

## Case Report

# Pathogenic characterization of a cervical lymph node derived from a patient with Kawasaki disease

Harutaka Katano<sup>1</sup>, Seiichi Sato<sup>2</sup>, Tsuyoshi Sekizuka<sup>3</sup>, Akiko Kinumaki<sup>3,4</sup>, Hitomi Fukumoto<sup>1</sup>, Yuko Sato<sup>1</sup>, Hideki Hasegawa<sup>1</sup>, Shigeru Morikawa<sup>5</sup>, Masayuki Saijo<sup>6</sup>, Tetsuya Mizutani<sup>6</sup>, Makoto Kuroda<sup>3</sup>

<sup>1</sup>Department of Pathology, <sup>3</sup>Laboratory of Bacterial Genomics, Pathogen Genomics Center, <sup>5</sup>Department of Veterinary Science, and <sup>6</sup>Virology 1, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640; <sup>2</sup>Division of Pediatrics and Neonatal Care Center, Niigata City General Hospital, 463-7 Shumoku, Chuo-ku, Niigata 950-1197; <sup>4</sup>Department of Pediatrics, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Received August 22, 2012; Accepted September 10, 2012; Epub October 1, 2012; Published October 15, 2012

**Abstract:** Kawasaki disease (KD) is the most common cause of multisystem vasculitis in childhood. Although cervical lymphadenitis is one of the major symptoms in KD, lymph node biopsy is rarely performed, because KD is usually diagnosed by clinical symptoms. A cervical lymph node biopsy was taken from a girl aged 1 year and 8 months who had suspected lymphoma, but she was diagnosed with KD after the biopsy. The cervical lymph node specimen was analyzed with multivirus real-time PCR that can detect >160 viruses, and unbiased direct sequencing with a next-generation DNA sequencer to detect potential pathogens in the lymph node. Histologically, focal necrosis with inflammatory cell infiltration, including neutrophils and macrophages, was observed in the marginal zone of the cervical lymph node, which was compatible with the acute phase of KD. Multivirus real-time PCR detected a low copy number of torque teno virus in the sample. Comprehensive direct sequencing of the cervical lymph node biopsy sample sequenced more than 8 million and 3 million reads from DNA and RNA samples, respectively. Bacterial genomes were detected in 0.03% and 1.79% of all reads in DNA and RNA samples, respectively. Although many reads corresponded to genomes of bacterial environmental microorganisms, *Streptococcus* spp. genome was detected in both DNA (77 reads) and RNA (2,925 reads) samples. Further studies are required to reveal any association of microbial or viral infection with the pathogenesis of KD.

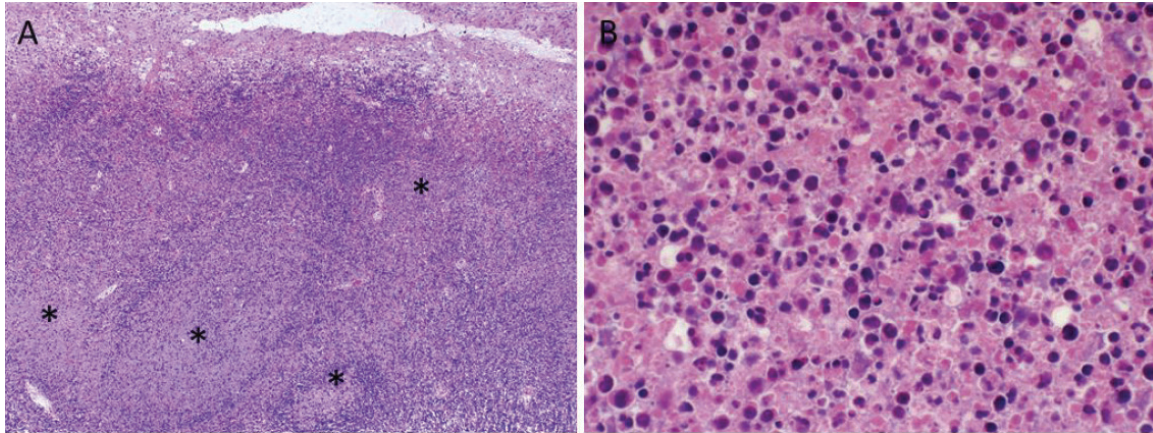
**Keywords:** Kawasaki disease, lymph node, next-generation sequencer, multivirus real-time PCR, torque teno virus, *streptococcus*

## Introduction

Kawasaki disease (KD) is an acute systemic vasculitis of infancy and early childhood that is characterized by prolonged fever, conjunctivitis, inflammation of the oropharynx, rash, erythematous induration of the distal extremities, and cervical lymphadenopathy [1]. Its etiology remains to be elucidated, and epidemiological studies have shown that it is more frequently observed in winter [2]. The disease occurs worldwide, but its incidence is 10 times higher in Japan than in western countries [3], suggesting that race-specific genotypes might be associated with the disease.

The resemblance of KD to toxic shock syndrome (TSS) suggests a causative role for as-

yet-uncharacterized infectious agents. TSS is caused by the superantigen (SAg) TSS toxin-1 [4]. SAg is a toxin of microbial or viral origin that cross-links antigen-presenting cells and T cells by simultaneously binding to class II major histocompatibility complex molecules [5-7] and the variable b-chain of T-cell receptors (TCR Vb) [8-10]. Although the etiology of KD is unknown, *Lactobacillus* cell wall [11], immunization with Bacillus Calmette-Guérin (BCG) [12], or *Candida albicans* fractions [13, 14] induce vasculitis and coronary arteritis in animal models, whereas tumor necrosis factor (TNF)- $\alpha$  has been suggested as necessary for this induction [15]. These observations suggest that, in addition to microbial SAg, infectious agents could be potential candidates for the onset of KD. To



**Figure 1.** Histopathological investigation of the lymph node sample from KD patient. A. Low-power view of the lymph node. Focal necrosis was observed in the marginal zone of the lymph node (asterisks). B. High-power view of the focal necrosis. Many necrotic ghost cells with neutrophils were found in the focal necrotic area.

date, a number of SAg-producing bacteria [16], including *Streptococcus pyogenes*, *Staphylococcus aureus* and *Yersinia pseudotuberculosis* [17, 18], as well as viruses, such as Epstein-Barr virus [19], have been speculated to be the causative agents of KD; however, there is a lack of consistency among reports. These disparate findings suggest that the inflammation observed in KD is not the result of a single agent, but rather from numerous infectious agents in genetically susceptible individuals [20-22].

Cervical lymphadenitis is one of the major symptoms in KD [1, 23]. A pathological study demonstrated that lymph node biopsy from KD patients showed focal necrosis with inflammatory cell infiltration [24]. This observation implied that lymphadenopathy is a symptom of the acute phase of KD, and suggested the association of bacterial or virus infection with the pathogenesis of KD. However, lymph node biopsy is rarely performed in KD patients, because KD is usually diagnosed by clinical symptoms [23].

In the present study, we analyzed a cervical lymph node biopsy from a girl aged 1 year and 8 months who had suspected lymphoma, but she was diagnosed with KD after biopsy. The recently developed next-generation sequencer is a powerful tool for detecting pathogen genomes in clinical samples. Comprehensive direct sequencing without any filtering preparation steps by a next-generation sequencer enables one to detect pathogens in representa-

tive and unbiased conditions in a small number of clinical samples [25-28]. The multivirus real-time PCR system developed recently by our group is another powerful tool to detect virus genomes in pathological samples. This system can detect >160 viruses in frozen or formalin-fixed paraffin-embedded (FFPE) tissues based on the techniques of the Taqman real-time PCR system [29]. To identify the potential pathogens in KD patients, the lymph node sample was analyzed with multivirus real-time PCR and comprehensive direct sequencing using a next-generation sequencer.

## Materials and methods

### Ethics statement

The study protocol was approved by the Institutional Medical Ethics Committee, National Institute of Infectious Diseases, Japan (Approval No. 295), and Niigata City General Hospital. The study was conducted according to the principles of the Declaration of Helsinki.

### KD patient

A girl aged 1 year and 8 months had prolonged fever and cervical lymphadenopathy. She was subjected to a lymph node biopsy for suspicion of malignant lymphoma; however, histological features were compatible with KD and suggested no malignancy (**Figure 1**) [24]. After the biopsy, conjunctivitis, skin rash, and “strawberry tongue” were observed in the patient. These symptoms met the diagnostic criteria for KD

established by the Japanese Kawasaki Diseases Research Committee [1, 23]. The patient was positive for 5 markers (fever, conjunctiva, exanthema, strawberry tongue, and lymphadenopathy) of the 6 KD symptom criteria at day 10 from the appearance of their earliest symptom. Administration of intravenous immunoglobulin and aspirin resulted in rapid decline of fever, and all symptoms disappeared at day 20 from the appearance of the earliest symptom.

### *Histopathology*

Hematoxylin–eosin (HE), periodic acid–Schiff (PAS), Gram, and Giemsa staining was performed on the paraffin sections. In immunohistochemistry, monoclonal or polyclonal antibodies to herpes simplex virus (HSV)-1 and -2 [30], varicella–zoster virus (VZV) [31], human cytomegalovirus (CMV) [32], human herpesvirus 6 (HHV-6) (P101; Millipore, Bedford, MA, USA), Kaposi's sarcoma-associated herpesvirus (KSHV) [33], and human papillomavirus (HPV) [34] were used as the primary antibodies. The labeled avidin–biotin method was used to detect virus antigens. *In situ* hybridization was performed to detect EBV-encoded small RNA (EBER) as described previously [35].

### *Total nucleic acid preparation from specimens*

Total DNA and RNA were prepared from a FFPE 7-mm cube of the autopsy lymph node tissue using QIAamp DNA extraction kit for FFPE (Qiagen, Hilden, Germany) and PureLink FFPE RNA isolation kit (Invitrogen, Carlsbad, CA, USA), respectively. Total RNA was treated with DNase (TurboDNase, Ambion, Austin, TX, USA).

### *Multivirus real-time PCR system*

The multivirus real-time PCR system that detects >160 viruses was used as reported previously [29]. Total DNA and RNA (each 1 µg) were applied to the system.

### *Short-read DNA sequencing*

The first-strand cDNA was prepared from ~0.5 µg total RNA using the random priming method with the RT2 First Strand kit (Supper Array Bioscience, Frederick, MD, USA), and double-strand cDNA (ds-cDNA) was synthesized with DNA polymerase I (*Escherichia coli*, 10 U/µl,

Invitrogen) with RNase H (2 U/µl; Invitrogen). The ds-cDNA was purified using a QIAquick PCR Purification Kit (Qiagen). A DNA library was prepared using a Genomic DNA Sample Prep Kit (Illumina, San Diego, CA, USA), and DNA clusters were generated on a slide using a Cluster Generation Kit (version 2) on an Illumina cluster station, according to the manufacturer's instructions. To obtain ~ $1.0 \times 10^7$  clusters for 1 lane, the general procedure, as described in the standard protocol (Illumina), was performed as follows: template hybridization, isothermal amplification, linearization, blocking, denaturation, and hybridization of the sequencing primer. All sequencing runs for 75–80 mers were performed with the Illumina Genome Analyzer IIx sequencing kit. Fluorescent images were analyzed using the Illumina base-calling pipeline 1.4.0 to obtain FASTQ-formatted sequence data. The detailed sequencing parameters are summarized in **Table 2**. The short read archives have been deposited in the DNA Data Bank of Japan (DDBJ; accession numbers: DRA000437).

### *Homology search analysis*

All of the obtained DNA sequence reads were aligned to a reference sequence of human genomic DNA, followed by quality trimming to remove low-quality reads, and further excluding reads with similarity to ambiguous human sequences by a BLASTN search with a cutoff value of  $e^{-8}$ . The remaining sequence reads were investigated using a BLASTN search against a nucleotide database. The results of the BLASTN search were analyzed and visualized using MEGAN v4.2.6 [36] with the following parameters: minimum support, 1 hit; minimum score 50 for the genus level.

## Results

### *Histopathological findings*

HE stain showed focal and severe necrosis mainly in the lymphoid follicle in the marginal zone of the lymph node (**Figure 1**). Lymph node structure was destroyed by necrosis, and no intact germinal center was found. The necrotic area contained many necrotic ghost cells with hemorrhage and infiltration of neutrophils, eosinophils and macrophages. Apoptotic cells were also frequently found around the necrotic area. The lymph node capsule was thickened with inflammatory cell infiltration. These obser-

## Pathogens in lymph node of Kawasaki disease

**Table 1.** Results of multivirus real-time PCR

	Results
DNA viruses	
Polyomavirus: JC virus, BK virus, Simian virus 40	-
Papillomaviruses: HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52,56, 58, 59, 66, 68, 73	-
Parvoviruses: adeno-associated virus 1, 2, 3 and 5; parvovirus B19; human bocavirus; adenovirus A-F	-
Herpes viruses: HHV 1-8, B virus	-
Poxviruses: variola virus, monkey pox virus, molluscum contagiosum virus	-
Anellovirus: TTV	+
Hepadnavirus: Hepatitis B virus	-
Others: mimivirus	
RNA viruses	
Filoviruses: Ebola virus, Marburg virus	-
Bunyaviruses: Crimean-Congo hemorrhagic fever virus, hemorrhagic fever with renal syndrome virus (Hantaan, Dohrava, Puumala, and Seoul), Rift valley fever virus, sin nombre virus	-
Arenaviruses: Lassa virus, Junin, Guanarito, Machupo and Sabia viruses	-
Togaviruses: equine encephalitis virus (Venezuelan, Eastern, and Western), Sindbis virus, Mayaro virus, Getah virus, Chikungunya virus, rubella virus	-
Enteroviruses: enterovirus 68 and 71; poliovirus 1-3; coxsackievirus A2-A6, A8-A10, A16, A21, A24, B1-B6; echovirus 5, 6, 7, 9, 11, 13-18, 25, 30; parechovirus 1 and 3; rhinovirus A and B; rotavirus; reovirus 1-4; Melaka virus; Colorado tick borne fever virus	-
Flaviviruses: dengue virus 1 and 2; Japanese encephalitis virus; Murray Valley encephalitis virus; St. Louis encephalitis virus; West Nile virus; Tick-borne encephalitis virus; yellow fever virus	-
Orthomyxoviruses: influenza virus A-C and H5N1	-
Paramyxoviruses: parainfluenza virus 1-3; Hendra virus; mumps virus; measles virus; Sendai virus; RS virus A and B; metapneumovirus; Nipah virus	-
Rabdoviruses: rabies virus, lyssavirus 5 and 6, Chandipura virus, Duvenhage virus	-
Coronaviruses: coronavirus OC43, 229E and NL63; SARS virus	-
Caliciviruses: sapovirus, Norwalk-like virus 1 and 2	-
Hepatitis viruses: hepatitis A virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, GB virus	-
Retroviruses: human immunodeficiency virus 1, human T cell leukemia virus 1 and 2	-
Others: astrovirus, Borna disease virus	-

vations were compatible with the acute phase of KD [24]. No bacteria or fungi were detected by PAS, Gram and Giemsa staining. HSV-1, CMV, HHV-6, VZV, KSHV and HPV antigens were not detected by immunohistochemistry, and EBER was negative by *in situ* hybridization.

### Multivirus real-time PCR

To detect viruses in the lymph node sample, we used the multivirus real-time PCR system, which can detect >160 DNA and RNA viruses (Table 1). RNA extracted from the lymph nodes was negative for all RNA viruses, while internal control RNA (hGAPDH mRNA) was detected. The multivirus real-time PCR system revealed that 30 ng DNA extracted from the lymph node contained 14 copies of torque teno virus (TTV).

All other DNA viruses examined in the system were negative.

### Sequencing reads by next-generation sequencer

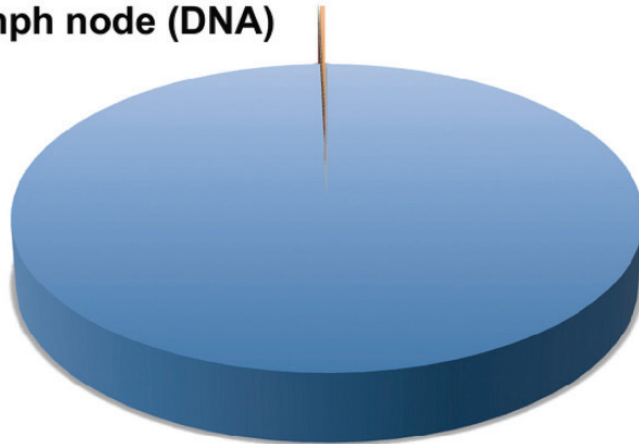
To determine the potential pathogens in the patient, direct sequencing was performed on the DNA or ds-cDNA from total RNA extracted from an FFPE sample of the lymph node biopsy. The Illumina Genome Analyzer Iix sequencing system produced several million 75-mer reads from the DNA or ds-cDNA libraries (Table 2). 99.59% and 77.43% of the sequences from the DNA and RNA samples were originated from a possible human source, respectively (Table 2 and Figure 2). The remaining reads were further analyzed using a BLASTN search against

## Pathogens in lymph node of Kawasaki disease

**Table 2.** Information of sequencing reads for the specimens

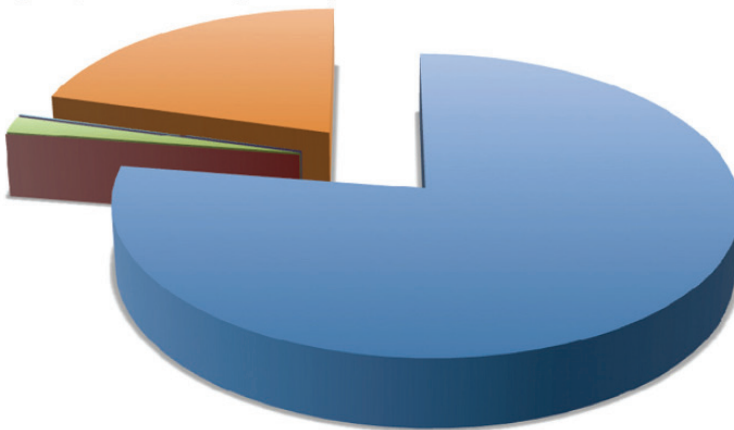
Sample	Total sequencing reads	Human	Archaea	Bacteria	Virus	Not assigned	No hits
DNA	8,151,996	99.6%	0.0001%	0.03%	0.0000%	0.02%	0.36%
RNA	3,644,872	77.4%	0.0047%	1.79%	0.0006%	0.11%	20.67%

### Lymph node (DNA)



Human	99.59%
Archaea	0.0001%
Bacteria	0.0273%
Virus	0%
Not assigned	0.0165%
No hits	0.3646%

### Lymph node (RNA)



Human	77.43%
Archaea	0.0047%
Bacteria	1.7936%
Virus	0.0006%
Not assigned	0.1061%
No hits	20.669%

**Figure 2.** Pie chart of the homology search results for all of the obtained reads from the lymph node biopsy sample. Percentage reads are shown in parentheses.

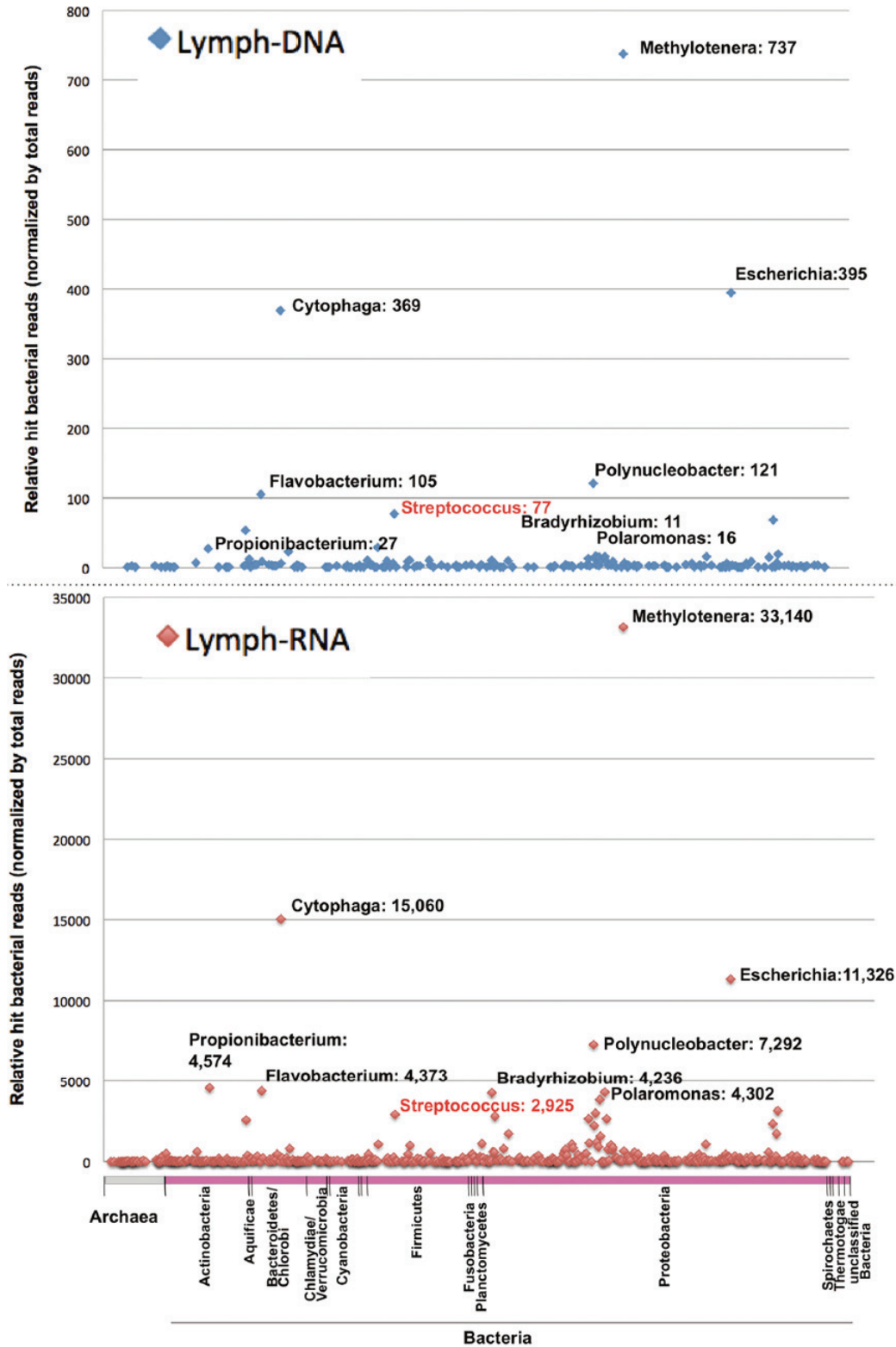
non-redundant databases, revealing potential bacterial and viral sequences (**Figure 2**). Regarding the bacterial hits, genus classification was determined from the results of a BLASTN search (**Figure 3**). The most abundant bacteria in both DNA and RNA samples were from the genera *Methylotenera*, *Cytophaga*, *Flavobacterium* and *Polynucleobacter*, which are well-known environmental microorganisms, but our other sequencing trials with clinical specimens suggested that these bacteria could be the result of contamination through the handling of FFPE samples, because these species

were always detected (data not shown). In addition, *Escherichia* and *Propionibacterium* spp. were frequently detected as possible contaminants. Sequencing of the lymph node showed that *Streptococcus* spp. was present, but it could not be confirmed as the specific pathogen related to the onset of KD (**Figure 3**; panel Lymph-RNA). No virus genome was detected by the direct sequencing.

### Discussion

Using a next-generation DNA sequencer and multivirus real-time PCR system, we detected

Pathogens in lymph node of Kawasaki disease



**Figure 3.** Dot plot of the short read numbers for archaea and all bacterial genera from the lymph node biopsy. The sequencing reads from lymph node DNA or RNA samples were analyzed by BLASTN homology search with a threshold score of 50.0, and the similarity results were classified into 11 and 653 genera of archaea and bacteria, respectively. The phylum taxonomy of the bacteria is shown below the dot plot. Notable genera and the corresponding number of sequencing reads are shown beside each dot.

possible pathogens from a cervical lymph node biopsy specimen, which is a tissue that is more likely to be associated with pathogens responsible for the pathogenesis of KD. Direct sequencing from DNA or RNA revealed that abundant environmental bacteria were unexpectedly identified, but variable bacterial genera were identified in the lymph node. Although we could not definitively identify the responsible pathogen for the lymphadenopathy, the presence of *Streptococcus* spp. in the lymph node suggests the association of streptococcal infection with the lymph node lesion in a KD patient.

Pathological findings in the lymph node from this patient, such as focal necrosis with abundant apoptotic cells, are compatible with previous findings in the lymph nodes of KD patients [24]. Focal necrosis with abundant apoptotic cells and severe inflammatory cell infiltration are frequently observed in any bacterial or viral infection, non-specifically. However, any specific pathogen has not been identified in the lymph node of KD patients so far. These previous findings suggest that etiological pathogens are already excluded by inflammation in the necrotic lesion, and some bacterial toxins are associated with formation of necrotic lesions in the lymph node. In such necrotic lesions, it is not easy to detect the pathogens. Another reason why the pathogen has not been identified in the lymph node of KD patients is that lymph node biopsy is not usually performed in such patients because the disease can be diagnosed from clinical symptoms. Thus, the lymph node sample in the present study is important for investigation of KD pathogenesis.

The next-generation sequencer identified *Streptococcus* spp. in the lymph node as one of the probable suspected pathogens for the pathogenesis of KD. Although detailed species classification could not be performed in our study, some oral *Streptococcus* spp. such as *Streptococcus salivarius*, *Streptococcus thermophilus*, *Streptococcus pneumoniae*, *Streptococcus mitis*, *Streptococcus sanguinis* and *Streptococcus dysgalactiae* are known to induce platelet activation [37]. In fact, increased platelet count is one of the significant parameters detected at the onset of KD [38, 39]. Streptococcal platelet activation is dependent on the species and strain. Some *S. sanguinis*

strains produce platelet-aggregation-associated protein [40], the adhesion of *S. pneumoniae* to platelets occurs as a consequence of platelet activation by thrombin [41], and although still controversial, *S. mitis* stimulates platelet aggregation in a strain-dependent manner [42]. In this study, we were unable to identify *S. pyogenes* using bacterial culture and comprehensive detection; however, it has been reported that the SAg genes of group A streptococci might be transferred among themselves [43]. It has been reported that the *Streptococcus* spp. isolated from KD patients induce expansion of T-cell populations expressing V $\beta$ 2 [44]. Thus, it is possible that an uncharacterized SAg gene could be transferred to the identified *Streptococcus* spp. Indeed, multiple SAGs have been identified in stool samples from KD patients [45]; however, this finding remains to be confirmed.

The multivirus real-time PCR revealed the absence of viruses, except TTV, in the lymph node from our patient. TTV was originally identified as an etiological agent of hepatitis [46, 47]. However, TTV has been detected frequently in various tissues and even from healthy individuals [48, 49]. Thus, TTV is thought to be a ubiquitous virus that is not associated with any specific diseases. TTV has been detected frequently even in samples from other children by the multivirus real-time PCR system (data not shown), therefore, it should not be thought of as an etiological agent of KD. However, increased copy numbers of TTV have been found in some disease tissues, suggesting that TTV is activated under certain conditions in some diseases [50-52]. Further studies are required to clarify the association of TTV infection and KD pathogenesis in the lymph node.

Finally, this study used multivirus real-time PCR and unbiased direct sequencing to identify possible causative agents of KD, and demonstrated the presence of TTV and *Streptococcus* spp. in the lymph node of a KD patient. It remains to be elucidated which species of streptococci are closely related to the etiology of KD. We are currently trying to identify additional specific isolates from KD patients, and whole genome sequencing of these isolates may identify a crucial virulence factor for KD pathogenesis, such as unique SAGs and platelet-activating factors.

**Acknowledgment**

This work was supported by a grant from the NPO Japan Kawasaki Disease Research Center (2008, to MK and TM) and a grant-in-aid for Scientific Research (C) and Challenging Exploratory Research from the Japan Society for the Promotion of Science (to MK, Grant No. 23590527 and to HK Grant No. 24659212). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Address correspondence to:** Dr. Harutaka Katano. Department of Pathology, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan. Tel: +81-3-5285-1111. ext. 2627; Fax: +81-3-5285-1189; E-mail: katano@nih.go.jp

**References**

[1] Kawasaki T. Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children. *Arerugi* 1967; 16: 178-222.

[2] Nakamura Y, Yashiro M, Uehara R, Sadakane A, Chihara I, Aoyama Y, Kotani K and Yanagawa H. Epidemiologic features of Kawasaki disease in Japan: results of the 2007-2008 nationwide survey. *J Epidemiol* 2010; 20: 302-307.

[3] Taubert KA, Rowley AH and Shulman ST. Seven-year national survey of Kawasaki disease and acute rheumatic fever. *Pediatr Infect Dis J* 1994; 13: 704-708.

[4] Bergdoll MS, Crass BA, Reiser RF, Robbins RN and Davis JP. A new staphylococcal enterotoxin, enterotoxin F, associated with toxic-shock-syndrome *Staphylococcus aureus* isolates. *Lancet* 1981; 1: 1017-1021.

[5] Fischer T, Wiegmann K and Pfizenmaier K. N-glycosidase treatment of Colo 205 cells interferes with hIFN-gamma induced HLA-DR expression. *Lymphokine Res* 1989; 8: 305-309.

[6] Fraser JD. High-affinity binding of staphylococcal enterotoxins A and B to HLA-DR. *Nature* 1989; 339: 221-223.

[7] Dellabona P, Peccoud J, Kappler J, Marrack P, Benoist C and Mathis D. Superantigens interact with MHC class II molecules outside of the antigen groove. *Cell* 1990; 62: 1115-1121.

[8] White J, Herman A, Pullen AM, Kubo R, Kappler JW and Marrack P. The V beta-specific superantigen staphylococcal enterotoxin B: stimulation of mature T cells and clonal deletion in neonatal mice. *Cell* 1989; 56: 27-35.

[9] Choi YW, Herman A, DiGiusto D, Wade T, Marrack P and Kappler J. Residues of the variable

region of the T-cell-receptor beta-chain that interact with *S. aureus* toxin superantigens. *Nature* 1990; 346: 471-473.

[10] Kappler J, Kotzin B, Herron L, Gelfand EW, Bigler RD, Boylston A, Carrel S, Posnett DN, Choi Y and Marrack P. V beta-specific stimulation of human T cells by staphylococcal toxins. *Science* 1989; 244: 811-813.

[11] Duong TT, Silverman ED, Bissessar MV and Yeung RS. Superantigenic activity is responsible for induction of coronary arteritis in mice: an animal model of Kawasaki disease. *Int Immunol* 2003; 15: 79-89.

[12] Nakamura T, Yamamura J, Sato H, Kakinuma H and Takahashi H. Vasculitis induced by immunization with *Bacillus Calmette-Guerin* followed by atypical mycobacterium antigen: a new mouse model for Kawasaki disease. *FEMS Immunol Med Microbiol* 2007; 49: 391-397.

[13] Nagi-Miura N, Shingo Y, Adachi Y, Ishida-Okawara A, Oharaseki T, Takahashi K, Naoe S, Suzuki K and Ohno N. Induction of coronary arteritis with administration of CAWS (*Candida albicans* water-soluble fraction) depending on mouse strains. *Immunopharmacol Immunotoxicol* 2004; 26: 527-543.

[14] Ohno N. Murine model of Kawasaki disease induced by mannoprotein-beta-glucan complex, CAWS, obtained from *Candida albicans*. *Jpn J Infect Dis* 2004; 57: S9-10.

[15] Hui-Yuen JS, Duong TT and Yeung RS. TNF-alpha is necessary for induction of coronary artery inflammation and aneurysm formation in an animal model of Kawasaki disease. *J Immunol* 2006; 176: 6294-6301.

[16] Matsubara K and Fukaya T. The role of superantigens of group A *Streptococcus* and *Staphylococcus aureus* in Kawasaki disease. *Curr Opin Infect Dis* 2007; 20: 298-303.

[17] Konishi N, Baba K, Abe J, Maruko T, Waki K, Takeda N and Tanaka M. A case of Kawasaki disease with coronary artery aneurysms documenting *Yersinia pseudotuberculosis* infection. *Acta Paediatr* 1997; 86: 661-664.

[18] Abe J, Onimaru M, Matsumoto S, Noma S, Baba K, Ito Y, Kohsaka T and Takeda T. Clinical role for a superantigen in *Yersinia pseudotuberculosis* infection. *J Clin Invest* 1997; 99: 1823-1830.

[19] Kikuta H, Matsumoto S and Osato T. Kawasaki disease and Epstein-Barr virus. *Acta Paediatr Jpn* 1991; 33: 765-770.

[20] Onouchi Y, Gunji T, Burns JC, Shimizu C, Newburger JW, Yashiro M, Nakamura Y, Yanagawa H, Wakui K, Fukushima Y, Kishi F, Hamamoto K, Terai M, Sato Y, Ouchi K, Saji T, Nariai A, Kaburagi Y, Yoshikawa T, Suzuki K, Tanaka T, Nagai T, Cho H, Fujino A, Sekine A, Nakamichi R, Tsunoda T, Kawasaki T and Hata A. ITPKC



- functional polymorphism associated with Kawasaki disease susceptibility and formation of coronary artery aneurysms. *Nat Genet* 2008; 40: 35-42.
- [21] Onouchi Y, Ozaki K, Buns JC, Shimizu C, Hamada H, Honda T, Terai M, Honda A, Takeuchi T, Shibuta S, Suenaga T, Suzuki H, Higashi K, Yasukawa K, Suzuki Y, Sasago K, Kemmotsu Y, Takatsuki S, Saji T, Yoshikawa T, Nagai T, Hamamoto K, Kishi F, Ouchi K, Sato Y, Newburger JW, Baker AL, Shulman ST, Rowley AH, Yashiro M, Nakamura Y, Wakui K, Fukushima Y, Fujino A, Tsunoda T, Kawasaki T, Hata A and Tanaka T. Common variants in CASP3 confer susceptibility to Kawasaki disease. *Hum Mol Genet* 2010; 19: 2898-2906.
- [22] Yeung RS. Kawasaki disease: update on pathogenesis. *Curr Opin Rheumatol* 2010; 22: 551-560.
- [23] Newburger JW, Takahashi M, Gerber MA, Gewitz MH, Tani LY, Burns JC, Shulman ST, Bolger AF, Ferrieri P, Baltimore RS, Wilson WR, Baddour LM, Levison ME, Pallasch TJ, Falace DA and Taubert KA. Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. *Pediatrics* 2004; 114: 1708-1733.
- [24] Giesker DW, Pastuszak WT, Forouhar FA, Krause PJ and Hine P. Lymph node biopsy for early diagnosis in Kawasaki disease. *Am J Surg Pathol* 1982; 6: 493-501.
- [25] Nakamura S, Yang CS, Sakon N, Ueda M, Tougan T, Yamashita A, Goto N, Takahashi K, Yasunaga T, Ikuta K, Mizutani T, Okamoto Y, Tagami M, Morita R, Maeda N, Kawai J, Hayashizaki Y, Nagai Y, Horii T, Iida T and Nakaya T. Direct metagenomic detection of viral pathogens in nasal and fecal specimens using an unbiased high-throughput sequencing approach. *PLoS One* 2009; 4: e4219.
- [26] Kuroda M, Katano H, Nakajima N, Tobiume M, Aina A, Sekizuka T, Hasegawa H, Tashiro M, Sasaki Y, Arakawa Y, Hata S, Watanabe M and Sata T. Characterization of quasispecies of pandemic 2009 influenza A virus (A/H1N1/2009) by de novo sequencing using a next-generation DNA sequencer. *PLoS One* 2010; 5: e10256.
- [27] Willner D, Furlan M, Haynes M, Schmieder R, Angly FE, Silva J, Tammadoni S, Nosrat B, Conrad D and Rohwer F. Metagenomic analysis of respiratory tract DNA viral communities in cystic fibrosis and non-cystic fibrosis individuals. *PLoS One* 2009; 4: e7370.
- [28] Pallen MJ, Loman NJ and Penn CW. High-throughput sequencing and clinical microbiology: progress, opportunities and challenges. *Curr Opin Microbiol* 2010; 13: 625-631.
- [29] Katano H, Kano M, Nakamura T, Kanno T, Asanuma H and Sata T. A novel real-time PCR system for simultaneous detection of human viruses in clinical samples from patients with uncertain diagnoses. *J Med Virol* 2011; 83: 322-330.
- [30] Kashiwase M, Sata T, Yamauchi Y, Minoda H, Usui N, Iwasaki T, Kurata T and Usui M. Progressive outer retinal necrosis caused by herpes simplex virus type 1 in a patient with acquired immunodeficiency syndrome. *Ophthalmology* 2000; 107: 790-794.
- [31] Muraki R, Baba T, Iwasaki T, Sata T and Kurata T. Immunohistochemical study of skin lesions in herpes zoster. *Virchows Arch A Pathol Anat Histopathol* 1992; 420: 71-76.
- [32] Maeda A, Sata T, Sato Y and Kurata T. A comparative study of congenital and postnatally acquired human cytomegalovirus infection in infants: lack of expression of viral immediate early protein in congenital cases. *Virchows Arch* 1994; 424: 121-128.
- [33] Katano H, Sato Y, Kurata T, Mori S and Sata T. High expression of HHV-8-encoded ORF73 protein in spindle-shaped cells of Kaposi's sarcoma. *Am J Pathol* 1999; 155: 47-52.
- [34] Iwasaki T, Sata T, Sugase M, Sato Y, Kurata T, Suzuki K, Ohmoto H, Iwamoto S and Matsukura T. Detection of capsid antigen of human papillomavirus (HPV) in benign lesions of female genital tract using anti-HPV monoclonal antibody. *J Pathol* 1992; 168: 293-300.
- [35] Howe JG and Steitz JA. Localization of Epstein-Barr virus-encoded small RNAs by in situ hybridization. *Proc Natl Acad Sci U S A* 1986; 83: 9006-9010.
- [36] Huson DH, Auch AF, Qi J and Schuster SC. MEGAN analysis of metagenomic data. *Genome Res* 2007; 17: 377-386.
- [37] McNicol A and Israels SJ. Mechanisms of oral bacteria-induced platelet activation. *Can J Physiol Pharmacol* 2010; 88: 510-524.
- [38] Chaiyarak K, Durongpisitkul K, Atta T, Soongswang J, Laohaprasitiporn D and Nana A. Clinical manifestations of Kawasaki disease: what are the significant parameters? *Asian Pac J Allergy Immunol* 2009; 27: 131-136.
- [39] Xiu-Yu S, Jia-Yu H, Qiang H and Shu-Hui D. Platelet count and erythrocyte sedimentation rate are good predictors of Kawasaki disease: ROC analysis. *J Clin Lab Anal* 2010; 24: 385-388.
- [40] Erickson PR and Herzberg MC. The *Streptococcus sanguis* platelet aggregation-associated protein. Identification and characterization of the minimal platelet-interactive domain. *J Biol Chem* 1993; 268: 1646-1649.

## Pathogens in lymph node of Kawasaki disease

- [41] Niemann S, Kehrel BE, Heilmann C, Rennemeier C, Peters G and Hammerschmidt S. Pneumococcal association to platelets is mediated by soluble fibrin and supported by thrombospondin-1. *Thromb Haemost* 2009; 102: 735-742.
- [42] Ohkuni H, Todome Y, Mizuse M, Ohtani N, Suzuki H, Igarashi H, Hashimoto Y, Ezaki T, Harada K, Imada Y and et al. Biologically active extracellular products of oral viridans streptococci and the aetiology of Kawasaki disease. *J Med Microbiol* 1993; 39: 352-362.
- [43] Vojtek I, Pirzada ZA, Henriques-Normark B, Mastny M, Janapatla RP and Charpentier E. Lyso-genic transfer of group A Streptococcus superantigen gene among Streptococci. *J Infect Dis* 2008; 197: 225-234.
- [44] Nagata S, Yamashiro Y, Ohtsuka Y, Shimizu T, Sakurai Y, Misawa S and Ito T. Heat shock proteins and superantigenic properties of bacteria from the gastrointestinal tract of patients with Kawasaki disease. *Immunology* 2009; 128: 511-520.
- [45] Suenaga T, Suzuki H, Shibuta S, Takeuchi T and Yoshikawa N. Detection of multiple superantigen genes in stools of patients with Kawasaki disease. *J Pediatr* 2009; 155: 266-270.
- [46] Naoumov NV, Petrova EP, Thomas MG and Williams R. Presence of a newly described human DNA virus (TTV) in patients with liver disease. *Lancet* 1998; 352: 195-197.
- [47] Nishizawa T, Okamoto H, Konishi K, Yoshizawa H, Miyakawa Y and Mayumi M. A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. *Biochem Biophys Res Commun* 1997; 241: 92-97.
- [48] Matsubara H, Michitaka K, Horiike N, Yano M, Akbar SM, Torisu M and Onji M. Existence of TT virus DNA in extracellular body fluids from normal healthy Japanese subjects. *Intervirology* 2000; 43: 16-19.
- [49] Hsieh SY, Wu YH, Ho YP, Tsao KC, Yeh CT and Liaw YF. High prevalence of TT virus infection in healthy children and adults and in patients with liver disease in Taiwan. *J Clin Microbiol* 1999; 37: 1829-1831.
- [50] Kurihara C, Ishiyama N, Nishiyama Y, Katayama K and Miura S. Changes of DNA titer and sequence variance of TT virus in hepatic disorders. *Hepatol Res* 2001; 19: 212-224.
- [51] Thom K and Petrik J. Progression towards AIDS leads to increased Torque teno virus and Torque teno minivirus titers in tissues of HIV infected individuals. *J Med Virol* 2007; 79: 1-7.
- [52] Zhong S, Yeo W, Tang MW, Lin XR, Mo F, Ho WM, Hui P and Johnson PJ. Gross elevation of TT virus genome load in the peripheral blood mononuclear cells of cancer patients. *Ann N Y Acad Sci* 2001; 945: 84-92.