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Parent-to-Child Transmission Is Relatively Common in the Spread of the Human Polyomavirus JC Virus

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JC polyomavirus (JCV), the causative agent of progressive multifocal leukoencephalopathy, is ubiquitous in the human population, infecting children asymptomatically and then persisting in renal tissue. In most adults, renal JCV replicates and the progeny are excreted in urine. We used this urinary JCV to elucidate the routes of JCV transmission. A 610-bp JCV DNA region (IG region) encompassing the 3′-terminal sequences of both T-antigen and VP1 (major capsid protein) genes was amplified by means of PCR from urine specimens collected from all members of seven families. JCV strains were then unequivocally identified by the nucleotide sequences of the amplified IG regions. We could identify 18 distinctive JCV strains from 27 individuals. Different JCV strains were detected from all unrelated persons. However, the same viral strain was detected from one (four families), two (one family), or three offspring (one family) as well as from the fathers (three families) or from the mothers (three families). In total, the JCV strains detected in half of the JCV-positive children were identified in their parents. Since most humans are infected during childhood, these findings indicated that JCV is transmitted frequently from parents to children. We roughly estimated that 50% of JCV transmission occurs by this route and that the other 50% occurs outside the family.

Human polyomavirus JC (JCV), the causative agent of progressive multifocal leukoencephalopathy (11), is widespread in the human population (10). Seroepidemiological studies (9, 13) have shown that JCV infection most frequently occurs in childhood. However, there was no information available regarding the route of transmission. This was mainly because no obvious symptom was correlated with JCV infection (10).

Recent studies have shown that after primary infection JCV persists in renal tissues (2, 14) and that in adults renal JCV is not latent but replicates to generate progeny in urine (6, 7). We developed a way of using this urinary JCV to elucidate the routes of JCV transmission in humans (7). We selected families in which both parents and children were excreting JCV in urine (that is, all of the children were adults). JCV DNA fragments were amplified by PCR and were compared by means of restriction fragment length polymorphism analysis. In the current study, we used nucleotide variation instead of restriction fragment length polymorphism analysis to improve the sensitivity of resolving different viral strains. We selected a 610-bp region, designated IG, that encompasses the 3′-terminal sequences of both the T-antigen and VP1 (major capsid protein) genes (1) (Fig. 1), because it contains abundant nucleotide variation compared with other regions (1). The strain distribution in each family further clarified the route of JCV transmission.

MATERIALS AND METHODS

Subjects. Seven families were studied. Families 1 to 3 resided in Tokyo, and families 4 to 7 resided in Tsubata-cho, Ishikawa Prefecture, Japan. The children of these families lived together throughout childhood, but most of them moved out because of marriage by the age of about 30 years. Families 1, 4, 5, 6, and 7 refer to families 7, 4, 5, 6, and 3, respectively, that were studied previously (7).

Extraction of DNA from urine. To minimize cross contamination during extraction, DNA was extracted from the urine of the parents at the Department of Viral Infection, Institute of Medical Science, University of Tokyo, and from the urine of the offspring at the Department of Urology, Faculty of Medicine, University of Tokyo. The procedure was essentially that described previously (6).

PCR amplification. The IG region was amplified as described by Ault and Stoner (1), with modifications. The PCR mixture (50 μl) containing 2.5 μl of sample DNA was amplified by 50 cycles at 94°C for 1.5 min, 55°C for 1.5 min, and 72°C for 2.5 min with the TSR-300 Thermal Sequencer (Iwaki Glass Co., Ltd., Tokyo, Japan). *Taq* DNA polymerase and reagents were obtained from Takara Shuzo Co., Ltd. (Kyoto, Japan) and were used as recommended by the supplier. Primer P-1 (5′-CACAAGCTTTTTTGGGACACTAACAGGAGG-3′) consisted of nucleotides (nt) 2107 to 2127 of the JCV genome (5) and a 5′-terminal nonanucleotide (underlined), which were ligated to create a *Hin*dIII site; primer P-2 (5′-GATTCTGCAGCAGAAGACTCTGGACATGG-3′) consisted of nt 2762 to 2742 and a 5′-terminal octanucleotide (underlined), which were ligated to generate a *Pst*I site. In performing PCR amplification, all precautions were taken to avoid cross contamination (8).

Molecular cloning. The PCR-amplified fragments were digested with a combination of *Hin*dIII and *Pst*I to excise the IG regions. The recovered fragments were ligated to *Hin*dIII- and *Pst*I-digested, alkaline phosphatase-treated pUC19 and were used to transform competent *Escherichia coli* HB101 cells (Takara Shuzo Co., Ltd.). Plasmids containing the IG regions were prepared by using the Wizard Midipreps DNA purification system (Promega Corporation, Madison, Wis.).

Sequencing. Plasmid pUC19 containing the IG region was sequenced with the AutoRead Sequencing kit and the ALF DNA Sequencer II (Pharmacia LKB Biotechnology AB, Uppsala, Sweden). Fluorescent primers for the sequencing reaction were forward and reverse primers provided by Pharmacia and fluorescein isothiocyanate-labeled primers S (5′-GGACCCCTAGAGTTGATGGG-3′) and G (5′-GGGTCCCCTGGAAGTTCCTCTG-3′) within the IG region (primers S and G span nt 2382 to 2401 and nt 2478 to 2457, respectively). Primers S and G were custom synthesized by Pharmacia. Both DNA strands were sequenced.

Nucleotide sequence accession numbers. All sequences reported in here have been submitted to the DDBJ, EMBL, and GenBank data libraries, and the accession numbers are D49930 (C-01), D49931 (C-02), D49932 (C-03), D49933 (C-04), D49934 (C-05), D49935 (C-06), D49936 (C-07), D49937 (C-08), D49938 (M-01), D49939 (M-02), D49940 (M-03), D49941 (M-04), D49942 (M-05),

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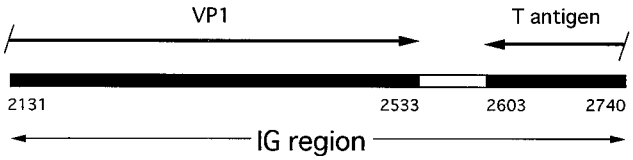

FIG. 1. IG region of JCV. The VT intergenic region (herein abbreviated IG) is shown diagrammatically. Closed rectangles indicate regions that code for VP1 and T antigen. The open rectangle indicates a noncoding region. Numbers below the closed rectangles denote, in nucleotides, the ends of the coding regions within the IG region. The nucleotide numbering is that of Frisque et al. (5).

D49943 (M-06), D49944 (M-07), D49945 (M-08), D49946 (M-09), and D49947 (M-10).

RESULTS

Consensus IG sequences in urine donors. We extracted DNA from two batches of urine from each donor and used it as the template for PCR amplification. Amplified fragments were cloned into pUC19, and one clone was sequenced from each DNA sample. Consequently, two sequences were obtained for one donor. Usually, these were consistent, and we therefore considered them to be the consensus IG sequence for the JCV in the donor.

However, in three donors (sibling F in family 1, sibling C in family 3, and the father in family 4) we found single-nucleotide mismatches in corresponding sequence data. For example, in sibling F in family 1, the nucleotide at position 2548 was T in one sequence and C in the other. For these samples, we sequenced two more clones for each DNA sample (four in total). All of these sequences and one of the previous ones were identical. Thus, we found that T was the consensus nucleotide at position 2548 in sibling F of family 1.

The nucleotide variation described above may have occurred within donors, or it may have been introduced during PCR amplification (12). Although the origin is not clear, the nonconsensus sequence was a minor component in the PCR products and was excluded as described above.

Identification of JCV strains by IG sequences. We used consensus IG sequences to identify JCV strains. Since the identified JCV strains were classified into two subtypes, subtypes CY and MY (15), with a subtype-specific nucleotide at six positions (boxed in Fig. 2), we designated a JCV strain belonging to subtype CY as C-*N* and that belonging to subtype MY as M-*N*, where *N* is a double-digit number. We identified 8 and 10 strains belonging to subtypes CY and MY, respectively (Fig. 2). All of these strains were judged to belong to type B on the basis of the presence of type-specific nucleotides within the IG region (1).

Pairwise comparisons among these strains are given in Table 1. In subtypes CY and MY, nucleotide differences ranged from 1 to 9 nt and 1 to 7 nt, respectively. In the comparison between strains of different subtypes, nucleotide differences ranged from 12 to 20 nt.

One strain (strain C-04) contained a dinucleotide insertion between nt 2599 and 2600 that was located in the noncoding region between VP1 and T-antigen genes (Fig. 1). This insertion was not found in the other strains analyzed in the study, and was therefore helpful for identifying this strain.

Strain distribution within the family. The strain distribution in each family is shown in the pedigree presented in Fig. 3 and is outlined as follows (the strains that were detected in multiple members of each family are indicated in Fig. 3).

In family 1, all members excreted JCVs which belonged to subtype MY. Sequencing of their IG regions resolved them into three strains, a common one detected both in the mother as well as in three siblings (siblings C, E, and F) and two unique ones detected in the father and sibling D.

In family 2, the father was not alive, and so urine could not be collected from him. However, the mother and sibling C excreted JCVs of subtype MY, while siblings E and F excreted JCVs of subtype CY. The IG sequences showed that the strain in the mother was identical to that in sibling C. The strains in siblings E and F were unique. Sibling D did not excrete JCV.

In family 3, the father and two siblings (siblings C and D) excreted JCVs of subtype CY. Their IG sequences indicated that they were all identical. The mother did not excrete JCV.

Strain	2161	2239	2251	2266	2311	2317	2326	2371	2380	2386	2404	2413	2416	2446	2470	2518	2534	2539	2540	2574	2584	2587	2593	2604	2606	2607	2642	2645	2654	2661	2662	2711	2712	2723	2726
C-01	C	G	C	A	T	A	G	A	G	C	G	T	C	T	A	G	T	A	G	G	C	T	G	T	C	T	A	G	G	T	A	A	T	T	A
C-02	C	G	C	A	T	A	G	A	G	C	G	T	C	T	A	G	T	A	G	C	C	T	G	T	C	T	A	G	G	A	T	A	G	T	A
C-03	C	T	C	A	T	A	G	A	A	C	G	T	C	G	A	G	T	G	G	G	C	T	A	T	T	T	A	G	G	T	A	A	G	T	A
C-04	C	G	C	A	T	A	G	A	A	C	G	T	C	T	A	G	T	A	G	G	C	T	G	T	C	T	A	G	G	T	A	A	T	T	A
C-05	C	G	C	A	T	A	G	G	A	C	G	T	C	T	A	G	T	A	G	C	C	T	G	T	T	C	A	G	G	T	A	A	G	T	A
C-06	C	G	C	A	T	A	G	G	A	C	G	T	C	T	A	A	T	A	A	G	C	T	G	T	C	T	A	G	A	T	A	A	G	T	A
C-07	C	G	C	A	T	A	G	A	A	C	G	T	C	T	T	A	T	A	G	G	C	T	G	T	C	T	A	G	G	T	A	A	G	T	A
C-08	C	G	C	A	T	A	G	A	A	C	G	T	C	T	A	G	T	A	G	C	C	T	G	T	C	T	A	G	G	T	A	A	G	T	A
M-01	C	T	A	A	T	C	G	G	A	C	G	T	T	T	A	A	T	A	G	G	C	T	A	T	T	T	G	A	G	T	A	A	A	C	A
M-02	T	T	A	A	T	C	G	G	A	C	G	T	T	T	A	A	T	A	G	G	C	C	A	C	T	T	G	A	G	T	A	A	A	C	A
M-03	T	T	A	A	T	C	G	G	A	C	G	T	T	T	A	A	T	A	G	G	C	T	A	C	T	T	G	A	G	T	A	A	A	C	A
M-04	T	T	A	A	T	C	G	G	A	C	G	T	T	T	A	A	C	A	G	G	G	T	A	C	T	T	G	A	G	T	A	A	A	C	A
M-05	C	T	A	G	T	C	A	G	A	C	G	T	T	T	A	A	T	A	G	G	C	T	A	T	T	T	G	A	G	T	A	G	A	C	A
M-06	C	T	A	A	T	C	A	G	A	C	G	T	T	T	A	A	T	A	G	G	C	T	A	C	T	T	G	A	G	T	A	A	A	C	G
M-07	C	T	A	A	T	C	A	G	A	C	G	C	T	T	A	A	T	A	G	G	C	T	A	C	T	T	G	A	G	T	A	A	A	C	G
M-08	C	T	A	A	G	C	G	G	A	C	G	T	T	T	A	A	T	A	G	G	C	T	A	C	T	T	G	A	G	T	A	A	A	C	A
M-09	T	T	A	A	T	C	G	G	A	T	A	T	T	T	A	A	T	A	G	G	C	T	A	C	T	T	G	A	G	T	A	A	A	C	A
M-10	T	T	A	A	T	C	G	G	A	T	G	T	T	T	A	A	T	A	G	G	C	T	A	C	T	T	G	A	G	A	A	A	A	C	A

FIG. 2. Nucleotide variations in the IG region among 18 JCV strains. The nucleotides shown are those at positions where differences were found among the strains identified in the present study. Nucleotides common to all strains belonging to subtype CY or MY are boxed. Strain C-04 contained a dinucleotide (AC) insertion between nt 2599 and 2600. The nucleotide numbering is that of Frisque et al. (5).

TABLE 1. Pairwise comparison of the JCV strains

Strain	No. of nucleotide differences																
	C-01	C-02	C-03	C-04	C-05	C-06	C-07	C-08	M-01	M-02	M-03	M-04	M-05	M-06	M-07	M-08	M-09
C-02	4																
C-03	7	9															
C-04	1	5	6														
C-05	6	6	7	5													
C-06	6	8	9	5	6												
C-07	4	6	7	3	6	4											
C-08	3	3	6	2	3	5	3										
M-01	13	16	11	12	12	12	12	13									
M-02	16	19	14	15	15	15	15	16	3								
M-03	15	16	13	14	14	14	14	15	2	1							
M-04	17	20	15	16	16	16	16	17	4	3	2						
M-05	16	19	14	15	15	15	15	16	3	6	5	7					
M-06	16	19	14	15	15	15	15	16	3	4	3	5	4				
M-07	17	20	15	16	16	16	16	17	4	5	4	6	5	1			
M-08	15	18	13	14	14	14	14	15	2	3	2	4	5	3	4		
M-09	17	20	15	16	16	16	16	17	4	3	2	4	7	5	6	4	
M-10	17	18	15	16	16	16	16	17	4	3	2	4	7	5	6	4	2

In family 4, both parents and sibling D excreted JCVs of subtype MY, each of which was unique upon sequencing of the IG regions. Sibling C did not excrete JCV.

In family 5, the father and sibling C excreted JCVs of subtype CY, while sibling E excreted a JCV of subtype MY. Sequencing of the IG regions revealed that the strain in the father was identical to that in sibling C. The mother and siblings D and F did not excrete JCV.

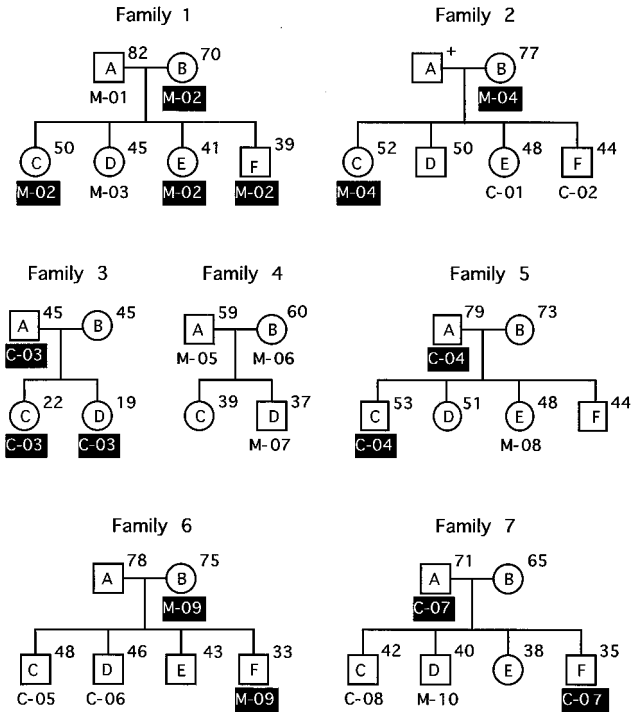

FIG. 3. Pedigrees showing JCV strains in urine excreted by the members of seven families. Families 1, 2, and 3 resided in Tokyo, and families 4 through 7 resided in Tsubata-cho, Ishikawa Prefecture. Males and females are indicated as boxes and circles, respectively, with ages given in years. JCV strains, identified as described in the text, are indicated below the boxes and circles (see text for naming of the strains). Common strains are shown by white letters on black backgrounds.

In family 6, the mother and sibling F excreted JCVs of subtype MY and two siblings (siblings C and D) excreted JCVs of subtype CY. The IG sequences showed that the strain in the mother was identical to that in sibling F. The strains in siblings C and D were unique. The father and sibling D did not excrete JCV.

In family 7, the father and two siblings (C and F) excreted JCVs of subtype CY, while sibling D excreted a JCV of subtype MY. Sequencing of their IG regions revealed that the strain in the father was identical to that in sibling F. The strains in siblings C and D were unique. The mother and sibling E did not excrete JCV.

In summary, we identified the same JCV strain in the offspring as well as in the fathers (three families) or mothers (three families) of the six families except family 4; in the children of six families except family 3, we identified unique strains that were undetected in the parents. In total, common strains were detected in half of the JCV-positive children (nine children), while unique ones were present in the remainder (nine children; JCV was not detected in four children). No identical strain was detected in a comparison among families.

DISCUSSION

In this and previous studies (7) we attempted to elucidate the routes of JCV transmission by tracing urinary JCV among the members of families. In the previous study, we used restriction fragment length polymorphism analysis to identify the subtypes of JCVs in individuals. The subtype distribution among the members of the families suggested that JCV transmission occurs both within and outside the family. Naturally, an identical subtype between two strains does not necessarily imply that they are the same strain. In the present study, therefore, we used nucleotide variations in the IG regions to distinguish different JCV strains detected in the members of seven families. According to the IG sequences, we could identify 18 different strains, 8 belonging to subtype CY and 10 belonging to subtype MY. This resolution of strains enabled us to further elucidate the route of JCV transmission. In short, we identified both common and unique strains in the children of most of the families studied. Common strains were those detected in children as well as in the child's father or mother;

unique strains were those detected only in children. This finding clarified several unique aspects of JCV transmission.

Since most individuals are infected during childhood (9, 13), the presence of common strains in children and parents indicates that transmission of JCV from parents to children occurs frequently. On the basis of the data presented in Fig. 3, we estimated that about 50% of the JCV transmission among humans occurs by this route. This route of JCV transmission should be distinguished from the vertical one, because the current data showed that JCV was transmitted not only from mothers but also from fathers. Furthermore, serological and seroepidemiological studies (3, 4, 13) have also ruled out the vertical transmission of JCV.

The unique strains in the offspring suggested that sources of JCV infection also exist outside the family. Since other polyomaviruses in urine or feces may enter the human body by oral or respiratory routes (10) and JCV is excreted in urine by most infected adult humans (6, 7), it is likely that the source of JCV for infection is urinary JCV. Because urinary excretion of JCV starts at early adulthood in most infected individuals (7), children have numerous chances to come into contact with urinary JCV which may be excreted by their parents, grandparents, babysitters, neighbors, and so on. Since, in general, children are in daily contact with their parents, children would most often be infected with JCV by this route. However, for some children, contact with JCV excreted by persons other than their parents may result in chance infection. Such children would not be superinfected with JCV from their parents, because superinfection with JCV in humans is very rare (7).

We found that in two families (families 1 and 3), multiple children excreted the same strain (Fig. 3). This suggested another route of JCV transmission, that is, transmission among siblings. A child who was infected by the JCV transmitted from its mother may have shed progeny JCV during primary infection. The progeny in turn may have caused the secondary infection in other children. However, this mode of JCV transmission seems to be rare, if it occurs at all, since children in many families were not infected with the same strain (Fig. 3). Alternatively, the children in families 1 and 3 may have been infected with JCV from their parents on different occasions.

Since JCV is phylogenetically related to the simian polyomavirus simian virus 40 (5), it is reasonable to assume that JCV has persisted in humans for a long time, probably since the emergence of human beings. For a virus to persist in the human population for generations, it is essential that the virus be passed to younger hosts, in whom it would persist and generate progeny viruses to be transmitted to the coming generation. Therefore, the mode of JCV transmission revealed in this report, that is, transmission from parents to children, may have allowed JCV to persist in the human population for a long time.

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REFERENCES

1. **Ault, G. S., and G. L. Stoner.** 1992. Two major types of JC virus defined in progressive multifocal leukoencephalopathy brain by early and late coding region DNA sequences. J. Gen. Virol. **73:**2669–2678.
2. **Chesters, P. M., J. Heritage, and D. J. McCane.** 1983. Persistence of DNA sequences of BK virus and JC virus in normal human tissues and in diseased tissues. J. Infect. Dis. **147:**676–684.
3. **Coleman, D. V., M. R. Wolfendale, R. A. Daniel, N. K. Dhanjai, S. D. Gardner, P. E. Gibson, and A. M. Field.** 1980. A prospective study of human polyomavirus infection in pregnancy. J. Infect. Dis. **142:**1–8.
4. **Daniel, R., K. Shah, D. Madden, and S. Stagno.** 1981. Serological investigation of the possibility of congenital transmission of papovavirus JC. Infect. Immun. **33:**319–321.
5. **Frisque, R. J., G. L. Bream, and M. T. Cannella.** 1984. Human polyomavirus JC virus genome. J. Virol. **151:**458–469.
6. **Kitamura, T., Y. Aso, N. Kuniyoshi, K. Hara, and Y. Yogo.** 1990. High incidence of urinary JC virus excretion in nonimmunosuppressed older patients. J. Infect. Dis. **161:**1128–1133.
7. **Kitamura, T., T. Kunitake, J. Guo, T. Tominaga, K. Kawabe, and Y. Yogo.** 1994. Transmission of human polyomavirus JC virus occurs both within the family and outside the family. J. Clin. Microbiol. **32:**2359–2363.
8. **Kwok, S., and R. Higuchi.** 1989. Avoiding false positives with PCR. Nature (London) **339:**237–238.
9. **Padgett, B. L., and D. L. Walker.** 1973. Prevalence of antibodies in human sera against JC virus, an isolate from a case of progressive multifocal leukoencephalopathy. J. Infect. Dis. **127:**467–470.
10. **Padgett, B. L., and D. L. Walker.** 1976. New human papovaviruses. Prog. Med. Virol. **22:**1–35.
11. **Padgett, B. L., D. L. Walker, G. M. Zurhein, R. J. Eckroade, and B. H. Dessel.** 1971. Cultivation of papova-like virus from human brain with progressive multifocal leukoencephalopathy. Lancet **i:**1257–1260.
12. **Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239:**487–491.
13. **Taguchi, F., J. Kojima, and T. Miyamura.** 1982. Prevalence rate and age of acquisition of antibodies against JC virus and BK virus in human sera. Microbiol. Immunol. **26:**1057–1064.
14. **Tominaga, T., Y. Yogo, T. Kitamura, and Y. Aso.** 1992. Persistence of archetypal JC virus DNA in normal renal tissue derived from tumor-bearing patients. Virology **186:**736–741.
15. **Yogo, Y., T. Kitamura, C. Sugimoto, T. Ueki, Y. Aso, K. Hara, and F. Taguchi.** 1990. Isolation of a possible archetypal JC virus DNA sequence from nonimmunocompromised individuals. J. Virol. **64:**3139–3143.