Journal of Clinical Virology 96 (2017) 67–72

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Full length article

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

Takako Suzuki[a], Jun-ichi Kawada[a], Yusuke Okuno[a], Satoshi Hayano[a], Kazuhiro Horiba[a], Yuka Torii[a], Yoshiyuki Takahashi[a], Syuichiro Umetsu[b], Tsuyoshi Sogo[b], Ayano Inui[b], Yoshinori Ito[a,*]

[a] Departments of Pediatrics, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi 466-8550, Japan
[b] Department of Pediatric Hepatology and Gastroenterology, Saiseikai Yokohamashi Tobu Hospital, 3-6-1 Shimosueyoshi, Tsurumi-ku, Yokohama, Kanagawa 230-0012, Japan

ARTICLE INFO

Keywords:
Next-generation sequencing
Acute liver failure
Acute hepatitis
Pathogen

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

* Corresponding author.
E-mail address: yoshi-i@med.nagoya-u.ac.jp (Y. Ito).

http://dx.doi.org/10.1016/j.jcv.2017.10.001
Received 25 August 2017; Received in revised form 26 September 2017; Accepted 1 October 2017

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.