Detection of JC virus DNA from whole blood and urine samples

H Ikegaya, H Motani, T Akutsu, K Sakurada

Citation

H Ikegaya, H Motani, T Akutsu, K Sakurada. *Detection of JC virus DNA from whole blood and urine samples*. The Internet Journal of Microbiology. 2006 Volume 3 Number 2.

Abstract

JC polyomavirus (JCPyV) is ubiquitous in humans and an etiological agent of progressive multifocal leukoencephalopathy (PML). The primary infection with this virus occurs in childhood and persists in the kidneys throughout life. Recently, detection of JC virus DNA from blood samples has been needed for forensic purposes. However, the reported detection rate of this virus from whole blood is unclear. Here, we detected JCPyV DNA from the whole blood and urine of 50 forensic cases who died from external injuries with no apparent illness. JCPyV DNA was detected in eight urine samples (16%) and one blood sample (2%). However, the case we detected from blood died in poor physical condition. Therefore, JCPyV may not present in the blood of healthy individuals and only re-spread into blood vessels in cases with immunosupressed status.

INTRODUCTION

JC polyomavirus (JCPyV) is known to be an etiological agent of progressive multifocal leukoencephalopathy (PML), a central nervous system demyelinating disease found in immunosuppressed patients such as those with AIDS and leukemia. Especially, in AIDS patients, around 10% of them suffer from PML ([1]). In those patients, JCPyV DNA is detected from urine and peripheral blood mononuclear cells (PBMC). According to serological studies, this virus is found to be a ubiquitous virus among humans worldwide. More than 75% of adults tested positive for antibodies ([2]). Primary infection of this virus occurs in childhood asymptomatically from their parents ([3]). The site of persistence in healthy humans is the kidney ([4,5,6]). Thus, JCPyV DNA is excreted into the urine of about 20-80% of adults ([7,8,9]).

Recently, the JCPyV genotype was found to be a good marker for revealing human birth origins in criminal investigation ([10,11,12]). For example, when we have a cadaver or a urine spot on the bed sheets at a crime scene, we cannot establish the crime victim's origins if there are no belongings, no eyewitnesses or no fingerprints on record. In such cases, we try to detect JCPyV DNA from the kidneys or the urine spot. From the worldwide genotype distribution, we can estimate the host's geographic origin. Therefore, detection of JCPyV DNA from whole blood samples is extremely important, as well as that from urine. Although there have been several reports on detecting JCPyV DNA from blood leukocytes, the detection rate is not clear. It ranged from 0% to 83% ([13,14,15]). However, most reports detected JCPyV DNA from hospitalized, immunocompetent individuals. Therefore, it is doubtful they can assume those patients to be “healthy” individuals, even if they do not suffer from immunocompromised-type diseases.

Here, we tried to detect JCPyV DNA from the whole blood and urine of 50 forensic cases who died from external injuries, not diseases. We compared the JCPyV sequences and genotypes detected from urine and whole blood DNA.

MATERIAL AND METHODS

WHOLE BLOOD AND URINE SAMPLES

Totally, 50 whole blood and urine samples were collected during forensic autopsies in Chiba University. The postmortem time was within 24 hours to guarantee fresh samples for this study. None of the present cases had any illnesses, including immunosuppressive diseases, such as AIDS and leukemia, and died from external injuries a judged from autopsy findings and police department reports. Table 1 shows the cause of death of positive subjects. All samples were stored at –20oC until use. DNA was extracted from 200μL of whole blood and urine using a QiaAmp DNA mini Kit (Qiagen GmbH, Hilden, Germany).

Figure 1

Table 1: JCPyV positive subjects

Case No.	age	sex	Cause of death	Detected sample (genotype)	
				blood	urine
1	63	M	Head injury	-	+(CY)
2	38	M	Drowning	-	+(CY)
3	56	M	Traffic accident	-	+(MY)
4	42	M	Drowning	-	+(CY)
5	71	M	Hip bone fracture	-	+(CY)
6	55	M	Traffic accident	-	+(CY)
7	83	M	Burned	-	+(CY)
8	79	F	Traumatic shock	+(CY)	+(CY)

CONVENTIONAL PCR

The 396-bp regulatory region and 610-bp typing region (IG region) ([16]) of JCPyV were PCR-amplified from DNA samples extracted from whole blood and urine, using ProofStart Taq DNA polymerase (QIAGEN). The 396-bp regulatory region was amplified using primers A1 and B3 ([17]). The IG region was amplified using primers P1 and P2 ([16]). The IG region is known to be a polymorphic region of the JCPyV genome. Enzyme activation and amplification of 20μL of reaction mixture containing a 1μL of sample DNA was performed according to the manufacturer's instructions. PCR conditions were 50 cycles of 20 s at 94°C, 30 s at 55°C, 1 min at 72°C after an initial 15 min of 95°C. PCR was performed using a Program Temp Control System Astec PC-320 (ASTEC Inc., Fukuoka City, Japan).

NESTED PCR

JCPyV regulatory regions were amplified by nested PCR using Proof Start DNA polymerase (QIAGEN) in which A1 and A2 were used as outer primers and B1 and B3 as inner primers ([17]). The first round was performed for 40 cycles, and the second round for 30 cycles using a Program Temp Control System Astec PC-320 (ASTEC). Other conditions were the same as conventional PCR method.

SEQUENCING OF AMPLIFIED FRAGMENTS

Amplified fragments were prepared using Gel Filtration Cartridges (Edge Biosystems, Gaithersburg, MD), and the purified fragments were used in a cycle sequencing reaction using a BicDYE Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems, Foster City, CA). Same promoters used for PCR were added as primers at a final concentration of 0.25 pmol/μL in a reaction volume of 10 μL. Cycle conditions were set as in the manufacturer's instructions.

Products for sequencing were purified on Gel Filtration Cartridges (Edge Bio,), and DNA sequencing was performed using an automated sequencer (3100-Avant DNA sequencer; Applied Biosystems).

PHYLOGENETIC ANALYSIS

JCPyV DNA IG sequences (610-bp) detected in this study and those representing each genotype of JCPyV ([18]) were aligned using the Clustal X program, Version 1.82 ([19]). From the aligned sequences, a neighbor-joining (NJ) phylogenetic tree ([20]) was constructed using Clustal X with Kimura's correction ([21]). The phylogenetic tree was visualized using the NJ plot program ([22]). To assess the confidence level of the phylogenetic tree, bootstrap probabilities were estimated with 1000 bootstrap replicates ([23]). Bootstrap probabilities larger than 70% were considered significant ([24]).

RESULTS

AMPLIFICATION OF THE CONTROL REGION AND IG REGION OF THE JCPYV GENOME

From the urine DNA samples, using the conventional PCR and nested PCR, the control region and the IG region were amplified in eight cases (16%). The age, sex and cause of death of these cases are shown in Table 1. There were no samples in which only the control region or the IG region were amplified. From the whole blood DNA samples, using the conventional PCR and nested PCR, the control region and the IG region was amplified in only one case (2%). Detected genotypes from the samples are shown in Table 1. Seven cases were genotype CY and one case was genotype MY. In this case, sequences of the control region and the IG region were also amplified from the urine sample.

A case in which the JCPyV genome was amplified from both urine and blood.

The sequences of the control region amplified from the urine and whole blood were completely identical. The sequence of the control region was also the same as the archetypical (CY strain ([25])) viral sequence. The PML type sequence (i.e. the rearrangements in the control region) ([26]) of the control region was not detected.

The sequences of the IG region amplified from the urine and whole blood were completely identical. The detected JCPyV genotype was the ‘CY' that is normally detected from healthy individuals in this geographical area.

DISCUSSION

Recently, the detection of JCPyV DNA has become an

efficient tool to estimate the geographical origins of unidentified cadavers in the field of forensic medicine ([10,11,12]). The JCPyV genotype is classified into 12 main genotypes ([19]). Each genotype distributes in type-specific areas worldwide. It is reported that JCPyV causes no super-infection ([27]) and no re-infection even if the host relocates. The same strain is maintained throughout life ([28]). Therefore, detection of the JCPyV genotype from the kidneys of unidentified cadavers or urine spots at crime scenes allows us to estimate the host's geographical origin medicine ([10,11,12]). In addition to the ethnic origin estimated from human DNA, the geographical origin estimated from the JCPyV DNA provides important information for profiling crime victims. The detection of JCPyV DNA from blood and bloodstain samples is very useful.

However, different from the detection of JCPyV DNA from urine and kidney samples, the reported detection rates of JCPyV DNA from blood leukocytes vary. In our study, JCPyV DNA was detected mostly from urine samples and it was very rare to detect JCPyV DNA from blood samples even in the urine of JCPyV-positive cases. We detected JCPYV DNA from both the blood and urine sample from one case. This cadaver was 79 years old female and was beaten by family members for more than six months until her death. In her postmortem blood analysis, a high CRP was noted. Although she had no illness, she may have been immunosuppressed and lacking the strength to maintain JCPyV in a persistent form. Therefore, we consider her to be a very special case.

Thus, we conclude that this virus persists in the kidneys after viremia from primary infection, but does not cause viremia in healthy individuals after the primary infection, so JCPyV DNA may not present in the blood of healthy individuals.

There was a report using samples taken from hospitalized patients, in which archetype JCPyV was found in urine samples, whereas PML type JCPyV was found in blood samples ([29]). However, we found no PML type JCPyV DNA from urine and blood samples taken from our case. It is unclear how many people have PML type JCPyV DNA in blood and how this rearrangement of the control region occurs in the blood. From our results, JCPyV persisting in renal tissue may re-spread into blood vessels and cause viremia again only in immunnosuppressed individuals.

ACKNOWLEDGEMENT

We are grateful to Dr. Yogo and Dr. Zheng for helpful discussions.

CORRESPONDENCE

Hiroshi Ikegaya, MD, PhD National Research Institute of Police Science 6-3-1 Kashiwanoha, Kashiwa, Chiba, 277-0882, Japan TEL: +81-4-7135-8001 FAX: +81-4-7133-9159 E-mail: Ikegaya-tky@umin.ac.jp, ikegaya@nrips.go.jp

References

1. Kuchelmeister K., Gullotta F., Bergmann M., Angeli. G, Masini T. 1993. Progressive multifocal leukoencephalopathy (PML) in the acquired immunodeficiency syndrome (AIDS). A neuropathological autopsy study of 21 cases. Pathol Res Pract. 189: 163-173.
2. Padgett BL, Walker DL. 1978. Natural history of human polyomavirus infections. In Stevens JG, Ed; Persistent Viruses. Proceedings of the 1978 ICN-UCLA Symposia on molecular and Cellular Biology held in Keystone, Colorado, February, 1978; Academic Press: New York, pp 751-758.
3. Kunitake T, Kitamura T, Guo J, Taguchi F, Kawabe K, Yogo Y. 1995. Parent-to-child transmission is relatively common in the spread of the human polyomavirus JC virus. J Clin Microbiol. 33: 1448-1451.
4. Dorries K. 1998. Molecular biology and pathogenesis of human polyomavirus infections. Dev Biol Stand. 94: 71-79.
5. Tominaga T, Yogo Y, Kitamura T, Aso Y. 1992. Persistence of archetypal JC Virus DNA in normal renal tissue derived from tumor-bearing patients. Virology. 186: 736-741.
6. Chesters PM, Heritage J, McCance DJ. 1983. Persistence of DNA sequences of BK virus and JCPyVirus in normal human tissues and in diseased tissues. J Infect Dis 147: 676-684.
7. Agostini HT, Ryschkewitsch CF, Stoner GL. 1998. JC virus Type 1 has multiple subtypes: three new complete genomes. J Gen Virol. 79: 801-805.
8. Chima SC, Ryschkzewitsch CF, Stoner GL. 1998. Molecular epidemiology of human polyomavirus JC in the Biaka Pygmies and Bantu of Central Africa. Mem Inst Oswaldo Cruz. 93: 615-623.
9. Kitamura T, Aso Y, Kuniyoshi N, Hara K, Yogo Y. 1990. High incidence of urinary JC virus excretion in nonimmunosuppressed older patients. J Infect Dis. 161: 1128-1133.
10. Ikegaya H, Iwase H, Sugimoto C, Yogo Y. 2002. JCPyVirus genotyping offers a new means of tracing the origins of unidentified cadavers. Int J Legal Med. 116, 242-245.
11. Ikegaya H, Iwase H. 2004. Trial for the geographical identification using JC viral genotyping in Japan. Forensic Sci Int. 139: 169-172.
12. Sakurada K, Ikegaya H, Motani H, Iwase H, Sekiguchi K, Akutsu T, Yoshino M, Takatori T, Sakai I. 2005. JC virus genotyping can be used to narrow down the native place of persons from urine stains. Jpn J Forensic Sci Tech. 111-117.
13. Sundsfjord A, Flaegstad T, Flo R, Spein AR, Pedersen M, Permin H, Julsrud J, Traavik T. 1994. BK virus and JC viruses in human immunodeficiency virus type a-infected persons: prevalence, excretion, viremia, and viral regulatory regions. J Infect Dis. 169: 485-490.
14. Azzi A, De Santis R, Ciappi S, Leoncini F, Sterrantino G, Marino N, Mazotta F, Laszlo D, Fanci R, Bosi A. 1996. Human polyoma virus DNA detection in peripheral blood leukocytes from immunocompetent and immunocompromised individuals. J Neurovirol. 2: 411-416.
15. Dorries K, Vogel E, Gunther S, Czub S. 1994. Infection

of human polyomaviruses JC and BK in peripheral blood leukocytes from immunocompetent individuals. Virology. 198: 59-70.
16. Ault GS, Stoner GL. 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.
17. Sugimoto C, Ito D, Tanaka H, Matsuda H, Saito H, Sakai H, Fujihara K, Itoyama Y, Yamada T, Kira J, Matsumoto R, Mori M, Nagashima K, Yogo Y. 1998. Amplification of JC virus regulatory DNA sequences from cerebrospinal fluid: diagnostic value for progressive multifocal leukoencephalopathy. Arc Virlo. 143: 249-262.
18. Sugimoto C, Kitamura T, Guo J, Al-Ahdal MN, Shchelkunov SN, Otova B, Ondrejka P, Chollet JY, El-Safi S, Ettayebi M, Gresenguet G, Kocagoz T, Chaiyarasamee S, Thant KZ, Thein S, Moe K, Kobayashi N, Taguchi F, Yogo Y. 1997. Typing of urinary JC virus DNA offers a novel means of tracing human migrations. Proc Natl Acad Sci U S A. 94: 9191-9196.
19. Jeanmougin F, Thompson JD, Gouy M, Higgins DG, Gibson TJ. 1998. Multiple sequence alignment with Clustal X. Trends Biochem Sci. 23: 403-405.
20. Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees.Mol Biol Evol. 4: 406-425.
21. Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 16: 111-120.
22. Perriere G, Gouy M. 1996. WWW-query: an on-line retrieval system for biological sequence banks. Biochimie. 78: 364-369.
23. Felsenstein J. 1985. Confidence limits on phylogenetics: an approach using the bootstrap. Evolution. 39: 783-791.
24. Hillis DM, Bull JJ. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst Biol. 42: 182-192.
25. Yogo Y, Kitamura T, Sugimoto C, Ueki T, Aso Y, Hara K, Taguchi F. 1990. Isolation of a archetypal JC virus DNA sequence from nonimmunocompromised individuals. J Virol. 64: 3139-3143.
26. Rentier-Delrue F, Lubiniecki A, Howley PM. 1981. Analysis of JC virus DNA purified directly from human progressive multifocal leukoencephalopathy brains. J Virol. 38: 761-769.
27. Ikegaya H, Iwase H, Yogo Y. 2004. Detection of identical JC virus DNA sequences in both human kidneys. Arch Virol. 149: 1215-1220.
28. Kitamura T, Sugimoto C, Kato A, Ebihara H, Suzuki M, Taguchi F, Kawabe K, Yogo Y. 1997. Persistent JC virus (JCV) infection is demonstrated by continuous shedding of the same JCV strains. J Clin Microbiol 35, 1255-1257.
29. Pietropaolo V, Videtta M, Fioriti D, Mischitelli M, Arancio A, Orsi N, Degener AM. 2003. Rearrangement patterns of JC virus noncoding control region from different biological samples. J Neurovirol. 9: 603-11.

Author Information

Hiroshi Ikegaya
(Departments of Legal Medicine), (Faculty of Medicine), National Research Institute of Police Science, (Chiba University)

Hisako Motani
Departments of Legal Medicine, Faculty of Medicine, Chiba University

Tokoko Akutsu
National Research Institute of Police Science

Koichi Sakurada
National Research Institute of Police Science