Progressive multifocal leukoencephalopathy (PML) is caused by the human polyomavirus JC (JCV), and there are at least 4 different genotypes of JCV in the United States. Type 1 strains are of European origin, whereas type 2 and 3 strains are of Asian and African origin, respectively. JCV type 4 strains are derived from a type 1/3 recombinant. In this study, the genotype distribution of JCV strains found in brain tissue or cerebrospinal fluid of 50 PML patients was compared with JCV genotypes excreted in the urine of 103 control subjects. Type determination was based on the polymerase chain reaction–amplified partial sequence of the VP1 coding gene and the noncoding region left of ori. Brain tissues from patients with PML were infected with a significantly higher proportion of JCV type 2 strains than were urine samples from the control group ($P = .004$). This evidence indicates a biologic difference between JCV genotypes and suggests a difference in their potential to cause PML.

JC virus (JCV) infects 70%–90% of the adult population [1]. The virus regularly persists in the kidneys [2], and by use of polymerase chain reaction (PCR), viral DNA can be detected in the urine of 30%–40% of healthy adults at any given time [3]. Peripheral blood lymphocytes also harbor the virus [4, 5]. In association with immunodeficiency, JCV establishes a lytic infection of oligodendrocytes and an abortive infection of astrocytes [6] and causes the central demyelinating disease progressive multifocal leukoencephalopathy (PML). For 25 years, only ~200 cases of PML were reported worldwide [7]; however, that number has increased rapidly with the spread of human immunodeficiency virus type 1 (HIV-1) infection [8]. PML is found in ~5% of autopsied AIDS patients [9].

The course and pathology of PML in persons with AIDS are similar to those as originally described in HIV-negative individuals [8, 9]. The most common initial symptoms of PML include motor weakness, ataxia, visual defects, and cognitive and speech deficits [7, 8, 10]. The outcome is usually fatal within 3 months to 1 year after onset [8]. Survival beyond 1 year is associated with manifestation of PML early in the course of AIDS, a higher CD4+ lymphocyte count, a prominent inflammatory response in biopsied lesions, and contrast enhancement on magnetic resonance imaging [11]. Lesions are most commonly found in white matter and can be present in any part of the central nervous system. The diagnosis of PML includes the detection of demyelinating lesions by magnetic resonance imaging [8] and the demonstration of the characteristic pathology or the presence of the virus in a brain biopsy [12]. PCR detection of JCV DNA in cerebrospinal fluid (CSF) is indicative of the presence of PML lesions [13, 14] and provides an additional approach to diagnosis.

The 5.1-kb super-coiled circular DNA of JCV encodes six or more proteins [15]. Viral genes are transcribed bidirectionally from the regulatory region in an early and a late phase (figure 1). In PML-affected tissue samples, the JCV regulatory region to the right of ori shows a rearranged pattern derived from the "archetypal" configuration found in urinary tract virus [17–19]. Each rearrangement is unique and consists of deletions and duplications within the promoter/enhancer. The archetypal kidney form is the infectious form of the virus [18, 19]. The early proteins, large (T) and small (t) antigens, regulate transcription of the VP1-3 genes following viral DNA replication [12, 20]. The mechanisms by which coinfection with HIV-1 may promote JCV replication are uncertain. One possibility is that the HIV-1 Tat protein transactivates transcription of the late region of JCV [21, 22].

Worldwide, there are different JCV genotypes, as determined by restriction fragment length polymorphism [23] or analysis
of the sequence variations within the major capsid protein gene (VP1) and the regulatory protein gene (T antigen) [24–26]. Of the 4 major genotypes known, JCV type 1 predominates in Europe, whereas JCV type 2 is found in Asia. A third genotype has also been characterized in urine from HIV-positive patients in Tanzania [26]. Type 3 infections are uncommon among the general population in the United States, although the prevalence of these African strains in a large cohort of African Americans remains to be determined. In addition to these 3 basic types, a fourth type comprises ~16% of the strains detected in the United States. These type 4 strains appear to have originated from a recombination event between JCV type 1 and a short segment of type 3 in the VP1 gene [3]. The present wide dissemination of type 4 strains in the United States suggests a selective advantage due to increased infectivity or enhanced replication.

In the noncoding regulatory region, distinction between JCV types 1 and 2 is possible only to the left of ori but not within the archetypal regulatory region right of ori (figures 1 and 2) [3]. In JCV type 3, there is a unique “C” at position 133 [26]. JCV types 1 and 4 are not distinguishable anywhere in the regulatory region, as the type 4 complete genome is very closely related to the type 1 sequence (unpublished data).

Urine is a valuable source of JCV for investigation of the epidemiologic aspects of this neurotropic virus and its genotypes. Previous studies have shown that a single JCV strain can infect multiple organs of an individual [16, 27]. Although the genotypes of JCV are well characterized, nothing has been known about possible type-specific biologic behavior. We therefore examined the genotype profile of JCV strains in brain tissue and CSF of PML patients and compared it to the genotype distribution of virus excreted in the urine of persons with and without HIV-1 infection. A significantly higher proportion of JCV type 2 strains was present in tissue samples with PML than in urine from controls. This suggests that infection with type 2 strains of JCV is more likely to cause PML than is infection with types 1 or 4.

Patients and Methods

PML tissue. A total of 41 JCV-positive clinical samples (35 brain, 6 CSF) were collected from 32 male and 7 female PML patients and sent to the National Institutes of Health (NIH) by different neurologic clinics and neuropathologic departments primarily in the Los Angeles area. Archival material from the central and eastern states was provided by Duard L. Walker (Department of Medical Microbiology, University of Wisconsin, Madison). Both brain tissue and CSF were available from 2 of these 39 patients. Of the 39 PML patients, 24 (62%) were between 30 and 40 years old (range, 11–57). In 10 persons, the immunodeficiency underlying PML was not related to AIDS: 2 had Hodgkin’s disease, 3 had lymphoproliferative diseases, and 1 each had reticulum cell sarcoma, systemic lupus erythematosus, pulmonary sarcoma, or combined immunodeficiency syndrome (in an 11-year-old boy). No definite underlying immunodeficiency was found in a 45-year-old African-American woman. The 11 other JCV strains from PML patients included in the statistical analysis have been characterized previously [24]. Table 1 shows the ethnic composition and median age of and the number of males and females in the patient and control cohorts.

Urine samples. Urine samples were collected at a neurologic clinic and an AIDS clinic in the Los Angeles area. Samples from Pennsylvania and Maryland came from a general medical clinic and from healthy volunteers in the NIH work force. In total, 213 HIV-negative individuals (reported in part in [3]) and 32 HIV-positive individuals were included. To assure ethnic comparability with the PML group, 84% of controls from different geographic regions were of European origin. Since most PML patients came from the area around Los Angeles, an ethnically comparable control group of 92 patients from Los Angeles with noninfectious disease were defined by positions 1843 and 1850 [3, 29]. The 50-cycle, two-step PCR program included 1 min for annealing and elongation at 63°C followed by 1 min for denaturation at 94°C. When the complete genome amplification (described below) was unsuccessful, the regulatory region, including three typing sites left of ori, was

Figure 1. Circular genome of JCV. Archetypal (kidney) or re-arranged (brain) part of regulatory region (a/r) is located to right of origin of replication (ori). DNA encoding viral proteins (arrows) is transcribed from divergent regulatory region. Squares indicate regions to left of ori and in VP1 gene that were analyzed to determine JCV genotypes. Agno = agnoprotein, VP1-3 = capsid proteins, T = large T antigen, t = small t antigen. Redrawn from [16].
amplified by primers JRR-25 and -28 from brain tissue and CSF from PML patients. Amplification of the regulatory region right of ori with primers JRR-1 and -8 yields a fragment that lacks the three typing sites left of ori but permits characterization of regulatory region rearrangements. All short-range PCRs were performed using Ultima DNA polymerase with 3′-5′ proofreading activity (Perkin-Elmer Cetus, Norwalk, CT) in a standard PCR buffer containing 1.5 mM Mg²⁺.

**PCR amplification of the complete JCV genome.** JCV-positive samples were further processed for complete PCR amplification of the 5.1-kb JCV genome as described previously [30]. In brief, the lysed sample was digested with the restriction enzyme BamHI before PCR. Viral DNA was amplified using primers BAM-1 and BAM-2 with a 5′ overlap at the restriction enzyme site (GGATCC; table 2). The combination of thermostable DNA polymerases with and without 3′-5′ exonuclease activity facilitates long-range PCR, and buffer additives enhance strand separation (GeneAmp XL PCR; Perkin-Elmer Cetus). The reaction was run for 39 cycles with increasing annealing and extension time (6–12 min) at 64°C and denaturation (30 s) at 94°C, followed by a final extension (10 min) at 72°C. The reaction was stopped at 4°C.

**Direct cycle sequencing.** After gel purification according to the Qiagen procedure (Qiagen, Chatsworth, CA), both short- and long-range PCR products were used as templates for direct cycle sequencing.

Table 1. Distribution of ethnicity, age, and sex in JCV-positive PML cohort and control cohorts.

<table>
<thead>
<tr>
<th>Cohort (no.)</th>
<th>EUAM</th>
<th>AFAM</th>
<th>HISP</th>
<th>UNK</th>
<th>F/M</th>
<th>Median age, years</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PML</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (50)</td>
<td>80</td>
<td>10</td>
<td>4</td>
<td>6</td>
<td>10/40</td>
<td>37</td>
</tr>
<tr>
<td>CA (26)</td>
<td>80</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>1/25</td>
<td>36</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (103)</td>
<td>84</td>
<td>9</td>
<td>7</td>
<td>0</td>
<td>23/80</td>
<td>48</td>
</tr>
<tr>
<td>HIV negative in PA, MD (43)</td>
<td>93</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>18/25</td>
<td>47</td>
</tr>
<tr>
<td>HIV negative in CA (48)*</td>
<td>79</td>
<td>13</td>
<td>8</td>
<td>0</td>
<td>5/43</td>
<td>48</td>
</tr>
<tr>
<td>HIV positive in CA (12)</td>
<td>75</td>
<td>0</td>
<td>25</td>
<td>0</td>
<td>0/12</td>
<td>36</td>
</tr>
</tbody>
</table>

**NOTE.** F/M = female/male, EUAM = American of European origin, AFAM = African American, HISP = Hispanic, UNK = unknown, CA = California, PA = Pennsylvania, MD = Maryland.

* Includes 42 patients with multiple sclerosis.
Table 2. JCV primers used for polymerase chain reaction and cycle sequencing of brain tissue samples and urinary cell pellets for JCV type determination.

<table>
<thead>
<tr>
<th>Code</th>
<th>5′–3′ Position</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>JRR-25</td>
<td>CATGGATTCCTCCTATTCCAGCA</td>
<td>4981–5003</td>
</tr>
<tr>
<td>JRR-1</td>
<td>CTTCTGAGTAAAGCTTGAGGAGG</td>
<td>5103–5125</td>
</tr>
<tr>
<td>JRR-8</td>
<td>GGCAGAAGACATGGCAGCTGGA</td>
<td>289–267</td>
</tr>
<tr>
<td>JRR-28</td>
<td>TCACAGAAGCTTTACGTGACAGC</td>
<td>313–291</td>
</tr>
<tr>
<td>JLP-1</td>
<td>CTCAATGTTGAGGCTG(T)ACTT²</td>
<td>1769–1790</td>
</tr>
<tr>
<td>JLP-4</td>
<td>ATGAAAGCTGGTGCCCTGCACT</td>
<td>1897–1876</td>
</tr>
<tr>
<td>BAM-1</td>
<td>GGGATCCTGTGTTTTCATCATGCACTGGC</td>
<td>4306–4333</td>
</tr>
<tr>
<td>BAM-2</td>
<td>AGGATCCCAACACTCTACCCACC</td>
<td>4313–4290</td>
</tr>
</tbody>
</table>

* Numbering based on JCV(Mad-1).

² Redundancy compensates for difference in sequence of JCV types 1 and 2.

sequencing as described previously [26]. In brief, primers JLP-1 or -4 for the VP1 coding region and JRR-25 and -28 or JRR-1 and -8 for the regulatory region were end-labeled with [33P]ATP (Amersham, Arlington Heights, IL). Radiolabeled primers were combined with 2%–5% of the cleaned template, DNA polymerase, and the chain-terminating deoxyribonucleotides (SequiTherm cycle sequencing; Epicentre Technologies, Madison, WI). The initial denaturation for 1 min at 95°C was followed by 30 cycles of 30 s at 95°C for denaturation and 1 min at 60°C (coding region) or 65°C (regulatory region) for annealing and elongation. Products were run on a 6% polyacrylamide gel containing 50% (wt/vol) urea (National Diagnostics, Atlanta), transferred to chromatography paper (3MM; Whatmann, Maidstone, England), dried under vacuum, and exposed to radiographic film for 16–48 h.

Reference sequences. Reference sequences, with the five-digit GenBank/EMBL Data Library accession numbers shown in square brackets, were JCV(Mad-1) [J02227] for the complete genome [16]; JCV(CY) [M35834] for the archetypal regulatory region [18]; and JLP-1 and -4 fragment of type 1 [U21842], type 2 [U21843], and type 3 [U21844] [26]. Numbering of the JCV coding region and the regulatory region left of ori is that of JCV(Mad-1).

Statistical methods and software. JCV type distribution in paired cohorts was analyzed in a 2 × 2 contingency table, using the x² statistic with Yates’s correction for continuity (SigmaStat program; Jandel Scientific, San Rafael, CA). If any cell of a 2 × 2 contingency table contained less than five expected observations, Fisher’s exact test was applied. For this retrospective study, the relative risk of developing PML as a result of infection with JCV type 2 was calculated as the product of type 2 strains in PML and non–type 2 strains in non-PML divided by the product of non–type 2 strains in PML and type 2 strains in non-PML. Primer design utilized the Oligo program (version 5.0; NBI, Plymouth, MN).

Results

JCV in Brain Tissue and CSF from PML Patients

JCV genotypes. The sequence of different JCV genotypes in the JLP-1– and -4–amplified VP1 fragment is illustrated in figure 3. JCV DNA was amplified by PCR with primers JLP-1 and -4 in 35 brain and 6 CSF samples from 39 PML patients. Both CSF and brain from 2 AIDS patients were infected with the same JCV genotype and were regarded as 1 isolate. Viral DNAs representing 2 different JCV types (1 and 2) were identified in the same brain sample from a male AIDS patient [31]. In addition, brain tissue from a female patient with Hodgkin’s disease had distinguishable subtypes of type 1 [31]. In total, 19 of the 41 viral sequences were identified as JCV type 1 in the JLP-1– and -4–amplified VP1 fragment (table 3). JCV type 2 and 4 were found in 20 and 2, respectively, of 41 viral sequences. Of 10 patients with non-AIDS related PML, 7 were infected with JCV type 1 strains and 3 with type 2 strains. In the case of 1 male AIDS PML patient, a JCV-positive urine sample was available and harbored the same genotype (type 2) as found in the brain biopsy sample.

JCV regulatory region. The viral regulatory region, in whole or in part, could be PCR-amplified and directly cycle-sequenced in 29 of the 41 JCV-positive clinical samples. All 29 regulatory regions analyzed right of ori were uniquely rearranged from the archetypal sequence found in urinary virus (for details see [32]). In 7 samples, the complete genome was amplified by PCR after restriction enzyme digestion, and the product was used for direct cycle-sequencing of the three typing sites left of ori (positions 5017, 5026, 5039) [24] (figure 2). In 12 other samples, the determination of JCV genotypes was confirmed by sequence analysis of the region left of ori by use of the PCR products amplified by JRR-25 and -28. The genotypes identified in these 19 samples confirmed the type determination in the VP1 fragment. In strain number 209, deoxyguanosinase at typing site 5039 in type 2 was replaced by deoxyadenosinase. Since deoxyadenosinase at this site is unique and the partial VP1 sequence was unambiguous, this strain was classified as type 2. Strain number 210 had a point mutation at position 5040, which is not a typing site (figure 2).

JCV Excretion in the Urine of Non-PML Patients

Controls without HIV-1 infection. The age of the 213 persons without known HIV-1 infection ranged from 10 to 94
Figure 3. Partial VP1 gene amplified by primers JLP-1 and -4 (see table 2). JCV genotypes are defined by 4 major typing sites (1804, 1818, 1869, 1870). Positions 1837, 1843, and 1850 allow subtype determination. K = G or T, R = G or A, and Y = T or C. Numbering based on JCV(Mad-1) [16]. Dashes indicate identity with type 1.

years. The group included 72 females and 141 males, of which a total of 91 (42.7%) tested positive for JCV excretion, as detected by PCR, in at least 1 urine sample. More than 1 urine sample was analyzed for 54 individuals. Excretion status was stable in 24 of the excreters and in 21 of the nonecreters. For 1 excretor over a 17-month period, 9 consecutive urine samples were analyzed for JCV genotype, with identical results. One nonecretor was tested seven times and remained negative. In 9 persons, the excretion status changed without a change in the JCV genotype excreted: 4 changed from JCV-positive to JCV-negative, 2 from negative to positive, 1 from negative to positive, and 2 from positive to negative to positive.

Controls with HIV-1 infection. Urine samples from 32 HIV-positive persons without PML (31 men, 1 woman; age range, 31–68 years) were tested by PCR for JCV DNA excretion: Samples tested with primers JLP-1 and -4 were positive in at least 1 sample in 12 male donors (38%). A second sample taken 1–6 months later was available from 13 patients: excretion of a specific genotype was confirmed in 3, excretion of JCV was transient in 3, and 7 initial nonecreters remained negative for JCV in the urine.

<table>
<thead>
<tr>
<th>JCV Type</th>
<th>Brain (PML patients)</th>
<th>HIV Negative</th>
<th>HIV Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19 (46)</td>
<td>55 (58)</td>
<td>8 (67)</td>
<td>63 (59)</td>
</tr>
<tr>
<td>2</td>
<td>20 (49)</td>
<td>22 (23)</td>
<td>3 (25)</td>
<td>25 (23)</td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>3 (3)</td>
<td>—</td>
<td>3 (3)</td>
</tr>
<tr>
<td>4</td>
<td>2 (4.9)</td>
<td>13 (14)</td>
<td>1 (8)</td>
<td>14 (13)</td>
</tr>
<tr>
<td>Unclassified</td>
<td>2 (2)†</td>
<td>2 (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>41 (100)</td>
<td>95 (100)</td>
<td>12 (100)</td>
<td>107 (100)</td>
</tr>
</tbody>
</table>

NOTE. HIV = human immunodeficiency virus type 1.
* Includes 45 JCV strains from 43 persons reported in [3, 31].
† All in African Americans.
* No presently defined genotype based on sequence data available.

Age and JCV excretion. The frequency of JCV excretion with increasing age in HIV-positive patients did not differ significantly from that in HIV-negative individuals; however, the proportion of individuals excreting JCV generally increased with age. In both groups, >40% of persons >30 years old tested positive for JCV DNA in a single urine sample. Infection with HIV-1 did not increase the number of individuals excreting JCV. For patients from whom a second urine sample was obtained, only results for the first samples were included in this age analysis.

JCV genotypes. On the basis of the sequence of the JLP-1– and -4–amplified fragment within the VP1 gene (figure 3), all 4 major JCV genotypes could be found in urine samples from persons without PML (table 3). Four males showed signs of coinfection with 2 different JCV genotypes: types 1 and 2 strains in 2 persons, types 2 and 3 in another, and 2 distinguishable subtypes of JCV type 1 in a 38-year-old man from Pennsylvania. Among a total of 107 detected strains, JCV type 1 was the most prevalent, accounting for 55 (58%) of 95 strains in HIV-negative persons and 8 (67%) of 12 strains in HIV-positive persons (total: 63/107 [59%]). JCV type 2 strains accounted for 22 (23%) of 95 strains in HIV-negative persons and 3 (25%)
of 12 in HIV-positive persons (total: 25/107 [23%]). JCV type 4 strains represented 13 (14%) of 95 strains in HIV-negative individuals and 1 (8%) of 12 in HIV-positive individuals (total: 14/107 [13%]). Three examples of a type 3–related sequence (3/107 [3%]) were found in the HIV-negative control group. Two isolates from 31- and 55-year-old Californian patients with multiple sclerosis could not be classified as one of the presently defined genotypes and, thus, await further analysis; they were not included in the statistical analysis of genotype distributions. When results for HIV-positive controls from Los Angeles were compared with results for HIV-negative controls also from Los Angeles, the excretion of JCV type 2 was marginally increased, and the frequency of type 4 was decreased in the HIV-positive cohort, but the genotype distribution of type 2 and non–type 2 strains was not significantly different ($P = 1.0$).

A comparison of the 107 JCV strains from the East (Pennsylvania and Maryland; total, 45 strains) and the West (California; total, 62 strains, with 2 unclassified) showed that type 1 dominates in both groups (64% in the East, 52% in the West), followed by type 2 (18% in the East, 28% in the West). Type 3 strains accounted for 2% (East) and 3% (West), and type 4 strains were similarly represented with 16% (East) and 11% (West). Therefore, the geographic origin of the urine samples from ethnically similar groups (table 1) did not significantly influence the JCV type distribution of type 2 and non–type 2 strains ($P = .35$).

Genotype distribution can vary with different ethnic groups. Of all classified JCV strains from both PML and control groups, 24 (17%) of the 144 isolates came from 9 Hispanic Americans and 15 African Americans. In the Hispanic-American group, JCV type 2 was identified four times, type 1 three times, and type 4 once. A 55-year-old male excreted an unclassified JCV strain. Among the African Americans, JCV type 1 was found five times, JCV type 4 was found four times, and JCV types 2 and 3 were found three times each. Of note, all three examples of type 3 strains in this study were from African Americans. As mentioned above, the ethnicity of the probands in the PML and control groups was comparable. The proportion of JCV-positive Americans of European, African, or Hispanic origins was (in percent) 93:7:0 in the Pennsylvania-Maryland control group, 78:10:12 in the California control group, and 80:10:4 in the PML group with 3 patients (6%) of unknown ethnicity.

**Statistical Significance of Differing JCV type Distributions**

Besides the 41 PML strains reported in this study, the statistical analysis included JCV strains from 11 PML patients described by Ault and Stoner [24]. These samples from 9 white and 2 African-American persons came from eastern and central states of the United States. This earlier study characterized JCV in brain tissue of 6 AIDS and 5 non-AIDS PML patients, of which 6 were positive for JCV type 1 and 5 were positive for JCV type 2. To determine whether the frequency of JCV type 2 and non–type 2 strains differed significantly in brain tissue from PML patients and urine samples from controls, the numbers of JCV strains were analyzed in a 2 × 2 contingency table, using the $\chi^2$ statistic. The type 2 proportion was significantly higher ($P = .004$) in strains from all PML tissues (AIDS PML and non-AIDS PML) than in strains from urine samples of all non-PML individuals (HIV-positive and HIV-negative) (table 4). By use of a 2 × 4 contingency table for the $\chi^2$ analysis of the distribution of JCV types 1–4 in all strains of PML and non-PML individuals, the significant difference in genotype distribution was confirmed ($P = .011$). To minimize the possible influence of geographic variation in the genotype distribution, the 27 JCV strains from PML patients living in the Los Angeles area (16 type 2, 11 non–type 2) were compared statistically with 60 urinary JCV strains (17 type 2, 43 non–type 2) of individuals with comparable ethnicity from the same geographic area. This comparison confirmed the significantly increased frequency of type 2 strains in PML tissue ($P = .012$).

As mentioned above, 6 persons (2 with PML, 4 controls) were infected with 2 distinguishable JCV strains. Repeating the above analysis in 2 × 2 contingency tables but excluding data from these doubly infected patients for all PML strains and all controls ($P = .002$) and the California cohorts ($P = .014$) underlines the significantly increased frequency of type 2 strains in PML patients.

The genotype distribution among 37 isolates from AIDS PML patients was 19 type 2 and 18 non–type 2 strains, compared with 6 type 2 and 9 non–type 2 strains in non-AIDS PML patients. The tendency toward an increased number of type 2 strains in AIDS PML was not statistically significant ($P = .66$).

**Discussion**

This study provides the first evidence that JCV type 2 can be identified more frequently in tissue from patients with PML than in urine samples from controls without PML. The other JCV genotypes found in the United States are types 1, 3, and 4. The higher proportion of JCV type 2 strains in PML-affected tissue indicates that infection with this genotype is associated

**Table 4.** JCV type distribution in brain tissue samples from patients with PML and in urine samples from controls without PML.

<table>
<thead>
<tr>
<th>Type</th>
<th>Brain (PML patients)</th>
<th>Urine (controls)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 2</td>
<td>25</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Non–type 2</td>
<td>27</td>
<td>80</td>
<td>107</td>
</tr>
<tr>
<td>Totals</td>
<td>52</td>
<td>105</td>
<td>157</td>
</tr>
</tbody>
</table>

NOTE. $P = .004$ ($<.05$ was considered significant).

* Includes 11 patients from [24].
with a 3-fold increased risk of developing PML compared with infection with non-type 2 strains.

As outlined here and elsewhere [17, 27], JCV strains from the urinary tract and central nervous system of a PML patient are of the same coding region genotype, but rearrangements of the viral regulatory region right of ori are invariably found in the patient’s brain. All 29 regulatory region sequences from PML-affected samples showed rearrangements characteristic of PML configurations described previously [19], and each was rearranged uniquely and differently from common laboratory strains [32]. Since JCV types 1 and 2 are not distinguishable within the rearranged region right of ori, type-specific biologic behavior seems unlikely to be a function of either the archetypal regulatory region or the rearranged (PML-type) regulatory region per se. With the exception of JCV type 3, the nucleotide polymorphisms described in the regulatory region [3, 18, 26, 33] are unrelated to the coding region genotypes that are the subject of this study. Furthermore, urinary type 4 strains, which have the same archetypal regulatory region as type 1 strains but differ in amino acid sequence in VP1, seem to have had a selective advantage in the US population [3]. Therefore, type-specific behavior may in large part reflect sequence differences between JCV coding region genotypes.

In this study, the control group consisted of 213 persons not infected with HIV-1 and 32 HIV-infected persons. JCV excretion in HIV-positive individuals confirms that JCV DNA can be detected in >40% of single urine samples from persons >30 years old [3]. HIV-1 infection did not appear to increase the overall frequency of JCV excretion. This agrees with previously published results in which neither HIV-positive patients with low CD4 lymphocyte counts [34, 35] nor multiple sclerosis patients treated with cyclosporine [29] showed an increased frequency of JC viruria compared with immunocompetent controls. Sixty-seven probands were tested more than once for JCV excretion in their urine, and in most, the excretion status was stable. Twelve persons had transient viruria, indicating that JCV excretion can be variable. It may therefore be useful to test serial samples in order to increase the probability of identifying the JCV genotype carried by a particular individual.

The assessment of the global prevalence of PML in relation to the distribution of JCV types in a population is of interest. In Africa, for example, PML is thought to be uncommon among AIDS patients [36], although initial studies have shown that JCV type 3 excretion and HIV-1 seropositivity are detectable at a high frequency in the same population groups [26, 37]. In the current study, we have established that in the United States, a significantly higher proportion of type 2 JCV strains are found in brain tissues from persons with PML than in urine specimens from persons without PML. The molecular biologic basis for this increase of type 2 strains in PML remains to be determined.

Acknowledgments

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References


