Genome Sequences of Five Microviruses and a Murine Norovirus

Rui Zhou,a Jun Yin,b Juan Lu,a Jianqiang Wang,c Wen Zhang,a Shixing Yang,a Xiaochun Wang,a Quan Shena

aSchool of Medicine, Jiangsu University, Zhenjiang, Jiangsu, People’s Republic of China
bNanjing Customs District, Nanjing, Jiangsu, People’s Republic of China
cIntensive Care Unit, Jintan District Hospital of Traditional Chinese Medicine, Changzhou, Jiangsu, People’s Republic of China

ABSTRACT Murine norovirus is a fecal-ora$l$ transmitted pathogen in mice which belongs to the same genus as human norovirus. Microviruses are bacteriophages with small circular single-stranded DNA genomes, belonging to the family Microviridae. Here, we report the genome sequences of five microviruses and one murine norovirus obtained from the intestinal content of a lab mouse.

Murine norovirus (MuNoV), a model used to study human norovirus, is a single-stranded positive-sense RNA (ssRNA) virus belonging to the Caliciviridae family (1). The Microviridae family, which is thought to be an important inhabitant of animal guts, can be divided into two subfamilies, Bullavirinae and Gokushovirinae, and at least two tentative subfamilies, including Pichovirinae and Alpavirinae, which have not been formally accepted by the International Committee on Taxonomy of Viruses (2–4).

The intestinal tissue of a laboratory mouse (BALB/c) with diarrhea from the Laboratory Center of the School of Medicine, Jiangsu University, was collected. The sample was resuspended in Dulbecco’s phosphate-buffered saline (d-PBS) and homogenized and filtered to remove eukaryotic and bacterial cell-sized particles (5). The filtrate was treated with a cocktail of DNases (Turbo DNase from Ambion, Baseline-ZERO from Epicentre, and Benzonase from Novagen) and RNase A (Fermentas) to digest unprotected nucleic acid at 37°C for 60 min, and then nucleic acid was extracted using the TaKaRa nucleic acid extraction kit. cDNA was synthesized using the SuperScript III first-strand synthesis system (Invitrogen) according to the instructions. The second strand of cDNA was synthesized using Klenow fragment DNA polymerase. Then, a library was constructed using the Nextera XT DNA sample preparation kit (Illumina) with dual barcoding for this pool. Sequencing was performed on the Illumina Miseq instrument using the MiSeq reagent kit version 2 (500 cycles) (6).

Raw data were processed according to the standard procedure, which included debarcoding, trimming, and assembling (7). In short, reads were debarcoded using vendor software from Illumina. Clonal reads were removed, and low-sequencing-quality tails were trimmed using a Phred quality score of 10 as the threshold. Adaptors were trimmed using the default parameters of VecScreen, which is NCBI BLASTn with specialized parameters designed for adaptor removal. The cleaned reads were de novo assembled with SOAPdenovo2 version r240 using a kmer size of 63 with default settings. Contigs and singlet reads were then compared against a customized viral proteome database using BLASTx with an E value cutoff of < 10^-5, where the virus proteome database was compiled using the NCBI virus reference proteome (ftp://ftp.ncbi.nih.gov/refseq/release/viral/), to which we added viral protein sequences from the NCBI nonredundant (nr) fasta file (only sequences taxonomically annotated as Virus kingdom). All tools were run with default parameters unless otherwise specified. Geneious software was used to check the circularity of the microviruses. The overlapping reads of the genomes at the start and end of the contigs confirmed their circular genomes. Five complete genomes of microviruses and one 6,131-bp-long partial genome of MuNoV with 5 gaps were generated. Seven sets of primers

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Address correspondence to Quan Shen, shenquan@ujs.edu.cn.
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TABLE 1  Primers used to amplify the gaps of the MuNoV strain

| Primer name | Sequence (5'-3') | Polaritya | Locationb | Length (bp) |
|-------------|-----------------|-----------|-----------|-------------|
| F1          | GTGAAAATGAGGATGGCAAC | +         | 1–19      | 324         |
| R1          | AGATAGCCTGTGAGACA |           |           |             |
| F2          | CAAACTTCCTCACCAAA | +         | 1118–1135 | 465         |
| R2          | CCAAGGGACGACAGAATT | –         |           |             |
| F3          | TGCACCTGTGAGAAGA + | 2232–2249 | 466       |
| R3          | CATCAGACTCTCTTCTGTC |           |           |             |
| F4          | TGGTTGTTAGGAGGCTTTG | +         | 3438–3455 | 396         |
| R4          | GTCCTCAAGGCGGTCTTC |           | 3816–3833 |             |
| F5          | ACCCTGAAAGGCCAGAAG + | 3834–3851 | 341       |
| R5          | CAAGTCTTCTCAGGCGCATC | –         | 4155–4174 |             |
| F6          | GTGCCAACCTGCAAGAGAT | +         | 5965–5984 | 698         |
| R6          | CCTTGTGCCGAAGCTTCCA | –         | 6644–6662 |             |
| F7          | CCCGGCCTTCCTTCAATGG | +         | 6619–6636 | 765         |
| R7          | AAAATGCATCTAAATTACCA | –         | 7364–7383 |             |

a +, forward primer; –, reverse primer.
b Location of the primer in the nucleotide residue of UJSM01 (GenBank accession number MW018367).

were used to close the gaps between sequences of MuNoV (Table 1). The Sanger sequences were assembled along with the initial contigs to bridge the gaps and obtain the complete genome using Geneious Prime software (version 2020.0.4). Putative open reading frames (ORFs) were predicted using Geneious Prime and NCBI ORFfinder. After multiple sequences were aligned with Clustal W, phylogenetic trees were generated with MrBayes version 3.2.7 with a mixed substitution model (8).

The genomes of the 5 microviruses and 1 norovirus are 4,737 bp, 5,121 bp, 5,211 bp, 5,254 bp, 4,722 bp, and 7,383 bp long with G+C contents of 41.5%, 43.7%, 45.9%, 45.9%,
47.9%, 48.8%, and 56.8%, respectively. The vertical coverages of the genomes are 193 \times, 339 \times, 132 \times, 128 \times, 101 \times, and 34 \times, respectively. The phylogenetic tree based on the amino acid of major capsid protein VP1 indicated that these five strains belong to three different subfamilies; UJSM7 and UJSM14 belong to Gokushovirinae, and UJSM1/UJSM3 and UJSM20 belong to two potential new subfamilies, respectively (Fig. 1A).

The genome sequence of this MuNoV strain (named UJSMN01) is 7,383 bp long with three ORFs (Fig. 1C). Based on the phylogenetic tree, UJSMN01 belongs to genogroup V (GV) and shares the highest identity (91%) with a South Korean strain (GenBank accession number FJ446719) (Fig. 1B).

Data availability. These data are available in GenBank under BioProject accession number PRJNA666281, BioSample accession number SAMN16287582, and SRA accession number SRR12756396. The genome sequences of MuNoV and five bacteriophages have been deposited in NCBI GenBank under accession numbers MW018367, MW073821, MW073822, MW073823, MW073824, and MW073825, respectively.

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REFERENCES

1. Nice TJ, Robinson BA, Van Winkle JA. 2018. The role of interferon in persistent viral infection: insights from murine norovirus. Trends Microbiol 26:510–524. https://doi.org/10.1016/j.tim.2017.10.010.
2. Krupovic M, Prangishvili D, Hendrix RW, Bamford DH. 2011. Genomics of bacterial and archaeal viruses: dynamics within the prokaryotic virosphere. Microbiol Mol Biol Rev 75:610–635. https://doi.org/10.1128/MMBR.00011-11.
3. Roux S, Krupovic M, Poullet A, Debros D, Enault F. 2012. Evolution and diversity of the Microviridae viral family through a collection of 81 new complete genomes assembled from virome reads. PLoS One 7:e40418. https://doi.org/10.1371/journal.pone.0040418.
4. Adams MJ, Lefkowitz EJ, King AM, Harrach B, Harrison RL, Knowles NJ, Kropinski AM, Krupovic M, Kuhn JH, Mushegian AR, Nibert M, Sabanadzovic S, Sanfalcon H, Siddell SG, Simmonds P, Varsani A, Zerbini FM, Gorbalenya AE, Davison AJ. 2016. Ratification vote on taxonomic proposals to the International Committee on Taxonomy of Viruses (2016). Arch Virol 161:2921–2949. https://doi.org/10.1007/s00705-016-2977-6.
5. Zhang W, Yang S, Shan T, Hou R, Liu Z, Li W, Guo L, Wang Y, Chen P, Wang X, Feng F, Wang H, Chen C, Shen Q, Zhou H, Hua X, Cui L, Deng X, Zhang Z, Qi D, Delwart E. 2017. Virome comparisons in wild-diseased and healthy captive giant pandas. Microbiome 5:90. https://doi.org/10.1186/s40168-017-0308-0.
6. Ling Y, Wang J, Yin J, Xu J, Wu Y, Zhou L, Lu J, Yang S, Wang X, Shen Q, Zhang W. 2020. Genomic organization of a Gamma-6 papillomavirus metagenomic discovered from vaginal swab samples of Chinese pregnant women. Virol J 17:44. https://doi.org/10.1186/s12985-020-01319-9.
7. Deng XT, Naccache SN, Ng T, Federman S, Li LL, Chiu CY, Delwart EL. 2015. An ensemble strategy that significantly improves de novo assembly of microbial genomes from metagenomic next-generation sequencing data. Nucleic Acids Res 43:e46. https://doi.org/10.1093/nar/gkv002.
8. Fonseca AA, Jr., Camargos MF, Barbosa AA, Gonçalves VL, Heinemann MB, Reis JK. 2016. Evolutionary diversity of Suid herpesvirus 1 based on U44 partial sequences. Intervirology 59:20–29. https://doi.org/10.1159/000446540.