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MicroRNA expression, genomic variations, subtypes, and cytopathic behavior of BK polyomavirus among kidney transplant recipients

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Abstract

BK polyomavirus-associated nephropathy (BKPyVAN) is a major cause of allograft dysfunction in kidney transplant recipients (KTRs), where viral microRNAs (miRNAs) and capsid variations may influence pathogenesis. This study aimed to investigate whether urinary bkv-miR-B1-3p and bkv-miR-B1-5p expression is affected by viral genomic variations, subtypes, and in vitro cytopathic behavior among kidney transplant recipients (KTRs) with BKPyV reactivation. Urine from 20 KTRs with BKPyVAN was used to quantify two BK viral miRNAs by real-time PCR, sequence and phylogenetically type the VP1 region, and inoculate Vero E6 cells for Cytopathic Effect (CPE) and PCR confirmation of infection. Data were analysed in GraphPad Prism and SPSS. The expression of bkv-miR-B1-3p was significantly higher than that of bkv-miR-B1-5p ($P < 0.0001$). A higher bkv-miR-B1-3p/5p ratio correlated positively with CPE appearance ($P = 0.01$), but not with the time frame progression ($P = 0.09$). BKPyV subtypes I and IV were found most frequently, respectively, with mutations clustered among amino acids 61–83. There was no significant correlation between the number or type of VP1 mutations and the miRNA expression ratio ($P > 0.05$). However, the E61N mutation was significantly associated with the CPE development time frame ($P = 0.05$). Higher expression of bkv-miR-B1-3p than bkv-miR-B1-5p among BKPyVAN patients supports the potential value of viral miRNAs as non-invasive biomarkers. Subtypes I and IV were derived predominantly, similar to other Middle Eastern countries. The VP1 E61N mutation, in association with the CPE development time frame, was previously described in relation to the persistence of BKPyV infection. Future research should pair genome sequencing with miRNA profiling against controls to clarify infection status and prognosis.

Highlights

• Urinary bkv-miR-B1-3p expression is significantly higher than 5p in BKPyVAN.

• Higher urinary bkv-miR-B1-3p/5p ratio correlates with earlier CPE appearance.

• BKPyV subtype I (85%) and subtype IV (10%) predominated in renal allografts.

• VP1 mutations clustered at residues 61–83 without affecting miRNA expression.

• The VP1 E61N substitution was associated with a prolonged CPE time frame.

Introduction

BK polyomavirus (BKPyV; family Polyomaviridae, genus Betapolyomavirus, species Betapolyomavirus hominis) has a double-stranded circular DNA genome of approximately 5 kbp in size enclosed in an icosahedral capsid shell with a diameter of 45–50 nm. The BKPyV genome is divided into early and late regulatory regions and encodes five major proteins. The capsid contains three structural proteins, VP1, VP2, and VP3. VP1, the major structural protein, accounts for approximately 80% of the total capsid

protein, whereas VP2 and VP3 play roles in nuclear entry of the virus via their nuclear localization signal [4]. Recent evidence indicates VP4 is likely not functional or essential for viral replication [13].

After primary infection, BKPyV remains latent mainly in the urinary tract [45]. This infection is highly prevalent globally, with seroprevalence of 80% to 90% in adults [36]. Primary infection is usually asymptomatic, and in immunosuppressed kidney transplant recipients, viral reactivation can lead to BKPyV-associated nephropathy (BKPyVAN), resulting in graft loss, rejection, and death in some cases [12]. This situation complicates early diagnosis and may lead to delays in appropriate clinical management [1, 2].

Although the first case of BKPyV infection was initially diagnosed in 1971, after almost 50 years, the management of this infection is still limited [11, 31]. While various treatment methods have been studied, none can be recommended due to their lack of effectiveness. The primary treatment for BKPyVAN is the reduction of immunosuppression, which allows the patients immune system to control the virus [1]. This method also increases the risk of allograft rejection [1].

Currently, definitive diagnosis of BKPyVAN relies on allograft biopsy with histological examination; however, false negatives remain a possibility [1]. In addition to allograft biopsy, quantitative nucleic acid testing (QNAT) for plasma BKPyV-DNA loads is the standard clinical tool used to screen for viral replication and identify probable or presumptive nephropathy in kidney transplant recipients and facilitate early intervention before overt nephropathy develops [20, 30]. The timing of BKPyV diagnosis is critical, as persistent BKPyV DNAemia is a dominant driver for allograft injury, and delays in viral clearance are directly associated with an increased risk of graft loss [12, 14]. Although measurement of BKPyV DNAemia by plasma quantitative PCR is more specific than urine-based testing, relying on plasma DNAemia alone may delay detection of early viral replication and increase the risk of graft loss [5].

Isolated BKPyV from different locations of the world are grouped into four main subtypes using serological and genotyping methods [32]. Subtype I is the globally dominant strain; subtype IV ranks second in frequency and is predominantly isolated from East Asian populations [22]. Although subtypes II and III are isolated worldwide, their overall frequencies are low [22]. Furthermore, according to the phylogenetic investigations, subtype I itself is divided into 4 subgroups, including subtypes Ia, Ib-1, Ib-2, and Ic; each with a distinct geographical distribution [22]. According to previous reports, there is no clear correlation between BKPyV subtype determination alone and clinical outcome [10, 40].

In recent years, microRNAs (miRNAs), typically consisting of 20 to 22 nucleotides, have become a promising diagnostic or prognostic marker for several diseases, including viral infections [21, 29]. miRNAs can regulate protein expression by directly binding to mRNA or by inhibiting translation through the RNA-induced silencing complex (RISC) [29]. BKPyV produces two mature miRNAs (bkv-miR-B1-3p and bkv-miR-B1-5p) during its replication cycle, with expression controlled by the non-coding control region (NCCR) [6, 24, 27]. In vivo, BKPyV miRNAs have been found in a variety of biological fluids, including urine, serum, plasma, and cerebrospinal fluid [23].

Recent studies have shown that detecting these two miRNAs in the urine of patients with BK virus nephropathy and kidney transplant recipients pointed out the priority of early molecular detection of the virus in urine and in association with expression levels in nephropathy patients [7, 15, 42, 44]. On the other hand, genetic variation in BKPyV, particularly within the VP1 capsid gene, is frequently observed and is associated with BKPyV disease in transplant recipients [25, 34, 39].

This study aimed to quantify the urinary expression of bkv-miR-B1-3p and bkv-miR-B1-5p in kidney transplant recipients diagnosed with BKPyVAN and to examine their relationship with VP1 genomic variation and the *in vitro* cytopathic behavior of the corresponding viral isolates.

Materials and methods

Patients and Ethical Approval

This cross-sectional study included 20 kidney transplant recipients from Tehran, Iran, with clinical and histological features of BKPyVAN diagnosed by nephrologists [1]. The study was conducted in accordance with the Declaration of Helsinki and its later amendments. All patients provided written informed consent to participate in the study. The study protocol was reviewed and approved by the Ethics Committee of the Urology and Nephrology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran (Approval No. IR.SBMU.UNRC.REC.1401.003).

Sample Collection and Processing

Urine samples were collected during 6 weeks after transplantation and transported under cold chain conditions, aliquoted, and stored at -70°C until nucleic acid extraction and virus isolation.

Nucleic Acid Extraction and miRNA cDNA Synthesis

Total viral nucleic acid was extracted from 200 µL of urine using a commercial spin-column kit (Roje, Yazd, Iran) according to the manufacturer's protocol. Extracted miRNAs were reverse-transcribed using a polyadenylation-based cDNA synthesis approach with BON-RT adaptor primers (Bonyakhteh, Tehran, Iran).

Relative quantification of bkv-miR-B1-3p and bkv-miR-B1-5p was performed using the BON-miR High-Specificity real-time PCR Core Reagent Kit (Bonyakhteh, Tehran, Iran) on a Rotor-Gene Q MDx instrument (Corbett, Australia). Expression levels were normalized to U6 small nuclear RNA (snRNA) as an endogenous control. Relative expression was calculated using the 2^-ΔCt method (Ct_gene of interest - Ct_U6) and log-transformed for statistical analysis.

BKPyV VP1 gene amplification and sequencing

DNA Integrity and BKPyV PCR

The integrity of the extracted DNA was confirmed by a conventional PCR for the human β-globin gene using primers GH20 (5′-GAAGAGCCAAGGACAGGTA-3′) and PC04 (5′-CAACTTCATCCACGTACC-3′) [16]. The thermal profile was 95°C for 5 minutes; 30 cycles of 95°C for 30 seconds, 65°C for 30 seconds, and 72°C for 1 minute; followed by a final extension at 72°C for 10 minutes. All 20 samples tested positive by beta-globin PCR and were subjected to BKPyV conventional PCR.

The BKPyV VP1 region was subsequently amplified using forward primer VP1-F (5′-CCAAGTGCCAAAACTAAT-3′) and reverse primer VP1-R (5′-TGCATGAAGGTTAAGCATGC-3′). The thermal profile was: 94°C for 5 minutes; 30 cycles of 94°C for 30 seconds, 53°C for 30 seconds, and 72°C for 1 minute; followed by a final extension at 72°C for 10 minutes.

Gel Electrophoresis, Sequencing, and Analysis

All PCR products were visualized by electrophoresis on a 1.5% agarose gel stained with DNA Safe Stain (SinaClon, Iran) and imaged under UV light. Expected band sizes were 268 bp for beta-globin and 287 bp for the BKPyV VP1 fragment. Positive BKPyV PCR products were purified and subjected to bidirectional Sanger sequencing by Applied Biosystems ABI-3130 Genetic Analyzer. Sequencing chromatograms were analyzed using Chromas, and sequences were aligned using BioEdit (v7.0.0). Nucleotide sequences were translated, and predicted amino acid changes in the VP1 region (amino acids 40–132) were identified by comparison to the reference strain (Accession: V01108.1).

Sequences obtained were aligned with published BKPyV genomes from GenBank using ClustalW within the MEGA11 software [38]. Simian virus 12 (Accession number: NC_007611) was designated as the outgroup. To establish phylogenetic relationships, phylogenetic trees were constructed using the neighbor-joining method based on the Maximum Composite Likelihood model, with pairwise deletion for gaps and missing data, and evaluated through 1,000 bootstrap replicates in MEGA11[38]. The final sequence data were deposited into GenBank with accession numbers PZ369782-PZ369801.

Cell Culture and Virus Isolation

Vero E6 cells were used for cell culture and virus isolation. Cells were maintained in DMEM (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) and penicillin-streptomycin.

The final pellet was resuspended in 1 mL of serum-free DMEM containing gentamicin and was used to infect Vero E6 cell monolayers at 40–50% confluency. After a 2-hour adsorption period at 37°C, the inoculum was removed and replaced with high-glucose DMEM supplemented with L-glutamine, 25 mM HEPES, and sodium pyruvate (Biosera, Cholet, France) containing 3% FBS at 37°C under 5% CO2 in a humidified incubator. Cultures were maintained for up to three weeks and monitored daily by inverted light microscopy for the appearance of CPE. During this time, culture media were changed daily. For each sample, one uninoculated flask maintained in DMEM with 3% FBS served as a negative control

throughout the viral inoculation and CPE monitoring period. CPE appearance was defined as the first day on which characteristic cytopathic changes were visible in the cell monolayer. The rate of CPE development time frame was defined as the number of days from CPE appearance to complete (> 90%) destruction of the monolayer cells.

CPE was characterized by the appearance of many vacuoles in the cytoplasm of infected Vero cells in the first phase, enlarged and swollen. Whereas the same cells are lysed and destroyed in the final step. When present, CPE were graded as follows: 0 = no CPE present, 1+ = ≤25% CPE, 2+ = >25% CPE, 3+ = >50% CPE, 4+ = >75% CPE. CPE was graded for all flasks on the first day on which any flask demonstrated CPE. Images of CPE were acquired using an inverted light microscope. For cultures with typical CPE, diagnosis was confirmed by BKPyV PCR, as described previously (Fig. 1).

Statistical Analysis

Statistical analyses were performed using SPSS (v20) and GraphPad Prism (v6). A paired t-test was used to compare the expression of bkv-miR-B1-3p and bkv-miR-B1-5p. Spearman correlation was used to assess the relationship between miRNA expression levels, genomic mutations, and cell culture CPE metrics. An independent t-test was used to compare expression levels based on the presence or absence of specific mutations. A p-value ≤ 0.05 was considered statistically significant.

Results

A total of 20 patients with BKPyV-associated nephropathy were included in this cross-sectional study. The study consisted of 12 females (60%) and 8 males (40%), with a mean age of 33.2 ± 20.7 years. All 20 urine samples tested positive for both the human beta-globin gene and BKPyV DNA.

bkv-miR-B1-3p expression was significantly higher than bkv-miR-B1-5p among 19 of the 20 samples (95%). The mean expression of bkv-miR-B1-3p (79.01 ± 93.03) was significantly higher than that of bkv-miR-B1-5p (5.08 ± 6.12) ($p < 0.0001$).

The 287 bp VP1 typing region was successfully amplified from all 20 samples using BKPyV-specific primers. The obtained sequences were compared against published BKPyV sequences spanning nucleotides 1,650–1,936 of the BKPyV Dunlop genome (GenBank accession V01108). Sequence analysis identified three BKPyV subtypes (I, II, and IV) among the 20 isolates (Fig. 2). The predominant subtype was I, with subgroups Ib1 and Ib2 detected in 4 (20%) and 13 (65%) of sequences, respectively. No representative sequences of subgroups Ia and Ic was detected. One sequence (5%) was classified as subtype II, and two (10%) classified as subtype IV.

Although all sequences were classified into specific subtypes or subgroups, nucleotide variation relative to the defining-subtype sequences was found at specific positions, involving substitutions in the VP1 protein (Fig. 3). Regarding subtype Ib1 and subtype II viruses, four variants characterized by the substitution E61D in comparison with the reference sequences. In subtype II and IV isolates, additional

substitutions, including F66Y, S71T, D75A, S77D, and E82D, were identified relative to the reference sequence. Among the two subtype IV variants, E61N, N62H, or N62D, K69R, and N74T were identified by comparison with the reference sequence. Furthermore, no significant association was found between different VP1 mutations and miRNA expression levels.

A significant positive correlation was found between the bkv-miR-B1-3p/5p ratio and the days to CPE appearance ($p = 0.01$). Additionally, the ratio showed no correlation with the rate of CPE development time frame ($p = 0.09$). The presence of the E61N mutation showed a significant correlation with the CPE development time frame ($p = 0.05$; Fig. 4).

Discussion

Viral miRNAs play a crucial role in cellular regulation, and viruses utilize them to modulate their own replication and host cell functions. BKPyV-encoded miRNAs have recently emerged as subjects of active research, with studies evaluating their roles in disease prognosis, viral activity, and the establishment of persistent infection [8, 19, 41, 42, 46].

The present findings showed that the expression level of bkv-miR-B1-3p was significantly higher than that of bkv-miR-B1-5p. This result is consistent with other studies. For instance, Huang et al. reported that bkv-miR-B1-3p expression was approximately one log higher than bkv-miR-B1-5p [15]. Similarly, Zhang et al. utilized both 3p and 5p arms in their diagnostic score but noted that the 3p arm has historically shown higher expression levels during active infection [44]. Consistent with these findings, Pietilä et al. (2015) reported a higher rate of bkv-miR-B1-3p than 5p among their samples [33]. Virtanen et al. (2018) also showed that expression of both miRNA arms was detected in the vast majority of samples (85.5% and 98.2%), with expression levels in nephropathy patients being 9.8- and 7.8-fold higher, respectively, than in controls [42]. Since these researchers had tracked the expression of the above genes in both plasma and urine of kidney transplant recipients, they also concluded that the urine of patients has a higher value and that the expression level of these genes is also associated with higher viral titers in urine. On the other hand, the study by Baptiste Demey in 2021, in addition to the importance of detecting these two miRNAs in the urine of patients with BK virus nephropathy and kidney transplant recipients, also pointed out the priority of early molecular detection of the virus in urine. The important role of examining the expression of these genes was described in order to monitor patients and help doctors to detect patients who are improving and, as a result, to increase the treatment of immunosuppressive drugs in a timely manner. Therefore, as mentioned, it can be noted that the diagnostic role of the expression of these miRNAs in the diagnosis of BK virus nephropathy is becoming more important. The methodology of the aforementioned studies differs from our study, as in the present study, gene expression was measured using a relative quantitative method, but the results of the present study can be considered consistent with the above studies. Demey et al. (2021) further emphasized the diagnostic value of urinary BKPyV miRNAs in BKPyVAN, noting that early molecular detection in urine is critical for monitoring patients and guiding timely adjustments to immunosuppressive therapy [7].

According to global epidemiological trends, the phylogenetic analysis identified BKPyV subtype I as the predominant circulating strain. However, sequence analysis of the VP1 hypervariable region revealed multiple distinct mutational profiles, particularly within subtypes II and IV. Specifically, the E61D substitution was detected in subgroups Ib1 and subtype II. Ledesma et al. reported this mutation across subtypes I, II, and III [22]. Similarly, the E61N mutation was identified within subtype IV, corroborating comparable findings in Ledesma et al. [22] and Pastrana et al. [32]. Furthermore, a cluster of substitutions, including E82D, F66Y, S71T, D75A, and S77D, was observed in the subtype II and IV isolates. Tremolada et al. reported the E82D polymorphism in subtype I isolates [39], while Ledesma et al. identified it across multiple subtypes, including Ib2, I, II, III, and IV [22].

Crucially, while our functional assays indicated that most variations (such as E61D and E82D) exhibited no significant association with miRNA expression levels, the E61N mutation in subtype IV was significantly associated with a prolonged CPE development time frame ($p = 0.05$). Taken together, these comparative analyses validate these mutational findings against independent global cohorts and reinforce the hypothesis that specific, high-risk substitutions within the hypervariable region rather than broad genotype classifications alone can directly modulate viral cytopathic effect and *in vitro* virulence.

In the present study, genomic analysis of the partial VP1 region revealed mutations that were more clustered between amino acid residues 61 to 83 and correspond to the hypervariable region and form the surface-exposed BC1 and BC2 loops. This finding is biologically significant, as the BC1 loop binds the host cell receptor, and mutations in this location can therefore alter viral cellular tropism [35, 37]. Among the mutation findings, the presence of E61N showed a significant correlation with the CPE development time frame among derived viruses and might be explained by the persistence of BKPyV infection *in vivo* [25]. Consistent with our study, researchers have reported K69R, E73Q, and D75N, D77E, E82D mutations in the BC loop and described these mutations as altering the structural properties of the virus in a way that allows it to evade the humoral immune response. By shifting the structure of the binding sites, the virus actively exploits holes in the neutralizing antibody repertoire, rendering the antibodies less effective at neutralizing the viral particles. Persistent high-level BK polyomavirus replication in KT recipients is therefore associated with the accumulation of VP1 mutations that can confer resistance to neutralization [25]. Also, two of the amino acid changes exclusively found in this study, K69R and D75N, were previously reported following analyses of BKPyVAN patients [3, 34, 39]. In vitro studies have shown that residue 69 of VP1 is important for virus viability, since an amino acid substitution at this position may induce a reduction of viral spread and receptor binding ability [9]. These findings might be considered as direct *in vitro* evidence that specific high-risk mutations, rather than overall mutational load, are key drivers of viral cytopathic behavior or virulence. In line with these observations, Tremolada et al. (2010) demonstrated that specific VP1 polymorphisms have a strong influence on viral growth and induce more pronounced CPE, linking viral diversity directly to pathogenesis and graft loss [40].

The lack of a statistical correlation between these VP1 mutations and the expression of either viral miRNA is consistent with the hypothesis that VP1 variation is independent of the pathways regulating

viral miRNA expression. Viral miRNA transcription is known to be modulated not by VP1, but by the non-coding control region (NCCR) and the subsequent expression of the Large T-antigen (TAg) [24]. In fact, recent single-cell analyses confirmed that host cell cycle and TAg expression are the dominant forces modulating miRNA output [26, 43].

This study faced several limitations. The small sample size (n = 20) reduced statistical power, potentially obscuring subtle correlations. Also, the cross-sectional design provided only a single sampling per patient; a longitudinal study is needed to assess correlations with disease progression over time. Furthermore, our genomic analysis was restricted to the VP1 gene. Importantly, sequencing data on the NCCR or TAg regions were not obtained, which are recognized as critical modulators of miRNA expression. Future studies should include these regions to fully investigate the genomic drivers of miRNA modulation. However, the routine clinical application of these biomarkers remains challenging due to their low abundance in circulation and the lack of standardized protocols for normalization and quantification [5, 44]. Similarly, urinary miRNA analysis faces unique hurdles, particularly the lack of a universal normalization strategy to account for drastic variations in urine concentration [17]. Another limitation was that, in contrast to bkv-miR-B1-5p, which is specific to BKPyV and has been the focus of many BKPyVAN studies, bkv-miR-B1-3p is more conserved between BKPyV and JCPyV [7]. An earlier study indicated that bkv-miR-B1-3p expression is higher during infection [8], and that while it shares sequence homology with JCPyV, the 5p arm differs by three single-nucleotide polymorphisms [23]. Therefore, further research on both bkv-miR-B1-5p and – 3p is necessary to determine if these molecules could serve as improved biomarkers alongside viral load testing to assist clinicians in distinguishing BKPyVAN patients before optimizing immunosuppressive therapy [18, 28].

Urinary bkv-miR-B1-3p was expressed at significantly higher levels than bkv-miR-B1-5p in KTRs with BK polyomavirus–associated nephropathy, supporting the potential value of viral miRNAs as non-invasive biomarkers. VP1 amino acid substitutions clustered within the hypervariable region, but neither the number nor the type of VP1 mutations correlated with miRNAs expression levels, suggesting that capsid diversity and miRNA regulation may be independent. In contrast, the bkv-miR-B1-3p/5p ratio was significantly associated with the time to CPE appearance, and the VP1 E61N mutation was associated with CPE development time frame, indicating that specific viral molecular signatures may relate to *in vitro* cytopathic effect. In addition, larger longitudinal studies including analysis of additional viral regions are needed to validate these findings and clarify clinical utility. However, comparison of the results of these studies with the control group is suggested.

This study provided theories about the effect of BKPyV pathogenesis on viral miRNAs expression. It has been demonstrated that miRNAs expression and VP1 mutations are independent factors that may correlate with different biological outcomes. This study provided *in-vitro* evidence that VP1 mutations, such as E61N, are linked to viral cytopathology, while the bkv-miR-B1-3p/5p ratio was linked to the timing of the lytic cycle.

While plasma viral load screening remains the standard of care for KTRs according to current guidelines. Strengthening the molecular evidence base for non-invasive biomarkers such as viral miRNAs may ultimately reduce the need for invasive allograft biopsies in patients with suspected BKPyVAN. However, this information, in conjunction with viral subtyping and genome diversity, could provide more detailed information on viral pathogenesis.

Declarations

CRediT authorship contribution statement

Saba Kianpour: Investigation, Resources, Data curation.

Neda Yousefi: Investigation, Resources, Data curation.

Ali Hosseini Sani: Formal analysis, Software, Data curation, Investigation, Writing – original draft, Writing – review & editing.

Shiva Samavat: Resources, Validation.

Baptiste Demey: Writing – review & editing.

Maryam Vaezjalali: Conceptualization, Supervision, Formal analysis, Project administration, Funding acquisition, Writing – review & editing.

All authors read and approved the final manuscript.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Urology and Nephrology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran (Approval No. IR.SBMU.UNRC.REC.1401.003). Written informed consent was obtained from all participants prior to study inclusion.

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Author Contribution

Saba Kianpour: Investigation, Resources, Data curation.Neda Yousefi: Investigation, Resources, Data curation.Ali Hosseini Sani: Formal analysis, Software, Data curation, Investigation, Writing – original draft, Main manuscript Drafting, Figures preparation, Writing – review & editing.Shiva Samavat: Resources, Validation.Baptiste Demey: Writing – review & editing.Maryam Vaezjalali: Conceptualization, Supervision, Formal analysis, Project administration, Funding acquisition, Writing – review & editing.All authors reviewed the manuscript.

Data Availability

The BK polyomavirus VP1 nucleotide sequences generated in this study have been deposited in the NCBI GenBank database under accession numbers PZ369782–PZ369801.

References

1. Al-Talib M, Welberry-Smith M, Macdonald A, Griffin S (2025) BK Polyomavirus-associated nephropathy - diagnostic and treatment standard. Nephrol Dial Transpl 40:651–661
2. Ambalathingal GR, Francis RS, Smyth MJ, Smith C, Khanna R (2017) BK Polyomavirus: Clinical Aspects, Immune Regulation, and Emerging Therapies. Clin Microbiol Rev 30:503–528
3. Baksh FK, Finkelstein SD, Swalsky PA, Stoner GL, Ryschkewitsch CF, Randhawa P (2001) Molecular genotyping of BK and JC viruses in human polyomavirus-associated interstitial nephritis after renal transplantation. Am J Kidney Dis 38:354–365
4. Bennett SM, Zhao L, Bosard C, Imperiale MJ (2015) Role of a nuclear localization signal on the minor capsid proteins VP2 and VP3 in BKPyV nuclear entry. Virology 474:110–116
5. Brochot E, Descamps V, Handala L, Faucher J, Choukroun G, Helle F, Castelain S, François C, Duverlie G, Touzé A (2019) BK polyomavirus in the urine for follow-up of kidney transplant recipients. Clin Microbiol Infect 25:112.e111-112.e115
6. Broekema NM, Imperiale MJ (2013) miRNA regulation of BK polyomavirus replication during early infection. Proc Natl Acad Sci U S A 110:8200–8205
7. Demey B, Descamps V, Presne C, Helle F, Francois C, Duverlie G, Castelain S, Brochot E (2021) BK Polyomavirus Micro-RNAs: Time Course and Clinical Relevance in Kidney Transplant Recipients. Viruses 13
8. Demey B, Bentz M, Descamps V, Morel V, Francois C, Castelain S, Helle F, Brochot E (2022) BK Polyomavirus bkv-miR-B1-5p: A Stable Micro-RNA to Monitor Active Viral Replication after Kidney Transplantation. Int J Mol Sci 23
9. Dugan AS, Gasparovic ML, Tsomaia N, Mierke DF, O'Hara BA, Manley K, Atwood WJ (2007) Identification of amino acid residues in BK virus VP1 that are critical for viability and growth. J Virol 81:11798–11808

10. Furmaga J, Kowalczyk M, Zapolski T, Furmaga O, Krakowski L, Rudzki G, Jaroszyński A, Jakubczak A (2021) BK Polyomavirus-Biology, Genomic Variation and Diagnosis. Viruses 13
11. Gardner S, Field A, Coleman D, Hulme B (1971) NEW HUMAN PAPOVAVIRUS (B.K.) ISOLATED FROM URINE AFTER RENAL TRANSPLANTATION. Lancet 297:1253–1257
12. Gately R, Milanzi E, Lim W, Teixeira-Pinto A, Clayton P, Isbel N, Johnson DW, Hawley C, Campbell S, Wong G (2023) Incidence, Risk Factors, and Outcomes of Kidney Transplant Recipients With BK Polyomavirus-Associated Nephropathy. Kidney Int Rep 8:531–543
13. Henriksen S, Hansen T, Bruun JA, Rinaldo CH (2016) The Presumed Polyomavirus Viroporin VP4 of Simian Virus 40 or Human BK Polyomavirus Is Not Required for Viral Progeny Release. J Virol 90:10398–10413
14. Hirsch HH, Randhawa PS (2019) BK polyomavirus in solid organ transplantation-Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl 33:e13528
15. Huang Y, Zeng G, Randhawa PS (2019) Detection of BKV encoded mature MicroRNAs in kidney transplant patients: Clinical and biologic insights. J Clin Virol 119:6–10
16. Jin L (2001) Molecular methods for identification and genotyping of BK virus. SV40 Protocols. Springer, pp 33–48
17. Jung SW, Cho W-H, Seo J-W, Kim Y-G, Moon J-Y, Kim JS, Kim C-D, Chung BH, Park JB, Kim YH, Lee S-H (2023) Urine Exosomal bkv-miR-B1-5p and BK Virus Nephropathy in Kidney Transplant Recipients. J Infect Dis 227:1185–1193
18. Kim J-M, Kwon HE, Han A, Ko Y, Shin S, Kim YH, Lee KW, Park JB, Kwon H, Min S (2025) Risk Prediction and Management of BKPyV-DNAemia in Kidney Transplant Recipients: A Multicenter Analysis of Immunosuppressive Strategies. Transpl Int Volume 38–2025
19. Kim MH, Lee YH, Seo J-W, Moon H, Kim JS, Kim YG, Jeong K-H, Moon J-Y, Lee TW, Ihm C-G, Kim C-D, Park JB, Chung BH, Kim Y-H, Lee S-H (2017) Urinary exosomal viral microRNA as a marker of BK virus nephropathy in kidney transplant recipients. PLoS ONE 12:e0190068
20. Kotton CN, Kamar N, Wojciechowski D, Eder M, Hopfer H, Randhawa P, Sester M, Comoli P, Tedesco Silva H, Knoll G, Brennan DC, Trofe-Clark J, Pape L, Axelrod D, Kiberd B, Wong G, Hirsch HH (2024) The Second International Consensus Guidelines on the Management of BK Polyomavirus in Kidney Transplantation. Transplantation 108:1834–1866
21. Lagatie O, Tritsmans L, Stuyver LJ (2013) The miRNA world of polyomaviruses. Virol J 10:1–9
22. Ledesma J, Bouza E, González-Nicolás MA, García de Viedma D, Rodríguez-Sánchez B, Muñoz P (2013) BK polyomavirus genotyping at inter- and intra-patient level in Spain. J Med Virol 85:1402–1408
23. Li JYZ, McNicholas K, Yong TY, Rao N, Coates PTH, Higgins GD, Carroll RP, Woodman RJ, Michael MZ, Gleadle JM (2014) BK Virus Encoded MicroRNAs Are Present in Blood of Renal Transplant Recipients With BK Viral Nephropathy. Am J Transplant 14:1183–1190

24. Martelli F, Wu Z, Delbue S, Weissbach F, Giulioli M, Ferrante P, Hirsch H, Giannecchini S (2018) BK Polyomavirus MicroRNA Levels in Exosomes Are Modulated by Non-Coding Control Region Activity and Down-Regulate Viral Replication When Delivered to Non-Infected Cells Prior to Infection. Viruses 10:466
25. McIlroy D, Hönemann M, Nguyen NK, Barbier P, Peltier C, Rodallec A, Halary F, Przyrowski E, Liebert U, Hourmant M, Bressollette-Bodin C (2020) Persistent BK Polyomavirus Viruria is Associated with Accumulation of VP1 Mutations and Neutralization Escape. Viruses 12
26. Needham JM, Perritt SE, Thompson SR (2024) Single-cell analysis reveals host S phase drives large T antigen expression during BK polyomavirus infection. PLoS Pathog 20:e1012663
27. Nog R, Mopidevi B, Sivankutty I, Rivera VB, Islam HK, Glicklich D, Diflo T, Chaturvedi V (2024) BK Polyomavirus microRNA in Kidney Transplant Recipient. Transpl Proc 56:1493–1495
28. Nourie N, Boueri C, Tran Minh H, Divard G, Lefaucheur C, Salmona M, Gressens SB, Louis K (2024) BK Polyomavirus Infection in Kidney Transplantation: A Comprehensive Review of Current Challenges and Future Directions. Int J Mol Sci. 10.3390/ijms252312801
29. O'Brien J, Hayder H, Zayed Y, Peng C (2018) Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. Front Endocrinol (Lausanne) 9:402
30. Pang XL, Doucette K, LeBlanc B, Cockfield SM, Preiksaitis JK (2007) Monitoring of polyomavirus BK virus viruria and viremia in renal allograft recipients by use of a quantitative real-time PCR assay: one-year prospective study. J Clin Microbiol 45:3568–3573
31. Parajuli S, Aziz F, Zhong W, Djamali A (2024) BK polyomavirus infection: more than 50 years and still a threat to kidney transplant recipients. Front Transplantation Volume 3–2024
32. Pastrana DV, Brennan DC, Cuburu N, Storch GA, Viscidi RP, Randhawa PS, Buck CB (2012) Neutralization serotyping of BK polyomavirus infection in kidney transplant recipients. PLoS Pathog 8:e1002650
33. Pietilä T, Nummi M, Auvinen P, Mannonen L, Auvinen E (2015) Expression of BKV and JCV encoded microRNA in human cerebrospinal fluid, plasma and urine. J Clin Virol 65:1–5
34. Randhawa PS, Khaleel-Ur-Rehman K, Swalsky PA, Vats A, Scantlebury V, Shapiro R, Finkelstein S (2002) DNA sequencing of viral capsid protein VP-1 region in patients with BK virus interstitial nephritis. Transplantation 73:1090–1094
35. Sharma PM, Gupta G, Vats A, Shapiro R, Randhawa P (2006) Phylogenetic analysis of polyomavirus BK sequences. J Virol 80:8869–8879
36. Sharma R, Zachariah M (2020) BK Virus Nephropathy: Prevalence, Impact and Management Strategies. Int J Nephrol Renovasc Dis 13:187–192
37. Sorin MN, Di Maio A, Silva LM, Ebert D, Delannoy CP, Nguyen NK, Guerardel Y, Chai W, Halary F, Renaudin-Autain K, Liu Y, Bressollette-Bodin C, Stehle T, McIlroy D (2023) Structural and functional analysis of natural capsid variants suggests sialic acid-independent entry of BK polyomavirus. Cell Rep 42:112114

38. Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evol 38:3022–3027
39. Tremolada S, Delbue S, Castagnoli L, Allegrini S, Miglio U, Boldorini R, Elia F, Gordon J, Ferrante P (2010) Mutations in the external loops of BK virus VP1 and urine viral load in renal transplant recipients. J Cell Physiol 222:195–199
40. Tremolada S, Delbue S, Larocca S, Carloni C, Elia F, Khalili K, Gordon J, Ferrante P (2010) Polymorphisms of the BK virus subtypes and their influence on viral in vitro growth efficiency. Virus Res 149:190–196
41. van Doesum WB, Gard L, Knijff LWD, Niesters HGM, van Son WJ, Stegeman CA, van den Berg A, Groen H, van den Born J, Riezebos-Brilman A, Sanders JS (2022) Longitudinal monitoring of BKPyV miRNA levels in kidney transplant recipients with BKPyV-related pathology reflects viral DNA levels and remain high in viremia patients after clearance of viral DNA. Transpl Infect Dis 24:e13927
42. Virtanen E, Seppälä H, Helanterä I, Laine P, Lautenschlager I, Paulin L, Mannonen L, Auvinen P, Auvinen E (2018) BK polyomavirus microRNA expression and sequence variation in polyomavirus-associated nephropathy. J Clin Virol 102:70–76
43. Weissbach FH, Follonier OM, Schmid S, Leuzinger K, Schmid M, Hirsch HH (2024) Single-cell RNA-sequencing of BK polyomavirus replication in primary human renal proximal tubular epithelial cells identifies specific transcriptome signatures and a novel mitochondrial stress pattern. J Virol 98:e0138224
44. Zhang Z, Cao C, Garcia Haider C, Wang Y, Zhu Y, Abdelhak O, Liu H, Yu C, Chang S, Zhang W, Jiang T, Zhou Y (2025) Diagnostic value of three urine miRNAs composite score in BK virus nephropathy. Clin Chim Acta 569:120162
45. Zhou X, Zhu C, Li H (2023) BK polyomavirus: latency, reactivation, diseases and tumorigenesis. Front Cell Infect Microbiol 13:1263983
46. Zou W, Vue GS, Assetta B, Manza H, Atwood WJ, Imperiale MJ (2020) Control of Archetype BK Polyomavirus MicroRNA Expression. J Virol 95

Figures

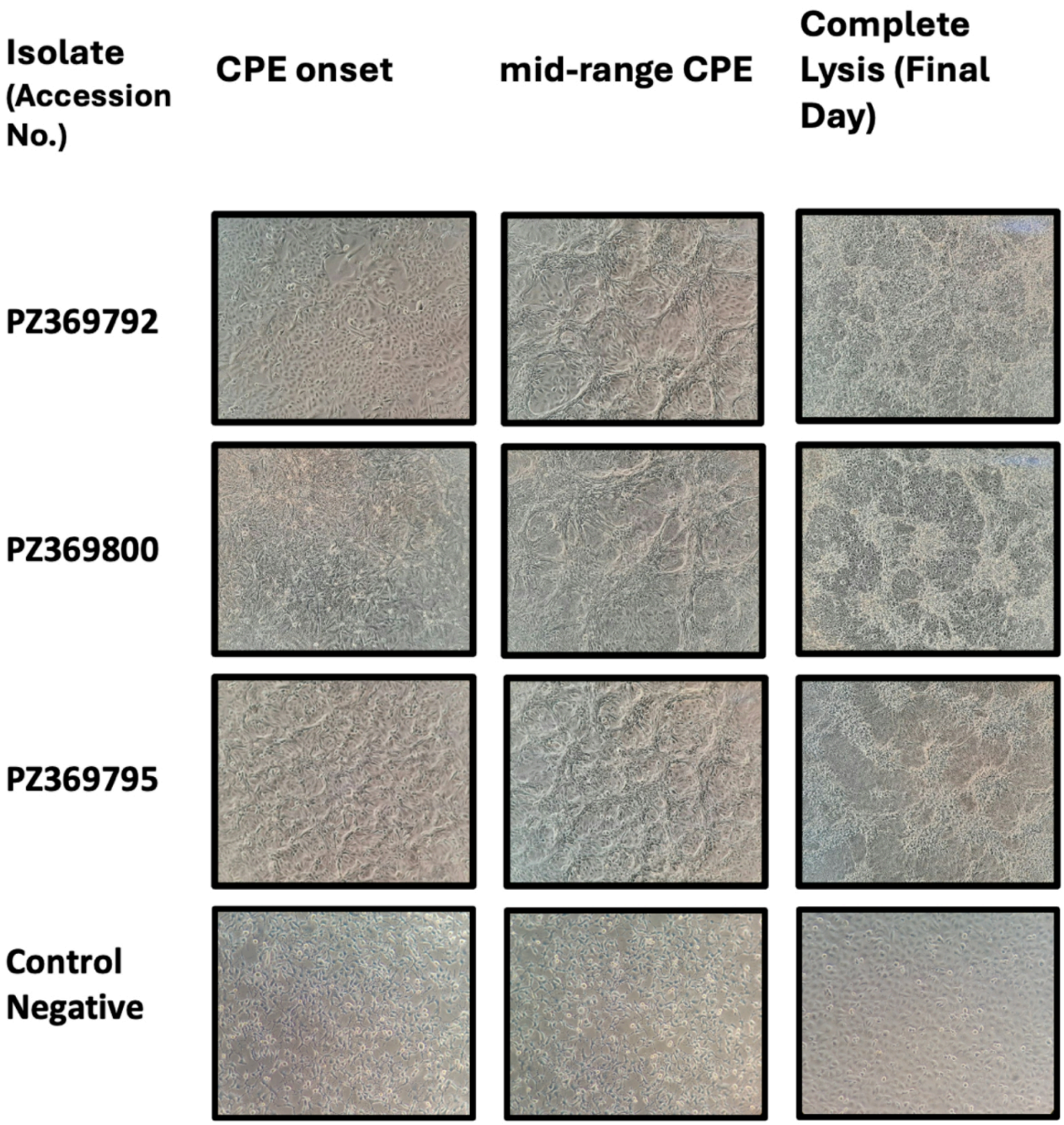

Figure 1

In vitro progression of cytopathic effects (CPE) induced by BK polyomavirus (BKPyV) isolates in Vero E6 cell monolayers. First row (PZ369792): Representative isolate harboring the VP1 E61D mutation. Second row (PZ369800): Representative isolate harboring the VP1 E61N mutation. Third row (PZ369795): Representative typical wild-type/reference-like viral strain. Fourth row (Control Negative): Uninfected Vero E6 cell control maintaining an intact monolayer throughout the observation period. The isolate

harboring the E61N substitution exhibited a prolonged CPE development timeframe of 12 days to reach complete monolayer destruction, compared to 3–5 days observed for the E61D and typical reference-like strains.

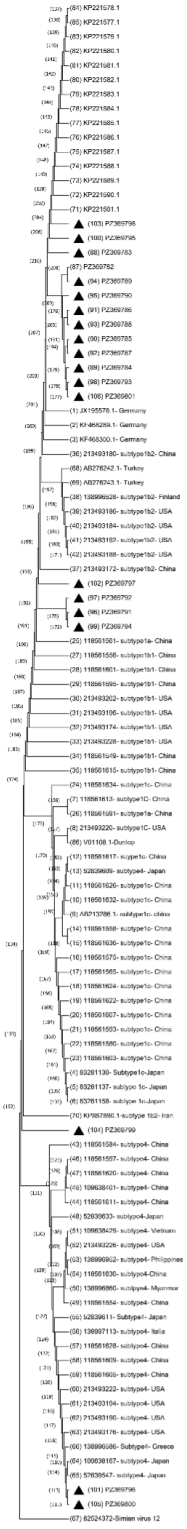

Figure 2

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 106 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1096 positions in the final dataset. Evolutionary analyses were conducted in MEGA11.

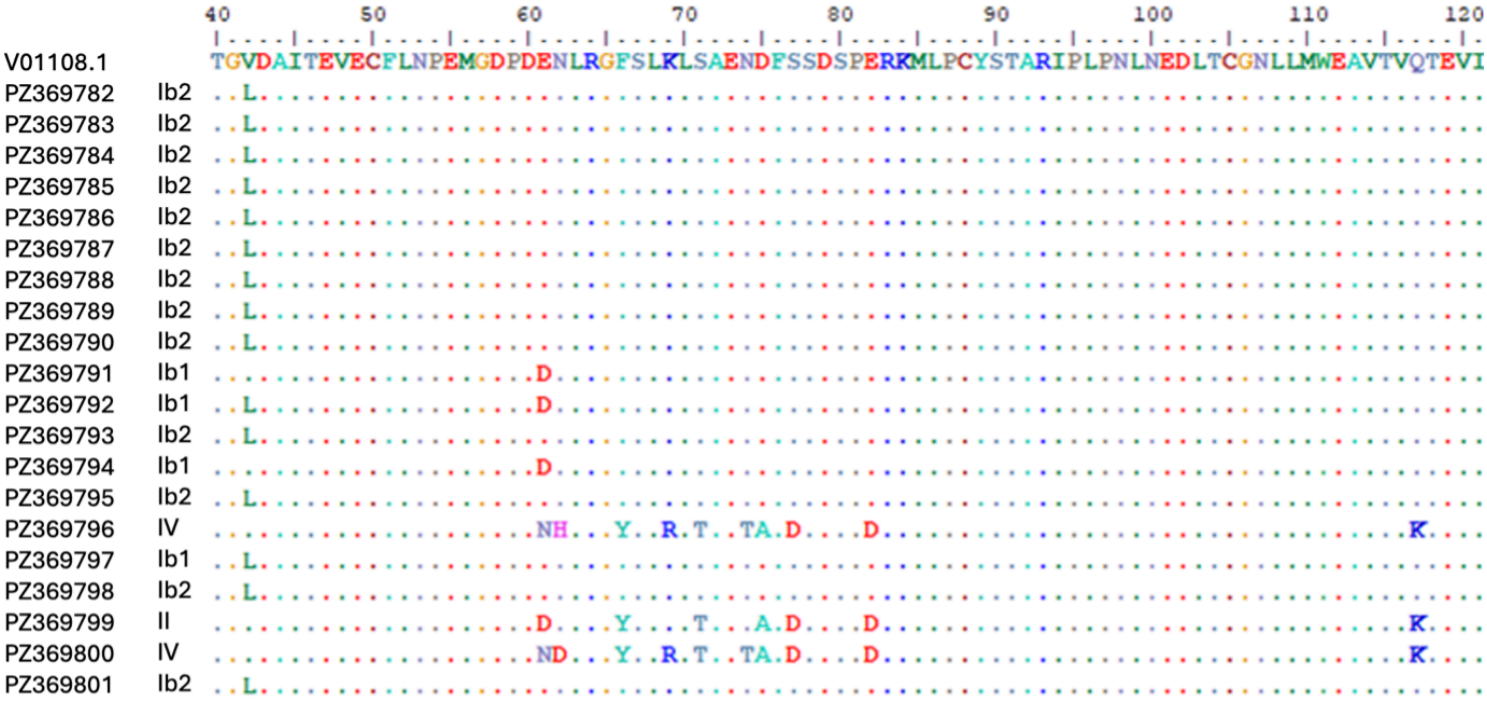

Figure 3

Amino acid alignment of the VP1 hypervariable region. Sequences from the 20 patient samples were compared to the reference strain (V01108.1, Dunlop). Mutations (indicated by letters) are visibly clustered between amino acid positions 61 and 83.

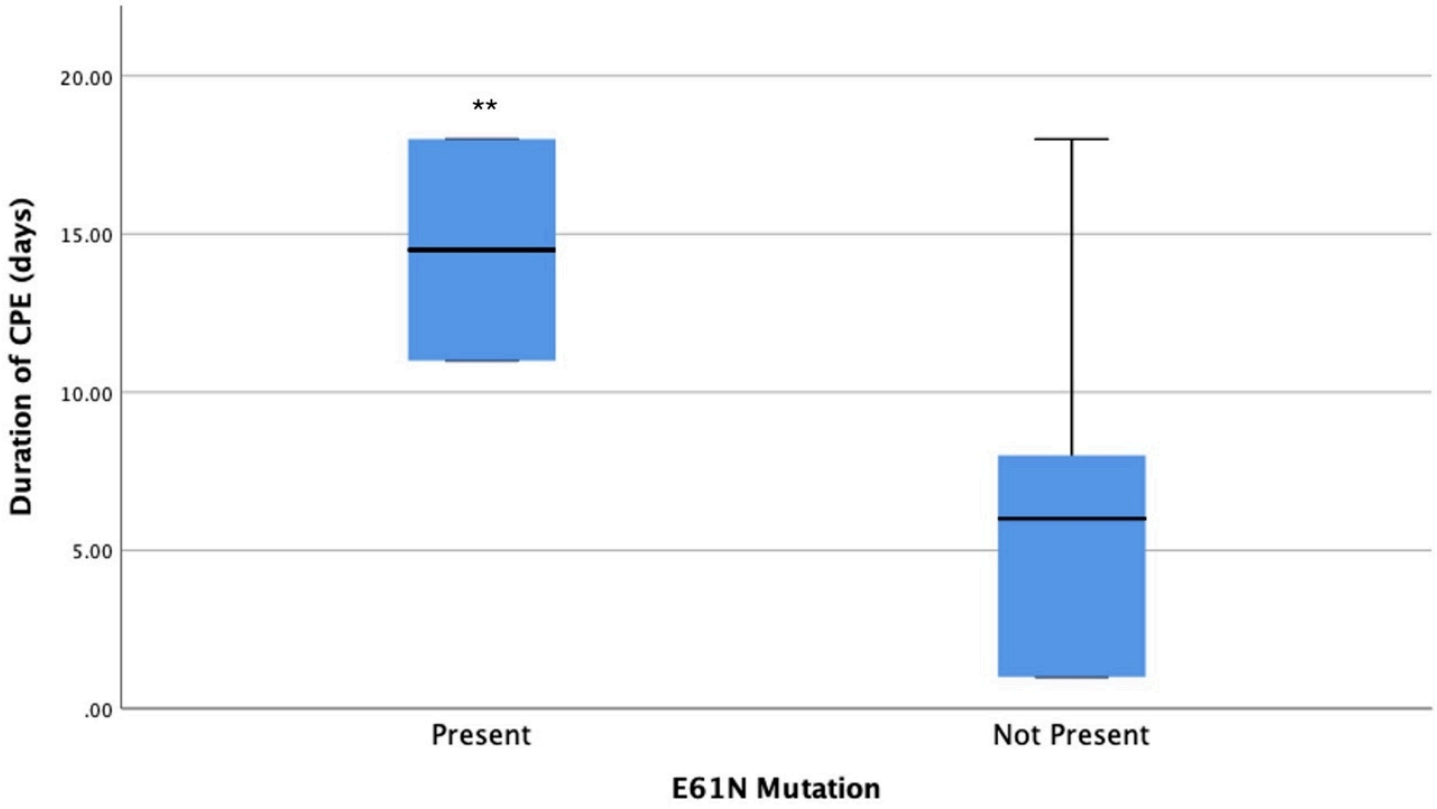

Figure 4

Association between the E61N mutation and rate of CPE development time frame. The boxplot compares the rate of CPE development time frame in viruses with the E61N mutation (Present) and those without (Not present). The presence of the mutation was significantly associated with CPE development time frame