RESEARCH ARTICLE

Molecular epidemiology of norovirus infections in children with acute gastroenteritis in 2017–2019 in Tianjin, China

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Abstract

Norovirus (NoV) is the leading cause of acute gastroenteritis (AGE) worldwide. Globally, the GII.4 Sydney 2012 strain has predominated since 2012, although GII.4 variant strains have caused AGE outbreaks in China. Recent patterns of NoV genotype distributions in 6011 children with AGE in Tianjin, China were investigated. NoV was detected using real-time reverse-transcriptase polymerase chain reaction and sequencing of partial sequences of the viral capsid gene. NoV genotypes were determined, and phylogenetic analysis was conducted. Epidemiological and clinical data were compared between children infected with different NoV genotypes. NoV was detected in 27.6% of the specimens tested. GII.4 strains comprised 49.4% infections, followed by GII.3 at 39.9%. Genotypes GII.2, GII.13, GII.17, GII.1, GII.6, and GII.14 were also detected. NoV was detected during most of the year, with a peak season of cases in the winter. Diarrhea, vomiting, fever, abdominal pain, and dehydration were present in patients with NoV infection. The main genotypes were GII.4 and GII.3, with a slight increase in GII.2, beginning in March 2017. Among the GII.4 strains, GII.4 Sydney 2012 was the only epidemic strain in Tianjin. Patients with GII.4 genotypes were more likely to present with diarrhea and vomiting than those with GII.3. Children with GII. Others were prone to suffered from dehydration and abdominal pain than those with GII.3. NoV GII has become the main cause of viral AGE in Tianjin, China. The predominant genotypes of NoV were GII.4 and GII.3. Identification of emerging genotypes is crucial for the prevention and control of NoV-caused AGE.

KEYWORDS
acute gastroenteritis, capsid, children, genetic diversity, genotype, norovirus

1 | INTRODUCTION

Norovirus (NoV) is considered to be the leading cause of human virus acute gastroenteritis (AGE) worldwide.[1] NoV was reported to be the second most important cause of AGE in children less than 5 years of age worldwide, following rotavirus.[2] It has been estimated that NoV is associated with 18% of all AGE cases and causes over 200,000 deaths in developing countries annually.[1] Its high morbidity in developing countries makes NoV infections an important public health issue with a substantial socioeconomic burden.[3] Following the

introduction of the rotavirus vaccine, NoV is now the leading cause of diarrheal illness requiring medical attention in children less than 5 years of age in the United States.[4]

The NoV single-stranded RNA genome is approximately 7.5 kb in length and is divided into three open reading frames (ORFs). ORF1 encodes the nonstructural viral proteins, including RNA-dependent RNA polymerase (RdRp). ORF2 encodes the major capsid protein (VP1), and ORF3 codes for the minor structural proteins (VP2).[5] NoV can be classified into at least seven recognized NoV genogroups (GI–GVII), based on the complete amino acid sequence of the capsid protein, with each genogroup subdivided into several genotypes.[6,7] Most infections in humans are caused by viruses of genogroups GI, which can be further divided into at least 9 genotypes, and GII, which includes 27 genotypes.[8]

Members of genogroup GII are the most predominant and strains of genotype GII.4 have caused the majority of NoV-related sporadic AGE worldwide since the mid-1990s.[9] Over the past two decades, new GII.4 variants have emerged every 2–3 years, which resulted in the six global epidemics including in United States 1995/96 in the late 1990s, Farmington Hills virus in 2002, Hunter virus in 2004, Den Haag 2006b virus in late 2007, New Orleans virus in 2009, and the current predominant GII.4 strain in circulation, Sydney 2012.[10] Some studies have found that non-GII.4 strains can also contribute significantly to the NoV disease burden. Recently, different genotypes (GII.17 and GII.2) emerged in China, Japan, and South Korea and replaced GII.4 strain predominance.[11–14] Meanwhile, related studies showed that GII.12, GII.1, and GI.6 viruses have been reported to cocirculate with GII.4 strains.[15]

To better estimate the burden of NoV infections in Tianjin, we examined the epidemiological and molecular characteristics of NoV in AGE patients in Tianjin, China. Our epidemiological surveillance was initiated in January 2017, with the aim of monitoring the frequency and diversity of variations of NoV genotypes in strains over time. In this study, data from the epidemiological surveillance of NoV infection among children with AGE in Tianjin between 2017 and 2019 were collected and analyzed. Findings from the study could be useful in the early detection of NoV infection, and in initiating rapid responses to potential NoV-AGE outbreaks.

2 | MATERIALS AND METHODS

2.1 | Patients and clinical samples

A total of 6011 stool specimens were collected from children who had been hospitalized with AGE at Tianjin Children's Hospital from January 2017 to December 2019. AGE was defined as follows: diarrhea (loose/watery stool) or vomiting with a frequency of three or more times in 24 h.[16] The age of the participants ranged from 1 month to 148 months. All stool specimens were stored at −80°C. The study was conducted according to the Helsinki guidelines and was approved by the Ethics Committee of Tianjin Children's Hospital.

2.2 | RNA extraction and viral detection

Stool samples were diluted to 10% suspension with phosphate-buffered saline and then clarified by centrifugation at 3000g for 10 min. Viral RNA was extracted using QIAamp Viral RNA Mini Kits (Qiagen) according to the manufacturer's instructions, and stored at −80°C. NoV was detected using the Probe real-time polymerase chain reaction (RT-PCR) Kit (Langde) on a 7500 Real-Time PCR platform (ABI). The final volume for each reaction was 50 μl. Each reaction contained 35.8 μl of the reaction mixture, 4.2 μl of enzyme mix, and 10 μl of RNA. The amplification conditions were set as follows: reverse transcription 42°C for 30 min, followed by 95°C for 3 min, then 40 cycles of 95°C for 10 s, 60°C for 1 min. Targets with cycle threshold values below 36 were considered to be positive for that particular sample.

2.3 | Genotyping of NoV

To identify the NoV genotype, positive samples were analyzed using conventional reverse- transcription polymerase chain reaction (RT-PCR), using TaKaRa Ex Taq Hot Start RT-PCR Kits (TaKaRa Shuzou). Partial capsid genes of NoV-positive samples were amplified using the primer sets G1SKF/G1SKFR, to amplify 331 bp for NoV GI, and G2SKF/G2SKR to amplify 345 bp for the GII genogroup.[17] Each 25 μl reaction mixture contained 2.5 μl of 10× Ex Taq buffer, 2 μl of dNTPs (2.5 mM), 1 μl of each primer, 0.25 μl of Taq DNA polymerase (5 U/μl), and 5 μl of complementary DNA (cDNA), with 13.25 μl of ddH$_2$O added, to reach a volume of 25 μl. PCR was performed under the following conditions: 94°C for 5 min followed by 35 cycles, 94°C for 3 min, 55°C for 45 s, 72°C for 1 min, and final extension at 72°C for 7 min. All RT-PCR products were analyzed using 1.5% agarose gel electrophoresis. The PCR products were purified and sent to the GENEWIZ Company for sequencing. The genotypes of all NoV strains successfully sequenced were determined by nucleotide sequence analysis using the online Norovirus Typing Tool Version 2.0 (https://www.rivm.nl/mpf/typingtool/norovirus/).

2.4 | Phylogenetic analysis

The phylogenetic relationships of NoV were analyzed by aligning sequences using ClustalW software with Kimura's parameter corrections for DNA or proteins. The phylogenetic tree of the nucleotide sequences of the capsid gene was constructed using the neighbor-joining method with MEGA Version 6.0 software and validated using 1000 bootstrap replicates.[18]

2.5 | Nucleotide sequence submission

The nucleotide sequence data used in this study had been submitted to the GenBank database and had been assigned accession

numbers MW8220022 to MW8220024, MW866524 to MW866528, MW866532 to MW866533, MW866536 to MW866537, MW866543 to MW866545, MW866554 to MW866556, and MW866541.

2.6 | Statistical analysis

Data analysis was carried out using SPSS software version 19.0. Categorical variables were compared using χ^2 tests, and continuous variables were compared using t tests. p values <0.05 were considered to be statistically significant.

3 | RESULTS

3.1 | Detection of NoV infection in AGE patients

In total, 6011 AGE patients were recruited to our study between January 2017 and December 2019. The detection rate of NoV was 27.6%. NoV-AGE occurred most frequently in the periods from November to February of the following year and reached a peak in December every year (Figure S1). For all 3 years, the genogroup of the AGE cases caused by NoV was GII (Table 1).

3.2 | Epidemiological and clinical characteristics of NoV infection in AGE patients

Among the 6011 children with AGE enrolled in the study, 3674 (61.1%) were male and 2337 (38.9%) were female. The median age of the children with AGE was 12 months. NoV was detected at a higher rate in males (27.7%, 1018/3674) than females (27.4%, 640/2337), but this difference was not significant ($p = 0.785$).

In the study, AGE cases were divided into five age groups: 0–6, 290 (22%); 7–12 months, 830 patients (32%); 13–36 months, 352 (30.1%); 37–84 months, 127 (20.2%); and over 85 months, 59 (19.3%). Among all age groups, the NoV detection rate was highest in the 7–12 months group, and the difference was statistically significant ($\chi^2 = 77.744$, $p < 0.001$).

The annual detection rates of NoV were 31.8% (241/758) in 2017, 28.5% (608/2137) in 2018, and 26% (809/3116) in 2019. NoV was more frequently detected in 2017 than in 2018 and 2019 ($\chi^2 = 11.631$, $p = 0.003$). According to the characteristics of the climate in China, 1 year is divided into four seasons: spring (March–May), summer (June–August), autumn (September–November), and winter (December-February). The seasonal pattern of NoV was analyzed, and GII NoV infection was prevalent in all seasons from 2017 to 2019. The highest NoV detection rate was in winter (39.8%, 788/1981), followed by autumn (29.2%, 335/1147), spring (25.6%, 429/1674), and summer (8.8%, 106/1209), and there was also a significant difference in NoV detection rate across seasons

TABLE 1 Genogroups of NoV detected in AGE patients from 2017 to 2019 in Tianjin, China

Date	AGE cases	AGE NoV infections	GII n	%
17-Jan	30	20	13	65
17-Feb	51	19	12	63.2
17-Mar	73	16	12	75
17-Apr	66	14	14	100
17-May	71	15	15	100
17-Jun	72	14	10	71.4
17-Jul	45	4	4	100
17-Aug	31	4	3	75
17-Sep	14	2	2	100
17-Oct	32	5	3	60
17-Nov	90	40	11	27.5
17-Dec	183	88	64	72.7
18-Jan	198	66	42	63.6
18-Feb	179	41	25	61
18-Mar	224	71	43	60.6
18-Apr	194	47	19	40.4
18-May	153	31	20	64.5
18-Jun	160	24	12	50
18-Jul	127	5	0	0
18-Aug	102	4	2	50
18-Sep	73	8	1	12.5
18-Oct	106	22	3	13.6
18-Nov	276	123	70	56.9
18-Dec	345	166	72	43.4
19-Jan	421	186	83	44.6
19-Feb	362	114	55	48.2
19-Mar	345	114	49	43
19-Apr	276	74	22	30
19-May	272	47	16	34
19-Jun	260	35	6	8.6
19-Jul	206	12	0	0
19-Aug	206	4	0	0
19-Sep	178	16	4	25
19-Oct	155	36	16	44.4
19-Nov	223	83	41	49.4
19-Dec	212	88	21	23.9
Total	6011	1658	785	47.3

Abbreviations: AGE, acute gastroenteritis; NoV, norovirus.

(χ^2 = 366.485, p = 0.000). Clinical and epidemiological data are given in Table 2.

strain was first detected in a case reported in January 2018 and was found only in 2018 (Figure 1).

3.3 | Composition of GII NoV genotypes in AGE patients

A total of 785 NoV strains among 1658 NoV-positive patients were further genotyped by sequencing the partial VP1. Eight genotypes were detected over the 3 years of the study (Table S1). The genotype diversity was dominated by the GII.4 strain at 49.4%, followed by GII.3 at 39.9%, GII.2 at 7.0%, GII.13 at 1.3%, GII.17 at 1.0%, GII.1 at 0.8%, GII.6 at 0.5%, and GII.14 at 0.1%. Genotype GII.4 Sydney 2012 was the only variant among GII.4 positive samples detected in the study from 2017 to 2019.

These strains dominated the NoV-AGE epidemics jointly or in turn. The annual distribution of genotypes did not show major fluctuations over time, except for an increase in GII.4 in 2017 and GII.3 in 2018. Although GII.4 was the dominant strain over all four seasons from 2017 to 2019 in Tianjin, an unusual pattern was observed in 2018, when the GII.3 genotype was the most prevalent. The GII.6

3.4 | Epidemiological and clinical characteristics of NoV-AGE patients with different genotypes

Of 785 NoV-positive samples successfully genotyped, the most common two genotypes, GII.3 and GII.4 were found from 2017 to 2019. The genotype distribution pattern differed according to the gender of the NoV-AGE patients, but the difference was not statistically significant (χ^2 = 2.440, p = 0.295). However, the genotype distribution was significantly different between the age groups (χ^2 = 75.2, p = 0.000) (Table 3).

The distribution of genotypes was differed significantly over time across the 3 years (χ^2 = 44.540, p = 0.000). GII.3 was mainly found in 2018, and GII.4 in 2019. Although NoV infections occurred throughout the year, the genotype distribution pattern showed significant seasonal variations across 3 years (χ^2 = 245.263, p = 0.000). A major seasonal trend was observed in NoV GII.4 and GII.3, which

TABLE 2 Demographic characteristics of the NoV-AGE patients in Tianjin, 2017–2019

	AGE patients (n)	NoV-AGE patients (n)	Detection rate (%)	χ^2	p value
Gender					
Male	3674	1018	27.7	0.074	0.785
Female	2337	640	27.4		
Age-group					
0–6 months	1317	290	22		
7–12 months	2590	830	32		
13–36 months	1169	352	30.1		
37–84 months	629	127	20.2	77.744	0.000
≥85 months	306	59	19.3		
Year					
2017	758	241	31.8		
2018	2137	608	28.5	11.631	0.003
2019	3116	809	26		
Season					
Spring (Mar–May)	1674	429	25.6		
Summer (Jun–Aug)	1209	106	8.8		
Autumn (Sep–Nov)	1147	335	29.2	366.485	0.000
Winter (Dec–Feb)	1981	788	39.8		
Total	6011	1658			

Note: The bold values indicates $p < 0.05$.

Abbreviations: AGE, acute gastroenteritis; NoV, norovirus
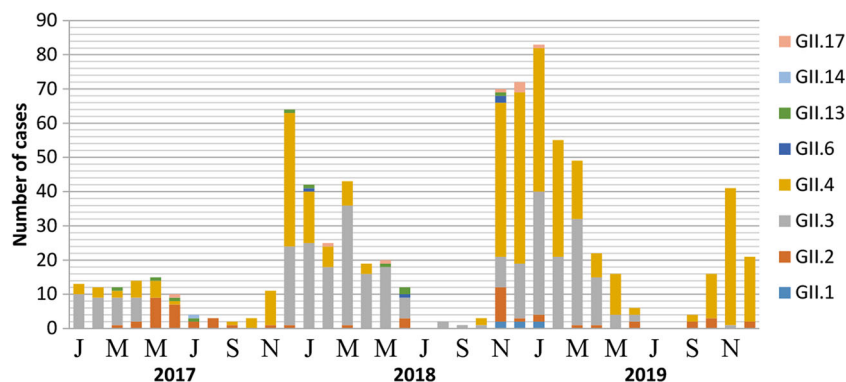
FIGURE 1 Monthly distribution of NoV genotypes in children with AGE in Tianjin from 2017 to 2019. NoV, Norovirus

TABLE 3 Demographic characteristics of GII NoV-AGE patients with different genotypes

	GII.3 (n, %)	GII.4 (n, %)	GII. Others (n, %)	χ^2	p value
Age-group					
0–6 months	76 (24.3)	35 (9.0)	8 (9.5)		
7–12 months	146 (46.6)	219 (56.4)	35 (41.7)	75.2	0.000
13–36 months	70 (22.4)	100 (25.8)	18 (21.4)		
37–84 months	14 (4.5)	28 (7.2)	12 (14.3)		
≥85 months	7 (2.2)	6 (1.6)	11 (13.1)		
Gender					
Male	192 (61.3)	237 (61.1)	44 (52.4)	2.440	0.295
Female	121 (38.7)	151 (38.9)	40 (47.6)		
Year					
2017	57 (18.2)	72 (18.6)	34 (40.5)		
2018	147 (47)	128 (33.0)	34 (40.5)	44.540	0.000
2019	109 (34.8)	188 (48.5)	16 (19.0)		
Season					
Spring (Mar–May)	133 (42.5)	58 (14.9)	19 (22.6)		
Summer (Jun–Aug)	10 (3.2)	3 (0.8)	24 (28.6)	245.263	0.000
Autumn (Sep–Nov)	12 (3.8)	116 (29.9)	23 (27.4)		
Winter (Dec–Feb)	158 (50.5)	211(54.4)	18 (21.4)		
Total	313	388	84		

Note: The bold values indicates $p < 0.05$.

Abbreviations: AGE, acute gastroenteritis; NoV, norovirus

peaked in the winter, while other GII genotypes were more prevalent in the summer (Table 3).

Further studies indicated that the genotype played a role in the severity of vomiting among the different groups ($\chi^2 = 13.581$, $p = 0.009$). Patients with genotype GII.4 were more likely to have diarrhea and vomiting than those with genotype GII.3 ($\chi^2 = 13.366$, $p = 0.01$; $\chi^2 = 25.804$, $p = 0.000$). However, children with other GII genotypes were more likely to suffer from dehydration and abdominal pain than those with GII.3 genotype ($\chi^2 = 12.475$, $p = 0.002$; $\chi^2 = 9.395$, $p = 0.009$) (Table 4).

3.5 | Phylogenetic relationships among GII strains of NoV

A phylogenetic tree was constructed with 41 sequences from the strains collected from the study and 51 additional reference sequences collected worldwide. The partial VP1 sequences of the majority of strains identified in the present study were highly similar to the corresponding sequences of NoV strains that circulated worldwide. Figure 2 shows that 41 representative strains were highly homologous and clustered together, whereas genotype-specific genetic clusters

TABLE 4 Clinical manifestation of GII NoV-AGE patients with different genotypes

	GII.3 (n, %)	GII.4 (n, %)	GII. Others (n, %)	χ^2	p value
Diarrheal					
Yes	164 (52.4)	254 (65.5)	45 (53.6)	13.366	**0.01**[a]
No	149 (47.6)	134 (34.5)	39 (46.4)		
Severity of diarrheal					
1–2 times per day	95 (57.9)	120 (47.2)	18 (40)		
3–4 times per day	28 (17.1)	64 (25.2)	14 (31.1)	7.985	0.092
>5 times per day	41 (25)	70 (27.6)	13 (28.9)		
Vomiting					
Yes	122 (39.0)	226 (58.2)	43 (51.2)	25.804	**0.000**[b]
No	191 (61.0)	162 (41.8)	41 (48.8)		
Severity of vomiting					
1–2 times per day	80 (65.6)	110 (48.7)	26 (60.5)		
3–4 times per day	23 (18.9)	44 (19.5)	5 (11.6)	13.581	**0.009**
>5 times per day	19 (15.6)	72 (31.8)	12 (27.9)		
Fever (>38°C)					
Yes	191 (61.0)	231 (59.5)	40 (47.6)	5.061	0.080
No	122 (39.0)	157 (40.5)	44 (52.4)		
Dehydration					
Yes	2 (0.6)	20 (5.2)	5 (6.0)	12.475	**0.002**[c]
No	311 (99.4)	368 (94.8)	79 (94.0)		
Abdominal pain					
Yes	7 (2.2)	22 (5.7)	**8 (9.5)**	9.395	**0.009**[d]
No	306 (97.8)	366 (94.3)	76 (90.5)		
Total	313	388	84		

Note: The bold values indicates $p < 0.05$.

[a]χ^2 test, $p < 0.05$ by Bonferroni method multiple comparison test with group, GII.3 versus GII.4.

[b]χ^2 test, $p < 0.05$ by Bonferroni method multiple comparison test with group, GII.3 versus GII.4.

[c]χ^2 test, $p < .0.05$ by Bonferroni method multiple comparison test with group, GII.3 versus GII.4, and GII.3 versus GII.other.

[d]χ^2 test, $p < 0.05$ by Bonferroni method multiple comparison test with group, GII.3 vs GII.Others.

were formed in GII.4, GII.3, GII.2, GII.17, GII.13, GII.6, GII.1, and GII.14 branches. The GII.4 Sydney 2012 strain dominated during 2017–2019, and the nine GII.4 Sydney 2012 strains were closely related to the reference strain KP784696.1, with 92.9%–99.3% nucleotide identity. Replacing the GII.4 Sydney 2012 strain, the GII.3 strain emerged in 2018 and was clustered closely with the reference strain KY433576.1, sharing 92.1%–99.6% nucleotide identity.

4 | DISCUSSION

Viral AGE remains an important cause of morbidity and mortality in developing countries. NoV is an important pathogen in humans, causing epidemics and sporadic AGE in both children and adults.[19]

In this study, 3 years of data on NoV prevalence, clinical outcomes, and genetic diversity among hospitalized children with AGE from January 2017 to December 2019 in Tianjin were analyzed. The combination of epidemiological, etiological, and molecular data analysis could provide comprehensive evidence to support NoV infection prevention and control measures.

Analysis of the data found that the proportion of NoV in AGE children was 27.6% in Tianjin, similar to results previously reported elsewhere in China.[20] NoV-AGE was prevalent throughout the year, and peaked in the winter, with a decline in summer, as previously reported.[21,22] A correlation between the seasonality of NoV infection and climatic conditions such as rainfall, humidity, and low temperatures has been proposed, due to the frequent transmission of NoV by waterborne sources, and high chances for human-to-human
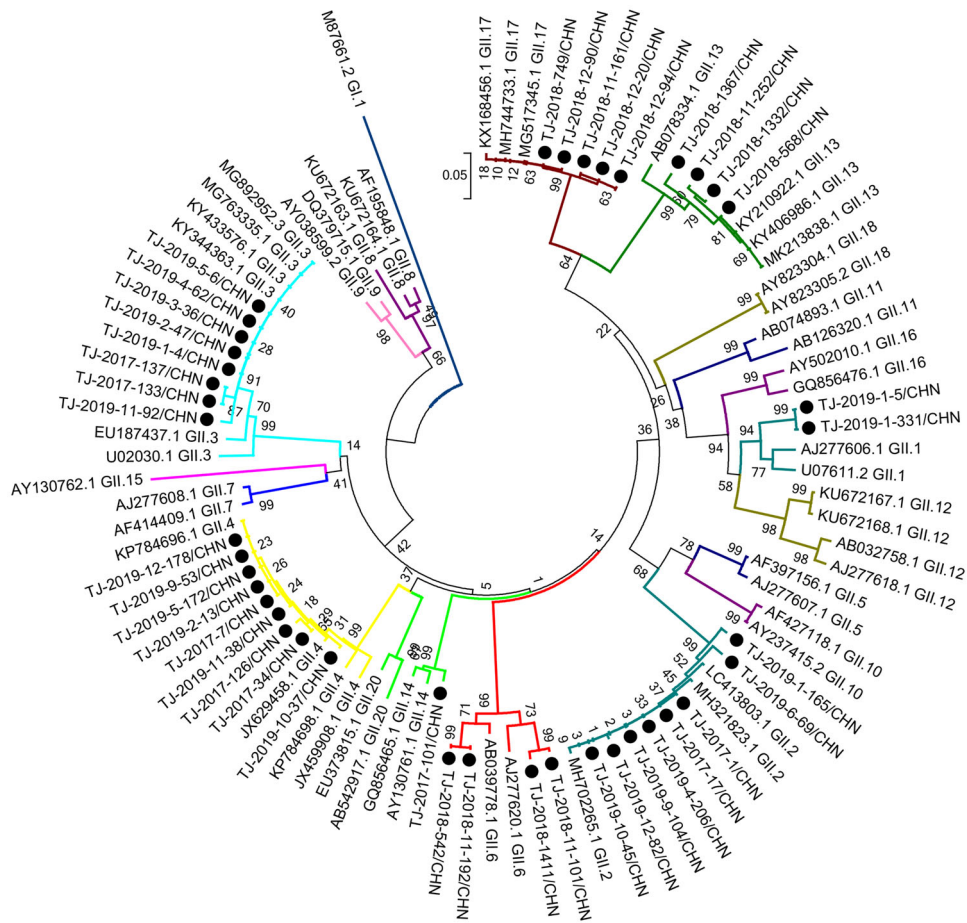
FIGURE 2 Phylogenetic analysis of partial nucleotide sequence of capsid gene of NoV strains detected in children with AGE in Tianjin, 2017–2019. The NoV strains detected in this study are indicated by black dots. The trees were constructed in MEGA 6.0 through the neighbor-joining method using the Kimura 2-parameter model. The bootstrap values (1000 replicates) are indicated in the phylogenetic tree. AGE, acute gastroenteritis; NoV, norovirus

transmission due to people crowding together.[23] In Tianjin, the seasonal increase of NoV-AGE started in October and usually lasted until March of the following year, consistent with the results of Xue et al.[24] In the study, NoV-positive patients less than 3 years old accounted for 88.9% of all age groups, and children aged from 7 to 12 months seemed particularly vulnerable to NoV infection, a finding which was consistent with previous studies.[25]

The circulating genotypes of NoV vary with time, place, and population in China, so these conditions might influence the size and severity of NoV-AGE epidemics. Since 2002, GII.4 viruses have been the most common genotypes circulating in China.[26] A new GII.4 NoV strain was identified in Australia in March 2012 and named GII.4 Sydney 2012. As the predominant strain of the GII.4 genotype, GII.4 Sydney 2012 and its variants have been widely reported since 2012,[27–29] and has caused AGE outbreaks in multiple countries.[30] This phenomenon might be explained by the fact that the GII.4 strain has a 1.7-fold higher rate of evolution on average within the capsid sequence, and a higher number of nonsynonymous changes than other NoVs, resulting in the emergence of a new strain of GII.4 every 2–3 years.[31] In this study, eight NoV GII genotypes—GII.4 (49.4%),

GII.3 (39.9%), GII.2 (7.0%), and GII.13 (1.3%)—were the four most prevalent strains in Tianjin from 2017 to 2019, a result which is similar to those reported from other studies.[26] Genotypic and phylogenetic analysis showed that GII.4 Sydney 2012 strains were highly homologous. They were closely related to South Africa strain KP784696.1 (GII.P4-GII.4), which indicated that GII.4 Sydney 2012 strains in our study might be recombinant strains.

Further studies were conducted to explore the epidemiological characteristics of the epidemic strains and provide a theoretical basis for the development of disease prevention strategies. Eight NoV genotypes were detected in this study. Each year, two to four genotypes cocirculated, with GII.4 strains detected most frequently. Children with GII.3 were concentrated into the 0–6 and 7–12 months groups, but children with GII.4 and GII. Others were more prevalent in the 7–12 and 13–36 months groups. The question of whether NoV genotype is linked to age-group in children remains to be answered. There were no significant differences in gender among children infected with different NoV genotypes, indicating that gender was not a susceptibility factor in NoV infection. NoV was detected throughout the year, but GII.4 and GII.3 dominated during the cold winter,

and GII. Others became the predominant genotypes during the summer. This trend is consistent with those identified in some of the published studies.[32] Patients infected with NoV in this study shared most of the common clinical symptoms of AGE, such as fever, vomiting, diarrhea, abdominal pain, and dehydration. However, the degree of severity varied between patients infected by different NoV genotypes. Patients with GII.4 genotypes were more likely to have diarrhea and vomiting than those with GII.3 genotypes. Children with GII. Others were more likely to suffer from dehydration and abdominal pain than those with GII.3 genotypes. Therefore, more clinical studies are necessary to conclusively identify the different clinical characteristics associated with infection with different NoV genotypes.

Our study had some limitations. First, NoV genotyping was conducted based only on the VP1 sequence. Other genes especially the RdRp gene should be analyzed to explain recombinant events and enhance the accuracy. Second, we only detected NoV in our study, other common gastroenteritis pathogens were not screened in our samples. Meanwhile, our study placed emphasis on molecular epidemiological analysis of NoV infection, which could only trace out the circulating patterns rather than capturing the genomic diversity. It would also become our research focus in the future.

In conclusion, this study reported an epidemiological and molecular investigation of NoV infection in children with AGE in Tianjin, China. NoV GII has been identified as the most frequent genogroup responsible for AGE in this area. Our study further found that genotype GII.4 was endemic, but genotypic diversity was high during the years of the study, suggesting that continuous surveillance is necessary, to monitor genotypes and the emergence of new strains, as a basis for the development of prevention and control strategies. Finally, further studies that include larger sample sizes from several regions of China are needed, to provide a better perspective into the strains circulating throughout the country.

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CONFLICT OF INTERESTS
The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS
All authors contributed to the intellectual content of this manuscript and approved the final manuscript as submitted. Yulian Fang designed the study, performed the analysis, and drafted the manuscript. Yanzhi Zhang and Hong Wang conceived and designed the experiments, analyzed all the data. Ouyan Shi and Mengzhu Hou performed a phylogenetic analysis of the sequence and statistical analysis. Jinying Wu, Lu Wang, and Wei Wang searched the literature and collected the clinical samples. Yu Zhao revised the article critically for important intellectual content.

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

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