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Comprehensive detection of viruses in pediatric patients with acute liver failure using next-generation sequencing

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ABSTRACT

Background: Pediatric acute liver failure (PALF) is a rare and severe syndrome that frequently requires liver transplantation. Viruses are one of the most frequent causes of this disease, however, pathogenic viruses are not determined in many patients. Recently next-generation sequencing (NGS) has been applied to comprehensively detect pathogens of infectious diseases of unknown etiology.
Objectives: To evaluate an NGS-based approach for detecting pathogenic viruses in patients with PALF or acute hepatitis of unknown etiology.
Study design: To detect virus-derived DNA and RNA sequences existing in sera/plasma from patients, both DNA and RNA sequencing were performed. First, we validated the ability of NGS to detect viral pathogens in clinical serum/plasma samples, and compared different commercial RNA library preparation methods Then, serum/plasma of fourteen patients with PALF or acute hepatitis of unknown etiology were evaluated using NGS.
Results: Among three RNA library preparation methods, Ovation RNA-Seq System V2 had the highest sensitivity to detect RNA viral sequences. Among fourteen patients, sequence reads of torque teno virus, adeno-associated virus, and stealth virus were found in the sera of one patient each, however, the pathophysiological role of these three viruses was not clarified. Significant virus reads were not detected in the remaining 11 patients.
Conclusions: This finding might be due to low virus titer in blood at the time of referral or a non-infectious cause might be more frequent. These results suggest an NGS-based approach has potential to detect viral pathogens in clinical samples and would contribute to clarification of the etiology of PALF.

1. Background

Pediatric acute liver failure (PALF) is a rare disease in which liver function is rapidly destroyed accompanied with coagulopathy and with or without altered mentation. In Japan, approximately 10 cases are annually registered and the estimated frequency is 20 cases per year [1]. The prognosis of PALF is severe; more than 70% of cases undergo liver transplantation with an estimated survival rate of 50–70% [1]. Patients are required to take immunosuppressive agents for a long time after liver transplantation, thus alternative treatments are needed.

The etiologies of Japanese PALF cases are similar to that of North American and Western European cases except that drug-induced cases are less common [1–4]. The most frequent causes of PALF are metabolic (25%) and viral causes (22%), however, the specific cause is not determined in 40–50% of patients [1]. Among viral causes, hepatitis B virus (HBV) and Epstein-Barr virus (EBV) account for 25% and 30% of pediatric fulminant hepatitis and 40% and 20% of severe PALF, respectively, in Japan [1], whereas HBV infection is uncommon in North America and Western Europe [2–4]. Delayed introduction of HBV universal vaccination might be the reason for high prevalence of HBV infection in Japan. Schwarz et al. identified herpes simplex virus (HSV) in 11.6% of PALF patients who underwent testing in a registry study in the United States, Canada, and the United Kingdom, however, many cases were not fully tested for viral causes [5]. GB virus A (GBV-A), GBV-B [6,7], GBV-C [8,9], torque teno virus (TTV) [10], and SEN virus [11] were also detected in some cases of hepatitis, however, the association between these viruses and hepatitis has been controversial. In indeterminate cases, some cases might be caused by specific pathogens

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that could not be detected by conventional methods such as virus-specific antibody, PCR for specific viruses, and viral culture. Therefore, identification of the causative virus of acute liver failure may contribute to clarification of disease pathogenesis and development of treatment.

Next-generation sequencing (NGS) has the ability to comprehensively detect the whole genome from both host and pathogen without the need for specific primers with relatively high sensitivity. Recently, NGS has been applied for detecting pathogens of infectious diseases of unknown etiology or discovery of novel viruses [12,13]. We also identified the etiological agent from pediatric acute encephalitis and encephalopathy using NGS in a previous study [14]. Ganova-Raeva et al. reported NGS-based detection of HBV and hepatitis E virus from hepatitis of unknown etiology [15]. In this study, we investigated serum or plasma samples from patients with PALF or acute hepatitis of unknown etiology using NGS to identify viral pathogens.

2. Methods

2.1. Patients and specimens

Fourteen pediatric patients who were diagnosed with acute liver failure or acute hepatitis of unknown etiology were enrolled in this study. Acute liver failure was defined as hepatitis with coagulopathy (prothrombin time-international normalized ratio > 1.5) in a patient with previously normal hepatic function. Patients with acute hepatitis with highly elevated aminotransferase levels (alanine aminotransferase > 1000 IU/ml and aspartate aminotransferase > 1000 IU/ml) were also included. Clinical characteristics of the patients are shown in Table 1. Patients with drug-induced hepatitis were excluded, and none of the patients were immunocompromised. Serology of common hepatitis viruses such as hepatitis A–C viruses were negative, and PCR for EBV, cytomegalovirus, and human herpesvirus 6 were negative in all patients. PCR for HSV was not performed at enrollment. Serum or plasma specimens were collected and stored at −30 °C until use.

2.2. NGS

Total nucleic acids were extracted from 140 µl of serum or plasma using the QIAamp UCP Pathogen Mini Kit (Qiagen, Hilden, Germany) and recovered in a 35 µl final volume. Before extraction, specimens were filtered through a 0.22 µm filter (Merck-Millipore, Temecula, CA, USA) to remove blood cells and bacteria. We quantified extracted DNA using a Qubit assay kit (Thermo Fisher Scientific, Waltham, MA, USA). RNA quality was not assessed because of low extraction volume. DNA- and RNA-sequencing libraries were prepared independently to detect DNA and RNA viruses, respectively. To prepare the DNA-sequencing library, the Nextera XT DNA Library Preparation kit (Illumina, San Diego, CA, USA) was used in accordance with the manufacturer's instruction.

Before preparation of the RNA-sequencing library, extracted nucleic acid was treated with Turbo DNase (Ambion, Darmstadt, Germany) to digest host DNA, and 17 µl of nucleic acid was used for reverse transcription with the Ovation RNA-Seq System V2 kit (NuGEN, San Carlos, CA, USA). cDNA was synthesized and amplified in accordance with the manufacturer's instructions and purified with the MinElute Reaction Cleanup kit (Qiagen). To eliminate contaminating sequences, such as primer dimers, the GeneRead Size Selection kit (Qiagen) was used. Purified cDNA was used to prepare the RNA-sequencing library with the Nextera XT DNA Library Preparation kit. The ScriptSeq V2 RNA-Seq Library Preparation kit (Illumina) and SMARTer Stranded RNA-Seq kit (Takara Bio USA, Mountain View, CA, USA) were also used in accordance with the manufacturers' instructions [14,16]. Library quality was assessed using Agilent 2200 TapeStation (Agilent, Santa Clara, CA, USA). Indexed libraries were pooled and sequenced on HiSeq 2500 (Illumina) using the 2 × 150 bp paired-end protocol.

2.3. Sequence data analysis

For metagenomics pathogen identification, the cloud-computing pipeline, MePIC v2.0 (National Institute of Infectious Diseases, Japan, as of 10/21/2016) was used [17]. In the pipeline, unnecessary bases such as adaptors and low-quality sequences were trimmed, and reads derived from the human genome were removed. For remaining reads, similar sequences were searched against the database of known nucleotide sequences including viruses with the MEGABLAST program. To summarize the taxonomic information, the metagenomics analyzer, MEGAN Community Edition (University of Tübingen, Tübingen, Germany) was used [18,19]. To avoid making calls based on potentially spurious alignments or contamination of sequences in the flow cell, such as hybridized sequences with other samples' sequences and sequences of samples of previous runs in the same lane, we considered the virus to be present if > 10 reads of sequences were aligned to the viral reference genome in this study. We also checked samples with few reads aligned to a viral reference genome. If the read count was > 2, then sequence data were also analyzed using a read mapping approach. Read mapping to each viral reference genome was analyzed using CLC Genomics Workbench 9.5 (CLC bio; Qiagen).

Table 1
Patient characteristics.

Patient no.	Age	Sex	Clinical diagnosis	Hepatic encephalopathy	PT-INR	AST (IU/L)	ALT (IU/L)	T-Bill (mg/dl)	Outcome
1	4 mo	M	Acute liver failure	Yes	6.56	1672	1036	30.6	Liver transplant
2	6 mo	M	Acute liver failure	Yes	2.61	1018	472	14.2	Liver transplant
3	9 mo	M	Acute liver failure	Yes	1.81	937	519	8.8	Recovery
4	2 yr	F	Acute liver failure	Yes	2.17	2251	1572	11.4	Recovery
5	2 yr	M	Acute liver failure[a]	No	1.64	1038	578	17.6	Recovery
6	3 yr	M	Acute hepatitis	No	1.01	1482	1381	2.9	Recovery
7	5 yr	M	Acute liver failure	Yes	1.59	2342	956	14.7	Recovery
8	10 yr	F	Acute liver failure[b]	No	2.02	1775	1836	14.6	Recovery
9	10 yr	M	Acute hepatitis[b]	No	1.11	1094	1217	1.6	Recovery
10	11 yr	F	Acute hepatitis	No	1.21	1022	1593	1.1	Recovery
11	12 yr	M	Acute hepatitis	No	1.19	1199	1335	17.7	Recovery
12	12 yr	F	Acute hepatitis	No	1.44	1286	1315	9.1	Recovery
13	14 yr	F	Acute liver failure	Yes	AUL[c]	9018	10,652	28.9	Liver transplant
14	14 yr	M	Acute liver failure	No	1.89	491	1043	21.3	Recovery

PT-INR prothrombin time-international normalized ratio, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *T-Bill* Total bilirubin.
[a] Patient 5 received a transfusion 10 days before the onset of pediatric acute liver failure.
[b] Patients 8 and 9 were diagnosed with hepatitis-associated aplastic anemia after recovery of acute hepatitis.
[c] Above the upper limit.

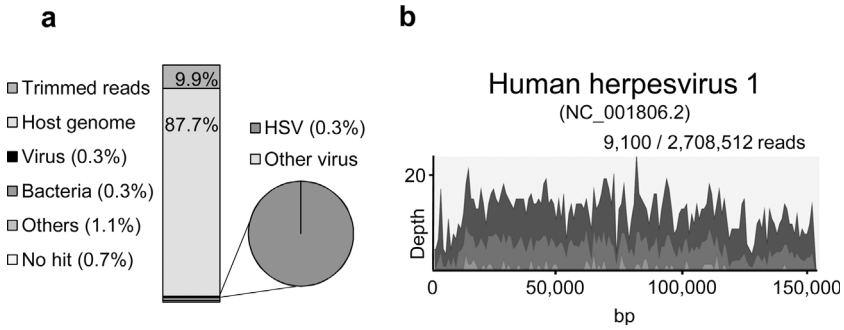

Fig. 1. Identification of DNA viral sequences with DNA sequencing. Sequencing results of the DNA-sequencing library prepared from the serum of a patient with neonatal herpes simplex virus (HSV) infection. (a) Cumulative bar chart shows classification of total reads into trimmed reads, host genome subtraction (human), viruses, bacteria, others, and unknown categories (no hit). Pie chart shows classification of viral sequence reads to each specific virus. (b) Total reads were mapped against the reference genome of human herpesvirus 1 using the CLC Genomics Workbench. Light gray, gray, and dark gray colors in the viral genome alignment represent minimal, average, and maximal coverage in aggregated 1 kbp region, respectively.

3. Results

3.1. Identification of DNA viral sequences with DNA sequencing

To validate the NGS-based approach to detect viral sequences in clinical samples, several types of clinical samples were examined. Sensitivity of NGS for detection of viral sequences was approximately 100 DNA copies/ml, which was equivalent to that of real-time PCR with a sequencing depth of 5,000.000 reads, and the number of reads was significantly correlated with viral DNA copies [examinations have been completed for human herpesvirus 6 [14]]. Fig. 1 shows a representative result of DNA sequencing of a serum sample from a patient with HSV-1 infection. Metagenomic analysis of DNA sequencing performed with MePIC v2.0 showed 2885 reads per million (0.3% of total reads) were aligned to the HSV-1 genome (Fig. 1a). Read mapping performed with CLC Genomics Workbench 9.5 showed high coverage of the viral genome (Fig. 1b).

3.2. Comparison of RNA-sequencing library preparation methods for identification of RNA virus

To detect RNA viral genomes with very low quantity, three methods for preparing RNA-sequencing libraries were compared using a serum sample from a patient with hepatitis C virus (HCV) infection. The Ovation RNA-Seq System V2 kit, which was reported to have high sensitivity [20], was compared to the ScriptSeq V2 RNA-Seq Library Preparation kit and SMARTer Stranded RNA-Seq kit (Fig. 2). These results indicated that Ovation RNA-Seq System V2 had > 10-fold higher sensitivity than the other two library preparation methods. Therefore, Ovation RNA-Seq System V2 was used in this study to detect RNA viral genome. Considering the HCV loads of this serum sample (4.0×10^6 IU/ml), the sensitivity for the detection of HCV reads by NGS using the Ovation RNA-Seq System V2 was approximately 50 IU/ml.

DNA and RNA sequencing of serum samples from two healthy controls were performed, which demonstrated no significant viral reads.

3.3. Detection of viral sequences in clinical samples of PALF/acute hepatitis

We next investigated plasma or serum samples of patients with PALF or acute hepatitis of unknown etiology. All RNA-sequencing libraries contained sequence reads aligned to the viral genome of Cafeteria roenbergensis virus and Equine infectious anemia virus, which were likely derived from the Ovation RNA-Seq System V2. A summary of viral sequences detected is shown in Table 2. Sequences that aligned to a specific viral genome with > 10 reads were detected in three of 14 patients. Torque teno virus 7 (TTV-7) was detected in both DNA and RNA sequencing from patient 5 (2.84 and 34.8 reads per million, respectively), who developed PALF 10 days after red blood cell transfusion.

The coverage plots of viral genome of adeno-associated virus (AAV) and TTV-7 (GenBank accession numbers AF_043303_AAV-2 and AF_261761_TTV-7, respectively) are shown in Fig. 3. As for stealth virus (GenBank accession number AF_191073_Stealth virus 1) detected from patient 9, an unexpectedly high number of sequence reads (196,698 reads) were mapped with the read mapping approach. A few sequence reads aligned to stealth virus were also detected in another 2 RNA-sequencing libraries by metagenomic analysis (data not shown). Thus, we examined read mapping for stealth virus in all RNA-sequencing libraries and found all of them contained mapped reads (3-196,698; median 2433). We also analyzed the RNA-sequencing library from patient 9 with read mapping to the reference genome of *Agrobacterium vitis* ribosomal RNA operon (GenBank accession number U_28505), which was reported to have high sequence homology to stealth virus [21]. A total of 165,205 reads were mapped to the bacterial ribosomal RNA, and 83.13% of the mapped reads extracted from the RNA-sequencing library were again mapped to the reference genome of stealth virus. Taken together, the sequence reads aligned to stealth virus were considered to be derived from bacterial genomes and/or contaminants during RNA sequencing.

4. Discussion

In our previous study, we showed that the sensitivity of NGS for detecting DNA virus using the Nextera XT DNA Library Preparation Kit was equivalent to that of real-time PCR [14]. However, the ScriptSeq V2 RNA-Seq Library Preparation kit used in a previous study for RNA sequencing contains substantial sequence reads of avian retrovirus (Fig. 2c) and thus may not have sufficient sensitivity to detect RNA virus. In this study, we compared three different RNA-sequencing library preparation methods using a clinical sample and revealed that the Ovation RNA-Seq System V2 kit produced more reads aligned to the HCV genome than the ScriptSeq V2 RNA-Seq or SMARTer Stranded RNA-Seq kit. Malboeuf et al. reported that the Ovation RNA-Seq System V2 kit produced increased amplification of viral RNA compared to several different RNA-sequencing library preparation kits because it had the lowest percentage of reads aligning to host contamination, particularly rRNA [20]. Because the initial total RNA quantity eluted from plasma or serum is very low, the Ovation RNA-Seq System V2 kit is more suitable for RNA-sequencing library preparation, although it is more costly and labor intensive [16].

We anticipated that DNA or RNA viruses, which are associated with hepatitis but not detected by conventional methods, would be identifiable by NGS. Sequence reads of several viruses such as TTV-7 were detected in some patients, however, it is unclear whether these viruses are causative agents of hepatitis. Three RNA sequencing reads aligned to human adenovirus 6 were detected in patient 12. However, adenovirus reads were not detected by DNA sequencing even though this virus is a DNA virus. In our previous study, we analyzed the serum of a patient with adenovirus hepatitis by NGS, which detected a large amount of reads of adenovirus by DNA sequencing [14]. Although the origin of adenovirus 6 sequencing reads detected in patient 12 is unclear, this result is not sufficient to confirm the presence of these

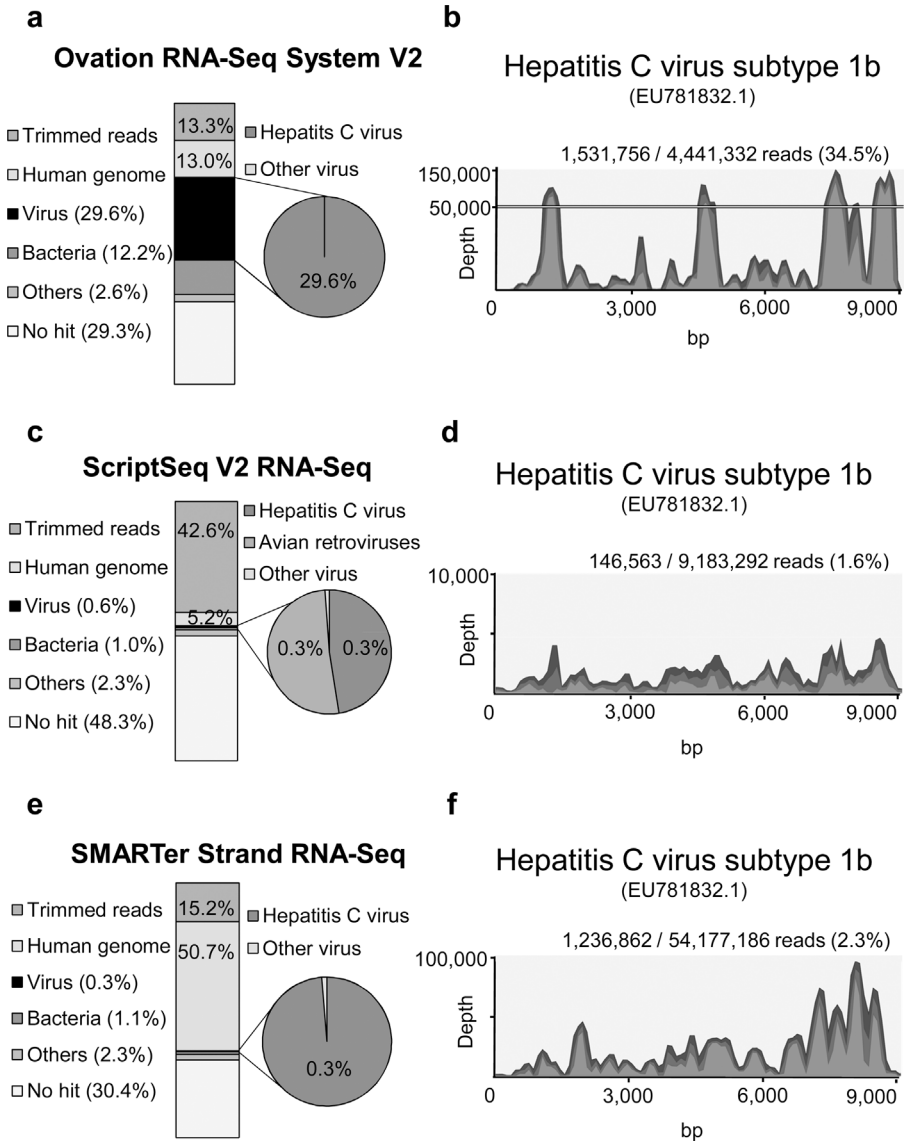

Fig. 2. Comparison of RNA-sequencing library preparation methods for identification of RNA virus. Sequencing results of the RNA-sequencing library prepared with the Ovation RNA-Seq System V2 kit (a, b), ScriptSeq V2 RNA-Seq kit (c, d), and SMARTer Stranded RNA-Seq kit (e, f) from the serum of a patient with hepatitis C virus (HCV) infection. Cumulative bar charts show classification of total reads from each library, and pie charts show classification of viral sequence reads to each specific virus. Sequencing reads were mapped to the reference genome of HCV genotype 1b. Light gray, gray, and dark gray colors in the viral genome alignments represent minimal, average, and maximal coverage in aggregated 100 bp region, respectively.

Table 2
Virus detection with next-generation sequencing.

Patient no.	DNA sequencing				RNA sequencing			
	Total reads	Detected virus	Readn count	Reads/million	Total reads	Detected Virus	Read count	Reads/million
1	10,058,088	N.D.	–	–	20,750,856	N.D.	–	–
2	16,682,520	N.D.	–	–	7,698,016	N.D.	–	–
3	16,347,490	N.D.	–	–	8,346,090	N.D.	–	–
4	17,180,686	N.D.	–	–	8,969,670	N.D.	–	–
5	12,310,840	TTV-7	35	2.84	14,539,070	TTV-7	506	34.8
6	13,769,570	AAV	10	0.73	14,475,574	N.D.	–	–
		HSV-2	3	0.22				
7	12,189,970	N.D.	–	–	17,130,766	N.D.	–	–
8	31,082,752	N.D.	–	–	8,438,582	N.D.	–	–
9	13,922,770	N.D.	–	–	7,117,112	Stealth virus	11	1.55
10	13,924,060	N.D.	–	–	22,614,664	N.D.	–	–
11	7,912,376	N.D.	–	–	12,757,640	N.D.	–	–
12	10,534,050	N.D.	–	–	24,024,650	Human adenovirus 6	3	0.12
13	4,229,046	N.D.	–	–	17,847,650	N.D.	–	–
14	10,249,842	N.D.	–	–	17,510,160	N.D.	–	–

N.D not detected, *TTV-7* torque teno virus 7, *AAV* adeno-associated virus, *HSV-2* herpes simplex virus 2.

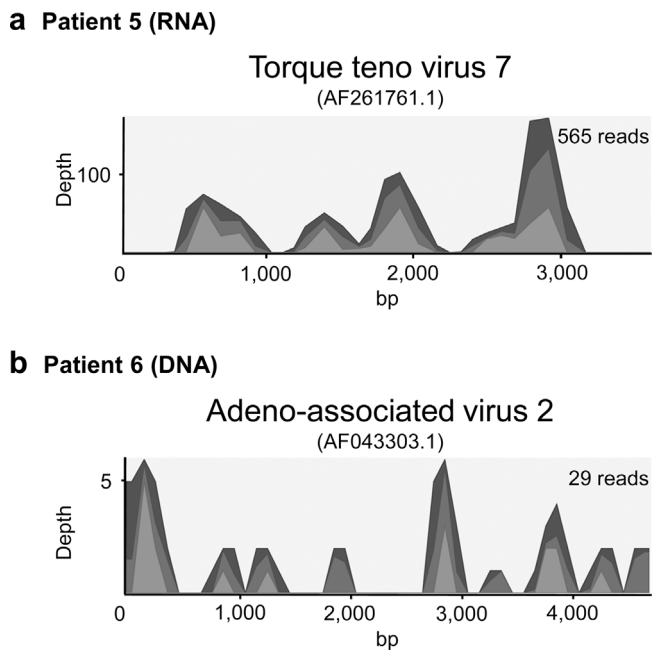

Fig. 3. Coverage plots of viral genomes. Sequencing reads detected in each patient were mapped to the reference genome of torque teno virus 7 (a), and adeno-associated virus 2 (b). Light gray, gray, and dark gray colors in the viral genome alignments represent minimal, average, and maximal coverage in aggregated 10 bp (torque teno virus 7) or 100 bp (adeno-associated virus 2) region, respectively.

viruses.

Compared to adult acute liver failure, metabolic causes are more frequent in PALF [1]. Therefore, it might be possible that metabolic causes that could not be diagnosed with present examinations were more frequent than viral causes in indeterminate cases. Conversely, patients might not be referred to the hospital during the active phase of infection because the first presentation of acute liver failure or hepatitis is obscure. The virus titer in blood might be decreased or undetectable at the time of referral, although hepatitis was triggered by viral infection.

TTV is reported to have some association to hepatitis, especially hepatitis after transfusion [10,22,23], although pathogenicity of the virus is considered to be weak [23]. The patient in whom TTV was detected developed ALF after receiving a transfusion and recovered without transplantation. Therefore, TTV might be associated to ALF in this case. However, TTV has an extremely high prevalence and was detected in > 50% of the general population in a study conducted in the early 2000s [24]. Thus, its pathogenicity remains controversial.

The pathogenicity of AAV also remains unknown, and AAV infection is considered to be asymptomatic. AAV is a satellite virus that requires co-infection with adenovirus or herpesvirus [25,26]. A few sequence reads were aligned to HSV-2 from the same sample, thus AAV infection might have occurred as a co-infection with HSV-2. However, HSV-2-associated hepatitis is unlikely in this case because the sequence reads of HSV-2 were too infrequent.

Reports about stealth virus are limited [21,27], and its pathogenicity is not clear. Stealth virus is a double-stranded DNA virus belonging to the family *Herpesviridae*. The virus was reported to have homology to not only human cytomegalovirus and simian cytomegalovirus but also some bacterial sequences such as bacterial ribosomal gene complex [21]. Malboeuf et al. reported bacterial ribosomal RNA as one of the artifacts of the Ovation RNA-Seq system V2 kit [20]. In this study, sequence reads aligned to stealth virus might have originated from bacterial genomes or could be artificial sequences produced during RNA sequencing.

In the present study, we applied NGS to identify viral pathogens in patients with PALF, however, significant viral reads were not detected in 11 of 14 patients. Karetnyi et al. reported human parvovirus B19 DNA was detected in liver tissue specimens from patients with acute fulminant liver failure but not in their sera using a PCR assay [28,29]. To investigate the causative pathogens of ALF and/or hepatitis, a liver biopsy specimen might be desirable. However, liver biopsy is not always performed in PALF patients due to the risk of bleeding. Furthermore, using patient specimens, about 90% of total sequence reads were aligned to the human genome. To effectively detect low titer virus using NGS, it is essential to eliminate human genome contamination before library sequencing. Although TTV is a DNA virus, TTV reads were detected more effectively from the RNA-sequencing library. RNA sequencing tended to contain fewer sequence reads of the human genome, probably because we treated extracted RNA with DNase before preparing the RNA-sequencing library. Therefore, effectively eliminating host genome contamination would contribute to successful detection of the pathogenic viral genome. Finally, when analyzing NGS data, we must consider contaminant sequences due to pooling of several libraries or repeatedly using the same lane. NGS is costly, thus, sequencing pooled libraries is a cost-saving method that is generally used. However, when sequencing with or after a library containing specific sequences with a high proportion, other libraries can be influenced and contain contaminated sequence reads in the data. Thus, pathogens with few sequence reads must be carefully considered.

In conclusion, we applied NGS to detect the causative agent in patients with PALF or acute hepatitis of unknown etiology. An NGS-based approach has potential for detecting pathogens in clinical samples and would contribute to clarification of the mechanism of infectious diseases.

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Conflict of interests

None declared.

Ethical approval

The study design and purpose were approved by the Institutional Review Board of Nagoya University Hospital (IRB number: 6503). Informed consent was obtained from all patients or their guardians.

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References

[1] A. Inui, S. Ida, R. Sumazaki, et al., Acute liver failure in japanese children: findings of acute liver failure study group in the Japanese pediatric hepatology research association, Nihon Fukubu KyuKyu Igakkai Zasshi (J. Abdom. Emerg. Med.) 29 (2009) 583–589.

[2] W.S. Lee, P. McKiernan, D.A. Kelly, outcome and prognostic indicators of childhood fulminant hepatic failure in the United kingdom, J. Pediatr. Gastroenterol. Nutr. 40 (2005) 575–581.

[3] R.H. Squires Jr., B.L. Shneider, J. Bucuvalas, et al., Acute liver failure in children: the first 348 patients in the pediatric acute liver failure study group, J. Pediatr. 148 (2006) 652–658.

[4] R.H. Squires, E.M. Alonso, Liver Disease in Children, 4th ed., Cambridge University Press, 2014.

[5] K.B. Schwarz, D. Dell Olio, S.J. Lobritto, et al., Analysis of viral testing in non-acetaminophen pediatric acute liver failure, J. Pediatr. Gastroenterol. Nutr. 59

(2014) 616–623.
[6] J.N. Simons, T.J. Pilot-Matias, T.P. Leary, et al., Identification of two flavivirus-like genomes in the GB hepatitis agent, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 3401–3405.
[7] G.G. Schaluder, G.J. Dawson, J.N. Simons, et al., Molecular and serologic analysis in the transmission of the GB hepatitis agents, J. Med. Virol. 46 (1995) 81–90.
[8] J.N. Simons, T.P. Leary, G.J. Dawson, et al., Isolation of novel virus-like sequences associated with human hepatitis, Nat. Med. 1 (1995) 564–569.
[9] J. Linnen, J. Wages Jr., Z.Y. Zhang-Keck, et al., Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent, Science 271 (1996) 505–508.
[10] T. Nishizawa, H. Okamoto, K. Konishi, et al., A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology, Biochem. Biophys. Res. Commun. 241 (1997) 92–97.
[11] Y. Tanaka, D. Primi, R.Y. Wang, et al., Genomic and molecular evolutionary analysis of a newly identified infectious agent (SEN virus) and its relationship to the TT virus family, J. Infect. Dis. 183 (2001) 359–367.
[12] N.L. Yozwiak, P. Skewes-Cox, M.D. Stenglein, et al., Virus identification in unknown tropical febrile illness cases using deep sequencing, PLoS Negl. Trop. Dis. 6 (2012) e1485.
[13] S. Prachayangprecha, C.M. Schapendonk, M.P. Koopmans, et al., Exploring the potential of next-generation sequencing in detection of respiratory viruses, J. Clin. Microbiol. 52 (2014) 3722–3730.
[14] J. Kawada, Y. Okuno, Y. Torii, et al., Identification of viruses in cases of pediatric acute encephalitis and encephalopathy using next-generation sequencing, Sci. Rep. 6 (2016) 33452.
[15] L. Ganova-Raeva, L. Punkova, D.S. Campo, et al., Cryptic hepatitis B and E in patients with acute hepatitis of unknown etiology, J. Infect. Dis. 212 (2015) 1962–1969.
[16] A. Alberti, C. Belser, S. Engelen, et al., Comparison of library preparation methods reveals their impact on interpretation of metatranscriptomic data, BMC Genom. 15 (2014) 912.
[17] F. Takeuchi, T. Sekizuka, A. Yamashita, et al., MePIC, metagenomic pathogen identification for clinical specimens, Jpn. J. Infect. Dis. 67 (2014) 62–65.
[18] D.H. Huson, N. Weber, Microbial community analysis using MEGAN, Methods Enzymol. 531 (2013) 465–485.
[19] D.H. Huson, S. Beier, I. Flade, et al., MEGAN community edition – interactive exploration and analysis of large-scale microbiome sequencing data, PLoS Comput. Biol. 12 (2016) e1004957.
[20] C.M. Malboeuf, X. Yang, P. Charlebois, et al., Complete viral RNA genome sequencing of ultra-low copy samples by sequence-independent amplification, Nucl. Acids Res. 41 (2013) e13.
[21] W.J. Martin, Bacteria-related sequences in a simian cytomegalovirus-derived stealth virus culture, Exp. Mol. Pathol. 66 (1999) 8–14.
[22] F. Piaggio, F. Dodi, G. Bottino, et al., Torque Teno virus–cause of viral liver disease following liver transplantation: a case report, Transplant. Proc. 41 (2009) 1378–1379.
[23] Z.J. Hu, Z.W. Lang, Y.S. Zhou, et al., Clinicopathological study on TTV infection in hepatitis of unknown etiology, World J. Gastroenterol. 8 (2002) 288–293.
[24] S. Spandole, D. Cimponeriu, L.M. Berca, et al., Human anelloviruses: an update of molecular, epidemiological and clinical aspects, Arch. Virol. 160 (2015) 893–908.
[25] W. Xiao, K.H. Warrington Jr., P. Hearing, et al., Adenovirus-facilitated nuclear translocation of adeno-associated virus type 2, J. Virol. 76 (2002) 11505–11517.
[26] M.A. Goncalves, Adeno-associated virus: from defective virus to effective vector, Virol. J. 2 (2005) 43.
[27] W.J. Martin, Stealth adaptation of an African green monkey simian cytomegalovirus, Exp. Mol. Pathol. 66 (1999) 3–7.
[28] A.N. Langnas, R.S. Markin, M.S. Cattral, et al., Parvovirus B19 as a possible causative agent of fulminant liver failure and associated aplastic anemia, Hepatology 22 (1995) 1661–1665.
[29] Y.V. Karetnyi, P.R. Beck, R.S. Markin, et al., Human parvovirus B19 infection in acute fulminant liver failure, Arch. Virol. 144s (1999) 1713–1724.