Table 1. JCV isolates whose complete DNA sequences were used for the phylogenetic analysis

Genotype	Isolate	Geographic origin (ethnic origin)	Accession no.[1]	Reference
Af1	#601	USA	AF015537	Agostini et al. (1998c)
Af1	GH-1	Ghana	AB038252	Kato et al. (2000)
Af1	GH-2	Ghana	AB038253	Kato et al. (2000)
Af1	GH-3	Ghana	AB048545	Sugimoto et al. (2002a)
Af1	GH-4	Ghana	AB048546	Sugimoto et al. (2002a)
EU-a	G2	Germany	AB038251	Kato et al. (2000)
EU-a	N2	Netherlands	AB048574	Sugimoto et al. (2002a)
EU-a	SW-3	Sweden	AB048575	Sugimoto et al. (2002a)
EU-a	UK-2	UK	AB048576	Sugimoto et al. (2002a)
EU-a	IT-5	Italy	AB048568	Sugimoto et al. (2002a)
EU-a	#123	USA	AF015527	Agostini et al. (1998a)
EU-a	#124	USA	AF015526	Agostini et al. (1998a)
EU-a	Mad-1	USA	J02226	Frisque et al. (1984)
EU-a/JK[2]	AT-2	Japan	AB048569	Sugimoto et al. (2002a)
EU-a/JK	AT-4	Japan	AB048570	Sugimoto et al. (2002a)
EU-a/JK	HR-5	Japan	AB048572	Sugimoto et al. (2002a)
EU-a/JK	HR-13	Japan	AB048571	Sugimoto et al. (2002a)
EU-a/JK	SD-9	Japan	AB048573	Sugimoto et al. (2002a)
EU-a/JK	SK-6	South Korea	AB183152	Yogo et al. (2004)
EU-a/Arc	ES-1	Canada (Inuit)	AB074578	Sugimoto et al. (2002b)
EU-a/Arc	ES-3	Canada (Inuit)	AB074579	Sugimoto et al. (2002b)
EU-a/Arc	KO-2	Siberia (Koryak)	AB074585	Sugimoto et al. (2002b)
EU-a/Arc	KO-3	Siberia (Koryak)	AB074586	Sugimoto et al. (2002b)
EU-a/Arc	KO-5	Siberia (Koryak)	AB074587	Sugimoto et al. (2002b)
EU-a/Arc	AN-12	Japan (Ainu)	AB092586	Yogo et al. (2003)
EU-b	GR-3	Greece	AB048563	Sugimoto et al. (2002a)
EU-b	MR-7	Morocco	AB048564	Sugimoto et al. (2002a)
EU-b	N25	Netherlands	AB048565	Sugimoto et al. (2002a)
EU-b	SP-1	Spain	AB048566	Sugimoto et al. (2002a)
EU-b	UK-1	UK	AB048567	Sugimoto et al. (2002a)
EU-b	#402	USA	AF015528	Agostini et al. (1998a)
EU-c	AM-5	Siberia (Nanai)	AB074576	Sugimoto et al. (2002b)
EU-c	AM-7	Siberia (Nanai)	AB074577	Sugimoto et al., (2002b)
EU-c	AM-18	Siberia (Nanai)	AB074575	Sugimoto et al. (2002b)
EU-c	SI-1	Siberia	AB074589	Sugimoto et al. (2002b)
EU-c	SI-7	Siberia	AB074590	Sugimoto et al. (2002b)
EU-c	AN-3	Japan (Ainu)	AB092579	Yogo et al. (2003)
EU-c	AN-5	Japan (Ainu)	AB092581	Yogo et al. (2003)
EU-c	AN-11	Japan (Ainu)	AB092585	Yogo et al. (2003)
Af2	#308	Tanzania	U73500	Agostini et al. (1997)
Af2	#311	USA (African American)	U73501	Agostini et al. (1997)
Af2	#312	USA (African American)	U73502	Agostini et al. (1997)
Af2	ET-3	Ethiopia	AB048547	Sugimoto et al. (2002a)
B1-a	ML-1	Malaysia	AB048548	Sugimoto et al. (2002a)
B1-a	C2	Taiwan	AB048549	Sugimoto et al. (2002a)
B1-a	CB-2	China	AB048550	Sugimoto et al. (2002a)
B1-b	MO-3	Mongolia	AB048551	Sugimoto et al. (2002a)
B1-b	MO-5	Mongolia	AB048552	Sugimoto et al. (2002a)
B1-b	SL-2	Sri Lanka	AB048553	Sugimoto et al. (2002a)
B1-b	#230	USA (African American)	AF015536	Agostini et al. (1998b)
B1-c	N4	Netherlands	AB048554	Sugimoto et al. (2002a)
B1-c	GS/K	Germany	AF004349	Loeber and Dörries (1988)
B1-c	GS/B	Germany	AF004350	Loeber and Dörries (1988)
B1-c	#223	USA (African American)	AF015532	Agostini et al. (1998b)
B1-c	#227	USA (European American)	AF015533	Agostini et al. (1998b)
B1-d	SA-3	Saudi Arabia	AB048555	Sugimoto et al. (2002a)
B1-d	SA-5	Saudi Arabia	AB048556	Sugimoto et al. (2002a)
B2	IN-6	India	AB048557	Sugimoto et al. (2002a)
B2	MU-3	Mauritius	AB048558	Sugimoto et al. (2002a)
B2	MU-9	Mauritius	AB048559	Sugimoto et al. (2002a)
CY	CY	Japan	AB038249	Kato et al. (2000)
CY	Tky-2a	Japan	AB038255	Kato et al. (2000)
CY	CB-3	Japan	AB048560	Sugimoto et al. (2002a)
CY	MO-1	Mongolia	AB048561	Sugimoto et al. (2002a)
CY	MO-6	Mongolia	AB048562	Sugimoto et al. (2002a)

Table 1. (continued)

Genotype	Isolate	Geographic origin (ethnic origin)	Accession no.[1]	Reference
MX	AN-9	Japan (Ainu)	AB092584	Yogo et al. (2003)
MX	AN-13	Japan (Ainu)	AB092587	Yogo et al. (2003)
MX	ANF	Japan (Ainu)	AB185020	This study
MY-a	Aic-1	Japan	AB081005	Zheng et al. (2003)
MY-a	AT-8	Japan	AB048577	Sugimoto et al. (2002a)
MY-a	JP-7	Japan	AB081016	Zheng et al. (2003)
MY-a	YI	Japan	AB081030	Zheng et al. (2003)
MY-b	Tokyo-1	Japan	AF030085	Agostini et al. (1998b)
MY-b	Tky-1	Japan	AB038254	Kato et al. (2000)
MY-b	HR-7	Japan	AB048578	Sugimoto et al. (2002a)
MY-b	MY	Japan	AB038250	Kato et al. (2000)
MY-b	SK-1	South Korea	AB081028	Zheng et al. (2003)
MY-b	SK-4	South Korea	AB081029	Zheng et al. (2003)
MY-b	AN-4	Japan (Ainu)	AB092580	Yogo et al. (2003)
MY-b	AN-6	Japan (Ainu)	AB092582	Yogo et al. (2003)
MY-b	AN-8	Japan (Ainu)	AB092583	Yogo et al. (2003)
MY-c	#224	USA (Hispanic)	AF015529	Agostini et al. (1998b)
MY-c	ME-5	Mexico (Tarahumalan)	AB081021	Zheng et al. (2003)
MY-c	ME-14	Mexico (Tarahumalan)	AB081018	Zheng et al. (2003)
MY-c	ME-16	Mexico (Tarahumalan)	AB081019	Zheng et al. (2003)
MY-c	GU-8	Guatemala (Mayan)	AB081015	Zheng et al. (2003)
MY-d	#225	USA (Navaho)	AF015530	Agostini et al. (1998b)
MY-d	#226	USA (Navaho)	AF015531	Agostini et al. (1998b)
MY-e	GU-4	Guatemala (Mayan)	AB081014	Zheng et al. (2003)
MY-e	GU-15	Guatemala (Mayan)	AB081011	Zheng et al. (2003)
MY-e	GU-21	Guatemala (Mayan)	AB081012	Zheng et al. (2003)
MY-e	GU-25	Guatemala (Mayan)	AB081013	Zheng et al. (2003)
MY-e	ME-8	Mexico (Tarahumalan)	AB081022	Zheng et al. (2003)
MY-f	PE-1	Peru (Andean)	AB081023	Zheng et al. (2003)
MY-f	PE-11	Peru (Andean)	AB081024	Zheng et al. (2003)
MY-f	PE-12	Peru (Andean)	AB081025	Zheng et al. (2003)
MY-f	PE-16	Peru (Andean)	AB081026	Zheng et al. (2003)
MY-f	PE-21	Peru (Andean)	AB081027	Zheng et al. (2003)
MY-g	#228	USA (Navaho)	AF015534	Agostini et al. (1998b)
MY-g	#229	USA (European American)	AF015535	Agostini et al. (1998b)
MY-g	CN-1	Canada (Beaver/Dene Tha')	AB081007	Zheng et al. (2003)
MY-g	CN-13	Canada (Beaver/Dene Tha')	AB081006	Zheng et al. (2003)
MY-g	CN-15	Canada (Beaver/Dene Tha')	AB081008	Zheng et al. (2003)
MY-g	CN-25	Canada (Beaver/Dene Tha')	AB081009	Zheng et al. (2003)
MY-g	CN-28	Canada (Beaver/Dene Tha')	AB081010	Zheng et al. (2003)
MY-x	AN-1	Japan (Ainu)	AB092578	Yogo et al. (2003)
MY-x	255A	South Korea	AY121910	Cui et al. (2004)
MY-x	256A	South Korea	AY121911	Cui et al. (2004)
MY	ME-4	Mexico (Tarahumalan)	AB081020	Zheng et al. (2003)
MY	ME-12	Mexico (Tarahumalan)	AB081017	Zheng et al. (2003)
SC	CW-2	China	AB048579	Sugimoto et al. (2002a)
SC	ID-1	Indonesia	AB048580	Sugimoto et al. (2002a)
SC	ML-6	Malaysia	AB048581	Sugimoto et al. (2002a)
SC	MO-11	Mongolia	AB048582	Sugimoto et al. (2002a)
2E	#233	New Britain (Tolai)	AF281605	Jobes et al. (2001)
2E	#234	Guam (Chamorro)	AF281606	Jobes et al. (2001)
8A	#801	PNG (Highlander)	AF281623	Jobes et al. (2001)
8A	#802	PNG (Highlander)	AF281624	Jobes et al. (2001)
8B	#803	PNG (Highlander)	AF281625	Jobes et al. (2001)
8B	#804	PNG (Highlander)	AF281626	Jobes et al. (2001)

[1] GSDB, DDBJ, EMBL, and NCBI accession numbers.
[2] EU-a/Arc has been renamed EU-a/JK (Yogo et al., 2004).

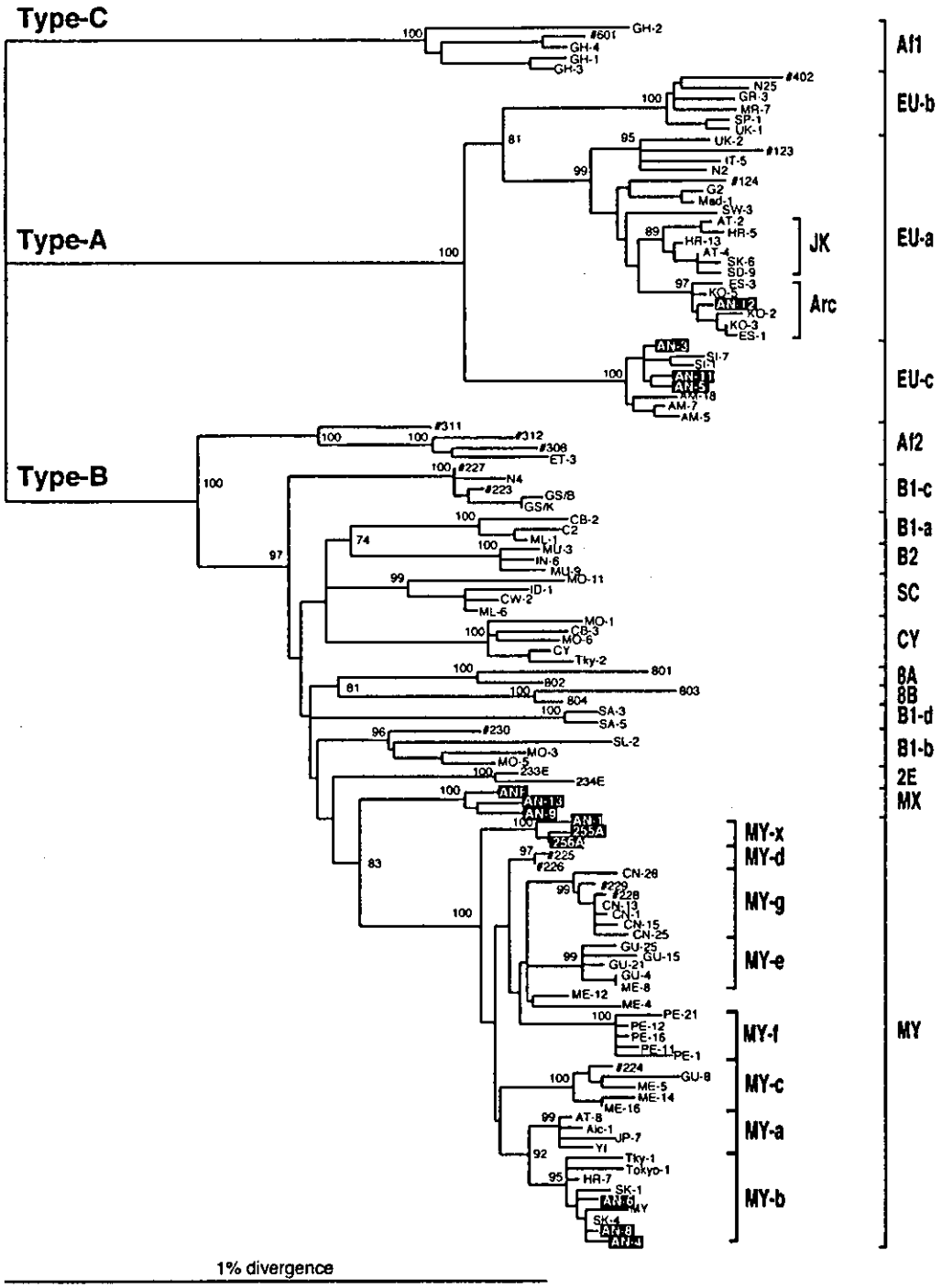

Figure 1. Neighbor-joining (NJ) phylogenetic tree relating 118 complete JCV DNA sequences. A phylogenetic tree was constructed from complete sequences, excluding regulatory sequences, using the NJ method. The phylogenetic tree was visualized using the TREEVIEW program (Page, 1996). The tree was rooted using Af1 as the outer group, as this genotype probably represents the ancestral type (Pavesi, 2003). The symbols for sequences are shown in Table 1. The number at each node in the tree indicates the bootstrap probability (BP, %) obtained from 1000 replicates (only values >70% are shown for major clusters). Superclusters (Types A, B, and C), genotypes (Af1, Af2, EU-a, EU-b, EU-c, B1-a, B1-b, B1-c, B1-d, B2, CY, MX, MY, 2E, 8A, and 8B), intra-EU-a subgroups (EU-a/Arc and EU-a/JK), and intra-MY subgroups (MY-a to MY-g and MY-x) are indicated. Isolates from the Ainu (Yogo et al., 2003; this study) and from Koreans (Cui et al., 2004) are shown in white on black backgrounds.

DNA extraction and analysis

About 5 ml of urine was collected from the subject's bladder and was used as the source for our extraction of viral DNA, as described by Kitamura et al. (1990). A 610-bp IG region was amplified by means of polymerase chain reaction (PCR) using the P1 and P2 primers (Kunitake et al., 1995). The entire JCV DNA was cloned into pUC19 at the unique *Bam*HI site, as described by Yogo et al. (1991), and the resultant clone, which carried the entire JCV DNA, was sequenced as described by Sugimoto et al. (2002a).

Reported complete DNA sequences of JCV

The complete JCV DNA sequences reported previously and used in this study are shown in Table 1, along with their GSDB, DDBJ, EMBL, and NCBI accession numbers.

Phylogenetic Analysis

The non-coding regulatory region of the JCV genome was excluded from our phylogenetic analysis, as this region is hypervariable, especially in JCV isolates derived from the brains of patients with PML (Yogo and Sugimoto, 2001). DNA sequences were aligned using the CLUSTAL W program (Thompson et al., 1994) with a gap-opening penalty of 15.00 and a gap-extension penalty of 6.66. To evaluate the phylogenetic relationships among DNA sequences, we used the neighbor-joining (NJ) method (Saitou and Nei, 1987) in CLUSTAL W. Divergences were estimated using Kimura's two-parameter method (Kimura, 1980). To assess the confidence limit of the branching patterns of the NJ tree, bootstrap probabilities (BPs) were estimated using 1000 bootstrap replicates (Felsenstein, 1985) in CLUSTAL W. BPs greater than 70% were considered to be significant (Hillis and Bull, 1993). A phylogenetic tree was then visualized using the TREEVIEW program (Page, 1996).

Results

As the urine sample from the Ainu subject gave positive amplification in the PCR process, we attempted to establish complete clones of the JCV DNA from the urine. We obtained a complete JCV DNA clone, which we designated ANF. We then sequenced this clone completely. Cui et al. (2004) recently reported various JCV isolates in Asia, including China, South Korea, and India. Among these isolates, two from Seoul (255A and 256A) attracted our attention because despite their Asian origin, they were described as a genotype (Type 2A2) formerly considered to be characteristic of Native Americans (Cui et al., 2004). We constructed an NJ phylogenetic tree from the complete DNA sequences of ANF, 255A, and 256A, together with 118 complete sequences that had been collected from around the world, including ten detected in the Ainu (Yogo et al., 2003). Based on the resulting tree (Figure 1), we conclude that ANF and two isolates (AN-9 and AN-13) that had previously been detected in the Ainu (Yogo et al., 2003) formed a distinct clade (designated MX), with a high bootstrap probability (100%). In contrast, the Korean isolates (255A and 256A) together with an isolate (AN-1) from an Ainu formed another distinct clade (MY-x) with a 100% BP (Figure 1). MY-x and the other MY subgroups (MY-a to MY-g) constituted a superclade, designated MY. As previously shown (Yogo et al., 2003), the MX and MY grouping has been demonstrated with a significantly high BP (83%; Figure 1).

Discussion

Yogo et al. (2003) detected MY-x and MX in only one or a few subjects. Therefore, the ethnic distribution of these genotypes required further study. In the present study, we demonstrated the presence of the MX genotype in a forensic subject whose origin was Ainu. MX was previously detected at two distant sites (Asahikawa and Shiraoi) on Hokkaido (Yogo et al., 2003). In the present study, we detected MX in a subject from another site on Hokkaido (i.e. Makubetsu), which suggests that the MX genotype of JCV is widespread among the Ainu, albeit at a lower frequency.

We found that two isolates from Seoul belonged to MY-x, a subgroup within genotype MY (Yogo et al., 2003). These isolates were previously described as belonging to the Native American subgroup of MY (named Type 2A2; Cui et al., 2004), but the result of our analysis suggests that this classification should be revised. Although MY-x was distributed in two distinct human populations (i.e. the Ainu and Koreans), further division of the MY-x isolates was not demonstrated. This suggests that peoples carrying the MY-x genotype migrated to the Korean Peninsula and Hokkaido relatively recently (i.e. not earlier than 10000 years ago).

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JC virus genotyping using formalin-fixed, paraffin-embedded renal tissues

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Abstract

Recently genotyping of JC virus (JCV) DNA in renal tissue was reported to be useful to identify the geographic origin of unidentified cadavers. In the above study, autopsied tissue samples without storage or stored in a frozen state were used. This study examined JCV DNA sequence modifications caused by formalin-fixation, in an attempt to elucidate whether formalin-fixed, paraffin-embedded tissue samples can also be used to determine the genotypes of JCV DNA in the kidney. In four cases, a 610 bp typing region of the JCV genome was PCR-amplified from renal tissues stored for 1 year in three different states: frozen at −80 °C [Amaker, B.H., Chandler, F.W., Huey, L.O., Colwell, R.M., 1997. Molecular detection of JC virus in embalmed, formalin-fixed, paraffin-embedded brain tissue. J. Forensic Sci., 1157–1159], formalin-fixed, paraffin-embedded [Ault, G.S., Stoner, G.L., 1992. Two major types of JC virus defined in progressive multifocal leukoencephalopathy brain by early and late coding region DNA sequences. J. Gen. Virol. 73, 2669–2678], and soaked in 5% formalin [Baksh, F.K., Finkelstein, S.D., Swalskey, P.A., Stoner, G.L., Ryschkewitsch, C.F., Randhawa, P.R., 2001. Molecular genotyping of BK and JC virus in human polyomavirus-associated interstitial nephritis after renal transplantation. Am. J. Kidney Dis. 38 (2), 354–365]. The amplified fragments were cloned, and the resultant clones were sequenced. In frozen samples, single sequences ('original' sequences) were detected in all cases. In formalin-fixed, paraffin-embedded samples, not only the original sequences but also those with 1–6 base substitutions were detected. From formalin-soaked samples, the original sequences and those with 1–5 and 10–13 substitutions were detected. The genotyping of JCV DNA was not hampered by the presence of 1–6 substitutions, but a shift in JCV genotypes was observed in sequences with 10–13 substitutions. Thus, it was concluded that the genotypes of JCV DNA in the kidney can be determined only with specimens stored in a frozen state or formalin-fixed for a short time.

Keywords: JC virus; Genotyping; Renal tissue; Formalin; Paraffin; Base substitution

1. Introduction

Although the number of unidentified cadavers is increasing throughout the world (Cattaneo et al., 2000), there is no reliable means of tracing their origins. Recently it is reported that genotyping JC virus (JCV) DNA in the kidney or urine provides useful information as to the geographic origin of unidentified cadavers (Ikegaya et al., 2002). JCV asymptomatically infects most humans during childhood and persists in the kidneys throughout life (Chesters et al., 1983; Ikegaya et al., 2002, 2004; Kitamura et al., 1997; Padgett and Walker, 1973; Tominaga et al., 1992). JCV DNA can be classified into more than 10 genotypes that occupy unique domains in different parts of the world (Sugimoto et al., 1997; Yogo et al., 2004).

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In the study noted above, autopsied tissue samples without storage or stored frozen were used to PCR-amplify and sequence a 610 bp region of the JCV genome usually used for the genotyping (Ault and Stoner, 1992). However, there are a considerable number of unidentified cadavers for which only formalin-fixed, paraffin-embedded or formalin-soaked tissue samples are available. If JCV DNA sequences in tissue samples stored under such conditions could be accurately determined, the application of the JCV genotyping method would be expanded greatly.

Indeed, JCV DNA fragments have been amplified by PCR from formalin-fixed, paraffin-embedded tissue samples (Amaker et al., 1997; Telenti et al., 1990), and their nucleotide sequences have been determined to classify the detected JCV DNA into subtypes (Baksh et al., 2001; Stoner and Ryschkewitch, 2000). However, to our knowledge, no studies have been conducted to examine nucleotide modifications that might be introduced into JCV DNA in tissue samples during formalin-fixation before being embedded in paraffin. To clarify this issue, we examined the influence of formalin-fixation by comparing DNA sequences detected in renal tissue samples stored differently: (a) frozen at −80 °C for a year, (b) fixed in formalin for 2 weeks and then embedded in paraffin, and (c) soaked in formalin for a year.

2. Materials and methods

2.1. Tissue samples

Both kidneys were excised from eight Japanese cadavers with various causes of death (Table 1). All cadavers had no immunosuppressive diseases. The partially excised kidney blocks were stored for 1 year in three states: (a) frozen (i.e. frozen at −80 °C), (b) formalin-fixed, paraffin-embedded (i.e. embedded in paraffin at 20 °C after fixation in 5% formalin for 2 weeks), and (c) formalin-soaked (i.e. soaked in 5% formalin at 20 °C). Slices (10 mm × 10 mm × 2 mm) were excised from two different portions of the medulla of each kidney stored in each state. Paraffin-embedded samples were deparaffinized, and formalin-soaked samples were deformalinated, by the conventional methods. All samples were minced with scissors and digested with proteinase K in the presence of 0.5% sodium dodecyl sulfate at 56 °C for 16 h. The digest was extracted once with phenol and once with chloroform/isoamyl alcohol (24:1 by volume); DNA was recovered by ethanol precipitation and then dissolved in 200 μL water.

2.2. PCR

The 610 bp IG region of the JCV genome (Ault and Stoner, 1992) was PCR-amplified from the DNA extracted from renal tissue samples, using primers P1 and P2 (Kunitake et al., 1995). The total reaction volume of 50 μL contained 2.5 μL of sample DNA, 125 units of HotStar Taq DNA polymerase (QIAGEN GmbH, Hilden, Germany), 200 μM of each dNTP, 1.5 mM $MgCl_2$, 0.5 μM primers and a PCR Buffer supplied by the manufacturer. After activation at 95 °C for 15 min, amplification was performed for 50 cycles. The cycle profile was 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min. Both activation and amplification were carried out in a PC-800 (Astec, Fukuoka, Japan). The size of the amplicons was 666 bp. PCR was conducted twice for each DNA sample.

2.3. Cloning and sequencing

The amplified fragments were digested with a combination of *Hin*dIII and *Pst*I, which excises the IG region (Kunitake et al., 1995). The digested DNA was ligated to *Hin*dIII- and *Pst*I-digested, alkaline phosphatase-treated pBluescript II SK (+) (Stratagene, La Jolla, USA), and used to transform *Escherichia coli* HB101-competent cells (Takara Shuzo Co. Ltd., Kyoto, Japan). Recombinant clones containing the IG region were selected by digestion with a combination of *Hin*dIII and *Pst*I followed by agarose gel electrophoresis. Purified recombinant plasmids were sequenced with an autosequencer (ABI Prism 3700 DNA Analyzer, Applied Biosystems, Foster City, CA, USA). Six clones from each DNA sample were sequenced.

2.4. Phylogenetic analysis

Neighbor-joining (NJ) phylogenetic trees (Saitou and Nei, 1987) were constructed using the CLUSTAL W Program Version 1.7 (Tompson and Higgins, 1994). Divergences were estimated by the two-parameter method (Kimura, 1980). Phylogenetic trees were visualized with TREEVIEW Program Ver-

Table 1
Eight cases studied

Case	Age (years)/gender	Post-mortem time (h)	Cause of death	Detection of JCV DNA[a]
1	65/M	24	Exsanguination	+
2	69/M	72	Frail chest	+
3	74/F	20	Pulmonary embolism	−
4	43/M	13	Exsanguination	−
5	64/M	24	General peritonitis	+
6	53/M	19	Burns	−
7	33/M	24	Traumatic shock	+
8	40/M	15	Strangulation	−

[a] See text.

sion 1.4 (Page, 1996). To assess the confidence of the NJ tree branching patterns, 1000 bootstrap replicates (Felsenstain, 1985) were performed with CLUSTAL W.

2.5. Statistical analysis

Statistical analysis was performed using the Bartlett test and the one-factor ANOVA test, with the significance level set at 5% using Microsoft Excel 2001 software.

3. Results

3.1. Detection of the IG region

As described in Section 2, we excised slices from two different portions of the medulla of each kidney. Using each slice, we performed PCR twice to amplify the IG region. Thus, we repeated PCR six times using renal tissue samples derived from the same cadaver and stored in the same state. If amplified products of about 670 bp were obtained at least once, we regarded the renal tissue as positive for the JCV IG region. The specificity of the amplified fragments was confirmed by analyzing their sequences, as described below.

In four cases (cases 1, 2, 5, and 7), we could amplify the IG region from renal tissue samples stored in the frozen state. The tissue samples excised from these cadavers but stored in paraffin-embedded and formalin-soaked states also gave positive amplifications. In cases where the IG region was not detected in frozen samples, the IG region was detected in neither paraffin-embedded nor formalin-soaked samples. Positive amplifications were frequently obtained with only one of the duplicated DNA samples from stored in not only frozen but also paraffin-embedded and formalin-soaked tissues. Thus, as judged from the appearance of amplified fragments, renal tissue samples stored in three different states gave rise to similar amplification results.

3.2. Sequence comparisons of amplified IG fragments

We cloned the amplified fragments into a plasmid vector, and purified clones were sequenced. We sequenced six clones for each JCV-DNA positive tissue sample. In total, we identified 25 different IG sequences (SEQ-1 to -25) (Fig. 1). Table 2 shows the tissue samples in which these sequences were detected.

A single sequence was detected in the frozen tissue sample in each case. We designated this sequence as the original sequence that had not undergone base modifications. The original sequences in cases 1 and 2 were SEQ-1, and those in cases 5 and 7 were SEQ-11 and -20, respectively.

In paraffin-embedded tissue samples, not only the original but also sequences with a lower rate of base substitutions (i.e. 1–6 substitutions) were detected in all four cases. For ex-

Cases	Sequence	2151	2152	2158	2161	2168	2178	2188	2190	2210	2239	2247	2251	2264	2296	2316	2317	2341	2371	2395	2416	2431	2451	2475	2513	2514	2518	2523	2563	2593	2604	2606	2619	2642	2645	2663	2665	2680	2687	2699	2712	2723	No. of substitutions	Genotypes
1	SEQ1	A	T	A	T	A	T	A	T	A	T	G	A	A	C	T	C	C	G	T	T	A	A	A	A	T	T	A	A	A	A	C	T	G	G	A	A	T	T	A	T	A	C	-	MY
	SEQ2	A	T	A	T	A	T	G	T	A	T	G	A	A	C	T	C	C	G	T	T	A	A	A	A	T	T	A	A	A	A	C	T	G	G	A	A	T	T	A	T	A	C	1	MY
	SEQ3	A	T	A	T	A	T	A	T	A	T	G	A	A	C	T	C	C	G	T	T	A	A	A	A	T	T	A	A	A	A	C	T	G	G	G	A	T	T	A	T	A	C	1	MY
	SEQ4	A	T	G	T	A	T	A	C	A	G	G	C	A	C	T	A	A	A	T	C	A	A	A	T	T	A	A	A	A	C	C	G	A	G	A	T	T	A	T	G	T	13	CY
	SEQ5	A	T	A	C	A	C	A	T	A	G	G	C	A	C	T	A	C	A	T	C	A	A	A	T	T	A	A	A	A	C	C	G	A	G	A	T	T	A	C	G	T	13	CY
2	SEQ1	A	T	A	T	A	T	A	T	A	T	G	A	A	C	T	C	C	G	T	T	A	A	A	T	T	A	A	A	A	C	T	G	G	A	A	T	T	A	T	A	C	-	MY
	SEQ6	A	T	A	T	A	T	A	T	A	T	G	A	A	C	T	C	C	G	T	T	A	A	G	T	T	A	A	A	A	C	T	G	G	A	A	T	T	A	T	A	C	1	MY
	SEQ7	A	T	A	T	A	T	A	T	A	T	G	A	A	T	T	C	C	G	T	T	A	A	A	T	T	A	A	A	A	C	T	G	G	A	A	T	T	A	T	A	C	1	MY
	SEQ8	A	T	A	T	A	T	A	T	G	T	G	A	A	C	T	C	C	G	T	T	A	G	A	T	T	A	A	A	A	C	T	G	G	A	A	T	T	A	T	A	C	2	MY
	SEQ9	G	T	A	T	A	T	A	T	A	T	A	A	A	C	T	C	C	G	T	T	A	A	A	T	T	A	A	A	A	C	T	G	G	A	A	T	T	A	T	A	C	2	MY
	SEQ10	A	T	A	C	A	T	A	T	A	G	G	C	A	C	C	C	C	G	T	T	A	A	A	T	T	A	A	A	A	C	T	G	G	A	A	T	T	A	T	A	C	4	MY
5	SEQ11	A	T	A	T	A	T	A	T	A	T	G	A	A	C	T	C	C	G	T	T	A	A	A	T	T	A	A	A	A	C	T	T	G	A	A	T	T	A	T	A	C	-	MY
	SEQ12	A	T	A	T	A	T	A	T	A	T	G	A	A	C	T	C	C	G	C	T	A	A	A	T	T	A	A	A	A	C	T	T	G	A	A	T	T	A	T	A	C	1	MY
	SEQ13	A	T	A	T	A	T	A	T	G	T	G	A	A	C	T	C	C	G	T	T	A	G	A	T	T	A	A	A	A	C	T	T	G	A	A	T	T	A	T	A	C	2	MY
	SEQ14	A	T	G	T	A	T	A	T	A	T	G	A	G	T	T	C	C	G	T	T	A	A	A	C	T	A	A	A	A	C	T	T	G	A	A	T	T	A	T	A	C	5	MY
	SEQ15	A	T	G	T	A	T	A	T	A	T	G	A	G	T	T	C	C	G	T	T	A	A	A	C	T	A	A	G	A	C	T	T	G	A	A	T	T	A	T	A	C	5	MY
	SEQ16	A	T	G	T	A	T	A	T	A	T	G	A	G	T	T	C	C	G	T	T	A	A	A	C	T	A	A	G	A	C	T	T	G	A	A	T	T	A	T	-	C	6	MY
	SEQ17	A	T	G	T	A	T	A	T	A	T	G	A	G	T	T	C	C	G	T	T	A	A	A	C	T	A	A	G	A	C	T	T	G	A	A	T	T	G	T	A	C	6	MY
	SEQ18	A	T	A	C	A	T	A	T	A	G	G	C	A	C	T	A	C	A	T	C	A	A	A	T	T	A	A	A	A	C	C	T	A	G	A	T	T	A	T	G	T	10	CY
	SEQ19	A	T	A	C	A	T	A	T	A	G	G	C	A	C	T	A	C	A	T	C	G	A	A	T	T	A	A	A	A	C	C	T	A	G	A	T	T	A	T	G	T	12	CY
7	SEQ20	A	T	A	C	A	T	A	T	A	G	G	C	A	C	T	A	C	A	T	C	A	A	A	T	T	A	A	A	G	C	C	T	A	G	A	T	T	A	T	G	T	-	CY
	SEQ21	A	T	A	C	A	T	A	T	A	G	G	C	A	C	T	A	C	A	T	C	A	A	A	T	T	A	A	A	G	T	C	T	A	G	G	T	T	A	T	G	T	2	CY
	SEQ22	A	T	A	C	A	T	A	T	A	G	G	C	A	C	T	A	C	A	T	C	A	A	A	T	T	A	A	A	G	C	C	T	A	G	A	C	C	A	T	G	T	2	CY
	SEQ23	A	C	A	C	A	T	A	T	A	G	G	C	A	C	T	A	C	A	T	C	A	A	A	T	C	G	T	A	G	C	C	T	A	G	G	T	T	A	T	G	T	5	CY
	SEQ24	A	T	A	T	A	T	A	T	A	T	G	A	A	C	T	C	C	A	T	C	A	A	A	T	T	A	A	A	G	C	C	T	G	G	A	T	T	A	T	G	T	5	CY
	SEQ25	A	T	G	T	A	T	A	T	A	G	G	C	G	C	T	A	C	G	T	T	A	A	A	C	T	A	A	G	G	C	T	T	G	A	A	T	T	A	T	A	C	12	MY

Fig. 1. Nucleotide variations among IG sequences detected from renal tissue samples stored in various states. The nucleotides shown are those at positions where differences were found. Nucleotide numbering is that of strain Mad-1 (Frisque et al., 1984). Nucleotides different from those of the original sequence (shown at the top line in each case) are underlined.

Table 2
IG sequences detected in renal tissue samples stored in various states

Cases	IG sequence	No. of base substitutions	No. of clones detected from tissue samples stored in various states			Accession number[a]
			Frozen[b]	Paraffin[c]	Formalin[d]	
1	SEQ-1	–[e]	6	4	4	AB185175
	SEQ-2	1	0	1	0	AB185176
	SEQ-3	1	0	1	0	AB185177
	SEQ-4	13	0	0	1	AB185178
	SEQ-5	13	0	0	1	AB185179
2	SEQ-1	–	6	3	4	AB185175
	SEQ-6	1	0	0	1	AB185180
	SEQ-7	1	0	1	0	AB185181
	SEQ-8	2	0	0	1	AB185182
	SEQ-9	2	0	1	0	AB185183
	SEQ-10	4	0	1	0	AB185184
5	SEQ-11	–	6	2	0	AB185185
	SEQ-12	1	0	1	0	AB185186
	SEQ-13	2	0	0	2	AB185187
	SEQ-14	5	0	0	1	AB185188
	SEQ-15	5	0	1	0	AB185189
	SEQ-16	5	0	1	0	AB185190
	SEQ-17	6	0	1	0	AB185191
	SEQ-18	10	0	0	2	AB185192
	SEQ-19	12	0	0	1	AB185193
7	SEQ-20	–	6	4	3	AB185194
	SEQ-21	2	0	0	1	AB185195
	SEQ-22	2	0	0	1	AB185196
	SEQ-23	5	0	1	0	AB185197
	SEQ-24	5	0	1	0	AB185198
	SEQ-25	12	0	0	1	AB185199

[a] GSDB, DDBJ, EMBL and NCBI accession numbers.
[b] Stored at −80 °C for 1 year.
[c] Fixed in 5% formalin for 2 weeks and embedded in paraffin for 1 year.
[d] Stored in 5% formalin for 1 year.
[e] Indicates the original sequence.

Table 3
Base substitutions detected in paraffin-embedded and formalin-stored renal tissue samples

Substitution type	No. (%) of substitutions in paraffin-embedded samples[a]	No. (%) of substitutions in formalin-stored samples[b]
A → T	1 (2.9)	0 (0.0)
A → G	16 (44.4)	25 (32.9)
A → C	2 (5.6)	5 (6.6)
T → A	0 (0.0)	0 (0.0)
T → G	1 (2.8)	4 (5.3)
T → C	8 (22.2)	18 (23.7)
G → A	1 (2.8)	10 (13.2)
G → T	1 (2.8)	0 (0.0)
G → C	0 (0.0)	0 (0.0)
C → A	1 (2.8)	5 (6.6)
C → T	5 (13.8)	9 (11.8)
C → G	0 (0.0)	0 (0.0)
Total	36 (100.0)	76 (100.0)
Transitions	30 (83.3)	62 (81.6)
Transversions	6 (16.7)	14 (18.4)

Twenty-four clones were examined for each storage conditions.
[a] Fixed in 5% formalin for 2 weeks and embedded in paraffin for 1 year.
[b] Stored in 5% formalin for 1 year.

ample, the original sequence (SEQ-1) and sequences (SEQ-2 and -3) with a single substitution were found in case 1. The original sequence (SEQ-1) was identified in three clones, and sequences (SEQ-7, -9 and -10) with 1–4 substitution were found in case 2. The original sequence (SEQ-11) and sequences (SEQ-12, -15, -16 and -17) with 1–6 substitutions were found in case 5. The original sequence (SEQ-20) and sequences (SEQ-23 and -24) with five substitutions were found in case 7.

In formalin-soaked tissue samples, the original sequences were detected in three cases but not in one case. Sequences with a lower rate of base substitutions were detected in cases 2, 5 and 7 and those with a higher rate of substitutions (i.e. 10–13 substitutions), were detected in cases 1, 3 and 5. For example, the original sequence (SEQ-1) and sequences (SEQ-4 and -5) with 13 substitutions were found in case 1. The original sequence (SEQ-1) and sequences (SEQ-6 and -8) with 1 or 2 substitutions were found in case 2 (a higher rate of substitutions were not detected). The original sequence was not detected in case 5, but sequences (SEQ-13 and -14) with 2 or 5 substitutions and a sequence (SEQ-18) with 10 substitutions were found. The original sequence (SEQ-20), sequences (SEQ-21 and -22) with two substitutions and a se-

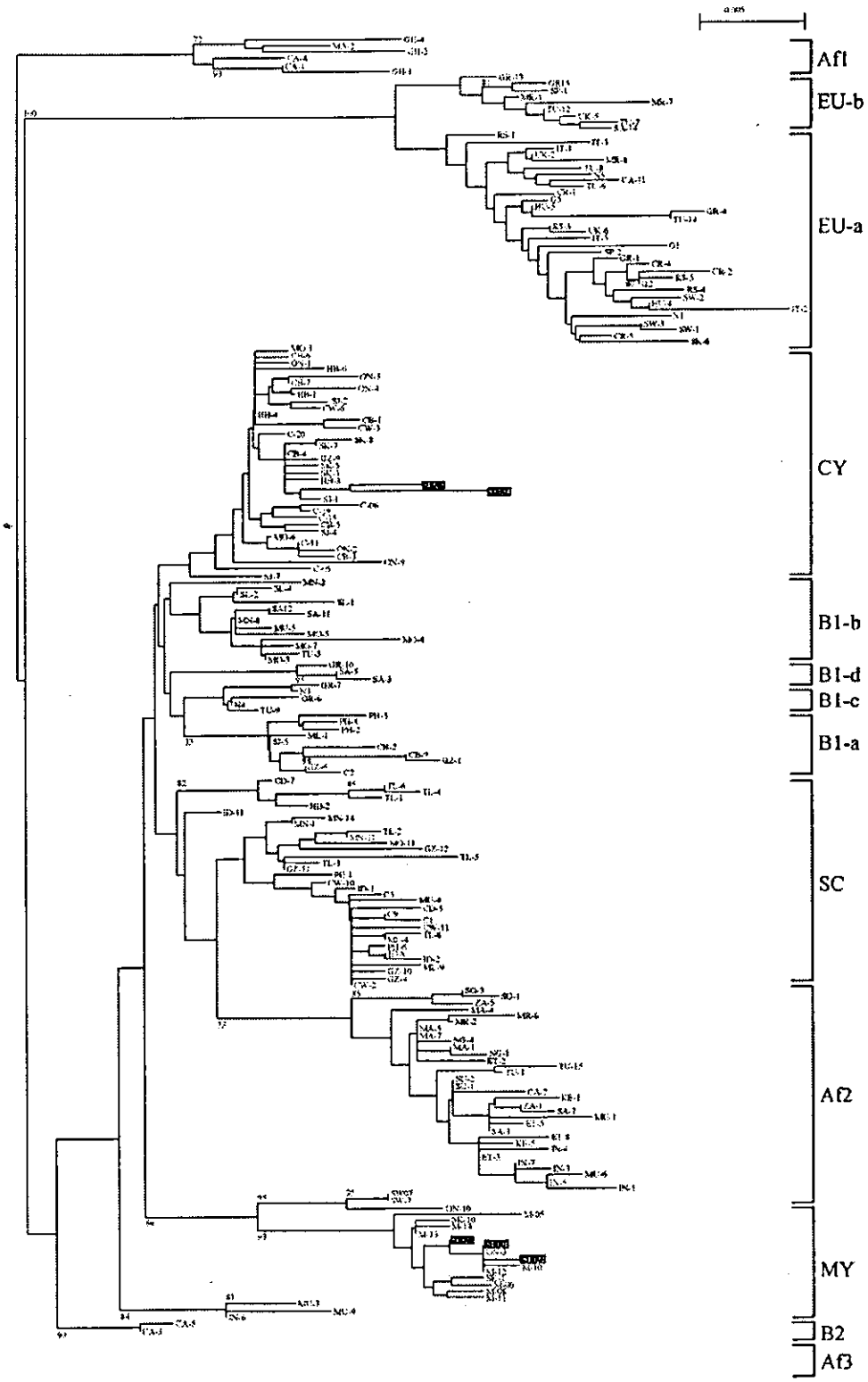

Fig. 2. An NJ phylogenetic tree showing the genotype shift detected in renal tissue samples stored in 5% formalin. An NJ phylogenetic tree was constructed from the five IG sequences detected in case 1 (see Fig. 1) and reference IG sequences reported previously (Sugimoto et al., 1997). The phylogenetic tree was visualized using TREEVIEW. The tree was rooted using genotype Af2 as the outgroup, since this genotype was suggested to indicate possible ancestral type (Pavesi, 2003). The symbols for reference sequences are described elsewhere (Sugimoto et al., 1997). The numbers at the tree nodes indicate the bootstrap confidence levels (percent) obtained with 1000 replications (only values ≥50% are shown). Genotypes are indicated on the right of the tree.

quence (SEQ-25) with 12 base substitutions were detected in case 7.

3.3. Base substitutions detected in paraffin-embedded and formalin-soaked tissue samples

The total number of base substitutions in paraffin-embedded samples was 36, while that in formalin-soaked samples was 76. This difference was statistically significant ($P < 0.05$), indicating that more base modifications occurred in formalin-soaked samples than in paraffin-embedded samples.

Table 3 lists all base substitutions detected in amplicons from paraffin-embedded and formalin-soaked tissue samples. In formalin-fixed, paraffin-embedded samples, base transitions ($n = 30$) were five times as frequent as transversions ($n = 6$), as in formalin-soaked samples.

3.4. Location of sequences with substitutions on phylogenetic trees

We examined whether the presence of lower and higher rates of base substitutions affected the genotypes of JCV. Five sequences (SEQ-1 to SEQ-5) detected in case 1 in paraffin-embedded and formalin-soaked tissue samples together with 197 IG sequences reported by Sugimoto et al. (1997) were used to construct an NJ phylogenetic tree. On the resultant trees (Fig. 2), the original sequence (SEQ-1) and sequences (SEQ-2 and -3) with a lower rate of base substitutions were found within the MY cluster, whereas sequences (SEQ-4 and -5) with a higher rate of base substitutions were located within the CY cluster. Similarly, we analyzed the distribution of sequences detected in cases 2, 5 and 7 on NJ phylogenetic trees (data not shown), and confirmed that the original sequences and those with a lower rate of base substitutions fell within the same genotypes (genotype MY in cases 2 and 5 and CY in case 7) (Fig. 2), and that sequences (SEQ-18, -19, -25) with a higher rate of substitutions were located within genotypes to which the original sequence did not belong (Fig. 2).

4. Discussion

For the first time, the effect of formalin-fixation on the nucleotide sequence of JCV DNA in tissue samples was analyzed in this study. The JCV DNA sequence detected in tissue samples stored in a frozen state served as the source of unaffected JCV DNA sequences (designated original ones). Original and variant sequences were detected in tissue samples that underwent formalin-fixation. A comparison between original and variant sequences allowed us to detect base substitutions.

When JCV DNA with some bases modified with formalin is used as the template for PCR amplification, the modified bases were recognized as one of the four bases, sometimes as a base different from the original one, generating base substitutions that can be identified by sequencing. The present finding that base transitions much more frequently occurred than transversions in paraffin-embedded as well as formalin-soaked tissue samples suggests that Taq DNA polymerase can distinguish between modified purines and pyrimidines with some ambiguity.

It has been reported that as the time of exposure to formalin increased, the PCR-amplification of DNA was more severely damaged (Inoue et al., 1996; Rogers et al., 1990). In this study, the paraffin-embedded tissue samples were previously fixed with formalin for 2 weeks, while the formalin-soaked samples were fixed for 1 year. Thus, based on our results, we may state that the duration of fixation affected the appearance of base substitutions. More substitutions were found after long-term fixation than after short-term fixation. As a result, we detected IG sequences with a higher rate of base substitutions (i.e. 10–13 substitutions per IG sequence) only in tissue samples undergoing long-term fixation. We found a genotype shift in sequences with a higher rate of substitutions. In contrast, we did not detect a higher rate of substitutions in tissue samples undergoing short-term fixation, but instead a lower rate of substitutions (i.e. 1–6 substitutions per IG sequence) was detected. Sequences with a lower rate of substitutions did not cause a genotype shift.

In summary, it was found that a significant number of base substitutions occurred in viral DNA sequences amplified from formalin-fixed, paraffin-embedded renal tissues, and that the rate of base substitutions increased in tissues soaked in formalin for a prolonged time (1 year). Thus, it was concluded that the genotypes of JCV DNA in the kidney can be determined only with specimens stored in a frozen state or formalin-fixed for a short time (2 weeks). The present findings also suggest that special care should be paid to the diagnosis of viral diseases using formalin-fixed, paraffin-embedded tissues for which formalin-fixation time is unknown.

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古西　満	AIDS関連カリニ肺炎の診断から治療までのコツ	斎藤　厚	感染症診療のコツと落とし穴	中山書店	東京	146-147	2004

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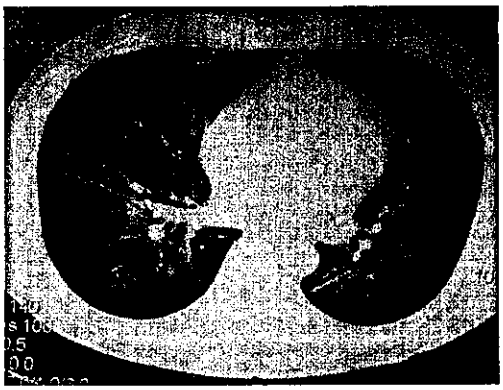
AIDS 関連カリニ肺炎の診断から治療までのコツ

古西 満（奈良医大感染症センター）

日本では HIV 感染者/AIDS 患者が着実に増えており，しかも AIDS 患者の多くは AIDS を発症するまで HIV 感染の事実を自らも把握していない．未治療の HIV 感染者が AIDS を発病する場合，カリニ肺炎を AIDS 指標疾患として発症することが比較的多い．カリニ肺炎を発症した AIDS 患者が HIV/AIDS 専門病院を初めから受診することは少なく，一般内科医や呼吸器科医を受診していることが多い．AIDS 関連カリニ肺炎を診断するためには，まず患者の HIV 感染を疑うことから始まる．

AIDS 関連カリニ肺炎は症状の進行が緩徐

自覚症状は発熱，咳嗽（乾性咳嗽のことが多い），労作時呼吸困難が多く，AIDS 関連カリニ肺炎に特異的なものはない．ただし他の免疫不全患者に発症するカリニ肺炎に比較して，多くの症例で症状進行が緩徐であることは特徴の一つである．



❶カリニ肺炎でよくみられる胸部 CT 所見
スリガラス状の肺野濃度上昇があり，カリニ肺炎で最も多いパターンである．

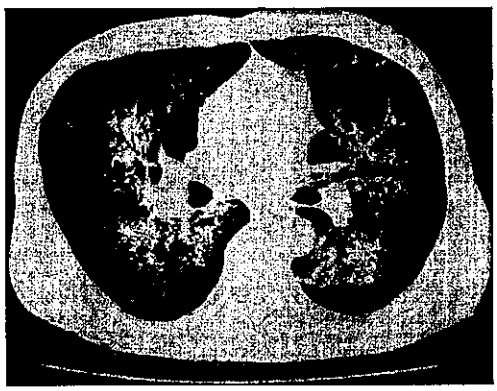
カリニ肺炎を発症する AIDS 患者ではしばしば口腔カンジダ症や脂漏性皮膚炎を認め，これが HIV 感染を疑う契機となるので診察時注意しておくべきである．口腔カンジダ症の病変には偽膜性，過形成性，紅斑性がある．偽膜性，過形成性は白色の病変で目立つが，紅斑性は口腔粘膜の赤っぽい斑点であり，時に口腔カンジダ症と認識しにくいことがあるので注意が必要である．脂漏性皮膚炎は眉間，頬部，顎髭部などの顔面にみられ，通常は左右対称性の桃色ないし赤色の皮膚炎で，明瞭な鱗屑を伴っている．

また性的接触による HIV 感染者では他の性感染症（STD）をしばしば合併する．梅毒，クラミジア感染症，性器ヘルペス，ウイルス性肝炎などの診断が HIV 感染症の診断に先行することもあるので，詳細な病歴聴取や診察が重要である．

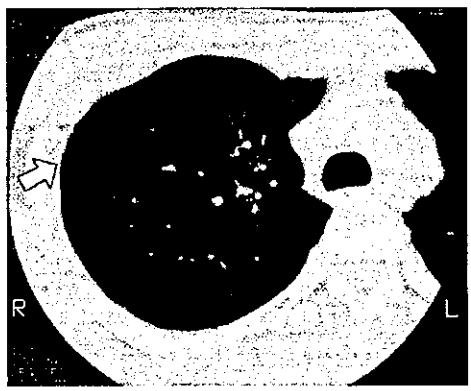
胸部所見としてスリガラス陰影を認めることが多い

AIDS 関連カリニ肺炎の胸部 X 線写真はびまん性スリガラス陰影であることが多いが，時に陰影濃度の濃い浸潤影がみられることもある．気胸は AIDS 関連カリニ肺炎ではしばしば合併する．

胸部 CT 所見は血管影を透過できるスリガラス状の肺野濃度上昇を認めることが多く（❶），その分布は区域性のない地図状分布が特徴的である．胸膜面に平行するような濃厚な帯状の濃度上昇や血管陰影を透過できない肺野濃度上昇域（❷）を伴ったりもする．気管支拡張や嚢胞形成（❸）も比較的よ



❷濃厚な肺野濃度上昇を認めた胸部 CT 所見



❸嚢胞形成を認めた胸部 CT 所見
しばしば嚢胞形成（矢印）を認め，本例は気胸を合併した．

く認められる所見である．

AIDS関連カリニ肺炎の検査所見の把握

CD4陽性リンパ球数が200/μL未満となると，AIDS関連カリニ肺炎を発症する危険性があるとされている．しかし実際にAIDS関連カリニ肺炎を発症している症例の大半はCD4陽性リンパ球数が100/μL以下である．CRPは陽性であることが多いが，その値は多くの症例で10mg/dL以下である．LDHは上昇していることが多く，動脈血酸素分圧と負の相関を認め，カリニ肺炎の重症度の指標ともなりうる．

*Pneumocystis carinii*は遺伝子学的解析で真菌に類似性が高く，シスト細胞壁の主要構成成分にβ-D-グルカンが含まれている．そのためカリニ肺炎時に血清β-D-グルカン値が上昇する．またKL-6は肺胞II型上皮細胞から産生，分泌され，肺胞II型上皮細胞の過形成を伴う間質性肺疾患の病勢指標の一つである．カリニ肺炎の病理学的所見では肺胞II型上皮細胞の増生を認めるとされ，カリニ肺炎発症時に血中KL-6値が上昇する．したがってβ-D-グルカン値とKL-6値とがともに上昇している場合にはカリニ肺炎を強く疑う所見であり，補助診断法として有用である．

第一選択薬SMX/TMPは投与量の再評価が必要

カリニ肺炎治療の第一選択薬はスルファメトキサゾール/トリメトプリム（SMX/TMP）であり，内服薬と点滴薬とが市販されている．AIDS関連カリニ肺炎での本薬剤の投与量はSMX 75～100mg/kg/日・TMP 15～20mg/kg/日が標準的である．しかし，われわれの施設では軽・中等症のAIDS関連カリニ肺炎症例に対して，この標準投与量の半量でも十分な治療効果を得ており，SMX/TMPの適切な投与量を再評価する必要があると考える．HIV感染者ではSMX/TMPの副作用発現頻度が50～60%であり，発熱，発疹，白血球減少，肝機能障害などが比較的早期（投与後7～10日目）に認められることが多い．SMX/TMPを減量しても副作用の発現率には差はないが，重篤な副作用が少なくなる印象がある．

SMX/TMPを用いることができない場合にはペンタミジン3～4mg/kg/日を1日1回点滴静注する．ペンタミジン吸入はカリニ肺炎予防には用いるが，治療目的の場合には効果が弱いので行うべきではない．軽・中等症の場合にはダプソン100mg/日の内服治療も可能である．治療期間は一般的に21日間とされているが，これは最短治療期間と考え，症状，検査所見（特に動脈血酸素分圧あるいは酸素飽和度），胸部画像所見などを総合的に判断して，治療を終了する．

大気圧下の動脈血酸素分圧が70 Torr未満の場合には副腎皮質ステロイド薬の併用投与が有効である．われわれの施設ではプレドニゾロン60～80mg/日で投与を開始し，4～5日ごとに減量し，2週間ほどで中止している．重症例の場合にはステロイドパルス療法を行うこともある．しかしAIDS関連カリニ肺炎ではしばしば気胸を合併し，副腎皮質ステロイド薬がその誘引となることも指摘されている．またカリニ肺炎を発症時にすでに他の日和見感染症を合併していたり，治療中に合併したりすることがあるので，漫然と副腎皮質ステロイド薬を投与することは厳に慎むべきである．

HAARTでAIDS関連カリニ肺炎予防の中止を考慮

HIV感染者ではカリニ肺炎未発症でもCD4陽性リンパ球数が200/μL未満の場合（一次予防）やカリニ肺炎治療後（二次予防）に予防投薬が必要である．予防薬は治療薬と同様で，SMX/TMP，ペンタミジン，ダプソンが用いられ，ペンタミジン吸入がオプションに加わる．

SMX/TMPはSMX 400mg・TMP 80mg（1錠あるいは1g）/日で投与するが，治療と同様に副作用を認めることが多い．発熱，発疹などのアレルギー機序が関与する副作用は脱感作療法で回避できるようになることがあるので試みる価値がある．またペンタミジン吸入は300mgを1回/4週で投与する方法で，しばしばカリニ肺炎の発症を認めることから，われわれの施設では300mgを1回/2週で吸入する方法を選択している．さらにペンタミジン吸入を3年以上の長期間行っている症例では呼吸機能検査上細気道障害の変化がみられることから，長期予防を避ける必要があると考えている．

強力な抗HIV治療（HAART）が行われ，免疫能が改善されるようになり，カリニ肺炎予防の中止が考慮されている．現在，CD4陽性リンパ球数が200/μL以上を3か月以上継続した場合，一次・二次予防とも中止が可能であるとされている．われわれの施設でも免疫能の改善に伴ってカリニ肺炎予防を中止した症例で，これまでにカリニ肺炎を発症したことはまったくない．

10. 当科におけるリポジストロフィー症例の現状

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はじめに

強力な抗HIV治療(HAART)によりHIV感染症の予後は改善している。その反面，新たな副作用が明らかになり，リポジストロフィーもその一つである。そこで今回われわれは当科での現状について検討したので報告する。

対象・方法

抗HIV治療開始時にはリポジストロフィーを認めず，同一治療を1年以上継続したHIV感染者29名(平均年齢43.7歳，男性27名・女性2名)を対象とした。本人・医療者ともが特徴的体型変化を認識した場合にリポジストロフィーと診断した。リポジストロフィーの発症状況を調べ，その臨床病態を評価した。

結　果

リポジストロフィーと診断した症例は7名(24.1%)であった。年齢・性別・HIV感染リスク・HIV感染症病態には非リポジストロフィー症例と有意差を認めなかった。薬剤はリポジストロフィー症例でd4T・RTVの服用率が高く，これまでのすべての抗HIV治療期間が長い傾向を認めた。

リポジストロフィー症例では非リポジストロフィー症例と比較して治療前後で有意な体重減少が

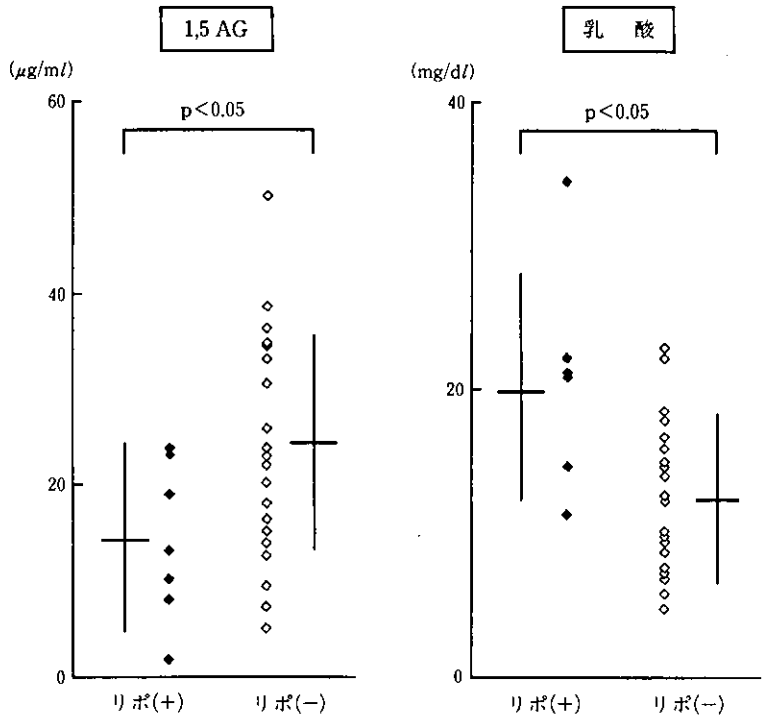

図1　リポジストロフィー症例と非リポジストロフィー症例における1,5アンヒドログルシトール(1,5 AG)値と乳酸値の比較

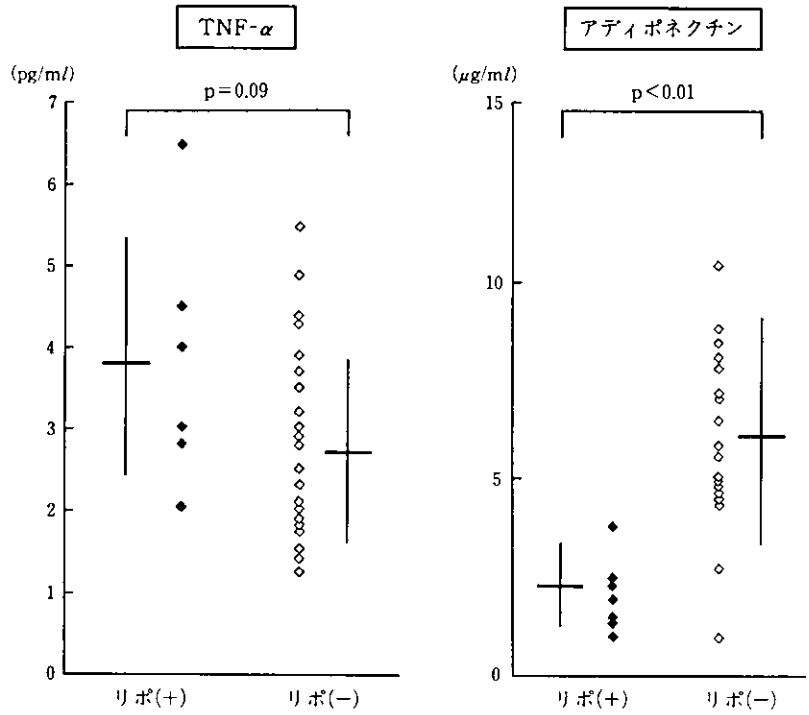

図2 リポジストロフィー症例と非リポジストロフィー症例におけるアディポサイトカイン(TNF-α・アディポネクチン)の比較

あり(p<0.01)，腹囲/臀囲比が有意に高値であった(p<0.01)。DXA(dual energy X-ray absorptiometry)で全身の脂肪分布を評価すると，体幹部/全身比はリポジストロフィー症例で61.2%，非リポジストロフィー症例で51.0%と有意差を認めた(p<0.01)。

治療後の脂質系検査をリポジストロフィー症例と非リポジストロフィー症例とで比較すると，それぞれの症例群で中性脂肪は327.0±106.7 mg/dl，230.5±146.2 mg/dl(p=0.06)，総コレステロールは231.3±44.5 mg/dl，183.3±48.9 mg/dl(p<0.05)，HDLコレステロールは44.9±16.7 mg/dl，39.5±9.6 mg/dl(p=0.3)であった。また，糖代謝の指標として1,5アンヒドログルシトール(1,5 AG)値を測定したところ，リポジストロフィー症例で有意に低値であり(p<0.05)，ミトコンドリア障害の指標と考えられる乳酸値はリポジストロフィー症例で有意に高値であった(p<0.05)(図1)。

脂肪組織から分泌される生物活性物質(アディポサイトカイン)に属するTNF-αとアディポネクチンの血清濃度をELISA法で測定した。リポジストロフィー症例でTNF-α値は高値の傾向があり(p=0.09)，アディポネクチン値は有意に低値であった(p<0.01)(図2)。

考　察

当科でのリポジストロフィーの発症率は欧米の報告に比べ低いものであったが，日本人HIV感染者にとっても重大な現象であることが示唆された。その発症は抗HIV治療と関連があり，特にd4TとRTVが重要な薬剤と考えられた。臨床的には体重変化と腹囲/臀囲比がリポジストロフィーの診断指標に役立つ可能性があり，DXAが客観的指標として利用できることが示された。リポジストロフィー症例では従来から指摘されているように脂質代謝異常・糖代謝異常・ミトコンドリア障害を認めた。また，リポジストロフィー症例では血清中TNF-α値・アディポネクチン値に

も影響が見られ，将来的に動脈硬化を発症するリスクが高いことが危惧された。

今後，さらに多くのリポジストロフィーに関する知見を集積し，有効な対応を早期に見いだす必要があると考える。

V. 臨床 2

11. 初回療法における EFV と LPV/r の使用成績調査

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目　的

当院における初回療法の組み合わせには，ヌクレオシド系逆転写酵素阻害剤(NRTI)の AZT/3TC 合剤(商品名：コンビビル)と非ヌクレオシド系逆転写酵素阻害剤(NNRTI)の EFV(商品名：ストックリン)またはプロテアーゼ阻害剤(PI)の LPV/r(商品名：カレトラ)を含む処方が選択される傾向にある。今回われわれは，EFV と LPV/r の初回療法における臨床効果および安全性につき比較検討を行ったので報告する。

EFV と LPV/r 使用成績調査

対象：初回療法にコンビビル+EFV or LPV/r の投薬を開始した患者。

期間：平成 11 年 12 月～平成 15 年 1 月。

調査内容：副作用発現の有無，症状，抗ウイルス効果，CD4 細胞数の変化。

結　果

患者背景を表 1 に示した。28 週を経過した時点でおのおのの評価項目について検討を行った。

EFV 群

服薬継続率は 76%であった。内訳は 44 例中，継続 28 例，副作用による中止 5 例，自己中断 2 例，転院・その他 9 例であった。副作用は 38 例，86%に認め，主な症状は，ふらつき，熱感，異夢などの精神神経症状であった(図 1)。なお，副作用により投薬を中止した症例は 5 例であり，中止までの期間は平均 18 週間であった。抗ウイルス効果は，HIV-RNA 量が 50 コピー未満となった症例の割合を基準として判定を行い，OT 解析(on treatment)100%，ITT 解析(intent to treat)75%の患者が 50 コピー未満を維持していた。CD4 細胞の増加数は，開始値が 50 以下群 97

表 1　患者背景

	EFV 群	LPV/r 群
例数	44 名	19 名
男女比	41：3	19：0
平均年齢	35.8 歳	35.3 歳
平均 HIV-RNA 量	1.2×10^5 copies/ml (0.04-7.5)	7.5×10^5 copies/ml (0.5-37.3)
平均 CD4 細胞数	208 cells/μl (4-496)	151 cells/μl (3-620)

研究●症例

Mycobacterium kansasii 感染症を合併したAIDSの2例

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要　旨

症例1は53歳・男性。不特定の女性と性交渉あり。呼吸不全で入院し，HIV抗体陽性のため転院となった。びまん性スリガラス影を認め，カリニ肺炎・CMV肺炎とともに *M. kansasii* 肺感染症と診断した。症例2は27歳・男性。非加熱血液製剤でHIVに感染し，抗HIV治療を受けていたが，CD4陽性細胞数は10/μl未満であった。発熱が持続し，左胸水貯留，肝・脾膿瘍を認め，播種性 *M. kansasii* 感染症を疑った。

免疫不全の進行したHIV感染者では *M. kansasii* 感染症を合併することがあり，肺感染症と播種性感染症との異なる病態があるので注意が必要である。

Key words：*Mycobacterium kansasii*，HIV感染症，肺感染症，播種性感染症/HIV infection，pulmonary infection，disseminated infection

1　はじめに

HIV（human immunodeficiency virus）感染症は免疫機能障害の程度により種々の日和見感染症を合併する。非結核性抗酸菌症はCD4陽性細胞数が50/μl未満の免疫機能障害が高度な症例に発症し，*Mycobacterium avium-intracellulare* complex（MAC）と *Mycobacterium kansasii*（*M. kansasii*）とが原因菌として重要である。今回われわれは *M. kansasii* 感染症を発症したAIDS

Two Cases of AIDS-related Mycobacterium Kansasii Infection

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