Development of a new method for BK polyomavirus genotyping: application to transplant recipients

Sonia BURREL [1,2], Maud Gits-Muselli [1,2], Zaïna AIT-ARKOUB [2], Stéphanie Nguyen [3], Nadia Arzouk [4], Henri Agut [1,2], David BOUTOLLEAU [1,2]

1 ER1 DETIV, University Pierre et Marie Curie - Paris 6, Paris, France
2 Virology Department, Pitié-Salpêtrière University Hospital, AP-HP, Paris, France
3 Hematology Department, Pitié-Salpêtrière University Hospital, AP-HP, Paris, France
4 Urology Department, Pitié-Salpêtrière University Hospital, AP-HP, Paris, France

INTRODUCTION

BK virus (BKV) is a non-enveloped virus with a circular double-stranded DNA belonging to the *Polyomaviridae* family. BKV infection may result in severe nephropathy in kidney transplant (KT) recipients or haemorrhagic cystitis in hematopoietic stem cell transplant (HSCT) recipients (Krumbholz et al., 2006). BKV strains are classified into 4 main genotypes (I to IV) and 10 additional subgroups within genotypes I and IV. BKV genotyping has been historically based on the analysis a 327-base pairs (bp) variable region of the gene coding for the major capsid protein VP1 (Jin et al., 1993). The aim of this work was to develop a new method for BKV genotyping based on the sequencing of the full-length gene coding for VP1 (1,089 bp) and to determine BKV genotypes among KT and HSCT recipients.

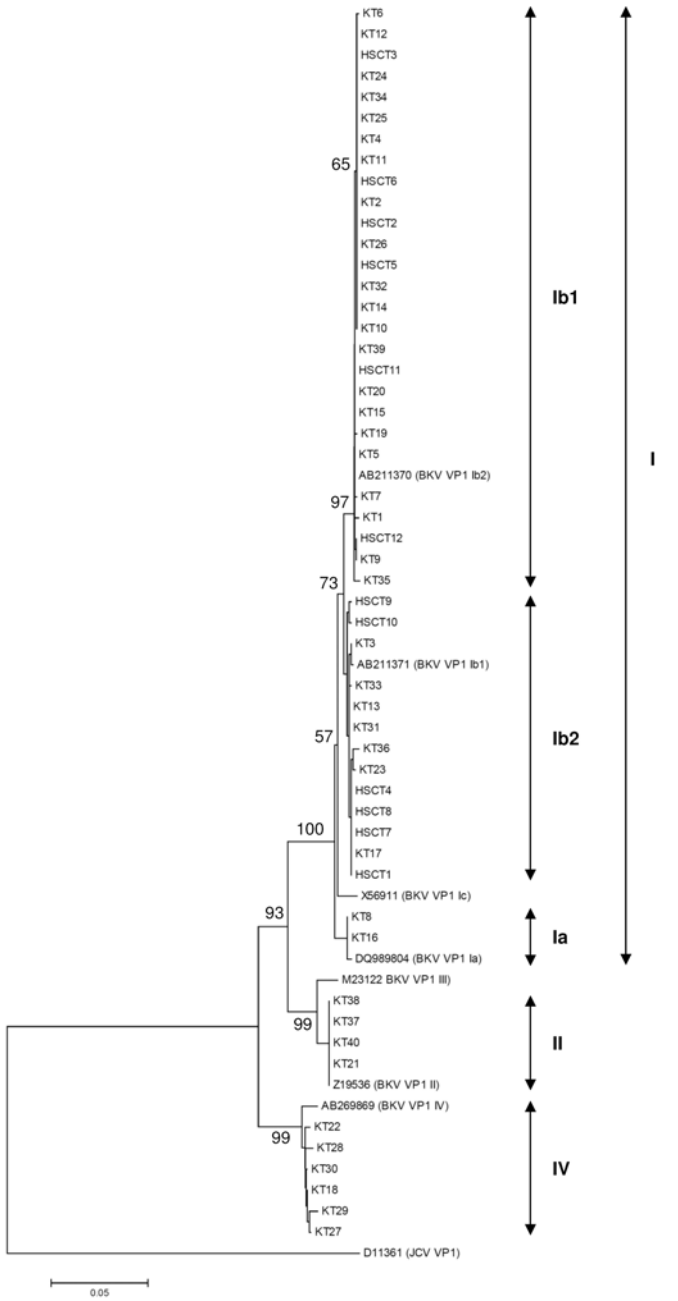

Figure 1. Phylogenetic tree constructed by the Neighbor-joining method using BK complete sequence of VP1 gene

Analysis was carried out for complete sequence from VP1 gene. All nucleotide sequencing results were compared with published sequence of the reference strain Dunlop (GenBank accession number V01108). Sequence alingments were performed with ClustalW using default parameters. Neighbor-joining trees were constructed with MEGA 4 and were visualized using MEGA 4 tree explorer. A bootstrap test with 1,000 replicates was applied to estimate the significance of the tree branching patterns. VP1 gene GenBank sequences DQ989804, AB211371, AB211370, X56911, Z19536, M23122 and AB269869 represent subtypes Ia, Ib1, Ib2, Ic, II, III and IV, respectively. The VP1 corresponding sequence from the JCV strain CY (GenBank accession number D11361) was used as the outgroup to root the tree. Bootstrap values are shown for major nodes. KT: kidney transplant recipients; HSCT: hematopoeitic stem cell transplant recipients.

CONCLUSION

We report here the development of a new method for the sequencing of the full-length VP1 coding region allowing BKV genotyping. Our results evidenced the high variability of VP1. This method constitutes a useful tool for further studies on BKV pathogenicity according to the genotypes.

MATERIALS AND METHODS

Primers were designed to amplify, by nested PCR, and to sequence the full-length VP1 coding region (1,089 bp). Amplified products were analyzed with the automated sequencer ABI 3100 Genetic Analyzer (Applied Biosystems). This new method was applied to 52 EDTA whole blood specimens obtained from 40 KT and 12 HSCT recipients (34 men, 18 women, median age: 48 years) experiencing BKV active infection (median BKV load in blood: 5.5 log). All nucleotide and amino acid VP1 sequences were aligned with SeqScape v2.5 software using BKV Dunlop strain as a reference (GenBank accession number V01108). A phylogenetic tree was constructed by the neighbor-joining method using ClustalW program, including the VP1 sequences from the different reference BKV strains representing the main genotypes obtained from GenBank.

RESULTS

The sensitivity of the VP1 nested PCR was 500 copies/mL. At the nucleotide level, the interstrain identity of VP1 gene ranged from 91.9 to 99.2%. At the amino acid level, a total of 45 amino acid changes were identified, that is 12.4% of the total codons of the protein. Each strain harboured a mean number of 8.8 amino acid changes (Table 1). Seven positions of polymorphism were identified among at least 50% of the BKV strains: V42L, E158D, S171T, D175E/Q, V210I, A219T and R340K/Q (Table 2). As a whole, the distribution of BKV genotypes among transplant recipients was as follows: I (81%), II (8%), III (0%), IV (12%). Among genotype I, BKV Ia, Ib1, Ib2, and Ic subgroups represented 5%, 31%, 64%, and 0%, respectively. Only BKV subgroups Ib1 and Ib2 were identified among HSCT recipients (Figure 1).

Table 1. VP1 variation, both at nucleotide and amino acid levels, among 52 clinical strains of BKV in comparison with Dunlop reference strain (GenBank accession no. V01108)

Parameter	BKV VP1
Nucleotide identity (%)	91.9 - 99.2
Nucleotide mutations (no.)	121
Frequency of nucleotide mutations per strain (mean)	9 - 88 (29.8)
Silent nucleotide mutations (%)	76 (62.8)
Amino acid identity (%)	92.8 - 99.2
Amino acid changes (no.)	45
Frequency of amino acid changes per strain (mean)	3 - 26 (8.8)
Variation of the total codons of the VP1 protein (%)	12.4

Table 2. Amino acid changes related to natural polymorphism of BKV VP1 identified in this study in comparison with Dunlop reference strain (GenBank accession no. V01108)

Amino acid position	Amino acid change	No. of strains	% of strains
42	V - L	26	50
60	D - N	2	4
61	E - D/S	5	10
61	E - N	5	10
62	N - D	6	12
66	F - Y	10	19
69	K - R/M	8	16
71	S - T	10	19
73	E - Q	1	2
74	N - T	7	13
75	D - A	10	19
77	S - D/N/K/E	12	23
82	E - D	12	23
83	R - K	3	6
117	Q - K	10	19
134	Q - K	1	2
139	H - N	11	21
145	I - V	4	8
158	E - D	52	100
171	S - T	48	92
175	D - E/Q	37	71
178	I - V	6	12
210	V - I	36	69
219	A - T	52	100
225	F - Y	10	19
284	A - P	6	12
291	L - M	1	2
305	L - F	1	2
306	L - I	1	2
316	R - K	4	8
340	R - K/Q	31	60
341	L - S	1	2
342	P - S	1	2
343	G - R	1	2
344	D - M	1	2
347	M - L	1	2
353	K - R	9	17
362	L - V	13	25

REFERENCES

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