Genetic Analysis of Norovirus Strains that Caused Gastroenteritis Outbreaks Among River Rafters in the Grand Canyon, Arizona

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ABSTRACT

Toilet solid waste samples collected from five outbreaks among rafters in the Grand Canyon were subjected to sequencing analysis of norovirus partial capsid gene. The results revealed that a GI.3 strain was associated with one outbreak, whereas the other outbreaks were caused by GII.5 strains whose sequences shared >98.9% homology.

Key words: Norovirus, gastroenteritis, PCR, phylogenetic analysis, genotype

TEXT

Noroviruses are the most significant pathogens associated with waterborne and foodborne outbreaks of nonbacterial acute gastroenteritis in humans worldwide. They are members of the family *Caliciviridae* and possess a positive-sense, single-stranded RNA genome with three open reading frames (ORFs) (Robilotti et al. 2015). Noroviruses show high genetic diversity and are currently divided into genogroups I (GI) to VI (GVI), of which GI, GII, and GIV infect humans (Robilotti et al. 2015). The strains in GI and GII can be further subdivided into genotypes.

Norovirus outbreaks among the Colorado River rafters in the Grand Canyon have been reported in the last few decades (Jones et al. 2009; Malek et al. 2009). In summer 2012, we experienced large recurrent gastroenteritis outbreaks among the Colorado River rafters and recently reported the results of our epidemiological investigation (Magill-Collins et al. 2015). In brief, we collected epidemiological information through confidential illness reports from ten trips (2.9% trip infection rate; out of 347 trips in total) that experienced gastroenteritis outbreaks in summer 2012. Fecal (composite solid waste from portable toilets) samples available from five out of the ten trips tested positive for norovirus-RNA, demonstrating that norovirus was probably the cause of gastroenteritis outbreaks (Magill-Collins et al. 2015). In the present study, we performed follow-up analysis of the norovirus-positive samples to characterize and relate norovirus strains associated with the multiple outbreaks among river rafters in the Grand Canyon, summer 2012.

Toilet solid waste samples collected from five outbreaks that previously tested positive for noroviruses by RT-qPCR (Magill-Collins et al. 2015) were subjected to genetic analysis. In brief, partial capsid gene of norovirus was amplified by semi-nested PCR assays using

G1SKF and G1SKR primers for GI and G2SKF and G2SKR primers for GII, as described previously (Kojima et al. 2002). The PCR products were separated by electrophoresis on 2% agarose gel and visualized under a UV lamp after ethidium bromide staining.

PCR products of expected size (330 bp for GI and 340 bp for GII) were excised from the gel and purified using the Zymoclean gel DNA recovery kit (Zymo Research, Irvine, CA, USA). Both strands of the purified PCR products were sequenced with a 3730 Genetic Analyzer (Applied Biosystems) at the University of Arizona Genetics Core (Tucson, AZ). Nucleotide sequences were assembled using the program Sequencher^{TM} version 4.2.2 (Gene Codes Corporation, AnnArbor, MI, USA) and aligned with Clustal W version 1.83 (http://clustalw.ddbj.nig.ac.jp/top-e.html).

The sequencing analysis revealed that the norovirus strains that caused the outbreaks were classified into two genotypes, GI.3 and GII.5 (Figure). The partial capsid gene nucleotide sequences of the GII.5 strains identified from four separate trips shared homology of greater than 98.9%, suggesting that genetically similar strains caused the recurrent norovirus outbreaks among the rafters over a period of more than a month (illness onset of May 30 to July 2; Figure).

As reported in Magill-Collins et al. (2015), screening of the presence of norovirus-RNA in the toilet solid waste samples using RT-qPCR demonstrated that one sample tested positive for GI and the other samples were positive for GII (no GI/GII coinfection was observed), while GIV was not detected from any samples (Magill-Collins et al. 2015). This result is consistent with other clinical studies on norovirus, which reported that GII accounts for the large majority of reported outbreaks and GI makes up the majority of the remaining cases, whereas GIV strains have rarely been detected from patients.

Genotype GI.3 is the most commonly found in outbreaks among GI genotypes, whereas GII.5 is relatively uncommon among GII genotypes as opposed to GII.4 that is the most prevalent norovirus genotype worldwide (Siebenga et al. 2010). In fact, GII.5 genotype caused only 0.3% (11/3,960) of all norovirus outbreaks reported in the United States between 2009 and August 2013 (Vega et al. 2014), which covers the study period of this work (i.e., summer 2012). More interestingly, the phylogenetic analysis revealed that the GII.5 strains identified from four trips were nearly identical, although three of them were conducted more than a month later than the other trip. This result suggests that these four trips shared the same virus origin (source) and/or the virus was amplified during the trip and propagated the illness between the multiple trips, whereas the outbreak in one trip due to GI.3 (illness onset of May 12, which was earlier than the other outbreaks) is seemingly unrelated to those outbreaks.

It should be noted that there have been a number of norovirus outbreaks among rafters in the Grand Canyon in the summer season, which are uncommon in a developed country where a peak of norovirus outbreaks is usually observed in colder months (Ahmed et al. 2013; Siebenga et al. 2010). This may be attributed to the nature of the environment in the rafting trips on which these norovirus outbreaks occur, such as up to 18 days of close contact international passengers in a semi-aquatic environment, which may provide optimal conditions for norovirus dispersal, making containment and prevention difficult to achieve. In the close-contact environment, person-to-person infection can occur via aerosolization of fecal material or vomitus in which they are widely dispersed by a nearby individual and use of shared camping sites that are widely used throughout the year.

For the past two decades, norovirus continues to be a presence on river rafting trips in the Grand Canyon during the summer seasons when tens of thousands of individuals embark

on a one to two week adventure. In the present study, we revealed the norovirus genotypes and molecular epidemiological feature of the outbreak strains in summer 2012. Various modes of transmission may have propagated the outbreak within the trip and to other trips in the same area of the Grand Canyon, demonstrating how easily the contagious virus can spread within one trip or to other trips in close proximity on the river. Molecular epidemiological evidence on high nucleotide sequence similarity among GII.5 strains identified from multiple trips suggested that there were indeed routes of cross contamination between the trips, although the exact routes of transmission still remain unidentified. In response to the recurrent norovirus outbreaks in the Grand Canyon over the last few decades, the National Park Service and Coconino Country Health District published the guidelines for norovirus outbreak prevention in an attempt to decrease the incidence of norovirus in rafting trips. As recommended in the guidelines, strict precautions, such as proper hand washing, sanitization, and quarantine procedures, must be continually encouraged and maintained in order to prevent the incidence and spread of this highly contagious virus among river rafters.

Nucleotide accession numbers

The nucleotide sequences determined in this study were deposited in GenBank under accession numbers LC150707–LC150711.

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REFERENCES

Ahmed, S. M., Lopman, B. A., & Levy, K. (2013). A systematic review and meta-analysis of the global seasonality of norovirus. *PLOS One*, *8*(10), e75922. doi:10.1371/journal.pone.0075922

Jones, E. L., Gaither, M., Kramer, A., & Gerba, C. P. (2009). An analysis of water quality in the Colorado River, 2003–04; an investigation into recurring outbreaks of norovirus among rafters. *Wilderness & Environmental Medicine*, *20*(1), 6–13.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, *16*(2), 111–120.

Kojima, S., Kageyama, T., & Fukushi, S. (2002). Genogroup-specific PCR primers for detection of Norwalk-like viruses. *Journal of Virological Methods*, *100*(1-2), 107–114.

Magill-Collins, A., Gaither, M., Gerba, C. P., Kitajima, M., Iker, B. C., & Stoehr, J. D. (2015). Norovirus outbreaks among Colorado River rafters in the Grand Canyon, summer 2012. *Wilderness & Environmental Medicine*, *26*(3), 1–7. doi:10.1016/j.wem.2015.02.007

Malek, M., Barzilay, E., Kramer, A., Camp, B., Jaykus, L., Escudero-abarca, B., et al. (2009). Outbreak of norovirus infection among river rafters associated with packaged delicatessen meat, Grand Canyon, 2005. *Clinical Infectious Diseases*, *30333*(1), 31–37. doi:10.1086/594118

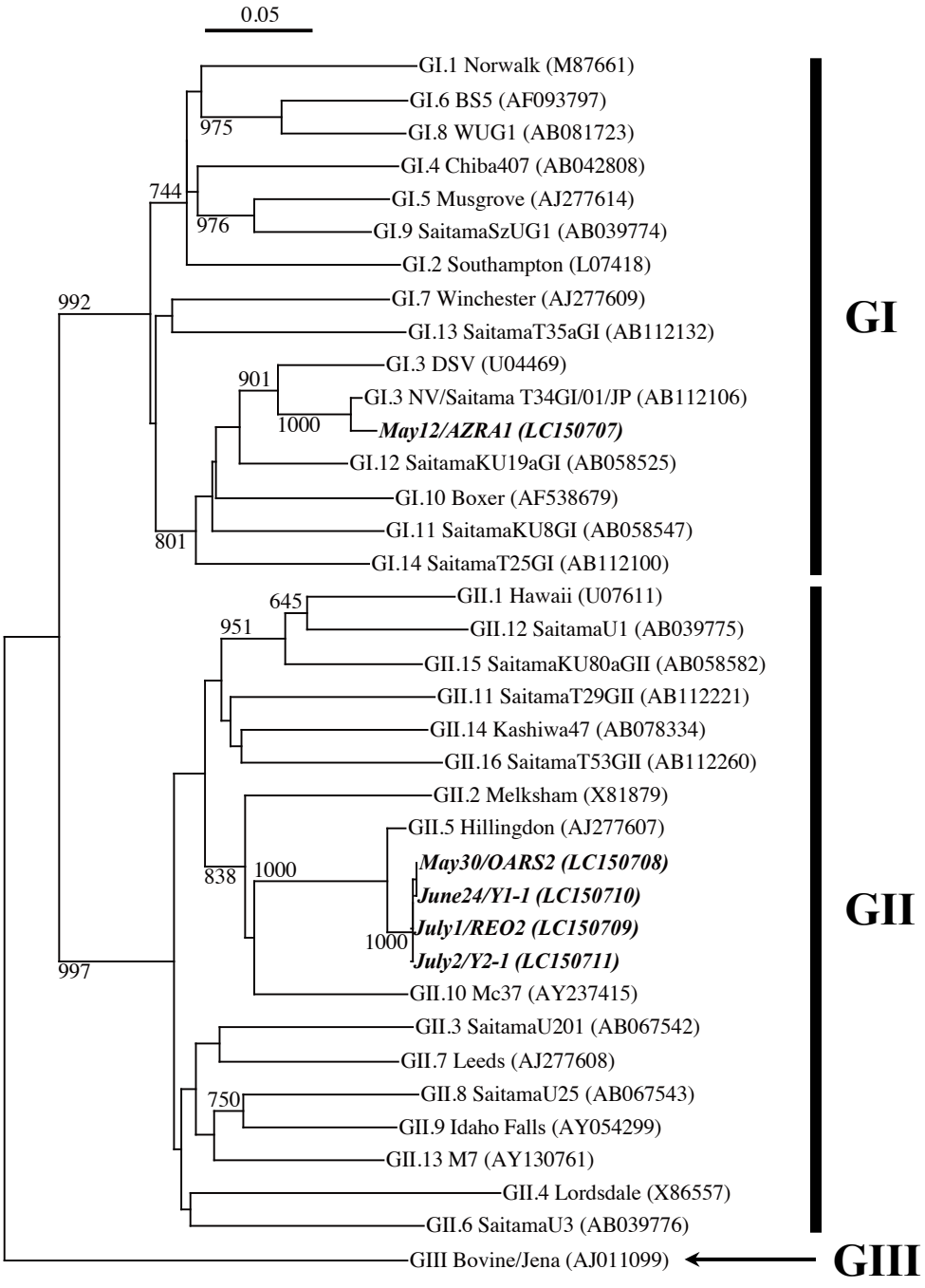
Robilotti, E., Deresinski, S., & Pinsky, B. A. (2015). Norovirus. *Clinical Microbiology Reviews*, *28*(1), 134–164. doi:10.1128/CMR.00075-14

Siebenga, J. J., Duizer, E., & Koopmans, M. P. G. (2010). Norovirus epidemiology. In G. S. Hansman, X. Jiang, & K. Y. Green (Eds.), *Caliciviruses: Molecular and Cellular Virology* (pp. 1–24). Norfolk, UK: Caister Academic Press.

Vega, E., Barclay, L., Gregoricus, N., Shirley, S. H., & Lee, D. (2014). Genotypic and epidemiologic trends of norovirus outbreaks in the United States, 2009 to 2013. *Journal of Clinical Microbiology*, *52*(1), 147–155. doi:10.1128/JCM.02680-13

FIGURE LEGENDS

Figure 1. Phylogenetic tree for norovirus strains using about 280 nucleotides of the partial capsid gene sequences; the distances were calculated using Kimura's two-parameter method (Kimura 1980), and the tree from a bootstrap analysis with 1000 replicates was generated by the neighbor-joining method with reference strains and the strains derived from the outbreak stool specimens. The numbers on each branch indicate the bootstrap values for the genotype, and bootstrap values of 950 or higher were considered statistically significant for the grouping. The scale represents nucleotide substitutions per site. Norovirus strains shown in ***italic bold*** are the strains identified in the present study, representing the onset of illness date, sample ID, and GenBank accession number.


0.05
GI.1 Norwalk (M87661)
GI.6 BS5 (AF093797)
975
GI.8 WUG1 (AB081723)
GI.4 Chiba407 (AB042808)
744
GI.5 Musgrove (AJ277614)
976
GI.9 SaitamaSzUG1 (AB039774)
GI.2 Southampton (L07418)
992
GI.7 Winchester (AJ277609)
GI.13 SaitamaT35aGI (AB112132)
GI.3 DSV (U04469)
901
GI.3 NV/Saitama T34GI/01/JP (AB112106)
1000
May12/AZRA1 (LC150707)
GI.12 SaitamaKU19aGI (AB058525)
GI.10 Boxer (AF538679)
GI.11 SaitamaKU8GI (AB058547)
801
GI.14 SaitamaT25GI (AB112100)
GI
645
GII.1 Hawaii (U07611)
951
GII.12 SaitamaU1 (AB039775)
GII.15 SaitamaKU80aGII (AB058582)
GII.11 SaitamaT29GII (AB112221)
GII.14 Kashiwa47 (AB078334)
GII.16 SaitamaT53GII (AB112260)
GII.2 Melksham (X81879)
GII.5 Hillingdon (AJ277607)
838
1000
May30/OARS2 (LC150708)
June24/Y1-1 (LC150710)
July1/REO2 (LC150709)
1000
July2/Y2-1 (LC150711)
997
GII.10 Mc37 (AY237415)
GII.3 SaitamaU201 (AB067542)
GII.7 Leeds (AJ277608)
750
GII.8 SaitamaU25 (AB067543)
GII.9 Idaho Falls (AY054299)
GII.13 M7 (AY130761)
GII.4 Lordsdale (X86557)
GII.6 SaitamaU3 (AB039776)
GII
GIII Bovine/Jena (AJ011099)
GIII