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ORIGINAL ARTICLE

Clinical and molecular epidemiology of norovirus infection in adults with acute gastroenteritis in Ji'nan, China

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Abstract Acute gastroenteritis caused by human noroviruses (NoVs) has become an important public health problem worldwide. This study was carried out to investigate the rates of NoV infections and the genetic characteristics of NoVs in adult outpatients with acute gastroenteritis in Ji'nan, a large eastern city in China. A total of 480 fecal samples were collected from outpatients at the Shandong University Qilu Hospital between June 2010 and May 2011. Of the collected samples, 42 (42/480, 8.75 %) were positive for NoVs by RT-PCR, and seven different genotypes were identified: GI-1, GI-4, GII-1, GII-3, GII-4, GII-6 and GII-13, of which GII-4 was the most prevalent (29/42, 69.0 %). Phylogenetic and Simplot analyses showed that three recombinant strains were detected: two GII-4 polymerase/GII-3 capsid recombinants and one GII-6 polymerase/GII-4 capsid recombinant. This study indicated that NoV was a common causative agent of sporadic acute gastroenteritis in adults in Ji'nan, China, and that NoV GII-4 was the predominant strain during this period. Three recombinant strains were identified in which GII-6 polymerase/GII-4 capsid was detected for the first time in China.

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Introduction

Noroviruses (genus *Norovirus*, family *Caliciviridae*) are the most common cause of both epidemic and sporadic non-bacterial acute gastroenteritis in children and adults [1–4]. NoV infection is characterized by sudden onset of diarrhea or vomiting, or both symptoms [5]. The viruses are transmitted by the fecal-oral pathway through a contaminated environment, water or food [6, 7]. The high attack rate of NoVs is due to their low infectious dose, high environmental stability and the short-term host immunity [6, 8]. Large outbreaks can occur in hospitals, cruise ships, schools and residential homes [9–11].

NoVs possess a single-stranded, positive-sense, polyadenylated RNA genome of 7.3-7.5 kb. The genome is divided into three open reading frames (ORFs): ORF1 encodes non-structural proteins, including the RNA-dependent RNA polymerase; ORF2 encodes the major structural proteins (capsid proteins); and ORF3 encodes a minor structural protein [12–14]. Based on sequence information, NoVs can be classified into five distinct genogroups (GI-GV). However, only GI, GII and GIV are associated with human infection, and GI and GII can be further divided into at least 8 and 17 genotypes, respectively [6, 15, 16]. NoVs showed high rates of recombination, and the most common breakpoint of recombination occurs in the region of the junction of ORF1 and ORF2 [17].

NoV has been recognized as a significant cause of epidemic viral gastroenteritis in all age groups. During the past several years, most studies of NoV infection in China have focused on children [6, 18, 19], and the role of NoVs in adults with acute gastroenteritis had not been well described. To achieve better control and prevention of NoV infections, it is necessary to understand the prevalence of

NoV infections in adults. In this study, we examine the clinical and molecular epidemiology of NoV infections in adults with acute gastroenteritis in Ji'nan, China.

Materials and methods

Fecal specimen collection

From June 2010 to May 2011, a total of 480 fecal specimens were collected from adult outpatients with acute gastroenteritis at the Department of Infectious Disease, Shandong University Qilu Hospital, Ji'nan, China. All outpatients who were enrolled met the following inclusion criteria: (1) they were older than 16 years, (2) they experienced vomiting or loose stools within a 24-h period and had undergone consultation at this hospital clinic for acute gastroenteritis, (3) they did not have pus or blood in their stools, and (4) they gave informed consent to participate in this study. All stool samples were freshly collected and immediately converted to 10 % (w/v) suspensions in phosphate-buffered saline, pH 7.2, containing 0.1 % diethylpyrocarbonate. The suspensions were centrifuged at 1,500 × g for 20 min, and the supernatants were collected and stored at −70 °C until they were used for viral RNA extraction.

Viral RNA extraction

Viral RNA was extracted from 200 µl of the stored fecal supernatant using a QIAamp Viral RNA Mini Kit according to the manufacturer's instructions. The extracted RNA was dissolved in 30 µl of RNase-free water and stored at −70 °C until used.

Detection of NoVs by RT-PCR

We carried out different RT-PCR assays for NoV genome characterization in which several sets of primers targeting the polymerase region and capsid region were used [20, 21].

The first RT-PCR targeted the polymerase region using primers JV12 (5'-TCATCATCACCATAGAAAGAG-3') and JV13 (5'-ATACCACTATGATGCAGATTA-3'). Reverse transcription (RT) was performed at 42 °C for 1 h using primer JV13 and SuperScriptII reverse transcriptase, followed by heating at 94 °C for 5 min and, finally, chilling at 4 °C. PCR was performed using JV12 and Taq DNA polymerase using the following steps: 94 °C for 3 min; 40 amplification cycles at 95 °C for 1 min, 37 °C for 90 s, and 74 °C for 1 min; and a final extension at 74 °C for 7 min.

The second RT-PCR targeted the capsid region using primers GI-SKF (5'-CTGCCCGAATTYGTAAATGA-3')/GI-SKR (5'-CCAACCCARCCATTRTACA-3') and COG2F (5'-CARGARBCNATGTTYAGRTGGATGAG-3')/G2-SKR (5'-CCRCCNGCATRHCCRTTRTACAT-3'). RT was performed at 50 °C for 1 h using a random primer and SuperScriptII reverse transcriptase. PCR was performed using the following steps: 94 °C for 3 min, 40 amplification cycles at 94 °C for 1 min, 55 °C for 30 s, and 72 °C for 60 s and a final extension at 72 °C for 7 min.

In order to authenticate the existence of recombinant strains, another RT-PCR was performed for three potential recombinants with primers JV12 and G2-SKR to amplify a fragment (1066 bp) covering the region encoding the RNA polymerase and capsid.

PCR products were detected by electrophoresis in a 2.0 % agarose gel and purified using a QIAquick PCR Purification Kit.

Analysis of sequence data

Amplification products were sequenced using a Big-Dye Terminator Cycle Sequencing Kit and an ABI 3730XL DNA Analyzer. All nucleotide sequences were compared with those of NoV strains deposited in the NCBI GenBank database, using the BLAST program. Multiple sequence alignments were analyzed using Clustal X (version 2.0). Phylogenetic trees were generated using the neighbor-joining method with MEGA (version 5.0). Simplot software (version 3.5.1) was used to compare sequences to identify potential recombinants.

Nucleotide sequences generated in this study were deposited in GenBank under the accession numbers JX666244-JX666327.

Results

NoV detection

A total of 480 stool specimens collected from outpatients with acute gastroenteritis between June 2010 and May 2011 were tested for the presence of NoVs, and 42 samples (42/480, 8.75 %) were positive for NoV based on analysis of partial sequences within the capsid region.

Age distribution and clinical manifestation

Infections with NoVs were found in every age group (16-25 years, 26-36 years, 37-57 years, >57 years). Of all of the patients infected with NoVs, the highest detection rate was in age group 26-36 years (19/42, 45.2 %), followed by >57 years (11/42, 26.2 %), 16-25 years (7/42, 16.7 %) and 37-57 years (5/42, 11.9 %).

Among the 42 patients infected with NoVs, the most common clinical manifestation was diarrhea (41/42,

97.6 %), followed by abdominal pain (17/42, 40.5 %), fever (12/42, 28.6 %) and vomiting (7/42, 16.7 %). The frequency of diarrhea and vomiting was 4-17 episodes/day and 1-5 episodes/day, respectively.

The highest rate of abdominal pain was detected in age group 26-36 years (12/19, 63.1 %). Fever and vomiting were more prevalent in age group 16-25 years (57.1 % and 42.8 %, respectively).

Seasonal distribution

A seasonal analysis of NoV infections was performed by calculating the positive rate of every month (Fig. 1). NoV infections were observed throughout the year. Higher activity of NoV infection was seen from September to December 2010, and lower positive rates were seen in June and July 2010.

Phylogenetic analysis

PCR products of 42 NoV-positive samples were sequenced and compared by phylogenetic analysis of the partial RNA sequences (Figs. 2 and 3). Two of the 42 positive samples (4.8 %) belonged to GI and the remaining 40 (95.2 %) were GII strains. One of the GI NoVs (JNHNV20100913) belonged to the GI-1 genotype within the same cluster as the reference strain GQ984296. The other GI NoV (JNHNV20101011) belonged to the GI-4 genotype within the same cluster as the reference strain GU012326. Among the 40 GII strains, GII-4 was the predominant genotype (29/40, 72.5 %). The remaining genotypes were GII-1 (2/40, 5 %), GII-3 (4/40, 10 %), GII-6 (1/40, 2.5 %), GII-13(1/40, 2.5 %) and recombinants (3/40, 7.5 %).

To investigate the presence of recombinant strains, phylogenetic analysis was performed using the partial capsid and RNA polymerase sequences, respectively. The results showed that there were two different potential recombinant types (Figs. 2 and 3): Two strains (JNHNV20101015, JNHNV20110405) that belonged to the GII-4 cluster based on the RNA polymerase sequence were classified as belonging to the GII-3 cluster based on the capsid sequence. One strain (JNHNV20101217) that belonged to the GII-6 cluster based on the RNA polymerase sequence was classified as belonging to the GII-4 cluster based on the capsid sequence.

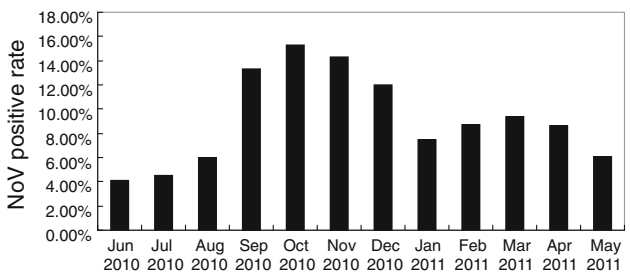

Fig. 1 Monthly distribution of norovirus infections between June 2010 and May 2011. The highest detection rate (15.3 %) was observed in October 2010. The lowest positive rate (4.1 %) was observed in June 2010. (Jun, 4.1 %; Jul, 4.5 %; Aug, 6.0 %; Sep, 13.3 %; Oct, 15.3 %; Nov, 14.3 %; Dec, 12 %; Jan, 7.5 %; Feb, 8.7 %; Mar, 9.4 %; Apr, 8.6 %; May, 6.1 %)

In order to eliminate the possibility that two NoV strains of different genotypes co-infected the same patient, leading to the observed differences in the branches of the phylogenic trees, a 1066-bp fragment was amplified and analyzed using SimPlot software. One SimPlot analysis for JNHNV1015 was performed with the Lorsdale (X86557, GII.4) and Mexico (U22498, GII.3) sequences. The other SimPlot analysis for JNHNV1217 was performed with the Saitama U3 (AB039776, GII6) and Lorsdale (X86557, GII.4) sequences (Fig. 4).

Discussion

Acute gastroenteritis caused by NoVs has become an important public-health problem worldwide. In this study, an investigation of clinical and molecular epidemiology of NoV was carried out in adult outpatients with acute gastroenteritis. The results showed that NoVs were detected in 8.75 % of all stool samples. The detection rate of NoVs in this study was lower than the rates from previous studies (8.9 %-16.1 %) [22–26] but was similar to that reported in children in China (7.6 %-8.9 %) [6, 18].

The most common clinical manifestation was diarrhea; some cases presented with abdominal pain, fever or vomiting. However, NoV infection resulted in a high rate of vomiting (>50 %) in children [27, 28]. Different seasonal patterns of NoV infections had been studied in various regions, with the peak in the spring, winter or rainy season [4, 23, 24, 29–32]. In this study, the seasonal peak occurred in autumn and early winter (from September to December), which was earlier and lasted longer than that observed for children [32, 33].

Genetic analysis revealed that most of the NoV strains in the present study belonged to the GII genogroup. GII-4 was the predominant genotype and circulated with other NoV genotypes (GI-1, GI-4, GII-1, GII-3, GII-6, GII-13) during this period in Ji'nan. This result is consistent with the report that GII-4 was the major genotype associated with most outbreaks and sporadic acute gastroenteritis [4, 18, 30, 32, 34].

RNA recombination is one of the important driving forces of NoV evolution [35]. Variation in the genome can strengthen the replication ability, virulence and/or environmental stability of the virus, giving rise to new strains capable of emergence in the human population. In our study,

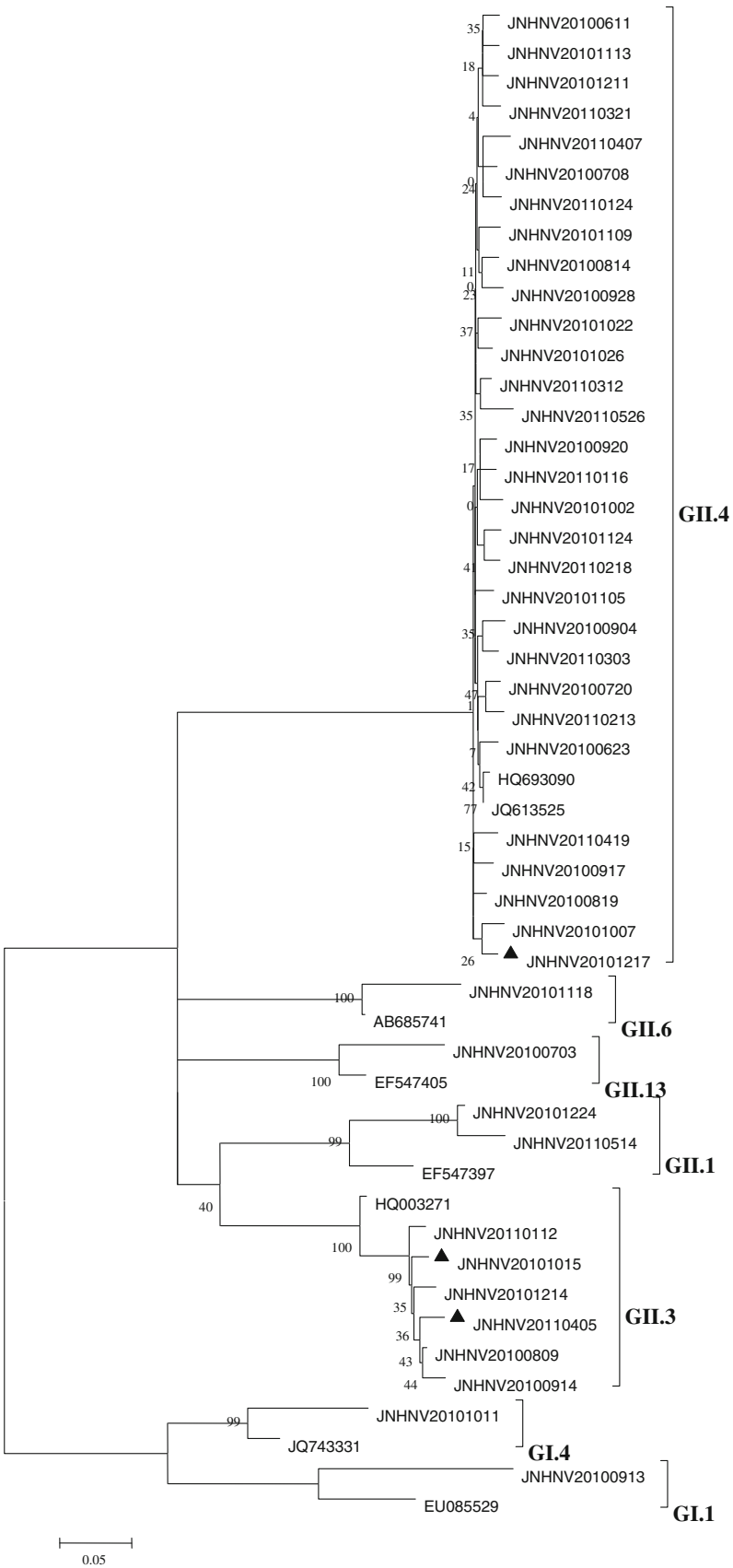

Fig. 2 Phylogenetic analysis based on partial capsid sequences (282 bp). All NoV-positive strains were genotyped. The tree was generated using the neighbor-joining method, and the bootstrap values from 1000 replicates are shown on each branch. The reference strains were from NCBI GenBank (HQ693090, JQ613525, AB685741, EF547405, EF547397, HQ003271, JQ743331, EU085529). Three strains with different genotypes based on analysis of polymerase sequences and capsid sequences are indicated by triangles

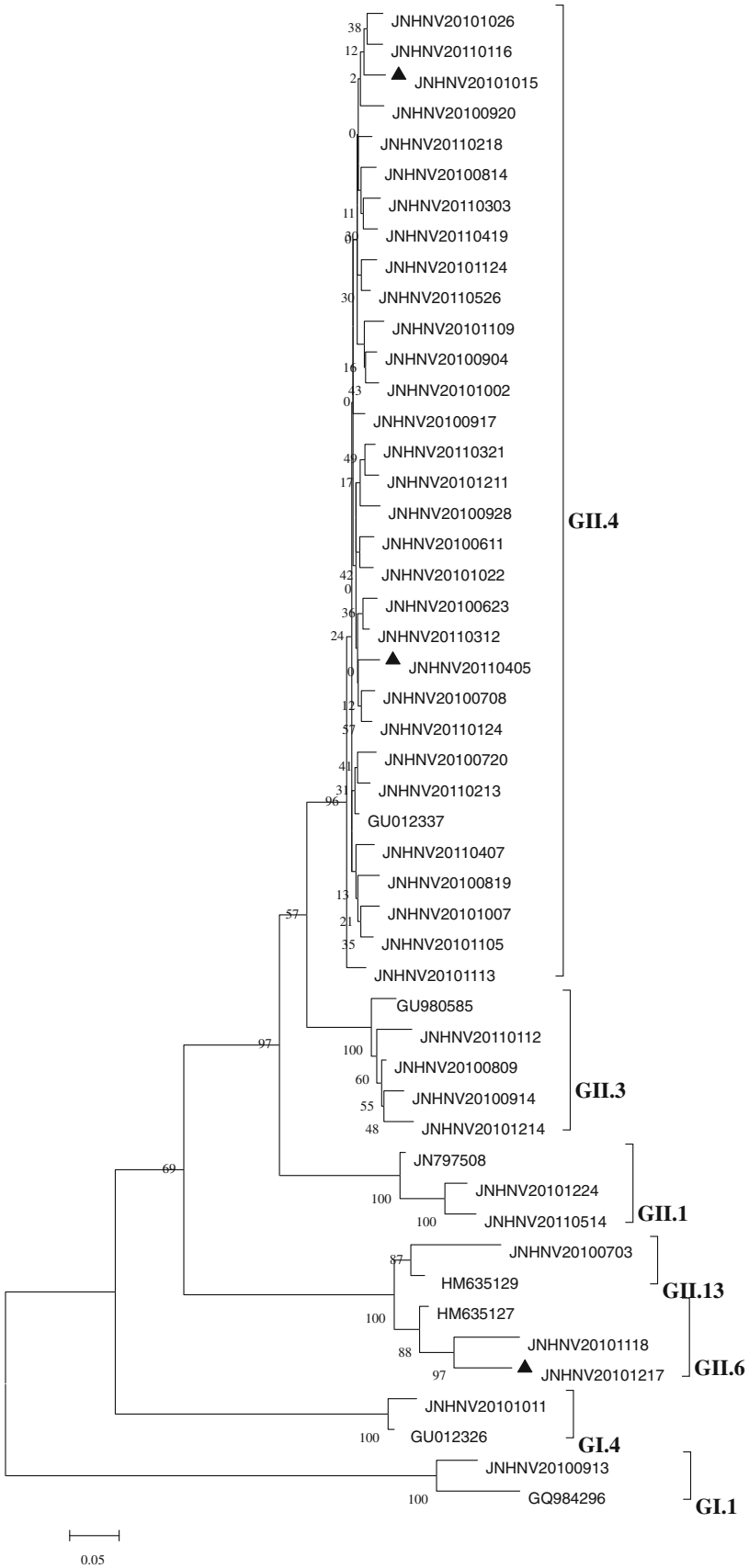

Fig. 3 Phylogenetic analysis based on partial polymerase sequences (298 bp). All NoV-positive strains were genotyped. The tree was generated using the neighbor-joining method, and the bootstrap values from 1000 replicates are shown on each branch. The reference strains were from NCBI GenBank (GU012337, HM635112, JN797508, HM635129, GU012326, HM635127, GQ984296). Three strains with different genotypes based on analysis of polymerase sequences and capsid sequences are indicated by triangles

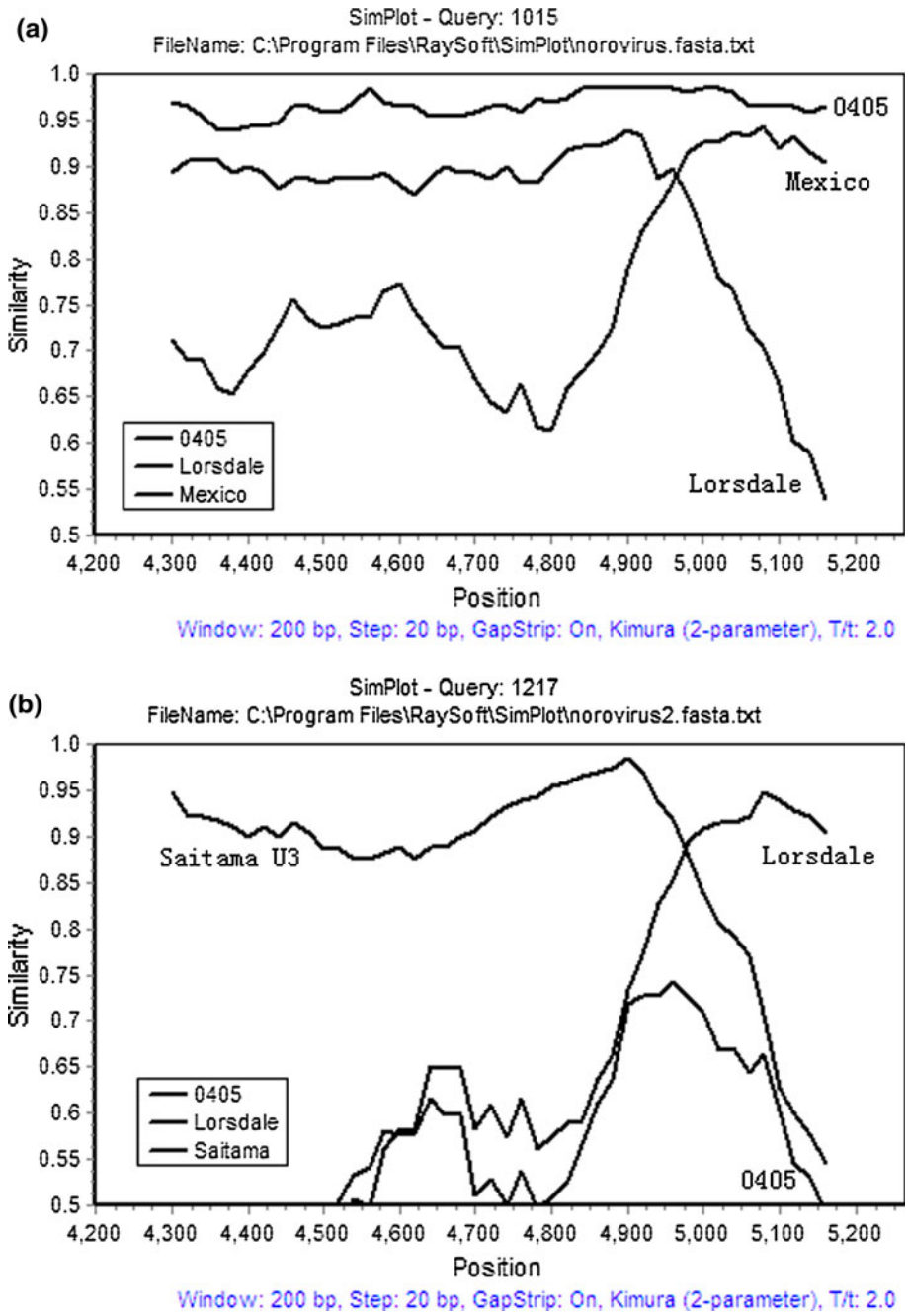

Fig. 4 Simplot analysis for identification of recombinants. The vertical axis indicates the nucleotide sequence similarity (%) between the query sequence and the parental strains. The horizontal axis indicates the nucleotide position. The site where the parental strains cross is the predicted site of recombination. **a** Detection of recombination in JNHNV1015. The Lorsdale and Mexico strains served as putative parental strains, and JNHNV0405 served as a control strain. **b** Detection of recombination in JNHNV1217. The Saitama and Lorsdale strains served as putative parental strains, and JNHNV0405 served as a control strain

we found two recombinant types (three strains) by phylogenetic analysis and Simplot analysis. JNHNV20101015 and JNHNV20110405 belonged to the same recombinant type, GII-4 polymerase/GII-3 capsid, which had been observed in children with NoV infection in China [6]. JNHNV20101217 belonged to another type, GII-6 polymerase/GII-4 capsid, which had not been reported previously in China. Further surveillance will be required, particularly on the recombinant strains, because the novel recombinant strains might have the ability to cause sporadic outbreaks of acute gastroenteritis [36–38].

In conclusion, the data from this study indicated that NoV infections were an important cause of acute gastroenteritis in adults in Ji'nan, China. Infected cases were found throughout the year with a peak from September to December. Of all of the isolates, the GII-4 strain was the dominant strain. Two recombinant types were seen: GII-4 polymerase/GII-3 capsid and GII-6 polymerase/GII-4

capsid. This study provides useful data for understanding the clinical and epidemiological features of NoV infection in Ji'nan, China.

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Conflict of interest The authors declare that they had no conflict of interest.

References

1. Junquera CG, de Baranda CS, Mialdea OG, Serrano EB, Sánchez-Fauquier A (2009) Prevalence and clinical characteristics of norovirus gastroenteritis among hospitalized children in Spain. Pediatr Infect Dis J 28:604–607
2. Johnston CP, Qiu H, Ticehurst JR, Dickson C, Rosenbaum P, Lawson P, Stokes AB, Lowenstein CJ, Kaminsky M, Cosgrove SE, Green KY, Perl TM (2007) Outbreak management and implications of a nosocomial norovirus outbreak. Clin Infect Dis 45:534–540
3. Sakon N, Yamazaki K, Yoda T, Tsukamoto T, Kase T, Taniguchi K, Takahashi K, Otake T (2007) Norovirus storm in Osaka, Japan, last winter (2006/2007). Jpn J Infect Dis 60:409–410
4. Victoria M, Carvalho-Costa FA, Heinemann MB, Leite JP, Miagostovich M (2007) Prevalence and molecular epidemiology of noroviruses in hospitalized children with acute gastroenteritis in Rio de Janeiro, Brazil, 2004. Pediatr Infect Dis J 26:602–606
5. Atmar RL, Estes MK (2006) The epidemiologic and clinical importance of norovirus infection. Gastroenterol Clin N Am 35:275–290
6. Jin M, Xie HP, Duan ZJ, Liu N, Zhang Q, Wu BS, Li HY, Cheng WX, Yang SH, Yu JM, Xu ZQ, Cui SX, Zhu L, Tan M, Jiang X, Fang ZY (2008) Emergence of the GII4/2006b variant and recombinant noroviruses in China. J Med Virol 80:1997–2004
7. O'Reilly CE, Bowen AB, Perez NE, Sarisky JP, Shepherd CA, Miller MD, Hubbard BC, Herring M, Buchanan SD, Fitzgerald CC, Hill V, Arrowood MJ, Xiao LX, Hoekstra RM, Mintz ED, Lynch MF (2007) A waterborne outbreak of gastroenteritis with multiple etiologies among resort island visitors and residents: Ohio, 2004. Clin Infect Dis 44:506–512
8. Rockx B, De Wit M, Vennema H, Vinj′e J, De Bruin E, Van Duynhoven Y, Koopmans M (2002) Natural history of human calicivirus infection: a prospective study. Clin Infect Dis 35(3):246–253
9. Lopman BA, Brown DW, Koopmans M (2002) Human caliciviruses in Europe. J Clin Virol 24:137–160
10. Mark PJ, Vipond IB, Regan FM, Wedgwood K, Fey RE, Caul EO (2003) A school outbreak of Norwalk-like virus: evidence for airborne transmission. Epidemiol Infect 131:727–736
11. Vinje J, Altena SA, Koopmans MP (1997) The incidence and genetic variability of small round-structured viruses in outbreaks of gastroenteritis in the Netherlands. J Infect Dis 176:1374–1378
12. Bertolotti CA, Crawford SE, Hutson AM, Estes MK (2003) The 3' end of Norwalk virus mRNA contains determinants that regulate the expression and stability of the viral capsid protein VP1: a novel function for the VP2 protein. J Virol 77:11603–11615
13. Green KY, Ando T, Balayan MS, Berke T, Clarke IN, Estes MK, Matson DO, Nakata S, Neill JD, Studdert MJ, Thiel HJ (2000) Taxonomy of the caliciviruses. J Infect Dis 181:S322–S330
14. Xi JN, Graham DY, Wang KN, Estes MK (1990) Norwalk virus genome cloning and characterization. Science 250:1580–1583
15. Wang QH, Han MG, Cheetham S, Souza M, Funk JA, Saif LJ (2005) Porcine noroviruses related to human noroviruses. Emerg Infect Dis 11:1874–1881
16. Zheng DP, Ando T, Fankhauser RL, Beard RS, Glass RI, Monroe SS (2009) Norovirus classification and proposed strain nomenclature 2006. Virology 346:312–323
17. Bull RA, Hansman GS, Clancy LE, Tanaka MM, Rawlinson WD, White PA (2005) Norovirus recombination in ORF1/ORF2 overlap. Emerg Infect Dis 11:1079–1085
18. Guo L, Song J, Xu X, Ren L, Li J, Zhou H, Wang M, Qu J, Wang J, Hung T (2009) Genetic analysis of norovirus in children affected with acute gastroenteritis in Beijing, 2004–2007. J Clin Virol 44:94–98
19. Xu J, Yang Y, Sun J, Ding Y (2009) Molecular epidemiology of norovirus infection among children with acute gastroenteritis in Shanghai, China 2001–2005. J Med Virol 81:1826–1830
20. Kojima S, Kageyama T, Fukushi S, Hoshino FB, Shinohara M, Uchida K, Natori K, Takeda N, Katayama K (2002) Genogroup-specific PCR primers for detection of Norwalk-like viruses. J Virol Methods 100:107–114
21. Vinje J, Koopmans M (1996) Molecular detection and epidemiology of small round-structured viruses in outbreaks of gastroenteritis in the Netherlands. J Infect Dis 174(3):610–615
22. De Wit MA, Koopmans MP, Kortbeek LM, Wannet WJ, Vinje J, van Leusden LF, Bartelds AI, van Duynhoven YT (2001) A population-based cohort study on gastroenteritis in the Netherlands: incidence and etiology. Am J Epidemiol 154:666–674
23. Fretz R, Herrmann L, Christen A, Svoboda P, Dubuis O, Viollier EH, Tanner M, Baumgartner A (2005) Frequency of norovirus in stool samples from patients with gastrointestinal symptoms in Switzerland. Eur J Clin Microbiol Infect Dis 24:214–216
24. Marshall JA, Hellard ME, Sinelair MI, Fairley CK, Cox BJ, Catton MG, Kelly H, Wright PJ (2003) Incidence and characteristics of endemic Norwalk-like virus-associated gastroenteritis. J Med Virol 69:568–578
25. Subekti DS, Tjaniadi P, Lesmana M, Simanjuntak C, Komalarini S, Digdowirogo H, Setiawan B, Campbell JR, Porter KR, Oyofo BA (2002) Characterization of Norwalk-like virus associated with gastroenteritis in Indonesia. J Med Virol 67:253–258
26. Yan G, Miao J, Xv C, Zhaojun D, Hui-ying L, Xiaolin G, Ying Z, Yanmin Z, Yi Z, Lai W (2011) Clinical and molecular epidemiologic analyses of norovirus-associated sporadic gastroenteritis in adults from Beijing, China. J Med Virol 83:1078–1085
27. Lopman BA, Reacher MH, Vipond IB, Saranqi J, Brown DW (2004) Clinical manifestation of norovirus gastroenteritis in health care settings. Clin Infect Dis 39:318–324
28. Buesa J, Collado B, López-Andújar P, Abu-Mallouh R, Rodríguez Díaz J, García Díaz A, Prat J, Guix S, Llovet T, Prats G, Bosch A (2002) Molecular epidemiology of caliciviruses causing outbreaks and sporadic cases of acute gastroenteritis in Spain. J Clin Microbiol 40:2854–2859
29. Bon F, Ambert BK, Giraudon H, Kaplon J, Le GS, Pommepuy M, Gallay A, Vaillant V, de Valk H, Chikhi BR, Flahaut A, Pothier P, Kohli E (2005) Molecular epidemiology of caliciviruses detected in sporadic and outbreak cases of gastroenteritis in France from December 1998 to February 2004. J Clin Microbiol 43:4659–4664
30. Dove W, Cunliffe NA, Gondwe JS, Broadhead RL, Molyneux ME, Nakagomi O, Hart CA (2005) Detection and characterization of human caliciviruses in hospitalized children with acute gastroenteritis in Blantyre, Malawi. J Med Virol 77:522–527
31. Iritani N, Seto Y, Kubo H, Murakami T, Haruki K, Ayata M, Ogura H (2003) Prevalence of Norwalk-like virus infections in cases of viral gastroenteritis among children in Osaka City, Japan. J Clin Microbiol 41:1756–1759

32. Medici MC, Martinelli M, Abelli LA, Ruggeri FM, Di Bartolo I, Areangeletti MC, Pinardi F, De Conto F, Izzi G, Bernasconi S, Chezzi C, Dettori G (2006) Molecular epidemiology of norovirus infections in sporadic cases of viral gastroenteritis among children in northern Italy. J Med Virol 78:1486–1492
33. Boga JA, Melon S, Nicieza I, De DI, Villar M, Parra F, De OM (2004) Etiology of sporadic cases of pediatric acute gastroenteritis in Asturias, Spain, and genotyping and characterization of norovirus strains involved. J Clin Microbiol 42:2668–2674
34. Siebenga JJ, Vennema H, Duizer E, Koopmans MP (2007) Gastroenteritis caused by norovirus GGII.4, the Netherlands, 1994–2005. Emerg Infect Dis 13:144–146
35. Worobey M, Holmes EC (1999) Evolutionary aspects of recombination in RNA viruses. J Gen Virol 80:2535–2543
36. Bull R, Tanaka M, White P (2007) Norovirus recombination. J Gen Virol 88:3347–3359
37. Roberto V, Patricia R, Veronica S, Jimena V, Xi J, David OM, Nora M, Valeria P, Mignel L (2006) Novel recombinant norovirus causing outbreaks of gastroenteritis in Santiago, Chile. J Clin Microbiol 44:2271–2275
38. Reuter G, Krisztalovics K, Vennema H, Koopmans M, Szucs G (2005) Evidence of the etiological predominance of norovirus in gastroenteritis in Hungary. J Med Virol 76:598–607