Genetic Diversity and Epidemiology of Norovirus in Children with acute sporadic gastroenteritis in Shanghai, China, 2012-2017

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Lijuan Lu
Children's Hospital of Fudan University

Huaqing Zhong
Children's Hospital of Fudan University

Menghua Xu
Children's Hospital of Fudan University

Liyun Su
Children's Hospital of Fudan University

Lingfeng Cao
Children's Hospital of Fudan University

Ran Jia
Children's Hospital of Fudan university

Jin Xu jinxu_125@163.com
Corresponding Author
ORCiD: 0000-0003-2876-2712

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Abstract

Background

Noroviruses are considered the important causes of acute gastroenteritis (AGE) across all age groups especially in children under five years. We investigated the prevalence and molecular epidemiology of norovirus in outpatient children from Children’s Hospital of Fudan University in Shanghai, China.

Methods

A total of 1433 stool specimens were collected from children under five years with acute gastroenteritis between January 2012 and December 2017. All the samples were analyzed by conventional reverse transcription-polymerase chain reaction (RT-PCR) for genogroup II targeting both the RNA-dependent RNA polymerase (RdRp) and partial capsid genes. Norovirus Genotyping Tool v.2.0 (https://www.rivm.nl/mpf/typingtool/norovirus/) was used for genotyping strains, and phylogenetic analyses were conducted by MEGA 6.0.

Results

During 2012 to 2017, NoVs were detected in 15.4% (220/1433) of the samples, with high detection rate in children aged 7-12 months (19.2%, 143/746) and in September (27.7%, 33/119). Based on genetic analysis of RdRp, GII.Pe (74.5%%, 137/184) was the most predominating RdRp genotype from 2013 to 2017 while GII.P4 played a dominant role in 2012 (55.6%, 21/36). The most prevalent NoVs genotype was GII.4 (73.6%, 162/220) during 2012 to 2017 among the capsid genotypes. According to genetic analysis of RdRp and capsid sequences, the strains were clustered into 19 RdRp/capsid genotypes, and 12 of them were discordant RdRp and capsid genotypes, such as GII.Pe/GII.4-Sydney_2012, GII.P12/GII.3, GII.P7/GII.6, GII.Pe/GII.3, GII.P16/GII.2. GII.Pe/GII.4-Sydney_2012 was completely instead of the pandemic of GII.P4-2006b/GII.4-2006b since 2013 and distributed across all age groups in children.
Conclusions

The present study shows high detection rates and genetic diversity of circulating NoVs genotypes in paediatric AGE samples from Shanghai. The findings emphasize the importance of continuous molecular surveillance of emerging NoVs strains.

Background

Despite substantial decreases in recent decades, gastroenteritis remains the second most common cause of morbidity and the fourth most common cause of mortality worldwide in children under the age of 5 years [1]. Noroviruses have been considered as the second most common virus cause of sporadic gastroenteritis after rotavirus in children [2-5].

*Noroviruses* (NoVs) have a non-segmented positive-strand RNA genome of approximately 7.6 kb that contains three ORFs [6]. ORF1 encodes a polyprotein that is cleaved by the virus-encoded protease into six nonstructural proteins, including the norovirus protease and RNA-dependent RNA polymerase (RdRp). ORF2 encodes the major structural protein (VP1), and ORF3 encodes a minor structural protein (VP2) [7]. NoVs are highly diverse and divided into seven genogroups (GI-GVII) of which GI, GII and GIV have been found among human, and GII is the most popular genogroup in children with acute diarrhea. GII NoVs have been subdivided into 29 genotypes and 23 genotypes based on the genetic diversity of ORF1 and ORF2, respectively [8, 9].

It is well known that natural recombination at the ORF1/ORF2 junction region is considered a common event between NoVs strains, and more and more combination or recombination norovirus strains are currently appearing in many areas, such as GII.Pe/GII.4, GII.P16/GII.4, GII.P17/GII.17 and GII.P16/GII.2 [10-17]. Consequently, a dual nomenclature system of NoVs have been proposed, taking the phylogenetic relationships of both partial ORF1 and ORF2 into account. This dual typing approach is correct to identify genetically different NoVs genotypes. In addition, combinant genotypes are better to understand the molecular
epidemiology of NoVs.

Previous studies in Shanghai mainly adopted either ORF1 or ORF2 nomenclature to understand the epidemiology of NoVs in children under five years [18-22]. Here, we conducted the current study to investigate the diversity of NoVs genotypes adopting a dual nomenclature system, based on both ORF1 and ORF2, in children suffering from acute gastroenteritis who visited Children’s Hospital of Fudan University in Shanghai from January 2012 to December 2017. Furthermore, we also assessed the overall frequency of NoVs, seasonal distribution of NoVs, and NoVs genotypes distribution by age group.

Methods

Study design

Fecal specimens were collected from 1433 children up to 5 years old who visited the outpatient department of Children’s Hospital of Fudan University and were diagnosed as acute sporadic gastroenteritis between January 2012 and December 2017 in Shanghai. The demographic information and clinical diagnosis were collected from medical history. The study was approved by the Institutional Review Board of Children’s Hospital of Fudan University.

Stool suspensions were prepared as 10% (w/v) in saline solution. Nucleic acid was extracted from 200 μL clarified stool suspensions using TIANamp Virus DNA/RNA Kit (TIANGEN Biotech (Beijing) Co., Ltd) according to the manufacturer’s recommendations. The extracted genetic material was submitted to reverse transcription (RT) with a random primer using the PrimeScript™ II Reverse transcriptase (Takara, Biotechnology (Dalian) Co., Ltd).

cDNA was amplified by PCR for the GII NoVs genotyping. PCR and sequencing for NoVs were performed using primers targeting the RdRp region of ORF1 (313 bp) and partial
capsid region of ORF2 (344 bp) (Table 1). PCR was performed under the following conditions: initial denaturation at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30s, 55 °C for 30 s, 72 °C for 30 s, and at 72 °C for 7min for a final extension. All amplified cDNA products were electrophoresed on 2.0% agarose gels containing 4S GelRed and visualized by Gel Doc EZ Imaging System (Bio-Rad Laboratories (Shanghai) Co., Ltd.). The amplifications of NoVs positive samples were subjected to nucleotide sequencing by Sangon Biotech (Shanghai) Co., Ltd.. Sequences generated from the PCR products of each strain were analyzed by using the Norovirus Genotyping Tool v.2.0 (https://www.rivm.nl/mpf/typingtool/norovirus/), where each sequence was used to assign to a NoVs genotype. Phylogenetic analysis on the nucleotide sequences obtained in our study and sequences data from GenBank, was also performed using the Maximum-Likelihood method (Kimura two-parameter model, 1000 bootstrap replications for branch support) in MEGA 6.0.

Statistical analysis

The difference between NoVs detection rates in boys and girls was compared using a two-sided chi-square test in SPSS Statistics Version 19.0 (IBM Corp., Armonk, NY, USA) and the P-value less than 0.05 was considered statistically significant.

Results

Detection and epidemiology of NoVs in children

A total of 1433 stool samples were collected from outpatient children with acute gastroenteritis during 2012 to 2017, of which 897 (62.6%) were boys and 536 (37.4%) were girls. Among them, 15.4% (220/1433) were infected with NoVs GII genogroup, and annual detection rate was 25.0% (36/144), 15.3% (22/144), 11.8% (17/144), 15.5%
(41/265), 18.8% (59/313) and 10.6% (45/423) from 2012 to 2017, respectively. No significant difference \((P>0.05)\) was obtained between boys (16.4%, 147/897) and girls (13.6%, 73/536) in NoVs detection rate.

The overall prevalence of NoVs infection in different age groups ranged from 5.8% to 19.2%. Children in the age group 7-12 months had the highest prevalence (19.2%, 143/746), followed by children aged 13 to 24 months (15.3%, 26/170) (Fig. 1). Most of the children infected with NoVs (93.6%, 206/220) were aged less than 2 years. NoVs infection was prevalent over the year with the lowest and highest detection rate in May (5.0%, 6/121) and September (27.7%, 33/119), respectively (Fig. 2).

**NoVs genotypes distribution based on RdRp region**

Concerning the polymerase region analyzed, a large genetic diversity of circulating NoVs strains was observed in the 220 NoVs positive samples. GII.Pe (74.5%, 137/184) was the most predominant RdRp genotype from 2013 to 2017 while GII.P4 played a dominant role in 2012 (55.6%, 21/36). The second prevalent genotype was various from year to year. GII.Pe and GII.P7 was separately the second prevalent genotype in 2012 and 2014 while GII.P12 was the second prevalent genotype in the other years. GII.P17 was sequenced successfully from eight samples from 2015 to 2017. Besides, many other RdRp genotypes were also detected, such as GII.Pg (1.4%, 3/220), GII.P16 (0.5%, 1/220) and GII.P8 (0.5%, 1/220). Three GII.P4 subtypes were detected only in 2012, including GII.P4-2006b (50.0%, 18/36), GII.P4-2006a (2.8%, 1/36) and GII.P4-New_Orleans_2009 (5.6%, 2/36) (Fig. 3, Table 2).

**NoVs genotypes analysis based on partial capsid region**
According to the partial capsid region, the majority of the NoVs strains were classified as GII.4 (73.6%, 162/220) during 2012 to 2017, followed by GII.3 (15.9%, 35/220). The other non-GII.4 genotypes included GII.17 (3.6%, 8/220), GII.6 (3.2%, 7/220), GII.1 (1.8%, 4/220), GII.2 (0.9%, 2/220), GII.7 (0.5%, 1/220) and GII.8 (0.5%, 1/220). GII.4 was divided into three subtypes by the Norovirus Genotyping Tool v.2.0. GII.4-Sydney_2012 (73.4%, 135/184) was the most frequent GII.4 variant during 2013 to 2017 while GII.4-2006b (50.0%, 18/36) was the main variant in 2012. Two sequences detected in 2012 belonged to GII.4-New_Orleans_2009 (Fig. 3, Table 2).

**Combination genotypes of NoVs with both RdRp/Capsid fragments gene**

Overall, 19 kinds of RdRp/Capsid genotypes were presented according to the dual nomenclature system of NoVs, and discordant RdRp and capsid genotypes were identified in 12 of them, such as GII.Pe/GII.4-Sydney_2012, GII.P12/GII.3, GII.P7/GII.6 and GII.P16/GII.2. GII.Pe/GII.4-Sydney_2012 (73.4%, 135/184) was the dominating RdRp/Capsid genotype in each year from 2013 to 2017 while GII.P4-2006b/GII.4-2006b (44.4%, 16/36) was the most frequent genotype in 2012. GII.P12/GII.3 was the second frequent combination genotype in 2013 (27.3%, 6/22), 2015 (34.1%, 14/41), 2016 (10.2%, 6/59) and 2017 (8.9%, 4/45) while GII.Pe/GII.4-Sydney_2012 (16.7%, 6/36) and GII.P7/GII.6 (17.6%, 3/17) was separately the secondary genotype in 2012 and 2014. Most of the uncommon combination genotypes were detected in 2012, for example, GII.Pe/GII.6, GII.Pg/GII.1 and GII.Pe/GII.4-2006b. Besides, GII.P17/GII.17 was only detected in eight children from 2015 to 2017 (Table 3).

**Distribution of NoVs Capsid/RdRp**
genotypes in children with different age

In this study, only GII.Pe/GII.4-Sydney_2012 genotype was distributed across all age groups in children. Almost all the NoVs Capsid/RdRp genotypes were detected in children aged from 7 to 12 months (63.6%, 143/220). Among them, GII.P2/GII.2, GII.Pg/GII.1, GII.Pe/GII.6, GII.P4-2006b/GII.1, GII.P4-2006b/GII.4-Sydney_2012, GII.P12/GII.4-Sydney_2012, GII.P7/GII.4-Sydney_2012 and GII.Pe/GII.4-2006b were only detected in 7-12 months. GII.P4-2006a/GII.4-Sydney_2012, GII.P6/GII.6, GII.P7/GII.7 and GII.P8/GII.8 were only distributed in children aged from 0 to 6 months and GII.P16/GII.2 was only detected in a child aged 60 months (Fig. 4).

Discussion

This study was a long-term monitoring of the epidemiology and molecular characteristics of the NoVs in children under 5 years with acute sporadic gastroenteritis in Shanghai. Herein, the total detection rate of norovirus positive cases (15.4%) among outpatients was lower than that in our previous monitoring data of NoVs infections in both outpatients and inpatients [20, 22]. Although the percentage of NoVs infection in 2012 (25.0%) was similar to our previous data during 2006 to 2011, annual detection rate of NoVs was in fluctuating reduction from 2012 to 2017 [22]. This may be associated with the increased propaganda on how to prevent NoVs infection among people because of the increasing outbreaks of NoVs infection in many areas in recent years.

During 2012 to 2017, children with NoVs infection were mainly aged less than 2 years which is concert with our previous study and other studies [20, 22-25]. No significant difference of NoVs detection rates was found in girls and boys. This may imply that gender is not a predisposing factor in NoVs infection in children less than 5 years. In accordance
with previous studies, which concluded that norovirus mainly peaked in cold seasons [20, 22, 26-28], our study demonstrated that the highest detection rate was found in September and over 70% of NoVs infection were detected in autumn and winter. However, in some other areas, NoVs-associated diarrhea has a summer peak or no apparent seasonal peak which may be connected with the increased contaminated water and food or other unknown reasons [21, 29].

A great diversity of NoVs genotypes were identified on the basis of RdRp region. Among the NoVs positive cases, GII.P4 was the predominant NoVs genotypes in 2012, which is consistent with our previous data on outpatient children from 2010 to 2011 and western China during 2010 to 2013 [17, 20]. It is interesting to note that, the prevalence of GII.P4 abruptly disappeared after 2012 while GII.Pe obviously became the dominant genotype diffusing in children under five years with acute sporadic gastroenteritis from 2013 to 2017. This unexpected increase of GII.Pe from 2013 was also found in Huzhou, China [30].

Based on those references, we speculated that GII.Pe, first detected in the norovirus outbreak of 2008 in Victoria, Australia, obviously replaced GII.P4 as the leading RdRp genotype in children with sporadic gastroenteritis in Shanghai since 2013 [31]. However, no GII.Pe was detected in Suzhou (China) and western China in 2013 [17, 32]. It should be paid close attention on the spread of this type in China in future. It was surprising that GII.P12 became the second main RdRp genotype instead of GII.Pb during 2012 to 2017. No GII.Pb was detected in this study while it was the second predominant RdRp genotype from 2010 to 2011 in Shanghai [20].

Since 2014, GII.17 was more and more popular in several major cities of mainland China and some other areas in Asia [33-36]. However, only eight GII.P17/GII.17 strains were detected during 2015 to 2017 in our study. The same detection situation of GII.P17/GII.17 in children was also reported in Huzhou and Shanghai [30, 36]. However, adults were more
susceptible to GII.P17/GII.17 than children [30, 36]. The reason for this infection difference in diverse age groups was still ambiguous and further studies need to be conducted to explore the mechanism.

In comparison with the NoVs RdRp genotypes, diverse NoVs genotypes were also detected according to the partial capsid gene. As similar with data from Korea (2013-2015), Japan (2008-2014), Chongqing and Suzhou in China (2010-2013), Lusaka Province in Zambia (2012-2013) and Vietnamese (2012-2015), GII.4 was still the predominant capsid genotype spreading among children during 2012 to 2017 [17, 32, 37-40]. The predominance of distinct genotype determined by both sequence-based typing methods suggested the importance of genotyping NoVs simultaneously by both capsid and RdRp genes, which could assist us to comprehensively understand the epidemiology and evolution of NoVs. Although GII.4-2006b was still the predominant GII.4 variant, GII.4-Sydney_2012 became the main and unique GII.4 subtype from 2013 to 2017. As GII.4-Sydney_2012 firstly reported in Australia, it was the major variant prevalent among children worldwide since 2012 [41]. As reported in Bangladesh (2010-2014), Chongqing (2010-2013) and Jiangsu (2010-2013) in China, Japan (2008-2012) and Vietnam (2012-2015), GII.3 was the second main capsid genotype in our study [17, 32, 38, 40, 42].

Combined genotypes of NoVs conducted in this study demonstrated that 19 kinds of RdRp/Capsid genotypes were determined, and 12 of them were distinct in RdRp genotypes and capsid genotypes. All of those discordant RdRp/Capsid genotypes were suspected as recombinant strains and most of them have been reported elsewhere [14, 16, 17, 23, 37, 41]. Although it needs more analysis on the junction of ORF1 and ORF2 to confirm the recombination site in our study, it obviously suggest that this phenomenon is very common in NoVs as observed elsewhere. Furthermore, we also observed a change in the circulation pattern of the RdRp/Capsid strains, with GII.P4-2006b/GII.4-2006b predominant
in 2012, followed by an emergence and predominance of GII.Pe/GII.4-Sydney_2012 since 2013. GII.Pe/GII.4-Sydney_2012, first reported in Australia in 2012, was widespread in South Africa (2012-2013), Iran (2015-2016), Botswana (2013-2015), Korea (2013) and some cities in China (2012-2015) after then [23, 31, 37, 41, 43-46]. It was surprising that GII.Pe/GII.4-Sydney_2012 was completely instead of the pandemic of GII.P4-2006b/GII.4-2006b since 2013 and close attention should be paid on the prevalence of this genotype. As the second most predominant RdRp/Capsid genotype in 2013 and from 2015 to 2017, GII.P12/GII.3 was also reported as the main NoVs genotype in Chongqing (2011-2013), China [17]. Interestingly, this type was mainly observed in Asia-Pacific region, implying that the pandemic of GII.P12/GII.3 may have regional characteristics [17, 47, 48]. GII.P7/GII.6, as the second prevalent genotype in 2014, was reported with a few cases elsewhere [46, 49]. Besides, many other rare RdRp/Capsid strains were detected in our study, such as GII.Pe/GII.3 and GII.P16/GII.2. Among them, GII.P16/GII.2 strain was observed only in 2017, however, this strain has become the main genotype in Japan, France, HongKong, Taiwan and other several cities in China during 2016 and 2017 [17, 50-54]. Consequently, it is necessary to continuously monitor those rare strains in Shanghai. In epidemiological investigations carried out in different age of children, the distribution of NoVs Capsid/RdRp genotypes varied in different age groups. In our study, GII.Pe/GII.4-Sydney_2012 was detected in all age groups while other genotypes were not. Some genotypes were only detected in one age group. Those results may imply that the infection of some NoVs Capsid/RdRp genotypes was age-specific. However, more and longtime surveillance of the epidemiology of NoVs in different age of children should be conducted to illustrate this phenomenon.

Conclusions

In conclusion, our study has demonstrated the epidemiology and great genetic diversity of
NoVs combination genotypes in outpatient children less than 5 years in Shanghai during 2012 and 2017. Many discordant RdRp/Capsid genotypes were also detected in our study and they were firstly reported in this area. Thus, continuous monitoring of NoVs RdRp/Capsid genotypes will be needed to predict the emergence of new pandemic strains and guide the selection of norovirus vaccine strain in Shanghai.

Abbreviations

NoVs: Noroviruses RdRp: RNA-dependent RNA polymerase AGE: acute gastroenteritis ORFs: Open reading frames

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Children’s Hospital of Fudan University. Because fecal specimens enrolled in this study were the remaining samples after routine examination, consent was not sought from the parent or legal guardians of the children.

Consent for publication

Not applicable.

Availability of data and material

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

Competing interests

The authors have no competing interest.

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Authors' contributions

JX and LJJ conceived of and designed the study. LJJ acquired and analyzed all the data, interpreted the data, drafted the manuscript and obtained funding. HQZ, MHH, LSY, LFC and RJ acquired the clinical data and coordinated in the design of the study. All authors provided critical comments or revisions, approved the final version of the article.

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References

1. Institute for Health Metrics and Evaluation (IHME). Global Burden of Disease. Global Health Data Exchange. 2013. https://vizhub.healthdata.org/irank/arrow.php.

2. 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.

3. Patel MM, Widdowson MA, Glass RI, Akazawa K, Vinjé J, Parashar UD. Systematic literature review of role of noroviruses in sporadic gastroenteritis. Emerg Infect Dis. 2008;14(8):1224-31.

4. Anderson EJ. Prevention and treatment of viral diarrhea in pediatrics. Expert Rev Anti Infect Ther. 2010;8(2):205-17.

5. Lopman BA, Steele D, Kirkwood CD, Parashar UD. The Vast and Varied Global Burden of Norovirus: Prospects for Prevention and Control. PLoS Med. 2016;13(4):e1001999.

6. Jiang X, Wang M, Wang K, Estes MK. Sequence and genomic organization of Norwalk
virus. Virology. 1993;195(1):51-61.

7. Thorne LG, Goodfellow IG, Norovirus gene expression and replication. J Gen Virol. 2014;95 (Pt 2):278-91.

8. Vinjé J, Advances in laboratory methods for detection and typing of norovirus. J Clin Microbiol. 2015;53(2):373-81.

9. Hoa Tran TN, Trainor E, Nakagomi T, Cunliffe NA, Nakagomi O. Molecular epidemiology of noroviruses associated with acute sporadic gastroenteritis in children: global distribution of genogroups, genotypes and GII.4 variants. J Clin Virol. 2013;56(3):185-93.

10. van Beek J, de Graaf M, Al-Hello H, Allen DJ, Ambert-Balay K, Botteldoorn N, et al. Molecular surveillance of norovirus, 2005-16: an epidemiological analysis of data collected from the NoroNet network. Lancet Infect Dis. 2018;18(5):545-53.

11. Eden JS, Tanaka MM, Boni MF, Rawlinson WD, White PA. Recombination within the pandemic norovirus GII.4 lineage. J Virol. 2013;87(11):6270-82.

12. Kroneman A, Vega E, Vennema H, Vinjé J, White PA, Hansman G, et al. Proposal for a unified norovirus nomenclature and genotyping. Arch Virol. 2013;158(10):2059-68.

13. Di Felice E, Mauroy A, Pozzo FD, Thiry D, Ceci C, Di Martino B, et al. Bovine noroviruses: A missing component of calf diarrhoea diagnosis. Vet J. 2016;207:53-62.

14. Supadej K, Khamrin P, Kumthip K, Kochjan P, Yodmeeklin A, Ushijima H, et al. Wide variety of recombinant strains of norovirus GII in pediatric patients hospitalized with acute gastroenteritis in Thailand during 2005 to 2015. Infect Genet Evol. 2017;52:44-51.

15. Andrade JSR, Fumian TM, Leite JPG, Assis MR, Bello G, Mir D, et al. Detection and molecular characterization of emergent GII.P17/GII.17 Norovirus in Brazil, 2015. Infect Genet Evol. 2017;51:28-32.

16. Fu JG, Shi C, Xu C, Lin Q, Zhang J, Yi QH, et al. Outbreaks of acute gastroenteritis associated with a re-emerging GII.P16-GII.2 norovirus in the spring of 2017 in Jiangsu,
China. PLoS One. 2017;12(12):e0186090.

17. Lu QB, Huang DD, Zhao J, Wang HY, Zhang XA, Xu HM, et al. An increasing prevalence of recombinant GII norovirus in pediatric patients with diarrhea during 2010-2013 in China. Infect Genet Evol. 2015;31:48-52.

18. Xue C, Pan L, Zhu W, Wang Y, Fu H, Cui C, et al. Molecular epidemiology of genogroup II norovirus infections in acute gastroenteritis patients during 2014-2016 in Pudong New Area, Shanghai, China. Gut Pathog. 2018;10: 7.

19. Pan L, Xue C, Fu H, Liu D, Zhu L, Cui C, et al. The novel norovirus genotype GII.17 is the predominant strain in diarrheal patients in Shanghai, China. Gut Pathog. 2016;8: 49.

20. Lu L, Jia R, Zhong H, Xu M, Su L, Cao L, et al. Molecular characterization and multiple infections of rotavirus, norovirus, sapovirus, astrovirus and adenovirus in outpatients with sporadic gastroenteritis in Shanghai, China, 2010-2011. Arch Virol. 2015;160(5):1229-38.

21. Zeng M, Xu X, Zhu C, Chen J, Zhu Q, Lin S, et al. Clinical and molecular epidemiology of norovirus infection in childhood diarrhea in China. J Med Virol. 2012;84(1):145-51.

22. Lu L, Zhong H, Xu M, Su L, Cao L, Dong N, et al. Molecular epidemiology of human calicivirus infections in children with acute diarrhea in Shanghai: a retrospective comparison between inpatients and outpatients treated between 2006 and 2011. Arch Virol. 2014;159(7):1613-21.

23. Farsi M, Roodbari F, Nejati B, Arashkia A, Jalilvand S, Nateghian A, et al. Prevalence and genetic diversity of norovirus genogroup II in children less than 5 years of age with acute gastroenteritis in Tehran, Iran. Med Microbiol Immunol. 2018;207(3-4):201-10.

24. Hassine-Zaafrane M, Sdiri-Loulizi K, Kaplon J, Salem IB, Pothier P, Aouni M, et al. Prevalence and genetic diversity of norovirus infection in Tunisian children (2007-2010). J Med Virol. 2013;85(6):1100-10.

25. Esteves A, Nordgren J, Tavares C, Fortes F, Dimbu R, Saraiva N, et al. Genetic diversity
of norovirus in children under 5 years of age with acute gastroenteritis from Angola, Epidemiol Infect. 2018;146(5):551-7.

26. Y Xue Y, Pan H, Hu J, Wu H, Li J, Xiao W, et al. Epidemiology of norovirus infections among diarrhea outpatients in a diarrhea surveillance system in Shanghai, China: a cross-sectional study. BMC Infect Dis. 2015;15:183.

27. Ahmed SM, Lopman BA, Levy K. A systematic review and meta-analysis of the global seasonality of norovirus. PLoS One. 2013;8(10):e75922.

28. Tan D, Deng L, Wang M, Li X, Ma Y, Liu W. High prevalence and genetic diversity of noroviruses among children with sporadic acute gastroenteritis in Nanning City, China, 2010-2011. J Med Virol. 2015;87(3):498-503.

29. Siafakas N, Zerva L, Hatzaki D, Lebessi E, Chronopoulou G, Paraskakis et al. Molecular epidemiology of noroviruses in children in South Greece, 2013-2015. J Med Virol. 2018;90(11):1703-11.

30. Zhang P, Chen L, Fu Y, Ji L, Wu X, Xu D, et al. Clinical and molecular analyses of norovirus-associated sporadic acute gastroenteritis: the emergence of GII.17 over GII.4, Huzhou, China, 2015. BMC Infect Dis. 2016;16(1):717.

31. Bruggink LD, Dunbar NL, Marshall JA. Emergence of GII.e as a major ORF 1 norovirus genotype and its associated ORF 2 GII.4 variant forms. Infect Genet Evol. 2014;22:157-63.

32. Fu JG, Ai J, Zhang J, Wu QB, Qi X, Ji H, et al. Molecular epidemiology of genogroup II norovirus infection among hospitalized children with acute gastroenteritis in Suzhou (Jiangsu, China) from 2010 to 2013. J Med Virol. 2016;88(6):954-60.

33. Xue L, Dong R, Wu Q, Li Y, Cai W, Kou X, et al. Molecular epidemiology of noroviruses associated with sporadic gastroenteritis in Guangzhou, China, 2013-2015. Arch Virol. 2016;161(5):1377-84.

34. Lu J, Sun L, Fang L, Yang F, Mo Y, Lao J, et al. Gastroenteritis outbreaks caused by
norovirus GII.17, Guangdong Province, China, 2014-2015. Emerg Infect Dis. 2015;21(7):1240-2.

35. Han J, Ji L, Shen Y, Wu X, Xu D, Chen L. Emergence and predominance of norovirus GII.17 in Huzhou, China, 2014-2015. Virol J. 2015;12:139.

36. Chen H, Qian F, Xu J, Chan M, Shen Z, Zai S, et al. A novel norovirus GII.17 lineage contributed to adult gastroenteritis in Shanghai, China, during the winter of 2014-2015. Emerg Microb Infect. 2015;4(11):e67.

37. Dang Thanh H, Than VT, Nguyen TH, Lim I, Kim W. Emergence of Norovirus GII.17 Variants among Children with Acute Gastroenteritis in South Korea. PLoS One. 2016;11(5):e0154284.

38. Nakamura N, Kobayashi S, Minagawa H, Matsushita T, Sugiura W, Iwatani Y. Molecular epidemiology of enteric viruses in patients with acute gastroenteritis in Aichi prefecture, Japan, 2008/09-2013/14. J Med Virol. 2016;88(7):1180-6.

39. Howard LM, Mwape I, Siwingwa M, Simuyandi M, Guffey MB, Stringer JS, et al. Norovirus infections in young children in Lusaka Province, Zambia: clinical characteristics and molecular epidemiology. BMC Infect Dis. 2017;17(1):92.

40. Hoa-Tran TN, Nakagomi O, Dao AT, Nguyen AT, Agbemabiese CA, Vu HM, et al. Molecular epidemiology of noroviruses detected in Vietnamese children with acute gastroenteritis from 2012 to 2015. J Med Microbiol. 2017;66(1):34-45.

41. Bruggink LD, Moselen JM, Marshall JA. Genotype analysis of noroviruses associated with gastroenteritis outbreaks in childcare centres, Victoria, Australia, 2012-2015. Epidemiol Infect. 2017;145(9):1933-41.

42. Rahman M, Rahman R, Nahar S, Hossain S, Ahmed S, Golam Faruque AS, et al. Norovirus diarrhea in Bangladesh, 2010-2014: prevalence, clinical features, and genotypes. J Med Virol. 2016;88(10):1742-50.
43. Mans J, Murray TY, Nadan S, Netshikweta R, Page NA, Taylor MB. Norovirus diversity in children with gastroenteritis in South Africa from 2009 to 2013: GII.4 variants and recombinant strains predominate. Epidemiol Infect. 2016;144(5):907-16.

44. Makhaola K, Moyo S, Lechiile K, Goldfarb DM, Keabaabetswe LP. Genetic and epidemiological analysis of norovirus from children with gastroenteritis in Botswana, 2013-2015. BMC Infect Dis. 2018;18(1):246.

45. Wu X, Han J, Chen L, Xu D, Shen Y, Zha Y, et al. Prevalence and genetic diversity of noroviruses in adults with acute gastroenteritis in Huzhou, China, 2013-2014. Arch Virol. 2015;160(7):1705-13.

46. Chen C, Yan JB, Wang HL, Li P, Li KF, Wu B, et al. Molecular epidemiology and spatiotemporal dynamics of norovirus associated with sporadic acute gastroenteritis during 2013-2017, Zhoushan Islands, China. PLoS One. 2018;13(7):e0200911.

47. Mahar JE, Kirkwood CD. Characterization of norovirus strains in Australian children from 2006 to 2008: prevalence of recombinant strains. J Med Virol. 2011;83(12):2213-9.

48. Phan TG, Kuroiwa T, Kaneshi K, Ueda Y, Nakaya S, Nishimura S, et al. Changing distribution of norovirus genotypes and genetic analysis of recombinant GIIb among infants and children with diarrhea in Japan. J Med Virol. 2006;78(7):971-8.

49. Bruggink LD, Moselen JM, Marshall JA. The Comparative Molecular Epidemiology of GII.P7_GII.6 and GII.P7_GII.7 Norovirus Outbreaks in Victoria, Australia, 2012-2014. Intervirology. 2016;59(1):60-5.

50. Nagasawa K, Matsushima Y, Motoya T, Mizukoshi F, Ueki Y, Sakon N, et al. Phylogeny and Immunoreactivity of Norovirus GII.P16-GII.2, Japan, Winter 2016-17. Emerg Infect Dis. 2018;24(1):144-8.

51. Bidalot M, Théry 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.

52. Kwok K, Niendorf S, Lee N, Hung TN, Chan LY, Jacobsen S, et al. Increased Detection of Emergent Recombinant Norovirus GII.P16-GII.2 Strains in Young Adults, Hong Kong, China, 2016-2017. Emerg Infect Dis. 2017;23(11):1852-5.

53. Liu LT, Kuo TY, Wu CY, Liao WT, Hall AJ, Wu FT. Recombinant GII.P16-GII.2 Norovirus, Taiwan, 2016. Emerg Infect Dis. 2017;23(7):1180-3.

54. Lu J, Fang L, Sun L, Zeng H, Li Y, Zheng H, et al. Association of GII.P16-GII.2 Recombinant Norovirus Strain with Increased Norovirus Outbreaks, Guangdong, China, 2016. Emerg Infect Dis. 2017;23(7):1188-90.

Tables

Table 1

Primers used for Noroviruses genotyping in this study

| Primers | Nucleotide position | Polarity | Target | Sequence(5’- 3’) | Size |
|---------|---------------------|----------|--------|------------------|------|
| G2SKF   | 5058                | F        | Capsid | CNTGGAGGGGC      | 344bp|
|         |                     |          |        | GATCGCAA         |      |
| G2SKR   | 5401                | R        | Capsid | CCRCCNGCATRH     |      |
|         |                     |          |        | CCRTTRTACAT      |      |
| 289H    | 4865                | R        | RdRp   | TGACGATTTTCAT    | 331bp|
|         |                     |          |        | CATCACCATAC      |      |
| 289I    | 4865                | R        | RdRp   | TGACGATTTTCAT    |      |
|         |                     |          |        | CATCCCGGTA      |      |
| 290H    | 4590                | F        | RdRp   | GATTACTCCAGG     |      |
|         |                     |          |        | TGGGACTCCAC     |      |
| 290I    | 4590                | F        | RdRp   | GATTACTCCAGG     |      |
|         |                     |          |        | TGGGACTCAAC     |      |
| 290J    | 4590                | F        | RdRp   | GATTACTCCAGG     |      |
|         |                     |          |        | TGGGAATCAAC     |      |
| 290K    | 4590                | F        | RdRp   | GATTACTCCAGG     |      |
|         |                     |          |        | TGGGAATCCAC     |      |
Table 2

NoV genotypes distribution according to the RdRp region of ORF1 or partial region of ORF2 in Children with AGE under five years in Shanghai, 2012-2017

| Genotypes                  | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | Total |
|----------------------------|------|------|------|------|------|------|-------|
|                            | n(m%)|------|------|------|------|------|-------|
| **RdRp region of ORF1**     |      |      |      |      |      |      |       |
| GII.P2                     | -    | -    | -    | -    | 1(1.7)| 1(0.5)|       |
| GII.P4-2006a               | 1(2.8)| -    | -    | -    | -    | -    | 1(0.5)|
| GII.P4-2006b               | 18(50.0)| -    | -    | -    | -    | -    | 18(8.1)|
| GII.P4-New Orleans_2009    | 2(5.6)| -    | -    | -    | -    | -    | 2(0.9)|
| GII.P6                     | 1(2.8)| -    | -    | -    | -    | -    | 1(0.5)|
| GII.P7                     | -    | 1(4.5)| 3(17.6)| 1(2.4)|      | 5(2.2)|       |
| GII.P8                     | 1(2.8)| -    | -    | -    | -    | -    | 1(0.5)|
| GII.P12                    | 2(5.5)| 6(27.3)| 1(5.9)| 14(34.2)| 7(11.9)| 4(8.9)| 34(15.4)|
| GII.P16                    | -    | -    | -    | -    | -    | 1(2.2)| 1(0.5)|
| GII.P17                    | -    | -    | -    | -    | 5(12.2)| 2(3.4)| 1(2.2)| 8(3.6)|
| GII.Pe                     | 8(22.2)| 15(68.2)| 13(76.5)| 21(51.2)| 49(83.0)| 39(86.7)| 145(65.9)|
| GII.Pg                     | 3(8.3)| -    | -    | -    | -    | -    | 3(1.4)|
| **Total**                  | 36(100)| 22(100)| 17(100)| 41(100)| 59(100)| 45(100)| 220(100)|
| **Partial region of ORF2** |      |      |      |      |      |      |       |
| GII.1                      | 4(11.1)| -    | -    | -    | -    | -    | 4(1.8)|
| GII.2                      | -    | -    | -    | -    | 1(1.7)| 1(2.2)| 2(0.9)|
### Table 3

NoV RdRp/Capsid combination distribution in Children with AGE under five years in Shanghai, 2012-2017

| Combination Genotypes   | 2012  | 2013  | 2014  | 2015  | 2016  | 2017  | Total |
|-------------------------|-------|-------|-------|-------|-------|-------|-------|
|                         | n(m%) |       |       |       |       |       |       |
| GII.P2/GII.2            |       |       |       |       | 1(1.7)|       | 1(0.5)|
| GII.P4-2006b/GII.1     |       |       |       |       |       |       | 1(0.5)|
| GII.P4-2006a/GII.4     |       |       |       |       |       |       | 1(0.5)|

**n:** NoV positive numbers

**m:** Constituent ratio of each genotype
| Virus Combination                        | Count | 7  | 9  | 11 | 13 | 15 | Total |
|-----------------------------------------|-------|----|----|----|----|----|-------|
| GII.P4-2006b/GII.4                     | 16(44.4) | -  | -  | -  | -  | -  | 16(7.3) |
| GII.P4-2006b/GII.4 - Sydney 2012      | 1(2.8)  | -  | -  | -  | -  | -  | 1(0.5)  |
| GII.P4-New Orleans 2009/GII.4-New Orleans 2009 | 2(5.6)  | -  | -  | -  | -  | -  | 2(0.9)  |
| GII.P4/New Orleans 2009/GII.4          | 1(2.8)  | -  | -  | -  | -  | -  | 1(0.5)  |
| GII.P6/GII.6                           | 1(2.8)  | -  | -  | -  | -  | -  | 1(0.5)  |
| GII.P7/GII.6                           | -      | -  | 3(17.6) | 1(2.4) | -  | -  | 4(1.8)  |
| GII.P7/GII.7                           | -      | 1(4.5) | -  | -  | -  | -  | 1(0.5)  |
| GII.P8/GII.8                           | 1(2.8)  | -  | -  | -  | -  | -  | 1(0.5)  |
| GII.P12/GII.4-Sydney 2012              | -      | -  | -  | 1(1.7) | -  | -  | 1(0.5)  |
| GII.P12/GII.3                          | 2(5.6)  | 6(27.3) | 1(5.9) | 14(34.1) | 6(10.2) | 4(8.9) | 33(15.0) |
| GII.P16/GII.2                          | -      | -  | -  | -  | -  | -  | 1(2.2)  |
| GII.P17/GII.17                         | -      | -  | -  | 5(12.2) | 2(3.4) | 1(2.2) | 8(3.6)  |
| GII.Pe/GII.3                           | -      | 1(4.5) | -  | -  | 1(1.7) | -  | 2(0.9)  |
| GII.Pe/GII.4-2006b                     | 1(2.8)  | -  | -  | -  | -  | -  | 1(0.5)  |
| GII.Pe/GII.4-Sydney 2012               | 6(16.7) | 14(63.6) | 13(76.5) | 21(51.2) | 48(81.4) | 39(86.7) | 141(64.1) |
| GII.Pe/GII.6                           | 1(2.8)  | -  | -  | -  | -  | -  | 1(0.5)  |
| GII.Pg/GII.1                           | 3(8.3)  | -  | -  | -  | -  | -  | -      |
| Total                                  | 36(100) | 22(100) | 17(100) | 41(100) | 59(100) | 45(100) | 220(100) |
n: NoV positive numbers

m: Constituent ratio each genotype

Figures

Figure 1
Age distribution of Norovirus infection in Children under five years in Shanghai, 2012-2017

Figure 2
Seasonal distribution of Norovirus infection in Children under five years in Shanghai, 2012-2017

Figure 3
Phylogenetic analysis of GII norovirus based on the partial nucleotide sequences of the polymerase (a) and capsid regions (b). The bootstrap values (1,000 replicates) are indicated in the phylogenetic tree, and values less than 70% are not represented

Figure 4
NoV Capsid/RdRp combination genotypes distribution in Children with acute diarrhea in different ages in Shanghai, 2012-2017