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Research Article

Changing predominance of norovirus strains in children with acute gastroenteritis in Shanghai, 2018–2021

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ABSTRACT

Norovirus (NoV) is a major pathogen that causes acute gastroenteritis (AGE) in people of all ages, especially in children. In this study, we investigated the molecular epidemiological characteristics of NoV in children with AGE in Shanghai from 2018 to 2021. The overall detection rate of NoV was 11.9% (181/1545), with annual detection rates of 9.4% (36/381), 13.6% (29/213), 5.8% (13/226) and 14.2% (103/725), respectively. Of note, the prevalence of NoV in 2020 was significantly lower than that in 2018–2019 (10.9%, 65/594) ($P = 0.023$) and 2021 (14.2%, 103/725) ($P = 0.000$). The 181 NoV strains identified in this study were classified into the GI group (1.1%, 2/181), GII group (98.3%, 178/181) and GIX group (0.6%, 1/181) according to the VP1 gene. The most common NoV VP1 genotype was GII.4 Sydney_2012 (63.5%, 115/181), followed by GII.3 (19.9%, 36/181) and GII.2 (9.4%, 17/181). For P genotypes, 174 strains were sequenced successfully according to the RdRp gene, and the predominant genotype was GII.P16 (44.8%, 78/174), followed by GII.P31 (25.9%, 45/174) and GII.P12 (21.3%, 37/174). Among the 174 cases, GII.4 Sydney_2012[P16] (36.8%, 64/174) was the dominant genotype, followed by GII.4 Sydney_2012[P31] (25.3%, 44/174), GII.3[P12] (20.1%, 35/174) and GII.2[P16] (8.0%, 14/174). In particular, the dominant genotypes in Shanghai changed from GII.4 Sydney_2012[P31] in 2018–2019 to GII.4 Sydney_2012[P16] in 2020–2021. This is the first report to describe the epidemiological changes in NoV infection before and during the COVID-19 pandemic in Shanghai. These data highlight the importance of continuous surveillance for NoV in children with AGE in Shanghai.

1. Introduction

Norovirus (NoV) is the predominant etiological agent of acute gastroenteritis worldwide and causes high morbidity and mortality (Bartsch et al., 2016; Kendra et al., 2022). Since it was first identified in 1972, NoV has been identified as a major pathogen in both outbreaks and sporadic cases of acute gastroenteritis (AGE) in people of all ages, particularly children under 5 years of age and elderly individuals (Robilotti et al., 2015; Lopman et al., 2016; Saupe et al., 2021). It has been reported that NoV is responsible for approximately 699 million cases of AGE and an estimated 219,000 deaths per year worldwide, with a substantial disease burden in developing countries (van Beek et al., 2018; Parra, 2019).

NoV belongs to the *Caliciviridae* family and is a small, non-enveloped, positive-sense, single-stranded RNA virus. The NoV genome contains three open reading frames (ORF1 to 3), of which ORF1 encodes a large polyprotein that is post-translationally cleaved into six nonstructural proteins, including RNA-dependent RNA polymerase (RdRp), which is critical for viral replication. ORF2 encodes the major structural protein (VP1), while ORF3 encodes the minor structural protein (VP2). VP1 consists of an N-terminal, a shell (S), and two protruding (P) domains (P1 and P2), with P2 having the most sequence variation and being critical in immune recognition and receptor binding (Campillay-Veliz et al., 2020). Based on the amino acid diversity of the complete *VP1* gene, NoV is further classified into 10 different genogroups comprising 48 genotypes [9 GI, 26 GII, 3 GIII, 2 GIV, 2 GV, 2 GVI and 1 genotype each for GVII, GVIII, GIX (formerly GII.15) and GX] (Chhabra et al., 2019, 2020). According to the nucleotide diversity in *RdRp*, NoV is classified into eight groups and 60 P genotypes (14 GI, 37 GII, 2 GIII, 1 GIV, 2 GV, 2 GVI, 1 GVII and 1 GX) (Chhabra et al., 2019). Of these, the GI, GII, GIV, GVIII

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and GIX groups can infect humans and cause AGE, with GI and GII are the most predominant genogroups associated with AGE in humans. Recently, a large number of studies have shown that NoV recombination often occurs near or within the ORF1 (RdRp)/ORF2 (VP1) junction, leading to the emergence of various recombinant strains worldwide (Kendra et al., 2022; Zheng et al., 2022; Vakulenko et al., 2023). For example, the recombinant NoV GII.2[P16] associated with AGE emerged in 2016–2017 and spread across Europe and many countries in Asia (Lu et al., 2017; Parra, 2019; Wang et al., 2021). During the same period, the new recombinant GII.4 Sydney_2012[P16] soon replaced GII.4 Sydney_2012 [P31] as the main cause of outbreaks and sporadic cases of AGE in North America and Oceania (Lun et al., 2018; Parra, 2019; Farahmand et al., 2022). Therefore, dual nomenclature using the genotypes of *RdRp* and *VP1* has been recommended for NoV genotyping to better understand the evolution and infection mechanisms of NoV.

From 2012 to 2017, the prevalent genotype of NoV among children with AGE in Shanghai was GII.4 Sydney_2012[P31] (Lu et al., 2019). However, studies on the prevalence of NoV among children with AGE in Shanghai after 2017 were scarce. To address this knowledge gap, we conducted this study to investigate the epidemiological characteristics of NoV in terms of the dynamics of NoV GI/GII strains, seasonal and age distributions, as well as clinical manifestations among NoV-infected children younger than 16 years with AGE in Shanghai from 2018 to 2021.

2. Materials and methods

2.1. Study population and stool sample collection

Between January 2018 and December 2021, 1545 stool samples were collected from children up to 16 years of age who visited the outpatient clinic for the first time during the course of their illness. These children were diagnosed with AGE at the Children's Hospital of Fudan University in Shanghai. AGE was defined by the presence of three or more loose, watery, thin stools with a pasty texture per day or the presence of mucous stools. These symptoms, possibly accompanied by vomiting, abdominal pain, and fever, persisted for less than two weeks. Demographic and clinical characteristics were collected from medical records.

2.2. Nucleic acid extraction, detection and sequencing of NoV

Stool suspensions were prepared at approximately 10% (w/v) in saline. Nucleic acids were extracted from 200 μL of clarified stool suspensions using the Viral Ex-DNA/RNA Kit (Xi'an TianLong Science and Technology Co., Ltd) and eluted in 80 μL of DEPC water according to the manufacturer's instructions. The extracted nucleic acids were subjected to random primer reverse transcription (RT) using the PrimeScript™ II Reverse Transcriptase [Takara, Biotechnology (Dalian) Co., Ltd]. For P genotypes, all the cDNA was amplified by PCR for GI/GII NoV using primers P289 and P290 targeting the partial region of the *RdRp* gene (Supplementary Table S1) (Lu et al., 2014). For *VP1* genotypes, the partial capsid region of the *VP1* gene was amplified in all the samples by PCR using two primer pairs, G1SKF/G1SKR for GI strains and G2SKF/G2SKR for GII/GIX strains (Supplementary Table S1) (Lu et al., 2019; Kojima et al., 2002). PCR was performed with an initial denaturation of 94 °C for 2 min, followed by 35 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s and 72 °C for 7 min with a final extension. All amplified PCR products were electrophoresed on 2.0% agarose gels containing Super GelBlue (Shanghai BioScience Co., Ltd.) and visualized using an automated gel image analysis system (Shanghai Tanon Life Science Co., Ltd.).

All amplifications of NoV-positive samples were sequenced by Sangon Biotech (Shanghai) Co, Ltd. Multiple sequence alignment was analyzed by MEGA 6.0 using the ClustalW method. Phylogenetic trees based on partial genes of norovirus *RdRp* and *ORF1* were generated using maximum likelihood (Kimura 2-parameter model) in MEGA 6.0. The reliability of the trees was assessed by 1000 bootstrap replicates.

2.3. Statistical analysis

Statistically significant differences in detection rates and proportions of categorical variables of different NoV genotypes were tested using Fisher's exact test, a two-tailed chi-squared test, or corrected chi-squared test in IBM SPSS Statistics software (version 20). Two-sided P values <0.05 were considered statistically significant.

3. Results

3.1. Detection of NoV in children with AGE

A total of 1545 stool samples were collected from children aged 0–16 years with AGE between 2018 and 2021. Of the 1545 children, 61.4% (949/1545) were male and 38.6% (596/1545) were female. Overall, 11.7% (181/1545) of samples were identified as NoV, with annual NoV detection rates of 9.4% (36/381) in 2018, 13.6% (29/213) in 2019, 5.8% (13/226) in 2020 and 14.2% (103/725) in 2021. Notably, the prevalence of NoV in 2020 (5.8%, 13/226) was significantly lower than that in 2018–2019 (10.9%, 65/594) ($P = 0.023$) and 2021 (14.2%, 103/725) ($P = 0.000$). The detection rates of NoV in male and female children were 11.7% (111/949) and 11.7% (70/596) ($P = 0.935$), respectively.

3.2. Age and seasonal distributions of NoV infections

Among the 181 NoV infections, 71.3% (129/181) occurred in children aged 0–36 months. The highest prevalence rate of NoV was 19.8% (73/369) among children aged 13–24 months (Fig. 1). The detection rate of NoV was similar among children aged up to 60 months (11.6%, 158/1362) and 61 months to 16 years (12.6%, 23/183) ($P = 0.713$). Further analysis revealed that NoV infections in Shanghai from 2018 to 2021 were predominantly in autumn and winter months (January–February and October–December), accounting for 63.5% (115/181) of all cases (Supplementary Fig. S1).

3.3. Distribution of NoV genotypes based on VP1 and RdRp genes

The VP1 capsid gene was successfully sequenced in all NoV-positive samples. Phylogenetic analysis showed that GII (98.4%, 178/181) was the most dominant genogroup, followed by GI (1.0%, 2/181) and GIX (0.6%, 1/181), based on the divergence of the *VP1* gene. Of all 181 NoV strains, nine VP1 genotypes were identified, including two GI genotypes (GI.1 and GI.3), six GII genotypes (GII.2, GII.3, GII.4, GII.6, GII.8 and GII.17) and one GIX genotype (GIX.1) (Fig. 2). Among the VP1

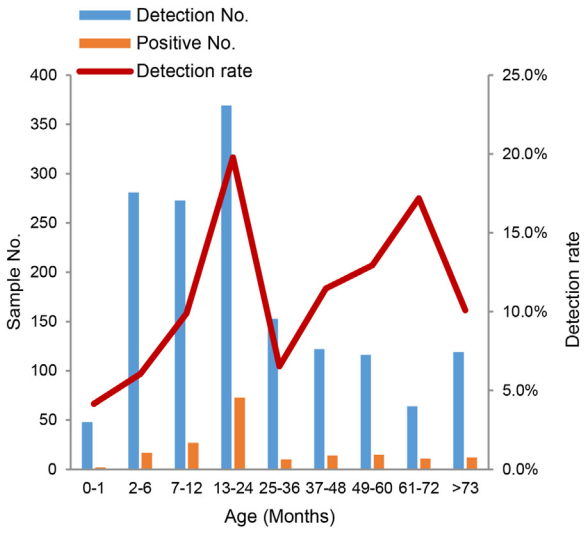

Fig. 1. NoV positive cases and detection rates among children with AGE in different age groups in Shanghai, 2018–2021.

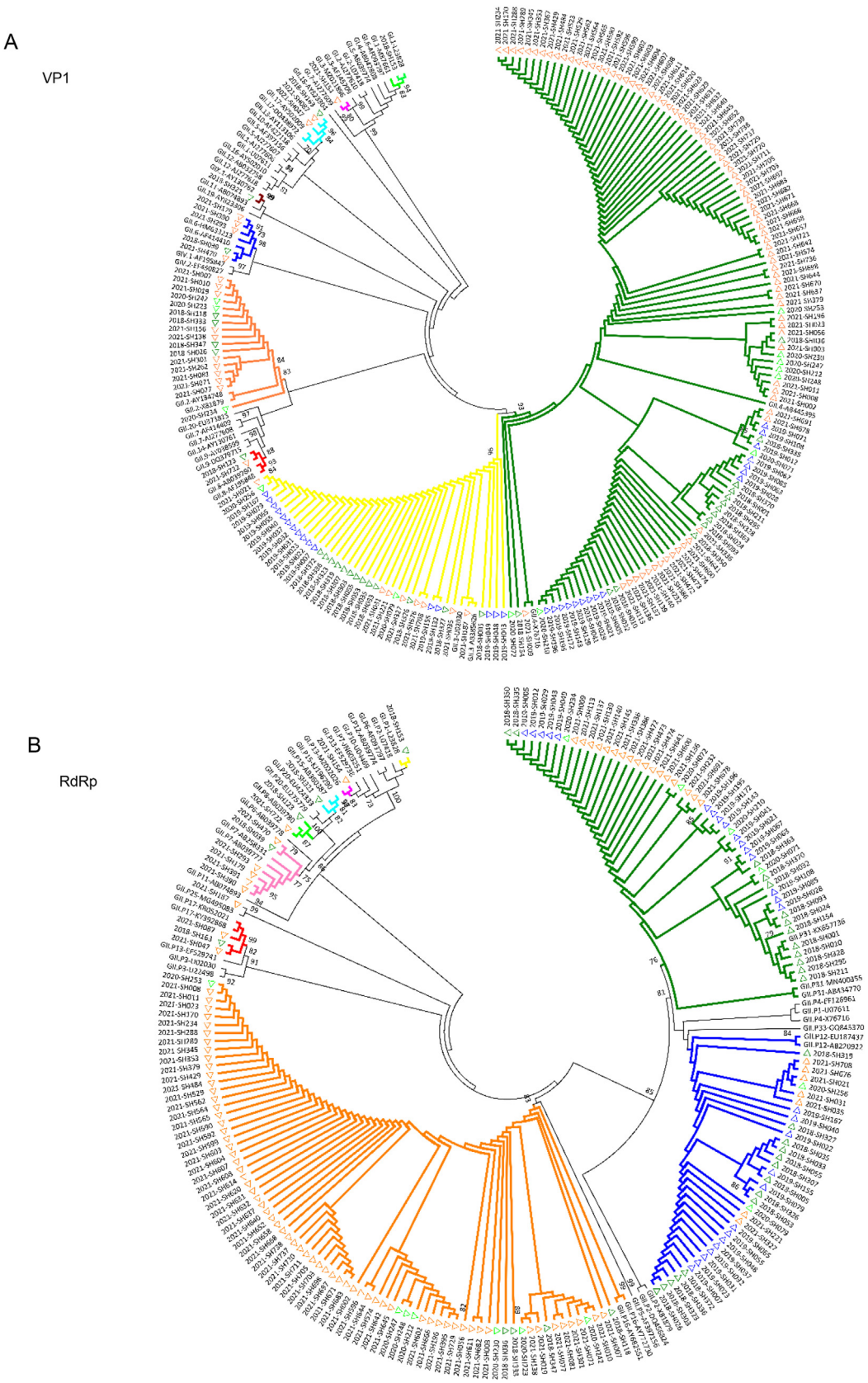

Fig. 2. Phylogenetic analysis of NoV according to partial sequences of the *VP1* (**A**) and *RdRp* genes (**B**). The trees were constructed in MEGA 6.0 through the maximum likelihood method using the Kimura 2-parameter model. The bootstrap values (1000 replicates) are indicated in the phylogenetic tree, and values less than 70% are not represented. Triangles represent the virus strains detected in this study.

genotypes, GII.4 was the most prevalent genotype (63.5%, 115/181), followed by GII.3 (19.9%, 36/181), and GII.2 (9.4%, 17/181) (Table 1). All of the GII.4 genotypes detected in this study were clustered into Sydney_2012 subtypes (Fig. 3). GII.4 Sydney_2012 was dominant each year from 2018 to 2021, while the other *VP1* genotypes showed a dynamic changing trend over the years. GII.3 was the second most dominant strain in 2018 (36.1%, 13/36) and 2019 (44.8%, 13/29), while the second most dominant strain in 2020 and 2021 was GII.2, accounting for 23.1% (3/13) and 9.7% (10/103), respectively (Table 1).

Nucleotide sequencing of the *RdRp* gene was successful in 174/181 (96.1%) of cases, of which 98.9% (172/174) were GII and 1.1% (2/174) were GI. The two GI genotypes were classified as GI.P1 and GI.P13, respectively. All 172 GII strains were genotyped into nine GII genotypes. The predominant genotype was GII.P16 (44.8%, 78/174), followed by GII.P31 (25.9%, 45/174) and GII.P12 (21.3%, 37/174). Notably, there was no evidence of GII.P4 prevalence in this study (Fig. 2). Due to the diversity of the P genotypes, the prevalence of P genotypes varied over the years. In 2018, eight P genotypes were identified, with GII.P12 (38.9%, 14/36) being the most common genotype, followed by GII.P31 (36.1%, 13/36) and GII.P16 (11.1%, 4/36). In contrast, only two definite P genotypes were detected in 2019, and the two genotypes of GII.P31 (51.9%, 14/27) and GII.P12 (48.9%, 13/27) had the same prevalence trend. To our surprise, GII.P16 became the most prevalent genotype in 2020 (53.8%, 7/13) and 2021 (68.4%, 67/98), whereas this genotype exhibited a low prevalence in 2018–2019. However, GII.P31 shifted to become the second most prevalent genotype in both 2020 (30.8%, 4/13) and 2021 (14.3%, 14/98) and was once the most prevalent genotype in Shanghai. In addition, GII.P12 was the third most common strain in both 2020 (15.4%, 2/13) and 2021 (8.2%, 8/98) (Table 1).

3.4. Genetic diversity of NoV strains in children with AGE

Of the 174 NoV strains that were successfully sequenced for both *RdRp* and *VP1* genes, 14 definite dual polymerase and capsid genotypes were identified, including 11 recombinant and three non-recombinant genotypes. The recombinant and non-recombinant strains accounted for 96.6% (168/174) and 3.4% (6/174), respectively. Among the 174 cases, GII.4 Sydney_2012[P16] (36.8%, 64/174) was the dominant genotype, followed by GII.4 Sydney_2012[P31] (25.3%, 44/174), GII.3 [P12] (20.1%, 35/174) and GII.2[P16] (8.0%, 14/174). Other genotypes exhibited sporadic prevalence, including GI.1[P1], GI.3[P13], GII.2 [P12], GII.2[P31], GII.3[P25], GII.4 Sydney_2012[P12], GII.6[P7], GII.8 [P8], GII.17[P17] and GIX.1[GII.P15]. Interestingly, similar to the prevailing trend of GII.4 Sydney_2012[P31], the GII.3[P12] strains were continuously detected throughout the study period (Table 1).

The predominant NoV strains varied each year from 2018 to 2021. In 2018, the detection rate of GII.3[P12] was the same as that of GII.4 Sydney_2012[P31], both accounting for 36.1% (13/36), followed by GII.2[P16] (8.3%, 3/36). GII.4 Sydney_2012[P31] (51.9%, 14/27) was the most common genotype in 2019, followed by GII.3[P12] (48.1%, 13/27). However, recombinant GII.4 Sydney_2012[P16] suddenly became the most dominant strain in 2020 (38.5%, 5/13) and 2021 (59.2%,

Table 1
Distribution of NoV genotypes detected in children with AGE in Shanghai in the years 2018–2021.

Genotypes	2018		2019		2020		2021		Total	
	n[a]	%[b]	n	%	n	%	n	%	n	%
VP1 genotypes										
GI.1	1	2.8	–	–	–	–	1	1.0	2	1.1
GII.2	4	11.1	–	–	3	23.1	10	9.7	17	9.4
GII.3	13	36.1	13	44.8	2	15.4	8	7.8	36	19.9
GII.4 Sydney_2012	14	38.9	16	55.2	8	61.5	77	74.8	115	63.5
GII.6	1	2.8	–	–	–	–	4	3.9	5	2.8
GII.8	1	2.8	–	–	–	–	1	1.0	2	1.1
GII.17	1	2.8	–	–	–	–	2	1.9	3	1.7
GIX.1	1	2.8	–	–	–	–	–	–	1	0.6
Total	36	100.0	29	100.0	13	100.0	103	100.0	181	100.0
RdRp genotypes										
GI.P1	1	2.8	–	–	–	–	–	–	1	0.6
GI.P13	–	–	–	–	–	–	1	1.0	1	0.6
GII.P6	–	–	–	–	–	–	4	4.1	4	2.3
GII.P7	1	2.8	–	–	–	–	–	–	1	0.6
GII.P8	1	2.8	–	–	–	–	1	1.0	2	1.1
GII.P12	14	38.9	13	48.1	2	15.4	8	8.2	37	21.3
GII.P15	1	2.8	–	–	–	–	–	–	1	0.6
GII.P16	4	11.1	–	–	7	53.8	67	68.4	78	44.8
GII.P17	1	2.8	–	–	–	–	2	2.0	3	1.7
GII.P25	–	–	–	–	–	–	1	1.0	1	0.6
GII.P31	13	36.1	14	51.9	4	30.8	14	14.3	45	25.9
VP1 [RdRp] genotypes										
GI.1[P1]	1	2.8	–	–	–	–	–	–	1	0.6
GI.1[P13]	–	–	–	–	–	–	1	1.0	1	0.6
GII.2[P12]	1	2.8	–	–	–	–	–		1	0.6
GII.2[P16]	3	8.3	–	–	2	15.4	9	9.2	14	8.0
GII.2[P31]	–	–	–	–	1	7.7	–		1	0.6
GII.3[P12]	13	36.1	13	48.1	2	15.4	7	7.1	35	20.1
GII.3[P25]	–	–	–	–	–	–	1	1.0	1	0.6
GII.4 Sydney_2012[P12]	–	–	–	–	–	–	1	1.0	1	0.6
GII.4 Sydney_2012[P16]	1	2.8	–	–	5	38.5	58	59.2	64	36.8
GII.4 Sydney_2012[P31]	13	36.1	14	58.9	3	23.1	14	14.3	44	25.3
GII.6[P7]	1	2.8	–	–	–	–	4	4.1	5	2.9
GII.8[P8]	1	2.8	–	–	–	–	1	1.0	2	1.1
GII.17[P17]	1	2.8	–	–	–	–	2	2.0	3	1.7
GIX.1[GII.P15]	1	2.8	–	–	–	–	–	–	1	0.6
Total	36	100.0	27	100.0	13	100.0	98	100.0	174	100.0

[a] n, number of NoV-positive.
[b] %, positive rates.

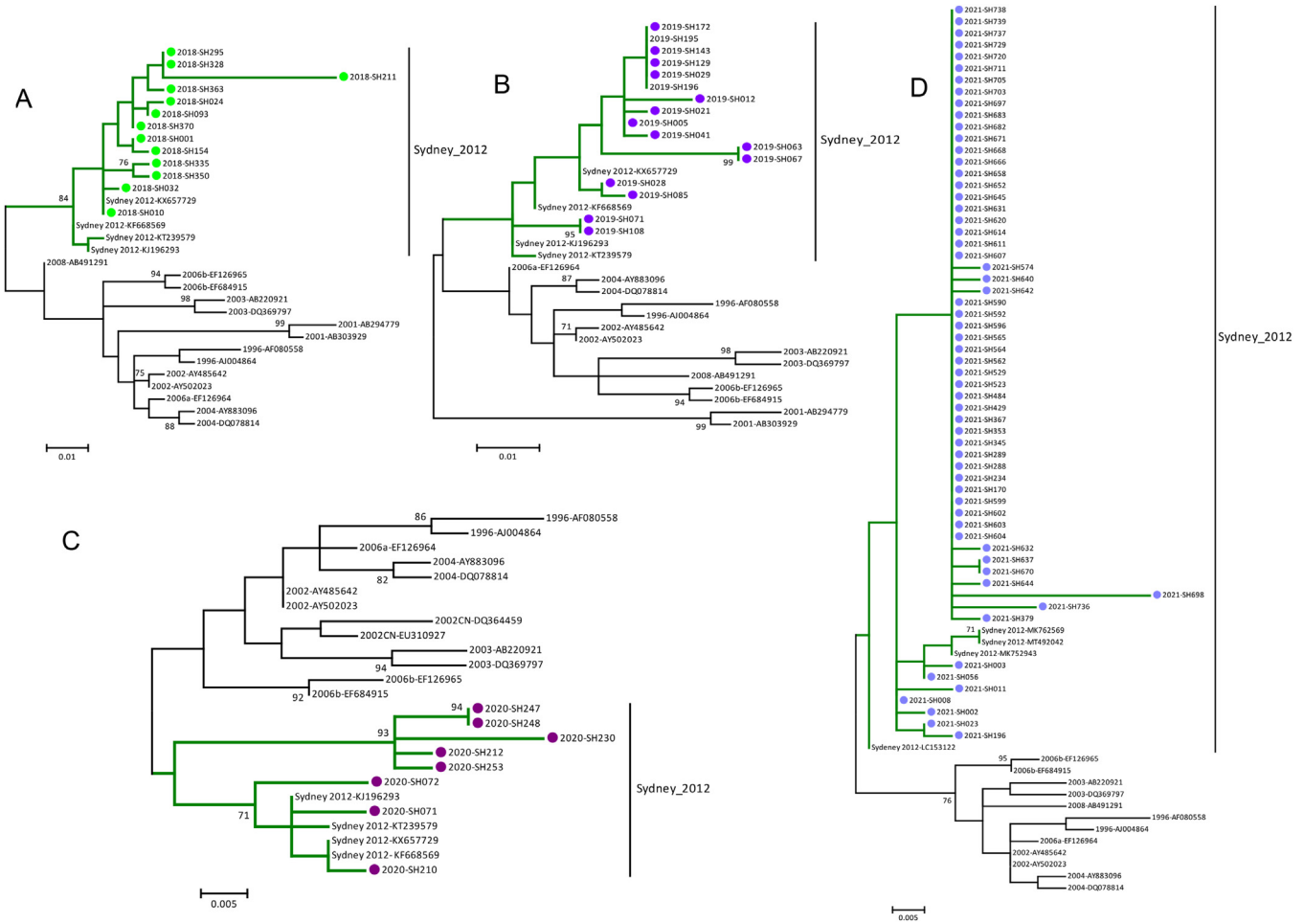

Fig. 3. Phylogenetic analysis of GII.4 variants based on the partial nucleotide sequences of the *VP1* gene. Solid circles represent the virus strains detected in this study. **A**, **B**, **C**, and **D** represent 2018, 2019, 2020, and 2021, respectively.

58/98), despite its initial identification in 2018. The secondary dominant genotype was GII.4 Sydney_2012[P31] in both 2020 (23.1%, 3/13) and 2021 (14.3%, 14/98). The remaining genotypes of NoV were sporadically prevalent throughout the study years (Table 1).

3.5. Gender and age distributions of NoV genotypes in children with AGE

There were 13 and 8 NoV genotypes detected in male and female children, respectively ($P = 0.135$). The predominant combination genotypes of NoV detected in males and females were similar (Supplementary Table S2). Regarding age distribution, the main prevalent genotypes GII.3[P12], GII.4 Sydney_2012[P31], GII.4 Sydney_2012 [P16] and GII.2[P16] were detected in almost all age groups, whereas the other unusual NoV genotypes were found sporadically in all age groups. Children between the ages of 13 and 24 months were infected with 12 genotypes, while fewer than 7 genotypes infected other age groups (Fig. 4).

3.6. Clinical characteristics of NoV-infected children

We analyzed the clinical presentation of 181 children infected with NoV between 2018 and 2021. All NoV-infected children presented with diarrhea, and half of the NoV-infected children presented with vomiting. The clinical manifestations varied among different age groups. Overall, the clinical characteristics of children infected with NoV aged 0–12 months were simpler than those of older children. There was no significant difference in the pattern of clinical signs between male and female children infected with NoV (Table 2). In addition, children infected with GII.4 Sydney_2012[P31] were more likely to present with diarrhea alone (56.8%, 25/44) compared to those infected with GII.4 Sydney_2012 [P16] (31.3%, 20/64) ($P = 0.010$) and GII.3[P12] (28.6%, 10/35) ($P = 0.000$), respectively.

4. Discussion

The present study illustrated the epidemiological characteristics and genetic dynamics of NoV in Shanghai before and during the coronavirus disease 2019 (COVID-19) pandemic. The overall detection rate of NoV in children in this study (11.7%) was lower than that worldwide from 2015 to 2020 (18.2%), and in China from 1999 to 2020 (16.7%) (Wei et al., 2021; Farahmand et al., 2022). In particular, the prevalence of NoV was significantly lower in 2020 (5.8%) than in 2018–2019 (10.9%) and 2021 (14.3%). After the COVID-19 pandemic spread in China at the end of 2019, non-pharmaceutical interventions (NPIs) (such as wearing masks, cordoning off areas, closing schools and daycare centers, ensuring physical distance from others, washing hands and using alcohol to disinfect hands) were strictly implemented to prevent the spread of COVID-19 in China (Zhang et al., 2020). These measures may also have been effective in preventing NoV infections. Another potential cause for the dramatic decrease in NoV infections in 2020 may be that children with mild AGE symptoms were not sent to the doctor because of the possibility of COVID-19 infection.

In line with the results of a meta-analysis on global NoV infection in patients with AGE, there was no significant difference in the detection rate of NoV between children under 5 and those aged 5 years of age in this study (Farahmand et al., 2022). In the current study, the children

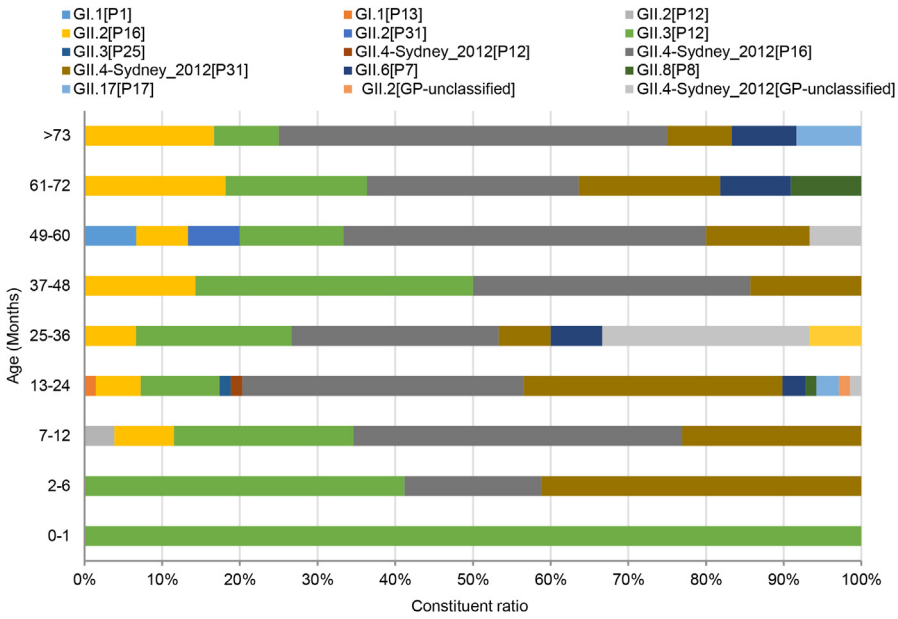

Fig. 4. Genotypic distribution of NoV in children of different age groups in Shanghai, 2018–2021.

aged 13–24 months emerged as the most susceptible to NoV infection. After a sharp decline in the detection rate of NoV in children aged 25–36 months, the detection rate of NoV increased in children aged 37 months and older. This may be related to the fact that children aged 3 years and older started attending kindergarten and living in groups, which may have increased the risk of NoV infection. Similar to the seasonal transmission pattern of NoV in most regions of China, the temporal distribution of NoV infections among children with AGE in Shanghai has a clear seasonal pattern, mainly concentrated in autumn and winter (Han et al., 2018; Fang et al., 2021).

Similar to previous global studies, this study identified GII NoV as the most predominant genogroup in Shanghai (Kendra et al., 2022). However, the detection rate of GI NoV in Shanghai was much lower than that in Congo (13.0%), Germany (6.2%) and Indonesia (3.4%) (Mikounou Louya et al., 2019; Ennuschat et al., 2021; Utsumi et al., 2021). This suggests that there are significant geographical differences in the prevalence of GI NoV. As GI NoV plays an important role in the occurrence of NoV outbreaks, the epidemiology of GI NoV in Shanghai needs to be closely monitored in the future.

In the present study, eight VP1 and 11 P genotypes were identified by phylogenetic analysis of *capsid* and *RdRp* sequences. Among the VP1 genotypes, GII.4 Sydney_2012 was the predominant capsid genotype in Shanghai each year from 2018 to 2021. In contrast, we observed marked fluctuations in the distribution pattern of P genotypes, with GII.P12 dominating in 2018, followed by GII.P12 and GII.P31 in 2019 and GII.P16 from 2020 to 2021. Consistent with our surveillance data, a growing body of research has shown that the main predominant NoV capsid genotypes have remained relatively stable over the past decade, while the P genotypes are more diverse (Zhou et al., 2017). Although the role of RdRp in the evolution of NoV is not clear, epidemiological data may suggest that RdRp plays an important role in NoV infection and transmission (Campillay-Veliz et al., 2020; Deval et al., 2017; Smertina et al., 2019; Ozaki et al., 2018). Therefore, the function of the RdRp region should be further investigated to better understand the mechanism of NoV infection.

The NoV recombination hotspot is close to the ORF1/ORF2 overlap region, and a number of recombinant strains have been reported in Shanghai and elsewhere (Lun et al., 2018; Lu et al., 2019; Kendra et al., 2022). This study identified 14 combination genotypes, including 11 recombinant genotypes. Among them, 96.6% of the NoV strains were recombinant strains, which is consistent with the global pattern that has been observed in many studies (Chen et al., 2018; Kendra et al., 2022; Han et al., 2018; Cao et al., 2021). According to our previous data, GII.4 Sydney_2012[P31] was the predominant genotype from its emergence in 2012 until 2019 in Shanghai (Lu et al., 2019). However, the main predominant strain changed from GII.4 Sydney_2012[P31] to GII.4 Sydney_2012[P16] in 2020–2021. The recombination of GII.4 with different RdRps may be an important mechanism for its long-term dominance in the population. All of these results may indicate that recombination events can increase the transmissibility of NoV.

Of note, combined with the surveillance data on NoV in previous studies from 2012 to 2017, GII.3[P12] strains were continuously detected in Shanghai from 2012 to 2021 (Lu et al., 2019). A similar observation was reported in Jiangsu Province from 2009 to 2019 (Fu et al., 2021). This suggests that GII.3[P12] may continue to evolve to maintain its epidemiological dominance in the population. Moreover, several studies have shown that GII.3[P12] strains continue to evolve rapidly, driven by intergenic recombination and amino acid substitutions (Mahar et al., 2013; Fu et al., 2021).

GII.4 Sydney_2012[P16] was first identified in NoV outbreaks in the United States in November 2015 (Cannon et al., 2017). Subsequently, this genotype gradually became the predominant strain in outbreaks and sporadic cases of AGE in many countries worldwide (Choi et al., 2017; Jones et al., 2018; Lun et al., 2018). However, during the same period, GII.4 Sydney_2012[P31] and GII.2[P16] were the most common norovirus genotypes in children with AGE in China (Ao et al., 2017; Chen et al., 2018; Lu et al., 2019). In this study, the first case of GII.4 Sydney_2012[P16] was detected in 2018, and it became the predominant genotype in Shanghai from 2020 to 2021. Unfortunately, epidemiological data on GII.4 Sydney_2012[P16] in children with AGE are still lacking in China. To date, only Hong Kong (21 cases, from 2012 to 2017), Tianjin (three cases, from 2019 to 2020), Hubei (17 cases, from 2017 to 2019), and Northern China (one case) have reported the low-level epidemiology of GII.4[P16] (Fang et al., 2021; Cheung et al., 2019; Chen et al., 2023; Li et al., 2023). Some evolutionary analyses have speculated that the GII.4 Sydney_2012[P16] genotype may be derived from GII.4 Sydney_2012[P31] and GII.2[P16] strains, but further studies are needed to investigate the transmission mechanism of this genotype as the predominant genotype in Shanghai (Lun et al., 2018; Parra, 2019). Furthermore, close attention should be given to the prevalence of GII.4 Sydney_2012[P16] in other regions of China.

Table 2
Clinical characteristics of NoV-infected children in different age groups and genders.

Clinical features	Ages (months) n (positive rates, %)								Gender n (positive rates, %)	
	0–1	2–6	7–12	13–24	25–36	37–48	49–60	61–72	>73	Male	Female
Diarrhea	2 (100.0)	12 (63.2)	13 (50.0)	24 (33.8)	3 (27.3)	5 (41.7)	2 (11.8)	2 (20.0)	3 (23.1)	39 (35.1)	27 (38.6)
Diarrhea and Vomiting	–	2 (10.5)	9 (34.6)	22 (31.0)	3 (27.3)	1 (8.3)	3 (17.6)	1 (10.0)	3 (23.1)	26 (23.4)	18 (25.7)
Diarrhea and fever	–	4 (21.1)	1 (3.8)	7 (9.9)	–	1 (8.3)	1 (5.9)	–	–	7 (6.3)	7 (10.0)
Diarrhea and abdominal pain	–	–	–	1 (1.4)	–	1 (8.3)	2 (11.8)	1 (10.0)	–	5 (4.5)	1 (1.4)
Diarrhea, fever and vomiting	–	1 (5.3)	3 (11.5)	10 (14.1)	2 (18.2)	2 (16.7)	2 (11.8)	2 (20.0)	4 (30.8)	17 (15.3)	9 (12.9)
Diarrhea, vomiting and abdominal pain	–	–	–	4 (5.6)	–	2 (16.7)	4 (23.5)	1 (10.0)	–	8 (7.2)	3 (4.3)
Diarrhea, fever and abdominal pain	–	–	–	2 (2.8)	–	–	2 (11.8)	–	–	2 (1.8)	2 (2.9)
Diarrhea, vomiting, fever and abdominal pain	–	–	–	1 (1.4)	3 (27.3)	–	1 (5.9)	3 (30.0)	3 (23.1)	8 (7.2)	3 (4.3)
Total	2 (100.0)	19 (100.0)	26 (100.0)	71 (100.0)	11 (100.0)	12 (100.0)	17 (100.0)	10 (100.0)	13 (100.0)	111 (100.0)	70 (100.0)

n: number of NoV positive samples.

Similar to Tianjin and the western region of China, the prevalence of GII.2[P16] was at a low level in Shanghai (Fang et al., 2021; Cao et al., 2021). GII.2[P16] was the most common genotype in Zhoushan and Huzhou in China in 2017 (Chen et al., 2018; Han et al., 2018). One study on NoV outbreak surveillance in China reported that more than 80% of all NoV outbreaks from 2016 to 2018 were genotyped as GII.2[P16] (Jin et al., 2020). This suggests that GII.2[P16] may be more likely to be transmitted during outbreaks of diarrhea caused by NoV. As the most common NoV genotypes worldwide in recent years are associated with GII.P16, GII.P16 may play a key role in the susceptibility of these recombinants, especially in combination with GII.4 Sydney_2012.

During this study, we also detected the formerly emergent virus GII.17[P17] in three samples (Fu et al., 2015). This emergent strain was first detected in Shanghai in 2015 with a consistently low prevalence (Lu et al., 2019). However, according to previous NoV epidemiological data, adults were more susceptible to GII.17[P17] than children (Lu et al., 2019; Fang et al., 2021). Therefore, we recommend focusing on the distribution of NoV genotypes in different age groups to provide a more accurate theoretical basis for NoV vaccine development and vaccination strategies.

Notably, some uncommon genotypes were detected for the first time in Shanghai, including GII.2[P12], GII.2[P31], GIX.1[GII.P15], GII.3 [P25] and GI.3[P13]. As some new variants have been the predominant NoV strains in recent years, close monitoring and in-depth analysis of the epidemiology of NoV is needed to prevent possible outbreaks of diarrhea caused by these genotypes in children (Iritani et al., 2012; Fu et al., 2015; Ao et al., 2017; Choi et al., 2017).

Furthermore, we found that the greatest diversity of NoV genotypes was found in children aged 13–24 months. We speculated that this phenomenon may be related to the fact that children in this age group have not developed immunity to NoV and are susceptible to multiple genotypes of NoV as they become more active outdoors. The clinical presentation of children infected with NoV was variable but mainly included diarrhea only or diarrhea and vomiting. The clinical feature of children infected with GII.4 Sydney_2012[P31] was more likely to be diarrhea only, compared to those infected with GII.3[P12] and GII.4 Sydney_2012[P16].

5. Conclusions

In conclusion, we illustrated the diversity of NoV genotypes and recombinant strains circulating in children with AGE in Shanghai from 2018 to 2021. The newly emerged strain GII.4 Sydney_2012[P16] circulated in Shanghai and became the predominant strain in 2020 and 2021. As this new variant is currently circulating in many countries worldwide, future studies are needed to further investigate the infection mechanisms of GII.4 Sydney_2012[P16] and whether new forms of GII.P16 recombination will emerge. These data provide useful information on the molecular epidemiology of NoV in Shanghai and raise concerns about vaccine development against recombinant NoV strains.

Data availability

All data generated or analyzed during this study are included in this published article. All the NoV sequences have deposited on the web of on the web of Data Repository of China Association for Science and Technology (https://cast.scidb.cn/en). The CSTR is https://cstr.cn/31253.11.sciencedb.cast.00006 and the DOI is https://doi.org/10.57760/sciencedb.cast.00006.

Ethics statement

Ethical approval for this study was granted by the Ethical Review Committee of the Children's Hospital of Fudan University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

Lijuan Lu: conceptualization, project administration, methodology, data curation, formal Analysis, investigation, writing-original draft, writing-review and editing. Yuanyun Ao: methodology, formal Analysis, investigation. Ran Jia: methodology, visualization. Huaqing Zhong: data Curation. Pengcheng Liu: methodology. Menghua Xu: formal Analysis. Liyun Su: investigation. Lingfeng Cao: data curation. Jin Xu: conceptualization, funding acquisition, resources, supervision, writing-review and editing.

Conflicts of interest

No potential conflict of interest was reported by the authors.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2023.08.005.

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