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Recombined sequences between the non-coding control regions of JC and BK viruses found in the urine of a renal transplantation patient

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Abstract Kidney cells are the common host for JC virus (JCV) and BK virus (BKV). Reactivation of JCV and/or BKV in patients after organ transplantation, such as renal transplantation, may cause hemorrhagic cystitis and polyomavirus-associated nephropathy. Furthermore, JCV and BKV may be shed in the urine after reactivation in the kidney. Rearranged as well as archetypal non-coding control regions (NCCRs) of JCV and BKV have been frequently identified in human samples. In this study, three JC/BK recombined NCCR sequences were identified in the urine of a patient who had undergone renal transplantation. They were designated as JC–BK hybrids 1, 2, and 3. The three JC/BK recombinant NCCRs contain up-stream JCV as well as down-stream BKV sequences. Deletions of both JCV and BKV sequences were found in these recombined NCCRs. Recombination of DNA sequences between JCV and BKV may occur during co-infection due to the relatively high homology of the two viral genomes.

Keywords Renal transplantation · JCV · BKV · DNA recombination

Human JCV and BKV belong to the *Polyomaviridae*. JCV and BKV were first identified in the brain tissue from a patient with progressive multifocal leukoencephalopathy (PML) [1] and the urine of a renal transplantation patient, respectively [2]. Primary infection of these viruses occurs in childhood. Although 70–90 % of human population is seropositive for these viruses [3], both JCV and BKV are usually latent in the kidney after infection [4, 5]. JCV may lytically infect oligodendrocytes and cause PML in patients with AIDS [6, 7]. On the other hand, reactivation of BKV is commonly found in patients after bone marrow transplantation and in renal transplant patients resulting in hemorrhagic cystitis and polyomavirus-associated nephropathy (PVAN) [8–10].

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Polyomavirus genome is a circular double-stranded DNA of 5 kb that contains a non-coding control region (NCCR), an early region, and a late region. The replication origin and the promoter/enhancer for early and late gene expression are located within the NCCR. The late region is responsible for the expression of structural proteins, VP1, VP2, and VP3, and a small agno peptide [11]. The early region is responsible for expression of regulatory proteins, the large and small tumor antigens. Based on the structure of the NCCR of JCV, it can be characterized into archetypal and rearranged genotypes. Archetypal sequences, such as CY [12], can be separated into A, B, C, D, E, and F boxes, with each box containing binding sites for various cellular nuclear factors involved in viral transcription [13–15]. WW genotype is one of BKV archetypes. The NCCR of WW BKV is divided into five sections: O142, P68, Q39, R63, and S63, where the numbers indicate the size of the units in bp [16–19]. Rearranged genotypes contain sequences that have deletions, duplications, and insertions when compared with the archetypal sequence [20]. Recombination between the NCCRs of JCV and BKV has not been reported previously. In this study, three sequences containing the recombination sequences of JCV and BKV NCCR were found in the urine of a renal transplantation patient.

Urine specimens were collected from a patient who had undergone renal transplantation for the detection of polyomavirus infection. The transplanted kidney appeared to be stable and not undergoing severe rejection based on the serum creatinine level of $\sim$1.5 mg/dl throughout the period of follow-up. When first identified a month after transplantation, four DNA fragments were detected by PCR using primers, JBR1 (5′-CCTCCACGCCCTTACTACTTCTGAG-3′) and JBR2 (5′-GTGACAGCTGGCGAAGAACCATGGC-3′), spanning the conserved sequences of the NCCRs of JCV and BKV [21]. The sizes of the DNA fragments were between 200 and 400 bp (Supplementary Fig. 1, lane 1). When the second urine sample was collected 1 month later for PCR amplification, only two DNA fragments were detected (Supplementary Fig. 1, lane 2). However, during the third and fourth amplifications, only a single DNA fragment was detected in the urine specimens as shown in Supplementary Fig. 1 (lanes 3 and 4). The urine specimens were collected approximately a month apart.

The DNA fragments were purified and sequenced on both strands. The exact sizes of the DNA fragments from the first urine samples determined by DNA sequencing were 340, 298, 260, and 219 bp. The sizes of DNA fragments from the second urine sample were 340 and 260 bp. The sizes of DNA fragment from the third and forth urine samples were both 340 bp. The sequencing results showed that the 340 bp DNA fragment was identical to that of CY genotype of JCV (Fig. 1). While the sequences of the 298, 260, and 219 bp DNA fragments were found to show recombination between the NCCRs of JCV and BKV (Fig. 1, JC–BK hybrids 1, 2, and 3). JC–BK hybrid 1 contains 97 bp from *ori* of the JCV NCCR sequence with a point deletion at nucleotide (nt) 82. The down-stream sequences of JC–BK hybrid 1 consists of BKV NCCR sequences from nt 34 to 295 with deletions from nt 73 to 81, 146 to 178, and 202 to 262 compared with the WW genotype (Fig. 1). JC–BK hybrid 2 contains JCV NCCR sequences from nt −45 to 97 with a deletion between nt 40 and 77, and a point deletion at nt 82. The BKV sequences in the JC–BK hybrid 2 were the same as those found in hybrid 1 except there was an additional nucleotide at the 5′-end (Fig. 1). Sequence of JC–BK hybrid 3 consists of nt −45 to 37 of JCV and nt 38 to 295 of BKV with deletions from nt 50 to 59, 73 to 81, 156 to 178, and 202 to 264 (Fig. 1). Accession numbers in GenBank for JC–BK hybrids 1, 2, and 3 are AF447050, AF447051, and AF447052, respectively. Furthermore, a pair of control designing specific primers, JBR1 (5′-CCTCCACGCCCTTACTACTTCTGAG-3′) and JBRR (5′-GCAGTTAATAGTGAAACCCC-3′), that overlap the chimeric regions was used to assess the real nature of the recombinant molecules and the dynamics of emergence and extinction of the recombinants in the urine samples. As shown in Supplementary Fig. 2, the sizes of the PCR DNA fragments were as expected to be 205, 237, 199, and 164 bp for CY, hybrids 1, 2, and 3, respectively. The results were consistent with that found in Supplementary Fig. 1.

The NCCR of human polyomavirus is highly diverse resulting in a range of different viral variants. The mechanism by which this diversity arises is still not clear. Different variants of the virus have been found in different tissues or co-infected with different viruses in the same tissue [22–24]. This is the first report to demonstrate that the NCCR regions of JCV and BKV that have undergone recombination can be detected in the urine of a patient who had undergone renal transplantation. No evidence of rejection was observed during the patient's routine follow-ups as prednisolone and cyclosporine had been prescribed after renal transplantation.

Members of the general human population are commonly infected with BKV and/or JCV. Such infections are usually symptomless when the individual is immuno-competent [25–27]. However, polyomavirus-associated nephropathy (PVAN) is often diagnosed among patients after renal transplantation due to reactivation of the two viruses. BKV seems to be more associated with cases of PVAN among renal transplantation patients, although JCV is occasionally detected in the urine of PVAN patients [28–32]. As co-infection of BKV with JCV has been reported [33], exchanges of viral genetic materials during co-infection may occur. Genetic materials exchanges have been reported in cetacean papillomaviruses [34] and HBV [35]. However, BKV was not detected in the urine samples. It may be due to the dynamics of emergence and disappearance of the virus.

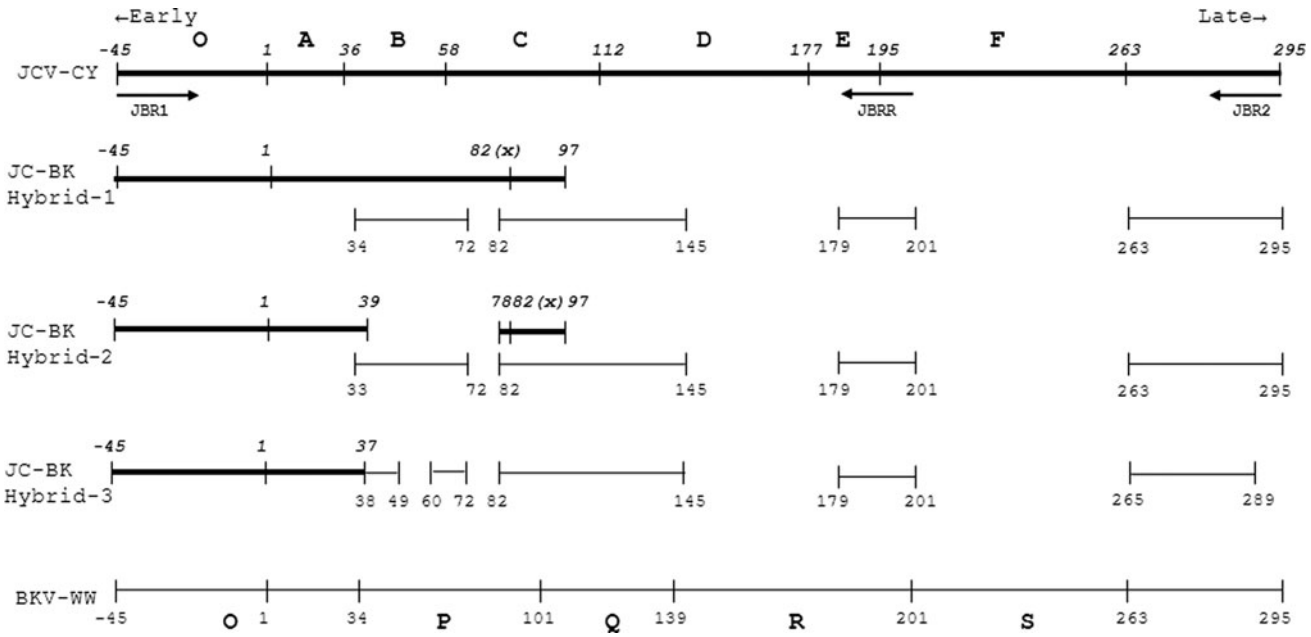

Fig. 1 Schematic representation of the JCV and BKV NCCRs identified in the examined urine samples. The NCCR of the CY genotype of JCV and the WW genotype of BKV are shown for comparison. The NCCR of the CY genotype is divided into sections O, A, B, C, D, and F. The NCCR of the WW genotype is divided into sections O, P, Q, R, and S. Sequences of JC–BK hybrid 1, JC–BK hybrid 2, and JC–BK hybrid 3 as identified from the urine samples of a patient who had undergone renal transplantation. The *thick line* represents the NCCR sequence of JCV. The *thin line* represents the NCCR sequence of BKV. The *spaces in between lines* represent the deleted sequences. The order of sequences within each recombinant NCCR represents the tandem repeated sequences. JBR1, JBR2, and JBRR are the primers used for PCR in this study

The kidney is the common tissue involved in both BKV and JCV infection. Therefore, kidney cells may be co-infected with both viruses in the same individual. There is ~70 % identity in DNA sequence between the NCCRs of BKV and JCV, as a result DNA recombination between the NCCRs of BKV and JCV is highly possible when there is persistent co-infection of kidney cells by these viruses. In the current study, three JC/BK NCCR recombined genotypes were identified in the urine of a patient who had undergone renal transplantation. The JC/BK NCCR recombined genotypes were transiently shed into the urine. Based on the medication history of the patient, the prescription of immunosuppression agents was persistent and the graft had remained stable during the viral genotype shift. The biological relevance of the JC/BK recombinants is not clear and needs to be further investigated.

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