Letter to the Editor

Norovirus Infection and Histo-blood Group Antigens in Children Hospitalized with Diarrhea in Lulong and Chenzhou in China[*]

SUN Xiao Man[1,2,^], YAN Xiao Feng[3,^], HE Zhi Gang[4,^], Li Jing Xin[1,2], GUO Ni Jun[1,2],
XU Zi Qian[1,2], JIN Miao[1,2], LI Dan Di[1,2], ZHOU Yong Kang[1,2], XIE Guang Cheng[1,2],
Pang Li Li[1,2], ZHANG Qing[1,2], LIU Na[1,2], and DUAN Zhao Jun[1,2,#]

Norovirus (NoV) is a pathogen that commonly causes viral diarrhea in children. Studies indicate that NoV recognizes human histo-blood group antigens (HBGAs) as cell attachment factors. In order to explore the correlation between of NoV infection and HBGAs, a cross-sectional study was conducted in children less than five years old who were hospitalized with diarrhea in two areas of China between November 2014 and February 2015. Of the paired stool and saliva samples taken from 424 children, NoV was detected in 24 (6%) children, with viral genotypes GII.3 (*n*=5), GII.4 (*n*=14), GII.12 (*n*=1), and GII.17 (*n*=4). All of the individuals having NoV infection were either secretors (Le^{a-b+}/Le^{x-y+}) or partial secretors (Le^{a+b+}/Le^{x+y+}) except one GII.3 infection of a non-secretor (Le^{a+b-}/Le^{x+y-}). These results suggest that secretor positive is associated with NoV infection, although non-secretors are not absolutely protected from NoV infection.

Norovirus (NoV) is one of the most common causes of acute gastroenteritis among young children worldwide[1]. NoV has a single-stranded, plus-sense RNA genome with three open-reading frames (ORFs). ORF2 encodes capsid protein VP1, while ORF3 encodes capsid protein VP2. NoVs can be divided into seven genogroups (GI to GVII). GI and GII NoVs are prevalent in humans and can be divided into 9 and 22 genotypes, respectively[1]. Moreover, GII norovirus are most frequently detected[1]. In China, GII.4 Sydney 2012 caused the majority of norovirus outbreaks during the 2013-to-2014 season, with GII.17 being the dominant strain during 2014-to-2015 season (unpublished data).

It has been shown that NoV can recognize histo-blood group antigens (HBGAs). The ABO and Lewis antigens are important for NoV infections[2]. Fucosyltransferase 2 (FUT2) is responsible for the expression of the H antigen and individuals with functional FUT2 are called secretors or partial secretors, while a homozygous mutation of the *FUT2* gene results in non-secretors. H-type antigen can be catalyzed by the specific A/B glycosyltransferase to synthesize A/B antigen. Fucosyltransferase 3 (FUT3), on the other hand, transfers fucose to the type-1/2 chain precursor or H-type 1/2 antigen generating Lewis a/x (Le$^{a/x}$) or Lewis b/y (Le$^{b/y}$).

Different NoV genotypes are reported to exhibit distinct HBGA binding patterns[2]. Binding studies with virus-like particles (VLPs) and wild-type viruses show that HBGA binding plays a role in host susceptibility to NoVs. Although several epidemiological studies have been performed in Caucasian, African, and Vietnamese populations[3-6], the genetic characteristics and the host interactions with NoVs prevalent recently in China deserve further research.

From November 2014 through February 2015, paired samples of feces, saliva, and buccal cells were randomly selected and collected from children under the age of five years who were hospitalized with acute diarrhea at the First People's Hospitals in Lulong in the Hebei province (surrounding Beijing in Northeast China) and Chenzhou in the Hunan province. In total, 202 and 222 paired samples were collected in Lulong and Chenzhou, respectively. Parents/guardians were asked to sign an informed consent form before collecting the samples. The

study protocol and consent form were approved by the ethical committee of the National Institute for Viral Disease Control and Prevention.

The NoV genotype was determined by RT-PCR (reverse transcription polymerase chain reaction) and sequencing was preformed as described previously[7]. In brief, RNA was extracted from fecal samples using the Qiagen Viral RNA mini Kit. RNA was reverse transcribed and the specific DNA fragments were then amplified and sequenced.

The HBGA phenotypes of A, B, H1, le^a, le^b, le^x, and le^y in saliva samples were detected using enzyme immunoassays (EIAs) according to published procedures[7]. 96-well plates were coated with saliva samples. The antibodies specific for A, B, H1, le^a, le^b, le^x, and le^y (BioLegend) were diluted at 1:300. For data analyses, Fisher's exact test (two-sided) was used to compare frequency variables between different groups. Differences were considered significant when P values were less than 0.05.

Of the 424 samples, 24 children were identified as positive for NoV. The prevalence rates of NoVs differ among different age groups. Children of six to eleven months were more frequently infected with NoV as compared to children under six months of age (8% versus 2%, P=0.196) and only one child more than three years old was infected (Table 1). All 24 NoV-positive samples were genotyped by sequencing. Genotype GII.4 (n=14, 58%) was the most prevalent strain followed by GII.3 (n=5, 21%) and GII.17 (n=4, 17%) (Table 2). In addition, all the GII.4 variants were classified to GII.4 2012 Sydney strain.

The HBGA phenotypes of the 424 children were 153 (36%) type A, 30 (7%) type AB, 104 (25%) type B, and 137 (32%) type O. Meanwhile, 263 (62%) were secretors, 150 (35%) were partial secretors and 11 (3%) were non-secretors (Table 3).

Table 1. Age Distribution of NoV Infection in Children <5 Years of Age Hospitalized with Diarrhea at the First People's Hospitals of Lulong and Chenzhou from November 2014 to February 2015

Age (month)	NoV No. (%)	Total No. of Diarrhea Cases
<6	1 (2)	51
6-11	13 (8)	155
12-23	7 (4)	185
24-35	2 (11)	18
36-60	1 (7)	15
Total	24 (6)	424

Table 2. Distribution of NoV Genotypes Detected at the First People's Hospitals of Lulong and Chenzhou

NoV Genotype	No. of Cases		
	Lulong Area	Chenzhou Area	Total
GII.3	4	1	5
GII.4	12	2	14
GII.17	3	1	4
GII.12	0	1	1

Table 3. Association between NoV Infection and Host HBGA Type among Children Hospitalized with Diarrhea at the First People's Hospitals of Lulong and Chenzhou

NoV Infection	No. of Cases							
	Total	A	AB	B	O	Secretor[a]	Partial Secretor[a]	Non-secretor[a]
NoV-positive								
GII.4	14	3	1	6	4	7	7	0
GII.3	5	2	1	1	1	3	1	1[b]
GII.17	4	2	0	0	2	2	2	0
GII.12	1	1	0	0	0	0	1	0
NoV-negative	400	145	28	97	130	251	139	10
Total[c]	424	153	30	104	137	263	150	11

Note. [a]Secretor, Le^{b+}, Le^{y+}, or H^+; Partial Secretor, Le^{a+b+} or Le^{x+y+}; Non-secretor, Le^{a+b-} or Le^{x+y-}. [b]P=0.129, compared with the NoV-negative group, Fisher's exact test. [c]Children with typed, paired stool-saliva samples were included.

To determine the correlation between HBGA and NoV infection, all of the 14 typed GII.4 cases were secretors/partial secretors (Table 3). Moreover, results indicate that for the ABO phenotype, GII.4 NoVs were more likely to infect blood B type than the non-GII.4 strains (6/14 *vs.* 1/10, $P=0.172$). This supports previous research (14/34)[3]. Among the five GII.3 cases, one was in a non-secretor. For the GII.17, all four cases were manifest in secretors/partial secretors.

In order to further describe the phenotypes of the NoV-infected children, profiles of the HBGAs were analyzed. Most children infected with NoV GII.4 expressed high levels of Le^y and Le^b, indicating an overwhelming prevalence in secretors (Figure 1A). The B antigen was co-expressed in almost 50% of the infected individuals. For the GII.3 NoVs, three children expressed high levels of Le^b and Le^y, while two partial secretors co-expressed Le^x and one non-secretor expressed high levels of Le^a and Le^x (Figure 1B). Meanwhile, one child showed low levels of all antigens with relatively higher abundances of Le^y and B. Of the four children infected with NoV GII.17, all expressed a high level of Le^y with one partial secretor co-expressing a high level of Le^x (Figure 1C).

Furthermore, in order to confirm the infection of the non-secretor, all eleven of the non-secretors identified by the EIA were further verified by *FUT2* gene amplification as previously described[8]. Four secretors and four partial secretors representing A, B, AB, and O types were chosen as a control, respectively. Genomic DNA was extracted from buccal cells using the QIAamp DNA Mini Kit (Qiagen, Germany) following the manufacturer's instructions. The *FUT2* coding region from position 152 to 543 was amplified and sequenced. The mutation G428A, common in European and American populations,[8] was not observed. Instead, the homozygous mutation A385T, common in Asian populations, was identified in all eleven non-secretors.

We performed a molecular epidemiological study of NoV in pediatric diarrhea cases in China during November 2014 and February 2015 and further
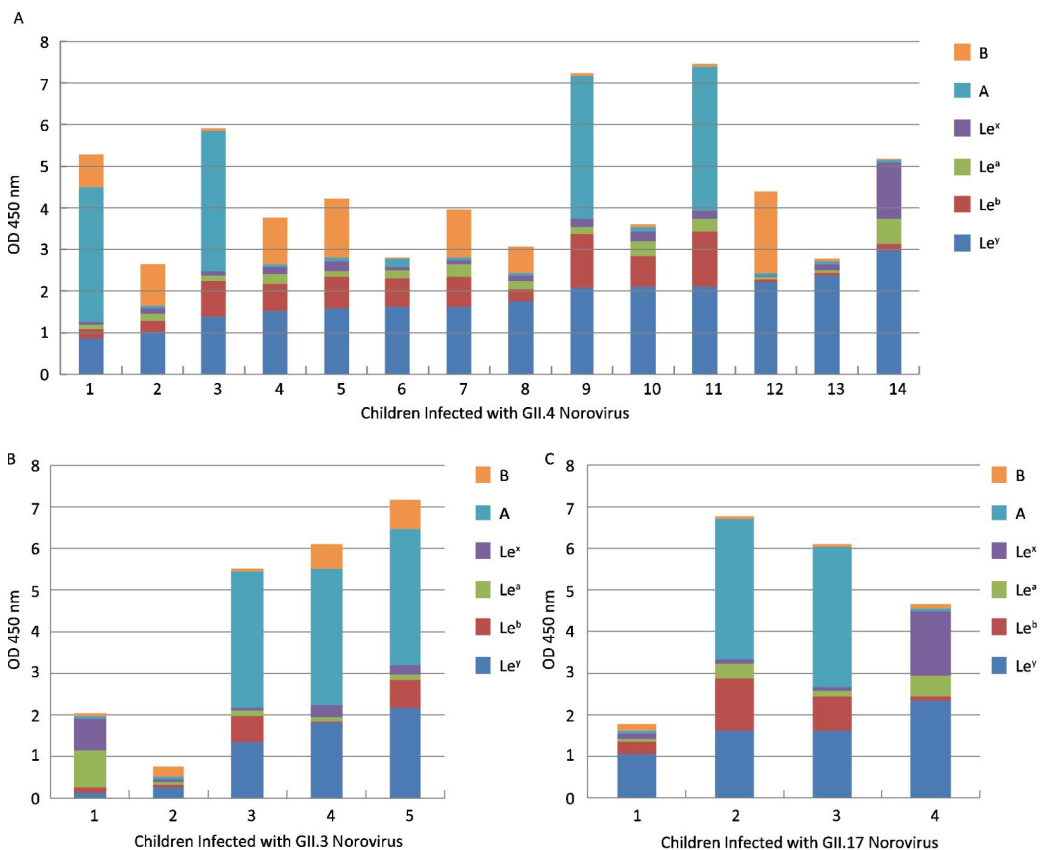

Figure 1. Profile of ABH and Lewis antigens in saliva samples from children infected with the three genotypes of norovirus (NoV): GII.4 (14 cases) (A), GII.3 (5 cases) (B), and GII.17 (4 cases) (C). OD, optical density.

correlated NoV infection with HBGA phenotype. The overall prevalence of NoV in pediatric patients with diarrhea was about 6%, which is lower than that of studies from Burkina Faso (12%) and Vietnam (43%)[3,6]. This may be due to the fact that the NoV was less prevalent during the period of sample collecting (according to NoV surveillance data from 2013 and 2014 in China, peak infection rates appeared in March and September, data not published). Moreover, for the Lulong and Chenzhou areas, the prevalence of NoV was 9% (19/202) and 2% (5/212), respectively (*P*=0.02). The reason for the relatively low prevalence in Chenzhou still needs further exploration. One possible cause may be attributed to the high prevalence of rotaviruses of these samples, which have been investigated in another study.

The most prevalent strain in this study was GII.4 and all GII.4-infected individuals were secretors/partial secretors. This indicates that GII.4 predominantly infected secretors and partial secretors. GII.3 NoVs were capable of infecting both secretors (*n*=4) and non-secretors (*n*=1). These data are consistent with former studies. Jin et al.[7] reported that a few non-secretors were also infected in two outbreaks caused by GII.3 and GII.4 NoVs in China in 2008. Furthermore, Liu et al.[9] found one GII.3 and one GII.4 infection in non-secretors among 34 NoV infections between 2009 and 2011. GII.17 NoV was sporadic in previous outbreaks but became predominant during the winter of 2014-2015 in China[10]. Four individuals were also GII.17 positive in this study, all of which were secretors/partial secretors, which indicates that GII.17 may also tend to infect secretors. However, with the limited cases of norovirus infection (*n*=24) and limited number of non-secretors (*n*=11), no significant differences were shown between the NoV-infected and non-infected groups (23/413 *vs*. 1/10, *P*=0.477). Further studies with more samples are needed to address the association between HBGA and NoV genotype.

In conclusion, NoV genotypes GII.4, GII.3, and GII.17 were detected in secretors and partial secretors with the exception of one GII.3 infection in a non-secretor. These data indicate that secretors may be more susceptible to NoV infection, which may provide clues for further investigations of host genetic factors and various NoV genotypes.

Author Contribution DZJ, LN, and LJX designed the experiments; YXF and HZG collected the samples; SXM, GNJ, and XZQ performed the experiments; SXM, JM, LDD, ZYK, PLL, and ZQ analyzed the data and results; SXM wrote the draft manuscript. XGC, JM, and DZJ revised the manuscript. All the authors reviewed the manuscript.

^These authors contribute equally to the work.

#Correspondence should be addressed to DUAN Zhao Jun, Tel: 86-10-58900898, E-mail: zhaojund@126.com

Biographical notes of the first authors: SUN Xiao Man, female, born in 1988, Assistant Professor, majoring in pathogenic microbiology; YAN Xiao Feng, female, Assistant, majoring in viral diarrhea; HE Zhi Gang, male, Director, majoring in Pediatrics.

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