# Title: BK Polyomavirus genotypes and nephropathy

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## Introduction

BK *polyomavirus*-associated nephropathy (BKPyVAN) is one of the most significant causes of graft dysfunction and loss in renal transplant recipients (1). BKPyV strains are classified in four subtypes (I-IV). Subtype I predominates in all geographical regions and is divided into four subgroups (Ia, Ib-1, Ib-2, and Ic) (2). Recently, it was demonstrated that the European subtype I/ subgroup Ib-2 is the most predominant BKPyV genotype circulating in Tunisia (3). However, until today the relationship between BKPyV genotypes and clinical severity is not yet very clear. In this study, we compared the genotypes of BKPyV in patients with BKPyVAN and those with an asymptomatic infection.

## MATERIALS AND METHODS

### Patients and samples

A total of 72 adult renal transplant recipients (46 males and 26 females) were included in this study. During the study two cases of BKPyVAN were approved by allograft biopsy. A volume of approximately 30 ml of urine or 5 ml of peripheral EDTA-blood were collected from each patient.

### BKPyV Detection and genotyping

DNA was extracted from 0.2 ml of urine and cell-free plasma using the QIAamp DNA mini kit (Qiagen). BKPyV DNA detection and quantification in urine and plasma samples was performed using a previously described real-time PCR (4). The genotyping was done through a nested PCR approach that amplifies the 287 bp typing region of the VP1 gene (5). Sequencing was done on the ABI310 genetic analyser. A phylogenetic tree was constructed by neighbor joining method .

## Results

### Detection of BKV DNA in urine and plasma samples

Polyomavirus DNAuria was detected in 54 (75%) of renal transplant recipients: 26 (36%) had BKPyV DNAuria, 20 (28%) had JCPyV DNAuria and 8 (11%) had a dual BKPyV/JCPyV DNAuria. BKPyV DNAemia was detected in 4 (5.5%) patients. BKPyVAN was observed and approved by biopsy in two patients. For one of them viremia was present twice on the 1st and 3rd month. For the second patient, the viremia was detected in the 9th month and the 12th month. These 2 patients reached the cut-off BKV DNAemia value of $10^4$ copies/ml proposed to be presumptive of BKPyVAN. The baseline characteristics of these two patients were summarized in Table 1.

**Table 1. Baseline characteristics**

| Patients With BKPyVAN | Patient 1 | Patient 2 |
|---|---|---|
| Age | 15 years | 28 years |
| Sexe | Male | female |
| Type of donor | living | living |
| Etiology of graft | Reflux disease of the kidneys | Reflux disease of the kidneys |
| Induction therapy | ATG | ATG |
| Maintenance therapy | MMF+Prednisolone+Tac | MMF+Prednisolone+Tac |
| Date of BKPyVAN* | J 112 | J 270 |
| Date of viremia appearance | 1st and 3rd month | 9th and 12th month |
| Viral load in plasma | 2,9 $\log_{10}$ copies/ml (1st months) | 5,2 $\log_{10}$ copies/ml (9th month) |
| | 4,7 $\log_{10}$ copies/ml (3rd month) | 4,6 $\log_{10}$ copies/ml (12th month) |
| Ttt after BKPyVAN | Reduction of Tac (10%) | Reduction of Tac (15%) |
| | Reduction of MMF (50%) | Reduction of MMF (50%) |
| Graft loss | no | yes |

ATG : Anti-thymocyte globulin, MMF: mycophenolate mofetil, Cys A: cyclosporine A, * the date of the graft biopsy confirming the BKPyVAN, Ttt: treatment

### BKV genotypes

The resulting tree (Figure 1) showed that the two patients with biopsy approved BKPyVAN clustered with subtype I/subgroup Ib-2 **(TUN 26 and TUN 30).** The majority of the other patients with asymptomatic infection clustered also with subtype I/subgroup Ib-2 (24 isolates). So, subtype I is the most prevalent in this study (79.5%) with the dominance of the subgroup Ib-2 (76.5%).

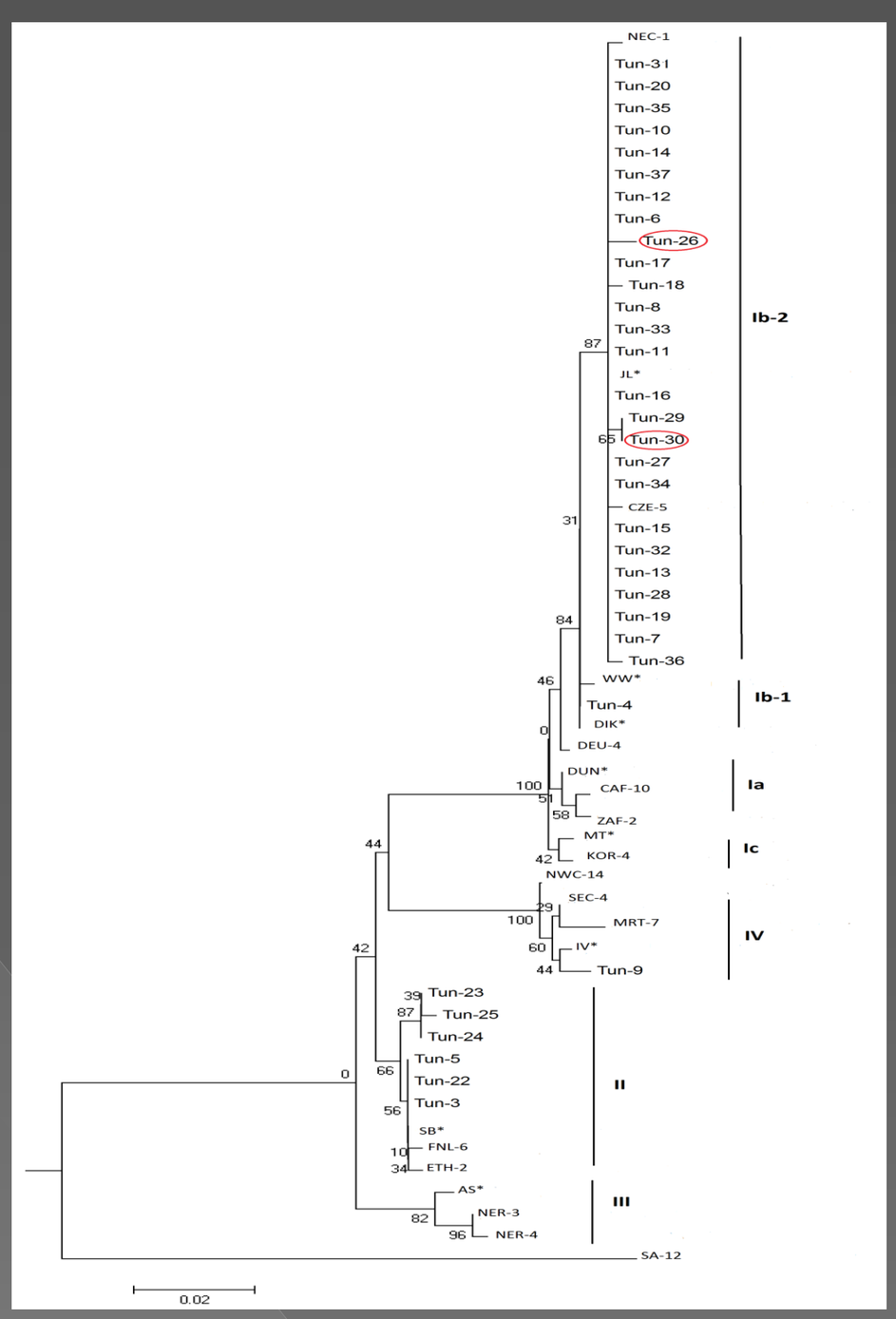


Figure 1. NJ phylogenetic tree clustering 34 Tunisian BKV sequences (TUN-number) plus 21 reference sequences from all over the world. The baboon polyomavirus SA12 (AY614708) was used as the outgroup. The circled isolates (TUN 26 and TUN 30) represent both patients with BKPyVAN.

## Discussion

In this study, BKPyVAN occurred in 2 (3%) patients; these latter reached the cut-off BKV DNAemia value of $10^4$copies/ml proposed to be presumptive of BKPyVAN (6). This viral nephropathy is associated with graft loss in over 50% of cases (7). One of the two BKPyVAN cases described in our study lost his graft (50%), which is consistent with bibliography data. According to the litterature, BKPyV subtype I predominates in all geographical regions (2). We also have detected genotype I in the majority (79.5%) of BKPyV isolates, including the two patients with BKVPyVAN.. Until today, the relationship between the BKPyV genotypes and the BKVPyAN or other histological complications remains unclear. A recent study found that different genotypes of BKPyV have different potential pathogenic in vivo (8). However, within the population of kidney transplant , BKPyV strains from patients with asymptomatic viruria did not show a complete separation of strains associated with viral nephropathy . This is in agreement with our study as we found that the two BKPyVAN cases clusterd with subtype I/subgroup Ib-2 like the most other patients with asymptomatic viruria. Our results may be also explained by the fact that subtype I is the most predominant in all geographical region, and that subtype I/subgroup Ib-2 is the most prevalent in Tunisia (3).

## Conclusion

This study demonstrates that the BKPyV subtypes do not play a direct role in the aggravation of the BKPyV infection and in the evolution to a BKPyVAN.

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