

## ORIGINAL ARTICLE

## JC virus detection in human tissue specimens

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*J Clin Pathol* 2007;60:787-793. doi: 10.1136/jcp.2006.040915

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Accepted 3 August 2006  
Published Online First  
19 January 2007

**Aim:** To clarify the advantages and disadvantages of different detection methods for JC virus in human tissue specimens.

**Methods:** Specimens of lung and gastric carcinomas, and normal lung tissue, gastric mucosa, and tonsil were examined for *T-antigen*, *VP* and agnoprotein of JC virus by nested PCR, Southern blotting and sequencing. JC virus load targeting *T-antigen* was evaluated by real-time PCR, and JC virus existence morphologically by immunohistochemistry, in-situ hybridisation (ISH) and PCR. For these experiments, the JCI cell line (JC virus cultured neuroblastoma cell line) was employed as positive control.

**Results:** In lung and gastric carcinomas, *T-antigen*, *VP* and agnoprotein of JC virus could be detected by nested PCR whose products were confirmed by Southern blots and sequencing. With real-time PCR, frozen samples of gastric carcinomas gave better detection of JC virus than their corresponding paraffin-embedded tissues ( $p < 0.05$ ). The positive rate of JC virus was high in lung carcinoma, compared with normal lung tissue ( $p < 0.05$ ). It was the same for JC virus copies in gastric carcinoma ( $p < 0.05$ ). Only the positive control exhibited JC virus in the nucleus by ISH and immunohistochemistry. In-situ PCR showed that JC virus genomic DNA was located in the nucleus of the carcinoma cell, some alveolar epithelial cells, and tonsil lymphocytes. In ISH and PCR, NBT/BCIP colouring was stronger than Fuchsin.

**Conclusions:** Nested PCR whose amplicons should be confirmed by Southern blot and sequencing was a comparatively sensitive approach to detect JC virus genomic DNA in human non-neural tissues. Real-time PCR might be employed to quantify copy number of JC virus. In-situ PCR was a good method to observe the JC virus location in cells, given appropriate modulation of amplification cycles. Combinations of various approaches will be adopted to explore the oncogenic roles of JC virus in malignancies.

JC virus constitutes a family of polyoma viruses, which are non-enveloped, have icosahedral capsids and contain small, circular, double-stranded DNA genomes. They feature early and late coding regions, whose transcription is initiated in opposite directions by a transcription control region between them. The early region is alternatively spliced to produce large *T-antigen* and small *t-antigen*.<sup>1</sup> *T-antigen*, a large nuclear phosphoprotein for viral DNA replication, binds to the viral replication region to promote unwinding of the double helix and recruitment of cell proteins that are required for DNA synthesis. The late region encodes the capsid structural protein VP1, VP2 and VP3 due to alternative splicing and the small regulatory protein known as agnoprotein.<sup>1,2</sup> VP proteins are essential to assemble with viral DNA to form virions.<sup>3</sup>

Serological studies have indicated an asymptomatic JC virus infection in about 90% of the adult population.<sup>4</sup> Because JC virus replication is restricted to glial and lymphoid cells, which contain the JC virus transcriptional factors, the virus enters through tonsillar stromal tissue and persists quiescent in the kidney and lymphoid tissue during latency<sup>3,5</sup>; however, it may be activated under immunosuppressive conditions, leading to the lethal demyelinating disease, progressive multifocal leucoencephalopathy (PML).<sup>1-5</sup> JC virus infection initiates binding to the JC virus-sensitive cell surface; JC virus capsids undergo endocytosis and are transported to the nucleus where the viral DNA is uncoated and the early region begins to be transcribed.<sup>2,3,6</sup> Under permissive infection, viral DNA can replicate, resulting in lytic infection with viral amplification; non-permissive cells do not allow viral replication, leading to an abortive infection or cell transformation.<sup>2-4</sup> Evidence from transgenic and infectious animal models indicated that JC virus can transform cells and cause various malignancies.<sup>1,7,8</sup> In

recent years, links have been suggested between JC virus and various types of human cancers, including colorectal, prostate and oesophageal cancers, brain tumours, bronchopulmonary carcinoma and B cell lymphoma,<sup>1,2,9-12</sup> confirming its role as an oncovirus; this was first hypothesised by Laghi *et al* who detected JC virus in colon mucosa and cancer by PCR.<sup>12</sup>

The molecular mechanisms of the oncogenic effects of JC virus mainly centre on its encoding products, such as T-antigen, and agnoprotein. JC virus T-antigen can serve as ATPase, helicase, and polymerase and orchestrates the assembly and function of cellular proteins such as replication protein A and DNA polymerase- $\alpha$ . T-antigen could inactivate p53 and members of the pRb family, and deregulate the Wnt signalling pathway to promote uncontrolled proliferation and immortal survival.<sup>3,13</sup> Meanwhile, T-antigen can induce structural chromosome aberrations and genomic instability as a mutator.<sup>2</sup> Recent findings indicate that JC agnoprotein inhibits the DNA repair after DNA damage and interacts with the DNA damage-induced cell cycle regulation.<sup>14</sup> Retention of JC virus can result in chromosomal instability, characterised by chromosomal breakage, dicentric chromosomes, and increasing ploidy. T-antigen, through interaction with several cellular regulatory proteins, including tumour suppressors and cell cycle regulators, promotes uncontrolled progression of cells through the cell cycle. Insertion of JC virus into genomic DNA of human cells will cause their malignant transformation and anaplasia.<sup>15,16</sup> Therefore, detection of JC virus in the cancer tissues should facilitate clarification of its oncogenic role in tumourigenesis. In the present study, we focused on the methodology for JC virus

**Abbreviations:** ISH, in-situ hybridisation; PML, progressive multifocal leucoencephalopathy

detection in cancers, determining their advantages and disadvantages, alone or in combination.

## MATERIALS AND METHODS

### Cases and controls

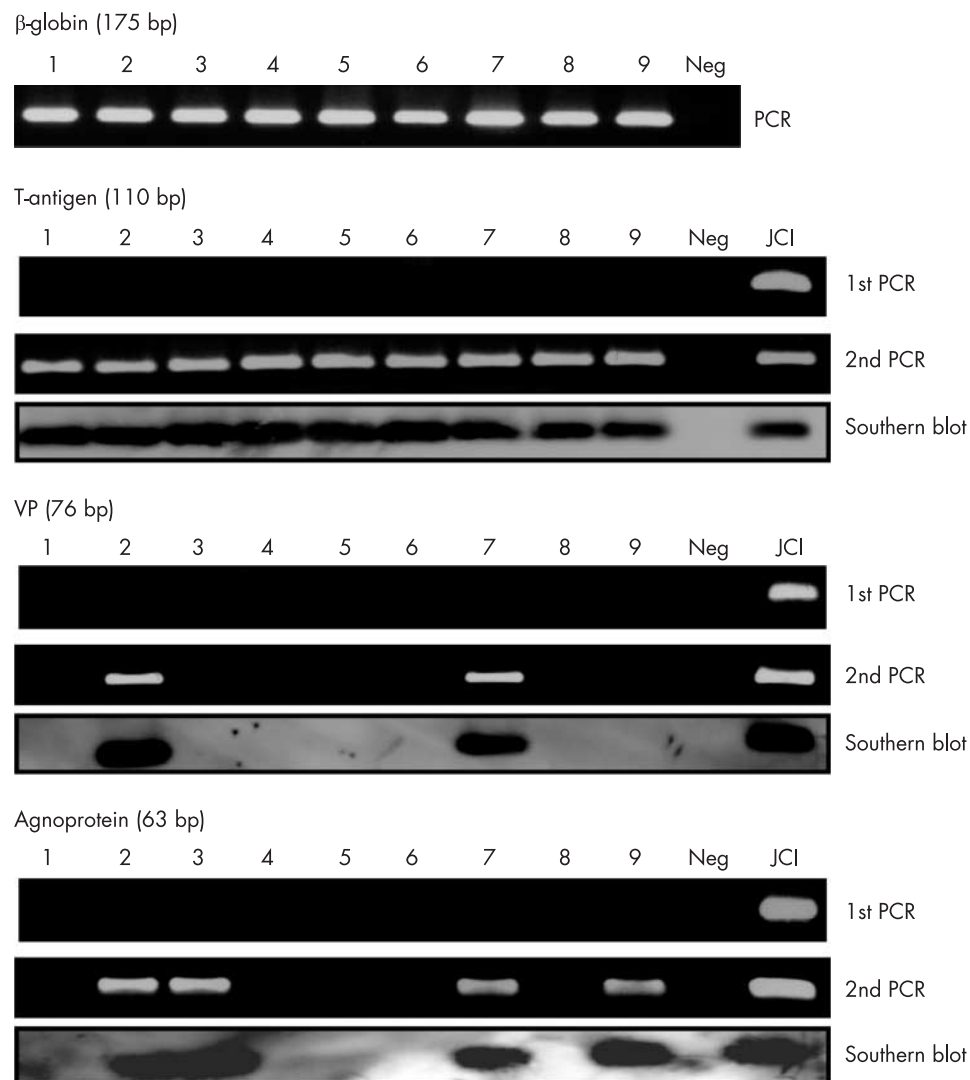
Lung (n = 50) and gastric carcinomas (n = 20), normal lung tissue (n = 20), normal gastric mucosa (n = 10), and tonsils (n = 2) were collected from operation or biopsy materials in our affiliated hospital and Kouseiren Takaoka Hospital after obtaining permission from patients. JCI cells (JC virus cultured neuroblastoma cell line, kindly provided by Associate Professor H Sawa, Department of Neuropathology, Hokkaido University, Graduate School of Medicine) served as a positive control. All tissues and cell lines samples were fixed with 4% neutralised formaldehyde, embedded in paraffin and incised into 4 µm sections. These sections were stained with H&E to confirm their histological diagnosis. Samples of gastric carcinoma and normal mucosa were frozen in liquid nitrogen for 6 hours and stored at -80°C until used. The ethics committee of the hospital gave approval for genetic experiments restricted to JC virus.

### DNA extraction and checking

Paraffin-embedded tissues were cut into 10 µm thick sections and microdissected with reference to H&E staining of consecutive sections, and subjected to deparaffinisation. DNA was extracted from cell lines, the deparaffined and frozen samples by standard proteinase K digestion and phenol/chloroform extraction. Before investigating paraffin-embedded samples, their DNA was amplified using β-globin primers (sense: 5' ACACAACCTGTGTTCACTAGC 3'; anti-sense: 5' GTC TCCTTAAACCTGTCTTG 3'; 175 bp) by 30 cycles of denaturation at 95°C for 30 s, annealing 55°C for 30 s, and extension at 72°C for 30 s to confirm integrity of the DNA.

### Nested PCR

PCR amplification was performed using three individual sets of primers for *T-antigen*, *agnoprotein*, and *VP*. For JC virus *T-antigen*, T1 and T2 (nucleotides 3049–3069 of the Mad-1 strain, 5' TGGCCTGTAAAGTCTAGGCA 3' and 3229–3207, 5' GCAG AGTCAAGGGATTACCTTC 3' respectively), which amplify sequences in the NH<sub>2</sub>-terminal region of the JC virus *T-antigen*, were used for the first PCR, whereas T1 and T3 (nucleotides



**Figure 1** Detection of β-globin, *T-antigen*, *VP* and *agnoprotein* in lung carcinoma. β-globin in all cases, *T-antigen* and *VP* and *agnoprotein* of JCI were positive by the first PCR. *T-antigen* was positive in all cases, *VP* in cases 2 and 7 and *agnoprotein* in cases 2, 3, 7 and 9 by the 2nd PCR. The Southern blot showed the same results as the nested-PCR. JCI: positive control; Neg: negative control.

3193–3171, 5' AGCAACCTTGATTGCTTAAGAGA 3') were used for the second PCR (110 bps). For the *VP1* capsid gene sequence, *VP2* and *VP3* (nucleotides 1828–1848, 5' TGTGCACTCTAATGGGCAAGC 3' and 2019–2039, 5' CTAGGTACGCCTTGTGCTCTG 5' respectively), were used for the first PCR, and both *VP3* and *VP4* (nucleotides 2004–1982, 5' GATTGC ACTGTGGCATTCTTTGG 3') were used for the second PCR (177 bps). Finally, for JC virus *agnoprotein*, AGNO1 and AGNO2 (nucleotides 458–438, 5' GTCTGCTCAGTCAAACCACTG 3' and 280–298, 5' GTTCTTCGCCAGCTGTAC 3' respectively), which amplify a region within the coding region of JC virus *agnoprotein* were used for the first PCR and AGNO1 and AGNO3 (nucleotides 395–415, 5' GCACAGGTGAAGACAGTGTAG 3') were used for the second PCR (64 bps). Each 25 µl of reaction mixture contained 0.125 µl Takara Ex Taq HS (TaKaRa) with 2.0 mM MgCl<sub>2</sub>, 2.5 µl ×10 PCR buffer, 2.5 µl dNTP mixture, 1 µM of each primer set (external primers), and 250 ng of template DNA. PCR conditions were denaturation at 95°C for 10 min, followed by 30 cycles of denaturation at 95°C for 15 s, annealing for 30 s, and extension at 72°C for 30 s. The annealing steps were performed at temperatures of 55°C for the *T-antigen* primers, 57°C for the *agnoprotein* primers, and 54°C for the *VP* primers. As a termination step, the extension time of the last cycle was increased to 7 min. Samples amplified in the absence of template DNA were employed as negative controls. Nested PCR was carried out as the first PCR cycles, using 1% (volume) of the first PCR product with the internal primers in each case.

### Southern blot

Southern blotting was performed by resolving 10 µl of each nested PCR product on 2% agarose gels stained with ethidium bromide. After gels were denatured, neutralised and blotted onto nylon membranes (Hybond N1, Amersham, UK), hybridisation was performed using 10 pmol/ml of digoxigenin-labelled oligonucleotide probes (nucleotide 3066–3101 for *T-antigen* 5' GGCACCTGAATATTCATTCATGGTTACAATTCCAGGT 3', nucleotide 1872–1891 for *VP* 5' AGCCAGTGCAGGGCACCAGC 3' and nucleotide 395–415 for *agnoprotein* 5' AAAGACAGAGACACAGTGGTT 3') at 48°C for 3 hours. After washing the membranes with 2× saline sodium citrate (SSC), 0.1% sodium dodecyl sulphate (SDS) and 0.1× SSC, 0.1% SDS at the same temperature as the hybrid temperature, membranes were subjected to incubation of the alkaline phosphatase-labelled anti-digoxigenin antibody for 30 min. Luminescence was detected with LAS1000plus (Fujifilm, Japan) using a Dig luminescent detection kit for nucleic acids (Boehringer Mannheim, Massachusetts, USA).

### DNA direct sequencing

The presence of JC virus was further confirmed by direct sequencing of nested PCR products. Amplified DNA fragments initially identified by Southern blot hybridisation were purified with MicroSpin SR-300 columns according to the manufacturer's instructions (Amersham Biosciences, Germany). After extraction, the DNA was quantified by Nanodrop ND-100 Spectrophotometer (Laboratory & Medical Supplies, Tokyo, Japan) and then sequenced using a BigDye Terminator v3.1 cycle sequencing kit for *T-antigen* (primer: T1) and *VP* (primer: *VP1*) or BigDye Terminator v1.1 cycle sequencing kit for the *agnoprotein* (primer: AGNO 1) by an ABI prism 3100 genetic analyser (Applied Biosystems, Foster City, California, USA). The sequence data were compared with the JC virus genomic DNA sequence (Access Number: NC\_001699) by GENETYX-SV/R(C) V.7.

### Real-time PCR

A real-time, fluorescence probe-based PCR method was used for quantitative JC virus of samples with the Mx3000P Real-

Time PCR system (Stratagene, La Jolla, California, USA). For establishment of the quantitative technique for estimating the JC virus copy number, a JC virus-containing plasmid (pBS-JC virusMad1) was serially diluted and served as a standard reference. This standard and DNA were subjected to PCR amplification of the 128-bp sequence of the *T-antigen* using RT-JC virus1, nucleotide 3492–3511, 5' GCCACCCCAGCCATATA TTG 3' and RT-JC virus-2, nucleotide 3619–3593, 5' GTTGACAGTATCCATATGACCAGAGAA 3' as the forward and reverse primers, respectively. Monitoring of amplicon development was performed using a double dye probe; RT-JC virus-3, nucleotide 3506–3530, 5' TAAAACAGCATT GCCA TGTGCCCA 3' was labelled with FAM at the 5' end and TAMRA at 3' end. The reactions were performed using the TaqMan Universal PCR master mix (Applied Biosystems). Each 25 µl of reaction mixture contained 12.5 µl TaqMan (×2) with 2.25 µl (10 µM) of each primer, 2.5 µl (2.5 µM) of double dye probe and 100 ng of template DNA. The protocol included the following parameters: an initial 10 min of incubation at 95°C for TaqMan DNA polymerase activation followed by 60 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 1 min, and extension at 72°C or 30 s.

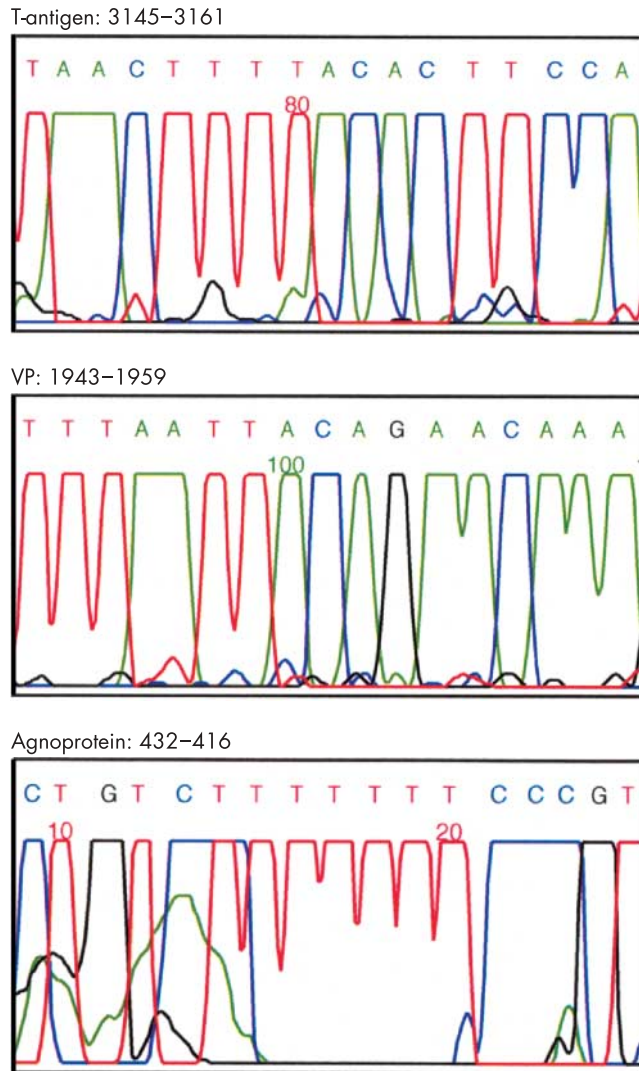
### Immunohistochemistry

Serial sections were deparaffinised with xylene, dehydrated with alcohol, and subjected to antigen retrieval by irradiating in target retrieval solution (Dako, Carpinteria, California, USA) for 5 min in a microwave oven (Oriental Rotor, Tokyo, Japan). Bovine serum albumin 5% was then applied for 1 min to prevent non-specific binding. The sections were incubated with anti-SV 40 T-antigen (Calbiochem, San Diego, California, USA; 1:100) for 20 min, then treated with anti-mouse Envison-PO (Dako) antibody for 20 min. All the incubation was performed in the microwave oven for intermittent irradiation as described previously.<sup>17</sup> After each treatment, the slides were washed with TBST (10 mM Tris-HCl, 150 mM NaCl, 0.1% Tween 20) three times for 5 min. All slides were developed for colouring with 3,3'-diaminobenzidine and counterstained with Mayer's haematoxylin. Omission of the primary antibody was used as a negative control.

### In-situ hybridisation

To perform DNA-DNA ISH for *T-antigen*, digoxigenin-labelled *T-antigen* probe was made through 100 µl of PCR solution consisting of 1 µM primer sets of RT-JC virus-1 and RT-JC virus-2 as for real-time PCR, 2.0 mM MgCl<sub>2</sub>, 10 µl ×10 PCR buffer, 200 µM DIG-11-dUTP (Roche Diagnostics, Penzberg, Germany), 100 ng template DNA from JCI cell line and 2U Taq polymerase, and followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s.

Sections (4 µm thick) were deparaffinised and digested with pepsin (0.05% in 0.2N HCl, pH 2.0) at 37°C for 30 min, dehydrated through graded ethanols and air-dried. Then 20 µl of a 1:50 digoxigenin-labelling probe dilution in hybridisation buffer (22 mM Tris-HCl, pH 7.4, 2.75 mM EDTA, 660 mM NaCl, 1× Denhardt solution, 5.5% dextran sulphate, 0.33% dimethyl sulphoxide, 0.55% ethoquad 18/25 and 44% deionised formamide) was added to each slide. After coverslipping, heating at 95°C for 5 min and incubation overnight in a humidified chamber at 37°C, sections were rinsed for 10 min in TTBS and incubated with anti-digoxigenin antibody conjugated with alkaline phosphatase (Roche Diagnostics) for 20 min at 37°C. The slides were then washed for 5 min and immersed in solution II (100 mM Tris-HCl, pH 9.5, 100 mM NaCl and 50 mM MgCl<sub>2</sub>) for 15 min at room temperature and incubated with an anti-digoxigenin antibody coupled to alkaline

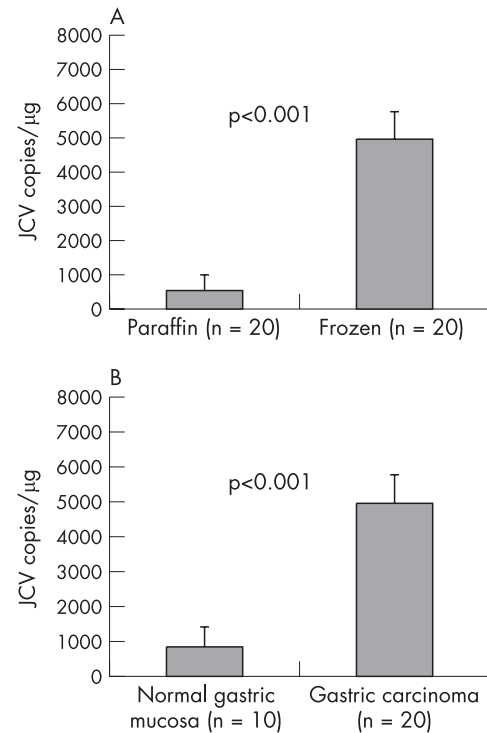


**Figure 2** Sequencing of nested PCR products of *T-antigen*, *VP* and *agnoprotein*.

phosphatase overnight and followed by NBT/BCIP or Fuchsin as chromogen. Finally, counterstaining of the tissue with nuclear fast red or methyl green respectively was performed.

#### In-situ PCR

Sections (10  $\mu$ m thick) was digested with proteinase K (20  $\mu$ g/ml, Dako) for 15 min at 37°C. After rinsing with phosphate-buffered saline (PBS), the tissue was fixed with 4% neutralised paraformaldehyde and washed with 2 $\times$  SSC. Then 125  $\mu$ l of PCR solution (0.2  $\mu$ M primers of JCT-1A and JCT-1AS, 0.125 nM DIG-11-dUTP, 2.5 mM MgCl<sub>2</sub>, 1 $\times$ PCR buffer, 6.25U Taq polymerase) was placed on the tissue under a membrane sealing and PCR consisting of 94°C for 3 min, followed by 15 cycles of 92°C for 15 s, 55°C for 15 s and 72°C for 30 s; finally 72°C for 5 min was performed on the slide griddle of a programmable thermal controller. These primers result in amplification of a 100 bp fragment of JC virus. The tissue was then washed with 2 $\times$ SSC and incubated with blocking solution (100  $\mu$ g/ml salmon testis DNA, 100  $\mu$ g/ml yeast tRNA, and 5% BSA in PBS) for 1 hour. Detection and counterstaining were the same as for ISH. The nucleotide sequences of JCT-1A and JCT-1AS were 5' AGGTAGGCCTTTGGTCTAA 3' (nucleotides 3009–3027) and 5' TGCCTAGAACTTTACAGG 3' (nucleotides 3069–3050) respectively.



**Figure 3** JC virus loads in gastric samples. (A) JC virus copies in frozen or corresponding paraffin-embedded samples of gastric carcinomas. (B) JC virus copies in frozen samples of gastric carcinomas and normal gastric mucosa.

#### Statistical analysis

Statistical evaluation was performed using Fisher's exact test to compare different rates and the Mann-Whitney U test to differentiate non-parametric means of different groups;  $p < 0.05$  was considered as statistically significant. SPSS V.10.0 software was used to analyse all data.

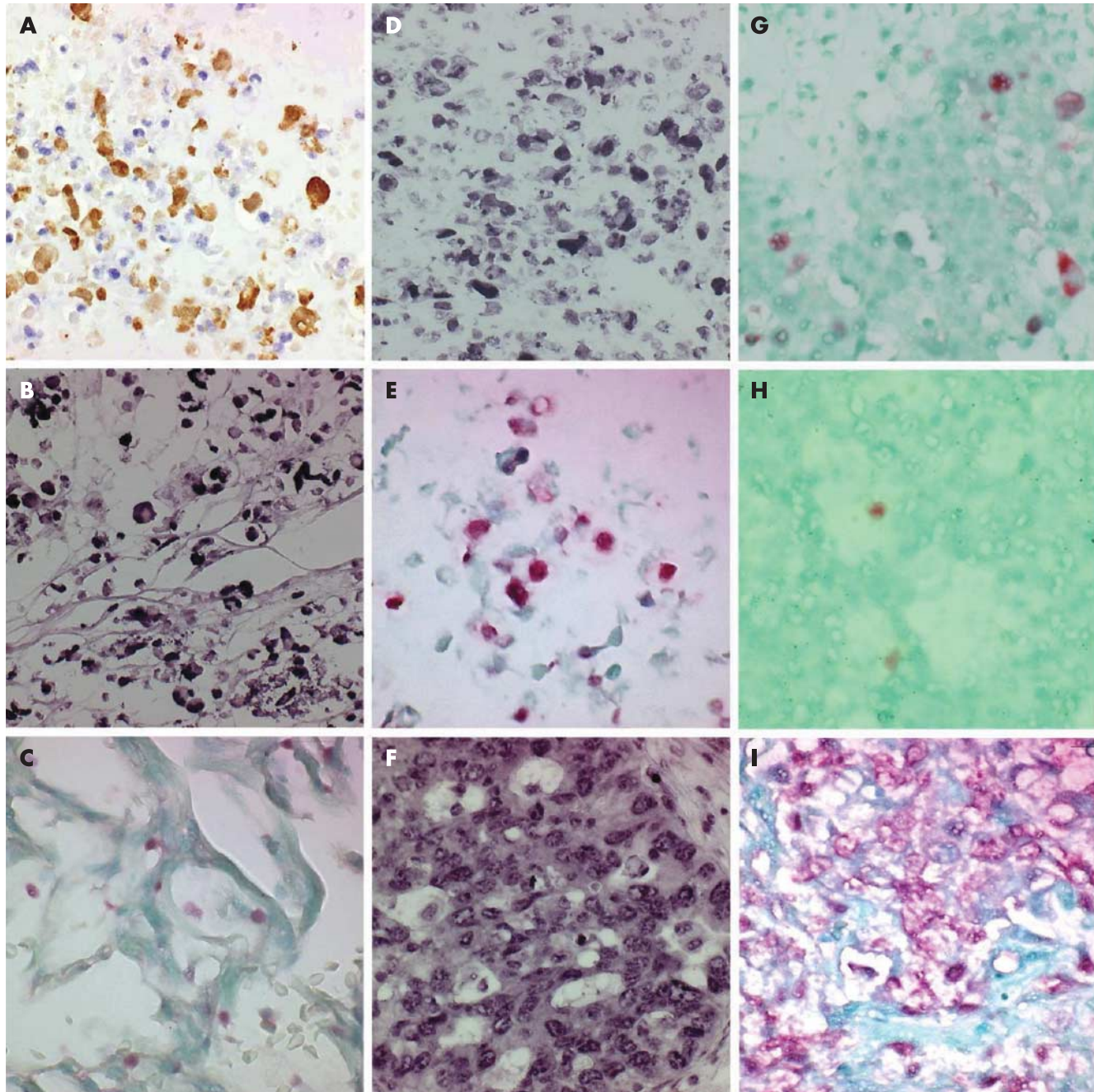
#### RESULTS

As shown in fig 1, clear amplification of  $\beta$ -globin was of high quality and appropriate for experimentation. *T-antigen*, *VP* and *agnoprotein* were not detected except in the JCI positive control by the first PCR, but were positive in lung carcinoma with the second PCR. Sequencing and Southern blot proved that amplified DNA sequences of *T-antigen*, *VP* and *agnoprotein* were correct in line with a previous report (Access Number: NC\_001699; figs 1–2). The positive rate of JC virus was higher in lung carcinomas than normal lung tissues by nested PCR followed by Southern blot targeting T-antigen ( $p < 0.001$ ) (table 1). JC virus copies were lower in paraffin-embedded gastric carcinomas than in frozen ones ( $p < 0.001$ ), which were higher than in frozen normal gastric mucosa ( $p < 0.001$ ) (fig 3).

In-situ PCR showed the JC virus genomic DNA location in the nucleus of the carcinoma cell, some epithelial cells and tonsil lymphocytes (fig 4). With ISH and immunohistochemistry, only the positive control exhibited the positive signal for JC virus in the nucleus. With in-situ PCR and hybridisation, NBT/BCIP colouring was stronger but Fuchsin provided the best contrast.

#### DISCUSSION

In the present study, *T-antigen*, *VP* and *agnoprotein* could be readily detected in the positive control (JC virus cultured neuroblastoma cell line) by the first PCR, and even in the lung carcinomas by the second PCR, in line with the neurotropicity



**Figure 4** Morphological examination of JC virus. Positive signals in in-situ hybridisation (ISH) and in-situ PCR are shown as a black colour by NBT/BCIP and as a red colour by Fuchsin. In the positive control, JC virus was detected in the nucleus by ISH (D,G) and PCR (B,E); T-antigen was detected in the nucleus by immunohistochemistry (A, brown). In-situ PCR showed that JC virus existed in the nucleus of cancer cells (F, lung carcinoma; I, gastric carcinoma), adjacent type-II alveolar epithelial cells (C) and tonsil lymphocytes (H).

**Table 1** JC virus existence in lung samples by nested PCR followed by Southern blot targeting T-antigen

Groups	n	T-antigen of JC virus		Positive rate
		-	+	
Normal lung tissues	20	18	2	10%
Lung carcinomas	50	21	29	58%*

\*Compared with normal lung tissue,  $p < 0.001$  (Pearson's  $r = 0.437$ ).

of JC virus and comparatively sensitivity of nested PCR.<sup>2 3 5</sup> JC virus is preferentially expressed in astrocytes and oligodendrocytes of human brain because these cells contain the JC virus transcriptional factors including Jun, NF-1, GF-1, Sp1, S $\mu$ bp-2, Pur $\alpha$ , and YB-1.<sup>3 5</sup> JC virus DNA has been detected from several organs, including the brain, lung, liver, kidney, spleen, bone marrow, bladder, prostate, tonsils, lymph nodes and leucocytes,<sup>12 18-20</sup> especially by the nested PCR, which greatly increases fold of amplification and amplification specificity or real-time PCR, which can quantify the copy number of JC virus. In the present study, we found a high frequent existence of JC

virus in lung and gastric carcinomas by nested or real-time PCR in accordance with other reports,<sup>2,6,21</sup> suggesting that JC virus genomic DNA insertion might be a molecular risk factor for carcinogenesis.

To enlarge the body of specimens available for JC virus examination, many investigators used formalin-fixed and paraffin-embedded cancer samples, but formalin fixation breaks DNA and may make it more difficult to recover sufficient quantities of intact JC virus DNA for amplification. It was necessary to design shorter amplicons (100–150 bp), which amplify well even from degraded DNA. However, nested and real-time PCR indicated that formalin fixation and paraffin embedding decreased the detection efficiency of JC virus T-antigen despite amplification of shorter amplicons in our study. However, it was difficult to sequence the short PCR products using general direct sequencing methods. For example, we sequenced the 64 bp amplicon using 21 bp primer and finally read at most 17 bp. Therefore, a combination of Southern blot and DNA sequencing is recommended to ensure the reliability of PCR results. Sequencing the amplicon is helpful to certify the DNA variation of JC virus and exclude DNA contamination because contamination results in the same PCR products. If short amplicons were designed, it would be difficult to find out the DNA variation, attributable to the sequencing bar. We therefore suggest the following: (1) appropriately choose the amplicon size to ensure PCR amplification and screen the DNA variation in the largest sense; (2) insert the amplicon into a plasmid for cloning sequencing to overcome the sequencing bar whether screening the aberrant PCR products or not.

In addition to the molecular biological approach, morphological examination of JC virus in human tissue needs to be considered. In the present study, in-situ hybridisation and immunohistochemistry targeting T-antigen were used; a positive signal of JC virus in lung and gastric carcinoma was not found (data not shown), in contrast to the case with JC virus cultured neuroblastoma cell lines. The failure of JC virus detection may be a result of its low copy number and expression levels in non-neural carcinoma tissue even if genetic changes occur in carcinoma cells. However, ISH and immunohistochemistry could be applied in neural tissue, cell lines and transgenic animal model.

The usefulness of standard ISH is occasionally limited by a detection sensitivity of about 1000 viral copies per cell.<sup>22</sup> Although researchers tried to improve threshold levels for detection in ISH studies, by using, for example, several antibody steps or increasing the absolute amount of hybridised probes (by using cocktails of oligonucleotide or multiple cRNA probes, for example<sup>23,24</sup>), its sensitivity is still less than that of in-situ PCR, which can detect even a few viral copies per cell.<sup>21</sup> In our study, JC virus has been detected by in-situ PCR using 15 cycles. If the cycles of PCR were increased to more than 20–25, the necrotic area would display some positive signal, due to the unspecific amplification. Because in-situ PCR is limited by its cycles, it is available for clinical JC virus examination in non-neural tissues as ISH and immunohistochemistry. Furthermore, NBT/BCIP colouring was found to be more sensitive than Fuchsin for both ISH hybridisation and PCR, although Fuchsin colouring and methyl green counterstaining could provide good results.

In-situ PCR showed that JC virus was present in the nucleus of both alveolar epithelial cells and lung carcinoma cells, as in gastric carcinoma. It is believed that exposure to JC virus, most likely through the upper respiratory tracts, causes the persistent infection.<sup>18</sup> Earlier surveys of raw sewage from urban areas have shown the detection of JC virus particles in sewage samples from widely divergent areas; the presence of JC virus DNA sequences in the upper human gastrointestinal tract suggests a potential re-entry of JC virus and/or viral DNA into the

### Take-home messages

- Nested PCR is a comparatively sensitive approach for detection of JC virus genomic DNA in human non-neural tissues. Southern blots and sequencing are necessary to confirm the identity of nested PCR products. Real-time PCR might be used to quantify the copy number of JC virus.
- In-situ PCR is a good method to observe JC virus location in cells, with appropriate amplification cycles. It may provide morphological evidence for JC virus transformation and infectious pathways.
- In-situ hybridisation and immunohistochemistry are not available to examine the existence and expression of JC virus in human non-neural tissues, but could be applied with transgenic mice and the transfected cell line of *T-antigen*.
- Although various methods can be used individually to detect JC virus, combinations are of importance to explore the oncogenic role of JC virus in malignancies.
- Detection of JC virus is very important to clarify its pathogenesis and related molecular mechanisms in diseases, especially in malignancies.

human population through the intake of virus-contaminated water and food.<sup>9,20</sup> Our findings also identified the respiratory and digestive tracts as doors for JC virus infection.<sup>18,20</sup> The existence of JC virus in tonsil lymphocytes supports the hypothesis of a quiescent state in lymphoid tissue during latency and infection of other cells on immune suppression.<sup>25,26</sup>

In conclusion, nested PCR, whose results should be confirmed by Southern blot and sequencing, was a comparatively sensitive approach to detect JC virus genomic DNA in human non-neural tissues. Real-time PCR might be used to quantify copy number of JC virus. In-situ PCR was a good method to observe the JC virus location in cells with appropriate amplification cycles. A combination of various approaches is necessary to determine its oncogenic role in malignancies.

### ACKNOWLEDGEMENTS

We thank Ms Kanako Yasuyoshi, Mr Hideki Hatta and Mr Tokimasa Kumada for their excellent technical support for tissue preparation and immunohistochemistry. We are also grateful to Yukari Inoue for secretarial assistance.

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**Funding:** This work was partially supported by the Japanese Ministry of Education, Science, Sports and Culture, Grant-in-Aid for Scientific Research 14770072 and 15922084, the 21st Century COE Program in Japan, Japanese Smoking Research Society and National Natural Science Foundation of China (30600286).

**Competing interests:** None.

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