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Case Report

Detection of trichodysplasia spinulosa-associated polyomavirus in a fatal case of myocarditis in a seven-month-old girl

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Abstract: Trichodysplasia spinulosa-associated polyomavirus (TSV) was identified in a seven-month-old girl with myocarditis. The number of TSV genomes detected was higher in the heart than in the other organs. The full-length TSV genome was cloned from the heart. This suggests a possible role of TSV infection in the pathogenesis of myocarditis in infants.

Keywords: Trichodysplasia spinulosa-associated polyomavirus, myocarditis

Introduction

Myocarditis is a rare but important cause of sudden death in childhood. Its etiology is wide ranging and often difficult to identify. Viral infection is thought to be one of the most frequent etiological agents of myocarditis [1]. Various viruses, including influenza virus, coxsackievirus B, parvovirus B19, adenoviruses, and cytomegalovirus, have been detected in myocarditis tissue samples. However, it remains unclear whether such viral infections are associated with the pathogenesis of myocarditis in all patients. In this report, we describe an autopsy case of fulminant myocarditis in which trichodysplasia spinulosa-associated polyomavirus (TSV), a human polyomavirus identified in 2010, was detected with next-generation sequencing [2].

Case description

In autumn 2012, a seven-month-old female infant suffering from respiratory discomfort at night attended our emergency outpatient department. She was admitted with acute, severe respiratory distress. She and her family had no appreciable past history, including asthma, congenital heart diseases, and immunodeficiencies. At the initial visit, her bodyweight was 6.3 kg, which was lower than the normal limit of Japanese seven-month-old female infants. Her developmental history was normal. She was appropriately immunized. She did not have any remarkable history of sick contact. Her body temperature was 36.0°C, pulse rate was 132/min, respiratory rate was 70/min, and oxygen saturation was 89% (ambient air). Her blood pressure was not measured. Marked wheezes in both lungs but no abnormal cardiac murmurs were heard on chest auscultation. Chest retraction and skin cyanosis were obvious. Laboratory findings were unremarkable, except for an elevated white blood cell count (14,510/μl) and a reduced hemoglobin level (9.3 g/dl). A venous blood gas analysis demonstrated mild acidemia and hypercapnea (pH 7.252, pCO_2 45.6 mmHg, base excess -6.9 mEq/l, HCO_3^- 19.4 mEq/l). No immunological test could be conducted because the patient came to the hospital at night, and no blood sample was preserved due to difficulty drawing

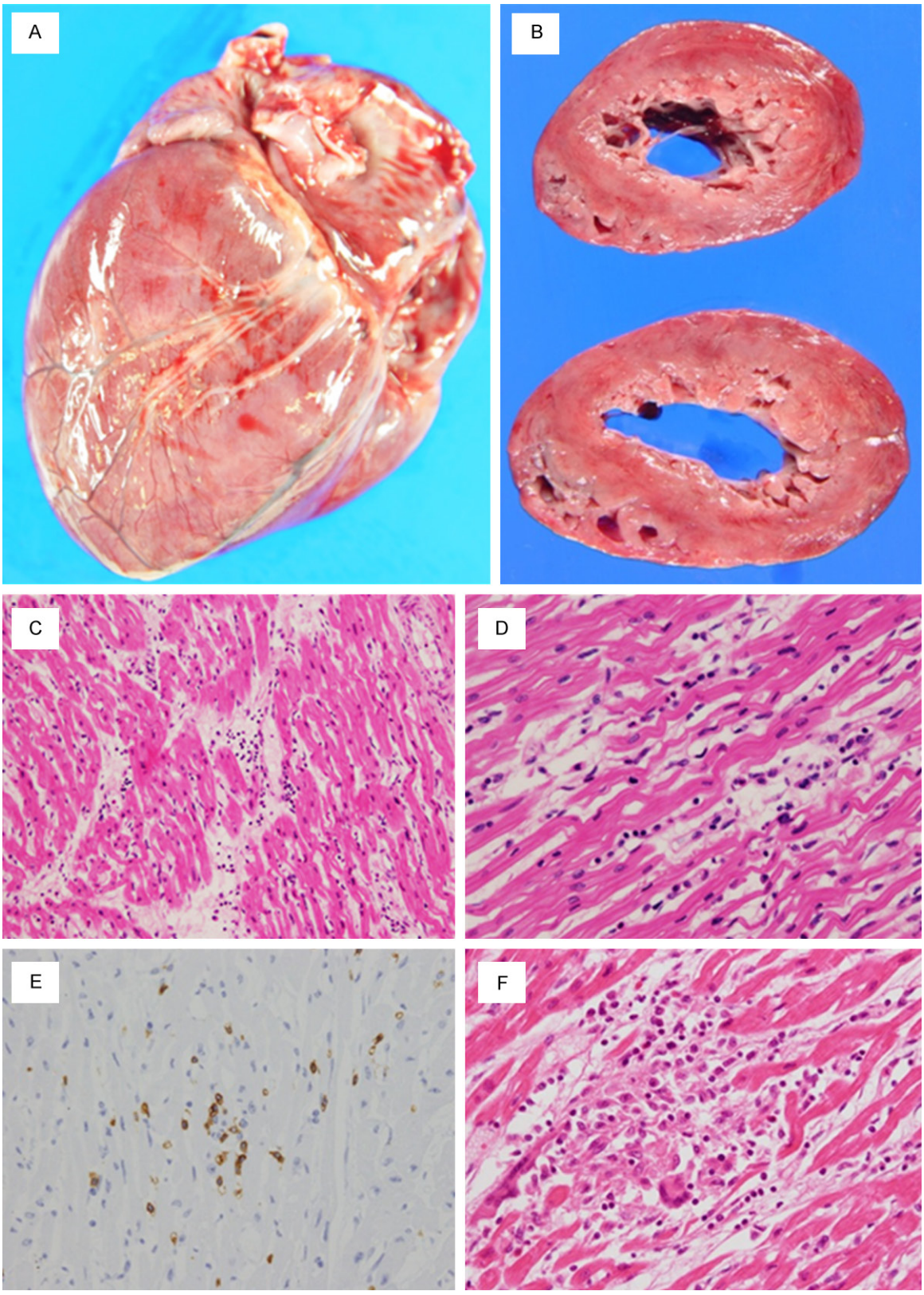

Figure 1. Macroscopic and histological findings in the heart. A, B. Macroscopic view of the heart. A. Petechial hemorrhage was observed on the surface. B. Left ventricle was enlarged and small petechiae were found on the cut surface. C-F. Histological views of the heart. C, D. Hematoxylin and eosin staining of the myocardial tissue. The

myocardial bundles were disturbed and edematous. Edema with infiltration by small lymphocytes and macrophages was observed between the myocardial bundles. E. Immunohistochemical analysis of CD8. F. Small granulomas with giant cells were found in the myocardium.

blood. Blood culture was conducted at the time of autopsy and demonstrated no significant bacterial growth. A chest radiograph showed abnormal infiltration in the right hilar area and lower lung field, and an increased cardiothoracic ratio. The lateral view also showed a posteriorly displaced major bronchus.

The patient was administered oxygen and inhaled salbutamol in the emergency room. Her respiratory rate and mode of respiration improved after inhalation, and she was diagnosed with a severe asthmatic attack complicated with a suspected lower respiratory infection and admitted to a pediatric ward. Three hours after admission, her respiratory condition deteriorated. Transfer to the intensive-care unit was determined and additional examinations and intubation was performed. A blood test revealed an extremely high brain natriuretic peptide level (6,788 pg/ml), whereas her creatine phosphokinase, lactate dehydrogenase, and aspartate aminotransferase levels were in the normal ranges. An echocardiogram was unremarkable, but an electrocardiogram was not performed. Cardiac arrest occurred suddenly after intubation. Resuscitation efforts were unsuccessful and the patient died 8 hour after admission. A morbid autopsy was performed within 6 hours of death. Macroscopically, the patient's heart was large and the left ventricle was dilated. There was no thrombus in the lumen or significant change in the four valves. Severe diffuse infiltration of small lymphocytes and macrophages was observed histologically in the myocardium (**Figure 1**). Immunohistochemistry showed that a large proportion of the small lymphocytes were $CD8^+$ T cells. Small granulomas with giant cells were found in the myocardium. There was no necrosis, and no disturbance of the myocardial array was observed. Small granulomas were also found in the lung and liver. Neither fungi nor acid-fast bacilli were found. In the both lungs, diffuse and mild lymphoplasmacytic infiltration of the bronchial and alveolar wall was observed.

Materials and methods

To identify any pathogen causing myocarditis, DNA and RNA samples extracted from a frozen sample of the heart were analyzed with a multi-virus real-time PCR system, which can examine 163 viruses simultaneously on a 96-well plate [3]. Although a low copy number of parvovirus B19 (36.1 copies per 100 ng of DNA) was detected in the bronchus, no viral genome was detected in the heart sample with this system. We then analyzed DNA and RNA samples extracted from frozen tissue from the heart with deep sequencing, using a next-generation sequencer (GAIIx, Illumina, San Diego, CA). RNA was reverse transcribed to cDNA, and both DNA and cDNA were applied to the GAIIx. In total, 3×10^7 reads were obtained with the GAIIx, and a BLAST search revealed that 244,965 80-bp reads were nonhuman sequences.

Results

Two of the reads corresponded to nucleotides (nt) 4,535-4,614 and nt 5,034-5,113 of the TSV genome (GU989205). No other significant pathogenic genome was identified. A real-time PCR analysis was performed to detect the TSV VP1 gene using the TSPyV-F primer (5'-cagtgctaatgacaaattggttgttc-3'), TSPyV-R primer (5'-ttagcttttgtttgtagtgaggattga-3'), and TSPyV-FAM probe (5'-FAM-cccaataaaacaccagagcacacaaggc-TAMRA-3'), targeting nt 1,841-1,923 of GU989205. The real-time PCR analysis detected the TSV genome in the heart (413.2 copies per 100 ng of DNA), lung (248.8), liver (81.6), spleen (103.7), bronchus (36.5), small intestine (58.1), and colon (24.1), indicating that the TSV genome copy number was higher in the heart than in the other organs.

Long PCR using KOD-FX DNA polymerase (Toyobo, Tokyo, Japan) amplified the entire TSV genome, with a length of 5,232 bp. We also successfully PCR amplified a 252-bp fragment including a putative replication origin of TSV using two primers (TSV-F5111 5'-agcctctgtgtgcctcaatt-3', and TSV-R131 5'-tcctcaggataacggtcttaa-3') suggesting the presence of a circular form of the TSV genome in the heart sample. The long PCR product of full-length TSV was cloned into the pCR2.1-blunt vector (Invitrogen, Carlsbad, CA). A sequencing analysis of four clones from independent colonies revealed

that the cloned PCR product was 99.4% homologous to the complete genome of previously reported TSV (GU989205 and JQ723730). Therefore, we designated the TSV clone strain TSV-TMC, and registered it in GenBank under accession number AB873001.

Discussion

Polyomaviruses are ~45 nm, nonenveloped, double-stranded DNA viruses with a small genome of ~5.2 kb. Until now, 10 polyomaviruses have been described in humans and the majority of human polyomaviruses (HPyVs) were identified in the last five years [4]. Generally, a primary infection with polyomavirus is thought to be asymptomatic, and the virus persists in immunocompetent individuals as a latent infection during their whole lives. JC polyomavirus and BK polyomavirus are considered to be pathogens that cause progressive multifocal leukoencephalopathy in AIDS patients and nephropathy in renal transplant recipients, respectively. Merkel cell polyomavirus (MCPyV) has been found to be integrated in a large proportion of Merkel cell carcinomas of the skin [5]. HPyV6 and HPyV7 productively infect the human skin, and infections with such skin-tropic polyomaviruses are very common in the general population [6]. A putative association between trichodysplasia spinulosa (TS) and TSV has been considered, but is not definitive [7]. TS is histologically characterized by the abnormal maturation and marked distention of the hair follicles, and is considered to be symptomatic only in immunocompromised patients. However, the actual etiology of TSV has not yet been established.

TSV was detected in the myocardial tissues of a seven-month-old girl with severe myocarditis. This report is notable for two points: (1) TSV was identified in a child who had no remarkable past history and would be immunocompetent; and (2) TSV was detected in tissues other than the skin. Although the seroprevalence of TSV in the general Japanese population is unknown, a high prevalence is predicted in Japan based on the seroprevalence data from European countries [8-11]. It has also been demonstrated that primary TSV infections frequently occur in early life, within 0-10 years after birth [8]. However, no report has described an association between primary TSV infection and any disease. We were unable to determine whether this was a primary infection, but the high copy number of the TSV genome and the presence of the circular form of TSV in the heart suggest a possible role of TSV as a causative agent of myocarditis in some infant cases. Although the molecular mechanism of TSV infection is unknown, this case serves a hypothesis of the presence of specific receptor for TSV in myocytes. Alternatively, TSV might be reactivated and produced in specific organs under the condition of severe inflammation. Histological changes in this case such as severe infiltration of lymphocytes and destruction of myocytes are observed commonly among other viral myocarditis like Coxsackievirus and enterovirus, not specific for TSV. It cannot be completely denied that such severe inflammation induced production of TSV which existed as a bystander there. To determine the association between myocarditis and TSV infection, serological studies and the immunohistochemical detection of the viral proteins in myocarditis samples will be required. Immunohistochemistry and in situ hybridization may be useful for detection of the virus protein or genome in tissue samples, but have not been established, yet, because there is no appropriate control sample or culture cell positive for TSV, so far. The direct relationship between granulomas and TSV infection was considered unlikely, because distribution of the granuloma was not correlated with TSV copy numbers. However, the presence of granulomas implies any immunological abnormality in the patient.

To the best of our knowledge, this is the first case report describing the detection of TSV in a myocarditis specimen from a child. Although we have previously reported that low copy numbers of MCPyV were detected in some tissue samples from myocarditis patients [12], few reports have described the association between polyomavirus infection and myocarditis. Therefore, further studies are required to clarify the association between TSV infection and the pathogenesis of myocarditis.

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Disclosure of conflict of interest

None.

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