Absence of Association between the Incidence of BK Virus and Sporadic Creutzfeldt-Jakob Disease

Byung-Hoon Jeong Jung-Hwa Lee Han-Jeong Cho Yong-Sun Kim

Ilsong Institute of Life Science, Hallym University, Gyeonggi-do, Republic of Korea

Key Words

Polyomavirus · Genotypes · BK virus · Prion disease · Creutzfeldt-Jakob disease · Phylogenetic tree

Abstract

Background: Many studies suggest that the virus-like particles are required for the infection of Creutzfeldt-Jakob disease (CJD). **Objective:** To determine the relationship between BK polyomavirus (BKV) and sporadic CJD. **Materials and Methods:** We investigated the prevalence of BKV in urine samples from 94 sporadic CJD patients and 54 other neurological disease (OND) patients using polymerase chain reaction. **Results:** BKV DNA was detected in 16 (17%) and 9 (16.7%) urine samples from sporadic CJD and OND patients, respectively. There was no significant difference in the incidence of BKV infection between Korean sporadic CJD and OND patients ($p = 0.9558$). In order to investigate the genotypes of BKV, we analyzed 22 BKV isolates obtained from Korean patients by DNA sequencing and nucleotide sequence analysis. Three distinct subtypes, namely I, III, and IV, were found in 66.7, 22.2, and 11.1% of 9 BKV isolates from OND patients, whereas subtypes I and IV were detected in 76.9 and 23.1% of 13 BKV isolates from sporadic CJD patients. Interestingly, subtype III was not detected in sporadic CJD patients.

Significant differences in the frequency of BKV genotypes were not observed between sporadic CJD and OND patients. **Conclusions:** These results indicate that BKV may not play an important role in the pathogenesis of prion diseases.

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Introduction

Transmissible spongiform encephalopathies or prion diseases, such as scrapie in sheep, Creutzfeldt-Jakob disease (CJD) and kuru in humans, and bovine spongiform encephalopathy in cattle, are fatal infectious neurodegenerative diseases, which are characterized by the accumulation of an abnormal form of prion protein (PrP^{Sc}), spongiform neurodegeneration, neuronal cell loss and astrogliosis in the brain [1]. CJD is classified into three types: sporadic, acquired, and inherited forms. The most common form of CJD is sporadic CJD, which occurs worldwide with an incidence rate of

B.-H.J. and J.-H.L. contributed equally to this work.

about 1 in a million per year. Although the conversion process of cellular prion protein (PrP^C) to PrP^{Sc} is the critical molecular event in the pathogenesis of sporadic CJD, the precise pathogenic mechanism and the nature of infectious agent(s) of sporadic CJD still remain unknown.

According to the protein-only hypothesis which is the most favored hypothesis, misfolded PrP^{Sc} itself is the infectious agent and certain co-factors including protein X may play an important role in the transition process of PrP^C to PrP^{Sc} [2]. Based on the virus theory, this infectious agent shows many essential biological and physical characteristics of viruses such as symbiotic and classical pathogenic viruses and can establish a long-term persistent infection without causing the neurodegenerative disorder for a few decades [3]. It has been reported that 20- to 35-nm virus-like particles and tubulofilamentous virus-like particles termed nemavirus have been found in transmissible spongiform encephalopathy-infected brains [4–9]. The intracellular 25-nm virus-like particles were observed in cells infected with scrapie and CJD agents [10, 11]. Herpes simplex virus (HSV) encephalitis has been reported as an initial presentation of CJD [12, 13]. Of 25 CJD patients, 3 had intrathecally synthesized antibodies against HSV in the cerebrospinal fluid and 2 against varicella zoster [14]. Also, DNA can convert PrP^C into the β -sheet conformation [15] and circular DNA sequences co-purified with infectivity in scrapie and CJD [3]. Our previous studies showed that endogenous murine leukemia virus titer was increased in the brain of senescence-accelerated mouse by the scrapie infection and the incidence rate of human endogenous retroviruses in cerebrospinal fluids from CJD patients was significantly higher than other neurological disease (OND) patients and normal controls [16, 17].

The BK polyomavirus (BKV), which is a member of the polyomavirus genus, can coexist asymptotically within the human body including brain [18]. The genome of approximately 5.1 kb circular DNA in length and the virion of about 40 nm in diameter are relatively smaller than other double-stranded viruses. Such properties prompted us to examine the incidence of BKV from urine samples of sporadic CJD patients.

In this paper, we investigated the relationship between BKV and sporadic CJD. In order to examine the incidence of BKV in Korean sporadic CJD and OND patients, we screened urine samples from 94 sporadic CJD and 54 OND patients and analyzed the genotype of BKV isolates.

Table 1. Characteristics of sporadic CJD patients and OND patients

	Sporadic CJD	OND
Number of subjects	94	54
Gender		
Male	42 (44.7%)	23 (42.6%)
Female	52 (55.3%)	31 (57.4%)
Mean age ($\pm$ SD) at disease onset, years	65.9 $\pm$ 7.9	66.2 $\pm$ 10.4
Male	65.5 $\pm$ 7.9	68.3 $\pm$ 9.2
BKV-positive	68.0 $\pm$ 2.0	67.0 $\pm$ 6.1
BKV-negative	65.3 $\pm$ 8.2	68.6 $\pm$ 10.0
Female	66.2 $\pm$ 8.0	64.6 $\pm$ 11.1
BKV-positive	68.6 $\pm$ 8.1	59.5 $\pm$ 14.4
BKV-negative	65.4 $\pm$ 7.8	65.3 $\pm$ 10.7

Material and Methods

Urine Samples

Urine samples were collected from 94 sporadic CJD and 54 OND patients between January 2002 and December 2007. We used previously established criteria [19] for diagnosis of sporadic CJD in Korea. The sporadic CJD cases in Korea have been reported previously [20–22]. The study was approved by the Ethical Committee of Hallym University Sacred Heart Hospital and informed consent was given by all subjects or their caregivers. All urine samples were frozen at -70° until used. The characteristics of subjects are shown in table 1.

DNA Extraction

40 ml of urine was centrifuged at 1,300 g for 10 min at 4° to generate pellets. The pellet was dissolved in 1.3 ml 10 mM Tris-HCl (pH 7.6) and 10 mM EDTA, and digested with 100 μ g/ml proteinase K in the presence of 0.5% SDS. The mixture was incubated at 56° for 1 h. DNA was extracted from the digest with phenol saturated with 10 mM Tris-HCl and chloroform-isoamyl alcohol (24:1, v/v) and then recovered by 5 M NaCl and cold ethanol and centrifuged at 30,000 rpm for 30 min at 4° . The pellet was dissolved in 80 μ l of sterilized water and used for polymerase chain reaction (PCR).

PCR Analysis

The DNA extracted from the urine was used to amplify BKV DNA. The 287-bp VP1 region of BKV was amplified by PCR using primers BKV1 and BKV2. Primer BKV1 (5'-CAAGTGCCAA-AACTACTAAT-3') spanned nucleotides 1630–1649 of the BKV genome and primer BKV2 (5'-TGCATGAAGGTTAAGCATGC-3') spanned nucleotides 1937–1956 [23]. The PCR reagents contained 50 pmol of each primer, 5 μ l of 10 $\times$ Taq DNA polymerase buffer, 1.5 mM MgCl₂, 0.2 mM of the dNTP mixture, and 2.5 units of Taq DNA polymerase (Promega). The PCR conditions were as follows: an initial denaturation step of 94° for 3 min, 40 cycles of 94° for 30 s, 56° for 30 s, and 72° for 2 min and a final extension at 72° for 10 min. The PCR products were separated on a 1.2% agarose gel and visualized with ethidium bromide staining under UV light.

Table 2. Summary of BKV prevalence in Korean sporadic CJD patients and OND patients

Subject groups	Urine samples examined, n	BKV DNA-positive samples, n (%)	BKV-positive number/total number of individuals (%)	
			male	female
OND	54	9 (16.7)	5/23 (21.7)	4/31 (12.9)
Sporadic CJD	94	16 (17.0)	3/42 (7.1)	13/52 (25.0)

Nucleotide Sequencing Analysis

The PCR products were purified for sequencing using a PCR purification kit (Qiagen). The DNA sequencing was carried out on an ABI 377 automatic sequencer using a *Taq* dideoxy terminator cycle sequencing kit (ABI). Nucleic acid sequences were assembled and edited using a combination of the ABI 377 DNA Sequencer Data Analysis Program and Sequence Navigator Software.

Statistical Analysis

Statistical analysis was performed using the SAS 8.2 software (SAS Institute, Inc.). Statistical parameters were analyzed by the χ^2 test or Fisher's exact test.

Phylogenetic Analysis

A neighbor-joining (NJ) phylogenetic tree was constructed using the Clustal W program and the TreeView 1.4 program. Divergences were estimated by the two-parameter method. The bootstrap test was applied to estimate the confidence of the branching patterns of the NJ tree.

Results

Detection of BKV DNA in Korean Sporadic CJD Patients

The general characteristics of the sporadic CJD patients and OND patients are shown in table 1. Urine samples were collected from 94 Korean CJD (mean age 65.9 years) and 54 OND patients (mean age 68.3 years). The mean age of BKV-positive sporadic CJD patients (65.5 years) in males was very similar to that of OND patients (68.3 years), whereas the mean age of BKV-positive sporadic CJD patients (68.6 years) in females was higher than that of OND patients (59.5 years) (table 1). To detect the presence of BKV, urine samples were screened by PCR using BKV1 and BKV2 primers. BKV DNA was detected in 16 (17.0%) of 94 Korean CJD patients and 9 (16.7%) of 54 OND patients (table 2). The detection rate of BKV was not significantly different in urine samples of sporadic CJD patients from those of OND patients ($p = 0.9558$). We have also examined whether there are significant dif-

Table 3. Incidence of subtypes of BKV in sporadic CJD patients and OND patients in South Korea

Subject groups	BKV isolates examined, n (%)	Isolates classified as indicated subgroup, n (%)			
		I	II	III	IV
OND	9 (100)	6 (66.7)	0	2 (22.2)	1 (11.1)
Sporadic CJD	13 (100)	10 (76.9)	0	0	3 (23.1)

ferences in the prevalence of BKV DNA between CJD and OND patients based on gender. There was no significant difference in BKV prevalence between CJD and OND patients in men ($p = 0.1193$) as well as in women ($p = 0.2631$). This result suggests that the prevalence of BKV is not associated with sporadic CJD in the Korean population.

BKV Subtypes in Korean Sporadic CJD Patients

To further characterize the genotypes of BKV detected in urine samples from Korean sporadic CJD and OND patients, we sequenced the 287-bp typing region of BKV DNA from 22 BKV isolates. The frequency of BKV subtypes in the sporadic CJD and OND patients is shown in table 3. The BKV-positive OND patients were classified into three subtypes, namely I, III, and IV, which were detected in 66.7, 22.2, and 11.1%, respectively. However, subtypes I and IV were detected in 76.9 and 23.1% of BKV-positive sporadic CJD patients, respectively. Interestingly, subtype III was not detected in sporadic CJD patients. In addition, subtype II was not detected in either OND or sporadic CJD patients. There is no significant difference in type frequency of BKV between Korean sporadic CJD and OND patients ($\chi^2 = 0.0031$, $p = 0.9558$). These results indicate that sporadic CJD may be not associated with BKV subtypes.

In order to identify the combined effects of BKV and prion protein gene (*PRNP*) on the pathogenesis of prion disease, BKV-positive data was stratified with *PRNP* codon 129 or with codon 219 genotypes (table 4). We found no significant differences in the frequency of BKV according to *PRNP* codon 129 or 219.

Phylogenetic Analysis of BKV Isolates Based on DNA Sequences

In order to investigate the phylogeny of BKV isolates, a phylogenetic tree was constructed using the NJ method from the DNA sequences of 22 BKV isolates derived from urine samples of Korean patients with sporadic CJD and

Table 4. Prevalence and genotyping of BKV according to *PRNP* codon 129 or 219 status

BKV subgroups	OND (n = 54)			Sporadic CJD (n = 94)		
	MM (n = 51)	MV (n = 3)	VV (n = 0)	MM (n = 94)	MV (n = 0)	VV (n = 0)
<i>PRNP</i> 129	8	1	0	13 ^a	0	0
I	6	0	0	10	0	0
II	0	0	0	0	0	0
III	1	1	0	0	0	0
IV	1	0	0	3	0	0
	EE (n = 51)	EK (n = 3)	KK (n = 0)	EE (n = 94)	EK (n = 0)	KK (n = 0)
<i>PRNP</i> 219	9	0	0	13 ^a	0	0
I	6	0	0	10	0	0
II	0	0	0	0	0	0
III	2	0	0	0	0	0
IV	1	0	0	3	0	0

^a Numbers except for unclassified 3 BKV isolates.

OND and those of other 18 BKV subtypes that were previously identified. According to the phylogenetic tree (fig. 1), of the 22 BKV isolates, 2 were subtype III, 4 in the subtype IV, and 16 in the subtype I cluster which belong to subgroup Ic.

Discussion

In this study, we examined the BKV subtype profile based on gender from the urine samples of Korean sporadic CJD and OND patients (table 2). The incidence of BKV DNA excretion in Korean sporadic CJD patients was similar to that in OND patients. In addition, there was no significant difference in the genotype frequencies of BKV. Our results suggest that the incidence of BKV and the virus genotype are not associated with sporadic CJD.

The BKV is responsible for the renal dysfunction in immunosuppressed patients [24] and hemorrhagic cystitis in bone marrow transplant patients [25]. This virus is widespread in the human population [26], infecting asymptotically during early childhood. It persists in the kidney throughout life [27]. The BKV genome consists of a circular double-stranded DNA. Genotype analysis of BKV revealed four distinct subtypes according to the DNA sequence variation of BKV VP1 region [28]. BKV subtype I is mainly distributed throughout the world. Based on the phylogenetic analysis, subtype I can be further subdivided

into four subgroups, such as subgroups Ia, Ib-1, Ib-2, and Ic [29–32]. BKV subtypes II and III are rarely distributed throughout the world. BKV subtype IV is mainly distributed in East Asians and divided further into six subgroups, such as subgroups IVa-1, IVa-2, IVb-1, IVb-2, IVc-1, and IVc-2. Similar to subtype I, each of subtype IV subgroups has a specific geographical distribution pattern [33]. The different subtypes of BKV are associated with human populations in different geographic regions [34].

In this study, the genotype distribution of BKV isolates found in urine samples of Korean CJD patients was compared with that of OND patients. There was no significant difference in the genotype distribution of BKV between OND and sporadic CJD (table 3). In addition, the distribution of BKV subtypes in controls is also very similar with that of OND and sporadic CJD patients in the Korean population. These results indicate that the distribution of BKV subtypes is not associated with the sporadic CJD.

Previously, the incidence of BKV in the urine of immunocompetent individuals in the Korean population has been reported [35]. 35% of the examined immunocompetent individuals were BKV-positive. The incidence of BKV in Korean normal controls was higher than that in sporadic CJD patients (17.0%, $p = 0.0016$) and OND patients (16.7%, $p = 0.0098$). This result may be due to the difference of the storage period or the age of the cases analyzed.

The compartmentalized replication of BKV in different organs is shown in many studies that identify isolates from different organs with differences in the genomic sequences [36]. Rearrangements have also been identified for BKV in brain and cerebrospinal fluid [37]. The BKV is present in brain tumors with a controversial result [38]. Our negative result in urine samples may not be generalized to sporadic CJD. Therefore, further studies are required to confirm whether the incidence and genotypes of BKV are associated with sporadic CJD by examining various samples including brain and cerebrospinal fluid.

PRNP plays a crucial role in conferring susceptibility to prion diseases. Polymorphisms of *PRNP* at codons 129 and 219 have been shown to be a genetic factor for sporadic CJD [39]. We investigated whether the combined effects between *PRNP* polymorphisms and BKV subtypes are responsible for the susceptibility to sporadic CJD. When BKV subtypes were stratified by *PRNP* codons 129 or 219, no significant correlation was found between the distribution of BKV subtypes and *PRNP* polymorphisms (table 4).

In conclusion, we determined the relationship between BKV infection and sporadic CJD by examining the

Fig. 1. Phylogenetic analysis of the BKV isolates from Korean CJD and OND patients. A NJ tree was constructed from Korean BKV isolates from this study and the reported BKV DNA sequences using the Clustal W and TreeView 1.4 program. The numbers at the nodes indicate bootstrap confidence levels obtained from 100 replicates. Only values greater than 50% are shown.

incidence rate and genotype of BKV from urine of Korean sporadic CJD patients. We found no correlation between the incidence of BKV infection and sporadic CJD. Also, there was no association in the frequency of BKV subtypes between Korean sporadic CJD and OND patients.

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