Prevalence of GII.4 Sydney Norovirus Strains and Associated Factors of Acute Gastroenteritis in Children: Winter 2019-2020 in Guangzhou

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Abstract

Background

Norovirus, the leading cause of non-bacterial acute gastroenteritis (AGE) worldwide, is constantly mutating. Continuous monitoring of the evolution of epidemic genotypes and emergence of novel genotypes is therefore necessary. This study determined the prevalence and clinical characteristics of norovirus strains in AGE in Guangzhou, China in 2019-2020 winter.

Methods

This study included children aged 2-60 months diagnosed with AGE in Guangzhou Women and Children Hospital, from August 2019 to January 2020. Norovirus was detected by real-time polymerase chain reaction and clinical data were obtained. Genotyping and phylogenetic analyses were performed with partial gene sequence fragments located at the open reading frames 1-2 junctions.

Results

During the study period, 168 children (61.3% males) were confirmed as norovirus infectious AGE. The main symptoms were diarrhoea and vomiting; and 38 patients (22.6%) had seizures. Norovirus was mainly prevalent in October and November, and GII.P31/GII.4 Sydney was the major genotype circulating in Guangzhou. The phylogenetic tree showed that the Guangzhou strains had high homology with the strains circulating in 2017-2019 worldwide.

Conclusions

GII.4 Sydney was the main prevalent norovirus genotype in Guangzhou from August 2019 to January 2020, which had more severe diarrhoea than those of other genotypes. These findings provide a valuable reference for the prevention, control, and treatment of norovirus in the future.

Introduction

Norovirus is the leading cause of non-bacterial acute gastroenteritis (AGE) worldwide, causing approximately 18% of AGE and 212,000 deaths every year [1, 2]. Norovirus causes considerable yearly losses to society, and more than half of the expenditure on norovirus, is due to the childhood infections [3]. Norovirus is mainly prevalent in winter, and often causes severe AGE outbreaks. The symptoms of patients with norovirus AGE mainly include vomiting, diarrhoea, and nausea. Most of these symptoms are mild and self-limiting, and generally last for 2-3 days. However, norovirus can cause serious clinical symptoms in some groups, especially children, the elderly, and immunocompromised patients [4, 5]. Once transmitted, the virus can remain in the patient for weeks or even years. Due to the lack of specific antiviral drugs and vaccines, supportive care and symptomatic treatment are the main therapeutic methods for norovirus infections. Moreover, norovirus has a strong ability to spread, and usually causes

AGE outbreaks in semi-enclosed areas, such as hospitals, schools, kindergartens, cruise ships, nursing homes, and prisons [1, 6].

Norovirus is a single-stranded positive-sense RNA virus belonging to the family Caliciviridae. The norovirus gene is approximately 7.5-7.7 kb long, and includes three open reading frames (ORFs). ORF1 encodes a polyprotein and is involved in viral replication. ORF2 and ORF3 encode the norovirus structural proteins VP1 and VP2, respectively. Norovirus is divided into 7 genogroups (GI to G□, but only GI, GII, and GIV can infect humans) based on the ORF2 gene, and further divided into more than 40 genotypes [7]. Because the mutation and recombination of norovirus mainly occur at the ORF1 and ORF2 junctions, the double genotype classification standard, based on ORF1 and ORF2, can well describe the epidemic potential of the virus, and has been widely accepted and applied in recent years [8].

The first norovirus outbreak occurred in Norwalk, USA, in 1968 [9]. Since 1995, GII.4 has become the most popular genotype worldwide, accounting for more than 90% of all the epidemics [7, 10]. In China, norovirus is also a major cause of non-bacterial AGE. It causes more than 20% AGE nationwide, affects about 800,000 people yearly [11]. However, previous studies have shown that non-GII.4 noroviruses(such as GII. 17 and GII. 2) have gradually replaced GII.4 to become the predominant genotypes in recent years [12, 13], and that norovirus is constantly mutating. Continuous monitoring of the evolution of epidemic genotypes and the emergence of novel genotypes is necessary to effectively control norovirus transmission.

In this study, we aimed to analyse the epidemiological and clinical characteristics of norovirus infection in children in Guangzhou in the winter of 2019-2020. Furthermore, we performed a phylogenetic analysis of the epidemic strains prevalent in Guangzhou to understand the trend of norovirus. These findings could provide important references for the control and treatment of norovirus, and for vaccine development, in the future.

Results

Epidemiological characteristics

From August 2019 to January 2020 in Guangzhou, China, a total of 168 children with norovirus AGE were included in this study (Figure 1). Among these patients, 159 were successfully genotyped while 9 failed because of low viral load. According to the RdRp gene on ORF1 and the VP1 gene on ORF2 (ORF 1-2 junctions), the majority of the norovirus genotypes were GII.P31/GII.4 Sydney and GII.P16/GII.4 Sydney, in 131 and 20 AGE cases, respectively. In addition, there were 6, 1, and 1 case of GII.P16/GII.2, GII.P7/GII.6, and GII.P21/GII.13, respectively.

As shown in Figure 2, the number of norovirus-infected children showed a monthly increase from 5 in August to 72 in October; in November, there were 55 infected children. The gradually decreasing trend started in November, with only four positive norovirus samples in January. GII.P31/GII.4 Sydney norovirus

was the dominant genotype in Guangzhou, Guangdong Province, in the winter of 2019. In October and November, GII.P16/GII.4 Sydney and GII.P16/GII.2 also increased slightly.

Demographic characteristics and clinical manifestations

The demographic characteristics and clinical manifestations of the 168 children with norovirus infection in this study are shown in Table. 1. Among these children, 103 (61.3%) were male and 45 (38.7%) were female. The median age was 19 (IQR 13.0-28.8) months, and 148 (88.1%) patients were under 3 years of age. A total of 80 (47.6%) children were hospitalised for a median of 5 (IQR 3.0-7.5) days. There were 56 (33.3%) children with fever, and the median body temperature during infection was 37 °C (IQR 37.0-37.9°C). Eighty-two (48.8%) patients had anorexia and 38 (22.6%) had seizures. Clinical data showed that the main symptoms shown by the patients with norovirus infection were diarrhoea (79%) and vomiting (83.9%), and no significant differences were found between the genotype groups. However, the duration and the maximum number of diarrhoea cases with GII.4 (GII.P31/GII.4 Sydney and GII.P16/GII.4 Sydney) were significantly higher than those in the other genotypes.

Table 1. Demographic characteristics and clinical manifestations of norovirus infected patients

	Total	GII.P31/ GII.4 Sydney	GII.P16/ GII.4 Sydney	Others [a]	Untyped
Number of patients	168(100.0%)	131(78.0%)	20(11.9%)	8(4.8%)	9(5.4%)
Sex(male)	103(61.3%)	80(61.1%)	12(60.0%)	6(75.0%)	5(55.6%)
Age(month）	19(13.0-28.8)	19(13.0-28.0)	19.5(14.3-38.5)	23(21.3-36.8)	16(11.5-29.0)
Hospitalisation(day)	80（47.6%）	63(48.1%)	8(40.0%)	3(37.5%)	6(66.7%)
Length of hospital stay(day)	5.0(3.0-7.5)	5.0(3-6)	4.5(3-9)	8(3-10)	9.5(3-12)
Fever	56(33.3%)	41(31.3%)	9(45.0%)	4(50.0%)	2(22.2%)
Body temperature(℃)	37(37-37.9)	37(37-37.8)	37(37-38)	37.5(37-39.73)	37(36.6-37.4)
Vomiting	141(83.9%)	111(84.7%)	17(85.0%)	7(87.5%)	6(66.7%)
Vomiting duration(day)	2.0(1.0-3.0)	2.0(1.0-3.0)	1.5(1.0-3.0)	1(1.0-1.8)	1(0.0-3.5)
Max. no. vomiting episodes/24 h	3.0(2.0-6.0)	3.0(2.0-6.0)	4.0(3.0-9.5)	4.0(1.5-4.8)	3.0(0.0-6.0)
Diarrhoea	132(79.0%)	104(80.0%)	15(75.0%)	4(50.0%)	9(100.0%)
Diarrhoea duration (day)	2.0(1.0-5.0)	2.0(1.0-5.0)	2.0(0.3-6.0)	0.5(0.0-1.0)*	2.0(1.0-3.0)
Max. no. diarrhoeal stools/24 h	3.0(1.0-6.0)	3(1.0-5.0)	5(0.3-8.8)	1(0.0-2.8)*	4.0(3.0-4.5)
Anorexia	82.0(48.8%)	70.0(53.4%)	8.0(40.0%)	2.0(25.0%)	2.0(22.2%)
Dehydration	15.0(8.9%)	13.0(9.9%)	1.0(5.0%)	1.0(12.5%)	0.0(0.0%)
Abdominal pain	22.0(13.1%)	15.0(11.5%)	4.0(20.0%)	2.0(25.0%)	1.0(11.1%)
Bloating	11.0(6.5%)	10.0(7.6%)	1.0(5.0%)	0.0(0.0%)	0.0(0.0%)
Nausea	21.0(12.5%)	15.0(11.5%)	3.0(15.0%)	1.0(12.5%)	2.0(22.2%)
Seizures	38.0(22.6%)	30.0(22.9%)	5.0(25.0%)	1.0(12.5%)	2.0(22.2%)

[a] The “Others” group contained three genotypes: GII.P16-GII.2, GII.P7-GII.6, GII.P21-GII.13

* *P<0.05*: Others vs. GII.P31-GII.4 Sydney and Others vs. GII.P16-GII.4 Sydney

Laboratory examinations

Laboratory examinations showed that the white blood cell (WBC), red blood cell (RBC), aspartate aminotransferase (AST), hydroxybutyrate dehydrogenase (HBDH), and creatine kinase isoenzyme (CK-MB) levels in the blood of patients with norovirus infection increased, but no significant differences were found between the genotype groups (Table 2). The levels of sodium (Na) (*P=0.002*) and chloride (Cl) (*P=0.024*) in the blood of patients infected with GII.4(GII.P31/GII.4 Sydney and GII.P16/GII.4 Sydney) were significantly higher than those of patients infected with other genotypes.

Table 2. Laboratory examinations of patients associated with the different norovirus genotypes

	Total	GII.P31/ GII.4 Sydney 2012	GII.P16/ GII.4 Sydney 2012	Others [a]	Untype
Total number of patients	168.0(100.0%)	131.0(78.0%)	20.0(11.9%)	8.0(4.8%)	9.0(5.4%)
hs-CRP (0-10 mg/L)	1.3(0.4-5.0)	1.2(0.3-5.1)	2.7(0.4-5.4)	1.1(0.5-6.2)	1.2(0.5-9.5)
WBC (3.5-9.5 × 109/L)	9.6(7.0-12.8)	9.67(6.9-13.4)	10.2(7.5-12.1)	12.38(8.1-20.1)	7.4(6.8-9.3)
NEUT (1.8-6.3 × 10^9/L)	5.1(2.64-9.21)	4.9(2.6-9.3)	5.4(2.8-8.1)	9.7(4.0-17.9)	4.0(1.2-6.7)
LYMPH (1.1-3.2 × 10^9/L)	3.0(1.9-4.5)	2.9(1.9-4.6)	3.9(2.9-4.3)	2.5(0.9-5.6)	2.1(1.4-4.2)
RBC (4.0-4.5 × 10^9/L)	4.7(4.5-4.9)	4.7(4.5-4.9)	4.6(4.4-4.9)	5.1(4.8-5.2)	4.65(4.6-4.9)
PLT (125-350 × 10^9/L)	325(272.5-392.5)	332(273.5-400.0)	314(268-438.3)	314(202.0-367.0)	329.0(234.0-368.0)
Na (137-147 mmol/L)	135.9(134.4-137.6)	135.95(134.3-137.6)	134.7(132.6-135.6)	139.2(137.1-140.7)*	135.55(133.7-137.3)
Cl (99-110 mmol/L)	102.25(100.3-105.0)	103(99.7-105.3)	102.3(99.9-105.1)	105.5(104.9-107.1)*	101.7(97.4-106.2)
LDH (180-430 U/L)	410.4(310.7-472.2)	413.6(329.4-488.7)	412(337.9-467.0)	340.2(291.1-427.9)	294.5(234.9-411.4)
AST (15-40 U/L)	41.0(34.0-48.0)	41.0(34.3-48.0)	39.0(31.5-51.0)	35(25.3-60.5)	40.5(30.3-59.0)
HBDH (72-182 U/L)	226.0(197.0-249.5)	229.0(208.5-259.0)	216.0(184.3-241.0)	222.5(166.0-262.5)	200.0(182.8-233.0)
CK-MB (0-25 U/L)	39.0(29.0-47.0)	40.0(30.5-47.0)	35.0(22.0-63.5)	27.5(20.0-86.5)	44.5(40.3-45.8)
FOBT	24.0(14.3%)	20.0(15.3%)	2.0(10.0%)	2.0(25.0%)	0.0(0.0%)

[a] The “Others” group contained three genotypes: GII.P16/GII.2, GII.P7/GII.6, GII.P21/GII.13

* P<0.05: Others vs. GII.P31/GII.4 Sydney and Others vs. GII.P16/GII.4 Sydney

Phylogenetic analysis

We performed phylogenetic analysis based on the partial gene sequence fragments (ORF1-2 junction), to investigate the genetic diversity of norovirus strains prevalent in Guangzhou in the winter of 2019. In Figure 3, the sequence alignment of 131 strains of GII.P31/GII.4 Sydney norovirus shows the identities of the nucleotides and amino acids as 94.87%-100.00% and 96.75%-100.00%, respectively. The nucleotide and amino acid identities between our strains and the GII.P31/GII.4 Sydney prototype strains (JX459908) were 96.36%-100.00% and 97.70%-99.55%, respectively. According to the phylogenetic tree, most of the GII.P31/GII.4 Sydney strains had high degree of homology with the 2017 to 2019 strains from China, the United States, and Thailand.

The nucleotide and amino acid identities of the 20 GII.P16/GII.4 Sydney strains were 95.81%-100.00% and 91.82%-100.00%, respectively. Those between our strains and the prototype strain of GII.P16/GII.4 Sydney 2012 (MG585767) were 95.31%-95.55% and 92.59%-94.87%, respectively, and had high homology with the 2016 to 2018 norovirus from China, Argentina, and Russia.

In addition, there were six GII.P16/GII.2 strains found in this study. they shared 98.87%-100.00% and 97.80%-100.00% of nucleotide and amino acid identities level respectively. Their nucleotide and amino acid identities with the prototype strains of GII.P16/GII.2 (AB662868) were 95.30%-95.78% and 93.26%-94.79%, respectively. The phylogenetic tree analysis showed that 5 of the strains were highly homologous to the 2017 to 2018 norovirus from Beijing, Huzhou, and Guangzhou, while that of the remaining one was highly homologous to the 2018 norovirus from Russia.

Discussion

Currently, norovirus is the main cause of non-bacterial AGE, and it often causes AGE outbreaks. In this study, we reported the detailed clinical and epidemiological characteristics of young children with norovirus AGE in Guangzhou City in the winter of 2019-2020. Our results revealed that norovirus was prevalent mainly in October and November during our study period, and GII.P31/GII.4 Sydney was the major genotype circulating in Guangzhou, followed by GII.P16/GII.4 Sydney and GII.P16/GII.2. Additionally, diarrhoea and vomiting were the main symptoms of norovirus AGE and GII.4 Sydney was associated with a more severe AGE presentation than other genotypes. These findings provide a better understanding of the clinical manifestations and aetiology of norovirus AGE, and can be helpful in the prevention, control, and treatment of norovirus AGE in the future.

Norovirus mutates and evolves rapidly, and it is classified into 7 genogroups and further divided into more than 40 genotypes based on the ORF2 gene [7]. These different norovirus genotypes are diverse in infectivity and virulence [14, 15]. More than 90% of non-bacterial AGE cases are caused by GII, with GII.4

as the major epidemic genotype in many regions of the world during the past two decades [10, 16]. GII.4 norovirus can be further classified into seven strains (GII.4 US95/96 strain, GII.4 Farmington Hills, GII.4 Hunter, GII.4 Den Haag, GII.4 New Orleans, and GII.4 Sydney) due to its continuous variations. These novel GII.4 epidemic strains that are associated with AGE outbreaks usually mutate every 2-3 years [17, 18]. This is because recombination frequently occurs between different strains and the breakpoints are usually at the junction of ORF1-2; hence, a dual typing based on both the RdRp of ORF1(P type) and the VP1 of ORF2 (genotype) of norovirus is widely used currently [8]. In our study, GII.P31/GII.4 Sydney was the main epidemic strain (78.0%) in Guangzhou in winter 2019-2020. This strain was first detected in Australia in 2012 and rapidly became the major epidemic strain in Europe, America, and Asia in 2012–2013 [19, 20]. In 2015, a novel recombinant norovirus strain, GII.P16/GII.4 Sydney, appeared and prevailed in Germany, France, USA, and China [21-23]. There were 20 cases (11.9%) of GII.P16/GII.4 Sydney during the study period. Previous studies have found that the RdRp gene of this novel strain is highly homologous to GII.P16/GII.2, and the VP1 gene is highly homologous to GII.P31/GII.4 Sydney [24, 25]. In addition, few strains of GII.P16/GII.2, GII.P7/GII.6, and GII.P21/GII.13 were also found in our study. GII.P16/GII.2 was the major epidemic strain in Guangdong Province in August 2016 and continued to circulate in 2016 and 2017 [13, 26]. Our study is the first to report that the main prevalent strains changed to GII.P31/GII.4 Sydney and GII.P16/GII.4 Sydney in Guangzhou in the winter of 2019-2020. The phylogenetic tree showed that the strains we obtained in Guangzhou had high homology with the strains circulating in 2017-2019 worldwide. This suggests that local strains may have become prevalent after the influx of the foreign strains. Overall, the epidemic strains of norovirus changes rapidly, and it is necessary to pay more attention to the monitoring of the norovirus outbreaks, which will help reduce the losses caused by norovirus infection.

Our study revealed October and November as the months when the norovirus infections were most prevalent in Guangzhou in the winter 2019-2020, which is different from the previous research that November to March of the following year is the prevalent month [27]. Research by Marshall et al. showed that the prevalence is positively related to the humidity and the amount of rainfall [28]. However, the weather in Guangzhou was characterised by continuous sunshine and drought from mid-October in 2019 [29], which was quite different from that of the previous years. The lack of rainfall in the winter of 2019 might be the reason why norovirus infections began to decrease in November. The molecular epidemiological data of this study demonstrated that norovirus circulating in Guangzhou in 2019 had, to a certain extent, sequence variations from the previous circulating strains, which may also change the prevalent characteristics; however, this needs further study. In addition, the strict control, wearing of face masks, and the increased awareness of hygiene by the citizens, associated with the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) at the beginning of 2020[30], not only effectively stopped the spread of SARS-COV-2, but may also have effectively reduced the opportunities of spread of norovirus.

In this study, male patients were more among the infected than females (103:65), which is consistent with previous studies [26, 27]. That the innate and acquired immunity in females are inherently stronger than in males may be the reason[31]. Children with norovirus infection are more likely to develop AGE,

especially children under 5 years. In our study, more than 88% of the patients were aged <36 months, with a median age of 19 (IQR 13-28.75) months. Fifty percent of infected patients were aged 12 to 23 months, which is consistent with previous reports[32-34]. These results suggest that children under 3 years of age are more susceptible to norovirus infection. Therefore, in the prevention and control of norovirus, more attention should be paid to children in this age group.

Vomiting and diarrhoea were the main symptoms of norovirus infection in our study. Compared with patients infected with non-GII.4 norovirus, patients with GII.4 were more likely to present with diarrhoea[20, 27]. In this study, we found that the duration of diarrhoea was significantly longer and the maximum frequency per day in patients with GII.4 infection was more ($P<0.05$) than that of other genotypes. In addition, laboratory examination results showed that the levels of Na and Cl in the blood of patients with GII.4 infection were lower than those with non-GII.4. These features also indicate that the GII.4 genotype infection is more serious, and effective measures, such as rehydration, are needed to prevent more serious complications. Furthermore, our study showed no significant differences in clinical symptoms and laboratory examination parameters between patients with GII.P31/GII.4 Sydney and GII.P16/GII.4 Sydney. The sequence alignment analysis demonstrated that these two strains were only different in the RdRp genes, while the VP1 genes were the same for the GII.4 Sydney. Previous studies have also proved that the VP1 gene did not differ between GII.P31/GII.4 Sydney and GII.P16/GII.4 Sydney [18]. The VP1 gene encodes a viral structural protein with the histo-blood group antigens (HBGAs)-binding sites, which can mediate the cellular interactions [35, 36]. The RdRp gene encodes a non-structural protein that participates in the transcription of norovirus, which can influence viral transmission [21, 24]. Perhaps the virulence of norovirus might correlate positively with its receptor-binding ability and be less correlated with its transmissibility. Further research is needed to prove this.

Our research showed nausea, abdominal pain, and dehydration as the complications of norovirus infection. In addition, we recorded that 38 (22.6%) patients had seizures, which was the main reason why some patients were admitted. Previous studies have also shown that the probability of seizures in norovirus infections is significantly higher than that in rotavirus infections [37-39]. Seizures, in this study, were similar to benign convulsions associated with mild gastroenteritis reported previously by Boman *et al.* [40]. The mechanism of norovirus-induced seizures in children is still unclear, and may be associated with the children's immature immune system [37]. Recently, some studies found that norovirus infection can cause encephalitis/encephalopathy [37, 41]. However, no case of encephalitis/encephalopathy was found in our study. In addition, the laboratory data showed that the levels of cardiac biomarkers, such as HBDH and CK, were elevated in most children. This suggests the need for clinical attention to myocardial damage in patients with norovirus infection.

There were several limitations to our study. For example, this study was not a multicentre or multi-year study; only cases occurring during the norovirus prevalent season (winter) were included in our study. In addition, we only analysed the epidemiological data of patients who visited the hospital, and patients who did not seek for medical advice at the hospital were not included in this study. In addition, we used

partial gene sequencing for the phylogenetic analysis; therefore, further analysis using whole-genome analysis is needed.

Conclusions

In conclusion, this study reported the epidemiological characteristics of norovirus from August 2019 to January 2020 in Guangzhou. The main genotypes of norovirus were GII.P31/GII.4 Sydney and GII.P16/GII.4 Sydney. Most of their strains had high homology with the epidemic strains of 2017-2019. We determined the clinical information of infected patients. Vomiting and diarrhoea were the main symptoms. These findings help us to understand the prevalence of norovirus strains in Guangzhou, and provide a valuable reference for the prevention, control, and treatment of norovirus in the future.

Material And Methods

Study population and specimen collection

A total of 168 children (age range: 2-60 months) diagnosed with AGE following norovirus infection at Guangdong Women and Children Hospital, from August 2019 to January 2020, were included in this study. AGE was defined as defaecation ≥3 times within 24 hours with trait changes (diluted watery stools), and/or vomiting ≥2 times within 24 hours [42]. All norovirus infection diagnoses were confirmed with molecular detection (described below).

Collection of clinical information

Clinical information (demographic characteristics, clinical symptoms, laboratory testing.) were collected on the participants.This study did not record any data about the patient's personal identity information since the collection process started and all patient names and other relative information were replaced with identifying numbers, therefore, the informed consent was not needed. The study was approved by the Ethics Committee of Guangdong Women and Children Hospital.

Norovirus RNA extraction and detection

Norovirus RNA was extracted from diarrhoea or vomitus samples using an RNA Extraction Kit (Tianlong, Xi'an, China), according to the manufacturer's instructions. Norovirus RNA was detected using a diagnostic kit for norovirus RNA (Land medical, Wuhan, China) on the ABI 7500 Fast Real-Time polymerase chain reaction (PCR) platform (Applied Biosystems, Foster, USA), and positive samples were further sequenced and typed.

Genotyping and phylogenetic analyses

Reverse transcription was performed using PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Beijing, China) to obtain norovirus cDNA. Norovirus belonging to genotypes GI and GII were amplified using primers MON432 and GISKF, MON431 and GIISKR, respectively [18]. The length of the PCR-

amplified products was expected to be 543bp for GI and 557bp for GII, including the partial RdRp gene located in ORF1 as well as the partial VP1 gene located in ORF2 (ORF1-2 junction) [8]. The amplified products were sent to Sangon Biotech (Sangon Biotech, Shanghai, China) for sequencing. To confirm the genotype of norovirus and to analyse the norovirus sequences at the ORF1-2 junction, we used the online Norovirus Typing Tool Version 2.0 (http://www.rivm.nl/mpf/norovirus/typingtool). The sequences were uploaded to GenBank (accession numbers: MT856488-MT856646) and are shown in the Additional file 1.

Multiple sequence alignment was performed using ClustalW in MEGA X, and the phylogenetic tree was constructed using the neighbour-joining method, with a bootstrap value of 1000 repetitions. All reference sequences were downloaded from GenBank.

Statistical analysis

The patient's clinical data were collated, imported, and analysed in IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, N.Y., USA). Qualitative data were expressed as frequency (percentage), and the chi-square test was used for analysis. Quantitative data were expressed as median and interquartile range (IQR), and t-test or one-way analysis of variance (ANOVA) was used to analyse data that conformed to the normal distribution, while the nonparametric rank-sum test was used for data that did not conform. Differences were defined as statistically significant when $P<0.05$.

Abbreviations

AGE, acute gastroenteritis; ORFs, open reading frames; PCR, polymerase chain reaction; IQR, interquartile range; ANOVA, analysis of variance; hs-CRP, high-sensitivity C-reactive protein; WBC, white blood cell; NEUT, neutrophils; LYMPH, lymphocytes; RBC, red blood cell; PLT, platelets; Na, sodium; Cl, chloride; LDH, lactate dehydrogenase; AST, aspartate aminotransferase (AST), HBDH, hydroxybutyrate dehydrogenase; CK-MB, creatine kinase isoenzyme; FOBT, foecal occult blood test; IQR, interquartile range; HBGAs, histo-blood group antigens

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of Guangdong Women and Children Hospital, Guangzhou, China.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the GenBank repository under accession numbers MT856488-MT856646. The datasets generated and/or analysed during the current study are also available in Additional files 1.

Competing interests

The authors declare that they have no competing interests.

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Author Contributions

Conceptualization and design, M.L., X.Y.; experiments performed, L.D., J.X.; sample collection, C.Z., H.Z.; clinical data collection and analysis, W.Z., H.L., M.W., Y.T.; experiments data analysis, M.L., L.D., J.X., X.Y., W.Z.; writing—original draft preparation, L.D., J.X.; writing—review and editing, M.L. All authors have read and agreed to the published version of the manuscript.

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References

1. Ahmed SM, Hall AJ, Robinson AE, Verhoef L, Premkumar P, Parashar UD, et al. Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. Lancet Infect Dis. 2014;14(8):725-30.
2. Pires SM, Fischer-Walker CL, Lanata CF, Devleesschauwer B, Hall AJ, Kirk MD, et al. Aetiology-Specific Estimates of the Global and Regional Incidence and Mortality of Diarrhoeal Diseases Commonly Transmitted through Food. PLoS One. 2015;10(12):e0142927.
3. Bartsch SM, Lopman BA, Ozawa S, Hall AJ, Lee BY. Global Economic Burden of Norovirus Gastroenteritis. PLoS One. 2016;11(4):e0151219.
4. Green KY. Norovirus infection in immunocompromised hosts. Clin Microbiol Infect. 2014;20(8):717-23.

5. O'Brien SJ, Donaldson AL, Iturriza-Gomara M, Tam CC. Age-Specific Incidence Rates for Norovirus in the Community and Presenting to Primary Healthcare Facilities in the United Kingdom. J Infect Dis. 2016;213 Suppl 1(Suppl 1):S15-8.
6. Wang X, Yong W, Shi L, Qiao M, He M, Zhang H, et al. An outbreak of multiple norovirus strains on a cruise ship in China, 2014. J Appl Microbiol. 2016;120(1):226-33.
7. Vinje J. Advances in laboratory methods for detection and typing of norovirus. J Clin Microbiol. 2015;53(2):373-81.
8. Kroneman A, Vega E, Vennema H, Vinje J, White PA, Hansman G, et al. Proposal for a unified norovirus nomenclature and genotyping. Arch Virol. 2013;158(10):2059-68.
9. Adler JL, Zickl R. Winter vomiting disease. J Infect Dis. 1969;119(6):668-73.
10. Bull RA, White PA. Mechanisms of GII.4 norovirus evolution. Trends Microbiol. 2011;19(5):233-40.
11. Zhou H-L, Zhen S-S, Wang J-X, Zhang C-J, Qiu C, Wang S-M, et al. Burden of acute gastroenteritis caused by norovirus in China: A systematic review. J Infect. 2017;75(3):216-24.
12. Jin M, Wu S, Kong X, Xie H, Fu J, He Y, et al. Norovirus Outbreak Surveillance, China, 2016-2018. Emerg Infect Dis. 2020;26(3):437-45.
13. Ao Y, Cong X, Jin M, Sun X, Wei X, Wang J, et al. Genetic Analysis of Reemerging GII.P16-GII.2 Noroviruses in 2016-2017 in China. J Infect Dis. 2018;218(1):133-43.
14. Haddadin Z, Batarseh E, Hamdan L, Stewart LS, Piya B, Rahman H, et al. Characteristics of GII.4 Norovirus versus other Genotypes in Sporadic Pediatric Infections in Davidson County, Tennessee, USA. Clin Infect Dis. 2020.
15. Karst SM, Wobus CE, Goodfellow IG, Green KY, Virgin HW. Advances in norovirus biology. Cell Host Microbe. 2014;15(6):668-80.
16. Siebenga JJ, Vennema H, Zheng D-P, Vinje J, Lee BE, Pang X-L, et al. Norovirus illness is a global problem: emergence and spread of norovirus GII.4 variants, 2001-2007. J Infect Dis. 2009;200(5):802-12.
17. Wang X, Wei Z, Guo J, Cai J, Chang H, Ge Y, et al. Norovirus Activity and Genotypes in Sporadic Acute Diarrhea in Children in Shanghai During 2014-2018. Pediatr Infect Dis J. 2019;38(11):1085-9.
18. Cannon JL, Barclay L, Collins NR, Wikswo ME, Castro CJ, Magana LC, et al. Genetic and Epidemiologic Trends of Norovirus Outbreaks in the United States from 2013 to 2016 Demonstrated Emergence of Novel GII.4 Recombinant Viruses. J Clin Microbiol. 2017;55(7):2208-21.
19. van Beek J, Ambert-Balay K, Botteldoorn N, Eden JS, Fonager J, Hewitt J, et al. Indications for worldwide increased norovirus activity associated with emergence of a new variant of genotype II.4, late 2012. Euro Surveill. 2013;18(1):8-9.
20. Leshem E, Wikswo M, Barclay L, Brandt E, Storm W, Salehi E, et al. Effects and clinical significance of GII.4 Sydney norovirus, United States, 2012-2013. Emerg Infect Dis. 2013;19(8):1231-8.
21. Ruis C, Roy S, Brown JR, Allen DJ, Goldstein RA, Breuer J. The emerging GII.P16-GII.4 Sydney 2012 norovirus lineage is circulating worldwide, arose by late-2014 and contains polymerase changes that

may increase virus transmission. PLoS One. 2017;12(6):e0179572.

22. Bidalot M, Thery L, Kaplon J, De Rougemont A, Ambert-Balay K. Emergence of new recombinant noroviruses GII.p16-GII.4 and GII.p16-GII.2, France, winter 2016 to 2017. Euro Surveill. 2017;22(15):30508.
23. Xue L, Cai W, Gao J, Zhang L, Dong R, Li Y, et al. The resurgence of the norovirus GII.4 variant associated with sporadic gastroenteritis in the post-GII.17 period in South China, 2015 to 2017. BMC Infect Dis. 2019;19(1):696.
24. Arias A, Thorne L, Ghurburrun E, Bailey D, Goodfellow I. Norovirus Polymerase Fidelity Contributes to Viral Transmission. mSphere. 2016;1(5):e00279-16.
25. Lun JH, Hewitt J, Yan GJH, Enosi Tuipulotu D, Rawlinson WD, White PA. Recombinant GII.P16/GII.4 Sydney 2012 Was the Dominant Norovirus Identified in Australia and New Zealand in 2017. Viruses. 2018;10(10):548.
26. Ao Y, Wang J, Ling H, He Y, Dong X, Wang X, et al. Norovirus GII.P16/GII.2-Associated Gastroenteritis, China, 2016. Emerg Infect Dis. 2017;23(7):1172-5.
27. Wang H, Wang DH, Chen C, Lu Y, Li MX, Li TG, et al. Epidemiologic characteristics of outbreaks of three norovirus genotypes (GII.2, GII.17 and GII.4 Sydney) in Guangzhou, China, from 2012 to 2018. Epidemiol Infect. 2019;147:e207.
28. Marshall JA, Bruggink LD. The dynamics of norovirus outbreak epidemics: recent insights. Int J Environ Res Public Health. 2011;8(4):1141-9.
29. Barreira DMPG, Fumian TM, Tonini MAL, Volpini LPB, Santos RP, Ribeiro ALC, et al. Detection and molecular characterization of the novel recombinant norovirus GII.P16-GII.4 Sydney in southeastern Brazil in 2016. PLoS One. 2017;12(12):e0189504.
30. Ludwig S, Zarbock A. Coronaviruses and SARS-CoV-2: A Brief Overview. Anesth Analg. 2020;131(1):93-6.
31. Jaillon S, Berthenet K, Garlanda C. Sexual Dimorphism in Innate Immunity. Clin Rev Allergy Immunol. 2019;56(3):308-21.
32. Shioda K, Kambhampati A, Hall AJ, Lopman BA. Global age distribution of pediatric norovirus cases. Vaccine. 2015;33(33):4065-8.
33. Zhirakovskaia EV, Tikunov AY, Bodnev SA, Klemesheva VV, Netesov SV, Tikunova NV. Molecular epidemiology of noroviruses associated with sporadic gastroenteritis in children in Novosibirsk, Russia, 2003-2012. J Med Virol. 2015;87(5):740-53.
34. Mathew S, Alansari K, K Smatti M, Zaraket H, Al Thani AA, Yassine HM. Epidemiological, Molecular, and Clinical Features of Norovirus Infections among Pediatric Patients in Qatar. Viruses. 2019;11(5):400.
35. Prasad BVV, Hardy ME, Dokland T, Bella J, Rossmann MG, Estes MK. X-ray crystallographic structure of the Norwalk virus capsid. Science. 1999;286(5438):287-90.

36. Mallagaray A, Creutznacher R, Dulfer J, Mayer PHO, Grimm LL, Orduna JM, et al. A post-translational modification of human Norovirus capsid protein attenuates glycan binding. Nat Commun. 2019;10(1):1320.
37. Chen YFE, Wang CY, Chiu CH, Kong SS, Chang YJ, Chen SY. Molecular epidemiology and clinical characteristics of norovirus gastroenteritis with seizures in children in Taiwan, 2006-2015. Medicine (Baltimore). 2019;98(40):e17269.
38. Chen S-Y, Tsai C-N, Lai M-W, Chen C-Y, Lin K-L, Lin T-Y, et al. Norovirus infection as a cause of diarrhea-associated benign infantile seizures. Clin Infect Dis. 2009;48(7):849-55.
39. Ma X, Luan S, Zhao Y, Lv X, Zhang R. Clinical characteristics and follow-up of benign convulsions with mild gastroenteritis among children. Medicine (Baltimore). 2019;98(2):e14082.
40. Chen B, Cheng M, Hong S, Liao S, Ma J, Li T, et al. Clinical outcome of recurrent afebrile seizures in children with benign convulsions associated with mild gastroenteritis. Seizure. 2018;60:110-4.
41. Sanchez-Fauquier A, Gonzalez-Galan V, Arroyo S, Roda D, Pons M, Garcia J-J. Norovirus-associated encephalitis in a previously healthy 2-year-old girl. Pediatr Infect Dis J. 2015;34(2):222-3.
42. Liao Q, Ran L, Jin M, Cui S, Yuan J, Ma H, et al. Guidelines on outbreak investigation, prevention and control of norovirus infection. Zhonghua Yu Fang Yi Xue Za Zhi. 2016;50(1):7-16.

Figures

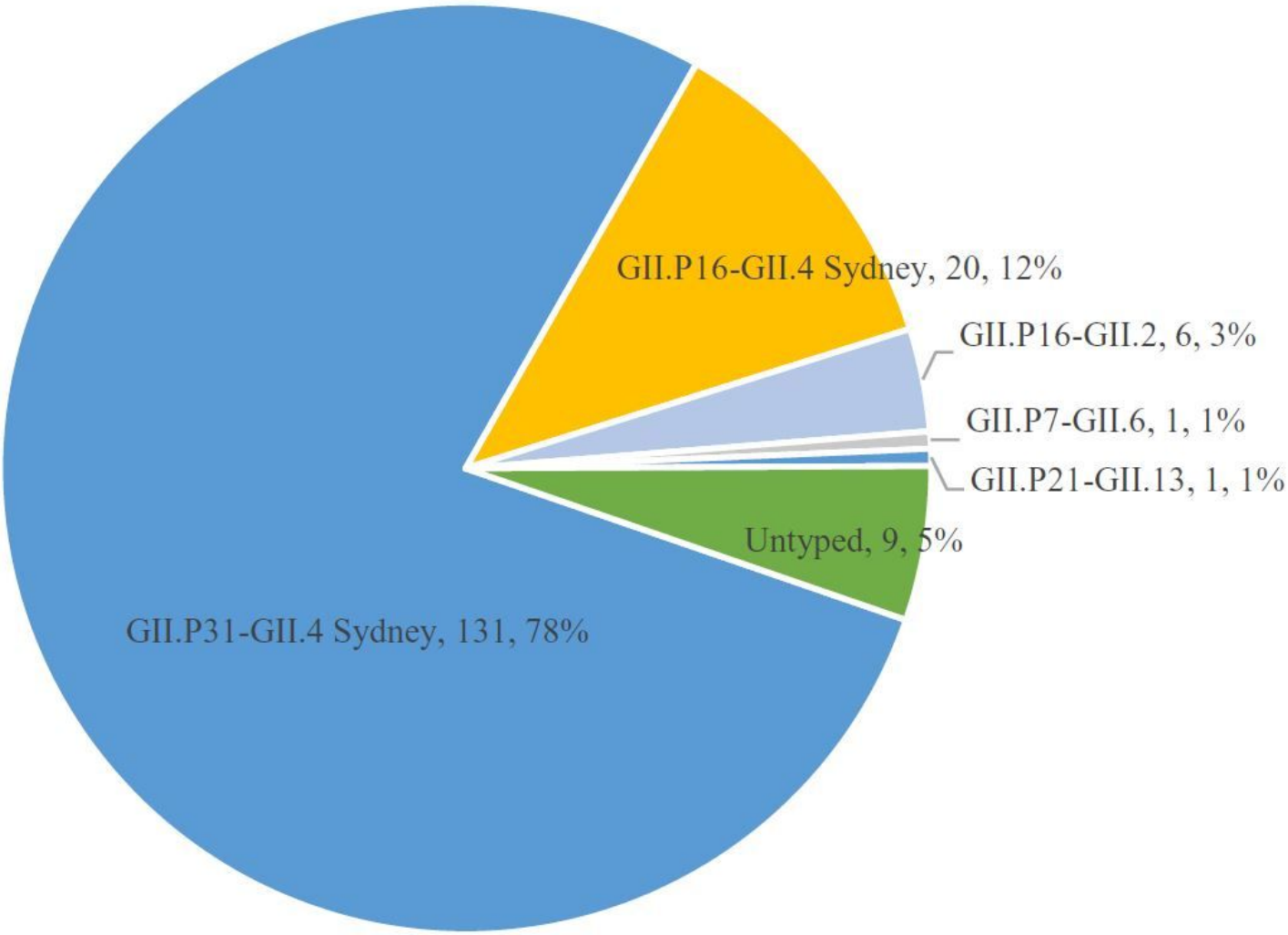

Figure 1

Distribution of norovirus genotypes associated with AGE in Guangzhou in winter 2019-2020

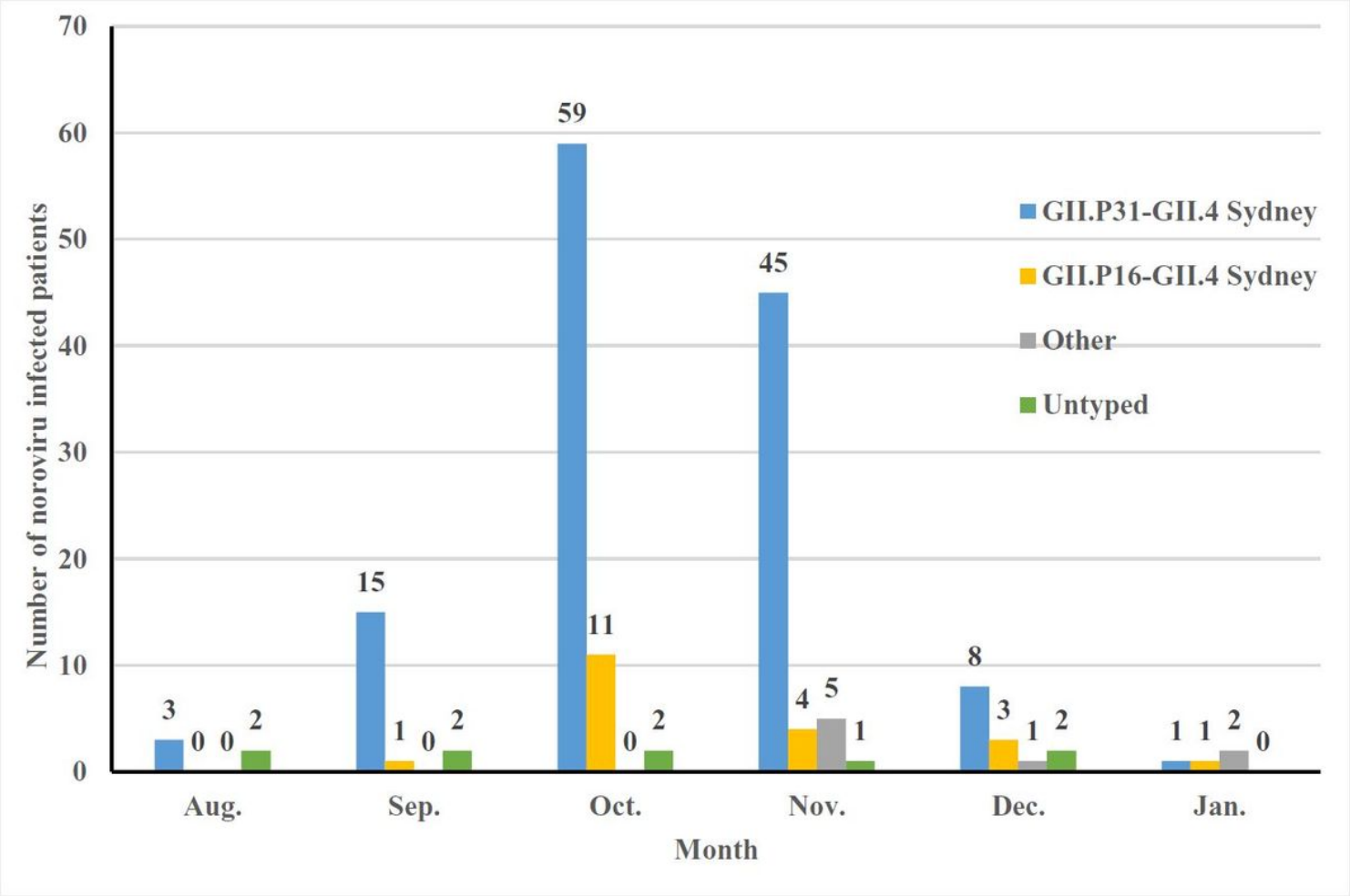

Figure 2

Monthly distribution of the children with norovirus infectious AGE in Guangzhou in winter 2019-2020. The “Others” group included three genotypes: GII.P16/GII.2, GII.P7/GII.6, and GII.P21/GII.13

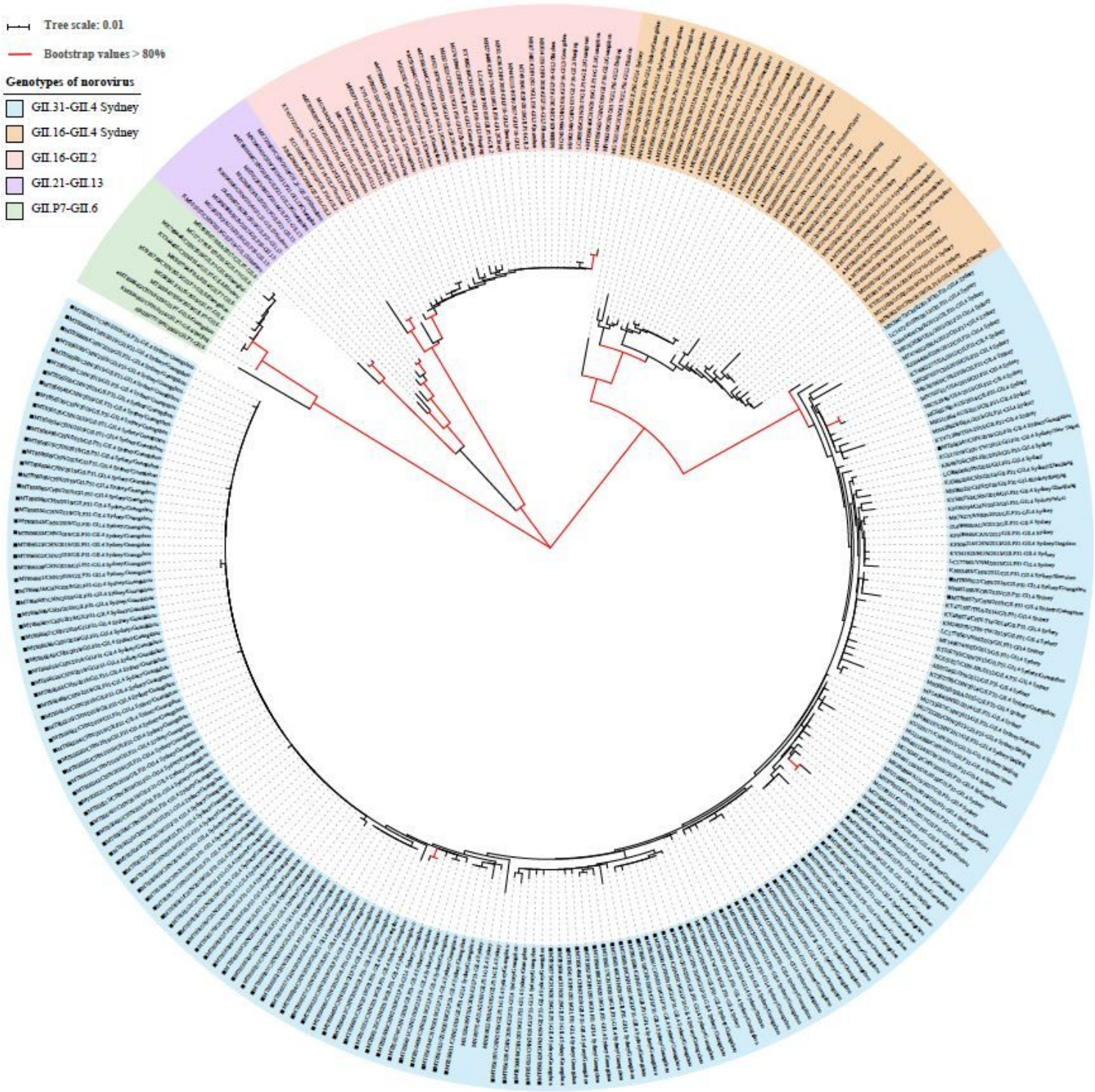

Figure 3

Phylogenetic analysis of the 445bp norovirus nucleotide sequence within the partial RdRp and VP1 genes. The norovirus strains detected in this study (MT856488-MT856646) and reference sequence retrieved from GenBank were constructed using the neighbour-joining method with bootstrap analysis of 1000 replicates using MEGA X. Bootstrap values >80% are shown in red. The nucleotide sequence in this study is presented as GenBank accession number/country/year of isolation/genotype. The sequences

obtained in this study are indicated as follows: ■, GII.P31-GII.4 Sydney; ▲, GII.P16-GII.4 Sydney; and ●, Others (including FII.P16-GII.2, GII.P21-GII13, GII.P7-GII.6).

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