Molecular Epidemiology of a Hepatitis C virus Outbreak in a Leprosy Sanatorium in Japan

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Abstract

Hepatitis C virus (HCV) outbreak that occurred between 1940-99 in a closed Leprosy Sanatorium located on a small island in Japan was analyzed. The analysis of 318 nucleotides in the NS5B region of HCV allowed us to establish the existence of at least three different HCV strains in this Sanatorium.
Since 1938, the National Sanatorium Oku-Komyo-En has been one of thirteen national leprosy sanatoriums in Japan. Because of the leprosy isolation policy in Japan (Leprosy Prevention Law of 1907) in the early years, leprosy patients were forced to live in closed and confined sanatorium, and once confined, patients were strictly isolated from the general public, even after death. The Leprosy Prevention Law remained in force until the end of 1996, and autopsies were routinely performed on almost all patients who had died in the sanatoriums, and the tissue samples usually formalin fixed, and archived. While reviewing the samples (TABLE 1), the pathological data showed a sharp rise in deaths between 1960-69 due to liver cirrhosis in patients.

We also noticed a sharp rise in deaths between 1980-99 from HCC with cirrhosis with statistically significance (FIG. 1). As 75% of all HCC cases in Japan are now reported to be from HCV infection (1), we examined the frequency of HCV infection in these archived tissue samples from leprosy patients, by using PCR type-specific primers (2) to detect HCV RNA (3).

In this study, we investigated the possibility of nosocomial infection in more detail, we examined the sequence similarity of HCV that were detected in these archived samples. Our study plan conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and was approved by the Ethics Committee, National Sanatorium Oku-Komyo-En, Kojin Hospital and Fujita Health University, School of Medicine, respectively. Tissues of 30 patients with leprosy (HCV genotype 1b) (TABLE 1) kept at the National Sanatorium Oku-Komyo-En (between 1940-99) were freshly embedded in paraffin, and followed by RNA extraction. Using the RNA as template for RT-PCR, we first planned to sequence the HVR1 (4) and NS5 (5) region of HCV, which has been used before for analogous studies. However, while the
amplification of the HVR region was unsuccessful (data not shown), the NS5B region readily amplified in 12 of the samples (TABLE 1). NS5B RT-PCR was performed with primers (Pr2; 5’-GGCGGAATTCTGGTCAT AGCCTCGTGAA-3’ and PR3; 5’-TATGAYACCGCTGYTTTGACTC-3’) (5). Part of the amplified products, were analyzed by electrophoresis, and the products were purified with GeneCLean II kit (MP Biomedicals, LLC), followed by cloning into the pTAC-1 vector (TA PCR Cloning Kit, BioDynamics Laboratory Inc.). Recombinant clones were then sequenced using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems) and the Applied Biosystem ABI 3100 Genetic Analyzer. Furthermore, phylogenetic analysis of NS5 sequences was carried out using the neighbor-joining method (MEGA 5 software) (6) by comparing the sequences obtained from the 12 Oku-Komyo-En samples with the 38 reported sequences of the HCV NS5 region in GenBank (FIG. 2). EF032892, EF032893 and EF032894 are samples from the same patient after infection 1, 5.5 and, 14 months, respectively (7). Even though this is identical in the NS5B region of the sequence, the distance is different (0, 0.00010, 0.00074) in the full sequences. Furthermore EF032891 is the donor of EF032892, EF032893 and EF032894 and, the different distance of 0.00629 for the NS5B sequences (316base/318base, 99% similarity), and 0.00658 is 8740 bp by comparison (EF03891 is reported to be 8740bp for partial sequence). Our data may suggest that at least three strains of HCV existed in this sanatorium. First group is Oku1967M, Oku1944M, Oku1984M (Group 1), the second group is Oku1981M, Oku1943M, Oku1947M, Oku1965M, Oku1961M (Group 2) and the third group is Oku1964M, Oku1964F, Oku1948M, Oku1987F (Group 3). In this phylogenetic tree, the difference in sequences of Oku1987F and EF032893 (USA) is 96%. These similarities may be because of the sequence alignment is based on a small
318bp region of NS5B that was amplified compared to the 9500bp full sequence.

It is generally accepted that the interval between initial HCV infection, and the development of cirrhosis and HCC is 20 and 30 years, respectively (9). Taken together, we can assume that horizontal transmission of the HCV occurred between 1940-1949 and, 20 and 30 years before cirrhosis and HCC, respectively because three groups contained samples from 1940s. This period is the same time frame as the establishment of the National Sanatorium Oku-Komyo-En. Most of the patients in the sanatorium had received regular intravenous drugs for the treatment for pain and subcutaneous injection of chaulmoogra oil for the treatment for leprosy using non-disposable syringes and needles. Furthermore, leprosy was dermatide disease and they needed to care for their skin with reused sharpeners and bandage, and many chances to deal with blood without adequate sterilization. Consequently, a study by Kiyosawa K et al. (1) reported that in Japan, sharp rises in death rates from primary liver cancer in men were observed around 1975, and for women this rise was more gradual and occurring much later in 1980.

However, in this leprosy sanatorium, we could not conclude that there was a sex difference primarily because the female samples are too small to conclude the difference of the sex. (TABLE 1). The reason for the male predominance in this sanatorium is that male are more at risk to contract leprosy than female (10) and it is similar to the ratio of the average number of the male and female in the resident patients (TABLE 1). In this leprosy sanatorium, HCV genotypes 1b and 2a in Oku-Komyo-En in toto were 85.7%, and 14.3%, respectively (3), while the subtypes of HCV in present Japan included 1b 69.4%, 2a 14.8% and 2b 5.5% (11). HCV genotype 1b was predominant but statistically it is not prominent compared with outside of present Japan. HCV genotype 1b was believed to have been introduced in Japan in 1882, and started to spread exponentially
in the 1920s and the 1930s according to the molecular clock theory (12). This sanatorium may show one of the HCV spread phenomenon in Japan.

Here we report evidence of transmission of HCV by constructing a phylogenetic tree of multiple NS5B sequences isolated from archive tissues from leprosy patients who were confined to the National Leprosy Sanatorium Oku-Komyo-En of Japan. This observation strongly suggests the horizontal transmission of HCV in the past 70 years in this leprosy sanatorium.
ACKNOWLEDGMENTS

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REFERENCES


FIG. 1. Prevalence of cirrhosis of the liver and HCC in autopsy samples archived at the National Sanatorium Oku-Komyo-En (1940-1999)

FIG. 2. Phylogenetic analysis of the NS5B region (318 bp) of HCV (genotype 1b)

12 isolates from the National Sanatorium Oku-Komyo-En (Oku1943M[cirrhosis 1943, male], Oku1944M[cirrhosis 1944 male], Oku1947M[cirrhosis 1947 male], Oku1948M[cirrhosis 1948 male], Oku1961M[cirrhosis 1961 male], Oku1964M[cirrhosis 1964 male], Oku1965F[cirrhosis 1964 female], Oku1965M[cirrhosis 1965 male], Oku1967M[cirrhosis 1967 male], Oku1981M[cirrhosis 1981 male], Oku1984M[cirrhosis 1984 male], Oku1987F[cirrhosis 1987 female]) and 38 unrelated sequences from GenBank (AB249644, AB442221, AF165058, AF165061, AF165062, AF207753, AF207754, AF207756, AF207761, AF207765, AF207767, AF207769, AF207770, AF207774, AY045702, D10934, D63857, EF032892, EF032893, EF032894, EU155362, EU155364, EU155366, EU155374, EU155382, EU234062, EU239714, EU255960, EU256045, EU256059, EU256065, EU256076, EU256077, EU256078, EU256088, EU256089, EU256090, EU256099).
### TABLE I. Summary Data of the National Sanatorium Oku-Komyo-En (1940-1999)

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of Resident Patients (Male/Female)</th>
<th>No. of Patients dead</th>
<th>Cirrhosis (Male/Female)</th>
<th>HCC+Cirrhosis (Male/Female)</th>
<th>HCV genotype 1b/2a (Male/Female)</th>
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<tr>
<td>1940-1949</td>
<td>918.5/year (mean) 668.1/250.4</td>
<td>604</td>
<td>8 (1.3%)</td>
<td>0 (0%)</td>
<td>0 (6/0)</td>
</tr>
<tr>
<td>1950-1959</td>
<td>937.4/year (mean) 617.9/319.5</td>
<td>62</td>
<td>0 (0%)</td>
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<td>0 (0/0)</td>
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<td>1960-1969</td>
<td>915.5/year (mean) 592/323.5</td>
<td>114</td>
<td>12 (10.5%)</td>
<td>1 (0.9%)</td>
<td>7 (6/1)</td>
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<td>1970-1979</td>
<td>740.9/year (mean) 461.8/279.1</td>
<td>46</td>
<td>3 (6.5%)</td>
<td>2 (4.3%)</td>
<td>4 (0/0)</td>
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<td>1980-1989</td>
<td>592/year (mean) 363/229</td>
<td>111</td>
<td>9 (8.1%)</td>
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<td>5 (3/12)</td>
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<td>1990-1999</td>
<td>431.3/year (mean) 251.7/179.6</td>
<td>59</td>
<td>4 (8.8%)</td>
<td>5 (8.5%)</td>
<td>5 (4/1)</td>
</tr>
<tr>
<td>Total</td>
<td>996</td>
<td>36</td>
<td>17</td>
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*P <0.05 in Fisher’s exact test (two-sided)*

### Pathological Data of Tissues

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FIG. 1
Retraction for Teramoto et al., Molecular Epidemiology of a Hepatitis C Virus Outbreak in a Leprosy Sanatorium in Japan

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Volume 49, no. 9, p. 3358–3360, 2011. We are retracting our manuscript at the request of the Ethics Committee of the National Sanatorium Oku-Komyo-En.

Our study was initiated in August 2005. Dr. Masanao Makino, the president of the Sanatorium and coauthor of our studies, requested analysis of formalin-stored autopsy material. In September 2006, Dr. Makino further tasked our group to sample internal organs from a total of 14 fetuses for histological and histochemical evaluation. The Hansen’s Disease Study Council of March 2005 allowed for the pathological examination of autopsied material upon receipt of permission from the President of the Sanatorium. Thus, we are confident that our research complied with the regulations in place at that time.

Following a presentation at the 85th Meeting of the Japanese Leprosy Association, the Patients’ Council became aware that fetal material had been examined in addition to adult autopsy materials. The President and Vice President of the Patient’s Council reviewed the manuscripts and stated that no permission had been provided by the Patients’ Council for the use of fetal tissues. Subsequently, in April 2014, the Patients’ Council requested that we halt our research and return all materials, including more than 800 adult specimens, to the Sanatorium. The Ethics Committee of the National Sanatorium Oku-Komyo-En convened in January 2015 and canceled the previous approval of our research. The cancelation of the approval is considered retroactive, and we were strongly advised to retract this publication.

Thus, we regretfully retract our article, although we stand by the science and importance of the paper. We sincerely apologize for any inconvenience to the readers.