Arch Virol (2011) 156:1641–1646
DOI 10.1007/s00705-011-1010-3

BRIEF REPORT

Molecular epidemiology of norovirus gastroenteritis in children in Jiangmen, China, 2005–2007

Ying-chun Dai · Gui-fang Hu · Xu-fu Zhang · Can-lei Song · Wen-long Xiang · Xian-bo Wu · Le-yi Wang · Xi Jiang · Jun Nie

Received: 15 December 2010 / Accepted: 21 April 2011 / Published online: 12 May 2011

Abstract Human noroviruses (NoVs) are an important cause of epidemic acute gastroenteritis. Their role in sporadic cases, however, is less clear. In this study, we performed a two-year surveillance (September 2005 to August 2007) of NoV gastroenteritis in outpatient clinics in a southern city of China, Jiangmen City. NoVs were detected in 115 patients (115/881, 13.1%) with 30 (26.1%) co-infections with rotaviruses. Sequence analysis showed that all 115 NoVs belonged to genogroup II, with GII.4 being the most predominant (87.8%). NoV-associated infection can be seen year-around, with autumn and winter peaks. This study provides basic information on sporadic cases of major NoV gastroenteritis in children in different seasons, which is valuable for future disease control and prevention.

Y. Dai and G. Hu contributed equally to this research.

Y. Dai · G. Hu · C. Song · W. Xiang · X. Wu · J. Nie (✉)
Department of Epidemiology, School of Public Health and Tropical Medicine, Southern Medical University, Tonghe Road, Guangzhou 510515, Guang Dong, People's Republic of China
e-mail: jun_nie@live.cn

X. Zhang
Department of Traditional Chinese Medicine, Southern Medical University, Guangzhou, People's Republic of China

L. Wang · X. Jiang (✉)
Division of Infectious Diseases, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229-3039, USA
e-mail: jason.jiang@cchmc.org

X. Jiang
Department of Pediatrics, College of Medicine, University of Cincinnati, Cincinnati, OH, USA

Keywords Norovirus · Calicivirus · Acute gastroenteritis · Diarrhea · Sporadic cases · Recombinant

Noroviruses (NoVs) are the leading cause of non-bacterial acute gastroenteritis in people of all ages in both developing and developed countries [1, 4, 7, 30, 37]. These viruses are highly infectious and stable in the environment and can cause large outbreaks in different institutional and community settings [28, 35, 39]. NoVs have a single positive-strand RNA genome of approximately 7.4-7.7 kb, enclosed in a non-enveloped protein coat with distinct cup-shaped depressions [14, 32]. The genome can be divided into three open reading frames (ORFs): ORF1 encodes six nonstructural proteins, including the RNA-dependent RNA polymerase, and ORF2 and ORF3 encode structural proteins [13]. NoV is genetically diverse, and human strains can be divided into three genogroups (GI, GII, and GIV), at least 25 genotypes, and numerous subgroups based on sequence information from the ORF1 or ORF2 region [31, 44]. The high genetic diversity of NoVs can be attributed to both point mutations during genome replication and RNA recombination between co-circulating strains [2]. At least 23 inter-genotype and 9 intra-genotype NoV recombinants have been identified worldwide [8, 11, 26, 27].

Acute gastroenteritis (AGE) is one of the most common illnesses in China as well as in other developing countries. In family settings, gastroenteritis is second in frequency only to the common cold [6, 22]. The role of human NoV in epidemic and sporadic AGE has been emphasized recently [9, 30, 37]. However, their role in sporadic gastroenteritis in children in China (especially southern China) is not well described. The present study was carried out to determine the prevalence and genetic variability of NoVs

in young children with AGE at outpatient clinics from a middle-sized southern city of China, Jiangmen City of Guangdong province.

AGE diagnosis was made according to the viral gastroenteritis diagnosis standard of 'The Chinese Diarrhea Diagnosis Treatment Plan (1993)'. Patients included in this study met the following criteria: [1] they presented with clinical symptoms of gastroenteritis, including vomiting, diarrhea and fever, and the illness lasted less than 7 days [2]. Stools from the patients were often watery, but without blood [3]. White blood cells were detected occasionally, but no red blood cells were detected. Stool specimens were collected from each of 881 children younger than 5 years of age who were admitted to the outpatient clinics of Children's Hospital of Jiangmen City for AGE from September 2005 to August 2007. Information about clinical characteristics was collected by reviewing the clinical records and interviewing the parents as well as follow-up at the clinics every day until the child recovered from the illness. All stool specimens were tested for rotavirus by using a colloidal gold device (Wantai, Beijing, China) within 24 h and then stored at −80°C before being tested for NoV. Informed consent was obtained from the parents of all children who provided specimens. The study protocol was approved by the ethics committees of the School of Public Health and Tropical Medicine of Southern Medical University.

Nucleic acid was extracted from 100 µl of 20% (w/v) stool-PBS suspensions of 881 stool specimens using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). Precipitated RNA was dissolved in 20 µl of RNase-free water. The first-strand cDNA was synthesized using reverse transcriptase Superscript III (Invitrogen, Calrsbad, CA) and random hexamers (TakaRa, Japan) at 50°C for 1 h followed by another incubation of 99°C for 5 min. The RdRp genes were amplified with oligonucleotide primers JV12Y and JV13I, and capsid genes were amplified with GISKF/GISKR and COCG2F/GIISKR by using methods described earlier [19, 38]. Then, strains that seemed likely to be recombinant because the genotype was different between the RdRp and the capsid were amplified by using primers JV12Y and GISKR for GI or primers JV 12 Y and GIISKR for GII. The amplified PCR products were 1120 bp long for GI and 1111 bp for GII [11].

Purified PCR products were sequenced by Invitrogen (Shanghai, China). The nucleotide sequences of the PCR amplicons were aligned with the corresponding NoV sequences from the GenBank database using BLAST. Multiple sequence alignments were created using Clustal X (version 1.83) [36]. Phylogenetic trees of NoVs were constructed using the neighbor-joining method in MEGA (version 4.1) [34]. SimPlot software (Version 3.5.1) was used to compare sequences to identify potential recombinant virus strains [23].

Of the 881 stool samples, 115 (13.1%) were positive for NoV. Rotavirus was detected in 368 samples (41.8%), which was consistent with the results from most other parts of China and in the surrounding countries [3, 4, 15, 18, 22, 41]. Co-infection with these two viruses was found in 30 cases (3.4%). The majority (96.5%) of the diarrhea samples were collected from young children from birth to 3 years of age. Of the 190 infants who were less than 6 months old, 17 (8.95%) were positive for NoV. The prevalence of NoV infection was 12.7% (35/275) in infants from 6 to 11 months. High detection rates were observed in children between 24 to 35 months (14/68, 20.6%, Fig. 1). Of the 115 children with NoV infection, 73 were boys, with an infection rate of 13.8% (73/530). Different seasonal patterns of NoV infections have been reported. Some studies showed a higher frequency in the winter, spring or rainy season, and others indicated no obvious peak season [15, 24, 29]. In this study, NoV was detected almost all year around except March and June 2006 and August 2008, and the highest detection rates were from September to January, the autumn/winter seasons (Fig. 2). Similar seasonal distributions were also found in Shanghai and in our previous study in Guangzhou [7, 41]. The onset symptoms included vomiting (40.9%), diarrhea (44.3%), fever (7.0%) and other symptoms (7.8%). The main symptoms during the diarrhea period also included vomiting (59.2%), fever (20%), stomach ache (13.0%), dehydration (19.1%) and cough (9.6%). The median (0–100%) duration of diarrhea was 3.5 [1–10] days, and the number of stools per day was 5 [2–15]. Approximately one-fifth of the children had mild fever (mean [SD] = 38.3°C [0.6°C]).

All 115 NoVs detected in this study belonged to genogroup II (GII), based on the RNA-dependent RNA polymerase (RdRp) and the capsid protein sequence (Fig. 3). Phylogenetic analysis of partial capsid sequences (241 bp) of GII NoV (Fig. 3b) indicated that the main cluster of GII.4 strains (80/101, 79.2%) belonged to the GII.4/2006b cluster, which shared 97.8-99.3%

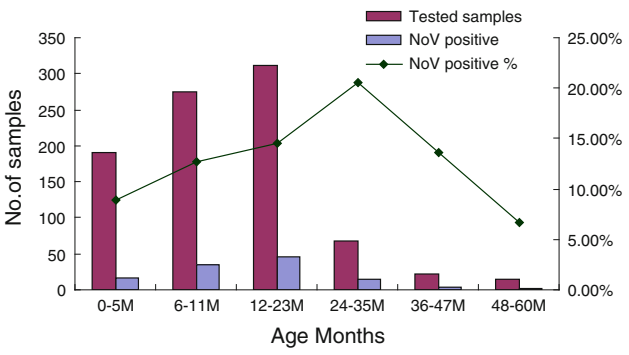

Fig. 1 Prevalence of NoV infections in children in Jiangmen, China, 2005-2007, by age group. Bars represent the number of detected cases, and lines represent the positive rate of NoV infections

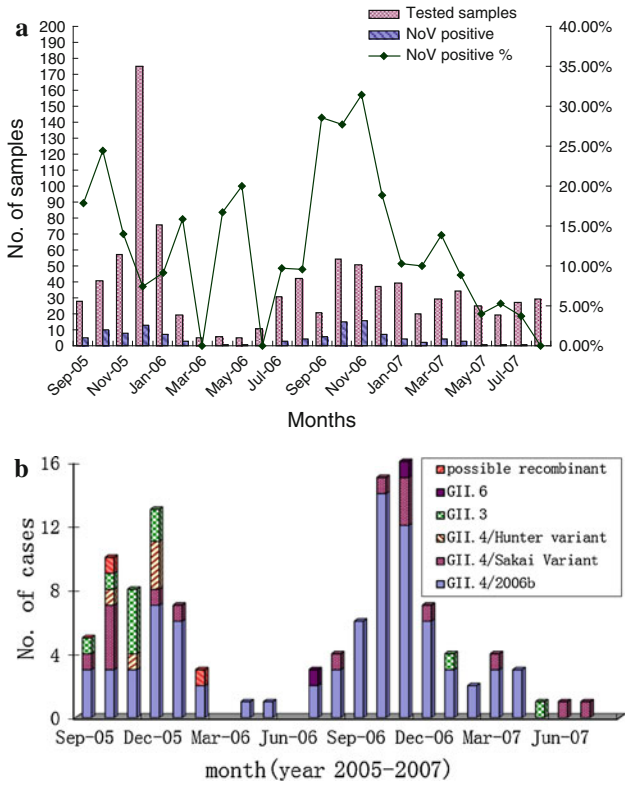

Fig. 2 Seasonal distribution of 115 NoV infections in Jiangmen city, China, 2005-2007. **a** Bars represent the number of detected cases, and lines represent the positive rate of NoV infections. **b** Bars represent the number of cases of the NoV genotype

nucleotide identity with Hu/GII-4/Aichi3/2006/JP (AB447446). One of the two minor clusters of GII.4 strains belonged to the GII.4/Hunter variant (2006a variant), which was associated with both outbreaks and sporadic cases throughout the world [33]. Another minor cluster of GII.4 showed 98% identity to virus strain Hu/GII-4/Sakai2/2006/JP, which was also found in Japan and Korea during the same time period [33, 43]. Ten of the 115 NoV strains were found to belong to GII.3 genotypes, clustering in the same linage with the Mexico strains. Two GII.6 strains showed high identity (99%) to the reference strain SaitamaU17 [17]. Moderate variations were observed in strains circulating over the two years of surveillance. In the first period, from September 2005 to August 2006, the NoV infection rate was 11.1% (55/496). Of 55 positive samples, 31 (56.4%) belonged to GII.4/2006b, 8 (14.5%) belonged to the Sakai strain, 5 (9.1%) belonged to the Hunter strain and 8 (14.5%) belonged to the GII.3 strain. In the second period (September 2006-August 2007), the NoV infection rate was slightly increased to 15.6% (60/385). Of the 60 positive samples, 49 (81.7%) belonged to GII.4/2006b, 8 (13.3%) belonged to the Sakai strain, and 2 (3.3%) belonged to the GII.3 strain.

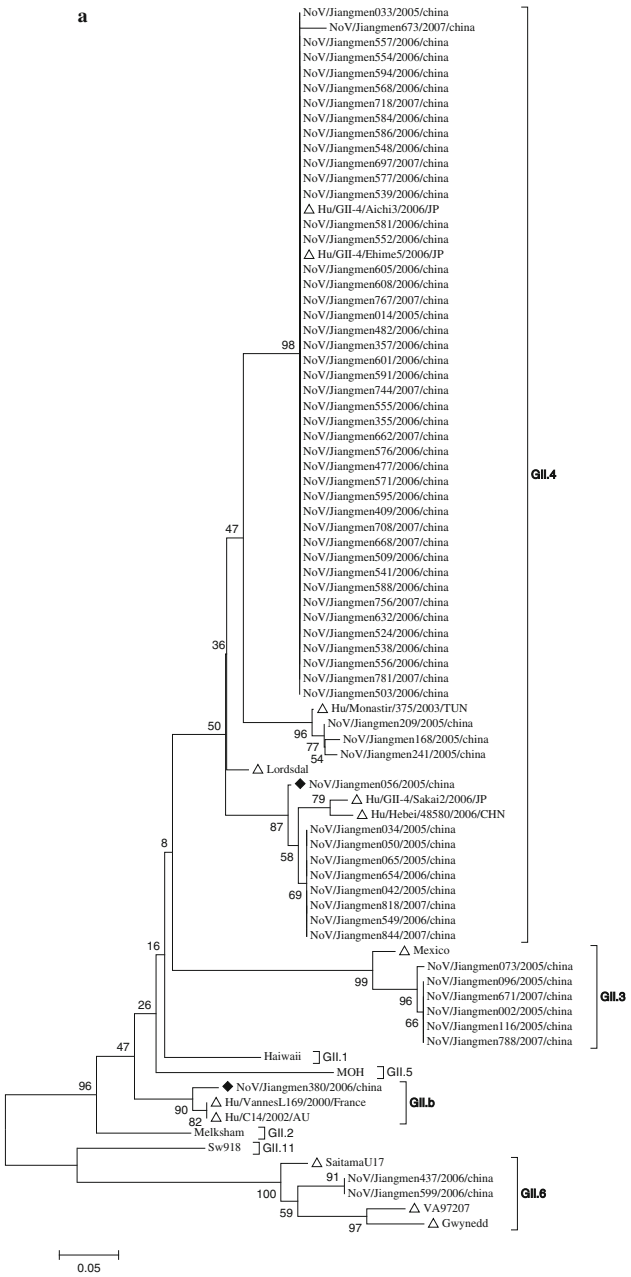

Fig. 3 Phylogenetic tree based on nucleotide sequences from 65 randomly selected representatives of the NoV strains in this study. The bootstrap values generated from 1000 replicates are shown at each node. **a** Nucleotide sequences were determined for a 209-bp fragment of the RDRP region (nt 4397-4605) corresponding to NVgz01 strain (GenBank DQ 369797). **b** Nucleotide sequences were determined for a 241-bp fragment of the capsid region (nt 5085-5324) corresponding to the NVgz01 strain. (The NCBI GenBank accession numbers are as follows: Lincoln House (DQ676865), Hunter (DQ78794), Hawaii (U07611), Melksham (X81879), Mexico (U22498), Lordsdal (X86557), MOH (AF397156), Gwyned (AF 414 409), SaitamaU25 (AB067543), Sw918 (AB 074893), SaitamaU1 (AB039775), Pont de Roide (AY682548), M7 (A Y130761), Tiffin (AY502010), CS-E1 (AY502009), SaKaeo-53 (AY646870), NLV/VannesL 169/2000/France (AY773210), Hu/C 14/2002/AU (AY845056), SaitamaU17 (AB03 9779), Hu/GII-4/Sakai2/2006/JP (AB447448), Hu/Hebei/48580/2006/CHN (EF670649)

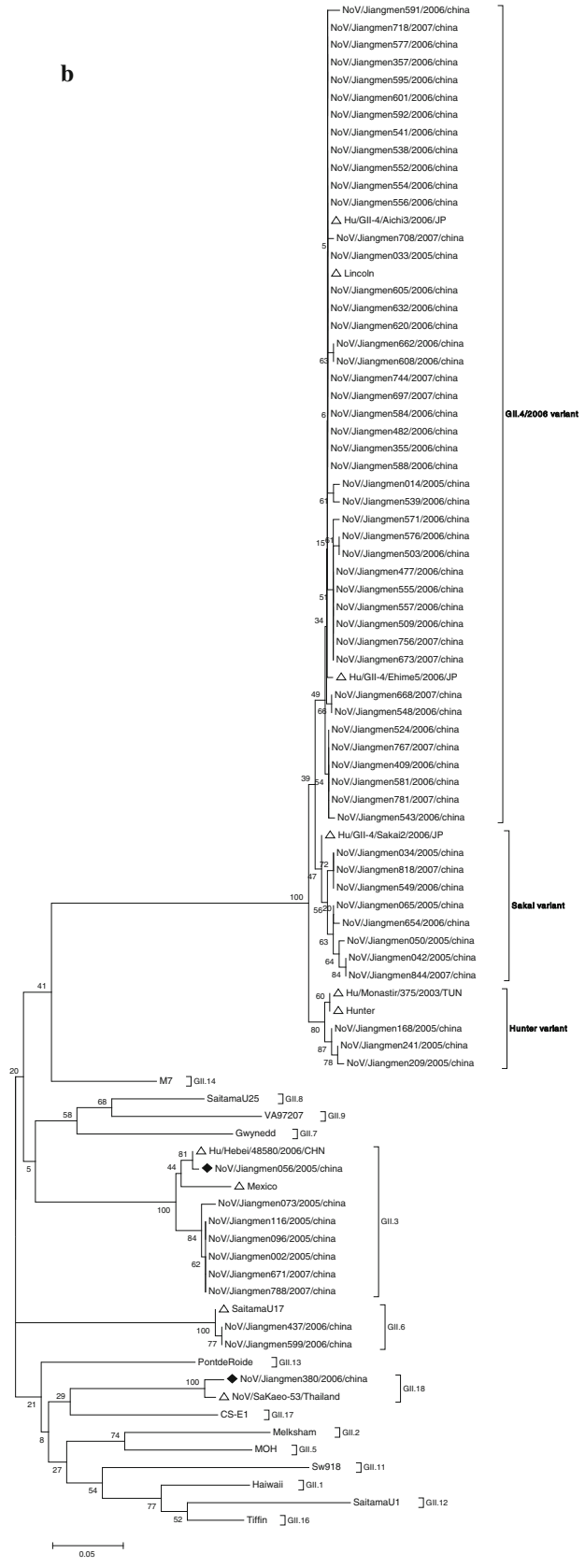

Fig. 3 continued

GII.4 was the predominant genotype (101 cases, 87.8%), which is consistent with reports from many countries, including China [3, 10, 15, 16, 18, 41]. The predominance of GII.4 also is believed to be attributable to the broad spectrum of GII.4 in recognizing human histo-blood group antigen receptors [42] . We also observed the occurrence of similar correlation patterns of major circulating GII.4 variants found in our study with those found in other countries. For example, in the past two decades, the circulating NoV GII.4 variants could be further divided into six clusters: Camberwell (GII.4–1987), Grimsby (GII.4–1997), Farmington Hills (GII.4–2002), Hunter (GII.4–2004), Sakai (GII.4–2005) and DenHaag (2006b variants) clusters [21], in which the GII.4/2006b variant was first isolated and accounted for 79.2% of GII.4 infections in Spain in 2005. This variant was then spread and became predominant in Europe, Japan, western India and China (Beijing) [4, 12, 15, 25]. In our study, the GII.4/2006b variant accounted for 56.4% of all NoV infections in 2005-2006 and 81.7% in 2006-2007. The Hunter cluster, which was dominant in 2004 in other countries, caused 9.1% of NoV infections in 2005-2006 but disappeared in 2006-2007 in our study.

We also found linkage of outbreak strains of GII NoVs in our study with those in other countries. For example, the GII.6 NoV was reported in the surrounding areas and countries [4, 40, 43] but was hardly detected in China, except for a GII.6 recombinant reported in 2008 [15]. Two GII.6 NoVs were found in our study, with high identities to those circulating in neighboring countries and cities, indicating a common source of infection. In addition, the GII.3 strain was the second predominant genotypes (14.5%) in 2005-2006 and decreased to 3.3% in 2006-2007 in our study, which was similar to that in the entire province of Guangdong [20], in which GII.3 was common in 2005 but was almost completely replaced by GII.4/2006b in 2006.

We also detected two recombinant NoVs in this study. The RdRp gene of strain NoV/Jiangmen380/2006/China had 97% identity to strain Hu/NLV/VannesL169/2000/France and Hu/C14/2002/AU of the GII.b strains. However, its capsid gene had 97% identity to NoV/SaKaeo-53/Thailand and 94% identity to the IF1998 strain, a “novel capsid type” described as GII.18 strains [31]. Because the RdRp sequence for NoV/SaKaeo-53/Thailand and IF1998 is not available, SimPlot analysis was performed with the reference strain Hu/NLV/VannesL169/2000/France (Fig. 4a). This recombinant has rarely been reported, and to our knowledge, this is the first report of the GII.b/GII.18 recombinant in China. Though rarely reported, this recombinant contributed to 37% of NoV infections in western India in 2005 [5]. Another strain, NoV/Jiangmen056/2006/China, had 97% identity in the RdRp but only 71.8% in the capsid

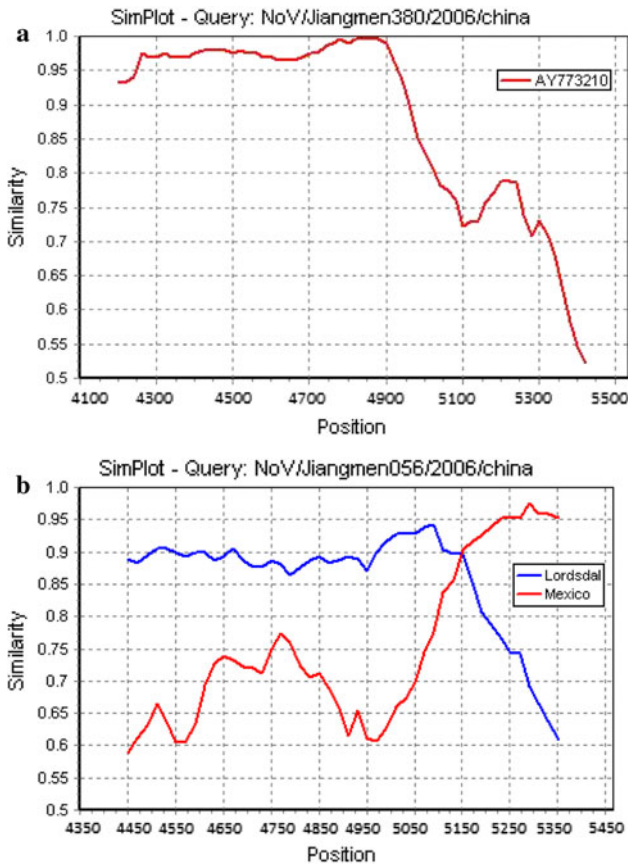

Fig. 4 SimPlot3.5.1 analysis of partial RdRp and capsid gene sequences of two possible recombinant strains. The window size was 100 bp, with a step size of 10 bp. **a** Simplot analyses of the NoV/Jiangmen380/2006/China strain with the reference VannesL169 strain (AY773210). **b** Simplot analysis of the NoV/Jiangmen056/2006/China strain with two reference strains, Lordsdal (X86557) and Mexico (U22498). The site where the two reference strains have equal identity to the recombinant is the predicted site of recombination

gene with strain Hu/GII-4/Sakai2/2006/JP, but the capsid gene had 95% identity to that of the Mexico strain (GII.3) (Fig. 4b).

In conclusion, the present study highlights the epidemiology, strain variations and putative recombinants of NoV in sporadic cases of AGE in children in China. The age and seasonal distribution data are valuable for future disease control and prevention. The predominance of GII.4 and its genetic variation as well as the variations in other GII NoVs and their potential relatedness to those of the surrounding countries found in our study provide further information for understanding the global distribution of NoV as an important cause of acute gastroenteritis.

Acknowledgments The authors are grateful to CDC of Jiangmen city for the specimens and data collection. This study was supported by the National Natural Science Foundation of China (Grants 30700716 and 30901992), Medical Scientific Research Foundation of Guangdong Province (B2010174), Doctoral Fund of Ministry of Education of China (No. 20104433120015), and Guangdong Province "211 Project" (Grant No. GW201006 and GW201106) of Southern Medical University. Dr. Jiang was supported by the NIH of the United States (R01 AI37093 and R01 AI55649).

Conflict of interest None.

References

1. Bon F, Ambert-Balay K, Giraudon H, Kaplon J, Le Guyader S, Pommepuy M, Gallay A, Vaillant V, de Valk H, Chikhi-Brachet R, 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
2. Bull RA, Tanaka MM, White PA (2007) Norovirus recombination. J Gen Virol 88:3347–3359
3. Cheng WX, Ye XH, Yang XM, Li YN, Jin M, Jin Y, Duan ZJ (2010) Epidemiological study of human calicivirus infection in children with gastroenteritis in Lanzhou from 2001 to 2007. Arch Virol 155:553–555
4. Chhabra P, Dhongade RK, Kalrao VR, Bavdekar AR, Chitambar SD (2009) Epidemiological, clinical, and molecular features of norovirus infections in western India. J Med Virol 81:922–932
5. Chhabra P, Walimbe AM, Chitambar SD (2010) Molecular characterization of three novel intergenotype norovirus GII recombinant strains from western India. Virus Res 147:242–246
6. Dai YC, Nie J, Zhang XF, Li ZF, Bai Y, Zeng ZR, Yu SY, Farkas T, Jiang X (2004) Seroprevalence of antibodies against noroviruses among students in a Chinese military medical university. J Clin Microbiol 42:4615–4619
7. Dai YC, Xia M, Zhan HC, Liu Y, Li JD, Chen Q, Yu SY, Nie J, Farkas T, Jiang X (2010) Surveillance and risk factors of norovirus gastroenteritis among children in a southern city of China in the fall-winter seasons of 2003–2006. J Paediatr Child Health 46:45–50
8. Dey SK, Phan TG, Mizuguchia M, Okitsua S, Ushijima H (2010) Novel recombinant norovirus in Japan. Virus Genes 40:362–364
9. Eden JS, Bull RA, Tu E, McIver CJ, Lyon MJ, Marshall JA, Smith DW, Musto J, Rawlinson WD, White PA (2010) Norovirus GII.4 variant 2006b caused epidemics of acute gastroenteritis in Australia during 2007 and 2008. J Clin Virol 49:265–271
10. Ferreira MS, Victoria M, Carvalho-Costa FA, Vieira CB, Xavier MP, Fioretti JM, Andrade J, Volotao EM, Rocha M, Leite JP, Miagostovich MP (2010) Surveillance of norovirus infections in the state of Rio De Janeiro, Brazil 2005–2008. J Med Virol 82:1442–1448
11. Fukuda S, Sasaki Y, Takao S, Seno M (2008) Recombinant norovirus implicated in gastroenteritis outbreaks in Hiroshima Prefecture, Japan. J Med Virol 80:921–928
12. 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
13. Hardy ME (2005) Norovirus protein structure and function. Fems Microbiol Lett 253:1–8
14. Jiang X, Wang M, Graham DY, Estes MK (1992) Expression, self-assembly, and antigenicity of the Norwalk virus capsid protein. J Virol 66:6527–6532
15. Jin M, Xie HP, Duan ZJ, Liu N, Zhang Q, Wu BS, Li HY, Cheng WX, Yang SH, Yu JM (2008) Emergence of the GII4/2006b variant and recombinant noroviruses in China. J Med Virol 80:1997–2004
16. Jin Y, Cheng WX, Yang XM, Jin M, Zhang Q, Xu ZQ, Yu JM, Zhu L, Yang SH, Liu N, Cui SX, Fang ZY, Duan ZJ (2009) Viral

agents associated with acute gastroenteritis in children hospitalized with diarrhea in Lanzhou, China. J Clin Virol 44:238–241
17. Katayama K, Shirato-Horikoshi H, Kojima S, Kageyama T, Oka T, Hoshino F, Fukushi S, Shinohara M, Uchida K, Suzuki Y, Gojobori T, Takeda N (2002) Phylogenetic analysis of the complete genome of 18 Norwalk-like viruses. Virology 299: 225–239
18. Khamrin P, Maneekarn N, Thongprachum A, Chaimongkol N, Okitsu S, Ushijima H (2010) Emergence of new norovirus variants and genetic heterogeneity of noroviruses and sapoviruses in children admitted to hospital with diarrhea in Thailand. J Med Virol 82:289–296
19. 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
20. Li H, Mo YL, Ke CW, Zheng HZ, Zhou XH, Guo RN, Chen QX, Fang L, Zou LR (2010) Molecular epidemiological characteristics of norovirus gastroenteritis outbreaks in Guangdong from 2005 to 2008. Bing Du Xue Bao 26:202–207
21. Lindesmith LC, Donaldson EF, Lobue AD, Cannon JL, Zheng DP, Vinje J, Baric RS (2008) Mechanisms of GII.4 norovirus persistence in human populations. PLoS Med 5:e31
22. Liu C, Grillner L, Jonsson K, Linde A, Shen K, Lindell AT, Wirgart BZ, Johansen K (2006) Identification of viral agents associated with diarrhea in young children during a winter season in Beijing, China. J Clin Virol 35:69–72
23. Lole KS, Bollinger RC, Paranjape RS, Gadkari D, Kulkarni SS, Novak NG, Ingersoll R, Sheppard HW, Ray SC (1999) Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. J Virol 73:152–160
24. Marshall JA, Hellard ME, Sinclair 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. Motomura K, Oka T, Yokoyama M, Nakamura H, Mori H, Ode H, Hansman GS, Katayama K, Kanda T, Tanaka T, Takeda N, Sato H (2008) Identification of monomorphic and divergent haplotypes in the 2006–2007 norovirus GII/4 epidemic population by genome wide tracing of evolutionary history. J Virol 82:11247–11262
26. Nayak MK, Balasubramanian G, Sahoo GC, Bhattacharya R, Vinje J, Kobayashi N, Sarkar MC, Bhattacharya MK, Krishnan T (2008) Detection of a novel intergenogroup recombinant Norovirus from Kolkata, India. Virology 377:117–123
27. Nayak MK, Chatterjee D, Nataraju SM, Pativada M, Mitra U, Chatterjee MK, Saha TK, Sarkar U, Krishnan T (2009) A new variant of Norovirus GII.4/2007 and inter-genotype recombinant strains of NVGII causing acute watery diarrhoea among children in Kolkata, India. J Clin Virol 45:223–229
28. Nenonen NP, Hannoun C, Olsson MB, Bergstrom T (2009) Molecular analysis of an oyster-related norovirus outbreak. J Clin Virol 45:105–108
29. Nguyen TA, Yagyu F, Okame M, Phan TG, Trinh QD, Yan H, Hoang KT, Cao AT, Le Hoang P, Okitsu S, Ushijima H (2007) Diversity of viruses associated with acute gastroenteritis in children hospitalized with diarrhea in Ho Chi Minh City, Vietnam. J Med Virol 79:582–590
30. Patel MM, Widdowson MA, Glass RI, Akazawa K, Vinje J, Parashar UD (2008) Systematic literature review of role of noroviruses in sporadic gastroenteritis. Emerg Infect Dis 14:1224–1231
31. Phan TG, Kaneshi K, Ueda Y, Nakaya S, Nishimura S, Yamamoto A, Sugita K, Takanashi S, Okitsu S, Ushijima H (2007) Genetic heterogeneity, evolution, and recombination in noroviruses. J Med Virol 79:1388–1400
32. Pletneva MA, Sosnovtsev SV, Green KY (2001) The genome of Hawaii virus and its relationship with other members of the caliciviridae. Virus Genes 23:5–16
33. Sdiri-Loulizi K, Ambert-Balay K, Gharbi-Khelifi H, Sakly N, Hassine M, Chouchane S, Guediche MN, Pothier P, Aouni M (2009) Molecular epidemiology of norovirus gastroenteritis investigated using samples collected from children in Tunisia during a four-year period: detection of the norovirus variant GGII.4 Hunter as early as January 2003. J Clin Microbiol 47:421–429
34. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599
35. ter Waarbeek HL, Dukers-Muijrers NH, Vennema H, Hoebe CJ (2010) Waterborne gastroenteritis outbreak at a scouting camp caused by two norovirus genogroups: GI and GII. J Clin Virol 47:268–272
36. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882
37. Tseng FC, Leon JS, MacCormack JN, Maillard JM, Moe CL (2007) Molecular epidemiology of norovirus gastroenteritis outbreaks in North Carolina, United States: 1995–2000. J Med Virol 79:84–91
38. Vinje J, Koopmans MP (1996) Molecular detection and epidemiology of small round-structured viruses in outbreaks of gastroenteritis in the Netherlands. J Infect Dis 174:610–615
39. Wadl M, Scherer K, Nielsen S, Diedrich S, Ellerbroek L, Frank C, Gatzer R, Hoehne M, Johne R, Klein G, Koch J, Schulenburg J, Thielbein U, Stark K, Bernard H (2010) Food-borne norovirus-outbreak at a military base, Germany, 2009. BMC Infect Dis 10:30
40. Wu FT, Oka T, Katayama K, Wu HS, Donald JD, Miyamura T, Takeda N, Hansman GS (2006) Genetic diversity of noroviruses in Taiwan between November 2004 and March 2005. Arch Virol 151:1319–1327
41. 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
42. Yang Y, Xia M, Tan M, Huang P, Zhong W, Pang XL, Lee BE, Meller J, Wang T, Jiang X (2010) Genetic and phenotypic characterization of GII-4 noroviruses that circulated during 1987 to 2008. J Virol 84:9595–9607
43. Yoon JS, Lee SG, Hong SK, Lee SA, Jheong WH, Oh SS, Oh MH, Ko GP, Lee CH, Paik SY (2008) Molecular epidemiology of norovirus infections in children with acute gastroenteritis in South Korea in November 2005 through November 2006. J Clin Microbiol 46:1474–1477
44. Zheng DP, Ando T, Fankhauser RL, Beard RS, Glass RI, Monroe SS (2006) Norovirus classification and proposed strain nomenclature. Virology 346:312–323