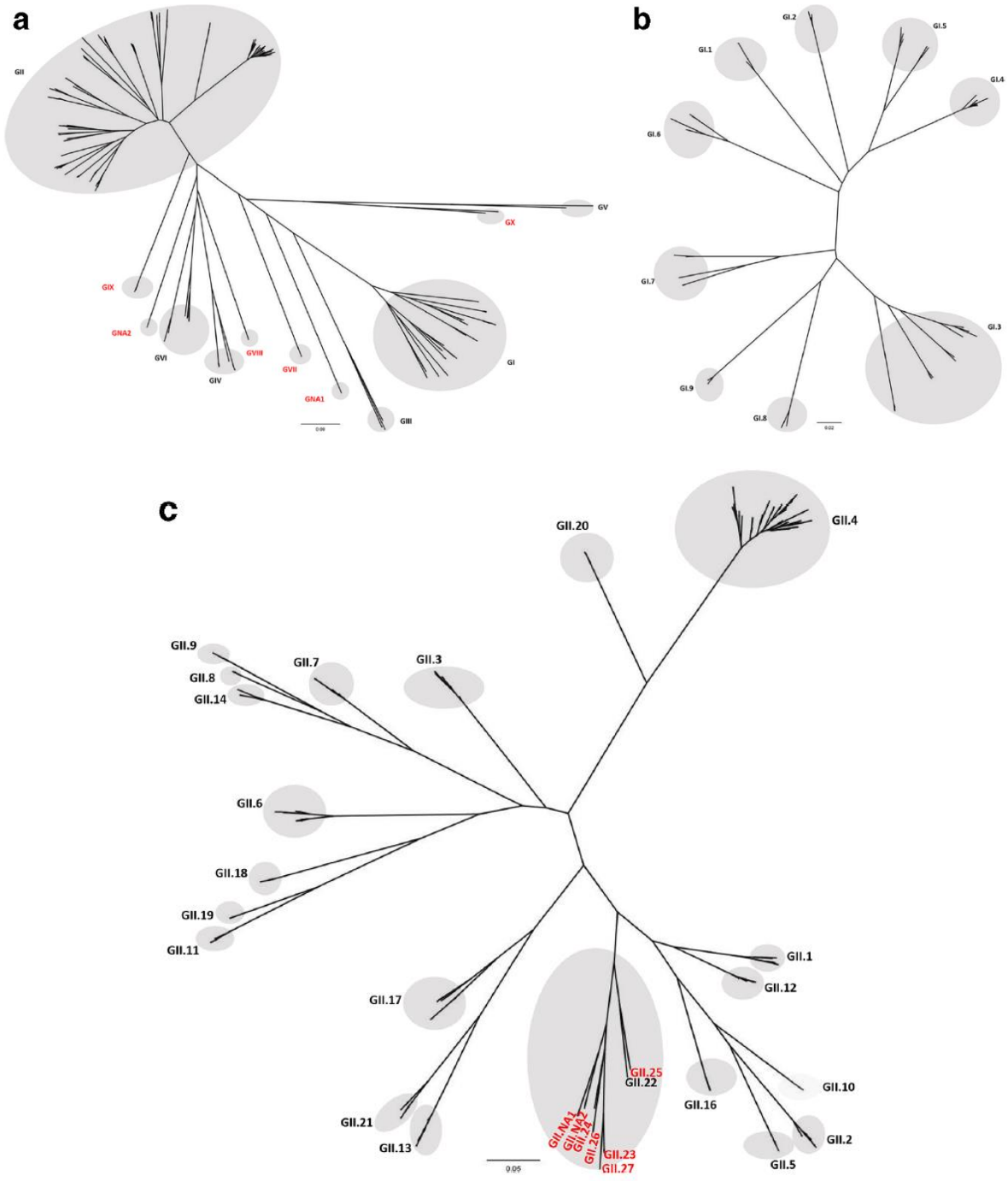

Figure 1.2. Phylogenetic trees of noroviruses based on VP1 amino acid sequences with 10 genogroups (a) two non-assigned (NA) genogroups, (b) GI genotypes, and (c) GII genotypes. Newly identified genogroups and genotypes are labelled in red (11).

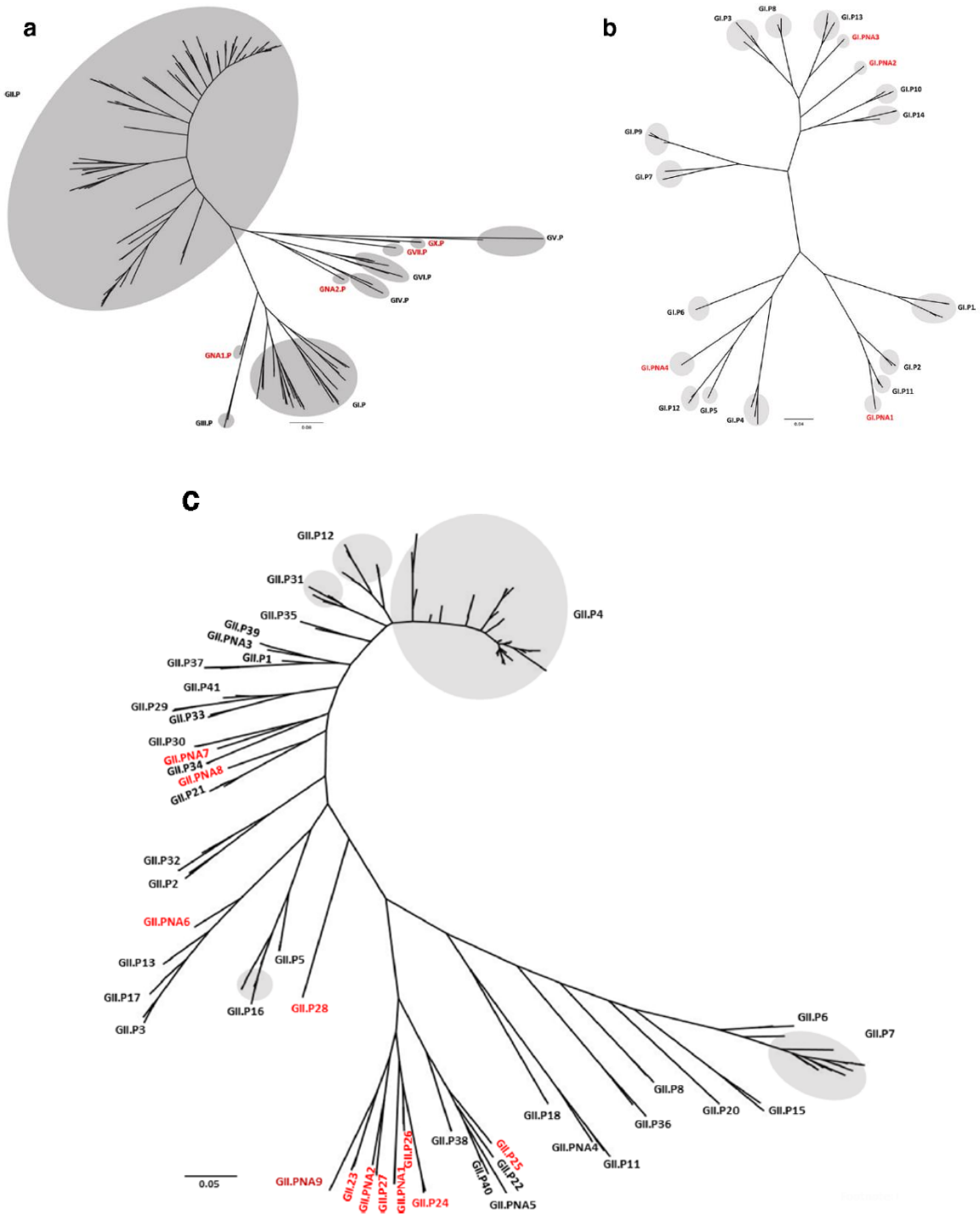

Figure 1.3. Phylogenetic trees of partial RdRp sequences of (a) norovirus genogroups, (b) GI P-types, and (c) GII P-types. Tentative P-groups and P-types with only a single sequence or multiple non-identified sequences from one geographic location are referred to as non-assigned (NA). Newly identified genogroups and genotypes are labelled in red. Highly divergent clusters are shown with a gray circle for Figure 2c (11).

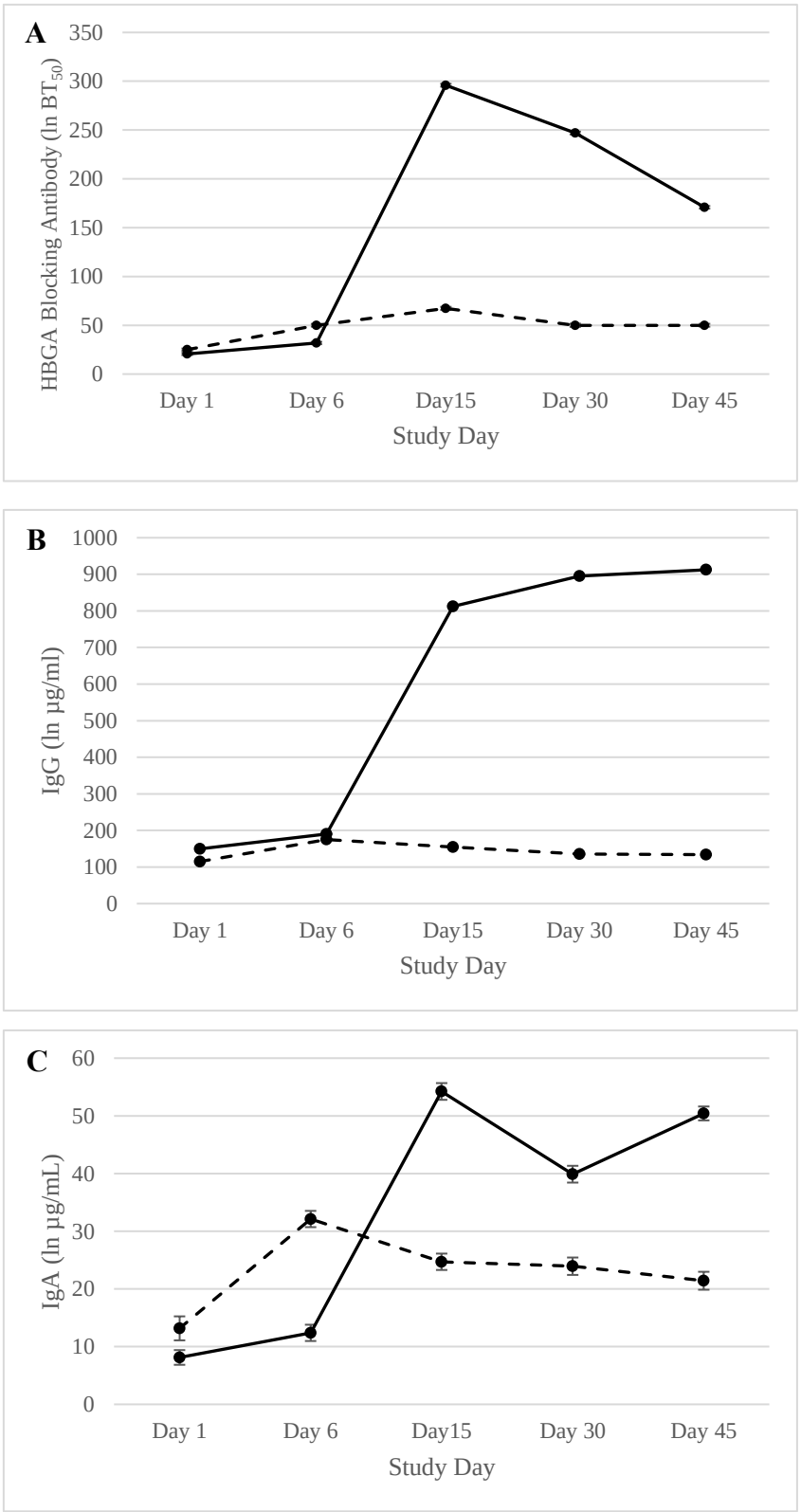

Figure 2.1. GMT of (A)HBGA-blocking antibody, (B) SMV-specific serum IgG, and (C) IgA levels. GMT results are from pre-challenge to post-challenge. Black solid line are infected individuals while the dashed line represents uninfected individuals. Error bars indicate one standard error.

CHAPTER III: SUMMARY, GLOBAL HEALTH IMPLICATIONS, FUTURE DIRECTIONS

A. SUMMARY

Noroviruses remain a major public health concern affecting millions of people around the world each year. Over the last several decades, the scientific community has gained better understanding of noroviruses through epidemiological studies, outbreak investigations, and human challenge studies. However, there are still much to learn about noroviruses and their impact on the human population. For example, there is still limited knowledge on SMV and the immune response associated with SMV infection. Compared to the GI.1 genotype, human challenge studies examining SMV are limited and currently, there are no vaccine candidates specifically for SMV. One reason for this may be due to inadequate knowledge of SMV immune response and pathogenesis. In recent years, reports of GII.2 outbreaks have been increasing worldwide. In some parts of the world, GII.2 infections have become more prevalent than other genotypes. This raises the urgency to understand the mechanism of, and response to SMV infections. Furthermore, to develop effective vaccines and prophylaxis against SMV, and more generally for noroviruses, it is critical to have a better understanding of the immune response against SMV infection.

This study found that infected individuals, when compared to uninfected individuals, had significantly higher HBGA-blocking antibody concentrations following SMV challenge. While both infected and uninfected individuals began the study with similar HBGA-blocking antibody concentrations, differences in concentrations became apparent by day 15 after challenge. The study also demonstrated that infected individuals experienced a significant increase in HBGA-blocking antibody concentrations after receiving the SMV challenge dose, with the most increase observed on day 15 post-challenge when compared to the pre-challenge day. As expected, a similar finding

was observed with SMV-specific serum IgG and IgA. Both immunoglobulins were significantly higher among infected individuals than uninfected individuals throughout the course of the observed post-challenge days.

On the other hand, this study was not able to support the hypothesis of pre-challenge HBGA-antibody titers being correlates of protection. Previous studies have demonstrated an association between HBGA-blocking antibody titers prior to inoculation and protection against disease, where those with high HBGA-blocking antibody levels did not develop infection and associated illness. In this study, however, this particular association between pre-challenge HBGA-blocking antibody levels and SMV-related infection nor illness could not be established.

B. GLOBAL HEALTH IMPLICATIONS

Currently, the dominant norovirus genotype circulating worldwide is the GII.4 genotype. While SMV (GII.2.1976) is often not associated with outbreaks, the number of outbreaks associated with GII.2 strains have been increasing over the last couple of years. In recent years, clusters of GII.2 outbreaks have been reported worldwide, even within high-income countries such as Japan and Germany (39, 148). Over the last two decades alone, there have been multiple years in which GII.2 has surfaced as the dominant genotype, causing multiple outbreaks throughout different parts of the world (38, 39, 148).

SMV is a public health concern not because of the frequent mutation seen with the GII.4 genotype, but because of its unpredictability of when an outbreak will occur in communities, the slow transition from an epidemic disease to an endemic disease, and the high frequency of infection observed among children under 5 years of age (149, 150). While the global health concern towards

SMV has been increasing in recent years, there is still lack of attention towards SMV in the medical and research communities. For example, the vaccines currently under development do not include SMV. Instead, the majority of norovirus vaccines being tested in clinical trials include either include GI.1 or GII.4 (101).

The results of this study can be applied in numerous ways for the global population at risk. One approach would be to utilize the results for vaccine development. The study results indicated that when compared to uninfected individuals, infected individuals had statistically significant increases in HBGA-blocking antibody titers across the post-challenge days when compared to the pre-challenge titer. If a vaccine could be formulated with optimal SMV VLPs to induce infection, but minimize illness onset, there would be a high chance for the antibody secreting cells in the vaccinated individual to acquire the mechanism to create HBGA-blocking antibodies needed against future infections. When an individual becomes exposed to SMV or other GII noroviruses (if assuming cross-reactivity) in the future, the adaptive immune response would be properly activated and prevent infection from occurring. Development of a SMV vaccine is critical for the global community especially because of the recent increase in SMV outbreaks and the trend of SMV becoming an endemic disease throughout parts of the world. With a readily available, effective vaccine, SMV outbreaks could be prevented and the number of individuals that are disproportionally affected by SMV would be reduced.

The global health benefits of developing a SMV-specific vaccine are significant. Prior SMV human challenge studies have observed that the HBGA-blocking antibodies, induced by SMV infection, are cross-reactive against other norovirus strains within the same genotype as well as within the

same genogroup (7, 93). More evidence is necessary, but if the HBGA-blocking antibodies can respond to other strains within the same genotype and genogroup, vaccines developed for SMV could provide protection against a diverse group of noroviruses circulating worldwide. Another benefit of developing a SMV-specific vaccine is the idea that the vaccine would not have to be replaced frequently due to the evolving pathogen. A study examining GII.2 strains that have been reported from 1976 until 2010 suggested that limited evolution had occurred within the GII.2 genotype (7). While some amino acid substitutions were observed over the 34 years, the noted residue changes were not significant enough to prevent the antibody from binding to the SMV VLPs. Studies with GII.4 strains and other RNA viruses have observed significant changes in neutralization potential with even only one amino acid change (151-153). Taking into consideration the idea that a vaccine would only have to be developed once and still have high vaccine effectiveness over a long period of time, investing in a SMV-specific vaccine may be worthwhile.

Another usage would be for screening and possibly genotype identification. In this study, at each post-challenge time point other than Day 6, infected individuals had significantly different HBGA-blocking antibody levels than uninfected individuals. This finding suggests that HBGA-blocking antibody levels can be utilized as a screening tool to determine whether an individual is infected with SMV. Utilizing this screening method may prevent the possibility of misclassifying individuals based on clinical symptoms. In this study, only 32% of those infected had AGE-associated symptoms. The observation suggests the importance of using a screening tool that can relatively quickly and easily determine infection status without relying solely on expression of clinical symptoms in settings with limited resources such as access to a PCR machine. HBGA-

blocking antibody levels could also potentially be used for genotype identification. If the HBGA-blocking antibody is elicited during an SMV infection, and is not cross-reactive with VLPs from strains within the same genogroup or across genogroups, the HBGA-blocking antibody measured in patient serum could potentially be used to notify medical personnel that the infection is caused by a GII.2 genotype norovirus. SMV-specific IgG was reported to be cross-reactive with VLPs from strains within the genogroup such as with Hawaii virus (HV) (93). If HBGA-blocking antibodies do not express a similar characteristic, HBGA-blocking antibody detection could provide critical information to medical and public health personnel about the infection.

C. FUTURE DIRECTIONS

Future studies should collect samples from a larger population in order to determine the immune response towards SMV. While in this study, the association between HBGA-blocking antibody titers and protection from illness could not be confirmed, a study with a larger sample size may provide different results. Future studies should also aim to address the issues of transportability. Since including children in human challenge studies would not be possible, it would be important for future studies to diversify the adult subjects included in the study instead. One of the goals of these human challenge studies is to be able to apply the results to different populations around the world. Thus, it would be critical to avoid underrepresentation in the study population. To study the HBGA-blocking antibody responses to SMV among children, other epidemiological studies such as case-control studies should be utilized.

Future studies should also determine the cross-reactivity of SMV specific HBGA-blocking antibody. Currently, SMV-specific serum IgG is cross-reactive with HV, another genogroup 2 norovirus, but not with NV, a genogroup 1 norovirus. It would be important to determine whether

SMV-elicited HBGA-blocking antibody will demonstrate cross-reactivity with VLPs of other genotypes or genogroups. This would be important to determine, since depending on the outcome, SMV-specific HBGA-blocking antibodies could become powerful screening tools to detect SMV infection in low resource settings.

Finally, the duration of HBGA-blocking antibody after challenge should be determined. In this study, HBGA-blocking antibody was measured up until 45 days post-challenge. A NV human challenge study, for example, was able to measure HBGA-blocking antibody levels up to 180 days post-challenge for infected subjects (122). It would be interesting to determine if similar results would be obtained for SMV. The results would also be helpful for vaccine development. In particular, when developing a vaccine against SMV, it would be important to determine if the vaccine could induce HBGA-blocking antibody activity.