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Molecular Analysis of JC Polyomavirus Genotypes Circulating Among Tribal Populations of North-Eastern West Bengal, India

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Abstract

There is a resurgence of interest in the study of occurrence, genotype and pathogenic associations of human Polyomaviruses in recent years. In the present study, we have ascertained the presence of human Polyomavirus JC (JCV) in the urine and peripheral blood leukocytes of tribal populations, for the first time in the North-Eastern part of West Bengal State of India. We have also characterized the prevalent genotypes of the non-coding control regions (NCCRs) of these natural isolates. The result suggests a high incidence of JCV reactivation in the populations assayed. Approximately 25% of the non-immunocompromized tribal men and women, tested positive based on polymerase chain reaction (PCR) analysis, and these results were further confirmed by sequencing of PCR products. Pairwise sequence comparison and alignment of the NCCR sequence of these Indian strains appeared to be comparable and related to the archetypal JCV (CY) and the Tibetan LH3 strains, with some alterations in few key positions. The sequence analyses were done with regard to transcription factor binding to DNA sequence elements of endemic JCV NCCRs.

Key words: JC Polyomavirus, NCCR, North-East India, TFBS

Introduction

JC virus (JCV), a member of the *Polyomaviridae* family, was first isolated from brain tissue of a 38 year old man with initials – JC, who was suffering from Hodgkin's disease for a quite a long time (Padgett *et al*., 1971). JC virus has simple genome containing a single molecule of covalently closed, circular double-stranded DNA of about 5000 base pair in length (Frisque *et al.*, 1984). The viral genome comprises early and late coding regions that are controlled by a common Non-Coding Control Region (NCCR), which lies between them. The early proximal side of the NCCR is highly conserved and contains the origin of viral DNA replication. The late proximal side of the NCCR contains the repetitive enhancer elements and undergoes rearrangements that account for most of the differences between different strains of the same virus.

Transcriptional regulation of the JCV early and late promoters during the viral lytic cycle is a complicated event that requires participation of both viral key proteins and cellular transcription factors. Several transcription factors are implicated in the regulation of JCV gene expression which include NF-κB (Ranganathan and Khalili, 1993), NFAT4 (Wollebo *et al*., 2012), upstream Target or up-TAR (Chowdhury *et al*., 1993), Tst-1 (Wegner *et al*., 1993), Sp-1 (Henson *et al*., 1992), Spi-B (Marshall *et al*., 2010), GBP-i (Raj and Khalili, 1994), Y-box binding protein 1 (YB-1) and Purα (Chen and Khalili, 1995), Nuclear factor 1 or NF-1 (Amemiya *et al.* 1989), CREB/ATF-1 (Lonze and Guinty, 2002), Activator Protein 1 (AP-1) family members (Sadowska *et al*., 2003), p53 (Ariza *et al*., 1994), Early growth response-1 protein or Egr-1 (Romagnoli *et al*., 2008), Bcl-2-associated athano gene-1 or BAG-1 (Devireddy *et al*., 2000) and CAAT/enhancer binding protein beta or C/EBPβ (Romagnoli *et al*., 2009).

JC polyomavirus is widespread in the human population and causes a rare fatal brain infection known as Progressive Multifocal Leucoencephalopathy (PML). PML occurs mainly in a limited number of individuals with suppressed immune system, especially in those with Human Immunodeficiency Virus (HIV) infection/ AIDS (Hou and Major, 2000; Khalili *et al*., 2006) and involves productive infection in both oligodendrocytes and astrocytes. However, there are reports where

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researchers have shown that JCV can even induce PML in non-immunocompromised individuals without AIDS, such as patients receiving monoclonal antibody natalizumab (Langer-Gould *et al*., 2009). JCV exists in ten or more geographically based genotypes identified in the United States, Africa, Europe and Asia (Agostini *et al*., 1997; Sugimoto *et al*., 2002). There appear to be several Asian subtypes and the Indian subtype has been designated as Type 2D (Cui *et al*., 2004).

In this study, we have screened urine and blood samples from tribal/ethnic human population of north-eastern part of West Bengal state of India to record for the first time the JCV reactivation status and to ascertain the prevalent nature of endemic viral NCCRs. Specific oligonucleotide primers were used to amplify the JCV NCCRs by PCR and then sequenced to analyze their genetic architectures. We have recorded about 25% of the subjects to be positive for JCV NCCRs. Analyses of predicted transcription factor binding sites (TFBS) revealed additional putative promoter elements in endemic NCCRs capable of binding to a diverse set of human/vertebrate transcription factors. Further *in vivo* and *in vitro* experiments are expected to substantiate these findings.

Experimental

Materials and Methods

Sample collection. The study methodology was approved by our institutional Human Ethical Committee. A total of 77 samples were collected from the tribal, non-immunosuppressed populations of the north-eastern part of West Bengal State of India with their prior informed consent. The samples included either urine or blood specimens from each individual. The collected samples were from two tribal groups of North Bengal – Oraon and Mundas of Kiran Chandra tea garden, Naxalbari, Darjeeling district (26°41'N Lat; 88°16'E Long) and Rabha or Rava population of Poro Busty, Tufanganj, Coochbehar district (26°18'N Lat; 89°39'E Long). The urine samples were collected in 1.5 ml eppendorf tubes and about 1 ml of blood samples were collected in vials containing EDTA. All the samples were brought to the laboratory and stored at –20°C refrigerator prior to DNA isolation.

DNA Isolation. DNA isolation from urine and blood samples was done using the High Pure Viral Nucleic Acid Kit (Roche Diagnostic GmbH, Germany) as per the manufacturer's instruction. In brief, 200 µl of freshly prepared working solution (carrier RNA-supplemented binding buffer) and 50 µl of Proteinase K solution was added to 200 µl of sample and incubated at 72°C for 10 minutes. Then 100 µl of binding buffer was added and centrifuged at 8,000 × g for 1min. The flowthrough liquid was discarded and 500 µl of inhibitor removal buffer was added and centrifuged again at 8,000 × g for 1 min. After discarding the flow through, samples were washed twice with 450 µl wash buffer. The last step included addition of 50 µl elution buffer followed by centrifugation at 8,000 × g for 1 min. The isolated DNA samples were stored in –20°C refrigerator until used for PCR amplification.

Standard Viral DNA. Plasmid pMITC-BSMKS containing the whole JCV genome (JCV Mad-1 strain) was a gift from Richard J. Frisque, Department of Biochemistry, Microbiology, Molecular & Cell Biology, Pennsylvania State University, USA. The plasmid pGMTW3JCV containing the whole genome of JCV (JCV Taiwan-3 strain) was gifted by Deching Chang, National Chung Cheng University, Taiwan.

PCR amplification and Electrophoretic analysis of endemic JCV NCCRs. Two oligonucleotide primers namely SDJ1 (5'-CCCTATTCAGCACTTTGTCC-3') and SDJ2 (5'-CAAACCACTGTGTCTCTGTC-3') were used for the amplification of the viral control region DNA. A final volume 50 µl PCR reaction mixture contained 20 pM of each primer, 200 µM dNTPs (NEB, USA), 10X standard Taq buffer containing $MgCl_2$ (NEB, USA) and Taq Polymerase enzyme (NEB, USA). Twenty microlitres of isolated DNA was used for each PCR amplification reaction. Amplifications were performed in an Applied Biosystems Thermal Cycler (Model: 2720 Thermal Cycler, Life Technologies, USA) programmed for initial heating at 94°C for 5 min, 25 cycles at 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min. A final extension was performed at 72°C for 5 min. The cloned JCV genome plasmids pMITC-BSMKS or pGMTW3JCV were used as positive control in each reaction and sterile distilled water was used as negative control. PCR products were run on 1% agarose gel containing 0.5 µg/ml of Ethidium bromide at 80 V for 1 hour approximately in a Bench Top Lab Systems (Model: BT-MS-300, Taiwan) electrophoresis apparatus and then visualized in a UV transilluminator (Spectroline BI-O-Vision UV/White Light Transilluminator, NY, USA).

Sequence Analysis. Sequencing of the JC virus NCCR PCR product from each positive sample was done at least twice by dye-dideoxy automated chain termination method (Biolinkk, New Delhi, India) and only six of them were deposited in NCBI public domain for GenBank accession. All the remaining NCCR sequences were similar to the ones deposited for GenBank accession. Submission IDs are as follows: NB1 (JX294575), NB2 (JX534216), NB3 (JX534217), NB4 (JX534218), NB5 (JX534219) and NB6 (JX534220). Nucleotide sequences of the endemic JCV NCCRs were aligned with the known NCCR sequences of JCV

strains CY, LH3, Tai3, IN-8 and Mad-1 using DiAlign alignment program of Genomatix suite v2.5 GmbH (Cartharius *et al*., 2005) to compare pairwise similarities (relative to the maximum similarity) among these sequences. All NCCR sequences (T-antigen coding start site to Agnogene coding start site) were again aligned in ClustalX ver. 2.0.3 (Thompson *et al*., 1997) using default parameters, curated in BioEdit ver. 7.0.9.0 (Hall, 1999) to generate an alignment showing deletion/mutation and common transcription factor binding sites derived from experimental data of other research groups. The NCCR sequences of NB3, 4 and 5 being identical only NB3 was included in the multiple sequence alignment. The MatInspector program of Genomatix Software suite v2.5 GmbH (Cartharius *et al*., 2005) was used to search for the predicted transcription factor binding sites (TFBS) in the aligned portion of endemic using a large library of weight matrices based on general core promoter and vertebrate promoter matrix families.

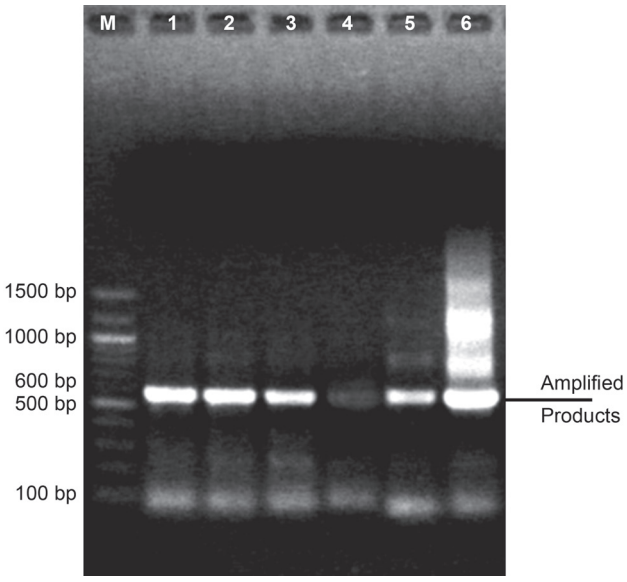

Fig. 1. Agarose gel showing amplified JCV Non Coding Control Region in tribal populations of North-East India.

Lane 1: 100 bp ladder; Lane 2: JCV NB1 NCCR; Lane 3: JCV NB2 NCCR; Lane 4: JCV NB3 NCCR; Lane 5: JCV NB4 NCCR; Lane 6: JCV NB5 NCCR; Lane 7: JCV NB6 NCCR.

Results

PCR amplification and Electrophoretic analyses. PCR amplification of JCV specific NCCR from all urine as well as from blood samples was done to check the prevalence of the virus in the North Bengal region of India. Seventy-seven samples of urine and blood were collected from the tribal, non immunosuppressed populations of this region. Twenty-four samples were from Kiran Chandra tea garden, Naxalbari, Darjeeling district and 53 samples were collected from Poro busty, Tufangunj, Coochbehar district of West Bengal. The two primers SDJ1 and SDJ2 specific for NCCR region amplified a product of approximately 586 bp of DNA. After PCR amplification, products were analysed by agarose gel electrophoresis (Fig. 1). Seven out of 24 assayable samples from the Oraon and Munda tribal groups in Kiran Chandra tea garden were found to be JCV positive showing an overall incidence of 29.17%. In Poro busty, Tufangunj, having mostly Rabha tribal population, 12 out of 53 samples supported positive amplification of the JCV NCCR region having an overall incidence of 22.64% (Table I). Thus the overall incidence of JCV control region DNA in urine and blood of non-immunosuppressed general tribal population was found to be 24.67% (19 out of 77 individuals) in this north-eastern part of India.

Pairwise Comparison of endemic JCV NCCRs with strains reported from North-East and South-East Asia. NCCR region is the variable segment of the viral genome except the early proximal part of the sequence which is highly conserved. In this study, DiAlign program of the Genomatix software suite (Table II) was used to check only the similarity of NCCR sequences among the six endemic isolates of JCV namely NB1, NB2, NB3, NB4, NB5 (from Oraon and Munda Tribes of Kiranchandra Tea Garden, Darjeeling District) and NB6 (from Rabha Tribes of Poro Busty, Coochbehar District) of this region and also with different strains from other regions such as archetypal CY, LH3, Tai3, IN-8 and

Table I
Summary of results obtained in different tribal groups showing incidences of JCV Non-Coding Control Region DNA detection

Location	Tribal group	Sex	Median age	Positive	Negative	Total
Kiran Chandra Tea Garden, Naxalbari, Darjeeling District [26°41'N Lat; 88 °16'E Long; Elev. 772 ft msl]	Oraon and Munda	F = 20 M = 4	F = 26 M = 42	F = 3 M = 4 7 (29.17%)	17	24
Poro Busty, Tufanganj, Coochbehar District [26°18'N Lat; 89 °39'E Long; Elev. 134 ft msl]	Rabha	F = 20 M = 33	F = 59 M = 40	F = 01 M = 11 12 (22.64%)	41	53
Total		F = 40 M = 37	F = 40 M = 26.5	19 (24.67%)	58	77

Table II
Pairwise similarities (relative to the maximum similarity) based on Non-Coding Control Regions (NCCRs) of endemic JCV strains NB1, 2, 3, 4, 5, 6 in comparison to archetype CY, Mad1, LH3, Tai3 and IN8 NCCRs using DiAlign alignment software of Genomatix suite v2.5 GmbH. The number of identical nucleic acids (% of shorter sequence) and maximum values (underlined) are shown

	NB2 JX534216 (372 bp)	NB3 JX534217 (372 bp)	NB4 JX534218 (372 bp)	NB5 JX534219 (372 bp)	NB6 JX534220 (383 bp)	CY AB038249 (384 bp)	LH3 AB262411 (382 bp)	Tai3 U61771 (374 bp)	IN8 AB126992 (384 bp)	Mad1 NC_001699 (393 bp)
NB1|JX294575 (372 bp)	0.943 98%	0.950 98%	0.950 98%	0.950 98%	0.896 94%	0.920 95%	0.927 97%	0.886 94%	0.920 95%	0.721 79%
NB2|JX534216 (372 bp)		0.962 99%	0.962 99%	0.962 99%	0.908 96%	0.921 97%	0.938 98%	0.889 93%	0.915 97%	0.683 80%
NB3|JX534217 (372 bp)			0.976 **100%**	0.976 **100%**	0.909 96%	0.936 97%	0.954 98%	0.904 94%	0.923 97%	0.684 78%
NB4|JX534218 (372 bp)				0.976 **100%**	0.909 96%	0.936 97%	0.954 98%	0.904 94%	0.923 97%	0.684 78%
NB5|JX534219 (372 bp)					0.909 96%	0.936 97%	0.954 98%	0.904 94%	0.923 97%	0.684 78%
NB6|JX534220 (383 bp)						0.962 98%	0.935 95%	0.912 97%	0.954 97%	0.676 78%
CY|AB038249 (384 bp)							0.981 97%	0.965 98%	**1.000** 99%	0.709 79%
LH3|AB262411 (382 bp)								0.961 98%	0.967 97%	0.695 79%
Tai3|U61771 (374 bp)									0.950 98%	0.667 74%
IN8|AB126992 (384 bp)										0.722 79%

* The similarity value 1.000 marks only the two most similar sequences; it does not necessarily mean that these sequences are identical.

Mad-1 strains. It was evident from the study that the NCCR sequences of NB3, 4 and 5 were identical. All the NCCR sequences (NB1-5) except the NB6 paired closely with each other (98–100% similar). Pairwise sequence comparison with other strains revealed that the NCCR sequence of NB1, 2, 3, 4 and 5 were almost similar to the Tibetan LH3 (97–98% similar) NCCR sequence and the NB6 NCCR sequence seemed almost identical with CY strain (98% similarity). These NCCR sequences including that of NB6 were divergent from Tai3, IN-8 and as expected from Mad-1 control regions.

Analyses of NCCRs for Transcription Factor Binding Sites (TFBS). Multiple sequence alignment of endemic JCV NCCRs and other strains reported earlier revealed a 10 nucleotide (169–178) and a di-nucleotide (454–455) deletions in NB1, 2, and 3 (and also in NB5 and NB6) but not in NB6 (Fig. 2). Point mutations in seven different sites within the NCCR of the endemic strains recorded are at 4, 13, 26, 27, 69, 226 and 452 nucleotide positions of the sequence alignment (Fig. 2).

Several transactivating factors have been experimentally shown to bind to JCV NCCR either individually or cooperatively with other cellular factors and/or virus encoded proteins. The binding motifs of NF-κB, NFAT4, upstream Target or up-TAR, Tst-1, Sp-1, Spi-B, GBP-i, Y-box binding protein 1, Purα, Nuclear factor 1 or NF-1, CREB/ATF-1, Activator Protein 1 (AP-1) family members, p53, Early growth response-1 protein or Egr-1, Bcl-2-associated athano gene-1 or BAG-1 and CAAT/enhancer binding protein beta or C/EBPβ are shown on the aligned sequences (Fig. 2). In an effort to check further the binding of putative transcription factors to this control region *in silico* we have searched a well defined general core and vertebrate transcription factor matrix families in MatInspector program of the Genomatix software suite (Cartharius *et al*., 2005). Supplementary Table I shows putative binding sites of transcription factors in an abridged form. Matches for transcription factors that are reported to be active in cells/tissues such as antibody-producing cells, antigen-presenting cells, bone marrow cells, hematopoietic and immune system, leukocytes, lymphocytes, monocytes, myeloid cells, phagocytes, brain, CNS, endocrine system, kidney, nervous system, urinogenital system are retained in the table list.

Discussion

We report here, for the first time, the incidence and sequence characterization of human polyomavirus JCV non-coding control/regulatory regions or NCCRs,

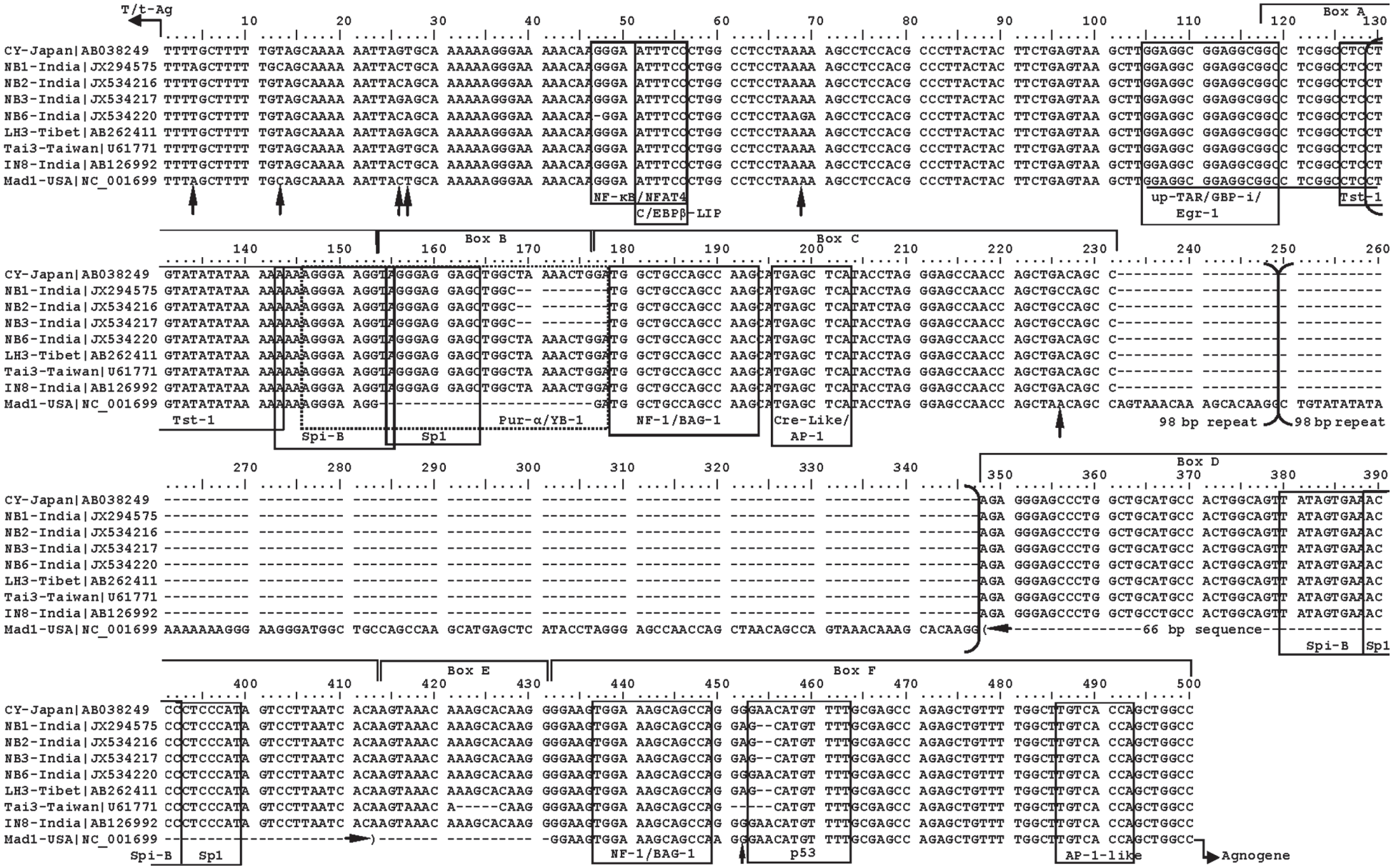

Fig. 2. Non-Coding Control Regions (NCCRs) of JCV endemic strains NB1, NB2, NB3 and NB6 were aligned against the CY, LH3, Tai3, IN-8 and Mad-1 strains, showing the common binding sites of known and reported transcription factors in square blocks. The 98 bp repeats of Mad-1 and 66 bp sequence of CY archetype strains are shown by large brackets and dotted arrows respectively. The NCCR is divided into six regions according to CY strain: Box A (36 bp), B (23 bp), C (55 bp), D (66 bp), E (18 bp) and F (69 bp). Nucleotide deletion (s) are marked by a dash (–) and nucleotide variation sites among endemic strains are marked with vertical arrows.

amplified directly from urine and blood samples of non-immunocompromized healthy tribal groups from north-eastern parts of West Bengal State of India. The objectives of the current investigation were (1) to document the prevalence or reactivation status of human polyomavirus JCV in non-immunosuppressed Indian tribal population, (2) to compare and contrast intergenic NCCR motifs of JCV variants found in healthy subjects with respect to transcription/transactivating factor binding and (3) to predict putative transcription factor binding sites within the endemic NCCR variants.

Initial infection with human polyomavirus seems to occur through the oral/respiratory route in childhood, as evidenced by detection of JCV DNA in infant tonsillar tissues, after which they remain in latent state in organs like kidneys, hematopoietic precursor cells, B-lymphocytes etc. till reactivation (Boldorini *et al*., 2003; Dorries *et al*., 1994; Monaco *et al*., 1998). Reactivation of both BKV and JCV infection with resulting urinary excretion of viruses readily occurs during conditions of immune suppression or immunocompromization. Conventional PCR has been used to detect and characterize both BKV and JCV DNA in urine of different patient groups, from different human tissues and from urine of pregnant women (Arthur *et al*., 1989; Markowitz *et al*., 1991). Regulatory regions of both BKV and JCV have been amplified and characterized from urine of bone marrow and renal transplant patients, HIV positive or negative individuals, patients with various autoimmune diseases. Full-length JCV genomes or DNA sequences have also been isolated and characterized from normal brain tissue, new born infants, immunocompetent older individuals and peripheral blood leucocytes of immunocompetent individuals (Kitamura *et al*., 1990; Elsner and Dorries, 1992, Dorries *et al*., 1994; Baksh *et al*., 2001).

Detection of JCV nucleic acid in urine (Tan *et al*., 2009; Husseiny *et al*., 2010), brain autopsy and CNS tissues (Delbue *et al*., 2008); serum/peripheral blood leukocytes (PBL) (Gu *et al*., 2003; Tan *et al*., 2009; Husseiny *et al*., 2010), bone marrow aspirates (Tan *et al*., 2009) of normal or non-immunocompromized human subjects, in different age groups, have been reported from many regions of the world. The JCV DNA occurrence in all these studies ranged from 0 to 20% based on variable sample sizes. Incidences of JCV viruria in different tribal populations showed variations with respect to the groups studied: 56 to 66% in Native Americans (Agostini *et al*., 1997), 48 to 67% in Myanmar tribals (Saruwatari *et al*., 2002) and 20 to 22% in African tribals (Chima *et al*., 1998). We have recorded a relatively high incidence of JCV DNA detection in non-immunosuppressed and healthy human tribal groups in our study (Table I). Out of 77 assayable urine and blood samples 19 (~25 %) were scored positive by gel electrophoresis and PCR product sequencing, and 58 (~75%) samples were scored negative based on the above mentioned criteria.

The non-coding control regions (NCCRs) of JC virus vary considerably among different natural isolates. Archetype like DNA, which lacks sequence repeats in the regulatory region and contains additional sequences, are generally not found in laboratory strains, but has been isolated by molecular cloning from several sources. Rearrangement of NCCRs occurs during passage in cell culture more readily. During reactivation in pregnancy, the shed viruses show archetype-like sequence rearrangement in their NCCRs (Markowitz *et al*., 1991; Markowitz *et al*., 1993). Viral strains with divergent NCCRs may have different tissue tropism and also aberrant potential for host cell transformation (Sundsfjord *et al*., 1994). It has been postulated that the rearrangement in the NCCRs may change the biological properties of polyomaviruses in the due course of a persistent infection, such as the ability to infect different target cells. Reactivation of BKV and/or JCV infection, with the resultant urinary excretion of virus, may occur in healthy individuals but occurs more frequently under conditions of immunosuppression, especially when T-cell functions are depressed (Chesters *et al*., 1983; Gardner *et al*., 1984). JCV infection has been demostrated in B-lymphocytes (Dorries *et al*., 1994) and JCV specific $CD4^+$ T-lymphocytes have been reported in healthy individuals (Gasnault *et al*., 2003). Healthy individuals have also been shown to harbour JCV-specific $CD8^+$ T-Lymphocytes, principally against $VP1_{p36}$ epitopes, in their peripheral blood mononuclear cells (Du Pasquier *et al*., 2004). Fragments of JCV DNA, but not proteins, have been detected in multiple regions of non-immunocompromized and non-PML normal brains (Perez-Liz *et al*., 2008). The authors have also hypothesized that JCV could spread within normal brain tissue through blood or infected immune cells like B-lymphocytes and remain as integrated or episomal forms within the oligodendrocytes and astrocytes till its initiation of lytic life cycle during immune impairment. Therefore this model raises the possibility of human brain being a secondary site of latency for JCV.

We have investigated the binding sites of known and reported transcription factors/transactivators in the endemic JCV NCCRs and also used computer software to search for additional transcription factors having potential to bind to this region of JCV genome. Two prominent features within the endemic JCV NCCR Box B are the absence, unlike JCV Mad-1, of full-length Purα/YB-1 binding pentanucleotide (5'-AGGGAAGGGA-3') (Chen and Khalili, 1995) and the presence of Sp1 binding site (GA Box) (5'-AGG-GAGGAGC-3') (Henson *et al*., 1992) in the same

Supplementary

Table I
Predicted transcription factor binding sites (TFBS) in the NCCRs of endemic JCV strains NB1, NB2, NB3, NB6 derived using MatInspector Release Professional 8.0.5, March 2011 of Genomatix Software suite v2.5 GmbH. Selected TFBS matches are shown as alphabetically arranged vertebrate matrix families. TFBS search involved both general core promoters (0.75/Optimized) and vertebrate (0.75/Optimized) promoter element groups of MatInspector matrix family library version 8.4 (June 2011).

JCV strains (NCCRs)	Matrix Family	Detailed Family Information	Tissue	Optimized Similarity Scores	TF Binding Sequence (Core Sequence in capital)
NB1, 2, 3, 6	O$VTBP	Vertebrate TATA binding protein factor	Ubiquitous	0.90	131-gtataTATAaaaaaaag-147
NB1, 2, 3, 6	V$AP4R	AP4 and related proteins	Ubiquitous	0.92	214-gctggcAGCTggttggc-230 (–) and 486-tggccAGCTggtgaca-500 (–)
NB1, 2, 3, 6	V$BRNF	Brn POU domain factors	Brain, CNS, Endocrines System, Neuroglia, Neuron	0.89	14-tttgctgTAATtttttgct-32 (–)
NB1, 2, 3, 6	V$CAAT	CCAAT binding factors	Ubiquitous	0.81	185-ccagCCAAgcatgag-199 and 211-ggagCCAAccagctg-225
NB1, 2, 3, 6	V$E2FF	E2F-myc activator/cell cycle regulator	Ubiquitous	0.84–0.85	458-ctctgGCTCgcaaaaca-474 (–) and 456-ctggctcgcAAAAcatg-472 (–)
NB1, 2, 3, 6	V$EGRF	EGR/nerve growth factor induced protein C & related factors	Brain, CNS, Endocrine System Kidney, Nervous System, Urinoogenital System	0.88	105-ggaggcggAGGCggcct-121 and 387-gactatGGGAggggttt-403 (–)
NB1, 2, 3, 6	V$ETSF	Human and murine ETS1 factors	Hematopoietic and Immune System, Leukocytes, Lymphocytes, Monocytes	0.88–0.96	424gcacaaggGGAAgtggaaagc-444 29-caaaaaagGGAAaaacaaggg-49 140-aaaaaaagGGAAggtagggag-160
NB1, 2, 3, 6	V$FKHD	Fork head domain factors	APCs, Blood Cells, Immune System, Leukocytes, Lymphocytes	0.89	132-tatataTAAAaaaaagg-148 and 410-cacaagTAAAcaaagca-426
NB2	V$GATA	GATA binding factors	Blood and Bone Marrow Cells Hematopoietic System, Immune System, Leukocytes, Lymphocytes	0.90	199-cctaGATAtgagc-211
NB1, 2, 3, 6	V$HICF	Krueppel-like C2H2 zinc finger factors hypermethylated in cancer	Erythropoiesis, control of cell proliferation, monocyte activation	0.88	180-ggcTGCCagccaa-192 and 221-agcTGCCagccag-349
NB1, 2, 3, 6	V$HOMF	Homeodomain transcription factors	Blood and Bone Marrow Cells, Endocrine System, Hemato poietic System, Immune System, Leukocytes, Lymphocytes	0.88-0.95	13-cagcaaaaAATTactgcaa-31 and 14-tttgcagtAATTttttgct-32 (–)
NB1, 2, 3	V$IKRS	Ikaros zinc finger family	Antibody-Producing Cells, Blood Cells, Hematopoietic System, Immune System, Leukocytes, Lymphocytes	0.84	43-acaagGGAAtttc-55
NB1, 2, 3, 6	V$IRFF	Interferon regulatory factors	Antibody-Producing Cells, APCs, Blood, Bone Marrow Cells, Hematopoietic and Immune System, Leukocytes, Lymphocytes, Monocytes Myeloid Cells, Phagocytes	0.85-0.87	376-cagttatagtGAAAcccctcc-396; 429-agggGAAGtggaaagcagcca-449; and 28-gcaaaaaaggGAAAaacaagg-48
NB1, 2, 3, 6	V$MAZF	Myc associated zinc fingers	Blood Cells, Immune System, Leukocytes	0.90	387-atggGAGGggttt-399 (–)
NB1, 2, 3, 6	V$MYBL	Cellular and viral myb-like transcriptional regulators	Blood and Bone Marrow Cells, Hematopoietic and Immune System, Leukocytes Lymphocytes	0.96	370-ctaTAACtgccagtg-384 (–)
NB1, 2, 3, 6	V$MZF1	Myeloid zinc finger 1 factors	Blood and Bone Marrow Cells, Hematopoietic and Immune System, Leukocytes Myeloid Cells	0.99	428-aaGGGGaagtg-438

Table I continued

JCV strains (NCCRs)	Matrix Family	Detailed Family Information	Tissue	Optimized Similarity Scores	TF Binding Sequence (Core Sequence in capital)
NB1, 2, 3, 6	V$NEUR	NeuroD, Beta2, HLH domain	Antibody-Producing Cells, Blood and Bone Marrow Cells, Brain, CNS, Hematopoietic System, Immune System, Leukocytes, Lymphocytes, Nervous System, Neuroglia, Neurons	0.95	216-caaccaGCTGcca-228; 488-tcacCAGCtggcc-500 and 489-ggcCAGCtggtg-500 (–)
NB1, 2, 3, 6	V$NF1F	Nuclear factor 1	Brain, Central Nervous System, Digestive System, Liver, Nervous System	0.81-0.92	161-gagctggctggctGCCAgcca-191 (+); 212-gagccaaccagctGCCAgcca-348; 433-ctcCTGGctgctttccacttc-453 (–); 477-gttTTGGcttgtcaccagctg-497; 161-tggcTGGCagccagccagctc-191 (–); 165-tggcTGGCtgccagccaagca-195 (+); 433-gaagTGGAaagcagccaggag-453; 171-aaactggatggctGCCAgcca-191 (NB6); 171-tggCTGGcagccatccagttt-191 (–) (NB6); 175-tggTTGGctggcagccatcca-195 (–) (NB6); 175-tggaTGGCtgccagccaacca-195 (NB6); 355-gcccTGGCtgcatgccactgg-375; 355-ccagTGGCatgcagccagggc-375 (–); 477-cagcTGGTgacaagccaaaac-497 (–)
NB1, 2, 3, 6	V$NFKB	Nuclear factor kappa B/c-rel	Blood and Bone Marrow Cells, Hematopoietic and Immune System, Leukocytes, Myeloid Cells, Phagocytes	0.87	45-aagggaatTTCCctg-59 and 45-cagggaaaTTCCctt-59 (–)
NB6	V$NOLF	Neuron-specific olfactory factor	Antibody-Producing Cells, Blood, Bone Marrow Cells, Hematopoietic, Immune, Nervous System, Leukocytes, Lymphocytes, Neurons	0.88	460-acatgtTCCCctggctgctttcc-438 (–)
NB2, 3, 6	V$P53F	p53 tumor suppressor	Ubiquitous	0.92	441-aagcagccaggggaaCATGtttt-463 (NB6) and 452-ctctggctcgcaaaaCATGttcc-474 (–) (NB6)
NB6	V$PAX3	PAX-3 binding sites	Embryonic Structures Muscle, Skeletal Muscles	0.93	187-gagctCATGgttggctggc-202 (–)
NB6	V$PAX5	PAX-2/5/8 binding sites	Antibody-Producing Cells, Blood Cells, Endocrine System, Hematopoietic System, Immune System, Kidney, Leukocytes, Lymphocytes	0.79	177-tatgagCTCAtggttggctggcagccatc-205 (–)
NB2, 6	V$PAXH	PAX homeodomain binding sites	Brain, CNS, Endocrine System, Nervous System Neurons	0.99	16-caaaaaATTAcagca-30
NB1, 2, 3, 6	V$PLAG	Pleomorphic adenoma gene	Brain, CNS, Nervous System	0.87	223-ccaggGCTCcctctggctggcag-361 and 348-agaggGAGCcctggctgcatgcc-370
NB1, 2, 3, 6	V$PURA	Pur-alpha binds both single-stranded and douple-stranded DNA in a sequence-specific manner	Brain, CNS, Nervous System, Neuroglia, Neurons	0.97	105-ggAGGCggaggcg-117
NB1, 2, 3, 6	V$RUSH	SWI/SNF related nucleo-phosphoproteins with a RING finger DNA binding motif	Ubiquitous	0.98	410-gtttACTTgtg-420 (–)
NB1, 2, 3, 6	V$SALL	Spalt-like transcription factors	Embryonic Structures, Kidney, Urogenital System	0.96	135-atATAAaaaaaag-147
NB1, 2, 3, 6	V$SP1F	GC-Box factors SP1/GC	Ubiquitous	0.88	151-aggtagGGAGgagctgg-167 386-actatgGGAGgggtttc-402 (–)
NB6	V$TALE	TALE homeodomain class recognizing TG motifs	Bone Marrow Cells, CNS, Embryonic Structures, Hema-topoietic System, Myeloid Cells, Nervous System, Neurons	0.95	219-ctctggcTGTCagctgg-351 (–) 217-aaccagctGACAgccag-349

Table I continued

JCV strains (NCCRs)	Matrix Family	Detailed Family Information	Tissue	Optimized Similarity Scores	TF Binding Sequence (Core Sequence in capital)
NB6	V$XBBF	X-box binding factors	Antibody-Producing Cells, Blood Cells, Immune System, Leukocytes, Lymphocytes	0.90	441-aagcagccaggGGAAcatg-459
NB6	V$YY1F	Activator/repressor binding to transcription initiation site	Embryonic Structures	0.82	166-ggcagCCATccagttttagcc-186 (–)
NB1, 2, 3, 6	V$ZF02	C2H2 zinc finger transcription factors 2	Blood Cells, Immune System Leukocytes, Lymphocytes	0.87	107-ggaggccgaggCCGCctccgcct-129 (–)

JCV NB1, NB2, NB3, NB6 showed 99, 140, 144, and 155 total matches respectively. JCV NB3, 5 and 6 being identical, only NB3 was included in the search. Matrix similarity column refers to an optimized similarity match above 0.80 score while a perfect match to the matrix is marked by a maximum score of 1.00. All matches below 0.80 score were excluded in the table. A range of scores for discrete sequences are shown where appropriate. Start and end positions of TF binding sites are numbered according to the aligned NCCR sequences as shown in Figure 1. Matching sequences found in negative strands are indicated as (–); however sequences are numbered along the plus strands. Matching sequences found only in NB6 are indicated within parentheses at the end of the sequences.

region. These features are also exhibited by the archetypal CY, Tibetan LH3, Taiwanese Tai3 and the Central-North Indian IN-8 strains (Fig. 2). In the light of these findings (Chen and Khalili, 1995) the significance of partial Purα/YB-1 binding sites within the NCCRs of the endemic circulating JCV vis-a-vis the level of viral T antigen expression within the tissues needs to be validated through cell culture-based gene expression studies. The terminal two nucleotide deletions in the Purα/YB-1 binding pentanucleotide and the concomitant presence of Sp1 binding GA-Box needs to be investigated with respect to glial cell specific gene expression studies. Cellular tumour suppressor protein p53 is reported to bind to JCV large T antigen to repress viral replication (Staib *et al*., 1996) and/or directly to and regulate JCV promoter (Ariza *et al*., 1994). We have found a di-nucleotide deletion within this p53 binding site of endemic JCV strains from Oraon/Munda group but not from the Rabha group (NB6) (Fig. 2). Implications of this deletion in the NCCRs of JCV NB1 to NB5 need to be evaluated in *in vivo* studies. The predicted TFBS depicted putative binding sites for transcription factors corresponding to thirty-three matrix families (Supplementary Table I). We retained the putative TFBS matches which are reported to be active in cells/tissues such as antibody-producing cells, antigen-presenting cells, bone marrow cells, hematopoietic and immune system, leukocytes, lymphocytes, monocytes, myeloid cells, phagocytes, brain, CNS, endocrine system, kidney, nervous system, urinogenital system. Although the TFBS were common to NB1 to NB6, on a closer look we can see few transcription factors (Neuron-specific Olfactory factor or NOLF, p53 tumor suppressor, TALE homeodomain class recognizing TG motifs or TALE, X-box binding factor or XBBF and Activator/repressor binding to transcription initiation site or YY1F) matching exclusively to either positive (+) or negative (–) strands of NB6 NCCR owing to its sequence differences with that of other endemic NCCRs. While the implications of these finding cannot be guessed at this juncture, the list is likely to provide a hint for a detailed *in vivo* binding studies to validate hypotheses regarding tissue-specific promoter activities in JCV.

In summary, we have reported for the first time the NCCR architecture of the endemic JCV strains in tribal population groups from north-eastern region of India and have compared their sequences with that of other key JCV strains from Asia. The NCCR sequence analyses were done with regard to transcription factor binding to DNA sequence elements of endemic JCV NCCRs.

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