JC Virus Genotypes in France: Molecular Epidemiology and Potential Significance for Progressive Multifocal Leukoencephalopathy


JC virus (JCV) induces progressive multifocal leukoencephalopathy (PML), especially in human immunodeficiency virus (HIV)-infected patients. Although JCV genotypes have primarily been associated with geographic patterns, a distinctive neuropathogenicity was recently attributed to genotype 2. A multicenter study was conducted to describe the distribution of JCV genotypes in France and to investigate correlations between genotypes and PML. Genotypes were determined by sequencing 494 bp in the VP1 capsid gene. Peripheral JCV was studied in 65 urine samples from 43 HIV-infected patients and from 22 control subjects. Genotypes 1, 4, 2, and 3 were detected in 52.3%, 30.8%, 12.3%, and 4.6% of the samples, respectively. In 56 brain or cerebrospinal fluid samples, PML-associated JCV of genotypes 1, 2, 4, and 3 was found in 66%, 19.7%, 8.9%, and 5.4%, respectively. Infection with JCV genotypes 1 or 2 was correlated with PML (odds ratio, 3.29). On the other hand, infection with JCV genotype 4 could represent a lower risk for PML.

Polyomavirus JC (JCV) is the causative agent of a rare central nervous system demyelinating disease known as progressive multifocal leukoencephalopathy (PML). This fatal brain infection of immunocompromised persons occurred in ~5% of AIDS patients before highly active antiretroviral therapies became available [1]. However, JCV infection is much more frequent: the virus infects 70%–90% of the adult population worldwide [2, 3]. After primary infection, which occurs during childhood without any known associated clinical manifestation, the virus persists lifelong in the kidneys. It is excreted in the urine of ~40% of the general population >30 years old [4–7].

The JCV genome is a 5.1-kb supercoiled circular DNA divided into 3 main regions: the noncoding regulatory region, which contains the origin of replication; the early region, which encodes viral regulatory proteins large T and small t; and the late region, which encodes the structural proteins VP1, VP2, VP3, and agnoprotein. The JCV noncoding regulatory region supports an extensive strain-to-strain variability. The sequences identified in PML-affected brains show a rearranged pattern by deletions or duplications derived from the “archetypal” form found in urinary tract virus [8–10]. In contrast, the coding regions are highly conserved. Polymerase chain reaction (PCR) and direct sequence analysis of the gene coding for the major capsid protein VP1 allow the distinction of ≥7 genotypes of JCV [5, 11–13]. The JCV genotypes evolved in specific geographic regions. JCV type 1 predominates in Europe and the United States, whereas type 2, which is found in Asia, is divided into 2 main subtypes—2a in northeastern Asia, including China and Japan, and 2b in western Asia. JCV types 3 and 6 are African genotypes, found in Tanzania and in Ghana, respectively [14, 15]. Type 4, which presently is found only in the United States, seems to be a recombinant of the major part of the type 1 genome and a short segment of the type 2 VP1 gene [5, 16]. JCV type 5 was shown to be a recombinant between type 2 and type 6 [17]. Type 7, which is closely related to type 2 [12], is the dominant strain in southern China and Southeast Asia.

Because JCV type 1 is most prevalent in other European countries, the first aim of this study was to determine which genotypes could be found in France. We therefore examined the genotype profile of JCV strains in 65 JCV-positive urine samples from 5 different French cities.

Previous studies in the United States suggested that JCV type 2, especially type 2b, was more likely to cause PML [16, 18].

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A different genotype distribution in France could prove helpful to confirm this biologic behavior difference between genotypes. Thus, we investigated the hypothesis of type-specific neuropathogenicity by determining the JCV genotypes in cerebral samples of 56 patients with PML and by comparing them with the urinary genotype distribution in 65 patients without PML.

Materials and Methods

Urine samples. Urine samples were collected in infectious diseases or internal medicine departments in hospitals in Bordeaux (southwestern France), Toulouse (south), Rennes (west), Rouen (north), and Paris. In total, 65 JCV-positive urine samples, taken from 54 immunocompromised patients with no sign of PML and from 11 immunocompetent persons, were included in this study. All samples were sent to the laboratories for diagnostic purposes. Among the immunocompromised patients, 43 were infected with human immunodeficiency virus (HIV), and 11 were organ or tissue graft recipients (bone marrow or kidney). Thirty-three patients came from Bordeaux, 13 from Rennes, 15 from Toulouse, 3 from Paris, and 1 from Rouen. The mean age of the patients was 41.6 years (range, 21–74 years; median, 39 years). Thirteen were women, and 52 were men. Ethnic origins were as follows: European, 93.8% (61); North African, 4.6% (3); and Asian, 1.5% (1).

Fresh urine samples (40–100 mL) were stored at 4°C until DNA extraction, with no previous treatment. Two different techniques, varying by the centers, were used for DNA extraction. In Bordeaux extraction, with no previous treatment. Two different techniques, recommended by the manufacturers. Both strands of the purified PCR products were sequenced with a ABI 377 automatic sequencer (PE Biosystems) with Sequencing Analysis 3.3 software (Applied Biosystems). The sequences were compared with references and with each other by use of Sequence Navigator software (Applied Biosystems).

The 494-bp amplified fragment of the VP1 gene contained 17 typing sites that differentiated the major JCV types and subtypes [5, 21–27]. However, it failed to distinguish between types 5 and 6.

Reference sequences. The sequences referred to were provided by GenBank and the European Molecular Biology Laboratory data library: complete genome JCV (Mad-1; accession no. J02227) [21]; VP1 sequence of type 1b (AF015527) [22], type 2a strain Tokyo-1 (AF030085) [23], type 2b strain GS/B (M20322) [24], type 2c (AF015534) [23], type 2d (AF015536) [23], type 3 (U73500) [25], type 4 (AF015528) [5], type 5 (AF015684) [23], type 6 (AF015537) [26], and type 7 strain Tai-3 (U61771) [27]. Numbering of the coding region is based on the JCV (Mad-1) sequence.

Statistical methods. JCV type distribution in brain and urine samples was analyzed in a 2 × 2 or 2 × 4 contingency table by use of the χ2 statistic with Yates’s correction for continuity. All variable analyses were done by use of Epi-Info (Centers for Disease Control and Prevention, version 6).

Results

JCV genotype distribution in urine. Table 1 illustrates type distribution among the 65 JCV strains excreted by 65 patients in 5 French cities. All 4 major types, types 1–4, were detected.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Rennes (n = 13)</th>
<th>Bordeaux (n = 33)</th>
<th>Toulouse (n = 15)</th>
<th>Paris (n = 3)</th>
<th>Rouen (n = 1)</th>
<th>Totala (n = 65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
<td>9 (13.8)</td>
</tr>
<tr>
<td>1b</td>
<td>4</td>
<td>11</td>
<td>9</td>
<td></td>
<td></td>
<td>24 (37)</td>
</tr>
<tr>
<td>1c</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>3 (4.6)</td>
</tr>
<tr>
<td>2a</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td>4 (6.2)</td>
</tr>
<tr>
<td>2b</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>4 (6.2)</td>
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<tr>
<td>2c</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td></td>
<td>3 (4.6)</td>
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<tr>
<td>2d</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>3 (4.6)</td>
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<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>3 (4.6)</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>12</td>
<td>3</td>
<td>2</td>
<td></td>
<td>20 (30.8)</td>
</tr>
</tbody>
</table>

aData are no. (%) of patients.
The dominant JCV type, type 1, was found in 52.3% (34/65) of the patients. Among type 1 viruses, 26.5% (9/34) belonged to subtype 1a and 70.6% (24/34) to subtype 1b. However, this study enabled us to describe a potential additional subtype, subtype 1c (table 2). This subtype differed in 2 typing sites, in positions 1940 and 2011. It was detected in 2 patients, once in urine and once in brain, in 2 different French cities.

The second most prevalent type was type 2, found in 30.8% (20/65) of the patients. Types 2 and 3 were found in 12.3% (8/65) and 4.6% (3/65) of the patients, respectively. Of the 8 JCV type 2 strains, 4 (50%) showed a subtype 2b sequence pattern, 2 (25%) a subtype 2c, 1 a subtype 2a, and 1 a subtype 2d. We did not identify any type 5 or 6 strains.

There was no correlation between JCV type distribution in urine and the geographic location of the patients, for the 3 hospitals which contributed mostly to this part of the study. The strains analyzed in 11 immunocompetent persons came from a single city. Comparison of these results with those obtained for 22 immunocompromised patients living in the same area showed no significant difference in genotype distribution (table 3).

The only 3 type 3 isolates were found in the 3 North African patients (Algeria and Morocco). The single Asian patient harbored the same genotype as the brain biopsy or cerebrospinal fluid samples (3 subtype 1b, 2 type 3, and 1 type 4).

### Statistical study of JCV type distribution

The existence of a correlation between JCV genotype and the occurrence of PML was first investigated individually for each genotype; no statistical link could be uncovered. In particular, infection with genotype 1 JCV was not associated with a significantly increased risk of PML, compared with other viral genotypes (odds ratio [OR], 1.74; 95% confidence interval [CI], 0.59–5.24) or genotype 1 infection (OR, 0.79; 95% CI, 0.25–2.45).

However, infection with genotype 1 or 2 was associated with a significant risk of developing PML, compared with other genotypes (OR, 3.29; 95% CI, 1.23–9).

### Discussion

JCV genotyping was never attempted in France before, although several studies provided data for other European countries. We took advantage of the existence of an Agence Nationale de Recherche sur le SIDA French Group for the Study of Polyomaviruses [28] to organize a nationwide study. Special attention was given to HIV-infected patients, because PML represents a dramatic opportunistic disease risk for them. This potential patient selection bias could have affected the interpretation of results. However, the comparison of JCV genotype...
significantly associated with PML (p < .001 nor was infection with genotype 2 alone was associated with a higher risk of developing PML, but neither infection with genotype 1 nor infection with genotype 2 alone was associated with a significant risk. Therefore, our observations indicated a link between genotype and neuropathogenicity, but they did not strictly support the genotype 2 hypothesis.

It was suggested that the rarity of genotype 2 viruses in West Africa was responsible for the lack of PML among AIDS patients in this region [31]. Such comments may be questionable, because patients in these regions of the world may die long before the onset of PML. The prevalence of PML in Asian developing countries would be interesting to analyze, because genotype 2 may represent most viral species among these patients.

On the other hand, the relative underrepresentation of type 4 JCV in the brain (3-fold less than in the periphery) was rather evocative. The possible limited pathogenicity of genotype 4 was suggested by Agostini et al. [18], but results did not reach significance. Although these data need to be confirmed, the presence of type 4 JCV in urine could represent a favorable factor for immunocompromised patients at risk of developing PML.

This potential link between neuropathogenicity and genotypes could be considered as artificial, because most control subjects in this study (Bordeaux) did not live in the same geographic area as did the patients with PML (Reims). However, Reims Hospital was for more than a decade the reference center for PML diagnosis in France and therefore tested samples from all French regions.

Although the connection between genotype and neuropathogenicity represents a controversial issue, its mechanism deserves further investigations. In addition to its structural function, VP1 plays other important roles in the polyomavirus life cycle. This protein possesses the receptor-binding domain, which makes it crucial in virus attachment and, consequently, in defining viral tropism and pathogenesis. As an example, a single-amino acid substitution in murine polyomavirus VP1 determines the plaque size and hemagglutination behavior of polyomavirus [32]. Subsequently, it has been shown that the presence of amino acid residue 92 at this position interferes with the binding of branched (2-6)-linked sialic acid chains, which serve as specific cell receptors for polyomavirus [33]. Infection of glial cells by human JCV is also mediated by an N-linked glycoprotein containing terminal (2-6)-linked sialic acids [34]. This initial interaction could be affected by amino acid variations of VP1 linked to the different genotypes and could modify the neuropathogenicity of those JCVs.
The need for multicenter studies, organized within a medical and scientific network such as ours, deserves to be underlined. However, because of the present efficacy of antiretroviral regimens, the potential link between neuropathogenicity and JCV genotypes, although it represents an interesting pathophysiological question, may not be determined easily in the future because of the limited number of patients now developing PML.

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References

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