High Prevalence of Distinct Human Herpesvirus 8 Contributes to the High Incidence of Non-acquired Immune Deficiency Syndrome-Associated Kaposi's Sarcoma in Isolated Japanese Islands

Ryoko Awazawa,1 Daisuke Utsumi,1 Harutaka Katano,2 Tsuyoshi Awazawa,1 Takuya Miyagi,1 Kentaro Hayashi,1 Shigetaka Matori,1 Hiroshi Uezato,1 and Kenzo Takahashi3

1Department of Dermatology, University of the Ryukyus, Graduate School of Medicine, Okinawa, Japan; 2Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan

Background. Non-acquired immune deficiency syndrome (AIDS) Kaposi's sarcoma (KS) is extremely rare in Japan but highly endemic in Okinawa, especially in Miyako Islands. We aimed to elucidate the exact incidence and cause of this high prevalence.

Methods. Non-AIDS KS cases in Okinawa Prefecture over the past 31 years were reviewed, and human herpesvirus 8 (HHV8) seroprevalence in Miyako Islands was determined. We examined whole-genome sequences of 3 HHV8 strains and performed whole-exome sequencing of 4 male patients from Miyako Islands.

Results. Approximately half of the non-AIDS KS cases in Okinawa Prefecture were from Miyako Islands. The age-adjusted incidence rate was 0.87/105 per year for Miyako Islands and 0.056/105 per year for the rest of Okinawa. Human herpesvirus 8 seroprevalence was 15.4% in Miyako Islands. The 3 HHV8 genomes isolated from Miyako islanders formed a phylogenetically branch distinct from those of previously sequenced HHV8 strains and shared specific mutations in 9 proteins. These mutations were verified in Okinawan patients other than those from Miyako Islands. Whole-exome sequencing of the 4 male Miyako Islanders did not reveal shared pathogenic mutations.

Conclusions. Miyako Islands are an endemic area of non-AIDS KS. The high rate of a distinct HHV8 may contribute to the high incidence of KS in the region.

Keywords. epidemiology; human herpesvirus 8; Kaposi’s sarcoma; Miyako Islands; Okinawa.

Kaposi's sarcoma (KS) is a multicentric, angioproliferative disease that is clinically divided into 4 categories: classic, iatrogenic, African (endemic), and acquired immune deficiency syndrome (AIDS)-related (epidemic). Although extremely rare, a hereditary form of the disease caused by mutations in several genes linked to immunocompetence has also been described [1–6]. Classic KS is particularly prevalent among elderly men from the Mediterranean region, Eastern Europe, and the Middle East [7]. Iatrogenic KS, which occurs in immunosuppressed patients, has been reported in regions where classic KS is prevalent [8].

Human herpesvirus 8 (HHV8) is involved in the development of KS of all 4 clinical types [9]. Unlike other human herpesviruses, the seroprevalence of HHV8 is relatively low and varies significantly worldwide [10].

In the Mediterranean region, HHV8 seroprevalence is 15% to 25% [11], whereas it is generally 10% or lower in the Americas, Northern Europe, and Asia [10]. Only a small proportion of individuals infected with HHV8 develop KS. Causative factors other than viral infection include immune status and genetic background [9]. The Xinjiang-Uygur Autonomous Region has a high incidence of KS. Both Uygur and Han populations in this region have a similar HHV8 seroprevalence of approximately 20%; however, KS is observed only in Uygur individuals [12].

HHV8 seroprevalence throughout Japan is low (1.4%); however, HHV8 seroprevalence among men who have sex with men (MSM) is higher, at 11.7% [13, 14]. Most cases of KS in Japan are MSM with AIDS. Non-AIDS-associated KS (non-AIDS KS), such as the classic and iatrogenic types, are extremely rare [15], except in Okinawa Prefecture, which is located in the southernmost region of Japan [16, 17]. Okinawa Prefecture comprises 3 major groups of islands: Okinawa Islands, where over 90% of the population lives, and the Miyako and Yaeyama Islands (Supplementary Figure 1).

Non-AIDS KS is particularly prevalent in Miyako Islands. The population of Miyako Islands represents only 0.05% of the total population of Japan and 4% of that of Okinawa Prefecture.
However, our epidemiological survey reported here indicated that approximately 20% of Japanese patients with non-AIDS KS are from Miyako Islands. Thus, we investigated the causes of this high prevalence of non-AIDS KS in Okinawa Prefecture, and in Miyako Islands in particular, by studying HHV8 seroprevalence, as well as viral and genomic mutations in patients.

METHODS

The present study was approved by the ethics committees of the University of the Ryukyus and Okinawa Prefectural Miyako Hospital. Written informed consent was obtained from all patients whose samples were used for whole-exome sequencing.

Review of Patients With Kaposi’s Sarcoma

Medical records of all KS cases diagnosed at the University of the Ryukyus Hospital and its affiliated hospitals in Okinawa Prefecture between January 1, 1984 and December 31, 2014 were collected and reviewed.

Sero logical Assays

Residual serum samples were collected from 132 patients who visited Okinawa Prefectural Miyako Hospital, Miyakojima City, between September 10 and October 12, 2012. Sera from 10 patients with non-AIDS KS who attended the University of the Ryukyus Hospital were used to confirm the validity of serological assays. Serum HHV8 antibody was detected using mixed-antigen enzyme-linked immunosorbent assay (ELISA) and was confirmed by immunofluorescence assay (IFA), as previously reported [13]. Mixed-antigen ELISA included purified recombinant glutathione S-transferase fusion proteins for the K8.1, ORF59, ORF65, and ORF73 domains of HHV8 as immunogens. This ELISA, developed by Katano et al [13] in 2000, showed 100% positivity in 26 KS patients in the original report. In the IFA, 12-O-tetradecanoylphorbol 13-acetate-induced TY-1 cells, an HHV8-infected cell line, were used as antigenic cells. Only sera that were positive in both ELISA and IFA were considered positive. ELISA and IFA are further detailed in the Supplementary Methods.

Statistical Analysis

Sex-based differences in HHV8 seroprevalence were analyzed by the χ² test. Mean ELISA values are shown together with standard deviations. Differences in ELISA values between ELISA-positive KS patients and HHV8 carriers in Miyako Islands were analyzed by the Wilcoxon rank-sum test.

Human Herpesvirus 8 Genome Sequencing

Deoxyribonucleic acid (DNA) was extracted from frozen tumor tissues from 4 patients with non-AIDS KS: 3 from Miyako Islands (1 patient with classic KS and 2 patients with iatrogenic KS) and 1 patient with classic KS from mainland Japan. HHV8 genomes were amplified by polymerase chain reaction (PCR) using 18 sets of primers (Supplementary Table 1). PCR products were purified using the NucleoSpin Gel and PCR Clean-up Kit (MACHEREY-NAGEL, Bethlehem, PA). PCR products were pooled at approximately equal molar masses, used for preparing sequencing libraries using the TruSeq Nano DNA LT Sample Prep Kit (Illumina, San Diego, CA), and sequenced (2 × 101 cycles, paired-end) on a HiSeq 2500 instrument using the TruSeq PE Cluster Kit and TruSeq SBS Kit (Illumina). De novo assembly was performed using CLC Genomic Workbench v8.5 (CLC Bio; QIAGEN, Venlo, Netherlands), and the reads were mapped against the registered HHV8 genome (AF148805.2) [18]. Obtained contigs lacked several regions (23–25, 117–119, and 124–127 kilobase pairs) because the next-generation sequencing data contained multiple repeated sequences. Therefore, we determined these sequences by direct sequencing with the Sanger method after PCR (Supplementary Table 2).

Multiple amino acid alignments were generated for each HHV8 gene using Clustal Omega. Nonsynonymous mutations that were detected in all 3 HHV8 strains from Miyako Islanders, but not in the mainland HHV8 strain, were selected. The mutations were confirmed by Sanger sequencing of each PCR product using the BigDye Terminator v3.1 Cycle Sequencing Kit (Supplementary Table 3).

Furthermore, DNA was obtained from frozen tumor samples of 3 patients with non-AIDS KS from the rest of Okinawa (1 patient with classic KS and 2 patients with iatrogenic KS). These mutations were confirmed by Sanger sequencing. HHV8 genome analysis is described in more detail in the Supplementary Methods.

Whole-Exome Sequencing of Patients With Non-AIDS KS From Miyako Islands

DNA was extracted from frozen tissue or peripheral blood mononuclear cells of 4 male patients with non-AIDS KS from Miyako Islands (2 patients with classic KS and 2 with iatrogenic KS), and exome sequencing libraries were prepared using the SureSelectXT Human All Exon v5 Kit (Agilent, Santa Clara, CA). The libraries were sequenced (2 × 101 cycles, paired-end) on a HiSeq 2500 using the TruSeq PE Cluster Kit and SBS Kit (Illumina). The sequence was aligned with the GRCh37 reference sequence of the human genome using the BWA aligner [19].

Downstream processing and variant calling were performed using the Genome Analysis Toolkit (GATK) [20], SAMtools [21], and Picard. Substitution and indel calls were made using the GATK Unified Genotyper; calls with QualByDepth score ≤2.0 were filtered out. Variants reported with a minor allele frequency ≤0.01 in public databases, including the Human Genetic Variation Database (http://www.hgvd.genome.med.kyoto-u.ac.jp/about.html) and ExAC (http://exac.broadinstitute.org/), were extracted using ANNOVAR.

RESULTS

Cases

Between 1984 and 2014, 39 cases of classic KS, 22 cases of iatrogenic KS, and 13 cases of AIDS-related KS were diagnosed in
Okinawa Prefecture (Table 1, Supplementary Table 4). Among the 61 patients with non-AIDS KS, 30 (49.1%) were from Miyako Islands, 21 (34.4%) from Okinawa Islands, 4 (6.56%) from Yaeyama Islands, and the place of birth could not be determined for 5 cases (8.20%) (Table 1, Supplementary Table 4). One patient (1.64%) was originally from Amami Islands in Kagoshima Prefecture (250 km northeast of Okinawa Islands); therefore, the total number of patients with non-AIDS KS from Okinawa Prefecture was 60. The number of reported cases of non-AIDS KS in Japan between 1984 and 2014 was 79, comprising 58 cases of classic KS and 21 cases of iatrogenic KS, after excluding cases from Okinawa (Table 1).

Incidence Rates of Non-AIDS KS Throughout Japan
The incidence rates for the entire 31-year period and for all ages were as follows: Miyako Islands: 0.87 (/10^5 per year), males: 1.9, females: 0.22; Okinawa Prefecture excluding the Miyako Islands: 0.056 (/10^5 per year), males: 0.085, females: 0.031; Japan excluding Okinawa Prefecture: 0.0015, males: 0.0022, females: 0.00090. The incidence rates for the entire 31-year period for individuals aged 50 and over were as follows: Miyako Islands: 4.6 (/10^5 per year), males: 8.3, females: 1.4; Okinawa Prefecture excluding Miyako Islands: 0.27, males: 0.33, females: 0.21; Japan excluding Okinawa Prefecture: 0.0054, males: 0.0082, females: 0.0031 (Table 2).

When we focused on shifts in the incidence rates from 1984–1999 to 2000–2014, the incidence rate for patients with non-AIDS KS aged 50 and over increased 8-fold in Miyako Islands and 3-fold in Okinawa Prefecture excluding Miyako Islands, indicating an increasing trend in incidence rates after 2000. There was no change in the incidence rates for mainland Japan.

**HHV8 Seroprevalence in Miyako Islands**
Of the 1132 individuals surveyed, 174 (15.4%) were seropositive for HHV8 (Table 3). There was no sex-based difference (male: 16.6%, female: 14.1%, P = .2534). The seroprevalence and mean ELISA value increased age-dependently. In 10 patients with non-AIDS KS, ELISA was positive for 9 patients (90%), with a mean ELISA value of 0.532, which is higher than that of HHV8 carriers from Miyako Islands (0.317; P = .00346, Wilcoxon rank-sum test). IFA results were positive in all 10 cases of non-AIDS KS.

**Non-AIDS KS Incidence Rate in HHV8 Carriers**
The yearly incidence rate of KS among HHV8 carriers aged 50 and over are presented in Table 2. Human herpesvirus 8 carriers from Miyako Islands and Okinawa Prefecture exhibited a markedly higher incidence rate than carriers from other parts of Japan.

### Table 1. Clinical Summary of Non-AIDS Kaposi’s Sarcoma (KS) Cases in Okinawa and Japan (1984–2014)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Okinawa Prefecture</th>
<th>Japan (Excluding Okinawa Prefecture)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total, n = 61</td>
<td>Classic KS, n = 39 (63.9%)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>42 (68.9)</td>
<td>29 (74.4)</td>
</tr>
<tr>
<td>Female</td>
<td>19 (31.1)</td>
<td>10 (25.6)</td>
</tr>
<tr>
<td>Male:Female</td>
<td>2:1</td>
<td>2.9:1</td>
</tr>
<tr>
<td><strong>Age at diagnosis (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>77.6</td>
<td>78.3</td>
</tr>
<tr>
<td>20–49</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>50–79</td>
<td>31 (50.8)</td>
<td>17 (43.6)</td>
</tr>
<tr>
<td>80–93</td>
<td>30 (49.2)</td>
<td>22 (56.4)</td>
</tr>
<tr>
<td><strong>Year of diagnosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1984–1999</td>
<td>10 (16.4)</td>
<td>4 (10.3)</td>
</tr>
<tr>
<td>2000–2014</td>
<td>51 (83.6)</td>
<td>35 (89.7)</td>
</tr>
<tr>
<td><strong>Origin (population)</strong>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Okinawa Prefecture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Okinawa Islands (1179671)</td>
<td>21 (34.4)</td>
<td>13 (33.3)</td>
</tr>
<tr>
<td>Miyako Islands (56719)</td>
<td>30 (49.1)</td>
<td>20 (51.3)</td>
</tr>
<tr>
<td>Yaeyama Islands (49568)</td>
<td>4 (6.66)</td>
<td>2 (5.13)</td>
</tr>
<tr>
<td>Unknown</td>
<td>5 (8.20)</td>
<td>3 (7.69)</td>
</tr>
<tr>
<td>Other areas of Japan</td>
<td>1 (1.64)</td>
<td>1 (2.56)</td>
</tr>
</tbody>
</table>

Abbreviations: AIDS, acquired immunodeficiency syndrome.

*Patients were counted by combining data from all proceedings and reported cases; it is therefore possible that the reported figures may underestimate the actual number of cases.

**Mean population estimate based on 1985 and 2010 census data.

**One patient of non-AIDS KS diagnosed in Okinawa prefecture originated from Kagoshima Prefecture; therefore, the total number of patients with non-AIDS KS from Okinawa Prefecture was 60.
HHV8 Genome Analysis

In the phylogenetic tree based on whole viral genome sequences, all 4 HHV8 strains derived from the non-AID KS cases formed a cluster that was distinct from the European/US and Zambian HHV8 strains (Figure 1). Among the 4 HHV8 that were analyzed, 3 strains derived from Miyako Island patients had a high degree of sequence similarity (99.9%). All 4 strains were categorized as K1 genotype C and K15 M allele (Supplementary Figure 2). The numbers of nonsynonymous mutations in HHV8, relative to GK18 (AF148805.2) [18], were 503, 507, and 505 from Miyako Islands and 517 from mainland Japan (except for ORF73) (Table 4). Many of the mutations shared among the 3 Miyako Islands-derived viruses were also shared with the mainland Japan-derived virus, although approximately 5% of the mutations were specific to Miyako Island strains and accumulated in only 9 of 86 viral proteins (Table 5). Their presence was additionally confirmed in HHV8 derived from 3 Okinawa Island patients.
On average, we obtained a sequence coverage of approximately \(57.7 \pm 2.1\times\). More than 98% of the targeted DNA bases were covered by >5 reads, and >89% were covered by >20 reads. There were no nonsynonymous mutations common to the 4 patients from Miyako Islands in 6 genes known to be responsible for hereditary or childhood-onset KS (\(TNFRSF4\), \(STIM1\), \(STAT4\), \(IFN\gammaR1\), \(CD40L\), and \(WAS\)) [1–6]. Only 1 male patient had a P460S (1378C>T) mutation in the \(WAS\) gene; however, this patient did not exhibit Wiskott-Aldrich syndrome symptoms.

The nonsynonymous mutations shared by the 4 male patients from Miyako Islands, which occurred with 1% or lower frequency in Japanese and global databases, were not on the X or Y chromosome.

### Table 3. HHV8 Seroprevalence in Miyako Islands in 2012

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>Mean ELISA Value in Positive Individuals (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10–19</td>
<td>0.0</td>
<td>15.4</td>
<td>8.7</td>
<td>0.168 (0.029)</td>
</tr>
<tr>
<td>20–29</td>
<td>33.3</td>
<td>0.0</td>
<td>3.6</td>
<td>0.118</td>
</tr>
<tr>
<td>30–39</td>
<td>0.0</td>
<td>1.7</td>
<td>1.2</td>
<td>0.168</td>
</tr>
<tr>
<td>40–49</td>
<td>3.0</td>
<td>8.5</td>
<td>6.3</td>
<td>0.214 (0.046)</td>
</tr>
<tr>
<td>50–59</td>
<td>10.2</td>
<td>11.0</td>
<td>10.6</td>
<td>0.292 (0.148)</td>
</tr>
<tr>
<td>60–69</td>
<td>14.7</td>
<td>20.2</td>
<td>16.9</td>
<td>0.298 (0.192)</td>
</tr>
<tr>
<td>70–79</td>
<td>22.7</td>
<td>19.7</td>
<td>21.4</td>
<td>0.354 (0.202)</td>
</tr>
<tr>
<td>80–89</td>
<td>23.9</td>
<td>18.9</td>
<td>22.0</td>
<td>0.322 (0.185)</td>
</tr>
<tr>
<td>90–97</td>
<td>16.7</td>
<td>25.0</td>
<td>20.8</td>
<td>0.326 (0.201)</td>
</tr>
<tr>
<td>Total</td>
<td>16.6</td>
<td>14.1</td>
<td>15.4</td>
<td>0.317 (0.186)</td>
</tr>
</tbody>
</table>

Abbreviations: ELISA, enzyme-linked immunosorbent assay; HHV8, human herpesvirus 8; SD, standard deviation.

The sample population in this study included few young people; there are no data for individuals under the age of 10. The youngest individual to test positive for HHV8 was 18 years old.

**Figure 1.** Unrooted maximum-likelihood phylogenetic tree of the nucleotide sequences of our 4 human herpesvirus 8 (HHV8) strains, ie, HHV8-Miyako1 (DDBJ accession number: LC200586), HHV8-Miyako2 (LC200587), HHV8-Miyako3 (LC200588), HHV8-Japan1 (LC200589), and 24 other HHV8 whole-genome sequences registered in GenBank, including 7 strains derived from European and US patients and cell lines (GK18 [AF148805.2] [18], KS [U93872.2] [23], BC-1 [U75698.1] [24], JSC-1 [GQ994935.1] [25], BCBL-1 [HQ404500.1] [26], DG-1 [JQ619843.1] [27], BrK.219 [KF588566.1] [28]), 16 strains derived from Zambian patients (ZM: KT271453–KT271468) [29], and a sequence derived from a cell line established from a Japanese patient with acquired immune deficiency syndrome-primary effusion lymphoma (SPEL: AP017458) [30]. The phylogenetic tree was generated using PhyML [31], with 1000 bootstrap replications, and was visualized using MEGA7 [32].
The incidence of non-AIDS KS in Miyako Islands and Okinawa Prefecture increased after 2000, and this increase cannot be explained by population aging. Because the percentage of individuals with iatrogenic KS did not rise, the higher incidence rates cannot be attributed to an increase in immunosuppressive therapy. In addition, it cannot be attributed to a rise in immigration, unlike in Israel [34]. Because this is the first survey of HHV8 seroprevalence in Miyako Islands, we cannot determine the change over time, despite its potential to explain the increasing incidence of non-AIDS KS after 2000.

Previously, HHV8 seroprevalence was reported to be 1.4% in the general population of Japan [13] and 1.43% in Naha City of Okinawa Islands [22], with the same method. Here, the seroprevalence in Miyako Islands was 15.4%, which is 11.0- and 10.7-fold higher than that in Japan and Okinawa Islands, respectively. There was no gender difference in seroprevalence, consistent with studies of other areas [11, 33].

The mean ELISA value of non-AIDS KS patients was significantly higher than that of HHV8 carriers in Miyako Islands; notably, the latter value tended to increase with age. An age-dependent increase in HHV8 antibody titers and high antibody titers among KS patients relative to HHV8 carriers have been reported [35–37]. The HHV8 detection rate from peripheral blood mononuclear cells and high-titer antibody retention rate against lytic antigen K8.1 were significantly higher in patients with classic KS than in premorbid HHV8 carriers in Italy [36]. K8.1 antibody titers increased as KS onset approached

Table 4. Summary of HHV8 Full Genome Sequencing Results

<table>
<thead>
<tr>
<th>HHV8 Strain</th>
<th>HHV8 Genome Size (bp)</th>
<th>Percentage Identity Compared With GK18</th>
<th>Total Number of Nonsynonymous Mutations*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miyako1 (LC200586)</td>
<td>136574</td>
<td>96.2</td>
<td>503</td>
</tr>
<tr>
<td>Miyako2 (LC200587)</td>
<td>136661</td>
<td>96.2</td>
<td>507</td>
</tr>
<tr>
<td>Miyako3 (LC200588)</td>
<td>136378</td>
<td>96.3</td>
<td>505</td>
</tr>
<tr>
<td>Japan1 (LC200589)</td>
<td>136429</td>
<td>94.5</td>
<td>517</td>
</tr>
</tbody>
</table>

Abbreviations: bp, base pairs; HHV8, human herpesvirus 8.

Over half of these nonsynonymous mutations were due to differences in the K15 subtype between GK18 (AF148805.2) [18] and the 4 types of HHV8 in Japan.

Nonsynonymous mutations relative to GK18 were counted, except for ORF73.

Y chromosome. Genetic mutations in autosomes were investigated similarly, but the shared mutations were neither homozygous nor heterozygous.

DISCUSSION

Japan (mean population over the past 31 years: approximately 123 million) has a low incidence of non-AIDS KS, except in Okinawa Prefecture (1.3 million). In addition, approximately half of all cases in Okinawa Prefecture originate from Miyako Islands (57,000), which have incidence rates comparable to those in the Mediterranean Islands, indicating that Miyako Islands are one of the global endemic areas for non-AIDS KS [33].

Table 5. Nonsynonymous Mutations Found in Three HHV8 Strains From Miyako Islands

<table>
<thead>
<tr>
<th>No.</th>
<th>Location in Reference Sequence (bp) (AF148805.2)</th>
<th>Gene</th>
<th>Coding Region Change</th>
<th>Amino Acid Change</th>
<th>Location in the Genome of HHV8-Miyako1 (LC200586)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>139</td>
<td>K1</td>
<td>c.35G &gt; A</td>
<td>p.Cys12Tyr</td>
<td>129</td>
</tr>
<tr>
<td>2</td>
<td>297</td>
<td>K1</td>
<td>c.193T &gt; G</td>
<td>p.Leu6Val</td>
<td>287</td>
</tr>
<tr>
<td>3</td>
<td>657</td>
<td>K1</td>
<td>c.553G &gt; C</td>
<td>p.Val185Leu</td>
<td>647</td>
</tr>
<tr>
<td>4</td>
<td>736</td>
<td>K1</td>
<td>c.632C &gt; A</td>
<td>p.Thr211Lys</td>
<td>726</td>
</tr>
<tr>
<td>5</td>
<td>20621</td>
<td>ORF70</td>
<td>c.416G &gt; T</td>
<td>p.Arg139Met*</td>
<td>20626</td>
</tr>
<tr>
<td>6</td>
<td>42480</td>
<td>ORF24</td>
<td>c.396G &gt; A</td>
<td>p.Cys133Tyr</td>
<td>42156</td>
</tr>
<tr>
<td>7</td>
<td>47214</td>
<td>ORF26</td>
<td>c.183A &gt; T</td>
<td>p.Gln61His</td>
<td>46890</td>
</tr>
<tr>
<td>8</td>
<td>47380</td>
<td>ORF26</td>
<td>c.349T &gt; C</td>
<td>p.Phe117Leu</td>
<td>47056</td>
</tr>
<tr>
<td>9</td>
<td>50787</td>
<td>ORF30</td>
<td>c.65T &gt; A</td>
<td>p.Phe227Tyr*</td>
<td>50460</td>
</tr>
<tr>
<td>11</td>
<td>93230</td>
<td>vIRF-2</td>
<td>c.879T &gt; A</td>
<td>p.Phe293Leu</td>
<td>92889</td>
</tr>
<tr>
<td>12</td>
<td>93267.93268</td>
<td>vIRF-2</td>
<td>c.841_842delTTinsGG</td>
<td>p.Leu281Gly*</td>
<td>92928..92927</td>
</tr>
<tr>
<td>13</td>
<td>93277</td>
<td>vIRF-2</td>
<td>c.832A &gt; G</td>
<td>p.Thr278Ala*</td>
<td>92936</td>
</tr>
<tr>
<td>14</td>
<td>93309</td>
<td>vIRF-2</td>
<td>c.800G &gt; T</td>
<td>p.Gly267Val*</td>
<td>92968</td>
</tr>
<tr>
<td>15</td>
<td>93346</td>
<td>vIRF-2</td>
<td>c.763G &gt; A</td>
<td>p.Val258Met</td>
<td>93005</td>
</tr>
<tr>
<td>16</td>
<td>93441</td>
<td>vIRF-2</td>
<td>c.668A &gt; G</td>
<td>p.Glu223Gly*</td>
<td>93100</td>
</tr>
<tr>
<td>17</td>
<td>123140</td>
<td>ORF72</td>
<td>c.676A &gt; G</td>
<td>p.Ser226Gly*</td>
<td>123072</td>
</tr>
</tbody>
</table>

Abbreviations: AIDS, acquired immunodeficiency syndrome; bp, base pairs; HHV8, human herpesvirus 8; KS, Kaposi’s sarcoma.

Non-synonymous mutations that were detected in all 3 HHV8 strains from Miyako Islands, but not in HHV8 strains from mainland Japan, were selected. All of these mutations were confirmed in 3 other HHV8 isolates from patients with non-AIDS KS in Okinawa Islands.

In ORF73 (latent-associated nuclear antigen), multiple deletions in the glutamine-rich sequence and the glutamic acid-rich region with a repeated leucine heptad motif, both of which are in the central repeated region, were observed in all 4 HHV8 strains analyzed in this study. Specifically, a 318-amino acid sequence, between Glu485 and Glu802 in the reference sequence (AF148805.2) [18], was truncated to 267 amino acids in the Japan-derived HHV8 and 182–183 amino acids in Miyako Islands-derived HHV8.

Mutations that were not previously reported: unique to HHV8 from Miyako Islands.

Previously, HHV8 seroprevalence was reported to be 1.4% in the general population of Japan [13] and 1.43% in Naha City of Okinawa Islands [22], with the same method. Here, the seroprevalence in Miyako Islands was 15.4%, which is 11.0- and 10.7-fold higher than that in Japan and Okinawa Islands, respectively. There was no gender difference in seroprevalence, consistent with studies of other areas [11, 33].

The mean ELISA value of non-AIDS KS patients was significantly higher than that of HHV8 carriers in Miyako Islands; notably, the latter value tended to increase with age. An age-dependent increase in HHV8 antibody titers and high antibody titers among KS patients relative to HHV8 carriers have been reported [35–37]. The HHV8 detection rate from peripheral blood mononuclear cells and high-titer antibody retention rate against lytic antigen K8.1 were significantly higher in patients with classic KS than in premorbid HHV8 carriers in Italy [36]. K8.1 antibody titers increased as KS onset approached

The incidence of non-AIDS KS in Miyako Islands and Okinawa Prefecture increased after 2000, and this increase cannot be explained by population aging. Because the percentage of individuals with iatrogenic KS did not rise, the higher incidence rates cannot be attributed to an increase in immunosuppressive therapy. In addition, it cannot be attributed to a rise in immigration, unlike in Israel [34]. Because this is the first survey of HHV8 seroprevalence in Miyako Islands, we cannot determine the change over time, despite its potential to explain the increasing incidence of non-AIDS KS after 2000.

Previously, HHV8 seroprevalence was reported to be 1.4% in the general population of Japan [13] and 1.43% in Naha City of Okinawa Islands [22], with the same method. Here, the seroprevalence in Miyako Islands was 15.4%, which is 11.0- and 10.7-fold higher than that in Japan and Okinawa Islands, respectively. There was no gender difference in seroprevalence, consistent with studies of other areas [11, 33].

The mean ELISA value of non-AIDS KS patients was significantly higher than that of HHV8 carriers in Miyako Islands; notably, the latter value tended to increase with age. An age-dependent increase in HHV8 antibody titers and high antibody titers among KS patients relative to HHV8 carriers have been reported [35–37]. The HHV8 detection rate from peripheral blood mononuclear cells and high-titer antibody retention rate against lytic antigen K8.1 were significantly higher in patients with classic KS than in premorbid HHV8 carriers in Italy [36]. K8.1 antibody titers increased as KS onset approached
in Ugandan human immunodeficiency virus-infected subjects [37]. Based on these results, it may be assumed that, as HHV8 carriers age, the number of viruses in the body increases, leading to an increased antibody titer.

Annual KS incidence rates among HHV8 carriers aged 50 and over in Sardinia are 0.018%–0.035% in males and 0.0080%–0.015% in females (male-to-female ratio: 2.3–3.5), as previously reported [33, 38]. In Miyako Islands, the rate was 0.045% in males and 0.0080% in females (male-to-female ratio: 5.7); in Okinawa, excluding Miyako Islands, the rate was 0.032% in males and 0.0089% in females (male-to-female ratio: 3.6). The incidence rate among HHV8 carriers in Miyako Islands and Okinawa Prefecture is therefore comparable to that in Sardinia; however, the male-to-female ratio in Miyako Islands is much higher. Differences between the incidence rate of non-AIDS KS in Miyako Islands and Okinawa Prefecture may be attributed to differences in HHV8 seroprevalence. However, the incidence rate among carriers in Miyako Islands/Okinawa Prefecture was approximately 100-fold (in men) and 60-fold (in women) greater than that in the rest of Japan. This discrepancy could be explained by HHV8 genome mutations distributed in the Miyako and Okinawa regions and genomic mutations in the population. This is the first study to report full-length HHV8 genomes from Japanese patients with non-AIDS KS. Overall similarity between the 4 Japanese HHV8 strains is high, and they form a phylogenetic branch distinct from those of strains from Western countries and Africa (Figure 1).

When we focused on the nonsynonymous mutations shared by 3 viruses from Miyako Islands that were not observed in the strain derived from mainland Japan, we found that of the 86 viral proteins [18], 9 proteins were mutated (Table 5). Among these, Arg139Met in ORF70, Phe22Tyr in ORF30, Arg136Gln in ORF42, Glu223Gly, Gly267Val, Thr278Ala, and Leu281Gly in K11, and Ser226Gly in ORF72 were not previously reported, ie, these mutations are unique to HHV8 strains from Miyako Islands.

K11:vIRF2 (viral interferon regulatory factor 2) [39] inhibits host interferon (IFN)-β transcription [40] and IFN-stimulated response element transactivation by suppressing the accumulation of functional IFN-stimulated gene factor-3 [41]. The mutated sites in the HHV8 genome from Miyako Islands occur in regions that are distinct from the vIRF2 DNA-binding site [42]. Although the precise effects of these mutations on vIRF2 function are not clear, we suppose that they might be involved in the high incidence of KS in HHV8-infected Miyako Islanders.

ORF72 (v-cyclin), like human cyclin, binds to cyclin-dependent kinase and promotes the phosphorylation of retinoblastoma protein [43]. In addition, v-cyclin of murine γ-herpes virus 68 contributes to the reactivation of the virus [44] and promotes tumor formation. However, its functional domains are not yet known; thus, we are unable to speculate on how the Ser226Gly mutation affects v-cyclin function.

All of the mutations shared by the 3 HHV8 strains from Miyako Islands were also detected in strains derived from 3 patients with non-AIDS KS in Okinawa Islands. Therefore, our findings indicate that the same HHV8 strain occurs in both Miyako Islands and the Okinawa area. It is not clear which, if any, of these HHV8 mutations contribute to their high oncogenicity; this remains to be elucidated in future studies.

Kaposi's sarcoma in HHV8 carriers from Miyako Islands occurs 5.7 times more frequently in men than in women; therefore, we investigated whether the factor responsible for this high incidence is associated with sex chromosomes. Unfortunately, neither shared low-frequency nonsynonymous mutations in the X or Y chromosome nor rare heterologous or homologous mutations in autosomal genes were found in the 4 male patients with non-AIDS KS from Miyako Islands.

Okinawa, which is located in the southernmost part of Japan, has experienced unique genetic and cultural developments [45]. Miyako Islanders are genetically distinct from the rest of the population, even within Okinawa, according to a genomewide single nucleotide polymorphism analysis [46]. This suggests that the prevalence of KS in Okinawa—and especially in Miyako Islands—is related to genetic factors. However, we were unable to identify a putative underlying mutation via exome analysis at this time.

Intriguingly, 23 of 79 (28%) patients with non-AIDS KS in Japan, excluding Okinawa, were from Hokkaido, the northernmost part of the country with a population of 5.5 million, representing 4.5% of the total population of Japan. However, there are no reports on HHV8 seroprevalence in Hokkaido, and HHV8 derived from patients with classic KS from Hokkaido are categorized into K1 subtypes D, E, and K15 P [47], which implies that HHV8 strains in Hokkaido and Okinawa are different. In Hokkaido, there is an ethnic minority population, the Ainu people, who are genetically closely related to the ancient Okinawa population [45]. It is not clear whether patients with non-AIDS KS from Hokkaido are of Ainu descent. However, the fact that non-AIDS KS is prevalent in 2 regions of Japan with genetically similar individuals indicates that the non-AIDS KS incidence may be attributed to common, specific, genetic susceptibility factors.

CONCLUSIONS

The results of the present study indicated a high incidence of non-AIDS KS in Miyako Islands and the Okinawa area. Although the exact reason for this high incidence, other than the high HHV8 seroprevalence in Miyako Islands, remains to be elucidated, we presume that characteristic HHV8 strains and ethnic background may influence KS development in these regions.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to
benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Financial support. This work was supported by a Grant-in-Aid for Young Scientists (grant numbers B 25860961 [to K. H.] and 16K19733 [to S. M.]) from the Japan Society for the Promotion of Science; a research grant from the Okinawa Medical Science Research Foundation (to R. A.); and the University of the Ryukyus Research Project Program Grant for Junior Researchers (to S. M.).

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


