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Review

Polymorphism in the genome of non-passaged human polyomavirus BK: implications for cell tropism and the pathological role of the virus

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Abstract

Worldwide studies have demonstrated that the human polyomavirus BK resides ubiquitously in the human population. After primary infection, which occurs mainly during childhood, the virus seems to establish a life-long harmless infection in the host. However, impaired immune functions may lead to reactivation of BK virus. The recent findings that associate BK virus with an increasing number of clinical conditions, including renal, pulmonary, ophthalmologic, hepatic, neurological, and autoimmune diseases, has resuscitated the interest in this virus as a pathogenic agent. This review focuses on polymorphisms in the genomes of non-passaged BK virus isolates from nonneoplastic tissues, with special focus on the transcriptional control region, the regulatory proteins large T-antigen and agnoprotein, and the major capsid protein VP1. The possible implications of genome diversity with respect to cell tropism, pathogenicity, and therapeutic strategies are discussed.

Keywords: Human polyomaviruses; Genomic polymorphism; Transcriptional control region; VP1; Large T-antigen; Agnoprotein; BKV-associated diseases

Introduction

The human polyomavirus BK (BKV) was originally isolated from the urine of a renal allograft recipient who developed ureteric stenosis (Gardner et al., 1971). Serological surveys have shown that BKV is distributed worldwide and has a high incidence among humans. Primary infection occurs predominantly during childhood and is reflected by the high prevalence of IgG antibodies in this group of individuals. By the age of 10, antibodies against the capsid proteins of BKV can be detected in 50% to 100% of the examined children (reviewed in Knowles, 2001). Primary infection appears to be asymptomatic, although few cases of BKV-induced mild respiratory or urinary tract diseases, pyrexia, fatal disseminated infection, and hemorrhagic cystitis have been reported in immunosuppressed patients. After primary infection, BKV establishes a harmless latent infection in healthy individuals (reviewed in Dörries, 2001). Despite its lack of obvious pathogenic properties in healthy individuals, BKV immediately received major scientific attention after its original isolation in 1971 because BKV, together with the simultaneously discovered JC virus (JCV), were the first human viruses that structurally and genomically strongly resembled the oncogenic polyomaviruses simian virus 40 and mouse polyomavirus. In accordance with these viruses, BKV was able to transform cells (also human cells) in vitro and to induce tumors in rodents. This encouraged researchers to examine a possible role of BKV in human cancers. Although BKV nucleic acid sequences or viral proteins have been detected in human tumors, a role for BKV in malignancy remains to be obtained (Corallini et al., 2001; White and Khalili, 2004). BKV has also been recognized as an important human pathogen in non-carcinogenic diseases. Active BKV infection has been associated with hemorrhagic and non-hemorrhagic cystitis, uretic stenosis, tubulointer-

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stitial nephritis, asymptomatic nephritis, interstitial desquamative pneumonitis, atypical retinitis, meningoencephalitis, hepatic dysfunction, and autoimmune diseases (reviewed in de Bruyn and Limaye, 2004; Hirsch and Steiger, 2003; Reploeg et al., 2001). The exact mechanisms of BKV reactivation remain elusive, but several factors may affect the latency state of BKV and/or the outcome of BKV-associated disease. The immune system of the host is pivotal to control viral infections, and both inherited and acquired immune dysfunctions increase the susceptibility to various infectious complications. Reactivation of BKV in individuals with inherited immune dysfunctions has been documented in two case reports. A 6-year-old boy with hyper-IgM syndrome, and a 5-year-old boy with cartilage-hair hypoplasia, an autosomal recessive disease that affects T cells and impairs humoral immune responses, both developed severe tubulointerstitial nephropathy or BKV-associated nephropathy (de Silva et al., 1995; Rosen et al., 1983). Furthermore, frequent BKV viruria is observed in patients with acquired immune deficiencies (AIDS) or allograft patients treated with immunosuppressive drugs compared to healthy controls (reviewed in Hirsch and Steiger, 2003; Knowles, 2001). The activation state of BKV may also be affected by modulators like co-infections with other viruses, or increased steroid hormone levels during pregnancy (Bendiksen et al., 2000; Bratt et al., 1999; Moens et al., 1994; Smith et al., 1998; and references therein). Host cellular determinants, such as receptors affinity for BKV and cell-specific transcription factors that can regulate viral expression, may explain the difference in cell tropism between different BKV strains. Finally, the composition of the transcription control region of BKV and mutations in the coding regions may determine the cell permissivity of the BKV strain. Whether polymorphisms in the genomes of non-cell propagated BKV strains influence BKV activity and BKV-associated diseases is the focus of this review.

Molecular biology of BKV

BKV, or polyomavirus hominis 1, belongs to the *Polyomaviridae*, a family of small non-enveloped DNA viruses. The virus has an icosahedral capsid with a diameter of 45 nm. The capsid is built up of the viral proteins VP1, VP2, and VP3. VP1 is the major protein and accounts for 80% of the total protein content of the virus particle. The viral capsid consists of 360 molecules of VP1 arranged in 72 pentamers. These pentamers reside at the outer surface of the viral capsid, while VP2 and VP3 reside in the inner part of the particles. The genome of BKV consists of a circular double-stranded DNA molecule of approximately 5.3 kbp that is packed with host cell histones. The genome can be divided in three functional domains: (1) the early region that encodes regulatory proteins, including the large T-antigen and small t-antigen; (2) the late region that encompasses the genetic information for the capsid proteins VP1, VP2, VP3, and the agnoprotein; and (3) the non-coding control region that spans the origin of replication (O-block, 142 base-pairs) and sequences involved in transcriptional regulation of both the early and the late genes, referred to as the transcriptional control region (TCR). The TCR of the proposed archetypal BK strain WW has been arbitrarily divided into four transcription factor binding sequence blocks, called P (68 base-pairs), Q (39 base-pairs), R (63 base-pairs), and S (63 base-pairs) as depicted in Fig. 1. The different BKV strains display a marked heterogeneity in their TCR due to point mutations, deletions, duplications, and rearrangements in this region (reviewed in Moens and Rekvig, 2001; Moens et al., 1995). These rearrangements, but also mutations in other parts of the genome, may offer advantages for the virus in its host. The possibility that mutant BKV strains with greater propensity for replication are circulating in clinical populations has been suggested (Stoner and Hübner, 2001). Recently, other genomic regions besides the TCR of BKV isolates that were not propagated in cell culture have been sequenced. This review analyzes in detail the variety of BKV sequences obtained from different tissues and different patients with distinct clinical conditions. We also discuss the possible implications of BKV genome variability on the pathogenic potentials of this virus in humans. The association of BKV with human tumors and the diversity of BKV sequences in neoplastic tissues have been recently reviewed elsewhere and are beyond the scope of this review (Corallini et al., 2001; White and Khalili, 2004).

BKV and polymorphism: the transcriptional control region

In vitro cell culture studies have shown that cell passage provokes rearrangements in the TCR of BKV. Viruses with rearranged TCR display different oncogenic potentials in animal models and altered replication properties in vitro (reviewed in Moens et al., 1995). The polymerase chain reaction technique allows the amplification of limited amounts of BKV DNA without cell propagation. The BKV DNA generated after PCR can then be directly sequenced. Hence, the sequence of TCR DNA present in clinical samples can be directly determined and mutations induced by cell passage is avoided. An overview of the TCR variants of BKV DNA that was directly obtained from clinical specimens is provided in Table 1. Archetypal strains with a linear PQRS anatomy, as well as rearranged strains with deletions or/and duplications of complete or part of the different blocks have been isolated from most tissues examined (Table 2). There seems to be a selection to preserve the P block as all described variants so far retain P sequences, while several TCR variants lack one or several of

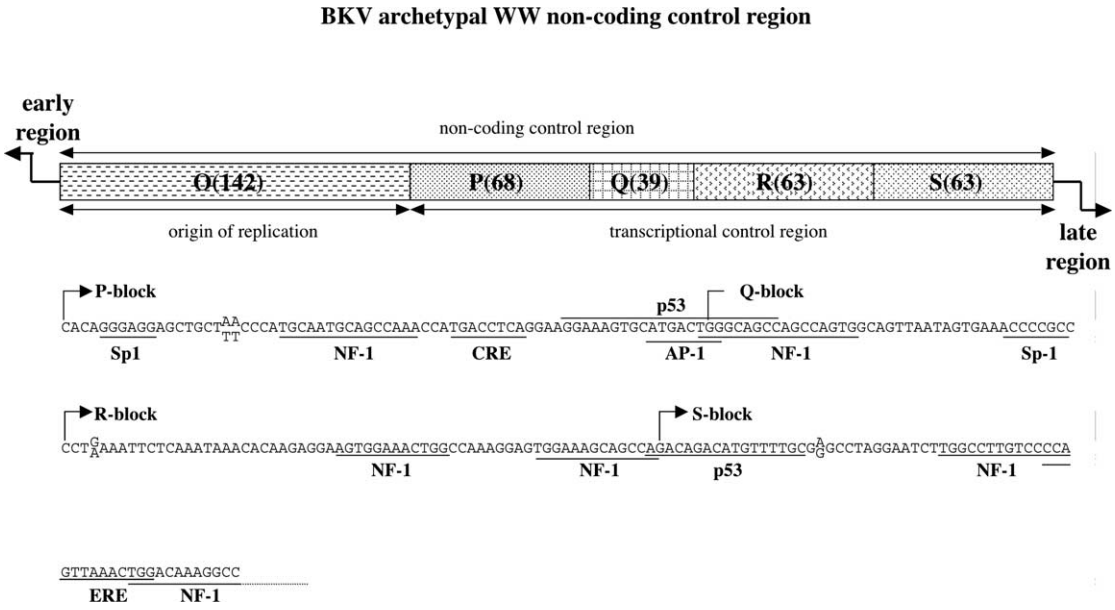

Fig. 1. Schematic presentation of the archetypal BKV (WW) non-coding control region using the O—P—Q—R—S nomenclature. The consensus sequence of the P, Q, R, and S block is given. The nucleotides $^{A}/_{T}$ in the P block (respectively $^{G}/_{A}$ in the R block, and $^{A}/_{G}$ in the S block) indicates that either one of these nucleotides is preferentially found at this site. The numbers in parentheses represent the number of base pairs in each block. A broken arrow indicates the early and late regions. Proven binding sites for transcription factors are depicted.

the other blocks (e.g., PQ, PQS, PPPS, PQR). All known variants, but two (T1R1.BK and T5R.BK clone 4; Li et al., 2002) have also retained S block sequences, indicating the importance of these sequences. In addition to the extensive changes, various point mutations have been characterized in the TCR. The point mutations in each BKV strain, as compared to the consensus sequence given in Fig. 1, are summarized in Table 3. Rearrangements as well as point mutations may affect putative binding sites for host transcription factors. This has been extensively reviewed previously and will not be further discussed here (Moens et al., 1995).

The conclusion whether specific BKV strains preferentially reside in specific tissues is hampered for two reasons. First, it is difficult to trace whether the detected strain is the original one that latently infected the host tissue or whether this strain was generated by recombination during BKV DNA replication after reactivation of the virus. Indeed, during latency, replication activity of the virus is absent or low and very few genome copies are present per cell, consequently reducing the possibility of recombination. During reactivation of the virus, replication activity, and therefore the number of genomes will increase. A higher number of genome copies may lead to recombination and creation of new TCR variants, often with more than one origin of replication (Hara et al., 1986; Rubinstein et al., 1991; Sugimoto et al., 1989; Watanabe and Yoshiike, 1986). Thus, while the archetypal strain may originally latently infect a cell, reactivation may lead to rearranged TCR variants that replicate better than the archetypal strain. These mutant strains may outgrow the parental virus. Secondly, only a few studies have addressed the diversity of TCRs in different tissues of the same person. In one study, Stoner et al. (2002) either PCR amplified or cloned the TCR DNA from brain, cerebrospinal fluid (CSF), kidney, lung, and urine from a patient with chronic lymphocyte leukemia with a fatal case of BKV tubulointerstitial nephritis. PCR-amplified TCR sequences from urine and lung possessed the archetypal PQRS anatomy, whereas rearranged PQSPQS TCR were obtained in brain and CSF samples. However, cloning of the TCR sequences isolated from CSF, kidney, lung and brain tissue revealed both the archetypal TCR, as well as rearranged TCR variants (Table 1). Cloned TCR DNA from brain and CSF from this patient exposed a PQSPQS TCR, while PQPQS and PQPQPQRS rearrangements were detected in his kidney samples. In another study, BKV (CNS) strain with rearranged PQRSPQRS anatomy was detected by PCR in CSF, brain, and eye tissue from an AIDS patient, while urinary shed virus had an archetypal PQRS TCR (Jørgensen et al., 2003). These findings are in agreement with Stoner et al. who by PCR observed rearranged TCRs in the kidney, while archetypal PQRS TCRs were detected in urine. Stoner et al., however, also found archetypal TCR in the kidney, after cloning the BKV DNA. Although the presence of different TCR variants in both patients may be biological, they may also be explained by the use of different methods to obtain the BKV sequences. In a biological specimen containing distinct amounts of related DNA molecules (e.g., BKV genomes), PCR will proportionally amplify the different DNA species. Subsequent cycle sequencing of the obtained PCR products will generate strong signals for the abundant PCR products, while scarcely represented DNA templates will produce minor signals that may not exceed above the noise ratio.

Table 1
Transcriptional control region variants found among non-passaged BKV strains directly isolated from nonneoplastic tissues and body fluids from healthy individuals and patients

Strain	Accession number	Anatomy	Point mutations	Specimen	References
WW and WW-like strains					
A191-2	AF218448	$P_{1–40,60–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	R_5:A→G R_{38}:C→G R_{39}:T→C R_{40}:G→A S_{22}:T→G S_{49}—T—S_{50}	renal transplant patient	Chang and Wang, unpublished
A250-1a	AF218447	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	R_5:A→G R_{38}:C→G R_{39}:T→A R_{41}:ΔG S_{22}:T→G S_{49}—T—S_{50}	renal transplant patient	Chang and Wang, unpublished
Azzi-U	AY273186	$P_{1–40,49–68}$—$Q_{1–27}$—$R_{2–63}$—$S_{1–63}$	R_{17}:A→T R_{41}:G→T S_{24}:G→A S_{49}—T—S_{50}	urine renal transplant patient	Azzi et al., unpublished
Azzi–K	AY272187	$P_{1–40,49–68}$—$Q_{1–25}$—$R_{13–63}$—$S_{1–63}$	R_{13}:A→T R_{41}:G→T S_{24}:G→A S_{49}—T—S_{50}	kidney from renal transplant patient	Azzi et al., unpublished
BMT-2,-7,-10, -12,-13,-15-19		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{56}:A→C or G ΔP_{57} R_5:A→G R_6:A→C R_{38}:C→G R_{40}:G→A R_{41}:C→T	urine from BMT[a] patients	Negrini et al. (1991)
BMT		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	Q_{36}:C→G Q_{38}:C→G R_2:C→T	urine from BMT patients	Priftakis et al. (2001, 2003)
CAL	AF317623	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{27}:A→G P_{31}:C→T P_{56}:A→G R_{13}:A→C R_{23}:A→C R_{35}:A→G R_{43}:C→A R_{47}:ΔG	not specified	Bhattacharjee and Chakraborty, unpublished
CAP–h2	AY628226	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{31}:C→T	heart from a patient with BKV-associated capillary leak syndrome	Chen et al. (2004)
CAP-h5	AY628228	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{31}:C→T	heart from a patient with BKV-associated capillary leak syndrome	Chen et al. (2004)

CAP-h8	AY628229	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{31}:C→T	heart from a patient with BKV-associated capillary leak syndrome	Chen et al. (2004)
CAP-h22	AY628227	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{31}:C→T	heart from a patient with BKV-associated capillary leak syndrome	Chen et al. (2004)
CAP-m2	AY628212	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{31}:C→T	striated muscle from a patient with BKV-associated capillary leak syndrome	Chen et al. (2004)
CAP-m5	AY628232	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{31}:C→T R_{29}:A→G	striated muscle from a patient with BKV-associated capillary leak syndrome	Chen et al. (2004)
CAP-m9	AY628233	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{31}:C→T	striated muscle from a patient with BKV-associated capillary leak syndrome	Chen et al. (2004)
CAP-m13	AY628230	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{31}:C→T	striated muscle from a patient with BKV-associated capillary leak syndrome	Chen et al. (2004)
CAP-m18	AY628224	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{31}:C→T	striated muscle from a patient with BKV-associated capillary leak syndrome urine SLE patient	Chen et al. (2004)
cl-108 G7RBK	AF374595	$P_{1–41,49–68}$—$Q_{1–39}$—$R_{3–63}$—$S_{1–63}$ $P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{14}:T→C	urinary cells from a FSGS[b] patient	Sugimoto et al. (1989), Li et al., unpublished
HC-u2	AY628234	P_{1-68}—$Q_{1–11}$—$R_{22–63}$—$S_{1–63}$		urine from healthy control	Chen et al. (2004)
HC-u5	AY628235	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$		urine from healthy control	Chen et al. (2004)
HC-u9	AY628236	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$		urine from healthy control	Chen et al. (2004)
HI-u5	AY628225	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{31}:C→T	urine from HIV-2 patient	Chen et al. (2004)
HI-u6	AY628237	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{31}:T→C R_{29}:A→G S_{18}:G→A	urine from HIV-2 patient	Chen et al. (2004)
HI-u8	AY628238	$P_{1–39,48–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{31}:C→T R_{29}:A→G	urine HIV-2 patient	Chen et al. (2004)
K8R2.BK 2+3	AF442896 + AY442897	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{31}:C→T	urine from a renal transplant patient	Li et al. (2002)
MT-1,cl-6,-8, -50,-87,-93		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	R_2:C→A R_{58}:C→T S_{22}:T→G S_{29}:C→G	urine SLE patient	Sugimoto et al. (1989)
PAT-A,PAT-E	S72998	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	Q_7:C→G	urine from AIDS patient (PAT-A) or from acute myeloid leukaemia patient (PAT-E)	Schätzl et al. (1994)
P1		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{31}:C→T P_{55}:G→T	urine from pregnant women	Bhattacharjee and Chakraborty, (2004)
RA	AF356531	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–4}$	P_{31}:C→T	sewage sample	Bofill-Mas et al. (2001)
S1,S2		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{31}:C→T R_{24}:A→C	urine from renal transplant patient	Bhattacharjee and Chakraborty, (2004)

(continued on next page)

Table 1 (*continued*)

Strain	Accession number	Anatomy	Point mutations	Specimen	References
WW and WW-like strains					
S3		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{27}:A→G P_{31}:C→T P_{56}:A→G R_{13}:A→C R_{23}:A→C R_{35}:A→G R_{43}:C→A R_{47}:ΔG	urine from renal transplant patient	Bhattacharjee and Chakraborty, (2004)
SA160900	AF356533	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–4}$[c]	P_{31}:C→T	sewage sample	Bofill-Mas et al. (2001)
SA090600	AF356532	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–4}$	P_{31}:C→T	sewage sample	Bofill-Mas et al. (2001)
Seq 1		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	R_5:A→G R_{40}:G→A S_{49}—T—S_{50}	urine from renal transplant and BMT patients	Takasaka et al. (2004)
Seq 2		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	S_{49}—T—S_{50}	urine from renal transplant patient	Takasaka et al. (2004)
Seq 3		$P_{1–41,50–68}$—$Q_{1–39}$—$R_{1–35}$—$S_{55–63}$	R_5:A→G	urine from renal transplant patient	Takasaka et al. (2004)
Seq 4		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{55}:G→C P_{58}:ΔA R_{40}:G→T R_{41}:G→T	urine from renal transplant and BMT patients	Takasaka et al. (2004)
Seq 5		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{31}:C→T R_{58}:C→T	urine from renal transplant and BMT patients	Takasaka et al. (2004)
Seq 7, 8		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	R_2:C→A R_{58}:C→T S_{23}:A→G S_{29}:C→G	urine from renal transplant and BMT patients	Takasaka et al. (2004)
Seq 9		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	R_{58}:C→T S_{23}:A→G S_{29}:C→G	urine from renal transplant patient	Takasaka et al. (2004)
Seq 10		$P_{1—41,50–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	Q_{34}:C→T R_{12}:C→T R_{58}:C→T S_{23}:A→G S_{29}:C→G	urine from renal transplant patient	Takasaka et al. (2004)
Shi 1-6		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	Q_7:C→G	urine HIV patients	Agostini et al. (1995)
T3R2.BK	AF442903	$P_{1–31,50–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	R_5:A→G R_{26}:A→G	urine from a renal transplant patient	Li et al. (2002)
T4R3.BK cl3	AF442905	$P_{1–38,48–68}$—$Q_{1–39}$—$R_{1–34}$—$S_{1–63}$	P_{31}:C→T	urine from a renal transplant patient	Li et al. (2002)
T2M.2BK	AF442894	$P_{1–41,50–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$		PBMC[d] from a renal transplant patient	Li et al. (2002)

TC-1		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{33}:G→A	urine from pregnant women and RA patients	Tsai et al. (1997); Chang et al. (1996a)
TC-2		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{33}:G→A Q_{18}:C→T Q_{27}:G→C R_2:C→G	urine from SLE and polymyositis patients	Tsai et al. (1997); Chang et al. (1996a); Chang et al. (1996b)
TIM.BK	AF442900	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{31}:C→T S_{44}:A→C	PBMC[d] from a renal transplant patient	Li et al. (2002)
U3	AF356280	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–4}$		sewage sample	Bofill-Mas et al. (2001)
USA5	AF356530	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–4}$		sewage sample	Bofill-Mas et al. (2001)
USA2	AF356529	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–4}$		sewage sample	Bofill-Mas et al. (2001)
V128-1	AF218446	$P_{1–41,50–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	R_{38}:C→G R_{40}:G→A S_{22}:T→G S_{49}—T—S_{50}	renal transplant patient	Chang and Wang, unpublished
V173-1	AF218445	$P_{1–41,50–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	R_5:A→G R_{38}:C→G R_{39}:T→C R_{40}:G→A S_{22}:T→G S_{49}—T—S_{50}	renal transplant patient	Chang and Wang, unpublished
WW	M15987	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{31}:C→T	urine from renal transplant patient	Rubinstein et al. (1987)
WW		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{31}:C→T R_{38}:C→G R_{40}:G→A S_{22}:T→G S_{49}—T—S_{50}	urine and PBMC from AIDS patient	Jørgensen et al. (2003)
WW		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1—63}$	P_{18}—T—P_{19} Q_8:C→G R_6:A→C R_7:A→T S_2:A→C	urine from pregnant women	Markowitz et al. (1991)
WW		$P_{13,50–68}$—$Q_{1–39}$—$R_{1–12,32–63}$—$S_{1–63}$		brain from patient with chronic lymphocyte leukaemia with fatal case of BKV tubulointerstitial nephritis	Stoner et al. (2002)
WW		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$		kidney sample A and C from patient with chronic lymphocyte leukaemia with fatal case of BKV tubulointerstitial nephritis	Stoner et al. (2002)
WW		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$		lung from patient with chronic lymphocyte leukaemia with fatal case of BKV tubulointerstitial nephritis	Stoner et al. (2002)

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Table 1 (*continued*)

Strain	Accession number	Anatomy	Point mutations	Specimen	References
WW and WW-like strains					
WW	AF123397–AF123428	$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{24}:T→C or A Q_{39}:ΔC R_5:A→G R_{10}:C→G R_{38}:C→G R_{40}:G→A S_{22}:T→G S_{49}—T—S_{50}	urine from SLE patients	Sundsfjord et al. (1999)
WWT		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$		urine from patient with chronic lymphocyte leukaemia with fatal case of BKV tubulointerstitial nephritis	Stoner et al. (2002)
WWT1		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	S_{29}:C→T	urine from AIDS and BMT patients	Flægstad et al. (1991)
WWT2		$P_{1–14,23–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$		urine BMT patient	Flægstad et al. (1991)
WWT3		$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	Q_7:C→G	urine from BMT patients	Flægstad et al. (1991)
16a,20		$P_{1–68}$—$Q_{1–26}$—$R_{1–8}$—$S_{1–8}$[c]		PBMC from healthy individuals	Chatterjee et al. (2000)
Rearranged strains					
AO	AF123403 + AF123404	$P_{1–67}$—$Q_{1–39}$—$R_{1–9}$—$P_{22–68}$—$Q_{1–32}$— $P_{54–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_5:ΔG P_{17}:ΔT P_{35}:C→A P_{62}:C→A P_{63}:A→C Q_2—T—Q_3 Q_{21}:T→G R_3—T—R_4	urine from SLE patients	Sundsfjord et al. (1999)
BK-OV1	AY573928	$P_{1–68}$—$Q_{1–6}$—$P_{20–68}$—$Q_{1–39}$—$S_{21–63}$	P_{19}:ΔA P_{24}—G—P_{25} S_{22}:T→G S_{49}—T—S_{50}	benign ovarian teratoma	Asomani et al., unpublished
BKV(Clin)		41 bp duplication and/or 48 bp deletion patient		kidney from AIDS	Smith et al. (1998)
BKV(CNS)	AY236489	$P_{1–68}$—$Q_{1–26}$—$R_{13–37}$—GTACCCG—$S_{23–49}$—T— $P_{37–68}$—$Q_{1–25}$—$R_{13–37}$—GTACCCG—$S_{23–63}$	S_{49}—T—S_{50}	brain, eye, and CSF from AIDS patient	Jørgensen et al. (2003)
BKV(Yale)		$P_{1–68}$—$Q_{1–27}$—$S_{20–33}$—$P_{39–68}$—$Q_{1–27}$—$S_{20–63}$		brain and CSF from patient with chronic lymphocyte leukaemia with fatal case of BKV tubulointestial nephritis	Stoner et al. (2002)
BKV(Yale)		$P_{1–68}$—$Q_{1–33}$—$P_{49–68}$—$Q_{1–37}$—$S_{18–63}$		kidney from patient with chronic lymphocyte leukaemia with fatal case of BKV tubulointestial nephritis	Stoner et al. (2002)

BKV(Yale)		$P_{1–68}$—$Q_{1–39}$—$P_{21–68}$—$Q_{1–39}$—$P_{21–68}$—$Q_{1–34}$—$R_{59–63}$—$S_{1–63}$		kidney from patient with chronic lymphocyte leukaemia with fatal case of BKV tubulointestial nephritis	Stoner et al. (2002)
BKVAN-1		$P_{1–68}$—$Q_{1–39}$—$R_{1–5}$—$P_{51–68}$—$Q_{1–36}$		kidney from patient with polyomavirus-associated nephropathy	Randhawa et al. (2003)
BKVAN-2		$P_{1–68}$—$Q_{1–28}$—$P_{14–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$		kidney from patient with polyomavirus-associated nephropathy	Randhawa et al. (2003)
BKVAN-3		$P_{1–30,49–68}$—$Q_{1–15}$—$P_{49–68}$—$Q_{16–39}$—$R_{1–63}$—$S_{1–48}$c		kidney from patient with polyomavirus-associated nephropathy	Randhawa et al. (2003)
BMT-14		$P_{1–31}$—$P_{9–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{56}:A→C P_{57}:ΔA R_6:A→C R_{40}:G→T R_{41}:G→T	urine BMT patient	Negrini et al. (1991)
cl-51,-54,-59		$P_{1–49}$—$P_{15–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$		urine from SLE patient	Sugimoto et al. (1989)
cl-97		$P_{1–39}$—$P_{55–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$		urine from SLE patient	Sugimoto et al. (1989)
cl-7,-47,-104,-111		$P_{1–40}$—$O_{41–142}$—$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$		urine from SLE patient	Sugimoto et al. (1989)
cl-32		$P_{1–68}$—$Q_{1–39}$—$R_{1–12}$—$O_{42–142}$—$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$		urine from SLE patient	Sugimoto et al. (1989)
cl-44		$P_{1–68}$—$Q_{1–39}$—$R_{1—31}$—$O_{49–142}$—$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$		urine from SLE patient	Sugimoto et al. (1989)
cl-9,-11,-18,-29, -34,-80,-120		$P_{1–68}$—$Q_{1–39}$—$R_{1–31}$—$O_{46–142}$—$P_{1–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$		urine from SLE patient	Sugimoto et al. (1989)
DDP	U91605	$P_{1–68}$—$Q_{1–39}$—$S_{1–63}$	Q_{15}:T→A	PBMC from healthy and HIV$^+$	Degener et al. (1999); Dolei et al. (2000)
Dunlop		$P_{1–68}$—$P_{1–7,25–68}$—$P_{1–64}$—$S_{4–63}$	P_8:ΔA P_{63}:A→G	PBMC from immuno-competent individuals	Dörries et al. (1994)
NP132		$P_{1–68}$—$Q_{1–26}$—$P_{20–68}$—$Q_{1–39}$—$S_{1–63}$		nasopharyngeal aspirates from children with respiratory diseases	Sundsfjord et al. (1994a)
NP164 (=proto-2)		$P_{1–68}$—$P_{1–9,27–68}$—$P_{1–68}$—$Q_{1–28}$—AA—$S_{7–63}$		urine and PBMC from AIDS patients, naso-pharyngeal aspirates from children with respiratory diseases	Sundsfjord et al. (1994a), (1994b)
PBMC-6		$P_{1–68}$—$Q_{1–31}$—$P_{24–68}$—$Q_{1–30}$—$P_{12–68}$—$Q_{1–4}$—$S_{1–8}$c		PBMC healthy individuals	Chatterjee et al. (2000)
PBMC-7		$P_{1–68}$—$Q_{1–31}$—$P_{17–68}$—$Q_{1–18,32–39}$—$S_{1–8}$c		PBMC healthy individuals	Chatterjee et al. (2000)
PBMC-9b,18,21		$P_{1–68}$—$Q_{1–26}$—$P_{20–68}$—$Q_{1–27}$—$R_{41–63}$—$S_{1–8}$c		PBMC healthy individuals	Chatterjee et al. (2000)
PBMC-9c				PBMC healthy individuals	Chatterjee et al. (2000)
PBMC-13		$P_{1–68}$—$Q_{1–39}$—$R_{1–12}$—$P_{16–68}$—$Q_{1–34}$—$S_{1–8}$c			
PBMC-16b		$P_{1–68}$—$Q_{1–39}$—$S_{1–5}$—$P_{16–68}$—$Q_{1–39}$—$S_{1–8}$c		PBMC healthy individuals	Chatterjee et al. (2000)
PBMC-17		$P_{1–68}$—$Q_{1–39}$—$R_{1–12}$—$P_{18–68}$—$Q_{1—44}$—$S_{1–8}$c		PBMC healthy individuals	Chatterjee et al. (2000)
PBMC-30a		$P_{1–68}$—$Q_{1–26}$—$R_{1–9}$—$P_{37–68}$—$Q_{1–25}$—$R_{1–9}$—$S_{1–8}$c		PBMC healthy individuals	Chatterjee et al. (2000)
PQ		$P_{1–68}$—$Q_{1–39}$—$S_{1–63}$	P_{43}:G→A Q_7:C→G Q_{15}:T→A	fetal brain and kidney, placenta	Pietropaolo et al. (1998)

(continued on next page)

Table 1 (*continued*)

Strain	Accession number	Anatomy	Point mutations	Specimen	References
Rearranged strains					
Seq 6		$P_{1–35}$—$P_{27–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_{31}:C→T R_{58}:C→T	urine BMT patient	Takasaka et al. (2004)
TU	M34049	$P_{1–68}$—$Q_{1–39}$—$R_{1–12}$—T—$P_{16–68}$—$Q_{1–34}$—$R_{52–63}$—$S_{1–63}$		urine AIDS patients	Sundsfjord et al. (1990); Sundsfjord et al. (1994b)
T1R1.BK	AF442901	$P_{1–49}$—T—$Q_{3–39}$—$R_{1–40}$	P_{31}:C→T P_{45}:ΔC P_{49}—T—Q_3	PBMC healthy individuals urine renal transplant patient	Chatterjee et al. (2000), Li et al. (2002)
T2R.1BK	AF442893	$P_{1–68}$—$Q_{1–5}$—$P_{24–68}$—$Q_{1–27}$—$R_{30–63}$—$S_{1–63}$		urine renal transplant patient	Li et al. (2002)
T2R.2BK	AF442892	$P_{1–68}$—$Q_{1–25}$—$R_{47–63}$—$Q_{1–25}$—$R_{47–63}$—$S_{1–63}$	P_{45}:C→G P_{64}:T→C	urine renal transplant patient	Li et al. (2002)
T2Rbkit2	AF442895	$P_{1–68}$—$Q_{1–5}$—$P_{24–68}$—$Q_{1—27}$—$R_{30—63}$—$S_{1—63}$		urine renal transplant	Li et al. (2002)
T2RBKit21-2	AF411593	$P_{1–68}$—$Q_{1–25}$—$R_{47–63}$—G—$P_{46–68}$—$Q_{1–25}$—$R_{47–63}$—$S_{1–63}$	P_{64}:T→C	urinary cells from renal transplant patient	Li et al., unpublished
T4R4.BK	AF442905	$P_{36–50}$—$Q_{4–10}$—$P_{21–45}$—TCAT—$Q_{3–37}$—TCCC—$S_{37–63}$	P_{31}:C→T P_{45}:ΔC P_{49}—T—P_{50}	urine from renal transplant patient	Li et al. (2002)
T5R.BK cl 4	AF442906	$P_{1–68}$—$Q_{1–28}$	Q_7:C→G	urine from renal transplant patient	Li et al. (2002)
TC-3	AF164514	$P_{1–68}$—$Q_{1–6}$—$P_{21–68}$—$Q_{1–39}$—$R_{1–63}$—$S_{1–63}$	P_2:ΔC R_2:C→A R_{58}:T→C S_{23}:A→G S_{29}:C→G	kidney from renal transplant patient	Chen et al. (2001)
URO1		$P_{1–68}$—$Q_{1–26}$—$P_{32–68}$—$Q_{1–26}$—$R_{56–63}$—$S_{1–6}$	P_4—CA—P_5	nonneoplastic and neo-plastic tissue of bladder, prostate, kidney, and ureter	Monini et al. (1995)
WL-1	S67523	$P_{1–40,61–68}$—$Q_{1–4}$—$O_{131–142}$—$P_{1–40,61—68}$—$Q_{1–39}$—$R_{1–49}$—$S_{7–57,63}$	R_5:A→G R_{38}:C→G R_{40}:G→A S_{18}:G→A S_{22}:T→G S_{49}—T—S_{50}	PBMC from immuno-competent individuals	Dörries et al. (1994)
WT501	E00430	$P_{1–68}$—$P_{1–17,26–68}$—$P_{1–68}$—$Q_{1–39}$—$S_{1–63}$	S_{10}:G→A S_{18}:G→A	not provided	Soeda and Yoshimura, unpublished
9a,30b,33			Q_7:C→G Q_{15}:T→A S_{29}:C→T	healthy blood donors	Chatterjee et al. (2000)

[a] Bone marrow transplant.
[b] Focal segmental glomerulosclerosis.
[c] Downstream sequence not determined.
[d] Peripheral blood mononuclear cells.

Table 2
Anatomy of the transcriptional control region variants found in different human tissues

Specimen	NCCR anatomy	Strain	References
Bladder	PQPQRS	URO1	Monini et al. (1995)
Brain	PQS	PQ	Pietropaolo et al. (1998)
	PQRS	BKV(Yale)	Stoner et al. (2002)
	PQSPQS	BKV(Yale)	Stoner et al. (2002)
	PQRSPQRS	BKV(CNS)	Jørgensen et al. (2003)
CSF	PQRPQRS	BKV(CNS)	Jørgensen et al. (2003)
	PQSPQS, PQPQPQRS	BKV(Yale)	Stoner et al. (2002)
Eye	PQRPQRS	BKV(CNS)	Jørgensen et al. (2003)
Heart	PQRS	CAP-h2,-h5,-h8,-h22	Chen et al. (2004)
Kidney	PQS	PQ	Pietropaolo et al. (1998)
	PQRS	Azzi-K, BKV(Yale)	Azzi et al., unpublished; Stoner et al. (2002)
	PQPQS	BKV(Yale)	Stoner et al. (2002)
	PQPQRS	URO1, TC-3, BKVAN Pat2 + Pat3	Monini et al., 1995; Chen et al. (2001); Randhawa et al. (2003)
	PQRPQ	BKVAN Pat1	Randhawa et al. (2003)
	PQPQPQRS	BKV(Yale)	Stoner et al. (2002)
	not specified	BKV (Cin)	Smith et al. (1998)
Lung	PQRS	BKV(Yale)	Stoner et al. (2002)
Muscle	PQRS	CAP-m2,-m5,-m9,-m13,-m15	Chen et al. (2004)
Nasopharyngeal aspirates	PQPQS	NP132	Sundsfjord et al. (1994b)
	PPPQS	NP164(=prototype)	Sundsfjord et al. (1994a)
Ovarium	PQPQRS	URO1	Monini et al. (1995)
PBMC	PQS	DDP,PQ, PBMC-9a,30b,33	Degener et al. (1999); Dolei et al. (2000); Chatterjee et al. (2000)
	PPPS	Dunlop,	Dörries et al. (1994)
	PQRS	TIM.BK,T2M.2BK,PBMC-16a,20,WW	Li et al. (2002); Chatterjee et al. (2000); Jørgensen et al. (2003)
	PPPQS	NP164 (=proto-2)	Sundsfjord et al., 1994
	PQPQS	PBMC-7	Chatterjee et al. (2000)
	PQPQRS	PBMC-9b,18,21	Chatterjee et al. (2000)
	PQRPQS	PBMC-9c	Chatterjee et al. (2000)
	PQSPQS	PBMC-16b	Chatterjee et al. (2000)
	PQRPQS	PBMC-17	Chatterjee et al. (2000)
	PQPQPQS	PBMC-6	Chatterjee et al. (2000)
	PQRSPQRS	PBMC-13	Chatterjee et al. (2000)
	PQRPQRS	PBMC-30a, TU	Chatterjee et al. (2000)
	PQOPQRS	WL-1	Dörries et al. (1994)
Placenta	PQS	PQ	Pietropaolo et al. (1998)
Prostate	PQPQRS	URO1	Monini et al. (1995)
Sewage	PQRS	SA160900,SA090600,RA;U2,U3,U5	Bofill-Mas et al. (2001)
Ureter	PQPQRS	URO1	Monini et al. (1995)
Urine	PQ	T5R.BK clone 4	Li et al. (2002)
	PQR	T1R1.BK	Li et al. (2002)
	PQRS	Azzi-U;BMT	Azzi et al., unpublished; Priftakis et al. (2001, 2003)
		G7RBK, K8R2.BK, T4R3	Li et al., unpublished,
		HC-u2,-u5,-u9, HI-u2,-u6,-u8;	Chen et al. (2004)
		MT-1,cl-6,-8,-50,-87,-93,-108	Sugimoto et al. (1989)
		S1,S2,S3,P1	Bhattacharjee and Chakraborty, (2004)
		Seq 1-5, 7-10	Takasaka et al. (2004)
		TC-1,TC-2; Pat-A, Pat-E	Tsai et al., Chang et al. (1996a, b); Schätzl et al. (1994)
		WW, WWT	Rubinstein et al. (1987); Sundsfjord et al. (1990); Flægstad et al. (1991); Markowitz et al. (1991); Negrini et al. (1991); Sundsfjord et al. (1999); Stoner et al. (2002); Jørgensen et al. (2003)
	PPPQS	NP164(=proto-2)	Sundsfjord et al. (1994a)
	PQPQS	T4R4.BK	Li et al. (2002)
	PPQRS	BMT-14 , cl-51,-54,-59,-97; Seq 6	Negrini et al. (1991); Sugimoto et al. (1989); Takasaka et al. (2004)
	PQPQRS	T2Rbkit2,T2R.1BK(=T2RBkit21-1)	Li et al. (2002)
	PQRQRS	T2R.2BK	Li et al. (2002)
	POPPQRS	cl-7,-47,-104,-111	Sugimoto et al. (1989)
	PQROPQRS	cl-9,-11,-18,-29,-32-34,-80,-120	Sugimoto et al. (1989)

Table 3
Point mutations in the P, Q, R, and S blocks compaired to the consensus sequence as shown in Fig. 1

Position	Mutation	Strain	Accession number	References
Mutations P-block				
P_4-P_5	insertion CA	URO1	U33549	Monini et al. (1995)
P_5	ΔG	AO	AF123403	Sundsfjord et al. (1999)
P_{14}	T→C	G7RBK	AF374595	Li et al. (2002)
P_{17}	ΔT	AO	AF123403	Sundsfjord et al. (1999)
P_{18}	T→A	WW	M15987	Rubinstein et al. (1987)
		WW(BMT-12,15,16,19)		Negrini et al. (1991)
		WW		Jørgensen et al. (2003)
		T4R4.BK	AF442905	Li et al. (2002)
		TIM.BK	AF442900	Li et al. (2002)
		K8R2.BK clones 2,3	AF442897 + AF442896	Li et al. (2002)
		SA160900	AF356533	Bofill-Mas et al. (2001)
		SA090600	AF356532	Bofill-Mas et al. (2001)
		RA	AF356531	Bofill-Mas et al. (2001)
	T→C	WW Pat-A and Pat-E	S72998	Schätzl et al. (1994)
P_{19}	ΔA	BK-OV1	AY573928	Asomani et al., unpublished
P_{18}—P_{19}	insertion T	WW		Markowitz et al. (1991)
P_{24}—P_{25}	insertion G	BK-OV1	AY573928	Asomani et al., unpublished
P_{24}	T→C	WW	AF123397	Sundsfjord et al. (1999)
			AF123410–AF123416	Sundsfjord et al. (1999)
	T→A	WW	AF123399	Sundsfjord et al. (1999)
P_{27}	A→G	BKV(Cal)	AF317623	Bhattacharjee and Chakraborty, unpublished
		S3		Bhattacharjee and Chakraborty, (2004)
P_{30}	C→T	BKV(Cal)	AF317623	Bhattacharjee and Chakraborty, unpublished
		WW	M15987	Rubinstein et al. (1987)
		WW		Jørgensen et al. (2003)
		T4R4.BK	AF442905	Li et al. (2002)
		T1R1.BK	AF442901	Li et al. (2002)
		TIM.BK	AF442900	Li et al. (2002)
		K8R2.BL clones 2 and 3	AF442896 + AF442897	Li et al. (2002)
		SA160900	AF356533	Bofill-Mas et al. (2001)
		SA090600	AF356532	Bofill-Mas et al. (2001)
		RA	AF356531	Bofill-Mas et al. (2001)
		S1,S2,S3		Bhattacharjee and Chakraborty (2004)
		P1		Bhattacharjee and Chakraborty (2004)
P_{31}	C→T	Seq 5, 6		Takasaka et al. (2004)
P_{33}	G→A	TC-1		Chang et al. (1996a, 1996b)
		TC-2		Chang et al. (1996b)
		AO	AF123404	Sundsfjord et al. (1999)
P_{35}	ΔC	URO1	U33549	Monini et al. (1995)
P_{43}	G→A	PQ		Pietropaolo et al. (1998)
P_{45}	C→G	T2R.2B	AF442892	Li et al. (2002)
	ΔC	T5R.BK4	AF442906	Li et al. (2002)
		T1R1.BK	AF442901	Li et al. (2002)
P_{47}	ΔT		E00430	Soeda et al., unpublished
P_{49}—P_{50}	insertion T	T5R.BK4	AF442906	Li et al. (2002)
P_{53}	A→C	BMT-7,14		Negrini et al. (1991)
	A→G	BMT-8,9,10,12,13,15, 16,17,18,19		Negrini et al. (1991)
P_{55}	G→T	P1		Bhattacharjee and Chakraborty (2004)
	G→C	Seq 4		Takasaka et al. (2004)
P_{56}	A→G	BKV(Cal)		Bhattacharjee and Chakraborty, unpublished
		P1		Bhattacharjee and Chakraborty (2004)
	ΔA	BMT-7,14		Negrini et al. (1991)
P_{58}	ΔA	Seq 4		Takasaka et al. (2004)
P_{62}	C→A	AO	AF123404	Sundsfjord et al. (1999)
P_{63}	A→C	AO	AF123404	Sundsfjord et al. (1999)
P_{64}	T→C	T2R.2B	AF442892	Li et al. (2002)
P_{66}	A→T		E00430	Soeda et al., unpublished

Table 3 (*continued*)

Position	Mutation	Strain	Accession number	References
Mutations in Q-block				
Q_2—Q_3	insertion T	AO	AF123404	Sundsfjord et al. (1999)
Q_7	C→G	WW Pat-E	S72998	Schätzl et al. (1994)
		T5R.BK4	AF442906	Li et al. (2002)
		WWT3		Flægstad et al. (1991)
				Randhawa et al. (2003)
		PQ		Pietropaolo et al. (1998)
		Shi 1-6		Agostini et al. (1995)
Q_8	C→G	WW		Markowitz et al. (1991)
				Randhawa et al. (2003)
Q_{15}	T→A	DDP	U91605	Dolei et al. (2000)
		PQ		Pietropaolo et al. (1998)
Q_{18}	C→T	TC-2		Chang et al. (1996b)
Q_{21}	T→G	AO	AF123404	Sundsfjord et al. (1999)
Q_{27}	G→C	TC-2		Chang et al. (1996b)
Q_{34}	C→T	Seq 10		Takasaka et al. (2004)
Q_{36}	C→G	BMT		Priftakis et al. (2001, 2003)
Q_{38}	C→G	BMT		Priftakis et al. (2001, 2003)
Q_{39}	ΔC	WW	AF123398	Sundsfjord et al. (1999)
Mutations in R block				
R_2	C→G	TC-2		Chang et al. (1996b)
	C→A	TC-3		Chen et al. (2001)
		TU	M34049	Sundsfjord et al. (1990)
		MT-1		Sugimoto et al. (1989)
		Seq 7, 8		Takasaka et al. (2004)
	C→T	BMT		Priftakis et al. (2001, 2003)
R_3—R_4	insertion T	AO	AF123403 + AF123404	Sundsfjord et al. (1999)
R_5	A→G	WL-1	S67523	Dörries et al. (1994)
		T3R2.BK	AF442903	Li et al. (2002)
		A191-2	AF218448	Chang et al., unpublished
		V173-1	AF218445	Chang et al., unpublished
		A250-1-a	AF218447	Chang et al., unpublished
		Seq 1, 3		Takasaka et al. (2004)
		WW	AF123401 + AF123402 + AF123422–AF123428	Sundsfjord et al. (1999)
R_6	A→G	WW		Markowitz et al. (1991)
		BMT13		Negrini et al. (1991)
	A→C	BMT7		Negrini et al. (1991)
R_7	A→T	WW		Markowitz et al. (1991)
R_{10} R	C→G	WW	AF123398	Sundsfjord et al., 1999
R_{12}	C→T	Seq 10		Takasaka et al. (2004)
R_{13}	A→C	BKV(CAL)	AF317623	Battacharjee and Chakraborty, unpublished
		S3		Battacharjee and Chakraborty, 2004
	A→T	Azzi-K	AY272187	Azzi et al., unpublished
R_{17}	A→T	Azzi-U	AY272186	Azzi et al., unpublished
R_{23}	A→C	BKV(CAL)	AF317623	Battacharjee and Chakraborty, unpublished
		S3		Bhattacharjee and Chakraborty (2004)
R_{24}	A→C	S1,S2,S3		Bhattacharjee and Chakraborty (2004)
R_{26}	A→G	T3R2.BK	AF442903	Li et al. (2002)
R_{29}	A→G	HI-u6,CAP-m5, HI-u8	AY628237, AY628232, AY628238	Chen et al. (2004)
R_{35}	A→G	BKV(CAL)	AF317623	
		S3		Bhattacharjee and Chakraborty (2004)
R_{38}	C→G	WL-1	S67523	Dörries et al. (1994)
		BMT-13		Negrini et al. (1991)
		WW		Jørgensen et al. (2003)
		A191-2	AF218448	Chang et al., unpublished
		V173-1	AF218445	Chang et al., unpublished
		V128-1	AF218446	Chang et al., unpublished
		WW	AF123401 + AF123402	Sundsfjord et al. (1999)
		WW	AF123422–AF123427	Sundsfjord et al. (1999)
	C→T	A250-1-a	AF218447	Chang et al., unpublished

(continued on next page)

Table 3 (*continued*)

Position	Mutation	Strain	Accession number	References
Mutations in R block				
R_{39}	T→C	A191-2	AF218448	Chang et al., unpublished
		V173-1	AF218445	Chang et al., unpublished
	T→A	A250-1-a	AF218447	Chang et al., unpublished
R_{40}	G→A	WL-1	S67523	Dörries et al. (1994)
		WW		Jørgensen et al. (2003)
		A191-2	AF218448	Chang et al., unpublished
		V173-1	AF218445	Chang et al., unpublished
		V128-1	AF218446	Chang et al., unpublished
		Seq 1		Takasaka et al. (2004)
		WW	AF123401 + AF123402 + AF123422–AF123428	Sundsfjord et al. (1999)
	G→T	BMT2,7,8,9,10,12,15, 16,17,18,19,13		Negrini et al. (1991)
		Seq 4		Takasaka et al. (2004)
R_{41}	G→T	Azzi-K, Azzi-U	AY272186 + AY272187	Azzi et al., unpublished
		Seq 4		Takasaka et al. (2004)
	G→A	BMT-13		Negrini et al. (1991)
	ΔG	A250-1-a	AF218447	Chang et al., unpublished
R_{42}	C→G	BMT2,8,9,10,12,13,15-19		Negrini et al. (1991)
	C→T	BMT7		Negrini et al. (1991)
R_{43}	C→A	BKV(CAL)	AF317623	Bhattacharjee and Chakraborty, unpublished
		S3		Bhattacharjee and Chakraborty (2004)
R_{47}	ΔG	BKV(CAL)	AF317623	Bhattacharjee and Chakraborty, unpublished
		S3		Bhattacharjee and Chakraborty (2004)
R58	C→T	TC-3		Chen et al. (2001)
		TU		Sundsfjord et al. (1990)
		MT-1		Sugimoto et al. (1989)
		Seq 5-10		Takasaka et al. (2004)
Mutations in S-block				
S_2	A→C	WW		Markowitz et al. (1991)
S_{10}	G→A		E00430	Soeda et al., unpublished
S_{22}	T→G	WL-1	S67523	Dörries et al. (1994)
		WW		Jørgensen et al. (2003)
		A191-2	AF218448	Chang et al., unpublished
		A250-1-a	AF218447	Chang et al., unpublished
		V128-1	AF218446	Chang et al., unpublished
		V173-1	AF218445	Chang et al., unpublished
		WW	AF123401; AF123402, AF123422–AF123428	Sundsfjord et al. (1999)
		MT-1		Sugimoto et al. (1989)
	T→C	BK-OV1	AY573928	Asomani et al., unpublished
S_{23}	A→G	TC-3		Chen et al. (2001)
		Seq 7-10		Takasaka et al. (2004)
S_{24}	G→A	Azzi-K, Azzi-U	AY272186 + AY272187	Azzi et al., unpublished
S_{29}	C→G	TC-3		Chen et al. (2001)
		MT-1		Sugimoto et al. (1989)
		Seq 7-10		Takasaka et al. (2004)
	C→T	DDP		Degener et al. (1999); Dolei et al. (2000)
		WWT1		Flægstad et al. (1991)
S_{44}	A→C	TIM.BK	AF442900	Li et al. (2002)
S_{49}-S_{50}	insertion T	WL-1	S67523	Dörries et al. (1994)
		BK-OV1	AY573928	Asomani et al., unpublished
		BKV(CNS)	AY236489	Jørgensen et al. (2003)
		WW		Jørgensen et al. (2003)
		Azzi-K,Azzi-U	AY272186 + AY272187	Azzi et al., unpublished
		A191-2	AF218448	Cheng et al., unpublished
		A250-1-a	AF218447	Cheng et al., unpublished
		V128-1	AF218446	Cheng et al., unpublished
		V173-1	AF218445	Cheng et al., unpublished
		Seq 1, 2		Takasaka et al. (2004)

Table 3 (*continued*)

Position	Mutation	Strain	Accession number	References
Mutations in S-block				
		WW	AF123401, AF123402, AF123422–AF123428	Sundsfjord et al. (1999)
S_{57}	ΔA	WL-1	S67523	Dörries et al. (1994)
S_{58}	ΔA	WL-1	S67523	Dörries et al. (1994)
S_{59}	ΔA	WL-1	S67523	Dörries et al. (1994)
S_{60}	ΔG	WL-1	S67523	Dörries et al. (1994)
S_{61}	ΔG	WL-1	S67523	Dörries et al. (1994)

Hence, the latter signals will not be detected on the electropherogram. Therefore, sequences of BKV genomes poorly presented in the biological samples may be overlooked due to technical limitations of the PCR-based sequencing methods. Less abundant DNA (e.g., rearranged TCR) are more likely to be picked up by cloning if sufficient colonies are analyzed. Another disadvantage with PCR that faces researchers is the problem of contamination with laboratory strains. The detection of BKV strains with TCRs present in passaged laboratory strains should be interpreted with care. Several distinct TCR variants were also obtained after cloning BKV DNA from urine of a single patient with systemic lupus erythematosus. In addition to archetypal PQRS (referred to as MT-1 by the authors), rearranged TCRs were found. Many of these TCR variants contained a duplication of part of the O block, which spans the origin of replication (Sugimoto et al., 1989). These findings are in agreement with in vitro studies that have demonstrated that serial passage of BKV at high multiplicities of infection resulted in the accumulation of rearranged genomes with often more than one replication origin (Hara et al., 1986; Rubinstein et al., 1991; Sugimoto et al., 1989; Watanabe and Yoshiike, 1986). The non-coding control region of BKV WL-1 consists of an O-block and a transcriptional control region with PQOPQRS anatomy. Up to date, BKV WL-1 is the only other non-passaged strain described that contains duplicated O block sequences in its non-coding control region. This BKV strain was detected by PCR in peripheral blood leukocytes from healthy adult individuals (Dörries et al., 1994).

The results outlined above show that different TCR variants circulate in different cell types of the same patient. However, several cases have reported the presence of a unique strain in different tissues of the same or distinct patients. Azzi et al. found archetypal BKV with the PQRS TCR in the kidney as well as in the urine of a renal allograft transplant patient. The urine isolate had an unique mutation of nucleotide 13 in the R block replacing an A by a T, while in the kidney isolate T at R_{17} was mutated into A. Both isolates had the mutations G→A at R_{41}, G→A at S_{24}, and an insertion of T between S_{49} and S_{50} [Azzi et al., GenBank accession nos. AY273186 and AY273187]. Identically rearranged variants have been detected in PCR amplified BKV DNA from neoplastic and nonneoplastic tissue from different patients. The new variant URO1 (PQPQRS anatomy) was found in bladder, kidney, ovarium, prostate, and ureter samples (Monini et al., 1995). Examining fetal brain, kidney from different aborted fetuses and placenta from different mothers revealed PCR-amplified BKV TCR sequences with a PQS anatomy in all positive cases (Pietropaolo et al., 1998). This PQ strain was later also identified by different groups as the DDP strain in peripheral blood cells of HIV-positive and -negative individuals and healthy donors (Chatterjee et al., 2000; Degener et al., 1999; Dolei et al., 2000). A PQ TCR variant with only P and Q block sequences (T5R.BK isolate) was detected in the urine of a renal transplant recipient (Li et al., 2002). In a recent study, 15 full-length BKV genomes were generated by PCR, cloned, and subsequently sequenced (Chen et al., 2004). Nine clones were derived from the heart (4 clones) and muscle (5 clones) tissue from a single renal transplant recipient who developed BKV vasculopathy that led to capillary leak syndrome (BKV_{CAP}). Three clones each were obtained from urine of a HIV-2-positive patient (BKV_{HI}) and from the urine of a healthy individual (BKV_{HC}). All clones had the archetypal PQRS control region, but one BKV_{HI} clone contained a 10 bp deletion in the P-block. Only a few point mutations were detected between the different clones (see Table 1). These results again illustrate that different tissues from the same patient, and urine from different patients contained similar BKV strains with respect to their TCR.

In summary, the results from the above mentioned studies suggest that analyzing the TCR cannot with certainty predict cell tropism or strain-specific BKV-associated disease. However, this conclusion may be preliminary as only a limited number of different patients and tissues were investigated. A larger number of tissues and patients need to be deliberated. Moreover, if sufficient material is available, cloning should be considered as PCR preferentially allows the identification of the most prominent species in a sample and renders the detection of polymorphisms difficult.

Polymorphism in the major capsid protein VP1

Despite nearly 35 years of intense BKV research, little is known about the transmission, route of infection, host cells, and mechanisms for spread of this virus. BKV, its

genome, viral transcripts, and viral proteins have been detected in most human tissues and organs, including bladder, blood, bone, bone marrow, brain, central nervous system, CSF, cervix, connective tissue, endothelial cells, eye, heart, kidney, lips, liver, lung, lymph nodes, nasopharynx, mesentery, penis, pituitary gland, placenta, prostate, saliva, skin, small intestine, sperm, spleen, stomach, tongue, tonsils, urethra, and vulvae [for a complete overview, see Rekvig and Moens, 2002]. The range of cell types permissive for BKV infection has been linked to cell-specific transcription factors involved in the expression of the viral genome. However, it is also becoming apparent that the presence of specific receptors contributes to the tropism of BKV. The primary receptor-binding determinant on BKV is the major capsid protein VP1, and has been mapped to amino acid residues 61–83. This region also encompasses the epitopes responsible for serotype differences between BKV isolates (Atwood, 2001; Randhawa et al., 2002a). It is conceivable that changes in the VP1 protein represent a strategy adapted by the virus to evade the host immune system, as well as to increase the affinity or to broaden the range for cellular receptors. Studies with polyomaviruses support a role for VP1 in cell tropism. The human polyomavirus JC has a narrow cell tropism and JC virions were unable to infect HeLa cells. However, viral expression was monitored in HeLa cells transfected with naked JCV DNA (Schweighardt and Atwood, 2001). These findings indicate that the lack of permissivity in these cells was at the level of virus adsorption/uptake. Another observation that underscores the importance of VP1 in the viral life cycle comes from a mouse polyomavirus VP1 mutant. Substitutions of residue 92 (corresponds to amino acid 82 in BKV VP1) reduced the plaque size and affected the hemagglutination behavior of the mutant virus (Freund et al., 1991). The cellular receptors for BKV have not been fully identified, but seem to be glycolipids containing ubiquitous α(2–3)-linked sialic acid modifications (Atwood, 2001). Amino acid changes in the VP1 protein could be responsible for conformational differences that may lead to preferential entry in specific cells. Sequence analysis of the VP1 region of BKV-positive biopsies has suggested that the viral genome is unstable and changes in nucleotide sequences occur as patients are followed over time (Randhawa et al., 2002b). On the contrary, VP1 sequence analysis of urinary BKV in 100 urine samples from 21 SLE patients and 75 pregnant women collected over a one-year period were predominantly stable (Bendiksen et al., 2000). The sites of mutations and mutation rates in the VP1 protein are represented in Fig. 2 and Table 4. It should be noted, however, that the mutation rate of a specific residue was defined as the number of mutations found at this position divided by the total number of published sequences of this residue. The actual mutation frequency in the human population may differ because several studies have reported new mutations without stating how many samples or patients that possessed this particular mutation. The most hydrophobic regions of VP1 are located in two small regions, at residues 61–83 and 214–236. Regions 61–83 are highly predisposed for mutations. This region, referred to as the BC-loop, forms the antigenic determinants of BKV subtypes and mediates adsorption to the host cell (Jin et al., 1993; Liddington et al., 1991). In addition, high mutation frequencies are observed at residues Q117, D175, V210, and L362. Other hydrophobic residues substitute the hydrophobic residues 210 and 362, while Q117 is replaced by a basic lysine. The substitution of D175 into E conserves the negative charge at this position in the protein. A few mutations have only been reported in BKV strains isolated from a particular tissue, but the number of individuals is far too low to draw any conclusions. For example, a BKV variant with the unique T4A, R170W and E330G mutations was isolated from the heart of a single patient, but as no other VP1 sequences are available from heart tissue from distinct patients, it is impossible to draw conclusions (see Table 4). Experimental data that may provide an indication of the biological significance of these mutations are lacking.

Polymorphism in the large T-antigen

Large T-antigen plays a pivotal role in the life cycle of BKV because it stimulates viral DNA replication and controls expression of both early and late genes (Moens and Rekvig, 2001). Moreover, large T-antigen affects the expression of a large number of cellular genes probably to create an optimal molecular environment for the virus (Moens et al., 1997). Large T-antigen DNA sequences have been detected in a wide variety of tissues (Rekvig and Moens, 2002), yet few studies have determined the sequences of large T-antigen. The BKV Cin-strain identified in an AIDS patient with end-stage renal disease possessed a Q169L mutation in the DNA-binding domain of large T-antigen. Studies with SV40 large T-antigen have suggested that this mutation may stabilize the binding of large T-antigen to DNA. Whether this mutation enhances BKV DNA replication remains to be investigated (Smith et al., 1998). Comparing the amino acid sequences of large T-antigen of the 15 different complete BKV isolates by Chen et al. (2004) revealed that all these 15 non-passaged BKV isolates had the I127T and the S260N substitutions compared to the cell-passaged BKV (Dunlop) strain. Additional substitutions that were found include, E20G, P28S, K37R, H42R, E63G, K133E, S142C, S182P, T267A, K273R, F288L, F323C, I327T, N334D, T354S, K373R, I430V, 471V, F475G, D504G, F584S, E603K, D629G, and Q675E. The mutations P28S, S142C, and T354S were only present in virus shed in the urine from a healthy individual, while the substitution Q675E was exclusive for the clones obtained from the urine sample of a HIV-2 positive patient. All other mutations were detected in single clones. The

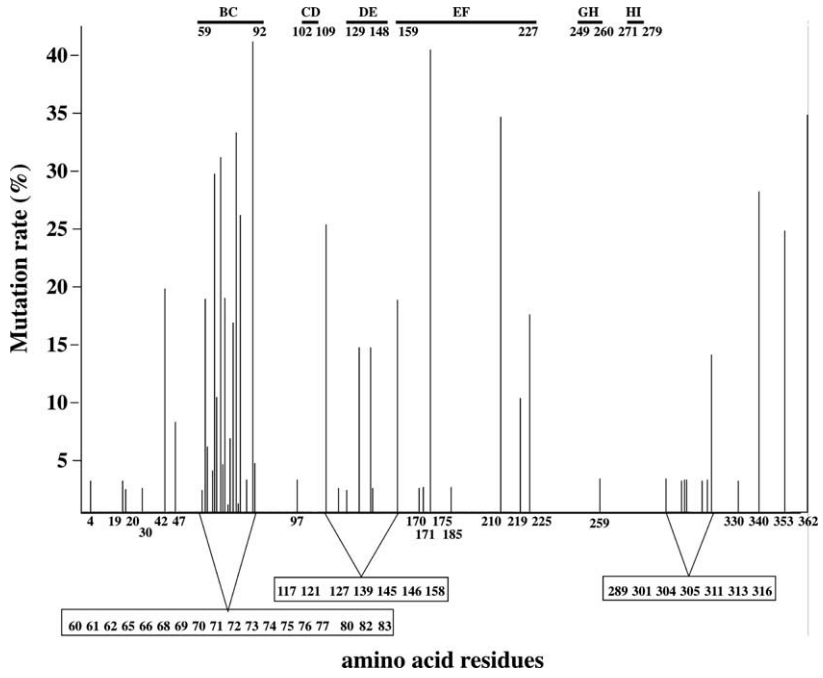

Fig. 2. Position and mutation rate of reported mutations in the VP1 protein of BKV. The numbers refer to the positions of the amino acid residues in the protein. The mutation rate for each residue (in %) was calculated as the number mutations reported at this side divided over the total number of sequences provided for this particular residue. The loops emanating from the β-sheet, as described in (Liddington et al., 1991), are indicated at the top of the diagram.

mutations in amino acids 20, 28, 133, 142, 182, and 267 correspond with the previously described mutations 20, 28, 131, 140, 180, and 265, respectively, in SV40. However, all of these SV40 large T-antigen mutations transformed and stimulated viral DNA synthesis with wild-type levels, whereas the K131T or R, the S140L, and the S180F mutants exhibited reduced transformation efficiency of Rat-1 cells, but retained wild-type DNA replication activity (Kalderon and Smith, 1984; Rutila et al., 1986). A K131M substitution resulted in an SV40 virus with slightly delayed growth properties in cell culture (Colledge et al., 1986). The biological consequences of the naturally occurring mutations in BKV large T-antigen have not been systematically explored, although they may be extrapolated from studies with the corresponding SV40 large T-antigen mutants because of the extensive homology between the proteins of these two viruses. One group examined the effect of mutating histidine residue 42 in the J domain of BKV large T-antigen. This domain shows extensive homology to the DnaJ family of molecular chaperone proteins and is required for efficient viral replication, specific interaction with heat shock protein hsc70, and transformation. Large T-antigen H42Q mutant protein retained the ability to bind members of the retinoblastoma family, but was severely impaired in both induction of the transcriptional activity of the E2F family of transcription factors and serum-independent growth of cells. Hence, substitutions of H42 may have an effect on cell proliferation as both the retinoblastoma proteins and the E2F transcription factors are involved in controlling cell cycle progression (Harris et al., 1998).

Polymorphism in the agnoprotein

The late region of BKV encodes a small phosphoprotein referred to as the agnoprotein (Rinaldo et al., 1998). Little is known about the function of this protein. The agnoproteins of BKV (66 amino acids), SV40 (62 amino acids), and JCV (71 amino acids) are highly identical, especially in the N-terminal 50 amino acid residues of the protein (41/50 identical for BKV-JCV and 36/50 identical for BKV-SV40). Studies with SV40 and JCV have suggested regulatory roles for their agnoproteins at the levels of viral transcription, replication, capsid protein translation, and assembly (Radhakrishnan et al., 2004). JCV agnoprotein was also shown to dysregulate the cell cycle by altering the expression of several cyclins and their associated kinases. The JCV agnoprotein can interact with p53 and the N-terminal 36 residues of the agnoprotein seem sufficient for this interaction (Darbinyan et al., 2002). Comparing this region with the corresponding domain of the BKV agnoprotein reveals only three substitutions (K9Q, S19T, and L29F), suggesting that BKV agnoprotein also may bind p53. Interestingly, the BKV agnoprotein was found to interact with an unidentified approximately 50 kDa protein (Rinaldo et al., 1998).

Few studies have addressed the expression of the agnogene in human tissues. Agnoprotein was detected in the kidney (tubular epithelial cells) and brain (ependyma cells) of a 26-year-old AIDS patient who developed menigoencephalitis, retinitis, and nephritis, but no sequence data were provided in this study (Bratt et al., 1999). Partial agnogene sequences were obtained from urine samples of an

Table 4
Amino acid substitutions and their mutation rates in the BKV VP1 protein

Residue	Mutation rate (%)	Specimen	Reference (GenBank accession no)
4T→A	1/28 (3.6)	heart	Chen et al. (2004) (AAT47371)
19K→E	1/28 (3.6)	muscle	Chen et al. (2004) (AAT47401)
20E→D	1/28 (3.0)	urine	Sugimoto et al. (1990) (CAA40235)
30ΔK	1/34 (2.9)	urine	Jin et al. (1993) (CAA79595)
42V→L	7/35 (20.0)	urine, kidney, brain, CSF, lung	Chen et al. (2004) (AAT47347; AAT47353; AAT47359); Sugimoto et al. (1990) (CAA40247); Touzé et al. (2001); Stoner et al. (2002); Kupper et al. (1993) (P72390)
47E→Q	3/34 (8.8)	urine	Chen et al. (2004) (AAT47419); Stoner et al. (2002); Kupper et al. (1993) (P72390)
60D→N	1/35 (2.9)	urine	Touzé et al. (2001)
61E→D	19/135 (14.1)	sewage, urine	Tavis et al. (1989) (P14996);Bofill-Mas et al. (2001) (AAL31866); Jin et al. (1995); Clewley, unpublished (S29968); Jin et al. (1993) (CAA79596); Kupper et al. (1993) (P72390)
61E→N	7/134 (5.2)	urine	Clewley, unpublished (S29965); CAA79595; Jin et al. (1995)
62N→D	8/132 (6.1)	urine	Clewley, unpublished (S29965); Jin et al. (1993) (CAA79595); Jin et al. (1995)
65G→D	6/155 (3.9)	kidney	Randhawa et al., 2002b
66F→Y	38/155 (24.5)	urine, kidney, sewage	Bofill-Mas et al. (2001) (AAL31866); Clewey, unpublished (S29965; S29968); Kupper et al. (1993) (P72390); Tavis et al. (1989) (P14996); Jin et al. (1993) (CAA79595; CAA79596); Jin et al. (1995); Randhawa et al., 2002b
66F→W	8/155 (5.2)	kidney	Baksh et al. (2001)
68L→Q	1/155 (0.6)	urine	Tavis et al. (1989) (P14996)
68L→R	8/155 (9.6)	kidney	Rhandawa et al., 2002b
69K→H	17/155 (11.0)	urine	Tavis et al. (1989) (P14996); Jin et al. (1995)
69K→R	29/155 (18.7)	kidney	Clewley, unpublished (S29965); Jin et al. (1993) (CAA79595); Jin et al. (1995); Baksh et al. (2001); Rhandawa et al., 2002b
69K→N	2/155 (1.3)	kidney	Baksh et al. (2001); Rhandawa et al., 2002b
70L→P	8/155 (5.2)	kidney	Rhandawa et al., 2002b
71S→T	30/155 (19.4)	sewage, urine, kidney	Bofill-Mas et al. (2001) (AAL31866); Clewley, unpublished (S29965; S29968); Kupper et al. (1993) (P72390); Jin et al. (1993) (CAA79595; CAA79596); Jin et al. (1995); Baksh et al. (2001); Rhandawa et al., 2002b
72A→P	1/155 (0.6)	kidney	Rhandawa et al., 2002b
72A→G	1/155 (0.6)	kidney	Rhandawa et al., 2002b
73E→Q	2/155 (1.3)	urine, heart	Chen et al. (2004) (AAT47425; AAT47431)
73E→K	3/155 (1.9)	urine, kidney	Sugimoto et al. (1990) (CAA40243;); Baksh et al. (2001); Rhandawa et al., 2002b
73E→D	5/155 (3.2)	kidney	Rhandawa et al., 2002b
73E→A	1/155 (0.6)	kidney	Rhandawa et al., 2002b
74N→T	25/155 (16.1)	urine, kidney	Jin et al. (1993) (CAA79595); Jin et al. (1995); Baksh et al. (2001); Clewley, unpublished (S29965); Rhandawa et al., 2002b
75D→A	48/155 (31.0)	urine, kidney, sewage	Bofill-Mas et al. (2001) (AAL31862); Tavis et al. (1989) (P14996); Clewley, unpublished; Jin et al. (1995); (S29965; S29968); Kupper et al. (1993) (P72390); Jin et al. (1993) (CAA79595; CAA79596); Baksh et al. (2001); Rhandawa et al., 2002b
75D→N	3/155 (1.9)	kidney	Baksh et al. (2001); Rhandawa et al., 2002b
76F→V	2/155 (1.3)	kidney	Rhandawa et al., 2002b
76F→D	1/155 (0.6)	kidney	Rhandawa et al., 2002b
77S→E	22/155 (14.2)	urine, kidney	Tavis et al. (1989) (P14996); Clewley, unpublished (S29965); Jin et al. (1993) (CAA79595); Jin et al. (1995); Baksh et al. (2001); Rhandawa et al., 2002b
77S→D	14/155 (9.0)	urine, kidney	Clewley, unpublished (S29968); Kupper et al. (1993) (P72390); Jin et al. (1993) (CAA79596); Jin et al. (1995); Baksh et al. (2001); Rhandawa et al., 2002b
77S→R	1/155 (0.6)	kidney	Rhandawa et al., 2002b
77S→K	2/155 (1.3)	urine	Jin et al. (1995)
77S→N	1/155 (0.6)	kidney	Baksh et al. (2001)
77S→Q	1/155 (0.6)	kidney	Baksh et al. (2001)
80S→D	2/155 (1.3)	kidney	Rhandawa et al., 2002b
80S→N	2/155 (1.3)	kidney	Rhandawa et al., 2002b
80S→Q	1/155 (0.6)	kidney	Rhandawa et al., 2002b

Table 4 (*continued*)

Residue	Mutation rate (%)	Specimen	Reference (GenBank accession no)
82E→D	61/155 (39.4)	urine, kidney, sewage	Bofill-Mas et al. (2001) (AAL31866); Tavis et al. (1989) (P14996); Clewley, unpublished (S29965; S29968); Kupper et al. (1993) (P72390); Jin et al. (1993) (CAA79595; CAA79596); Jin et al. (1995); Baksh et al. (2001); Rhandawa et al., 2002b
82E→K	2/155 (1.3)	kidney	Baksh et al. (2001); Rhandawa et al., 2002b
82E→G	2/155 (1.3)	urine	Jin et al. (1995)
83R→K	8/155 (5.2)	urine, kidney	Jin et al. (1993) (CAA79596); Jin et al. (1995); Clewley unpublished (S29968); P72390; Baksh et al. (2001); Rhandawa et al., 2002b
97P→I	3/77 (3.9)	urine	Jin et al. (1995)
117Q→K	9/36 (25.0)	urine	Bendiksen et al. (2000) (AAG23641; AAG23642); Tavis et al. (1989) (P14996); Kupper et al. (1993) (P72390); Clewley, unpublished (S29965; S29968); Jin et al. (1993) (CAA79595; CAA79596); Jin et al. (1995)
121I→T	1/33 (3.0)	muscle	Chen et al. (2004) (AAT47413)
127L→P	1/33 (3.0)	muscle	Chen et al. (2004) (AAT47395)
139H→N	4/34 (11.8)	urine	Tavis et al. (1989) (P14996); Jin et al. (1993) (CAA79596); Clewley, unpublished (S299967)
139H→T	1/34 (2.9)	kidney	Kupper et al. (1993) (P72390)
145I→V	5/34 (14.7)	urine	Tavis et al. (1989) (P14996); Jin et al. (1993) (CAA79596); Clewley, unpublished (S29968); Kupper et al. (1993) (P72390)
146Q→R	1/34 (2.9)	urine	Chen et al. (2004) (AAT474419)
158D→E	6/34 (17.6)	urine, kidney, brain, CSF, lung	Seif et al. (1979) (NP_041397); Sugimoto et al. (1990) (CAA40235; CAA40239; CAA402476); Stoner et al. (2002)
170R→W	1/33 (3.0)	heart	Chen et al. (2004) (AAT47431)
171T→S	1/33 (3.0)	urine	Seif et al. (1979) (NP_041397)
175D→E	9/33 (27.3)	urine, kidney, muscle, heart	Chen et al. (2004) (AAT47347; AAT47353; AAT47359; AAT47419; AAT47425; AAT47431); Sugimoto et al. (1990) (CAA40247; CAA40235); Touzé et al. (2001)
175D→Q	4/32 (12.5)	urine	Tavis et al. (1989) (P14996); Jin et al. (1993) (CAA79596); Clewley, unpublished (S29968)
175D→H	1/32 (3.1)	sewage	Bofill-Mas et al. (2000) (AAF24120)
185A→V	1/33 (3.0)	sewage	Bofill-Mas et al. (2000) (AAF24120)
210V→I	10/29 (34.5)	urine	Chen et al. (2004) (AAT47347; AAT47353; AAT47359); Tavis et al. (1989) (P14996); Sugimoto et al. (1990) (CAA40247; CAA40235); Jin et al. (1993) (CAA79596); Clewley, unpublished (S29968); Touzé et al. (2001)
219T→A	3/29 (10.3)	urine	Seif et al. (NP_041397); Sugimoto et al. (1990) (CAA24307); Touzé et al. (2001)
225F→Y	4/28 (14.3)	urine	Tavis et al. (1989) (P14996); Jin et al. (1993) (CAA79596); Clewley, unpublished (S29968)
225F→L	1/28 (3.6)	urine	Sugimoto et al. (1990) (CAA40235)
259S→G	1/27 (3.7)	muscle	Chen et al. (2004) (AAT47413)
289I→T	1/27 (3.7)	muscle	Chen et al. (2004) (AAT47413)
301P→L	1/28 (3.6)	urine	Sugimoto et al. (1990) (CAA24307)
304F→S	1/28 (3.6)	muscle	Chen et al. (2004) (AAT47413)
305L→S	1/28 (3.6)	urine	Chen et al. (2004) (AAT47419)
311N→T	1/28 (3.6)	heart	Chen et al. (2004) (AAT47389)
313R→G	1/28 (3.6)	urine	Chen et al. (2004) (AAT47347)
316R→K	4/28 (14.3)	urine	Tavis et al. (1989) (P14996); Jin et al. (1993) (CAA79596); Clewley, unpublished (S29968)
330E→G	1/28 (3.6)	heart	Chen et al. (2004) (AAT47371)
340R→Q	6/28 (21.4)	urine	Tavis et al. (1989) (P14996); Jin et al. (1993) (CAA79594; CAA79596); Clewley, unpublished (S29967; S29968)
340R→K	2/29 (6.9)	urine	Sugimoto et al. (1990) (CAA40235); Touzé et al. (2001)
353K→R	7/28 (25.0)	urine, muscle	Tavis et al. (1989) (P14996); Jin et al. (1993) (CAA79594; CAA79596); Clewley, unpublished (S29967; S29968); Chen et al. (2004) (AAT47365)
362L→V	10/28 (35.7)	urine	Chen et al. (2004) (AAT47347; AAT47353; AAT47359); Jin et al. (1993) (CAA79594; CAA79596); Tavis et al. (1989) (P14996); Sugimoto et al. (1990) (CAA40247); Clewley, unpublished (S29967; S29968)

The VP1 sequence of BKV Dunlop was used as reference except for residues 153, 171, and 210 where D, T, and V, respectively were chosen as these are most common in other BKV strains. The mutation rate of a specific residue is calculated as the number of reported substitution over the total number of sequences provided for this amino acid residue.

AIDS patient (Pat-A) and from an acute myeloid leukemia patient (Pat-E). In the N-terminal 46 amino acids, substitutions V14L, E43K, and V46D were found in Pat-A, while only the latter mutation was present in Pat-E when compared with the agnoprotein sequence of BKV Dunlop (Schätzl et al., 1994). Full-length BKV agnosequences were successfully amplified by PCR from kidney, urine, lung, CSF, and brain of a patient with chronic lymphocytic leukemia. Sequence analyses of the amplicons revealed no mutations compared to the BKV (Dunlop) sequences (Stoner et al., 2002). The agnoprotein of the BKV (Cal) strain had two mutations compared to Dunlop: G42S and K49E [GenBank accession no. AF317623; Bhattacharjee and Chakraborty, unpublished]. In a recent study, the sequences of 15 full-length BKV genomes were determined in heart, muscle, and urine of different patient groups (Chen et al., 2004). Comparing the Dunlop agnoprotein sequence with those 15 non-passaged BKV isolates revealed that the three clones obtained from urine of a healthy control had the V14L mutation, while all the clones obtained from the HIV-2-positive patient and from the patient with BKV-associated capillary leak syndrome possessed the substitution of S15G. The Q9H mutation was unique for a strain cloned from the heart of the patient with BKV-associated capillary leak syndrome (CAP-h5 clone). One clone from a urine specimen of the HIV-2-positive individual (clone HI-u6) contained a deletion removing amino acid residues 9–12 (Chen et al., 2004). The V14L mutation had previously been reported in urinary specimens of renal transplant recipients [GenBank accession nos. AF411593, AF442892, AF442893, AF442895, and AF442903; Li et al., 2002], while the substitution S15G was also present in the non-passaged BKV strains MT-I and WW (Sugimoto et al., 1990). Functional studies exploring the effect of the mutations in the BKV agnoprotein on the life cycle of the virus are lacking. The cell propagated AS strain, with an 8 amino acid insertion between residues 1 and 2, and the S15G mutation has been successfully propagated in a variety of cells, suggesting that mutations in the BKV agnogene are tolerated (Tavis et al., 1989). Cell-culture infection studies with SV40 agnogene deletion mutants or RNA interference that inhibits the expression of JCV agnoprotein resulted in reduced virus yield, indicating a important, but not crucial role for this protein [reviewed in Moens and Rekvig, 2001; Orba et al., 2004; Radhakrishnan et al., 2004].

The agnoprotein of BKV is a phosphoprotein with putative phosphorylation sites Ser-7, Ser-11, Thr-21, and Ser-64 for protein kinases like PKA, PKC, calmodulin kinase-II, casein kinases-I and -II, and glycogen synthase kinase-3. The phosphorylation and biological consequences of these putative phosphoacceptor sites have not been confirmed, but it can be assumed that phosphorylation/dephosphorylation events may affect the activity of the agnoprotein. The fact that Ser-7 and Ser-11 are conserved among BKV, JCV and SV40, while Thr-21 is conserved between BKV and JCV, and that no mutations have been reported in these sites so far, strengthens this assumption.

Future perspectives

BKV is increasingly being recognized as an important human pathogen, and has been associated with a wide variety of renal, pulmonary, ophthalmologic, hepatic, autoimmune, and even neurological diseases (reviewed in Reploeg et al., 2001). It has been suggested that mutant BKV strains with greater propensity for replication are circulating in clinical populations and that BKV-associated diseases could be linked to particular viral variants (Stoner and Hübner, 2001). Detailed sequence information may therefore provide valuable information on the virulent potencies of the different BKV strains. The development of highly sensitive PCR techniques has led to the identification of novel rearranged TCR and mutations in viral proteins such as large T-antigen, VP1, and agnoprotein. To date, sequence information of complete viral genomes of non-passaged BKV is, however, scanty. This urges to systematically examine the complete genome of non-passaged strain directly obtained from a large diversity of different tissues obtained from patients with a variety of clinical conditions. A complete genomic identification of BKV isolates may allow us to obtain a more profound picture of genotypes associated with the pathophysiology of this virus. Aimed experiments to address whether a novel strain is more virulent in humans are hampered due to obvious reasons. However, appropriate human cell cultures or animal models may be used as alternatives, and may provide some indications, but will never approach the real in vivo situation.

Expanding our knowledge of the BKV genome polymorphism and understanding the adaptation of the virus genome to its host may provide helpful information when designing specific drugs that prevent or quench viral reactivation and spread of the virus. Some feasible strategies, which require a detailed knowledge of the BKV genomes could include:

(1) The design of specific antibodies or inhibitors that prevent viral adsorption. This requires a fine mapping of the VP1 amino acids crucial for host cell receptor interaction.
(2) The development of specific inhibitors for large T-antigen that can prevent viral replication or/and viral gene expression.
(3) The use of small interfering RNA technique. Viral protein expression and virus production were successfully blocked in cell cultures transiently transfected with siRNA against JCV large T-antigen and agnoprotein (Orba et al., 2004; Radhakrishnan et al., 2004). Although it was proven that siRNA against

the JCV agnogene was very effective, it was also very specific. The siRNA directed against JCV agnogene differed only in two nucleotides from the BKV agnogene, yet it was completely unable to inhibit BKV agnoprotein expression. This exemplifies that a detailed knowledge of the circulating BKV genomes in the human population is necessary because genome diversity may lead to the unwanted design of ineffective siRNA molecules. The proven specificity and efficiency of the siRNA technique in cell culture should encourage its clinical applications in patients. SiRNA delivery in patients may be facilitated by the findings that recombinant polyomavirus VP1 is able to self-assemble into virus-like particles and deliver plasmid DNA into cells (Kimchi-Sarfaty et al., 2003; Ou et al., 1999; Touzé et al., 2001). In vitro packaging of siRNA-encoding plasmids using VP1 should therefore be considered in gene transfer trials. Detailed knowledge of the VP1 epitopes and receptor binding sites may circumvent unwanted host immune responses and increase the uptake by target cells when applying BKV-like particles as a gene transfer vector in patients.

(4) Immunization with attenuated strains could also be considered and has been successfully applied for animal polyomavirus (Ritchie et al., 1998).

The application of specific and efficient anti-viral therapeutics could prevent exacerbation of the course of the disease and improve the condition of patients suffering from an active BKV infection.

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