Norovirus GII.P16/GII.2 Strain in Shenzhen, China: A Retrospective Study

Jing Wang

Wuchang Hospital <https://orcid.org/0000-0002-7074-8351>

Miao Jin

National Institute for Viral Disease Control and Prevention

Hailong Zhang

Shenzhen Center for Disease and Prevention

Yanan Zhu

Department of Epidemiology, School of Public health, Southern Medical University

Hong Yang

Shenzhen Center for Disease Control and Prevention

Xiangjie Yao

Shenzhen Center for Disease Control and Prevention

Long Chen

Shenzhen Center for Disease Control and Prevention

Jun Meng

Shenzhen Center for Disease Control and Prevention

Guifang Hu

Department of Epidemiology, School of Public Health, Southern Medical University

Yaqing He (✉ heyaqing1019@126.com)

Shenzhen Center for Disease Control and Prevention <https://orcid.org/0000-0001-6608-8541>

Zhaojun Duan

National Institution for Disease Control and Prevention, Chinese Center Control and prevention

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Abstract

Background

Norovirus (NoV) is the main cause of non-bacterial acute gastroenteritis (AGE) outbreaks worldwide. From September 2015 through August 2018, 203 NoV outbreaks with 2,500 patients were reported to the Shenzhen Center for Disease Control and Prevention.

Methods

Fecal specimens were collected from the 203 outbreaks and epidemiological data were collected through the AGE outbreak surveillance system in Shenzhen. The genotypes were determined by sequencing analysis. To gain a better understanding of evolutionary characterization of NoV in Shenzhen, the molecular evolution was analyzed by time-scale evolutionary phylogeny and amino acid mutations.

Results

Most of these outbreaks were associated with NoV GII.P16/GII.2 strain (45.3%,92/203) and occurred in school settings (91.6%,186/203). The timescale phylogeny suggested that the GII.P16/GII.2 strain was recombination strain and were still stable. The amino acid mutations suggested that the nonstructural proteins of the recombination strain might play a more significant role than VP1 gene in these GII.P16/GII.2 recombination strain outbreaks.

Conclusions

This study illustrated the characteristics of the molecular epidemiological patterns in Shenzhen, China during September 2015 to August 2018 and provided the evidence that the GII.P16/GII.2 strain was static and the epidemic trend had fade.

Background

Norovirus (NoV), which is the main cause of non-bacterial acute gastroenteritis (AGE) around the world and could infect all age groups, especially children under 5 years of age. According to estimation, NoV was associated with 900,000 clinic visits amongst children in industrialized countries and up to 200,000 deaths of children in developing countries annually [1, 2].

NoV belongs to the *Caliciviridae* family and could be divided into seven genogroups (GI ~ GVII). The GI and GII genogroups are the most common genogroups that infect people, which could be further divided into 9 genotypes and 22 genotypes[3, 4]. The full-length of the single stranded RNA is around 7.5 ~ 7.7 kb and there are three open reading forms (ORFs)[5]. The first 5 kb sequence nearly to 5' is ORF1, it encodes

non-structure polyprotein, including N terminal protein (p48), NTPase, 3A protein (p22), VPg (viral genomic junction protein), 3 C-like protein (Pro) and RNA-dependent RNA polymerase (RdRp)[6]. P48 was reported to localize to intracellular vesicles and disrupts intracellular protein trafficking[7]. P22 contains an endoplasmic reticulum export signal that is proposed to promote P22 uptake into the coat protein complex II vesicles, which leading to vesical mislocalization and Golgi body disassembly[8]. NTPase encodes both ATP dependent RNA helices activity which unwinds RNA helices and ATP-independent RNA-chaperoning activity that can remodel structured RNAs and facilitate strand annealing[9]. Pro cleaves the polyprotein translated from ORF1[10]. The RNA-dependent RNA polymerase (RdRp) of norovirus is a key enzyme which responsible for transcription and replication of the viral genome[11]. These proteins are important for replication of NoV. ORF2 is 1.6 kb and encodes major structural protein VP1, which composes main capsid structure and responsible for infectivity and antigenicity of NoV[12]. The protein VP1 consists of a well-conserved shell (S) domain and a protruding (P) domain. The latter is divided into two sub-domains, P1 and P2[13]. Furthermore, the P2 region is considered to be the hypervariable part of the genome since the domain encodes the receptor binding domain, which responsible for histoblood group antigen (HBGA) binding, and important epitopes targeted by antibodies that inhibit binding[14, 15]. ORF3 is 0.6 kb and encodes minor structural protein (VP2) [16].

NoV is rapidly evolving RNA virus and via accumulation of point mutations or recombination to be predominant globally[1, 17]. Generally, the dominant epidemic variant strain was GII.4 and several worldwide NoV pandemic events were caused by GII.4 sub-variants, including Hunter2004, Yerseke2006a, Den Haag2006b, New Orleans2009 and Sydney 2012 [18]. GII.2 was poorly reported in the past decades years, although it had been recombined with multiple different polymerase regions (i.e., GII.P2, GII.P12, previous GII.P16, GII.P21, GII.Pc, GII.Pe and GII.Ph)[19]. However, the reemergence GII.P16/GII.2 caused rapidly increasing AGE outbreaks in China in winter 2016 [20]. And during a short time, GII.P16/GII.2 recombination strain swept Japan, Italy, Germany and other countries[21–23].

In this report, the retrospective study aimed to illustrate the genotypic diversity of NoV strains in outbreaks and genetic characteristics of GII.P16/GII.2 strain in the Shenzhen, China, from September 2015 to August 2018.

Methods

Sample and RNA extraction

All the fecal specimens were derived from the AGE outbreaks which were inspected to Shenzhen Center for Disease Control and Prevention (Shenzhen CDC) by districts Center for Disease Control and Prevention (districts CDC). The outbreaks were identified as more than 5 cases within 3 days. The viral RNA of fecal specimens were extracted by Viral Nucleic Acid Extraction Kit II (Geneaid, Taiwan, China) and stored at -80°C. And the NoV outbreaks were confirmed by two positive samples in per outbreak using real-time reverse transcription polymerase chain reaction (qRT-PCR)[24] (S1 Table).

Reverse transcription and PCR

Sequence amplification was used one-step RT-PCR Kit (QIAGEN, Germany). And before October 2016, the primer set G1SKF/G1SKR and COG2F/G2SKR were used for VP1 typing to detect GI(330bp) and GII(387bp), respectively [25](S1 Table). After October 2016, the primer set MON432/ G1SKR and MON431/G2SKR were used to augment both of partial RdRp region and VP1 gene of GI (543 bp) or GII (557 bp), respectively [26](S1 Table). And genotypes were confirmed by the Blast and an automated online NoV genotyping tool offered by the Netherlands National Institute for Public Health and the Environment (<http://www.rivm.nl/mpf/norovirus/typingtool>)[27]. Reverse transcription of viral RNA via SuperScript III kit (invitrogen, USA). Then cDNA were used Polymerase Chain Reaction (PCR) to retrieve nearly full-length sequence. The sequence of primers were shown in S1Table. The F of GII.P16/GII.2 strain represented forward primer and R represented reverse primer.

Phylogenetic analysis of RdRp region and VP1 gene

To evaluate the evolution of the NoV GII.P16/GII.2 strain in Shenzhen, the full-length RdRp region or VP1 gene from this study and GenBank were collected and phylogenetic trees of the genes were constructed by the MCMC method. The best substitution model was selected by MEGA6.0 using the BIC method. The analysis of phylogenetic data would be confirmed until the effective sample sizes greater than 200 by the Tracer. The final result would be visualized by the FigTree v1.4.0 program.

Recombination variant

To evaluate the impact the intergenic recombination of nonstructural region and capsid region, the amino acid (aa) mutations of nonstructural region and capsid region among different genotypes were analyzed by MEGA6.0.

Statistical analysis

The difference between GII.2 NoV detection rates in dominant setting distribution was compared using Fisher's Exact Test in SPSS Statistics v.22.0 (IBM Corp., Armonk, NY, USA), and a P-value less than 0.05 was considered statistically significant.

Nucleotide sequence accession numbers

The GenBank accession numbers for GII.P16/GII.2 strain sequences obtained in this study are MK729081, MK681452, MK614124-MK614161, MK720506-MK720583, MK692738-MK692654.

Results

Epidemiological characteristics of NoV outbreaks

There were 203 NoV outbreaks from September 2015 to August 2018 in Shenzhen. Of 203 outbreaks, 62 (30.5%) outbreaks were from Nanshan district, 42 (20.7%) outbreaks were from Futian district, 32 (15.8%) outbreaks were from Longgang district, 29 (14.3%) outbreaks were from Luohu district, 23 (11.3%)

outbreaks were from Longhua district, 5 (2.5%) outbreaks were from Baoan district, 4 (3.9%) outbreaks were from Pinshan district, 4 (3.9%) outbreaks were from Dapeng district and 2 (1.0%) were from Guangming district (Figure 1A). Information on the outbreak size was reported for 197 outbreaks (97.0%), ranging from 5 to 115 cases per outbreak (Table 1). Of the 203 outbreaks, 91.6% of outbreaks occurred in school settings including child care center (143, 70.4%), primary school (23, 11.3%), middle schools (7, 3.4%), university (4, 2.0%), nine-year school (8, 4.0%) and fifteen-year school (1, 0.5%). 8.4% of outbreaks occurred in non-school settings, including company (5, 2.5%), hotel (4, 2.0%), institution (3, 1.5%), hospital (2, 1.0%), restaurant (2, 1.0%) and community (1, 0.5%) (Table 2). The reported outbreaks peaked in cold season, especially during November to March (Figure 1B).

Distribution Characteristics of NoV genotypes

Of 203 outbreaks detected as NoV positive by Real-time RT-PCR from September 2015 to August 2018, 150 outbreaks were genotyped successfully. Of these 150 outbreaks with genotype information, 137 (91.3%, 137/150) were classified into GII genogroup and 12 (8.0%, 12/150) were classified into GI genogroup. There was 1 (0.6%, 1/150) outbreak comprised both GI- and GII-positive samples. A total of 15 capsid genotypes and 15 polymerase genotypes were detected. Among the 150 genotyped outbreaks, the capsid types of GI genogroup outbreaks were GI.2 (3, 2.0%), GI.3 (3, 2.0%), GI.6 (3, 2.0%), GI.5 (2, 1.3%), GI.1 (1, 0.7%) and the polymerase types were GI.Pb (3, 2.0%), GI.P2 (3, 2.0%), GI.P5 (2, 1.3%), GI.Pd (1, 0.7%), GI.P1 (1, 0.7%). The predominant capsid types of GII genogroup outbreaks were GII.2 (92, 61.3%), followed by GII.3 (13, 8.7%), GII.6 (8, 5.3%), GII.17 (8, 5.3%) and GII.4 Sydney 2012 (7, 4.7%). The other GII capsid types including GII.4, GII.8, GII.13 and GII.21 were detected in 3 (2.0%, 3/150), 2 (1.3%, 2/150), 1 (0.7%, 1/150) and 2 (1.3%, 2/150), respectively. The predominant polymerase types of GII outbreaks were GII.P16 (92, 61.3%), followed by GII.P12 (8, 5.3%), GII.P7 (6, 4.0%), GII.P17 (3, 2.0%), GII.Pe (2, 1.3%), GII.P8 (1, 0.7%) and GII.P21 (1, 0.7%). In addition, we found an novel recombination genotype GII.Pe/GII.17 that had not been found in Shenzhen before (Table 3).

Genotype distribution and outbreak characteristics

For outbreaks caused by GII.2 strain, 73 (79.3%, 73/92) outbreaks occurred in child care center, 7 (7.6%, 7/92) outbreaks in primary school, 4 (4.3%, 4/92) outbreaks in middle school, 4 (4.3%, 4/92) outbreaks in nine-year school, 1 (1.1%, 1/92) outbreak in university, 1 (1.1%, 1/92) outbreak in hospital, 1 (1.1%, 1/92) outbreak in company and 1 (1.1%, 1/92) outbreak in fifteen-year school. The dominant setting distribution (child care center, primary school, middle school) of GII.2 infection have showed no significantly different (Fisher's Exact Test=3.595, $p=0.177$). The 13 GII.3 strain outbreaks, 11 (84.6%, 11/13) outbreaks occurred in child care center, followed by 1 (7.7%, 1/13) outbreak in primary school and 1 (7.7%, 1/13) outbreak in nine-year school.

Phylogenetic Analysis of RdRp region and VP1 gene

In this study, all the GII.2 NoV were GII.P16/GII.2 strain. To examine the evolution of the strains, 52 full-length RdRp region sequences of GII.P16/GII.2 strain from Shenzhen and 95 reference sequences from

GenBank were collected to analyze. The best substitution model was TN93 (Tamura-Nei)+G (Gamma). After MCMC chains were run for 1.0×10^8 steps for the RdRp region sequences and the first 10% state was burn-in, effective sample sizes greater than 200 were accepted. The MCC tree shown that the evolutionary rate of the RdRp region of GII.P16/GII.2 strain was estimated as 2.1×10^{-3} substitutions/site/year (95% HPD interval, 1.7×10^{-3} - 2.5×10^{-3} substitutions/site/year). The common ancestors of GII.P16/GII.2 strain from Shenzhen and GII.P16/GII.4 Sydney 2012 diverged during 2011 to 2012, and the prototype of RdRp region of GII.P16/GII.2 strain from Shenzhen were formed during 2012 to 2013. The phylogenetic analyses suggested the RdRp region come from GII.P16/GII.4 Sydney 2012 from USA (Figure 2A).

Simultaneously, 72 full-length VP1 gene sequences of GII.P16/GII.2 strain retrieved from Shenzhen and 146 reference sequences from GenBank were used to explore evolutionary rate. The best substitution model was TN93 (Tamura-Nei)+G (Gamma)+I (Invariant). After MCMC chains were run for 2.0×10^8 steps for the VP1 gene and the first 10% state was burn-in, effective sample sizes greater than 200 were accepted. And the MCC tree shown that the evolutionary rate of the VP1 gene of GII.P16/GII.2 strain was estimated as 2.7×10^{-3} substitutions/site/year (95% HPD interval, 2.4×10^{-3} - 3.1×10^{-3} substitutions/site/year). The common ancestors of GII.P16/GII.2 strain from Shenzhen and previous GII.P16/GII.2 diverged during 2003 to 2004, and the prototype of VP1 gene of GII.P16/GII.2 strain in Shenzhen was formed during 2013 to 2014. The phylogenetic analyses suggested the VP1 gene come from GII.P16/GII.2 (2010) from Japan (Figure 2B).

Nonstructural Polymerase Region of GII.P16/GII.2 recombination strain

To explore the aa mutations of nonstructural region within recombination strains, 14 nearly full-length nonstructural polymerase sequences and 22 full-length reference sequences, including GII.P16/GII.17 (2002), GII.P16/GII.2 (2009-2014), GII.P16/GII.2 (2010-2012), GII.P16/GII.13 (2015), GII.P16/GII.3 (2012-2013), GII.P16/GII.4 (2015-2016), GII.P16/GII.17 (2016-2018), from GenBank were aligned. Sequences data indicated there were 102 (6%) parsimony-informative sites revealed, but no aa mutation in nonstructural region of GII.P16/GII.2 recombination strain. Furthermore, 6 aa substitutions (*77E, R750K, P845Q, H1310Y, K1546Q, T1549A) were found only in recent strains (GII.P16/GII.4 Sydney 2012 and GII.P16/GII.2 recombination strain), 2 aa sites (A644P, A1521V) achieved substitution in GII.P16/GII.2 recombination strains and 1 aa site (S/T753T) returned to anterior aa site. RdRp region could be divided into three highly conserved ingredients according to function and structure, including the fingers, thumb, and palm sub-domains, which could be organized into Motifs A to G [11]. The result shown 1310 aa site (Motif G) was substituted (Table 4).

HBGA-Binding Profile, Epitopes Predicted and Epitope A to E sites of GII.P16/GII.2 recombination strain

To explore HBGA-Binding profile, epitopes predicted and epitope A to E sites of GII.P16/GII.2 recombination strain, 72 full-length VP1 gene sequences from this study and 65 reference sequences, including GII.Pc/GII.2 (1976-1978), GII.Ph/GII.2 (1997), GII.P2/GII.2 (1987-2015), GII.P12/GII.2 (2004-2006), GII.P21/GII.2 (2010), GII.Pe/GII.2 (2014), GII.P16/GII.2 (2010-2012), GII.P16/GII.2 (2008-2014) and

GII.P16/GII.2 (2016-2018), from 1975 to 2018 were collected and aligned. Sequences data shown 29 (5.3%) parsim-informative sites were revealed but there were no mutations in the amino acids of the HBGA-Binding profile, epitopes predicted and epitope A to E of GII.P16/GII.2 strain (S2 Table)

Discussion

In this study, NoV-associated AGE outbreaks in Shenzhen, China, from September 2015 to August 2018 were analyzed. There were 203 NoV outbreaks were reported to Shenzhen Center for Disease Control and Prevention. The NoV infection was initially described as "winter vomiting disease" due to its seasonal preference[31], monthly distribution also indicated that the peak of the outbreak in Shenzhen was during the November to March. Previous study have found a link between NoV's increased number and climate or weather[32, 33]. Especially, rain, humidity, and temperature changes are important factors that influence the seasonal increase in NoV outbreaks, and it is suspected that meteorological factors may have significant influence on the activity and transmission of NoV. The peak in this study was in December, when Shenzhen began to turn cold, and March, when temperature began to turn warm, which is suspected that climate changes have an impact on NoV transmission. The NoV outbreak usually occurs in hospitals, nursing homes, schools, child care centers, hotels and other semi-enclosed places [34–36]. A study in United States reported 3,960 NoV outbreaks between 2009 and 2013 and found that long-term care homes were the most frequent sites of NoV outbreaks[37]. Another study from Qin [38] shown that middle schools were the most important setting of NoV outbreaks in China, followed by primary schools between 2006 and 2016. In this study, we classified the outbreak settings into 12 categories, and the results shown that most outbreaks were occurred in child care centers, followed by primary school. This suggests that schools remain the focus of NoV outbreaks in Shenzhen, but that the current high incidence is among children of lower school age. Combining on the results of monthly distribution of NoV outbreaks in Shenzhen, we suspect that the decrease in the number of NoV outbreaks in January and February is related to school holidays. When the scale of the outbreak was analyzed, the average number of people involved per outbreak in Shenzhen was 9, smaller than the 18 persons reported in the United States[37]. Shenzhen is one of the cities where the economy are most developed This may benefit from local high public health system and high effective handling of public health emergencies in Shenzhen (<http://www.szemo.gov.cn>). In genotype detection, both GI and GII genogroups were detected, furthermore, 15 capsid types and 15 polymerase types were found. Among the genotypes, the most was GII.2, followed by GII.3. GII.4 Sydney2012 was only account for 3.4%. In 2016, NoV outbreaks were associated with GII.P16/GII.2 strains were reported in multiple regions in China [39, 40]. In this study, we identified the GII.2 strain were NoV GII.P16/GII.2, and was recombinant strain as other regions in China [39, 40]. This study found the first outbreak identified as GII.P16/GII.2 recombinant strain was September 30, 2016 in Shenzhen and then the GII.P16/GII.2 strain caused steep rise in acute gastroenteritis in Shenshen in the following months. In current study of NoV evolution, the recombination was thought to be important and common for virus evolution. Most recombination often occurs within ORF1/ORF2 overlapping regions or near the RdRp region, resulting in different capsid and RdRp genotypes. In the study, we calculated the evolutionary rates of RdRp region and VP1 gene, which were 2.1×10^{-3}

substitutions/site/year and 2.7×10^{-3} substitutions/site/year, respectively, indicating that the polymerase and capsid regions of NoV GII.P16/GII.2 strains had evolved independently, which was consistent with the results of previous studies [41]. The evolution rate of NoV GII.2 was much lower than that of GII.4 NoV ($4.4\text{--}7.4 \times 10^{-3}$ substitutions/site/year) [42], it suggested GII.2 was still stable in Shenzhen. The evolutionary divergence time indicated that the GII.P16/GII.2 strains from Shenzhen might have been recombinant in 2013–2014. This provided us a better understanding of the formation of GII.P16/GII.2 recombination strains in Shenzhen.

The result of sequences alignment shown that important sites of VP1, including HBGA-Binding profile, epitopes predicted and epitope A to E sites, were not mutated. This suggested that the reason of prevalence of NoV GII.P16/GII.2 strains in population was different from that of the previous pandemic NoV GII.4, which mainly due to changes in capsid region leading to changes in blocking antibody epitopes to cause population among people [43, 44]. Parra [45] analyzed the GII.2 capsid sequences over a 40-year period and found only small differences, which our results agree with, indicating that GII.2 strain is more genetically stable than GII.4 strain. As the same time, lack of variation in antigen regions of strains may also explain their short duration. These results suggested that the presence of a structure other than the VP1 contributes significantly to the prevalence of GII.P16/GII.2 stain[19]. This could learn something from the epidemic reasons of GII.P17/GII.17 that caused the outbreak of acute gastroenteritis in many countries in the winter of 2014–2015. Tohm [46] summarized the epidemic reasons of GIIP17/GII.17 in the population and believed to have a relationship with unstructured region. And amino acid substitutions were found in the nonstructural regions, including P48, NTPase, P22 and RdRp in this study. These nonstructural polymerases played important roles in norovirus replication, which could destroy host cells and promote virus synthesis by interfering with intracellular protein transport, vesicle misorientation and golgi disintegration [8–11]. The results of this experiment suggested that the unstructured region may provide more materials for virus replication, accelerate the apoptosis of host cells' golgi bodies and enhance the fitness by changing the interaction mode. Another study also reported that GII.P16/GII.2 strain had a higher viral load than that of GII.Pe/GII.4 and GII.P17/GII.17 in patients [47]. But not all changes in unstructured region would cause epidemic. The study of Tohma calculated the amino acid substitution sites in the RdRp region of GII.P2/GII.2 and found that the replacement rate of GII.P2 was higher than that of GII.P16 [19]. however, no great GII.P2/GII.2 outbreaks were found. It indicated that GII.P16 played a crucial role in GII.P16/GII.2 epidemic and the further research of mechanism was limited.

This study shown the GII.P16/GII.2 outbreaks had reduced in Shenzhen, while the continuous surveillance to monitor genotypes is still necessary to identify new variants in time. The limitations of this study were as followed: first, genotyping was only successful for 150 (73.9%) in our study within positive NoV cases. Second, our study lack of clinical information and epidemiological data are incomplete within outbreaks. In future studies, epidemiological surveillance should be better perfected and molecular analysis for different NoV genotypes would be developed.

Conclusions

In conclusion, this study reported the epidemiological patterns and genetic characteristics of NoV in Shenzhen from September 2015 through August 2018 and the main cause was GII.P16/GII.2 strain. This study also provided the evidence that the NoV GII.P16/GII.2 strain was static in Shenzhen.

Abbreviations

AGE Acute gastroenteritis

NoV Norovirus

RdRp RNA-dependent RNA polymerase

HBGA Histoblood group antigen

Declarations

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JW, MJ, YQH and GFH designed the study. ZHL and ZYN participated in the Norovirus detection. HY, XJ Y,LC, JM participated in the Sequence analysis and phylogenetic analysis. ZJD coordinated in the design of the study.

All authors read and approved the final version of the article.

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Author details

¹ Wuhan Wuchang Hospital, Wuchang Hospital Affiliated to Wuhan University of Science and Technology, Wuhan, 430063, China

² NHC Key Laboratory of Medical Virology and Viral Diseases, National Institute for Viral Disease Control and Prevention, Chinese Center Control and Prevention, Beijing 102206, China

³ Shenzhen Center for Disease Control and Prevention, Shenzhen 518055, China

⁴ Department of Epidemiology, School of Public Health, Southern Medical University, Guangzhou 510515, China

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Availability of data and materials

The datasets used in the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Ethics committee of Chinese Center Control and Prevention. Informed consent for the fecal specimens was obtained from the patients or their guardians.

Consent for publication

Not applicable.

Contributor Information

Jing Wang, Email:wangjing5561@126.com

Miao Jin, Email:jinmiao37@126.com

Hailong Zhang, Email: 22899360@qq.com

Yanan Zhu, Email: 1963805943@qq.com

Hong Yang, Email:78641474@qq.com

Xiangjie Yao, Email:Yaoxiangjie26@qq.com

Long Chen, Email:chen_l_2011@163.com

Jun Meng, Email:269380997@qq.com

Guifang Hu, Email:hgf@smu.edu.cn

Yaqing He, Email: heyaqing1019@126.com

Zhaojun Duan, Email:zhaojund@126.com

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Tables

Table 1 Number of people with NoV gastroenteritis per outbreaks according to genotype

Genotype	No.of ill people¶range¶	No.of outbreaks involved ill people	Median no. of ill people¶range¶
GII.2	3-73	92	10
GII.3	3-45	13	7.5
GII.4Sydney 2012	9-14	7	11
GII.4	3-13	3	11
GII.6	4-8	8	6
GII.8	11-13	2	12
GII.17	3-12	8	5.5
GII.13	14	1	-
GII.21	11-23	2	17
Multiple genotype	3-10	2	6.5
GI.1	7	1	-
GI.2	5-9	3	7
GI.3	5-84	3	6
GI.5	3-5	2	4
GI.6	6-64	3	12
GII	3-115	53	9
Total	3-115	203	9

Table 2 Genotype distribution of norovirus outbreaks from 2015 to 2018 according to setting

	Total	No.(%) of outbreaks											
		child care centers	primary school	middle school	university	hospital	hotel	restaurant	company	Nine- year school	Fifteen- year school	Institution	Community
GII.2	92(45.3)	73(79.3)	7(7.6)	4(4.3)	1(1.1)	1(1.1)	-	-	1(1.1)	4(4.3)	1(1.1)	-	-
GII.3	13(6.4)	11(84.6)	1(7.7)	-	-	-	-	-	-	1(7.7)	-	-	-
GII.4Sydney 2012	7(3.4)	6(85.7)	1(14.3)	-	-	-	-	-	-	-	-	-	-
GII.4	3(1.5)	3(100.0)	-	-	-	-	-	-	-	-	-	-	-
GII.6	8(3.9)	5(62.5)	2(25.0)	-	-	-	-	-	1(12.5)	-	-	-	-
GII.8	2(1.0)	1(50.0)	1(50.0)	-	-	-	-	-	-	-	-	-	-
GII.17	8(3.9)	2(25.0)	1(12.5)	-	-	-	1 (12.5)	1(12.5)	2(25.0)	-	-	1(12.5)	-
GII.13	1(0.5)	1(100.0)	-	-	-	-	-	-	-	-	-	-	-
GII.21	2(1.0)	-	-	-	-	-	1 (50.0)	-	-	-	-	1(50.0)	-
Multiple genotype	2(1.0)	-	-	-	1(50.0)	-	1 (50.0)	-	-	-	-	-	-
GI.1	1(0.5)	1(100.0)	-	-	-	-	-	-	-	-	-	-	-
GI.2	3(1.5)	1(33.3)	1(33.3)	-	-	-	-	-	-	1(33.3)	-	-	-
GI.3	3(1.5)	1(0.7)	1(4.3)	1(14.3)	-	-	-	-	-	-	-	-	-
GI.5	2(0.1)	2(100.0)	-	-	-	-	-	-	-	-	-	-	-
GI.6	3(1.5)	2(66.7)	-	-	-	-	-	-	-	1(33.3)	-	-	-
GII	53(26.1)	34(64.2)	8(15.1)	2(3.8)	2(3.8)	1(1.9)	1(1.9)	1(1.9)	1(1.9)	1(1.9)	-	1(1.9)	1(1.9)
Total	203(100)	143(70.4)	23(11.3)	7(3.4)	4(2.0)	2(1.0)	4(2.0)	2(1.0)	5(2.5)	8(3.9)	1(0.5)	3(1.5)	1(0.5)

Note: Nine-year school indicated schools which included the primary school and middle school (especially junior high school). And the Fifteen-year school indicated schools which included occurred in schools which include primary school and middle school (junior high school and senior high school). Institution indicated units that belong to government.

Table 3 Genotype distribution of identified NoV strains in Shenzhen, September 2015–August 2018

Genotype	2015.09-2016.08	2016.09-2017.08	2017.09-2018.08
	Number(percentage)	Number(percentage)	Number(percentage)
Capsid			
GI.3	1(4.8)	1(0.9)	-
GII.3	4(19.0)	1(0.9)	-
GII.4	-	1(0.9)	1(1.4)
GII.4 Sydney2012	4(19.0)	3(2.7)	-
GII.6	-	1(0.9)	1(1.4)
GII.8	-	1(0.9)	-
GII.17	3(14.3)	-	-
GII.21	2(9.5)	-	-
RdRp/Capsid			
GI.P1/GI.1	-	1(0.9)	-
GI.Pb/GI.6	-	-	3(4.3)
GI.P2/GI.2	-	-	3(4.3)
GI.Pd/GI.3	-	-	1(1.4)
GI.P5/GI.5	-	-	2(2.8)
GII.P16/GII.2	-	73(65.2)	19(27.1)
GII.P12/GII.3	-	2(1.7)	6(8.6)
GII.Pe/GII.17	-	1(0.9)	-
GII.P17/GII.17	-	2(1.7)	2(2.8)
GII.P7/GII.6	-	1(0.9)	5(7.1)
GII.Pe/GII.4	-	-	1(1.4)
GII.P8/GII.8	-	-	1(1.4)
GII.P21/GII.13	-	-	1(1.4)
Multiple genotype	1(4.8)	-	1(1.4)
GII	6(28.6)	24(21.4)	23(32.9)
Total	21(100)	112(100)	70(100)

Table 4 Nonstructural polymerase of GII.P16/GII.2 recombination strain

	P48	NTpase	P22			RdRp			
Variants	77	644	750	753	845	1310	1521	1546	1549
GII.17(2002)	*	A	K	S	P	H	V	K	T
GII.2(2009-2014)	*	A	K	S	P	H	V	K	T
GII.2(2010-2012)	*	S	K	I	P	H	V	K	T
GII.3(2012-2013)	*	P/S	K	V/I	P	H	V	K	T
GII.13(2015)	*	S	K	T	P	H	V	K	T
GII.4(2015-2016)	E	P/S	R	T	Q	H/Y	V/I	Q	A
GII.2(2016-2018)	E	P	R	T	Q	H/Y	I	Q	A

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Additional File

Additional file 1: Table S1.

Primes used in this study

HBGA-Binding profile and epitopes predicted of Reemerging NoV GII.P16/GII.2 were compared with previous GII.2 strain.

Additional file 3: Reference sequences

Reference sequences used in this study

Figures

Figure 1

Epidemiological characteristics of NoV outbreaks in Shenzhen. (A) Regional distribution of NoV outbreaks in Shenzhen. The map was created by an online tool offered by Dituhui (<http://c.dituhui.com/apps>). (B) Monthly distribution of NoV outbreaks in Shenzhen by genotypes. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

A **B** **Figure 2**

A) Phylogenetic tree for the RdRp region of NoV GII.P16/GII.2 The time shaft was shown under the figure. The scale bars represent the length of time. Different NoV strains was use graphic legends to explain on the left side. B) Phylogenetic tree for the VP1 gene of NoV GII.P16/GII.2 The time shaft was shown under the figure. The scale bars represent the length of time. Different norovirus strains was use graphic legends to explain on the left side.

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