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Next-generation sequencing shows marked rearrangements of BK polyomavirus that favor but are not required for polyomavirus-associated nephropathy

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ABSTRACT

Background: BKPyV is associated with polyomavirus-associated nephropathy (PVAN), a major cause of graft rejection in kidney transplant recipients (KTRs). Mutations occur in the transcriptional control region (TCR) of BKPyV, but whether they are required for the development of PVAN is not completely understood. To this end, we characterized BKPyV TCRs from KTRs to assess whether TCR mutations are associated with PVAN.

Study design: We analyzed urine and plasma samples of fifteen KTRs with biopsy-confirmed PVAN, presumptive PVAN, or probable PVAN in order to explore the contents of the BKPyV virome. BKPyV TCRs were amplified and deep sequenced to characterize the viral strains. Alterations in block structures and transcription factor binding sites were investigated.

Results: The majority of sequences in both urine and plasma samples represented archetype BKPyV TCR. Minor populations harboring rearranged TCRs were detected in all patient groups. In one biopsy-confirmed PVAN patient rearranged TCRs predominated, and in another patient half of all reads represented rearranged sequences.

Conclusions: Although archetype BKPyV predominated in most patients, highest proportions and highest numbers of rearranged strains were detected in association with PVAN. TCR mutations seem not necessary for the development of PVAN, but immunosuppression may allow increased viral replication giving rise to TCR variants with enhanced replication efficiency.

1. Background

Polyomavirus-associated nephropathy (PVAN) is due to lytic infection of kidney tubular cells by reactivated BK polyomavirus (BKPyV). Prolonged immunosuppression may result in reactivation of BKPyV and replication in the kidneys leading to tissue damage characterized by interstitial inflammation, tubular atrophy, and fibrosis [1–3]. PVAN is a serious post-transplant complication of kidney transplantation: 4.2–8.0% of kidney transplant recipients (KTRs) develop PVAN which, if left untreated, leads to graft rejection in approximately 15% of cases [4–6]. In the lack of prophylactic or curative treatment for PVAN, monitoring and early identification of patients at risk is the only option.

In order to prevent PVAN, monthly monitoring of BKPyV replication in KTRs up to nine months post-transplant and thereafter every three months at least until the second post-transplant year is recommended [7]. Prolonged high-level BKPyV replication in blood (>1.0E + 04 copies/mL) and urine (> 1.0E + 07 copies/mL) always precedes PVAN onset. Additionally, excretion of BKPyV-infected uroepithelial cells with intranuclear inclusion bodies, i.e. decoy cells, in urine denotes BKPyV reactivation in the kidneys [3,7]. A definitive diagnosis, however, requires positive immunohistochemical staining for polyomavirus large T antigen and the presence of typical cytopathic changes indicative of

Abbreviations: BKPyV, BK polyomavirus; KTR, kidney transplant recipient; NCCR, non-coding control region; PVAN, polyomavirus-associated nephropathy; TCR, transcriptional control region

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polyomavirus infection in kidney biopsy [7]. Approximately 30% of KTRs experience BKPyV viruria and decoy cell shedding within a year from transplantation; of those 7-17.3% will further develop BKPyV DNAemia [5,8,9].

While the role of genomic rearrangements in the neurotropic JC polyomavirus in progressive multifocal leukoencephalopathy are well established [10,11], the role of BKPyV genome rearrangements in PVAN is pending, despite intensive studies. Archetype BKPyV is assumed to cause primary infection and establish persistence in the kidneys of up to 90% of individuals already in the childhood [12]. The noncoding control region (NCCR) of archetype BKPyV, such as the WW strain [13], regulates viral replication and gene expression. It can be divided into arbitrary sequence blocks O (142 base pairs, bp), P (68 bp), Q (39 bp), R (63 bp), and S (63 bp), respectively [14]. The NCCR harbors a number of cellular transcription factor binding sites (TFBSs) for Sp1, AP-1, NFAT, NF-1, and p53 [15–20]. The P, Q, R, and S blocks constitute the transcriptional control region (TCR) [14] that is prone to mutations such as rearrangements, deletions, and duplications. PVAN patients frequently have mutations within the TCR that seem to increase viral pathogenicity in vitro [21,22]. Also, mutations may enhance viral microRNA expression or modify the action of microRNAs on viral gene expression [23].

In the present study we performed deep sequencing of BKPyV TCRs present in paired plasma and urine samples of KTRs with or without histological and clinical evidence of PVAN. The aim was to study whether rearrangements within the TCR are associated with kidney function and the development of PVAN.

2. Materials and methods

2.1. Kidney transplant recipients

Fifteen plasma and urine samples, both collected on the same day, from 15 KTRs (5 females, 10 males; median age: 58 years, range: 23–72) were included in the study. The patients received a kidney transplant between August 2009 and March 2014 at the Medical University of Vienna, Austria. The patients were screened for BKPyV replication according to current recommendations [7]. Detailed clinical information is presented in Supplementary material.

2.2. Quantitative BKPyV and JCPyV real-time PCR

DNA was extracted from 200 µl plasma or urine using the automated NucliSens EasyMag extractor (bioMérieux, Marcy l'Etoile, France) and eluted into a final volume of 70 µl. BKPyV and JCPyV DNA were quantified using real-time Taqman PCR with primers and probes located within the minor capsid protein VP3. Amplification was performed in two separate reactions using the same forward primer (5′-TGC TCC TCA ATG GAT GTT GC-3′), the same fluorescence labelled probe (5′-FAM-CGG GAC TGT AAC ACC TGC TCT TGA AGC-TAMRA-3′), and either a BKPyV-specific reverse primer (5′-AGC TGC CCC TGG ACA CTC-3′) or a JCPyV-specific reverse primer (5´-CAC GGG GTC CTT CCT TTC-3´). PCR was run in a total volume of 25 µl containing 5 µl of template DNA and TaqMan universal PCR master mix (PE Applied Biosystems, Waltham, MA). Uniform PCR cycling conditions were: 3 min at 50 °C, 10 min at 95 °C followed by 45 cycles of 95 °C for 15 s, 55 °C and 72 °C each for 30 s. For quantification standard proficiency panels distributed by the Quality Control for Molecular Diagnostics (QCMD, Glasgow, UK) were used.

2.3. BKPyV TCR amplification

Total nucleic acids from plasma (180 µl) were extracted using the Easymag Nuclisens instrument and nucleic acid isolation kit (bioMérieux, Marcy l'Etoile, France). From six patients either 100 µl (5, 7, 9) or 80 µl (3, 4, 6) of original plasma was available. From urine samples (140 µl) nucleic acids were isolated using the Qiagen viral RNA mini kit (Qiagen, Hilden, Germany). Nucleic acids were eluted in 25 µl (plasma) or 60 µl (urine) elution buffer. Amplification of the complete TCR was performed as described previously [23].

2.4. Sequencing

Complete BKPyV TCRs (~319 bp) were sequenced in single reads using Illumina MiSeq sequencing (Illumina, San Diego, CA). For library generation the amplicons were polished, A-tailed, adapter-ligated, and purified. PCR amplification of the libraries was performed using Phusion Hot Start II DNA Polymerase (Thermo Fisher Scientific), Index P7 primers, and full-length P5 adapter primers. After pooling and size-selection the library pool was subjected to paired-end sequencing on a MiSeq Sequencer using the v3 600 cycle kit (Illumina).

2.5. Sequence analysis

The TCR sequence data were analyzed as described previously [23]. Only full-length TCR sequences containing both forward and reverse primer sequences were clustered. The structure of each cluster was confirmed by aligning cluster sequence with cluster reference sequence using the Tablet tool [24] (Supplementary Figs. 1 and 2). Nucleotide numbering is according to the TCR of BKPyV WW strain [13] (AB211371.1; nucleotides 35…267), which was used as the archetype BKPyV reference strain in sequence comparisons. Details of sequencing analysis are given in Supplementary Material.

3. Results

3.1. Patient characteristics

In this study fifteen KTRs with BKPyV DNAemia and DNAuria (>1.0E + 03 DNA copies/mL) were included (Table 1). Two patients (1, 2) had no clinical evidence of PVAN progression. Eight patients (3–10) had high plasma BKPyV DNA loads (median: 2.50E + 04, range: 1.0E + 04 – 3.80E + 05) and were considered to have presumptive PVAN [7]. Five patients (11–15) had PVAN confirmed by kidney histology performed within one week pre- or post-sampling (proven PVAN [7];) and had high-level BKPyV DNAemia (median: 1.70E + 06, range:1.40E + 04 – 4.20E + 08) accompanied by high decoy cell shedding (median: 70%, range: 50-98%) and drop in kidney function. Detailed clinical information is presented in Supplementary material.

3.2. Archetype BKPyV predominated in all but two patients with biopsy-confirmed PVAN

High amounts of original paired-end sequencing data were obtained from all KTRs, and comparable amounts of post-analytic sequences were gained from plasma and urine (Table 2). In plasma and urine of KTRs with either presumptive or probable PVAN, the majority of sequences, on average 99.49%, represented archetype TCRs. Archetype TCR was also frequent in plasma and urine of three (12–14) out of five patients with histologically confirmed PVAN. In five presumptive PVAN patients (3, 5, 6, 7, 9), and in one patient with proven PVAN (15) exclusively archetype TCR sequences exceeded the cutoff of 1000 reads in both plasma and urine.

The archetype BKPyV TCR sequences obtained from plasma and urine of each individual patient were always identical and shared high nucleotide identity (>97.01%) with archetype BKPyV WW reference strain. A total of sixteen nucleotide point mutations (P block: A52 T, A53 T, T65C, del88_89, A90C; R block: A145 G, A146 G, A147C, C179 G, G181A, G182 T; S block: A222 G, T226 G, G228A, C239 G, insT253_254) were identified in all patients with biopsy-confirmed PVAN, in three patients with presumptive PVAN (3, 8, 9), and in two

Table 1
Clinical characteristics of kidney transplant recipients with proven PVAN, presumptive PVAN, or probable PVAN. JC polyomavirus copy numbers remained below qPCR detection level (< 100 copies/mL) in the majority of samples.

Patient	Age (gender)	Time from KTx at sampling (days)	Duration of BKPyV viremia at sampling (days)	BKPyV load in urine (copies/mL)	BKPyV load in plasma (copies/mL)	JCPyV load in urine (copies/mL)	JCPyV load in plasma (copies/mL)	Decoy cells (%)	Drop of kidney function	Histological finding	Kidney status
1	58 (M)	140	0	3.10E + 07	5.40E + 03	BDL	BDL	< 5	no	N/A	Probable PVAN
2	59 (M)	159	77	3.10E + 08	2.00E + 03	BDL	BDL	< 5	no	N/A	Probable PVAN
3	23 (M)	468	0	2.50E + 08	2.70E + 04	BDL	BDL	< 5	no	N/A	Presumptive PVAN
4	72 (M)	207	71	3.70E + 07	1.00E + 04	4.90E + 07	1.50E + 02	5	no	N/A	Presumptive PVAN
5	52 (M)	40	0	3.50E + 10	1.10E + 04	8.70E + 01	BDL	< 5	no	N/A	Presumptive PVAN
6	27 (M)	126	28	1.20E + 10	1.40E + 05	BDL	BDL	40	no	N/A	Presumptive PVAN
7	60 (M)	303	0	1.10E + 08	2.30E + 04	BDL	BDL	90	yes	N/A	Presumptive PVAN
8	59 (M)	100	28	2.80E + 08	1.90E + 04	BDL	BDL	80	yes	N/A	Presumptive PVAN
9	45 (F)	127	22	5.10E + 08	3.80E + 05	BDL	BDL	70	no	N/A	Presumptive PVAN
10	39 (F)	108	78	1.00E + 10	1.10E + 05	BDL	BDL	50	no	N/A	Presumptive PVAN
11	67 (F)	90	90	1.90E + 11	4.20E + 08	3.90E + 07	8.60E + 04	60	yes	PVAN, SV40 LTag positive	PVAN
12	69 (M)	225	14	1.60E + 11	1.10E + 07	1.60E + 03	BDL	> 90	yes	PVAN, SV40 LTag positive	PVAN
13	58 (F)	224	131	2.90E + 10	4.70E + 05	BDL	BDL	70	yes	PVAN, SV40 LTag positive	PVAN
14	44 (M)	235	148	2.00E + 11	1.70E + 06	BDL	BDL	> 98	yes	PVAN, SV40 LTag positive	PVAN
15	49 (F)	69	14	6.30E + 07	1.10E + 04	BDL	BDL	50	yes	PVAN, SV40 LTag positive	PVAN

KTx, kidney transplantation; BKPyV, BK polyomavirus; JCPyV, JC polyomavirus; BDL, below detection level; N/A, not applicable; PVAN, polyomavirus-associated nephropathy; LTag, large T antigen.

Table 2
Characterization of BK polyomavirus transcriptional control regions from urine and plasma of kidney transplant patients with proven PVAN, presumptive PVAN, or probable PVAN. BKPyV WW strain (AB211371.1) was used as the archetype reference strain.

Patient	Kidney status	Source	BKPyV load (copies/mL)	Original read amount	Post-analytic read amount	Phenotype	TCR architecture	TCR lenght (bp)	Sequence identity with archetype TCR (%)	Proportion of all sequences in the sample (%)
1	Probable PVAN	Urine	3.10E + 07	194,648	125,244	Archetype	P-Q-R-S	234	97.01	97.19
						Rearranged	P-(Q)-(R)-S	184	76.10	1.76
		Plasma	5.40E + 03	195,179	124,213	Archetype	P-Q-R-S	234	97.01	98.17
						Rearranged	P-(Q)-(R)-S	207	85.90	1.93
2	Probable PVAN	Urine	3.10E + 08	173,797	121,460	Archetype	P-Q-R-S	233	98.50	99.72
		Plasma	2.00E + 03	206,449	126,478	Archetype	P-Q-R-S	233	98.50	96.01
						Rearranged	(P)-(Q)-R-S	188	79.40	3.98
3	Presumptive PVAN	Urine	2.50E + 08	179,658	129,518	Archetype	P-Q-R-S	233	98.50	99.87
		Plasma	2.70E + 04	147,503	85,959	Archetype	P-Q-R-S	233	98.50	99.97
4	Presumptive PVAN	Urine	3.70E + 07	197,551	134,458	Archetype	P-Q-R-S	233	100	98.72
						Rearranged	P-(Q)-(S)	144	61.80	0.74
		Plasma	1.00E + 04	179,504	102,914	Archetype	P-Q-R-S	233	100	98.90
						Rearranged	P-(Q)-(P)-Q-R-S	279	83.50	0.97
5	Presumptive PVAN	Urine	1.00E + 10	209,807	128,873	Archetype	P-Q-R-S	233	100	98.10
		Plasma	1.10E + 04	197,996	137,845	Archetype	P-Q-R-S	233	100	99.97
6	Presumptive PVAN	Urine	1.00E + 10	156,320	109,510	Archetype	P-Q-R-S	233	100	98.76
		Plasma	1.40E + 05	174,402	111,574	Archetype	P-Q-R-S	233	100	99.97
7	Presumptive PVAN	Urine	1.10E + 08	186,056	121,698	Archetype	P-Q-R-S	233	100	99.26
		Plasma	2.30E + 04	210,522	139,579	Archetype	P-Q-R-S	233	100	99.81
8	Presumptive PVAN	Urine	2.80E + 08	205,336	131,536	Archetype	P-Q-R-S	234	97.01	92.37
						Rearranged	P-(Q)-(R)-S	185	76.10	3.88
		Plasma	1.90E-04	170,259	107,301	Archetype	P-Q-R-S	234	97.01	98.84
9	Presumptive PVAN	Urine	5.10E + 08	203,181	147,722	Archetype	P-Q-R-S	233	98.13	98.31
		Plasma	3.80E + 05	201,098	142,933	Archetype	P-Q-R-S	233	98.13	98.82
10	Presumptive PVAN	Urine	1.00E + 10	171,575	118,488	Archetype	P-Q-R-S	233	98.50	96.10
						Rearranged	P-(Q)-(R)-S	184	77.70	0.89
						Rearranged	(P)-Q-R-S	215	91.0	0.51
		Plasma	1.10E + 05	197,232	135,637	Archetype	P-Q-R-S	233	98.50	95.16
						Rearranged	P-(Q)-(R)-S	184	77.70	0.89
						Rearranged	P-(Q)-(R)-S	215	91.0	0.51
11	PVAN	Urine	1.90E + 11	289,372	217,378	Rearranged	(P)-Q-R-S	216	90.67	27.6
						Rearranged	P-(P)-Q-R-S	266	84.30	18.17
						Rearranged	(P)-Q-R-S	226	91.60	15.18
						Archetype	P-Q-R-S	234	96.58	8.05
						Rearranged	P-(Q)-(R)-S	185	74.70	7.67
						Rearranged	P-(P)-Q-R-S	256	87.60	6.67
						Rearranged	P-(Q)-(P)-(Q)-(R)-S	266	57.60	5.52
						Rearranged	P-(Q)-(P)-Q-R-S	256	86.90	2.30
		Plasma	4.20E + 08	322,959	231,178	Rearranged	(P)-Q-R-S	216	90.67	46.93
						Rearranged	P-(P)-Q-R-S	266	84.30	7.19
						Rearranged	P-(Q)-(P)-(Q)-(R)-S	266	57.60	7.14
						Rearranged	P-(Q)-(R)-S	208	84.40	5.19
						Rearranged	P-(Q)-(R)-(S)-(Q)-(R)-S	232	65.20	2.88
						Rearranged	P-(Q)-(R)-(Q)-(R)-S	225	59.30	2.60
						Archetype	P-Q-R-S	234	96.58	2.60
						Rearranged	P-(Q)-(P)-(Q)-S	198	48.80	2.38
						Rearranged	P-(Q)-(P)-Q-(R)-(S)	249	63.60	2.01
						Rearranged	P-(Q)-(P)-Q-(R)-(S)	187	49.60	1.82
						Rearranged	P-(Q)-(P)-(Q)-(R)-S	248	61.40	1.56
						Rearranged	P-(Q)-(R)-(P)-(Q)-(R)-S	230	68.0	1.51
						Rearranged	P-(Q)-(P)-Q-R-S	256	86.90	1.38
						Rearranged	P-(Q)-(P)-Q-(R)-S	205	48.60	1.10
						Rearranged	P-(P)-Q-R-S	256	87.60	0.95
12	PVAN	Urine	1.60E + 11	293,225	233,812	Archetype	P-Q-R-S	234	97.01	73.22

(*continued on next page*)

Table 2 (*continued*)

Patient	Kidney status	Source	BKPyV load (copies/mL)	Original read amount	Post-analytic read amount	Phenotype	TCR architecture	TCR lenght (bp)	Sequence identity with archetype TCR (%)	Proportion of all sequences in the sample (%)
						Rearranged	P-(Q)-(R)-S	185	74.70	6.48
						Rearranged	P-(Q)-(R)-S	208	84.40	4.58
						Rearranged	P-(Q)-(R)-S	174	70.0	1.63
						Rearranged	P-(Q)-(S)	115	22.80	1.34
						Rearranged	P-(Q)-(R)-S	185	74.70	1.17
						Rearranged	P-(Q)-(R)-S	164	67.20	0.99
						Rearranged	P-(Q)-(R)-S	166	68.50	0.99
						Rearranged	P-(Q)-(S)	148	60.90	0.93
		Plasma	1.10E + 07	352,815	262,639	Archetype	P-Q-R-S	234	97.01	57.11
						Rearranged	P-(Q)-(R)-S	185	74.70	4.75
						Rearranged	P-(Q)-(R)-S	208	84.40	4.38
						Rearranged	P-(Q)-(S)	148	60.90	2.47
						Rearranged	P-(Q)-(R)-S	166	68.50	2.32
						Rearranged	P-(Q)-(R)-S	185	74.70	1.90
						Rearranged	P-(Q)-(R)-S	174	70.0	1.33
						Rearranged	P-(Q)-(R)-S	180	73.0	1.14
						Rearranged	P-(Q)-(R)-S	171	69.90	0.82
						Rearranged	P-(Q)-S	167	68.90	0.76
						Rearranged	P-(Q)-(S)	163	67.20	0.65
13	PVAN	Urine	2.90E + 10	294,844	237,925	Archetype	P-Q-R-S	233	98.50	98.77
		Plasma	4.70E + 05	360,608	263,356	Archetype	P-Q-R-S	233	98.50	46.68
						Rearranged	P-(Q)-(S)	141	59.70	3.32
						Rearranged	P-(Q)-(S)	152	59.80	3.12
						Rearranged	P-(Q)-(R)-S	206	85.80	2.85
						Rearranged	P-Q-(R)-S	225	90.80	2.47
						Rearranged	P-(Q)-(S)	148	60.90	2.47
						Rearranged	P-Q-(R)-S	201	82.20	2.28
						Rearranged	P-Q-(R)-(S)	168	68.60	1.71
						Rearranged	P-(Q)-(S)	131	55.40	1.52
						Rearranged	P-Q-(R)-(S)	140	57.40	1.33
						Rearranged	P-(Q)-(S)	113	47.60	1.14
						Rearranged	P-(Q)-(R)-S	203	85.80	1.14
						Rearranged	P-Q-(R)-(S)	130	53.20	0.99
						Rearranged	P-(Q)-(R)-S	185	77.40	0.95
						Rearranged	P-(Q)-S	165	68.80	0.91
						Rearranged	P-(Q)-(R)-S	181	74.80	0.87
14	PVAN	Urine	2.00E + 11	308,733	251,022	Archetype	P-Q-R-S	233	98.50	34.26
						Rearranged	(P)-Q-R-S	215	90.60	17.33
						Rearranged	P-(Q)-(R)-S	184	77.70	7.97
						Rearranged	P-(Q)-(S)	113	47.60	6.10
						Rearranged	P-(P)-(Q)-(S)	145	41.90	5.18
						Rearranged	P-(Q)-(R)-(P)-(Q)-(R)-S	226	69.40	4.78
						Rearranged	P-(Q)-(R)-S	173	73.0	3.98
						Rearranged	P-(P)-Q-R-S	265	84.30	2.19
						Rearranged	P-(P)-Q-R-S	265	84.30	1.87
						Rearranged	P-(Q)-(R)-S	207	87.60	1.57
						Rearranged	(P)-Q-R-S	225	94.80	0.72
						Rearranged	P-(Q)-(R)-S	155	65.20	0.64
						Rearranged	P-(Q)-(R)-S	166	70.0	0.56
						Rearranged	P-(Q)-(R)-(S)	130	53.20	0.52
		Plasma	1.70E + 06	322,959	231,178	Archetype	P-Q-R-S	233	98.50	46.27
						Rearranged	P-(Q)-(R)-S	184	77.70	12.86
						Rearranged	P-(Q)-(R)-(P)-(Q)-(R)-S	226	69.40	10.81
						Rearranged	P-(Q)-(R)-S	173	73.0	5.41
						Rearranged	P-(P)-(Q)-(S)	145	41.90	5.19
						Rearranged	(P)-Q-R-S	215	90.60	4.28
						Rearranged	P-(Q)-(S)	113	47.60	2.60
						Rearranged	P-(Q)-(R)-S	206	87.10	3.94
						Rearranged	P-Q-(R)-(S)	130	53.20	1.30
15	PVAN	Urine	6.30E + 07	288,023	230,811	Archetype	P-Q-R-S	234	97.01	99.71
		Plasma	1.40E + 04	281,712	217,932	Archetype	P-Q-R-S	234	97.01	99.42

BKPyV, BK polyomavirus; TCR, transcriptional control region; PVAN, polyomavirus-associated nephropathy.

probable PVAN patients (1, 2) (Supplementary Fig. 1). Some point mutations were located in NF-1 TFBSs (T65C, C179 G, G181A, G182 T, C239 G) and p53 (del88_89, A90C).

3.3. Rearranged BKPyV TCRs were found particularly in patients with biopsy-confirmed PVAN

In contrast, rearrangements were common among confirmed PVAN patients. The majority of sequences obtained from plasma and urine of one KTR with histologically verified PVAN (patient 11) represented

rearranged sequences (84.64% of sequences in plasma, 83.11% in urine). A considerable proportion of strains harbored an 18-bp deletion in the P block (P32-49). Half of the sequences from both plasma (46.39%) and urine (53.41%) of another PVAN patient (14) represented rearranged TCR regions.

Small proportions of rearranged sequences were found in all PVAN subgroups (Table 2, Supplementary Fig. 2). Rearranged TCRs were found in both plasma and urine samples of one probable PVAN patient, two patients with presumptive PVAN, and three patients with confirmed PVAN; in the plasma samples of one probable and one confirmed PVAN patient; in the urine sample of one presumptive PVAN patient. Rearranged strains were similarly frequent in plasma and urine. In PVAN patients the average proportion of sequences representing rearranged TCR was higher (urine: 45.03%, plasma: 33.57%) than in presumptive (2.01%, 1.19%) or probable (1.76%, 2.96%) PVAN patients. The highest degree of variation in TCR architecture was observed in association with biopsy-confirmed PVAN: while presumptive and probable PVAN patients had at the most two TCR variants in both urine and plasma, the number of different TCR variants ranged from 7 up to 15 in patients with proven PVAN (Table 2).

The most frequent individual TCR mutation in all three patient groups was partial deletion of Q (Q13-39) and R blocks (R1-22) (Table 2, Supplementary Fig. 2): in three proven PVAN patients, it was present in on average 13.05% and 12.46% of all sequences in urine and plasma, respectively. Another PVAN-associated TCR mutation was partial deletion of Q and S blocks: it was found in urine and plasma (average 3.72% and 2.86%, respectively) of two, and in plasma (11.57%) of one patient with biopsy-confirmed PVAN, and also in the urine of one presumptive PVAN patient. Duplications of both P (P47-58) and R (R47-63) blocks were found in two proven PVAN patients. Finally, urine and plasma samples (average: 16.62% and 4.28%, respectively) of two proven PVAN patients and the urine of one patient with presumptive PVAN (0.51%) harbored TCR sequences with either 8-bp (P42-49) or 18-bp deletion (P32-49) within the P block.

3.4. Mutations affected a number of TFBSs

More detailed analysis of rearranged BKPyV TCR regions revealed changes in a number of TFBSs for AP-1, Sp1, NFAT, NF-1, and p53 (Supplementary Table). In all rearranged strains of patients with proven PVAN and of two patients with presumptive PVAN, the number of binding sites for NF-1 and Sp1 was decreased, mostly owing to deletions affecting the archetypal Q and R blocks. The number of binding sites for AP-1 was less affected in all patient groups. Both patients with probable PVAN had viral strains with decreased number of binding sites for transcriptional activators NFAT and NF-1.

4. Discussion

PVAN is characterized by enhanced replication of BKPyV in the kidneys enabled by prolonged immunosuppression. Patient-specific factors such as inflammation, genetic background, and cellular immune response play essential roles in the outcome. Viral factors include rearrangements within the BKPyV TCR, which may increase pathogenicity by allowing enhanced replication and further emergence of mutations, although their association with severe disease has remained elusive [11,21,25–28]. To this end, we performed detailed characterization of BKPyV TCR populations in fifteen KTRs with either proven, presumptive or probable PVAN.

In this small cohort we found that the majority of sequences represented archetype TCR in all but two confirmed PVAN patients, suggesting that TCR mutations may favor but are not be necessary for the pathogenesis of PVAN. In archetype viral strains the individual mutations frequently disrupted NF-1 and p53 TFBSs. All but two p53 (del88_89, A90C) and one NF-1 (C239 G) binding site mutations have been previously detected in PVAN patients [18,22,23,26]. Single nucleotide point mutations are commonly observed in association with PVAN [23,26,29] but also in immunocompetent individuals [30]. Mutations located in the binding sites for NF-1 or p53 might per se decrease BKPyV replication indirectly by inhibiting viral early gene expression [19] or by inhibiting the binding of large T antigen to the NCCR [31]. In one PVAN patient an 18-bp deletion in the P block resulting in loss of a NF-1 binding site was found, reported earlier in kidney patients [26], and in all proven PVAN patients the binding sites for NF-1 and Sp1 were affected, mostly due to Q or R deletion, which was frequent in all patient groups. Similar modifications seem to occur frequently in BKPyV strains associated with PVAN [20–22,26]. As many of the individual mutations would not favor BKPyV replication, we assume that the high replication activity is a result of the overall composition of the viral strains, and possibly deletion of putative inhibitory sequences within the Q and R blocks, which was frequent among our patients. High-level replication of archetype BKPyV has been previously suggested to allow genetic alterations with further enhanced viral replication efficiency and increased viral load [11,21,25,32]. In the present work, the proportion and variety of rearranged strains increased along with viral loads towards confirmed PVAN cases, and this could only be verified using highly sensitive sequencing techniques.

To our knowledge, this is the first detailed next-generation sequencing analysis of BKPyV TCR variation in paired plasma and urine samples of KTRs with varying kidney status, albeit on a limited number of patients. Samples with high BKPyV loads were selected for this study to examine the multiplicity of strain variation and minute populations among the individual BKPyV virome. We cannot completely rule out the role of JC polyomavirus, observed in some patients, as JC polyomavirus can be a rare cause of PVAN [33,34]. Our results imply that rearranged BKPyV is frequently found in PVAN, but agree with previous studies proposing that rearranged BKPyV is not required for PVAN [9,23,35]. The presence of rearranged viral strains in most patients indicates, however, that enhanced viral replication after prolonged immunosuppression may allow accumulation of mutations within the TCR. Further studies on the emergence and accumulation of rearranged strains are warranted to understand the pathogenesis of PVAN, and to help earlier diagnosis and improve patient management.

Emergence of mutations within the body seems to apply to several human polyomaviruses, as has been established for JCPyV (reviewed in [36]) and recently described for Trichodysplasia spinulosa associated polyomavirus TSPyV [37]. Whether these mutations are required for pathogenesis (JCPyV and progressive multifocal leukoencephalopathy, or PML), favor pathogenesis (BKPyV and PVAN), or are implicated in pathogenesis (TSPyV and trichodysplasia spinulosa skin disease) remains a topic for further studies.

In the present study we aimed to characterize the BKPyV populations in individual patients to elucidate the association between viral strain variation and the pathogenesis of PVAN. High mutation rates in a DNA virus are uncommon and the mechanisms are not well understood. The majority of mutations are supposedly random, and many of them would be disadvantageous, and the strains would thus not be detected by sequencing. Viral strains with enhanced replication capacity causes lytic infection and tissue damage, and they, albeit not required, are associated with disease.

Ethical approval

The use of patient samples was approved by the ethics committee of the Medical University of Vienna (EK2064/2016). Because the samples had been acquired for virological diagnosis and in the distant past, the ethics committee concluded that no written informed consent was required from the patients (EK1035/2016).

Author Contributions

Hanna Liimatainen: conceptualization and design of the study, acquisition of data, analysis and interpretation of data, data curation, drafting and revising the article; Lukas Weseslindtner: design of the study, acquisition and interpretation of data, drafting and revising the article; Robert Strassl: design of the study, acquisition of data, drafting and revising the article; Stephan Aberle: design of the study, acquisition of data, drafting and revising the article; Gregor Bond: design of the study, acquisition of data, drafting and revising the article; Eeva Auvinen: conceptualization and design of the study, acquisition of data, drafting and revising the article.

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Declaration of Competing Interest

None declared.

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Appendix A. Supplementary data

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