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Donor origin of BKV replication after kidney transplantation

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ABSTRACT

Background: BK virus associated nephropathy (BKVN) leads to renal allograft dysfunction and loss. However, it is still unclear whether BKV replication in the transplant recipient is a result of reactivation in the recipient's native kidneys or whether BKV originates from the donor kidney.
Study design: Urine of 249 donor/recipient pairs was investigated for the presence of BKV-DNA by qPCR before living transplantation (Tx) and consecutively after Tx. In BKV positive samples, the VP1 typing region (TR) and, in case of the presence of sufficient amount of DNA, the complete VP1 gene, the NCCR and a fragment of the Large T-antigen were sequenced and compared between donors and corresponding recipients before and after Tx.
Results: In 20 pairs, sequencing of the BKV TR succeeded in donors and corresponding recipients after Tx. The derived sequences were completely identical in donor and post-Tx recipient samples. For comparison, identical TR sequences were detected in only 24% of 1068 randomly assembled pairs. This difference was statistically highly significant ($p < 0.0001$, Fisher's exact test). Furthermore, all VP1, Large T-antigen and NCCR BKV sequences were also identical between donors and corresponding post-Tx recipients. In two of the 20 donor/recipient pairs, VP1 TR sequencing was also successful from the recipient before Tx. In both cases the sequence differed from the sequence detected in donor and recipient after Tx giving further evidence that recipient BKV was replaced by donor BKV after Tx.
Conclusions: Our study for the first time provides evidence of BKV donor origin in renal transplant recipients.

1. Background

BK virus (BKV) associated nephropathy (BKVN) is a leading cause of renal allograft dysfunction and loss after kidney transplantation [1,2]. However, it is still unclear whether BKV replication is a result of reactivation in the recipient's native kidneys or whether the virus originates from the allograft. Donor origin of BKV was supposed in studies that were able to demonstrate a correlation between positive serostatus of the donor and the risk of BKV replication in the recipient [3,4], whereas no correlation could be detected between positive serostatus of the recipient and occurrence of viruria or BKVN [1,5]. The observation that removal of the allograft leads to a rapid decline of blood BKV load [6,7] together with the fact that retransplantation after BKVN is often successful [8–10] further corroborates the hypothesis of donor origin. Only two small studies intended to elucidate BKV origin by analyzing BKV sequences in six and four recipient pairs, respectively, that had received the kidney from the same deceased donors [3,11]. But due to the lack of donor derived BKV sequences, transmission from the donor could only be assumed because of identical sequences in corresponding recipients. Moreover, the phylogenetic approach is generally hampered by the low genetic diversity of BKV. Sequencing of the 287 nt typing region (TR), coding for a fragment of the capsid protein VP1, is the gold standard for typing of BKV resulting in four subtypes [12,13]. Additionally, other regions of the genome or the whole genome itself have been compared between strains [14,15] but only the *noncoding control region*, NCCR, has been shown to display a certain degree of sequence variation due to mutation, insertion/deletion events and rearrangements [16]. Unfortunately, these events seem to occur frequently even during the course of infection within one single person [17,18] thus limiting the usefulness of this region in terms of tracing transmission chains.

Objectives: In this study, we investigated the origin of BKV replication in kidney transplant recipients by analyzing virus sequences

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Table 1
Primers for PCR and sequencing used in this study.

Primer	Sequence	Target	Primer position[c]	Fragment finally used for sequence analysis[c]	Reference
F1n[a]	5′-CTTGAAGCATATGAAGATGGC-3′	VP1 TR	1548–1568	1650–1936	This study
R1n[a]	5′-GACCCTGCATGAAGGTTAAGC-3′	VP1 TR	1941–1961	1650–1936	This study
F2[a]	5′-TTACAGCACAGCAAGAATTC-3′	VP1	1827–1846	1564–2652	This study
R3[a]	5′-AACACCACCCCCAAAATAAC-3′	VP1	2721–2740	1564–2652	This study
F4[b]	5′-TTCCCCCAGTACTTCATGTG-3′	VP1	2255–2274	1564–2652	This study
R4[b]	5′-GGCTATCAGCTTTACAAAGAG-3′	VP1	2321–2341	1564–2652	This study
BKTT5[a]	5′-GAGCTCCATGGATTCTTC-3′	NCCR	5115–5132	1–387	[18]
BKTT6[a]	5′-CCAGTCCAGGTTTTACCA-3′	NCCR	429–446	1–387	[18]
BKTT7[a]	5′-CCCTGTTAAGAACTTTATCCATTT-3′	NCCR	5132–2	1–387	[18]
BKTT8[a]	5′-AACTTTCACTGAAGCTTGTCGT-3′	NCCR	408–429	1–387	[18]
LT-fw[a]	5′-CCAGCCTTTCCTTCCATTC-3′	Large T	2994–3012	3047–3583	This study
LT-rev[a]	5′-CACTGTTTGCTACCTAAAATG-3′	Large T	3601–3021	3047–3583	This study

[a] Primers used for PCR and sequencing.
[b] Primers used for sequencing only.
[c] Nucleotide position in Dunlop numbering, GenBank #V01108.1.

of a large cohort of 249 living related donor–recipient pairs complemented with a statistical approach.

2. Study design

2.1. Study design

All patients and their living related donors evaluated for kidney transplantation between March 2008 and October 2012 at Hannover Medical School were enrolled in this study. Arbitrary numbers were assigned to each donor–recipient pair. Urine samples that were routinely obtained from all patients and donors before transplantation (pre-Tx) were analyzed for the presence of BKV DNA by quantitative PCR (qPCR). In BKV positive donor and recipient pre-Tx samples, the VP1 typing region (TR) was sequenced and compared to BKV sequences derived from the first available BKV positive urine after Tx (median 62 days, range 15–667 days after Tx) of the corresponding recipients. In samples with sufficient viral loads, the complete VP1 gene, a fragment of the Large T gene and the NCCR were sequenced.

2.2. Viral DNA extraction and purification

DNA was extracted from 200 μl urine with the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) on a QIAcube platform (Qiagen) according to the manufacturer's instructions. To increase BKV DNA recovery from weakly positive pre-Tx urine samples, 20 ml urine was ultracentrifuged at 4 °C at 100,000 × *g* for 3 h, the pellet was resuspended in 200 μl of the supernatant and DNA was extracted as described above.

2.3. PCR and sequence analysis

Real-time quantitative PCR (qPCR) was performed with the affigene® BKV trender kit (Cepheid, Sunnyvale, USA) on an Mx3000P TM PCR platform (Stratagene, La Jolla, USA). For sequencing, respective fragments were amplified with the HotStarTaq Master Mix Kit (Qiagen) and the primers described in Table 1. PCR products were sequenced on an ABI 3130 sequencer. Sequences were deposited in GenBank with accession numbers KF468230–KF468362.

2.4. Phylogenetic analysis

Phylogenetic analysis was performed with the neighbor joining (NJ) option the MEGA software version 4.0.2 [19]. Distances were estimated by the Kimura two-parameter method [20].

2.5. Determination of BKV serostatus

BKV-specific antibody titers were measured in a modified anti-VP1 GST capture ELISA in serum samples of donors and pre-Tx recipients (median one day before Tx, range 0–3386 days). A detailed protocol for the polyomavirus ELISA has been published before [21] and was adapted for BKV. For details please see Supplementary material.

2.6. Statistical analysis

To assess the significance of identical BKV TR sequences in donor and corresponding recipient pairs, all 32 donor sequences were randomly paired with all 34 recipient sequences resulting in 1068 random non-related pairs in addition to the 20 corresponding donor/recipient pairs. The number of random pairs with identical BKV TR sequence was calculated and compared to corresponding pairs in a 2×2 contingency table. Two-tailed Fisher's exact test was calculated with GraphPad (http://www.graphpad.com/quickcalcs/).

3. Results

3.1. Patient cohort

For characteristics of 249 kidney recipients and their kidney donors please see Table 2. BKV DNA was detected in the urine of 55 donors and 37 recipients before Tx and 75 recipients post Tx by qPCR. Details regarding the number of analyzed samples and the prevalence of BKV replication are summarized in Table 3.

Table 2
Patient and donor characteristics.

	Donors	Recipients
Median age at Tx (range)	54 (29–77)	46 (18–73)
Sex		
Female	153	89
Male	96	160
Relationship donor to recipient		
Spouse	101 [41%]	
Parent	89 [36%]	
Sibling	30 [12%]	
Others[a]	17 [7%]	
Unknown	12 [5%]	

[a] Cousin, sister-in-law, grandmother, daughter, aunt, stepmother, friend.

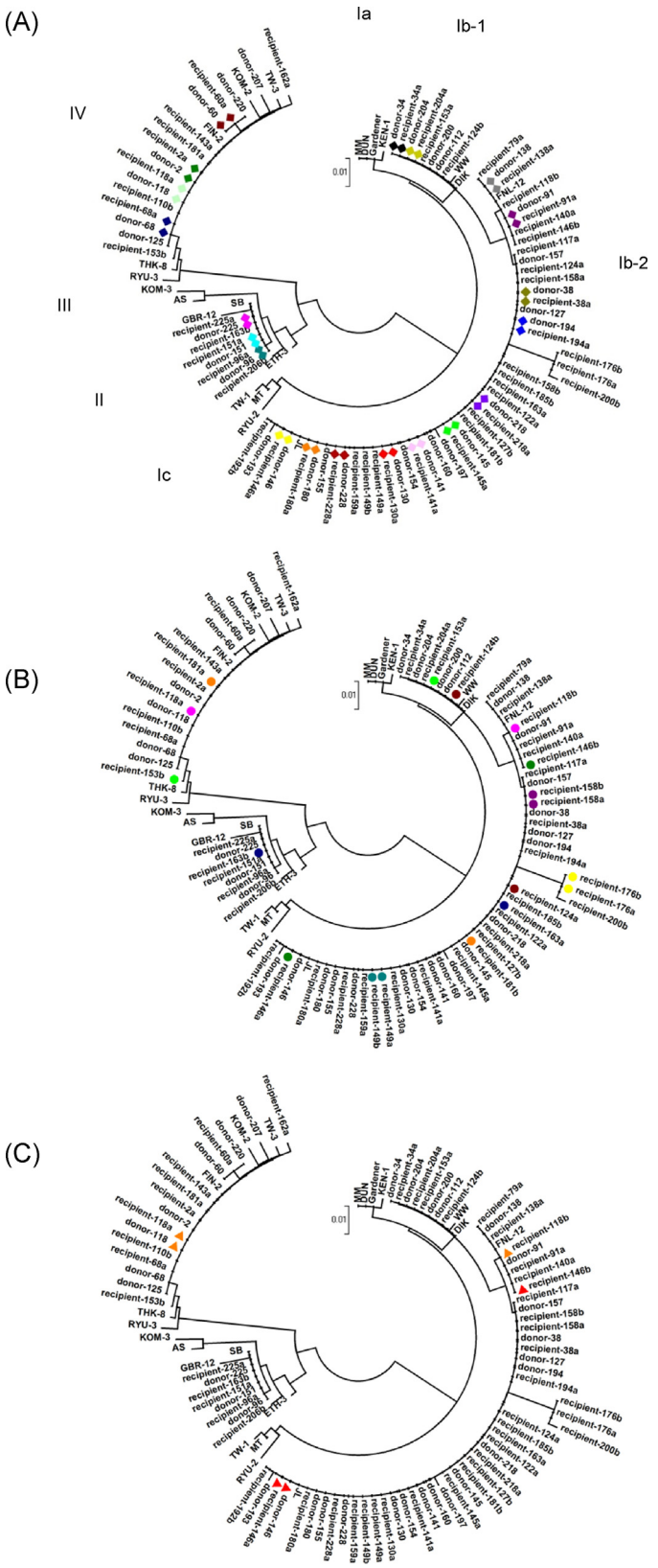

Fig. 1. Phylogenetic (neighbor joining) trees of the BKV VP1 typing region (TR) from donor and recipient samples in comparison to GenBank reference sequences for BKV subtype determination. All available sequences derived from donors, recipients before and after Tx were included in the analysis. Identical colors indicate (A) sequences from donors and corresponding recipients after Tx (Roman numerals indicate subtypes), (B) sequences from recipients before and after Tx (a = after Tx, b = before Tx), (C) sequences from donors and corresponding recipients before and after Tx. GenBank accession numbers of reference strains used for subtype clustering were as follows: NC_001538 (DUN), Z19534 (Gardener), AB211369 (Dik), AB211371 (WW), AB211370 (JL), AB211372 (MT), V01109 (MM), AB211381 (TW-1), AB263926 (KEN-1), AB263918 (FNL-12), AB211377 (RYU-2), Z19536 (SB), AB263916 (ETH-3), AB263920 (GBR-12), M23122 (AS), AB211386 (KOM-3), AB211389 (RYU-3), AB211390 (THK-8), AB211391 (TW-3), AB211387 (KOM-2), AB260033 (FIN-2). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 3
Prevalence of BKV replication and number of sequences available for analysis in donors and recipients.

	Donors	Recipients before Tx	Recipients post Tx
Number of available urine samples	249	220	208
BKV positive urine samples in qPCR	55 [22%]	37 [17%]	75 [36%]
BKV sequences determined			
VP1 TR	32	15	34
VP1 complete	17	2	28
Large T (partial)	15	–	15
NCCR	11		11

3.2. BKV subtype distribution

Sequencing of the 287 bp VP1 typing region (TR) succeeded in 34 recipients post Tx, 15 recipients before Tx and 32 donors (Table 3). Subtypes were assigned by alignment including published reference strains of the particular subtypes (Fig. 1). The majority of sequences (57/81 [70%]) could be assigned to subtype I with subgroup Ib-2 being most prevalent in our study population (49/81 [60%]), subtype IV was found in 16 of 81 samples (20%) whereas subtype II was only detected samples 8 of 81 (10%). Subtypes Ia, Ic and subtype III were absent in our study population. One TR sequence could not unambiguously assigned to one of the four subtypes, however, it seemed to be most closely related to subtype II (Fig. 1, recipient-206b).

3.3. Comparison of BKV TR sequences from donors and corresponding recipients after Tx

Out of the 32 donors with determinable TR sequence (Table 3), 20 corresponding recipients developed BK viruria after transplantation within the study period. In 20 of 20 donor–recipient pairs, the sequence of the TR was identical between the donor and the corresponding recipient after transplantation (Fig. 1A).

As identical TR sequences were also found in samples from unrelated donors and patients as well as in Genbank sequences (Fig. 1), the significance of identical sequences in donors and corresponding recipients after Tx was calculated with a 2×2 contingency table in comparison to randomly assembled pairs. Only 255 of 1068 random pairs [24%] had an identical sequence compared to 20 of the 20 real donor-recipient sequence pairs [100%]. This difference was highly significant ($p < 0.0001$, Fisher's exact test). Moreover, identical sequences in donors and corresponding recipients after Tx (20/20 [100%]) were also found more frequently compared to donors and corresponding recipient before to Tx (1/4 [25%] ($p < 0.01$, Fisher's exact test)).

3.4. Comparison of BKV VP1, Large T and NCCR sequences from donors and corresponding recipients after Tx

In order to overcome the problem of highly conserved BKV sequences in elucidating BKV origin, the complete VP1 gene was additionally sequenced. In each of the 16 pairs with determinable VP1 sequence, an identical sequence was detected in the donor and

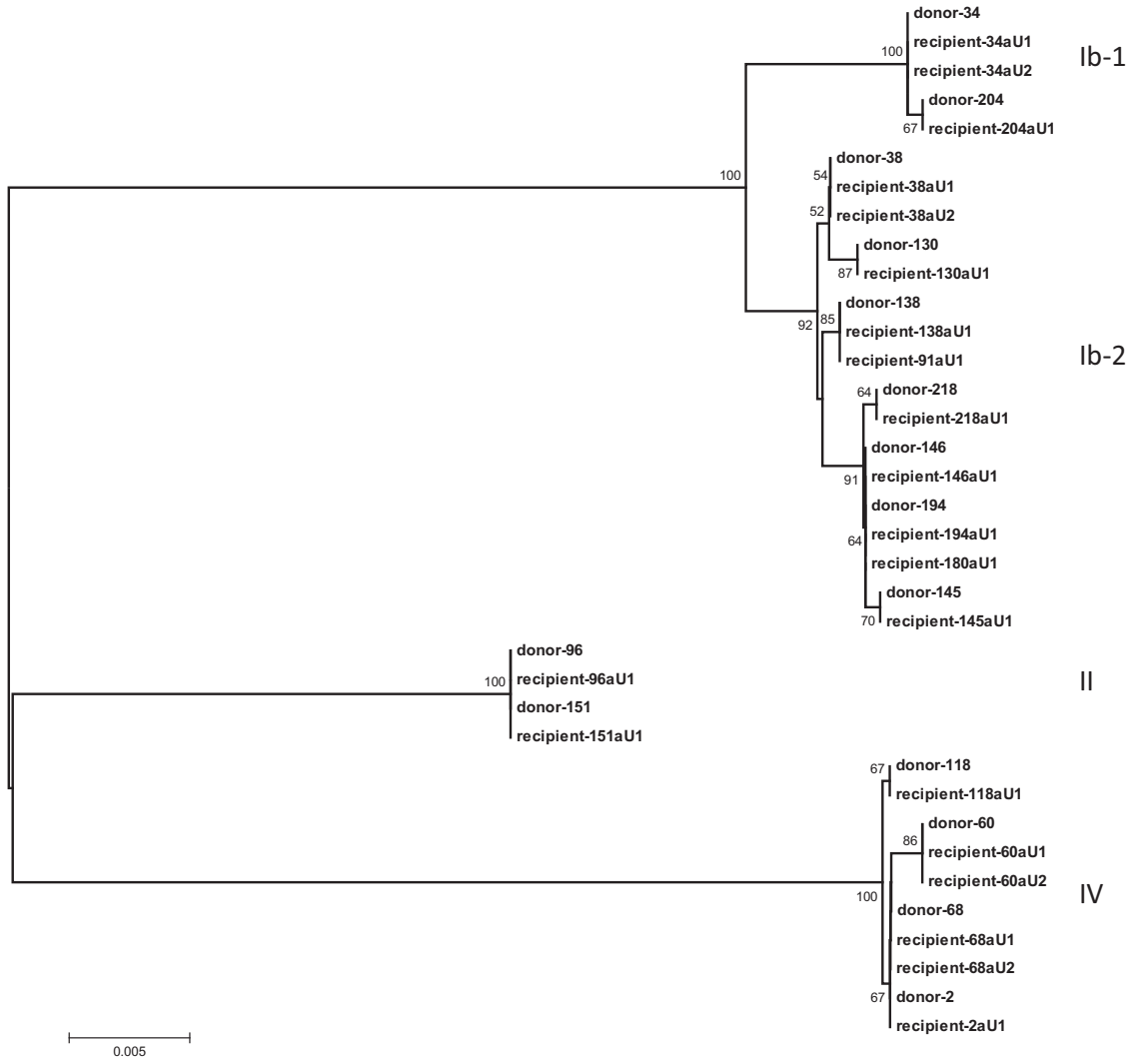

Fig. 2. Phylogenetic (neighbor joining) tree of concatenated sequences of the complete VP1 gene and the 536 bp fragment of the Large-T gene. Sequences were available for comparison from 15 donors and at least one BKV positive urine of their corresponding recipients after Tx. Additional sequences derived from consecutive urine samples of post-Tx recipients were included if available (a: after Tx, U1/U2: first/second urine sample analyzed after Tx). The sequences of recipient 91 and recipient 180 were included although the VP1 and Large-T sequences could not be derived from the corresponding donors due to low viral load in the respective samples. Subtype assignment based on the sequence of the VP1 TR were indicated with roman numerals. All major nodes were labeled with bootstrap values out of 1000 replicates (only values of 50% and higher are shown).

the corresponding recipient after Tx. Similarly, the fragment of the Large T gene was identical in all 15 donors–recipient pairs with determinable sequence (Fig. 2). In addition, the NCCR sequence that was determined in 11 donors and corresponding recipients after Tx was found to be identical in all corresponding pairs (data not shown). Unfortunately, identical sequences were also found in samples from unrelated donors and patients as well as in GenBank sequences, thus precluding a direct phylogenetic proof of BKV transmission.

3.5. Comparison of BKV TR sequences from recipients before and after Tx

In 9 recipients, the TR sequence could be analyzed before and after transplantation with 6 out of 9 recipients showing different TR sequences before and after Tx. The fact that an identical TR sequence was detected in 3 out of 9 recipients before and after transplantation does not rule out donor origin in these cases because in all 3 recipients a donor sequence was not available for comparison and, moreover, the widely distributed BKV subtype Ib-2 was found (Fig. 1B).

3.6. Comparison of BKV TR sequences from donors with sequences from recipients before and after Tx

In two donor/recipient pairs (pair 118 and pair 146), BKV TR sequences from the donor as well as from the recipient before and after Tx were available. In both cases, the TR sequence of the recipient before Tx differed from the sequence found to be identical in the donor and recipient after Tx thus giving evidence that recipient BKV was replaced by donor BKV in these cases (Fig. 1C).

3.7. Association between serostatus in donors and recipients and BKV replication in recipients

187 donor- and 136 recipient pre-Tx sera were available for the determination of BKV serostatus. BKV seroprevalence was similar in donors (88%) and recipients (91%). One recipient and three donors were tested seronegative but BKV DNA was concurrently

Table 4
BKV serostatus and detection of BKV DNA in donor and recipient pairs.

Serostatus donor/recipient	Number of donor–recipient pairs	BKV PCR positive donors	BKV PCR positive recipients before Tx	BKV PCR positive recipients after Tx
+/+	106	28	17	32
+/−	10	2	1	4
−/+	14	1	1	1
−/−	2	0	0	0
Total	132	31	19	37

+/− denotes positive or negative serostatus, respectively.

detected in urine thus suggesting false negative results of the BKV-IgG assay or very low antibody titers in the respective persons.

In 132 pairs, the BKV serostatus could be determined in both donor and corresponding recipient, respectively (Table 4). BKV replication after Tx was observed in 37 recipients (of these 132 pairs). BKV replication after Tx was significantly more frequent in recipients with seropositive donor (36/37 [97%]) than in recipients with seronegative donor (1/37 [3%]) ($p = 0.04$, Fisher's exact test). In contrast, no statistical association between seropositivity of the recipient before Tx and BKV replication after Tx was detected ($p = 0.73$, Fisher's exact test).

In only 12 donor–recipient pairs (12/132 [9%]) the recipient was seronegative pre-Tx. In 4 of these 12 seronegative recipients, BKV replication was detected after Tx indicating BKV transmission from the donor.

4. Discussion

As already demonstrated in 1999, BKV replication represents a threat to allograft survival in kidney transplant recipients [22]. However, it is still unclear, whether the virus originates from the donor allograft or the recipient's native kidney. In this study, we compared for the first time both donor- and recipient derived BKV sequences in a study population of 249 kidney recipients and their corresponding living related donors.

In 20 of the 249 donor/recipient pairs BKV sequencing was achieved both in the donor and in the recipient post transplantation. In all of these pairs, donor and recipient had identical TR sequences. In a contingency table analysis this sequence identity in real donor/recipient pairs (100%) vs. random donor/recipient pairs (24%) was highly significant ($p < 0.0001$) indicating donor to recipient transmission of BKV. However, a higher percentage of identical BKV strains in donor/recipient pairs cannot be excluded due to possible pre transplantation intra family transmission, as donors and recipients were close relatives or spouses. Previously, phylogenetic analysis has been successfully applied in multiple studies on genomic diversity of BKV on a population level [13,23–28]. In this study, a direct phylogenetic proof of BKV donor origin was not feasible because BKV sequences were highly conserved within one subtype. Thus, an individual proof of donor origin can only be drawn in case of the additional availability of recipient derived pre-Tx BKV sequences demonstrating replacement of recipient BKV by donor BKV. In two donor–recipient pairs BKV sequences could be derived from the recipient before as well as after Tx, and from the donor. Donor origin of BKV was proven in both cases by identical BKV sequences in donor and recipient after Tx, whereas the BKV sequence detected in the recipient before Tx was different. In 7 additional recipients, BKV sequences were available from before and after Tx (but not from the donor) with 4 recipients revealing different subtypes before and after Tx. This finding gives additional albeit indirect evidence of donor origin of BKV replication in the transplant recipients. Donor origin of BKV replication after transplantation even in case of BKV replication in the recipient before Tx may be explained by an ineffective immune surveillance in case of substitution of the recipient's BKV strain by a different BKV strain of the donor. However, Chen et al. demonstrated that BKV epitopes recognized by cytotoxic T lymphocytes were highly conserved, at least within the 23 different BKV strains analyzed [29].

In order to investigate the relevance of BKV transmission for BKV DNAemia, BKV sequences were also analyzed in blood samples. Ten recipients were positive in the VP1 PCR and 8 recipients in the less sensitive LT and NCCR PCR (data not shown). Sequence identity of blood borne virus to urine derived BKV (and donor derived sequences) was demonstrated in all recipients thus suggesting the relevance of BKV transmission for BKV DNAemia. An on going study will analyze the relevance of BKV transmission for BKVN and graft survival (Schwarz et al., in preparation).

In spite of the low genetic diversity of BKV on the subtype level, which hampered a phylogenetic proof of BKV transmission in the present study, a high variability of BKV coding sequences resembling a viral quasispecies has been described [30,31]. A few nucleotide polymorphisms were detected in the VP1 gene in samples of two recipients (data not shown) even with direct sequencing of PCR products in the present study: a minority sequence with four nucleotide substitutions was detected in the VP1 gene in a recipient post-Tx. One of these four nucleotide positions (position 217) may represent a "hot spot" of substitution events in the VP1 gene [31]. In the second recipient, a polymorphism was detected at only one position (position 248) in the VP1 gene. Interestingly, the same polymorphism was already present in the BKV sequence from the pre-Tx sample which, at least in the absence of a donor derived sequence, might be interpreted as recipient derived BKV in this patient. This latter case is exceptional and does not challenge the other findings in our study as this recipient had undergone lung transplantation as well as autologous bone marrow transplantation 10 years earlier and presented already with an extremely high BKV load (>10^9 copies/ml) in urine before kidney transplantation. As the patient's native kidneys were not removed during transplantation, high level BKV replication appeared to continue unchanged after Tx.

The BKV seroprevalence of 89% and the subtype distribution determined was similar to published data in healthy blood donors [32]. Our finding that positive serostatus of the donor but not of the recipient significantly correlated with occurrence of BKV replication after Tx corroborated findings in earlier studies [1,3–5]. However, as the recipient tested seronegative before Tx in only 9% of the donor–recipient pairs analyzed in our study and with only 4 seronegative recipients developing BK viruria after Tx, donor transmission could not be proven serologically.

In conclusion, this is the first study giving statistical evidence of donor origin of BKV infection in renal transplant recipients by comparing BKV sequences from donors and recipients in living related kidney transplantation. Though BKV seroprevalence is too high to exclude seropositive donors from kidney donation, the potential high risk constellation (BKV shedding in donors) should be analyzed for clinical outcome in comparison to other risk factors for reduced transplant survival [33–37].

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Competing interests

None declared.

Ethical approval

Donor and recipient had to give informed consent before testing of urine and blood. The study was approved by the local Ethical Committee #6116.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2013.11.009.

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