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TECHNICAL NOTE

H. Ikegaya · H. Iwase · C. Sugimoto · Y. Yogo

JC virus genotyping offers a new means of tracing the origins of unidentified cadavers

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Abstract There has been no reliable means of tracing the origins of unidentified cadavers but the recent finding that JC virus (JCV) can serve as a means of elucidating human migrations suggested that this virus may also be useful to trace the origins of unidentified cadavers. DNA samples extracted from renal tissue and urine were used as the template for PCR amplification of a 610 bp region (IG region) of the viral genome. We detected JCV DNA in 45% of the renal samples and in 33% of the urine samples and was detectable even 10 days after death. The sequences of the amplified IG regions could be used to determine the genotypes. We conclude that the JC virus genotype is a new marker useful for tracing the origins of unidentified cadavers.

Keywords Forensic medicine · Unidentified cadaver · JC virus · Phylogenetic analysis · Genotypes · Geographic origin

Introduction

In spite of the progress in the methods of scientific investigation, the increase in the number of unidentified cadavers is a growing problem throughout the world [1, 2]. Recently, the relationships between several human DNA markers and human populations have been investigated [3, 4, 5], but these markers were of little use to elucidate the geographic origins of unidentified cadavers. The recent finding that JC virus (JCV) can serve as a means of tracing human migrations [6, 7] suggested that this virus may also be useful to elucidate the origins of unidentified cadavers. JCV asymptomatically infects most humans during childhood [8, 9] and the same viral strain persists in the kidneys throughout life [10, 11, 12]. JCV DNA can be classified into at least 12 genotypes that occupy distinct domains in different parts of the world [6, 13] (Table 1). Here, we report that a 610 bp region (IG region) of the JCV genome [14], usually used for the identification of JCV genotypes, can efficiently be amplified by PCR from the kidneys or urine of cadavers, and that the sequences of the amplified IG regions can be used to determine the genotypes.

H. Ikegaya (✉) · H. Iwase
Department of Forensic Medicine,
The Graduate School of Medicine, The University of Tokyo,
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
e-mail: ikegaya@m.u-tokyo.ac.jp,
Fax: +81-3-58413367

C. Sugimoto · Y. Yogo
Laboratory of Viral Infection,
Department of Microbiology and Immunology,
The Institute of Medical Science, The University of Tokyo

Materials and methods

Subjects

A total of 64 renal tissue and 36 urine samples were obtained during forensic autopsies in our department. The origins of the cadavers were all known.

Table 1 Distribution of JCV genotypes in the Old World

JCV genotype[a]	Geographic region where an indicated genotype is mainly detected[b]
EU (Types 1 and 4)	Europe, Mediterranean areas
B1-c (Type 2B)	Europe
Af1 (Type 6)	Central and western Africa
Af2 (Type 3)	Africa, western Asia
Af3	Central Africa
B1-a	China
B1-b (Type 2D)	Central and western Asia
B1-d	Saudi Arabia, Greece
B2	India, Mauritius
CY	Northeastern Asia
SC (Type 7)	Southeastern Asia, southern China
MY (Types 2A and 2C)	Japan, South Korea

[a]Genotypes designated by Sugimoto et al. [6] and Guo et al. [13] are shown, and those designated by Jobes et al. [32] are shown in parentheses.
[b]Domains are indicated according to Sugimoto et al. [6] and Guo et al. [13]. Major JCV genotypes in the New World: EU, B1-c, Af1, Af2, MY [7, 32].

Extraction of DNA from kidney and urine

A 10×10×2 mm slice was excised from three different medullar regions in each of the bilateral kidneys. DNA was extracted as described previously [15]. Extraction of DNA from urine was also performed essentially as described [15].

Amplification of the IG region

Amplification of the 610 bp IG region was conducted using Pwo DNA polymerase with proof-reading activity (Boeringer Mannheim, Germany) as described [16].

Sequencing

The PCR-amplified fragments were cloned into pBluescript II SK(+) (Stratagene, La Jolla, Calif.) as described [12]. Recombinant plasmids were sequenced using an autosequencer ABI 3700 (Perkin-Elmer, Foster City, Calif.).

Phylogenetic analysis

Neighbor-joining phylogenetic trees [17] were constructed using the CLUSTAL W program [18].

Results

JCV DNA was usually detected only in part of the tissue specimens derived from the same cadaver (Table 2). JCV DNA was detected in both kidneys in 15 cases, in the left kidney only in 7 cases, and in the right kidney only in 8 cases. Thus, JCV DNA detection had no bias towards one of the bilateral kidneys. JCV DNA was efficiently detected in older age groups (Table 3) and was also efficiently detected up to 72 h after death (Table 4). We also attempted to detect JCV DNA in cadaveric urine specimens but this was less efficient than from kidneys (Table 4). We examined the correlation between the JCV genotype and the origins of cadavers. JCV genotypes detected in the kidney or urine specimens from cadavers are shown for each geographic region in Table 5. Two major JCV genotypes, CY and MY were detected in Japanese cadavers, CY was detected in all cadavers originating from northern China and CY and MY were detected in cadavers from Korea.

Discussion

In this study, we obtained the following findings as to the detection of the JCV DNA from the kidney of cadavers:

1. JCV DNA was detected in both kidneys
2. JCV DNA was efficiently detected from elderly cadavers aged 25 years or more at death
3. JCV DNA was not detected at all sites in the kidneys.

The last two findings were similar to those in living subjects [11, 15, 19, 20, 21], but the first finding has not been reported in studies using living subjects.

From the findings in this study we concluded that the JC virus genotype is a new marker useful for tracing the origins of unidentified cadavers. Many genetic markers, including mtDNA, Y chromosome, and microsatellites, have been used to analyse the relationships among various human populations. However, although these markers are useful in population-based studies, they hardly provide reliable information on the ethnic origin of individuals.

Table 2 Detection of JCV DNA in various areas of bilateral kidneys

	Not detected	Detected	No. of positive samples/no. of samples analysed (%)					
			1/6	2/6	3/6	4/6	5/6	6/6
No. of cadavers	35	29 (100)	7 (24)	6 (21)	9 (31)	5 (17)	1 (3)	1 (3)

Table 3 Detection of JCV DNA from cadaveric kidneys and urine in various age groups

Tissue	No. of positive cadavers/no. of cadavers examined (%) in age group (years)				Total
	0–25	26–50	51–75	>76	
Kidney	0/5 (0)	11/32 (34)	14/21 (67)*	4/6 (67)	29/64 (45)*
Urine	1/5 (20)	4/13 (31)	5/16 (31)	1/2 (50)	11/36 (31)

*$P<0.05$ (vs. urine in the same age group).

Table 4 Detection of JCV DNA in the cadaveric kidneys and urine at various post-mortem times

Tissue	No. of positive cadavers/no. of cadavers examined (%) at time post-mortem (hours)				Total
	0–24 h	25–48 h	49–72 h	>73 h	
Kidney	19/44 (43)	6/11 (55)	3/6 (50)	1/3 (33)[a]	29/64 (45)
Urine	6/24 (25)	2/7 (29)	2/4 (50)	1/1 (100)[b]	11/36 (31)

[a]JCV DNA was detected in a single cadaver 7 days after death.
[b]JCV DNA was detected in a single cadaver 10 days after death.

Table 5 JCV genotypes in cadavers of various origins

Origins of cadavers	No. of JCV DNA positive cadavers	No. of cadavers with genotype	
		CY	MY
Japan			
Southwestern area[a]	4	3	1
Northeastern area[a]	5	0	5
Intermediate area[a]	26	15	11
Out side Japan			
Northern China[b]	3	3	0
South Korea	2	1	1

[a]According to Kitamura et al. [31] Japan was divided into 3 areas, southeastern, northeastern and intermediate areas.
[b]According to Guo et al. [13] China was divided into 2 areas, northern and southern areas.

Therefore, at present the JCV genotype is the most useful marker that can be used to elucidate the origins of unidentified cadavers. The JCV marker cannot precisely locate the origin of cadavers, but can restrict the area to be investigated to trace the origin. The information concerning the JCV genotypes existing in cadavers could be one of the most important individual elements to be considered in the investigation of unidentified cadavers. Furthermore, if the geographic distribution of JCV genotypes is clarified in detail in the future, the application of the current method will be expanded. In combination with other methods [22, 23, 24, 25, 26, 27, 28, 29, 30], this new method should make the investigation of unidentified cadavers both more efficient and economical.

To emphasise the usefulness of our method, we present the following examples in which the detection of JCV genotypes was helpful to identify the cadavers. In the first case, a cadaver was found in Tokyo, which was apparently Japanese, but there were no belongings which could have been useful to determine the geographic origin of the cadaver. We detected the MY genotype in this cadaver and based on this information, an investigation to search for the origin of this cadaver was first performed in the northeast area of Japan, where MY is more predominant [31]. This cadaver was identified among the missing in the northeast area of Japan. In the second case the SC genotype which is rare in Japan was detected in a cadaver found in Tokyo. Since SC is rarely found in Japanese [6, 31], the investigation was extended abroad to southern China and southeastern Asia where this genotype is predominant. This cadaver was identified among the missing persons from southern China.

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