



HAL
open science

Prevalence of JC polyomavirus genomic sequences from the large T-antigen and non-coding control regions among Bulgarian patients with primary brain tumours

Iliya Tsekov, Dilyan Ferdinandov, Ventzeslav Bussarsky, Svetlana Hristova, Zlatko Kalvatchev

► To cite this version:

Iliya Tsekov, Dilyan Ferdinandov, Ventzeslav Bussarsky, Svetlana Hristova, Zlatko Kalvatchev. Prevalence of JC polyomavirus genomic sequences from the large T-antigen and non-coding control regions among Bulgarian patients with primary brain tumours. *Journal of Medical Virology*, 2011, 83 (9), pp.1608. 10.1002/jmv.22130 . hal-00657574

HAL Id: hal-00657574

<https://hal.science/hal-00657574>

Submitted on 7 Jan 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Prevalence of JC polyomavirus genomic sequences from the large T-antigen and non-coding control regions among Bulgarian patients with primary brain tumours

Journal:	<i>Journal of Medical Virology</i>
Manuscript ID:	JMV-10-2192.R1
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	25-Apr-2011
Complete List of Authors:	Tsekov, Iliya; National Center of Infectious and Parasitic Diseases, Virology Ferdinandov, Dilyan; St. Ivan Rilski University Hospital, Clinic of Neurosurgery Bussarsky, Ventzeslav; St. Ivan Rilski University Hospital, Clinic of Neurosurgery Hristova, Svetlana; University Hospital Alexandrovska, Medical Faculty, Department of general and clinical pathology, Central cytopathology laboratory Kalvathev, Zlatko; National Center of Infectious and Parasitic Diseases, Virology
Keywords:	brain tumours, JC virus, oncogenesis, progressive multifocal lekoencephalopathy

SCHOLARONE™
Manuscripts

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

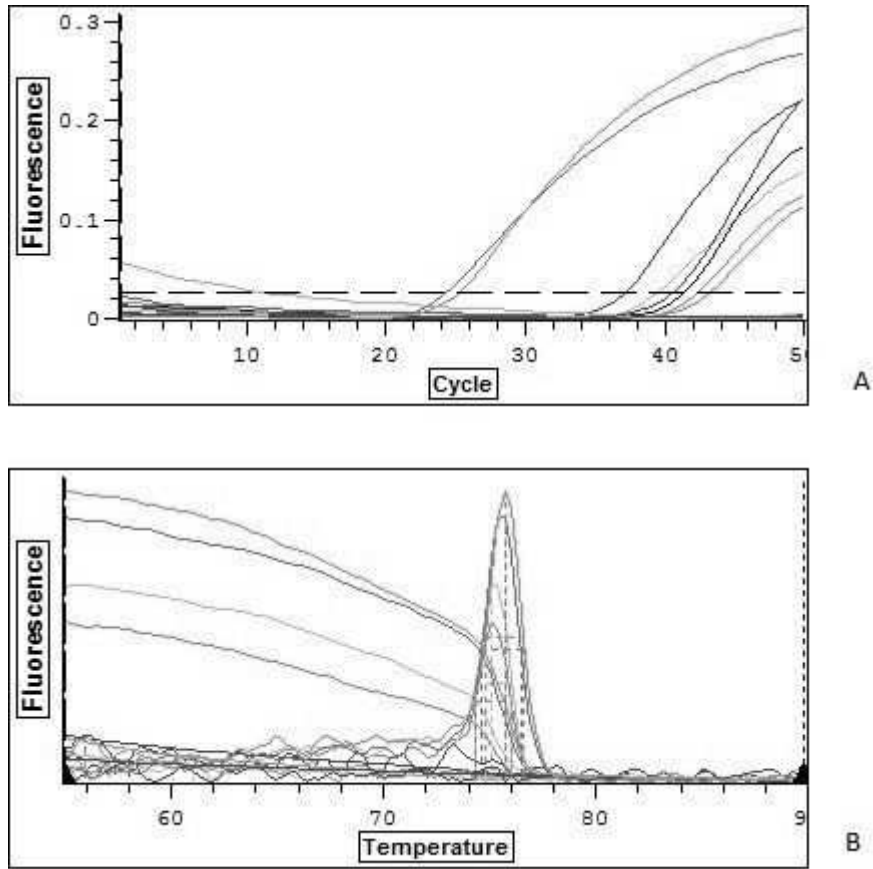


Fig. 1. Real-time SYBR Green polymerase chain reaction assay
A. An amplification plot showing positive control graphs with a threshold at 25th cycle and several positive samples with a threshold in the range from cycle 36 to 44.
B. Melting curve analysis showing a characteristic profile of the positive control at 75.7C identical to the profile of the positive samples
117x118mm (96 x 96 DPI)

Table I

Presentation of the patients according to their histopathological diagnosis, gender, age and prevalence of viral sequences

Tumor/Lesion type	Number	Gender (M:F)	Age (years) mean; range	LT* positive	Mean Ct**; range	NCCR***	
						Positive	Range of copies
Glioblastoma	39	22:17	53 (2-80)	19 (48.7%)	44 36-49	7 (17.5%)	10-100
Astrocytoma	19	10:9	34 (6-61)	6 (31.6%)	41 37-41	3 (15%)	10-100
Oligodendroglioma	12	7:5	46 (32-62)	4 (33.3%)	38 37-40	2 (16.7%)	10-100
Oligoastrocytoma	3	3:0	40 (33-51)	2 (66.7%)	43-44	none	N/A
Medulloblastoma	5	2:3	28 (3-48)	2 (40.0%)	49	none	N/A
Ganglioglioma	5	3:2	24 (11-54)	2 (40.0%)	39-41	3 (60.0%)	10-100
Ependimoma	5	2:3	45 (37-53)	none	N/A	2 (40.0%)	10-100
Pineocytoma	3	2:1	27 (7-59)	1 (33.3%)	40	2 (66.7%)	10-100
Radiotherapy induced	2	2:0	50 (45, 56)	2 (100%)	40	1 (50.0%)	10

alterations							
Metastases	6	4:2	50 (2-65)	1 (16.7%)	48	none	N/A
Rare brain tumors	6	4:2	34 (4-73)	None	N/A	1 (16.7%)	10
Vascular and other malformations	7	4:3	37 (17-62)	1 (14.3%)	43	none	N/A

*LT: Large T antigen encoding genomic sequences detected by SYBR Green real-time polymerase chain reaction;

**Ct: threshold cycle determined during the SYBR Green real-time polymerase chain reaction;

***NCCR: DNA sequences from the non-coding control region determined by quantitative assay;

Table II

Presentation of the patients who were determined as both LT and NCCR positive

Tumor type	Sex and age	Ct	Number of NCCR copies
Glioblastoma	57/F	36	10-100
Glioblastoma	67/M	50	10
Radiotherapy induced alterations in a patient with Glioblastoma	45/M	39	10
Astrocytoma	51/F	37	10-100
Astrocytoma	58/M	41	10
Oligodendroglioma	41/F	38	10-100
Ganglioglioma	25/F	41	10-100
Pineocytoma	15/F	40	10-100

1
2
3
4
5
6 **1 Prevalence of JC polyomavirus genomic sequences from the large T-antigen and**
7
8 **2 non-coding control regions among Bulgarian patients with primary brain tumours**
9
10
11
12
13
14

4 Iliya Tsekov¹, Dilyan Ferdinandov², Ventzeslav Bussarsky³ Svetlana Hristova⁴ and
5 Zlatko Kalvatchev^{1*}

6 ¹Molecular Virology Laboratory, National Centre of Infectious and Parasitic Diseases,
7 Department of Virology, Sofia, 1233, Bulgaria

8 ²Clinic of Neurosurgery, St. Ivan Rilski University Hospital, Sofia, 1431, Bulgaria

9 ³Central cytopathology laboratory, University Hospital Alexandrovska, Medical
10 Faculty, Department of general and clinical pathology, Sofia, 1431, Bulgaria

11 *Correspondence to: Prof. Zlatko Kalvatchev, Laboratory of Molecular Virology,
12 National Center of Infectious and Parasitic Diseases, Stoletov blvd. 44-A, 1233 Sofia,
13 Bulgaria

14 Phone: (+359 2) 931-07-13 ext. 232

15 E-mail: kalvatchev@gmail.com

16 Fax: +359 2 943 3075

17
18 **Running head:** Prevalence of JCV in primary brain tumours
19
20
21
22
23

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
1 ABSTRACT

2 A total of 111 fresh brain biopsies from patients with primary brain tumours were
3 examined for JC polyomavirus (JCV) sequences from the Large T antigen encoding
4 region (LT) and the viral non-coding control region (NCCR). SYBR Green and TaqMan
5 real-time polymerase chain reaction assays were used. In the glioblastoma group of 39
6 patients 48.7% were positive for LT sequences. Among the astrocytoma group (19
7 patients) and the oligodendroglioma group (12 patients) 31.6% and 33.3% were also
8 positive. The prevalence of LT genomic sequences among the other groups was as
9 follows: in 2 out of 3 oligoastrocytomas; in 3 out 5 gangliogliomas; in 2 out of 5
10 meduloblastomas; in 1 out 3 pineocytomas; and in none of the tested 5 ependimomas.
11 All positive samples had a late threshold cycle that varied from 36 to 49, indicative of
12 very low starting viral number. Only 21 of all the 111 samples were positive for NCCR.
13 Low copy number in range of 10 to 1000 was present. Notably, only 8 of all NCCR
14 positive specimens were also LT positive. It might be suggested that the disproportion
15 between the results for LT and NCCR is either due to clonally integrated LT fragments,
16 with loss of genetic material, or changes in the NCCR. The latter would alter the
17 productive course of the infection and may establish a premise for continuous
18 interaction of viral regulatory proteins with cell molecules that are responsible for the
19 control of the cell cycle. This may lead subsequently to malignant transformation.

20
21 **Key Words:** brain tumours; JC virus; oncogenesis; progressive multifocal
22 leukoencephalopathy

INTRODUCTION

Human polyoma viruses (HPoV) are small oncogenic viruses, comprised of an icosahedral capsid around a circular, double-stranded DNA molecule. Their genome is approximately 5100 base pairs in length and can be divided into a non-coding control region (NCCR) and overlapping genes encoding the viral regulatory and structural proteins. There are no genes encoding viral replication enzymes. Therefore, the replicative strategy of HPoV relies on the induction of an S phase environment and stimulation of the cellular machinery for specific enzyme production. This is a complex process involving mainly the so called large T-antigen (LT) [Imperiale and Major, 2007]. This peptide can interact with several important regulatory cell molecules by binding to tumours suppressor proteins like pRb and p53 [Khalili et al., 2006; White and Khalili, 2004] on the one hand; and to proto-oncogene products such as IRS-1 and β -catenin, [Lassak et al., 2002],[Gan and Khalili, 2004] on the other. It is believed that this interference with the cell cycle regulation might be a key factor for induced malignant transformation by viruses.

The primary infection with JC viruses (JCV), from the HPoV family, has been established to occur during childhood. The presence of specific JCV antibodies is pointed out by zur Hausen to be up to 98% of the human population [zur Hausen, 2008]. The infection remains latent but under certain conditions a viral reactivation is possible. The production of mature JC viral particles, followed by the death of the infected cells can lead to progressive multifocal leucoencephalopathy [Imperiale and Major, 2007]. If the infected cells die, any possible disregulation has no consequence for cellular

1 transformation. However, when the viral replication is in some way impaired and
2 associated with the survival of the affected cell, the events causing disturbances in
3 regulation contribute to the process of cellular transformation [Nevins, 2007]. JC virus
4 has a marked oncogenic potential when cultured *in vitro*. The development of tumours
5 in inoculated or transgenic experimental animals has also been demonstrated [Miller et
6 al., 1984].

7 JCV possess oncogenic potential, but still their role concerning the induction of
8 primary brain neoplasia is unclear and findings reported in the literature vary widely.
9 Investigations on the effect of JCV cultured *in vitro* and on laboratory animals rise
10 evidence associating this virus with human tumours and offer possible mechanisms of
11 JCV-mediated cell transformation [Del Valle et al., 2008]. In contrast, some researchers
12 fail to identify genomic sequences in human tumours [Herbarth et al., 1998; Rollison et
13 al., 2005]. Although a possible “hit-and-run” mechanism is discussed, they put under
14 question an association of these viruses with malignant transformation. There are
15 obstacles that hamper the establishment of a causal role between JCV and human
16 neoplasia. For instance, the discrepancy between the high prevalence of HPoV in nature
17 and the relatively low frequency of JCV-associated cancer has been raised as an
18 argument against a direct association between the two phenomena. Most often the
19 primary JCV infection is subclinical, making it difficult to establish the exact moment
20 and mechanism of infection. The incubation period between the onset of the infection
21 and the appearance of the tumours might be extended to several decades. In addition, a
22 productive JCV infection cannot be reproduced in laboratory animals, limiting
23 investigations aimed to give answers to the above questions. Environmental (e.g., co-
24 carcinogens) or host factors (e.g., immune system status) can additionally modify the

1
2
3
4
5
6 1 viral pathogenesis, making it even more difficult for experimental research [Khalili et
7
8 2 al., 2006].
9

10 3 Criteria about the etiologic role of an infectious agent and a neoplastic disease
11
12 4 have been proposed: [zur Hausen, 2006]
13
14

- 15 5 1. Epidemiological plausibility and evidence that the viral infection is a risk factor
16
17 6 for the development of a certain tumours type;
18
19 7 2. Regular identification and persistence in the cells of a particular tumours of the
20
21 8 respective infectious agent's nucleic acids;
22
23 9 3. Cell proliferation stimulation following transfection with given genomes or
24
25 10 sequences in the corresponding tissues and cell types;
26
27 11 4. Demonstration that the proliferative induction and the malignant phenotype of a
28
29 12 certain tumours cells depend on functions executed by the persisting nucleic
30
31 13 acids of the respective infectious agent.
32
33
34
35

36 14 There is evidence that JCV meets at least the last three criteria presented [Khalili et al.,
37
38 15 2006].
39
40

41 16 Differences in the patient populations and the used laboratory techniques have
42
43 17 been suggested as possible explanations for inconsistencies in the results across
44
45 18 different researchers [Del Valle et al., 2008; Rollison et al., 2005]. It has been generally
46
47 19 considered that additional facts are needed as data about the presence of polyoma virus
48
49 20 JC in primary brain tumours samples differ among the studied groups worldwide.
50
51

52 21 The aim of this study was to determine the prevalence of JC polyomavirus
53
54 22 sequences among patients with primary brain tumours and to evaluate the positive
55
56 23 samples in terms of viral copy number.
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 Project approval from the Ethics Committee of Medical University of Sofia was
2 given and all specimens were collected after a signed informed consent.

For Peer Review

MATERIALS AND METHODS

Study group

A total of 111 fresh brain biopsy materials were collected from patients who underwent surgical procedures of the central nervous system.

Ninety patients had primary brain tumours, six were diagnosed with a brain metastasis, 6 were with other rare tumours localized in the brain and seven patients had vascular and other malformations. Brain tumour type was verified as part of the routine histopathological examination in St. Ivan Rilski University Hospital by two independent neuropathologists. The study group is presented in details in Table I.

Sample collection

During the surgical procedure biopsy samples were placed in a sterile 1.5 ml tubes containing 0.6 ml of transport medium (Cat. No. K-1-1/A, Sacace, Como, Italy) and were immediately frozen. Specimens were kept at -20°C for a maximum of 48 hours prior to transportation to the virology laboratory. The collected samples were washed three times with a sterile phosphate buffered saline and were stored at -80°C until an extraction procedure was carried out.

Extraction procedure

DNA was obtained from 3-7 mg of tumours tissue subjected to extraction with AquaPure DNA Kit (Cat. No. 732-6340EDU, Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. An internal extraction control (Cat. No. Int-DNA, Primer Design, Southampton, UK) was added to each sample in order to verify the

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 presence of amplifiable DNA during the quantitative real-time assay. In addition at the
2 end of the extraction the quality and quantity of DNA was determined with a
3 spectrophotometer and agarose gel-electrophoresis.

5 **Detection of LT sequences**

6 All samples were analyzed for the presence of viral sequences that are part of the gene
7 encoding the LT by SYBR Green real-time polymerase chain reaction (PCR) assay. JCT
8 1 and JCT 2 primers [MacKenzie et al., 2003] for detection of JCV were used as a part
9 of a modified system. Cycling reactions and fluorescent detection was carried out in an
10 Opticon 2 instrument (MJ Research/Bio-Rad, Hercules, CA, USA). Briefly, the PCR
11 mixture consisted of 25 μ l 2X master mix (Platinum SYBR-Green qPCR SuperMix-
12 UDG, Invitrogen, Carlsbad, CA, USA), 20 pmol of each primer, 1 μ l ROX (as a
13 reference dye) and approximately 0.5÷1.2 μ g of total DNA in a 50 μ l total reaction
14 volume. Best signal strength and lowest level of background fluorescence was achieved
15 with the next protocol: 50⁰C for 2 min, 94⁰C for 10 min, followed by 50 cycles of
16 denaturation at 95⁰C for 15 sec and annealing/extension at 60⁰C for 30 sec with a plate
17 read at the end of each cycle (Fig. 1A). A dissociation profile was run after the
18 amplification stage in order to verify the specificity of the products. The samples were
19 gradually heated with an increment of 0.3⁰C and the change of the fluorescent signal
20 was determined at every step. Both negative (DNA free) and positive (JCV genomes,
21 partially sequenced and included in GenBank under accession no. GU222438 and
22 GU145384) samples were used in each run. Specimens were considered positive when a
23 significant increase of the fluorescence above the threshold line was obtained and the
24 determined specific melting temperature was corresponding to the positive control

1
2
3
4
5
6 1 profile with a maximal decrease in the intensity of the signal ($-dI/dT$) at 75.7°C (Fig.
7
8 1A, Fig. 1B).
9

10 3

11 4 **Quantitation of NCCR sequences**

12
13
14
15 5 Samples were analyzed further for the NCCR. Detection and quantitation was
16
17 6 performed with the Primer Design TaqMan[®] based JCV Advanced Kit (Cat. No. Path-
18
19 7 JCV, Primer Design, Southampton, UK). A total number of 8 quantity control dilutions
20
21 8 with the respective control viral genomes, covering a spectrum of 10^8 down to 10
22
23 9 starting copies per reaction, were used in each run. All samples contained an internal
24
25 10 amplification control and results were interpreted according to the manufacturer's
26
27 11 guidelines.
28
29
30
31
32
33

34 12
35 13 Both qualitative and quantitative PCR experiments were repeated at least twice
36
37 14 for verification of the results.
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

RESULTS

All collected specimens were determined to contain sufficient amplifiable DNA in the range of 0.2-1.2 $\mu\text{g}/\mu\text{l}$.

The results from the SYBR Green[®] assay targeted at LT, revealed the presence of JC viral sequences in a total of 41 out of the 111 tested samples. The distribution of LT positive patients among the different groups varied in a broad range. Notably, 48.7% (n=19) from the tested 39 glioblastoma patients were positive for LT sequences. Another six in the astrocytoma group of 19 subjects, and four positive samples in the oligodendroglioma group of 12 were determined. These formed a prevalence of 31.6% and 33.3%, respectively. Two out of three samples from patients with oligoastrocytoma were also positive for LT, as well as two out of five in the groups of medulloblastoma and ganglioglioma patients. None of the tested five ependimomas and only one from the three pineocytoma harboured LT genomic sequences. Two patients, who underwent radiotherapy following neurosurgery for an astrocytoma and glioblastoma are presented separately. The result from the LT PCR was positive for both cases. The prevalence of JCV sequences among the study groups is summarised in details on Table I. Notably, 48.7% from the tested 39 glioblastoma patients were positive for LT sequences. The prevalence of JCV sequences among the study groups is presented in details on Table I. Two patients, who underwent radiotherapy following neurosurgery for an astrocytoma and glioblastoma are presented separately. The result from the LT PCR was positive for both cases.

In comparison to the primary brain tumours several (n=6) rare tumours with brain localisation (primary plexus choroideus carcinoma, central neurocytoma, estesionevrioblastoma, germinoma, teratoma with malignant transformation and

1
2
3
4
5
6 1 retinoblastoma) were also tested and none of them was found to yield a PCR
7
8 2 amplification for LT. Out of these tumours only in the retinoblastoma patients NCCR
9
10 3 was amplified. Six other tumours were diagnosed to be CNS metastases with different
11
12 4 place of origin. Only one sample (metastasis from a papillary carcinoma) was evaluated
13
14
15 5 as LT positive. JCV sequences were also found in a brain biopsy from a patient who
16
17 6 underwent surgery because of an arterio-venous malformation (Table I).

18
19
20 7 All positive samples had an increase in the fluorescent signal during the late
21
22 8 cycles of the PCR, with a threshold cycle that varied from 36 to 50 (Table I).

23
24
25 9 The subsequent TaqMan[®] quantitative assay showed that most of the samples
26
27 10 did not yield a NCCR product, although the detection limit of the system was 10
28
29 11 starting copies. Only 21 of all the 111 samples were documented as NCCR positive
30
31 12 (Table I). Low copy number in the range of 10 to 1000 was present. Notably, only eight
32
33 13 of all NCCR positive specimens were also LT positive (Table II).

1
2
3
4
5
6 1 “bizarre astrocytes” and formation of brain tumours in progressive multifocal
7
8 2 leukoencephalopathy patients [Richardson, 1961]. Thus, the virus is considered a
9
10 3 plausible etiologic agent for the development of primary brain tumours [Del Valle et al.,
11
12 4 2008]. If replication of the viral genome and formation of mature particles are altered
13
14 5 and incomplete, it might be expected that viral sequences would be in low numbers
15
16 6 and/or only partial genomic fragments would be present.
17
18

19
20 7 The presence of NCCR is essential for the proper replication of HPoV.
21
22 8 Amplification failure or low NCCR copy number might confer a prerequisite for
23
24 9 incomplete or non-lytic course of the infection. It has been shown that JCV sequences
25
26 10 originating from progressive multifocal leukoencephalopathy patients carry NCCR
27
28 11 rearrangements compared to the archetypal strain [Miller et al., 1984]. Similarly, it is
29
30 12 possible that in brain tumour cells changes altering the function of NCCR may occur
31
32 13 leading to inability for mature virions to be formed. Indeed, it has been shown that
33
34 14 under the circumstances of continuous LT gene expression malignant transformation
35
36 15 may occur [Del Valle et al., 2001a]. A similar mechanism of viral-induced oncogenesis
37
38 16 was proposed for Kaposi's sarcoma associated herpesvirus [Zhao et al., 2007]. The
39
40 17 authors described that the disrupted control of lytic replication genes led to a non-lytic
41
42 18 course of the infection and a subsequent tumorigenesis. However, exact molecular
43
44 19 interactions and its significance *in vivo* are so far not well understood.
45
46
47
48
49

50
51 20 The histopathological diagnosis revealed one of the studied tumours to be
52
53 21 subependymal giant cell astrocytoma. This is a rare type of brain tumour that commonly
54
55 22 occurs in the setting of the familial syndrome of tuberous sclerosis complex and
56
57 23 hereditary factors are considered to explain its development [Adriaensen et al., 2009].
58
59 24 Therefore, as expected viral sequences were not detected. Similarly, in other rare brain
60

1
2
3
4
5
6 1 tumours JCV sequences were also not found, as probably human genetic factors might
7
8 2 have a prevalent impact for the development of malignant transformation.
9

10 3 Samples were often processed and tested prior the definitive histological
11
12 4 diagnosis, so six tumours, were later determined to be actually metastases. It might be
13
14 5 expected that these would be JCV negative, as it was the case with five of them.
15
16 6 However, one sample was positive for LT. The logic behind this remains unclear so far.
17
18

19 7 Two of the patients had alterations that were induced by the radiotherapy
20
21 8 following the surgical resection for fibrilar astrocytoma and glioblastoma. Their
22
23 9 biopsies were determined to be LT positive. Also in a non tumour disease, where
24
25 10 surgery was performed due to arterio-venous malformation JCV LT fragments were
26
27 11 found. These results bring into concern a question regarding the role of the immune
28
29 12 system in controlling viral re-activation. It has been suggested that JCV is already
30
31 13 present in a latent state in the brain tissue [Lam et al.]. Most probably under conditions
32
33 14 of impaired cellular immunity viral reactivation may occur. If a severe immune
34
35 15 deficiency is present progressive multifocal leukoencephalopathy might develop.
36
37 16 However, it can be speculated that the immune response can help restrain the viral
38
39 17 reproduction and bring the viral infection again to a latent state. If it is assumed that
40
41 18 several reactivation-restriction cycles might be repeated there is a risk that viral
42
43 19 genomic element would be altered. Consequentially, uncontrolled expression of
44
45 20 regulatory elements such as LT might occur. If virions, which would normally trigger
46
47 21 the immune system, do not appear, this could result in a premise for malignant
48
49 22 transformation of the infected cell. This hypothesis is also supported by a recent
50
51 23 investigation of polyomaviruses-induced tumours in mice, where NK cells and gammadelta
52
53 24 T cells mediated resistance to formation of the tumours [Mishra et al.]. Although this
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 investigation did not perform an expression assay in order to show the activity of LT,
2 other researchers have also demonstrated that LT is expressed in brain tumour tissue
3 [Del Valle et al., 2001b].

4 It remains unclear if JCV has a decisive role in the process of brain cell
5 malignant transformation. The current results suggest that JCV might induce or play
6 role in the subsequent stages of malignant transformation.
7 Further data in support of the infectious causes of cancer present a new concept that
8 warrants the need for advanced investigations.

ACKNOWLEDGMENTS

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 Grant support received from the Council for Medical Science at the Medical University
2 of Sofia, Bulgaria (Research project Contract No. 34/2010).

For Peer Review

24 **REFERENCES**

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1 Adriaensen ME, Schaefer-Prokop CM, Stijnen T, Duyndam DA, Zonnenberg BA, Prokop M.
2 2009. Prevalence of subependymal giant cell tumors in patients with tuberous sclerosis
3 and a review of the literature. *Eur J Neurol* 16:691-696.
- 4 Del Valle L, Gordon J, Assimakopoulou M, Enam S, Geddes JF, Varakis JN, Katsetos CD, Croul S,
5 Khalili K. 2001a. Detection of JC virus DNA sequences and expression of the viral
6 regulatory protein, T-antigen, in tumors of the central nervous system. *Cancer Res*
7 61:4287-4293.
- 8 Del Valle L, Gordon J, Assimakopoulou M, Enam S, Geddes JF, Varakis JN, Katsetos CD, Croul S,
9 Khalili K. 2001b. Detection of JC virus DNA sequences and expression of the viral
10 regulatory protein T-antigen in tumors of the central nervous system. *Cancer Res*
11 61:4287-4293.
- 12 Del Valle L, White MK, Khalili K. 2008. Potential Mechanisms of the Human Polyomavirus JC in
13 Neural Oncogenesis. *J Neuropathol Exp Neurol* 67:729-740.
- 14 Ferrer I, Armstrong J, Capellari S, Parchi P, Arzberger T, Bell J, Budka H, Ströbel T, Giaccone G,
15 Rossi G, Bogdanovic N, Fakai P, Schmitt A, Riederers P, Al-Sarraj S, Ravid R,
16 Kretzschmar H. 2007. Effects of formalin fixation, paraffin embedding, and time of
17 storage on DNA preservation in brain tissue: A BrainNet Europe study. *Brain Pathol*
18 17:297-303.
- 19 Gan DD, Khalili K. 2004. Interaction between JCV large T-antigen and beta-catenin. *Oncogene*
20 23:483-490.
- 21 Herbarth B, Meissner H, Westphal M, Wegner M. 1998. Absence of polyomavirus JC in glial
22 brain tumors and glioma-derived cell lines. *Glia* 22:415-420.
- 23 Imperiale MJ, Major EO. 2007. Polyomaviruses. In: Knipe DM, Howley PM, editors. *Fields*
24 *Virology*. 5 ed. Philadelphia: Lippincott Williams & Wilkins. p 2266-2290.
- 25 Kalvatchev ZN, Tsekov ID, Slavov SN, Draganov PI. 2010. Effective Light-Up-on-Extension Real-
26 Time PCR Primer Systems for Rapid Detection of Human Viruses. *LabMedicine* 41:150-
27 155.
- 28 Khalili K, Gordon J, White MK. 2006. The polyomavirus, JCV, and its involvement in human
29 disease. In: Ahsan N, editor. *Polyomaviruses and Human Diseases*. New York: Springer.
30 p 274-287.
- 31 Lam WY, Leung BW, Chu IM, Chan AC, Ng HK, Chan PK. 2010. Survey for the presence of BK, JC,
32 KI, WU and Merkel cell polyomaviruses in human brain tissues. *J Clin Virol* 48:11-14.
- 33 Lassak A, Del Valle L, Peruzzi F, Wang JY, Enam S, Croul S, Khalili K, Reiss K. 2002. Insulin
34 receptor substrate 1 translocation to the nucleus by the human JC virus T-antigen. *J*
35 *Biol Chem* 277:17231-17238.
- 36 MacKenzie J, Wilson KS, Perry J, Gallagher A, Jarrett RF. 2003. Association between simian virus
37 40 DNA and lymphoma in the United Kingdom. *J Natl Cancer Inst* 95:1001-1003.
- 38 Miller NR, McKeever PE, London W, Padgett BL, Walker DL, Wallen WC. 1984. Brain tumors of
39 owl monkeys inoculated with JC virus contain the JC virus genome. *J Virol* 49:848-856.
- 40 Mishra R, Chen AT, Welsh RM, Szomolanyi-Tsuda E. 2010. NK Cells and gammadelta T Cells
41 Mediate Resistance to Polyomavirus-Induced Tumors. *PLoS Pathog* 6:e1000924.
- 42 Nevins JR. 2007. Cell Transformation by Viruses. In: Knipe DM, Howley PM, editors. *Fields*
43 *Virology*. 5 ed. Philadelphia: Lippincott Williams & Wilkins. p 213.
- 44 Richardson EP. 1961. Progressive multifocal leukoencephalopathy. *N Engl J Med* 265:815-823.
- 45 Rollison DE, Utaipat U, Ryschkewitsch C, Hou J, Goldthwaite P, Daniel R, Helzlsouer KJ, Burger
46 PC, Shah KV, Major EO. 2005. Investigation of human brain tumors for the presence of
47 polyomavirus genome sequences by two independent laboratories. *Int J Cancer*
48 113:769-774.

- 1
2
3
4
5
6 1 White MK, Khalili K. 2004. Signaling pathways and polyomavirus oncoproteins: Importance in
7 2 malignant transformation. *Gene Ther Mol Biol* 8:19-30.
- 8 3 Zhao J, Punj V, Matta H, Mazzacurati L, Schamus S, Yang Y, Yang T, Hong Y, Chaudhary PM.
9 4 2007. K13 blocks KSHV lytic replication and deregulates vIL6 and hIL6 expression: a
10 5 model of lytic replication induced clonal selection in viral oncogenesis. *PLoS One*
11 6 2:e1067.
- 12 7 zur Hausen H. 2006. *Infections causing human cancer*. Weinheim: WILEY-VCH Verlag GmbH &
13 8 Co.
- 14 9 zur Hausen H. 2008. Novel human polyomaviruses-re-emergence of a well known virus family
15 10 as possible human carcinogens. *Int J Cancer* 123:247-250.
- 16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review