## Letter to the Editor

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

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**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norovirus outbreaks during the 2013-to-2014 season, with GII.17 being the dominant strain during 2014-to-2015 season (unpublished data).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

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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.