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
polymavirus 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 leucoencephalopathy 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 (TFBBSs) 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’Étoile, 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 in 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-CCG GAC TGT ACC TGG TCT TGA AGC-TAMRA-3’), and either a BKPyV-specific reverse primer (5’-AGC TGG 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’Étoile, 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 decay cell shedding (median: 70%, range: 50-98%) and drop in kidney function. Detailed clinical information is presented in Supplementary material.

3.2. Archetypetype 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. Archetypetype 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: A52T, A53T, T65C, del88_89, 90C; R block: A145G, A146G, A147C, C179G, G181A, G182T; S block: A222G, T226G, G228A, C239G, 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.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (gender)</th>
<th>Time from KTx at sampling (days)</th>
<th>Duration of BKPyV viremia at sampling (days)</th>
<th>BKPyV load in urine (copies/mL)</th>
<th>BKPyV load in plasma (copies/mL)</th>
<th>JCPyV load in urine (copies/mL)</th>
<th>JCPyV load in plasma (copies/mL)</th>
<th>Decoy cells (%)</th>
<th>Drop of kidney function</th>
<th>Histological finding</th>
<th>Kidney status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58 (M)</td>
<td>140</td>
<td>0</td>
<td>3.10E + 07</td>
<td>5.40E + 03</td>
<td>BDL</td>
<td>BDL</td>
<td>&lt;5</td>
<td>no</td>
<td>N/A</td>
<td>Probable PVAN</td>
</tr>
<tr>
<td>2</td>
<td>59 (M)</td>
<td>159</td>
<td>77</td>
<td>3.10E + 08</td>
<td>2.00E + 03</td>
<td>BDL</td>
<td>BDL</td>
<td>&lt;5</td>
<td>no</td>
<td>N/A</td>
<td>Probable PVAN</td>
</tr>
<tr>
<td>3</td>
<td>23 (M)</td>
<td>68</td>
<td>0</td>
<td>2.50E + 08</td>
<td>2.70E + 04</td>
<td>BDL</td>
<td>BDL</td>
<td>&lt;5</td>
<td>no</td>
<td>N/A</td>
<td>Probative PVAN</td>
</tr>
<tr>
<td>4</td>
<td>72 (M)</td>
<td>297</td>
<td>71</td>
<td>3.70E + 07</td>
<td>1.00E + 04</td>
<td>4.90E + 07</td>
<td>1.50E + 02</td>
<td>5</td>
<td>no</td>
<td>N/A</td>
<td>Probative PVAN</td>
</tr>
<tr>
<td>5</td>
<td>52 (M)</td>
<td>40</td>
<td>0</td>
<td>3.50E + 10</td>
<td>1.10E + 04</td>
<td>8.70E + 01</td>
<td>BDL</td>
<td>&lt;5</td>
<td>no</td>
<td>N/A</td>
<td>Probative PVAN</td>
</tr>
<tr>
<td>6</td>
<td>27 (M)</td>
<td>126</td>
<td>28</td>
<td>1.20E + 10</td>
<td>1.40E + 05</td>
<td>BDL</td>
<td>BDL</td>
<td>40</td>
<td>no</td>
<td>N/A</td>
<td>Probative PVAN</td>
</tr>
<tr>
<td>7</td>
<td>60 (M)</td>
<td>303</td>
<td>71</td>
<td>1.10E + 08</td>
<td>2.30E + 04</td>
<td>BDL</td>
<td>BDL</td>
<td>90</td>
<td>yes</td>
<td>N/A</td>
<td>Probative PVAN</td>
</tr>
<tr>
<td>8</td>
<td>59 (M)</td>
<td>100</td>
<td>28</td>
<td>2.80E + 08</td>
<td>1.90E + 04</td>
<td>BDL</td>
<td>BDL</td>
<td>80</td>
<td>yes</td>
<td>N/A</td>
<td>Probative PVAN</td>
</tr>
<tr>
<td>9</td>
<td>45 (F)</td>
<td>127</td>
<td>22</td>
<td>5.10E + 08</td>
<td>3.80E + 05</td>
<td>BDL</td>
<td>BDL</td>
<td>70</td>
<td>no</td>
<td>N/A</td>
<td>Probative PVAN</td>
</tr>
<tr>
<td>10</td>
<td>39 (F)</td>
<td>108</td>
<td>78</td>
<td>1.00E + 10</td>
<td>1.10E + 05</td>
<td>BDL</td>
<td>BDL</td>
<td>50</td>
<td>no</td>
<td>N/A</td>
<td>Probative PVAN</td>
</tr>
<tr>
<td>11</td>
<td>67 (F)</td>
<td>90</td>
<td>90</td>
<td>1.90E + 11</td>
<td>4.20E + 08</td>
<td>3.90E + 07</td>
<td>8.60E + 04</td>
<td>60</td>
<td>yes</td>
<td>PVAN, SV40 LTag positive</td>
<td>PVAN</td>
</tr>
<tr>
<td>12</td>
<td>69 (M)</td>
<td>225</td>
<td>14</td>
<td>1.60E + 11</td>
<td>1.10E + 07</td>
<td>1.60E + 03</td>
<td>BDL</td>
<td>&gt;90</td>
<td>yes</td>
<td>PVAN, SV40 LTag positive</td>
<td>PVAN</td>
</tr>
<tr>
<td>13</td>
<td>58 (F)</td>
<td>224</td>
<td>131</td>
<td>2.90E + 10</td>
<td>4.70E + 05</td>
<td>BDL</td>
<td>BDL</td>
<td>70</td>
<td>yes</td>
<td>PVAN, SV40 LTag positive</td>
<td>PVAN</td>
</tr>
<tr>
<td>14</td>
<td>44 (M)</td>
<td>235</td>
<td>148</td>
<td>2.00E + 11</td>
<td>1.70E + 06</td>
<td>BDL</td>
<td>BDL</td>
<td>&gt;98</td>
<td>yes</td>
<td>PVAN, SV40 LTag positive</td>
<td>PVAN</td>
</tr>
<tr>
<td>15</td>
<td>49 (F)</td>
<td>69</td>
<td>14</td>
<td>6.30E + 07</td>
<td>1.10E + 04</td>
<td>BDL</td>
<td>BDL</td>
<td>50</td>
<td>yes</td>
<td>PVAN, SV40 LTag positive</td>
<td>PVAN</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Kidney status</th>
<th>Source</th>
<th>BKPyV load (copies/mL)</th>
<th>Original read amount</th>
<th>Post-analytic read amount</th>
<th>Phenotype</th>
<th>TCR architecture</th>
<th>TCR length (bp)</th>
<th>Sequence identity with archetype TCR (%)</th>
<th>Proportion of all sequences in the sample (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Probable PVAN</td>
<td>Urine</td>
<td>3.10E+07</td>
<td>194,648</td>
<td>125,244</td>
<td>Archetype</td>
<td>P-Q-R-S</td>
<td>234</td>
<td>97.01</td>
<td>97.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plasma</td>
<td>5.40E+03</td>
<td>195,179</td>
<td>124,213</td>
<td>Archetype</td>
<td>P-Q-R-S</td>
<td>184</td>
<td>76.10</td>
<td>1.76</td>
</tr>
<tr>
<td>2</td>
<td>Probable PVAN</td>
<td>Urine</td>
<td>3.10E+08</td>
<td>173,797</td>
<td>121,460</td>
<td>Archetype</td>
<td>P-Q-R-S</td>
<td>233</td>
<td>98.50</td>
<td>99.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plasma</td>
<td>2.00E+03</td>
<td>206,449</td>
<td>126,478</td>
<td>Archetype</td>
<td>P-Q-R-S</td>
<td>233</td>
<td>98.50</td>
<td>96.01</td>
</tr>
<tr>
<td>3</td>
<td>Presumptive PVAN</td>
<td>Urine</td>
<td>2.50E+08</td>
<td>179,658</td>
<td>129,518</td>
<td>Archetype</td>
<td>P-Q-R-S</td>
<td>233</td>
<td>98.50</td>
<td>99.87</td>
</tr>
<tr>
<td>4</td>
<td>Presumptive PVAN</td>
<td>Plasma</td>
<td>2.70E+04</td>
<td>147,503</td>
<td>85,959</td>
<td>Archetype</td>
<td>P-Q-R-S</td>
<td>233</td>
<td>98.50</td>
<td>98.06</td>
</tr>
<tr>
<td>5</td>
<td>Presumptive PVAN</td>
<td>Plasma</td>
<td>3.70E+07</td>
<td>197,551</td>
<td>134,458</td>
<td>Archetype</td>
<td>P-Q-R-S</td>
<td>234</td>
<td>100</td>
<td>98.72</td>
</tr>
<tr>
<td>6</td>
<td>Presumptive PVAN</td>
<td>Plasma</td>
<td>1.00E+10</td>
<td>209,807</td>
<td>128,873</td>
<td>Archetype</td>
<td>P-Q-R-S</td>
<td>233</td>
<td>100</td>
<td>98.10</td>
</tr>
<tr>
<td>7</td>
<td>Presumptive PVAN</td>
<td>Plasma</td>
<td>1.40E+05</td>
<td>174,402</td>
<td>111,574</td>
<td>Archetype</td>
<td>P-Q-R-S</td>
<td>233</td>
<td>100</td>
<td>99.97</td>
</tr>
<tr>
<td>8</td>
<td>Presumptive PVAN</td>
<td>Plasma</td>
<td>2.30E+04</td>
<td>210,522</td>
<td>137,597</td>
<td>Archetype</td>
<td>P-Q-R-S</td>
<td>234</td>
<td>97.01</td>
<td>92.37</td>
</tr>
<tr>
<td>9</td>
<td>Presumptive PVAN</td>
<td>Plasma</td>
<td>1.90E+04</td>
<td>170,259</td>
<td>107,301</td>
<td>Archetype</td>
<td>P-Q-R-S</td>
<td>234</td>
<td>97.01</td>
<td>98.84</td>
</tr>
<tr>
<td>10</td>
<td>Presumptive PVAN</td>
<td>Plasma</td>
<td>3.80E+05</td>
<td>201,098</td>
<td>142,933</td>
<td>Archetype</td>
<td>P-Q-R-S</td>
<td>233</td>
<td>98.13</td>
<td>98.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plasma</td>
<td>1.10E+05</td>
<td>197,232</td>
<td>135,637</td>
<td>Archetype</td>
<td>P-Q-R-S</td>
<td>233</td>
<td>98.50</td>
<td>96.10</td>
</tr>
<tr>
<td>11</td>
<td>PVAN</td>
<td>Urine</td>
<td>1.90E+11</td>
<td>289,372</td>
<td>217,378</td>
<td>Archetype</td>
<td>P-Q-R-S</td>
<td>216</td>
<td>90.67</td>
<td>27.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plasma</td>
<td>4.20E+08</td>
<td>322,959</td>
<td>231,178</td>
<td>Archetype</td>
<td>P-Q-R-S</td>
<td>216</td>
<td>90.67</td>
<td>46.93</td>
</tr>
</tbody>
</table>

(continued on next page)
probable PVAN patients (1, 2) (Supplementary Fig. 1). Some point mutations were located in NF-1 TFBSs (T65C, C179G, G181A, G182T, C239G) 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

<table>
<thead>
<tr>
<th>Table 2 (continued)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient</strong></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>13 PVAN</td>
</tr>
<tr>
<td>14 PVAN</td>
</tr>
<tr>
<td>15 PVAN</td>
</tr>
</tbody>
</table>

BKPyV, BK polyomavirus; TCR, transcriptional control region; PVAN, polyomavirus-associated nephropathy.
rarely 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 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 one third patients 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 archetype 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 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 (C239G) 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 Weselindtner: design of the study, acquisition of data, drafting and revising the article; Robert Strassi: design of the study, acquisition of data, drafting and revising the article; Stephan Aaberle: 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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