

Asian Genotypes of JC Virus in Japanese-Americans Suggest Familial Transmission†

Makoto Suzuki,^{1,2*} Huai-Ying Zheng,^{2,3} Tomokazu Takasaka,³ Chie Sugimoto,³
Tadaichi Kitamura,² Ernest Beutler,¹ and Yoshiaki Yogo³

*Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, California 92037,¹ and
Department of Urology, Faculty of Medicine, The University of Tokyo, Bunkyo-ku, 113-8655 Tokyo,² and
Laboratory of Viral Infection, Department of Microbiology and Immunology, The Institute of
Medical Science, The University of Tokyo, Minato-ku, 108-8639 Tokyo,³ Japan*

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To examine the mode of JC virus (JCV) transmission, we collected urine samples from second- and third-generation Japanese-Americans in Los Angeles, Calif., whose parents and grandparents were all Japanese. From the urine samples of these Japanese-Americans, we mainly detected two subtypes (CY and MY) of JCV that are predominantly found among native Japanese. This finding provides support for the hypothesis that JCV is transmitted mainly within the family through long-term cohabitation.

JC virus (JCV), the etiological agent of progressive multifocal leukoencephalopathy (PML), is ubiquitous in the human population, infecting children asymptotically (26). Serological studies (10, 29, 36, 39) have shown that children are infected with JCV after birth (i.e., JCV transmission is categorized as horizontal [28]).

JCV strains worldwide can be classified into more than 10 genotypes according to phylogenetic analyses of viral DNA sequences (6, 13, 14, 17, 33, 34). Each of these genotypes occupies a unique domain in the eastern hemisphere (6, 13, 14, 17, 33, 34). The geographic distribution patterns of JCV genotypes indicates the existence of a correlation between JCV genotypes and human populations. To explain how this correlation was produced, Kunitake et al. (24) examined the possibility that JCV is transmitted preferentially within the family. A 610-bp JCV DNA region (IG region) from urine specimens collected from all members of seven families was amplified by PCR. JCV strains were identified by the nucleotide sequences of the amplified IG regions. The JCV strains detected in half of the JCV-positive children were identified in their parents. Thus, the authors concluded that JCV is transmitted frequently from parents to children. Nevertheless, since JCV strains with identical IG sequences often occur in the same geographical region (8, 14, 23, 33, 34), the detection of the same IG sequence in a child as well as in a parent does not necessarily indicate that a JCV strain was transmitted from the parent to the child.

Kato et al. (18) studied whether JCV is transmitted from the American population to the Japanese population existing on the same small island, Okinawa, Japan. No American JCV genotypes were detected in the Japanese population. On the basis of this finding, Kato et al. (18) proposed a unique mode of JCV transmission, i.e., horizontal transmission from parents

to children during long-term cohabitation. However, although the Japanese and American populations coexisted on Okinawa, they were not in daily contact with each other, and the proposed mode of JCV transmission required further examination with appropriate populations.

In this study, we examined the JCV genotypes in second- and third-generation Japanese-Americans living around Los Angeles, Calif. Japanese-Americans account for 2 to 3% of the total population in the area. Their homes are scattered throughout the area, and with the exception of their internment during the Second World War, most have lived in the area all their lives. Japanese-American children cohabited with their Japanese-American parents, but their homes were in American communities and they played with non-Japanese-American friends and attended kindergartens and primary schools with non-Japanese-American teachers. Thus, we thought that the Japanese-American population would be a good study group in which to examine the hypothesis that JCV is transmitted from parents to children during long-term cohabitation. If JCV was efficiently transmitted within the family, then the Japanese JCV genotypes, CY and MY (23), that were probably carried by Japanese immigrants would be detected at a high rate in both second- and third-generation Japanese-Americans.

We collected urine samples from Japanese-Americans (second and third generations) and from other Southern Californians. The Japanese-American urine donors were patients at the Nikkei Medical Center in the Little Tokyo neighborhood of Los Angeles. These patients were second- or third-generation Japanese-Americans whose parents and grandparents were all Japanese. All Japanese-American donors gave their informed consent prior to their inclusion in the study. The urine samples from other Southern Californians were those collected from the general patient population at the Scripps Clinic for clinical examinations. We used these samples, after they were stored for a month at the Department of Pathology, the Scripps Clinic, with the patients' name labels removed. The Japanese-American and Southern Californian urine donors were aged 30 years or more. This study was approved by the

* Corresponding author. Mailing address: Department of Urology, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, 113-8655 Tokyo, Japan. Phone: 81 3 5800 8662. Fax: 81 3 5800 8917. E-mail: msuzuki@scripps.edu.

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TABLE 1. JCV genotypes detected in various populations

Genotype ^a	Major domain in the eastern hemisphere ^b	Incidence (%) of genotype ^c in:		
		Southern Californians	Second-generation Japanese-Americans	Third-generation Japanese-Americans
EU (types 1 and 4)	Europe, Mediterranean areas	17/33 (52) ^e	1/35 (3)	0/18 (0)
Af2 (type 3)	Africa, West Asia	0/33 (0)	1/35 (3)	1/18 (6)
B1-c (type 2B)	Europe	1/33 (3)	0/35 (0)	0/18 (0)
B1-d	Saudi Arabia, Greece	1/33 (3)	0/35 (0)	0/18 (0)
CY (type 7B)	Japan, South Korea, North China, Mongolia	2/33 (6)	25/35 (71) ^f	12/18 (67) ^f
MY (type 2A)	Japan, South Korea	6/33 (18)	8/35 (23)	4/18 (22)
SC (type 7A)	Southeast Asia, South China, Pacific Islands	4/33 (12)	0/35 (0)	0/18 (0)
Other	ND ^d	2/33 (6)	0/35 (0)	1/18 (6)

^a Genotypes designated by Guo et al. (13) and Sugimoto et al. (34) and those (in parentheses) designated by Agostini et al. (6) are indicated.

^b References 13, 31, and 34.

^c Identified according to a phylogenetic tree (not shown) constructed from IG sequences.

^d ND, not detected in the eastern hemisphere.

^e Significantly different from values obtained with second- and third-generation Japanese-Americans ($P < 0.01$).

^f Significantly different from value obtained with Southern Californians ($P < 0.01$).

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DNA was extracted from urine samples as previously described (21). From extracted DNA, the 610-bp IG region of the viral genome (7) was PCR amplified by using primers P1 and P2 (24) and *Pwo* DNA polymerase with proofreading activity (Roche Diagnostics GmbH, Mannheim, Germany). The amplified fragments were cloned into pBluescript II SK(+) (Stratagene, La Jolla, Calif.) (24), and purified recombinant plasmids were sequenced with an autosequencer (ABI PRISM 3700 DNA analyzer; Applied Biosystems, Foster City, Calif.). Entire JCV DNAs were cloned into pUC19 at the unique *Bam*HI site as described previously (41). The resultant complete JCV DNA clones were prepared by using a QIAGEN Plasmid Maxi kit (QIAGEN GmbH, Hilden, Germany). Purified plasmids were sequenced as described previously (35).

The noncoding regulatory region of the JCV genome was excluded from phylogenetic analysis, as this region is especially hypervariable in JCV isolates derived from the brains of PML patients (42). The determined and reference sequences were aligned by using the CLUSTAL W program (37). The aligned sequences were subjected to phylogenetic analysis by using the neighbor-joining (NJ) method (32). Phylogenetic trees were constructed by using CLUSTAL W, and divergences were estimated by the two-parameter method (20). Phylogenetic trees were visualized by using TREEVIEW (30). To assess the confidence of branching patterns of the NJ trees, 1,000 bootstrap replicates were performed (11).

JCV genotypes in Southern Californians and Japanese-Americans. We detected JCV DNA in urine samples by PCR amplification of the 610-bp IG region. The detection rates were 39 of 218 (18%), 37 of 61 (61%), and 21 of 49 (43%) for the Southern Californian, second-generation Japanese-American, and third-generation Japanese-American populations, respectively. The detection rate for Southern Californians was significantly lower than those for the second- and third-generation Japanese-American populations. It appeared that JCV DNA in some urine samples from Southern Californians was partially damaged during storage (see above), but it is unlikely that this significantly affected the proportion of JCV genotypes detected in the population (see below).

We determined the sequences of IG fragments amplified in

the three populations, and from the obtained IG sequences and those previously detected in both the eastern hemisphere and the United States (7, 18, 34), we constructed an NJ phylogenetic tree (32). According to the resultant tree (not shown; similar trees can be seen in references 14, 33, and 34), the JCV isolates detected in the three populations were classified into seven genotypes, EU, Af2, B1-c, B1-d, CY, MY, and SC. The major domains of these genotypes in the eastern hemisphere are indicated in Table 1.

Profiles of JCV genotypes in Southern Californians and Japanese-Americans. The profile of the JCV genotype in Southern Californians (Table 1) can be summarized as follows. (i)

TABLE 2. Twenty JCV isolates whose complete DNA sequences were determined in this study and analyzed by using the whole-genome approach

Population	Isolate	Genotype ^a	Accession no. ^b
Second-generation Japanese-Americans	J2-10	CY	AB081600
	J2-24	MY	AB081601
Third-generation Japanese-Americans	J3-3	MY	AB081604
	J3-7	CY	AB081605
	J3-8	MY	AB081606
	J3-9	CY	AB081607
	J3-11	CY	AB081602
	J3-13a	CY	AB081603
Southern Californians	LA-2	SC	AB081611
	LA-4	SC	AB081616
	LA-11	MY	AB081608
	LA-13	EU	AB081609
	LA-17	CY	AB081610
	LA-27	MY	AB081612
	LA-28	B1-d	AB081613
	LA-29	MY	AB081614
	LA-31	EU	AB081615
Native Japanese ^c	MS	CY	AB081654
	SI	CY	AB081617
	UA	CY	AB081618

^a Determined according to a phylogenetic analysis using the whole-genome approach (Fig. 1).

^b GSDB, DDBJ, EMBL, and NCBI nucleotide sequence databases.

^c Reported previously (40).

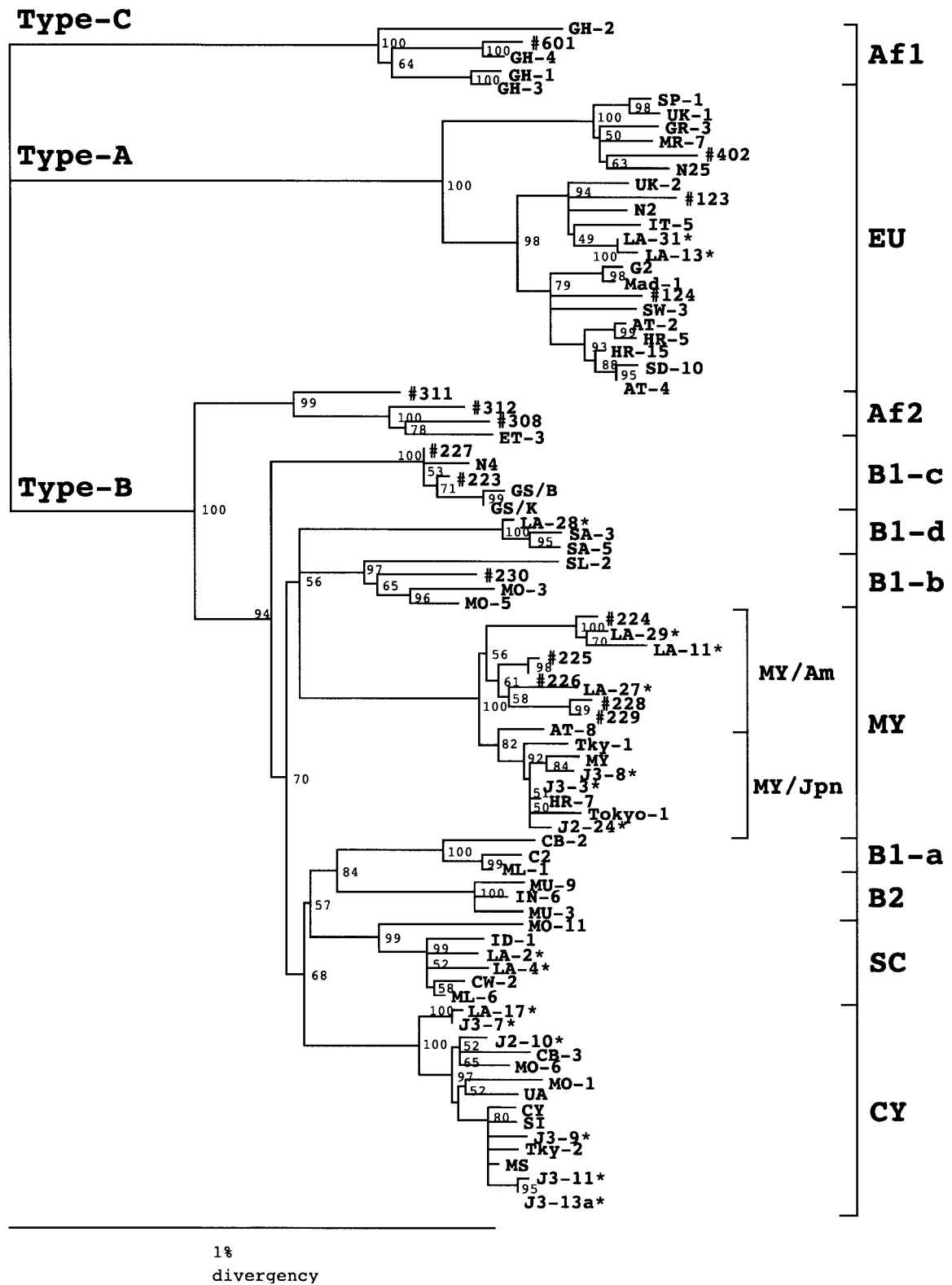


FIG. 1. NJ phylogenetic tree relating 84 complete JCV DNA sequences. An NJ phylogenetic tree was constructed from the complete sequences, excluding regulatory sequences, reported in this and previous studies. The phylogenetic tree was visualized by using TREEVIEW. As there is no reliable out-group, the tree was rooted by using subtype Af1 as the out-group. The symbols for isolates are shown in Table 2 and have been described elsewhere (2-5, 12, 19, 25, 35). Isolates identified in Southern Californians and second- and third-generation Japanese-Americans are indicated by asterisks. The numbers at the nodes in the tree indicate the bootstrap confidence levels (percent) obtained by 1,000 replicates (only values of >50% are shown). Superclusters (types A, B, and C) (35) and subtypes (Af1, Af2, EU, etc.) are indicated. MY/Jpn, a cluster containing MY isolates from native Japanese and Japanese-Americans; MY/Am, clusters containing MY isolates from Southern Californians and other Americans.

EU (the major European and Mediterranean genotype) (34) was most frequently detected, accounting for about 50% of the analyzed isolates. In addition, a minor European genotype (B1-c) (13, 34) was detected, albeit at a low frequency. (ii) Various genotypes probably derived from Native Americans (MY), Asians (B1-d, CY, MY, and SC) (13, 34), and Pacific Islanders (SC) (34) were detected at lower rates. (iii) No genotype of African origin was detected. As suggested by this JCV genotype profile, the ethnic composition of the Southern Californians roughly agreed with the proportions of the ethnic groups in San Diego, Calif., including the area where the urine samples were collected (38).

The profile of the JCV genotype in second- and third-generation Japanese-Americans (Table 1) can be summarized as follows. (i) We mainly detected two genotypes (CY and MY) that are predominantly carried by native Japanese (23), accounting for about 94 and 89% of the isolates from second- and third-generation Japanese-Americans, respectively. (ii) The detection ratio for CY was three- to fourfold higher than that for MY for both the second and third generations. (iii) Some genotypes found rarely or not at all in native Japanese (e.g., EU and Af2) (23) were detected at low rates, ranging from 3 to 6%.

Phylogenetic analysis using the whole-genome approach. In the phylogenetic analysis based on IG sequences, the clustering of some genotypes was not supported by high bootstrap probabilities (data not shown). Therefore, we analyzed representative isolates from the three populations by using the whole-genome approach, with which a highly reliable phylogeny of JCV can be reconstructed (15, 16, 35). From the Japanese-American and Southern Californian urine samples, respectively, we obtained eight and nine complete JCV DNA clones that belonged to various genotypes of JCV (Table 2). We sequenced all of these complete JCV DNA clones. Furthermore, we sequenced three complete JCV DNA clones (all of the CY type) (Table 2) identified in Japan (40), as only two complete CY sequences had previously been reported in Japan (19). The complete JCV DNA sequences determined in this study, together with those reported previously (2–5, 12, 19, 25, 35), were aligned and used to construct an NJ tree (the hyper-variable regulatory sequences were excluded). The resultant tree is shown in Fig. 1, and the following features of the tree are relevant to the present study (the general branching pattern of JCV isolates worldwide has previously been described in detail [35]). (i) All genotypes (i.e., EU, Af2, B1-c, B1-d, CY, MY, and SC) detected in the Japanese-Americans and Southern Californians (Table 1) formed distinct clusters with high bootstrap probabilities (99 or 100%). This finding confirms the validity of the IG sequence-based classification of JCV strains. (ii) All MY isolates from native Japanese and Japanese-Americans formed an intra-MY subcluster (designated MY/Jpn) with a bootstrap probability of 82%, and most of the MY/Jpn isolates, excluding AT-8, were clustered with a bootstrap probability of 92%. In contrast, isolates from Southern Californians and other Americans were widespread in the MY cluster, forming some subclusters with significantly high bootstrap probabilities (MY isolates from Southern Californians and other Americans were collectively designated MY/Am). We concluded that there is a phylogenetic distinction between MY/Jpn and MY/Am.

Although there were no data directly showing the JCV genotypes in first-generation Japanese-Americans, it can be inferred that they carried the same JCV genotypes as those prevalent in Japan. Since most Japanese immigrants reached adulthood before emigrating to the United States and JCV infection generally occurs during childhood (29, 36, 39), most of the immigrants should have been infected with JCV in the homeland. The infecting JCV strains should have accompanied the emigrants to the United States, since after primary infection, JCV strains persist in individuals throughout their lives (22). In this connection, it is worthwhile to mention the biased detection of genotype CY in Japanese-Americans (Table 1). This result is probably a reflection of the historical fact that Japanese from western Japan emigrated to the United States in greater numbers than those from eastern Japan (27), as CY occurs more frequently in western than in eastern Japan (23). Therefore, from the high detection rate for the Japanese JCV genotypes in the second- and third-generation Japanese-Americans, we concluded that the Japanese JCV strains were efficiently transmitted from generation to generation in Japanese-American families. This conclusion provides strong support for the hypothesis that JCV is mainly transmitted from parents to children through long-term cohabitation.

Two general modes of viral transmission have been recognized (28). The first mode, vertical transmission, involves viral transmission from the mother to the offspring across the placenta, in the birth canal, and through milk. The second mode, horizontal transmission, involves general viral transmission from infected hosts after birth. Previous studies (10, 29, 36, 39) have clearly indicated that JCV follows the horizontal transmission route. Nevertheless, the present study demonstrates that JCV is efficiently transmitted from parents to children. Thus, the mode of JCV transmission is vertical in effect, although JCV is transmitted horizontally.

It appears that the mode of JCV transmission permits a low level of crossover of JCV genotypes. Indeed, JCV genotypes (EU, Af2, and MY/Am) found rarely or not at all in native Japanese (23) were detected at a low rate (10% in total) in both second- and third-generation Japanese-Americans. These genotypes were apparently derived from other Americans, because all were detected in American populations (1, 7, 9, 18; the present study). It is likely that some Japanese-American children have daily contact with non-Japanese-Americans who shed JCV. Non-Japanese JCV genotypes may have been transmitted from these neighbors to the Japanese-American children. Therefore, although most children are infected with JCV within the family, some are infected outside the family by their daily contact with JCV-positive members of the local community.

Nucleotide sequence accession numbers. The complete DNA sequences determined in this study for 20 JCV isolates have been deposited in the GSDB, DDBJ, EMBL, and NCBI nucleotide sequence databases, and their accession numbers are listed in Table 2.

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