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Absence of JC polyomavirus in stool samples of patients with multiple sclerosis despite high anti-JCV antibodies in serum

Finja Schweitzer [a], Anne Ladwig [a], Sarah Opala [a], Sarah Laurent [a], Michael Schroeter [a], Susan Goelz [b], Gereon R. Fink [a,c], Ulrike Wieland [d], Steffi Silling [d,1], Clemens Warnke [a,1,*]

[a] Department of Neurology, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany
[b] Oregon Health and Sciences University, Portland, OR, USA
[c] Cognitive Neuroscience, Institute of Neuroscience and Medicine (INM-3), Research Centre Jülich, Jülich, Germany
[d] National Reference Center for Papilloma- and Polyomaviruses, Institute of Virology, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany

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ABSTRACT

Background: Natalizumab is an effective treatment for relapsing multiple sclerosis (MS). During therapy, individuals are at increased risk of developing progressive multifocal leukoencephalopathy (PML). So far, the relevant reservoir for PML-type JC polyomavirus (JCV) remains elusive. We here tested if the detection of JCV-DNA in stool of persons with MS treated with natalizumab could be a future tool for PML risk assessment.
Methods: The presence of JCV-DNA in stool, urine, and whole blood of MS patients treated with natalizumab and known serum anti-JCV antibodies index values (IV) was studied. Different DNA extraction methods, real-time (RT) and droplet digital (dd) PCR techniques were compared. JCV isolates were screened for PML-associated variants by sequencing.
Results: Thirty MS patients treated with natalizumab were screened. For 21 patients, blood, stool, and urine samples were available. These patients were stratified according to their serum anti-JCV antibody IV (high (>1.5, $n = 12$); medium (1.5–0.9, $n = 2$); low (<0.9, $n = 1$); negative ($n = 6$)). JCV-DNA could not be detected in the whole blood or stool samples. Four urine samples had measurable JCV-DNA, ranging from 1.71×10^4–1.07×10^8 international units (IU)/mL detected by RT-PCR, corresponding to 4.62×10^4–9.85×10^6 copies/mL measured by ddPCR. All JCV variants were wild-type and derived from patients with high antibody IV.
Conclusion: Stool-specific DNA extraction methods provided the highest quality of DNA, while the sensitivity of ddPCR and RT- PCR was comparable. Our findings do not support assessing stool samples for PML risk stratification in persons with MS. Further studies are needed to explore where PML-associated viral variants arise.

1. Introduction

JC polyomavirus (JCV or JCPyV), also referred to as human polyomavirus-2 (HPyV-2), establishes an asymptomatic persistent infection in 50–70 % of the adult population as determined by the presence of anti-JCV antibodies in serum. Primarily, in association with immunosuppression, JCV can cause progressive multifocal leukoencephalopathy (PML), a potentially fatal demyelinating infection of the brain (Schweitzer et al., 2023). In patients with multiple sclerosis (MS), PML can be a severe adverse event of natalizumab treatment. Natalizumab guidelines include a risk assessment based on JCV serology, with an ELISA-based technique that detects serum antibodies directed towards capsid protein VP1 (Stratify-JCV®) (Major et al., 2018; Plavina et al., 2014).

Despite intensive research, the relevant JCV reservoir for developing of PML-associated variants remains to be identified. In some asymptomatic individuals, and up to 50 % of natalizumab-treated MS patients, JCV excretion in urine is regularly observed (Delbue et al., 2015).

Abbreviations: CI, confidence interval; dd, droplet digital; HPV, Human papillomavirus; HPyV-2, Human Polyomavirus 2; IU, international units; IV, index value; JCV/JCPyV, JC polyomavirus; MadCAM-1, mucosal addressin cell adhesion molecule-1; NCCR, non-coding control region; OD, optical density; PML, progressive multifocal leukoencephalopathy; RT, real-time; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late antigen-4; VP1, major capsid protein VP1.

* Corresponding author at: Clinik und Polyclinic for Neurology, University Hospital Cologne, Kerpener Straße 62, 50937, Cologne, Germany.
E-mail address: clemens.warnke@uk-koeln.de (C. Warnke).

[1] Authors contributed equally.

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However, PML-type JCV variants, characterized by the rearrangement of sequence elements in the non-coding control region (NCCR) and sequence substitutions in major capsid protein VP1 (VP1), have not yet been found in urine, arguing against uroepithelial cells as the relevant reservoir for JCV from which PML develops (Reid et al., 2011; Gorelik et al., 2011). Furthermore, in the context of natalizumab-associated PML, the bone marrow has been proposed as a potentially relevant reservoir, as hematopoietic progenitor and B-cell numbers increase in the peripheral blood during natalizumab therapy, and the B-cell DNA recombination machinery might allow JCV to acquire pathogenic genetic changes (Warnke et al., 2014; Zohren et al., 2008; Major et al., 2013). However, strong evidence supporting this hypothesis is still lacking (Warnke et al., 2011).

The detection of JCV-DNA in the upper and lower gastrointestinal tract of patients with colorectal cancer points in the direction that the gut might be a PML-relevant reservoir for JCV (Ricciardiello et al., 2000). JCV shedding in stool has been previously described in adults (Vanchiere et al., 2009), however, without a link to PML risk stratification. As a humanized monoclonal antibody, natalizumab acts by targeting the α4-subunit of the α4β1 integrin VLA-4 (very late antigen-4) expressed on leukocytes, hampering the interaction of VLA-4 with endothelial counterparts such as VCAM-1 (vascular cell adhesion molecule-1). Consequently, the transmigration of activated leukocytes across the blood-brain-barrier is reduced, thus ameliorating brain inflammation in MS (Yednock et al., 1992). Importantly, natalizumab also targets the α4β7 integrin on T-cells, inhibiting their interaction with the mucosal counterpart MadCAM-1 (mucosal addressin cell adhesion molecule-1) in the gut. This inhibition reduces T-cell trafficking into the gut, as demonstrated by the effective treatment of inflammatory Crohn's disease with natalizumab (Van Assche et al., 2005). Besides inhibiting the influx of pathogenic T-cells, natalizumab may also reduce gut immune surveillance, which could allow local JCV replication leading to an accumulation of viral genome alterations, including the formation of PML-type JCV variants. Once present, these neurotropic variants could result in symptomatic brain infection.

In the present study, we evaluated stool samples, requiring only non-invasive sampling, to screen the gut as a potential reservoir for PML-associated JCV infection by assessing the presence of viral DNA in stool samples of MS patients with known serum anti-JCV antibody index values. Stool sampling could be used for the purpose of a more individualized PML risk prediction if fecal shedding of PML-type JCV variants could be detected in people with MS at risk of developing PML.

2. Materials and methods

2.1. Participants

Patients with MS treated with natalizumab and known serum anti-JCV antibody index values (Major et al., 2018; Plavina et al., 2014) above 18 years were eligible for the study after providing written informed consent. Patients were recruited at the MS center at the University Hospital Cologne. The study was approved by the institutional review board of the University of Cologne (vote number 19–1007).

2.2. Sample preparation

DNA from EDTA whole blood (200 μL) and urine (200 μL) samples was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, eluting extracted DNA in 50 μL elution buffer. 5–35 g of stool per patient was collected. DNA from stool samples was extracted using QIAamp DNA Mini Kit (Qiagen), NucleoSpin DNA Stool Kit (Macherey Nagel, Düren, Germany), QIAamp PowerFecal Pro-DNA Kit (Qiagen) (200 mg), and the semi-automated platform innuPREP AniPath DNA/RNA Kit (Analytik Jena, Jena, Germany) (100 mg). DNA extraction efficiency was assessed by determining DNA quantity (ng/μL) and quality (an OD ratio of 260/280 nm between 1.8 and 2.0 was considered as good quality), and the quantification of human cellular DNA (beta-globin gene copies per 1 mL) by real-time PCR.

2.3. Spiking experiment

DNA extraction efficiency from stool samples was further evaluated by spiking a JCV-negative sample (case 16) with five different amounts of JCV quantification standard (100,000 international units (IU), 50,000 IU, 20,000 IU, 10,000 IU or 5000 IU) (RealStar JCV PCR Kit 1.0; Altona Diagnostics, Hamburg, Germany), and 5 μL of internal control (Altona Diagnostics). Additionally, as spiking control, 5 μL of an human papillomavirus 16 (HPV16)-positive CaSki cell culture stock (8.4×10^6 copies HPV16) were added to each sample to provide an alternative target evaluating DNA extraction and detection methods.

DNA was then extracted using QIAamp PowerFecal Pro-DNA Kit (Qiagen) and the semi-automated platform innuPREP AniPath DNA/RNA Kit (Analytik Jena). JCV-DNA, β-globin gene DNA and HPV16-DNA were quantified as described below.

2.4. Quantitative real-time PCR

JCV DNA was quantified using RealStar JCV PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany), including internal control and quantification standards, using 10 μL of extracted DNA according to the manufacturer's instructions.

HPV16 load in spiking experiments was determined using quantitative real-time PCR, using a protocol previously published (Herberhold et al., 2017). The cellular content of samples was determined by quantification of the human single-copy beta-globin gene using a PCR protocol by van Duin and colleagues (van Duin et al., 2002). All JCV, beta-globin gene, and HPV16 quantitative real-time PCRs were performed on a LightCycler 480 II (Roche).

2.5. Droplet digital PCR (ddPCR)

JCV-DNA detection and quantification in urine and stool samples was performed using ddPCR Supermix for probes (Bio-Rad, Feldkirchen, Germany) according to the manufacturer's instructions, adapting a real-time PCR protocol published by Ryschkewitsch et al. (Ryschkewitsch et al., 2004) and 5 μL template DNA. JCV-specific primers (JCT-1 and JCT-2) were used at a final concentration of 900 nM and the probe (JCT-1.1) at 250 nM. Cycling conditions were as follows: 95 °C for 10 min, then 40 cycles of 95 °C for 30 s, 55 °C for 1 min, a final enzyme deactivation of 98 °C for 10 min. All ddPCRs were performed in duplicates on a QX200™ Droplet Digital™ PCR system (Bio-Rad). ddPCR data were analyzed using QuantaSoft analysis software (Bio-Rad) to assess the number of JCV-DNA copy numbers, considering only samples with more than 10,000 overall events, and setting thresholds manually.

2.6. Sequencing of JCV NCCR and VP1

JCV NCCR and VP1 coding sequences were amplified using PrimeSTAR® Max DNA Polymerase (Takara Bio, Saint-Germain-en-Laye, France), and external and internal primer pairs as previously published for NCCR (Monaco et al., 1998) and VP1 (Reid et al., 2011; Van Loy et al., 2013). Magnetic bead-purified PCR products were sequenced by next-generation sequencing at the Institute of Immunology and Genetics, Kaiserslautern, Germany. For NCCR analyses, FASTA sequences were aligned and mapped to wild-type and PML-type reference strains (Yogo et al., 1990; Frisque et al., 1984). For VP1 analyses, FASTA sequences were translated into a protein sequence using Expasy (SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland) for alignment with reference strains using Clustal Omega (EMBL-EBI, Hinxton, Cambridge, UK).

3. Results

3.1. Patient characteristics

Thirty MS patients treated with natalizumab and known serum anti-JCV antibody indices were screened. For twenty-one patients (16 female, 5 male) whole-blood, urine, and stool samples were available. These patients were included for further analysis. Their mean age was 42.3 ± 10.6 years. Six patients had no detectable JCV antibodies, as determined by Stratify-JCV®, and one had an antibody index below 0.9. Twelve patients had an antibody index above 1.5. Two had an antibody index between 0.9 and 1.5 (Table 1).

3.2. Evaluation of different DNA extraction methods

The highest-quality template DNA from stool samples was achieved using QIAamp PowerFecal Pro-DNA Kit (Qiagen). This was determined by optical density (OD) 260/280 nm ratio (1.87 [1.77–1.91]), quantity of extracted DNA (273.2 ng/μL [93.4–630.0]), and the detection of the highest number of β-globin gene copies (1000 β-globin gene copies/mL [0–68500]) (Table 2).

3.3. Spiking experiment

A JCV/HPV16-negative stool sample was spiked with different concentrations of JCV quantification standards and a solution of the HPV16-positive CaSki cell line. DNA extracted using the QIAamp PowerFecal Pro-DNA Kit (Qiagen) revealed a higher quality (OD 260/280: 1.90 [1.89–1.91] vs. 2.23 [2.18–2.24]) at a lower concentration (249.9 ng/μL [213.6–270.7] vs. 536.6 ng/μL [299.7 – 571.0]), compared to the semi-automated innuPREP AniPath DNA/RNA Kit (Analytik Jena).

JCV-DNA, HPV16-DNA, and β-globin gene DNA could be detected in all spiked samples at varying recovery rates (supplementary information, Table 1): While for DNA extracted using the QIAamp PowerFecal Pro-DNA Kit (Qiagen) 71 % [52–86 %] and 5 % [3–5 %] of spiked JCV and HPV16 DNA could be recovered, respectively, a much lower percentage could be recovered when using DNA extracted using the semi-automated innuPREP AniPath DNA/RNA Kit (Analytik Jena) as the template (JCV: 3 % [2–15 %]; HPV16: 0.1 % [0.04–1.38 %]). Using QIAamp PowerFecal Pro-DNA Kit (Qiagen), the lowest percentage of recovered JCV-DNA standard could be obtained for the sample spiked with the lowest amount of quantification standard. In contrast, samples spiked with higher amounts of quantification standard showed higher percentages of recovery (Table A.1).

Table 1
Patient characteristics.

Case	Gender	Age decade	Serum anti-JCV antibody index
Serum anti-JCV antibody index: <0.90			
1	F	41–50	neg
2	F	41–50	neg
3	F	31–40	neg
4	F	51–60	neg
5	F	31–40	neg
6	M	21–30	neg
7	F	41–50	0.30
Serum anti-JCV antibody index: 0.90–1.50			
8	M	41–50	0.90
9	F	61–70	1.41
Serum anti-JCV antibody index: >1.50			
10	F	21–30	1.57
11	F	31–40	1.58
12	M	31–40	2.25
13	F	41–50	2.26
14	F	31–40	2.53
15	F	31–40	2.86
16	M	61–70	2.88
17	F	41–50	3.43
18	F	41–50	3.53
19	F	51–60	3.56
20	M	41–50	3.93
21	F	21–30	4.34
		mean ± SD: 42.33 ± 10.57	median [range]: 1.58 [0.00–4.34]

F, female; M, male; neg, negative; SD, standard deviation.

3.4. Detection and quantification of JCV

All EDTA whole blood samples were negative for JCV-DNA (analytical sensitivity = 1.365 copies/μl [95 % confidence interval (CI): 0.568 - 5.831 copies/μl], as per manufacturer), while the internal control was detectable in all samples, except one. JCV DNA was detectable in four urine samples, ranging from 1.71×10^4 IU/mL to 1.07×10^8 IU/mL. These four patients had high serum anti-JCV antibody indices of >1.5. JCV-DNA could also be detected in all these samples by ddPCR, ranging from 4.62×10^4–9.85×10^6 copies/mL. All stool samples were negative for JCV-DNA, as determined by RT-PCR and ddPCR, while the internal control was detectable in all samples except one in the RT-PCR assay (Table 3).

3.5. Sequencing of JCV NCCR and VP1

The organization of JCV NCCR DNA amplified from four urine samples classified these isolates as wild-type (Yogo et al., 1990) (Fig. 1). None of the PML-associated point mutations within VP1 could be detected (supplementary information, Fig. 1 A.1).

4. Discussion

In the present study, we evaluated stool as a proxy for the gut as a relevant reservoir for persistent JCV infection, playing a role in PML development. Contrary to our hypothesis, we could not detect JCV-DNA in stool samples of MS patients.

Although the findings should be interpreted cautiously, this study has several strengths. Different DNA extraction protocols were used to ensure the presence of high-quality DNA. The positive results of the beta-globin gene PCR and HPV16 spiking controls, and the amplification of the internal controls, minimize the likelihood of technical failure. Another key strength of our study was the inclusion of individuals with high serum anti-JCV antibody index values indicating a previous or ongoing infection, thereby increasing the probability of detecting JCV-DNA in the clinical samples.

Nonetheless, several limitations need to be considered. First, the current study was based on a small sample of participants with only a single sampling time point. As expected, in consequence of female preponderance and relatively young age of MS patients treated with natalizumab, the male gender and persons of higher age were underrepresented in our study. Also, we were confronted with the fact that JCV-DNA was seen in the urine of only 19 % of the overall study population (4/21) and 27 % of seropositive patients (4/15). This is a lower percentage than expected based on other reports of natalizumab-treated MS patients, where up to 50 % urine secretion in at least one sample over four years has been reported (Delbue et al., 2015). Furthermore, regarding our analytical methods, assay sensitivity could have been impacted by the abundance of PCR inhibitors in stool samples, shown by our spiking experiments, in which lower spikes were associated with lower recovery rates. Furthermore, we cannot exclude that handling differences of clinical samples might also have contributed to a lack JCV DNA detection in stool.

In conclusion, our study suggests that stool sampling at a single time point in a relatively young, predominately female MS population treated with natalizumab may not be a tool for PML risk assessment. We were unable to detect JCV DNA in any of the stool samples studied, even if high anti-JCV antibody levels in blood were present. Despite our comprehensive efforts to minimize the risk of false negative findings, we are aware that negative findings could result from unresolved technical

Table 2
Evaluation of quality of extracted DNA.

Case	EDTA whole blood			Urine			Stool											
	QIAamp DNA Mini Kit, Qiagen			QIAamp DNA Mini Kit, Qiagen			QIAamp DNA Mini Kit, Qiagen			InnuPREP AniPath DNA/RNA Kit, Analytik Jena			NucleoSpin DNA Stool Kit, Macherey Nagel			QIAamp PowerFecal Pro-DNA Kit, Qiagen		
	260/ 280 ratio*	conc. [ng/ µL]*	β-globin gene / mL	260/280 ratio*	conc. [ng/ µL]*	β-globin gene / mL	260/ 280 ratio*	conc. [ng/ µL]*	β-globin gene / mL	260/ 280 ratio*	conc. [ng/ µL]*	β-globin gene / mL	260/ 280 ratio*	conc. [ng/ µL]*	β-globin gene / mL	260/ 280 ratio*	conc. [ng/ µL]*	β-globin gene / mL
Serum anti-JCV antibody index: <0.90																		
1	1.72	45.5	1.80×10^{6}	1.27	4.1	637	1.19	5.3	not det.	2.36	24.9	not det.	N/A	N/A	N/A	1.88	323.7	1473
2	1.72	20.1	8.03×10^{5}	1.01	6.5	12,550	1.04	4.4	not det.	2.19	31.6	not det.	N/A	N/A	N/A	1.86	504.0	68,500
3	1.73	41.3	1.85×10^{6}	0.86	4.9	2290	1.27	4.4	not det.	2.49	36.2	not det.	N/A	N/A	N/A	1.88	367.6	not det.
4	1.52	13.3	3.70×10^{5}	0.91	4.5	2178	1.14	3.9	135	1.88	38.5	not det.	N/A	N/A	N/A	1.89	370.6	2550
5	1.80	13.9	5.35×10^{5}	0.79	7.4	4275	1.31	2.6	not det.	2.75	58.9	not det.	N/A	N/A	N/A	1.84	217.2	not det.
6	1.53	26.3	7.88×10^{5}	1.50	2.0	135	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1.85	568.3	250
7	1.66	34.7	1.27×10^{6}	0.94	8.6	63,000	0.95	6.1	not det.	2.42	32.3	not det.	N/A	N/A	N/A	1.86	98.5	not det.
Serum anti-JCV antibody index: 0.90–1.50																		
8	1.78	26.6	1.01×10^{6}	0.92	3.7	135	1.91	3.7	not det.	N/A	N/A	not det.	N/A	N/A	N/A	1.87	256.5	not det.
9	1.83	35.9	1.44×10^{6}	0.92	4.6	5175	1.33	3.6	not det.	2.49	25.3	not det.	1.84	77.6	600	1.91	199.6	2800
Serum anti-JCV antibody index: >1.50																		
10	1.60	28.0	9.98×10^{5}	0.98	7.6	2355	1.13	4.3	not det.	2.04	36.6	not det.	1.70	13.1	800	1.87	204.8	2000
11	1.14	25.0	2.06×10^{5}	1.39	3.1	1730	1.10	4.4	not det.	2.45	31.7	not det.	1.86	78.7	400	1.87	231.9	2400
12	1.36	22.4	4.40×10^{5}	1.31	2.9	818	1.28	3.8	not det.	N/A	N/A	not det.	N/A	N/A	N/A	1.77	318.5	350
13	1.68	21.8	8.33×10^{5}	0.79	5.0	918	1.22	10.6	not det.	N/A	N/A	not det.	1.73	84.5	not det.	1.86	301.4	200
14	1.26	33.9	2.18×10^{4}	1.07	3.3	1610	1.14	2.8	not det.	2.26	24.4	not det.	1.40	98.2	not det.	1.84	158.8	3800
15	1.92	6.0	1.64×10^{5}	0.77	7.3	1100	1.14	3.6	not det.	2.53	61.2	not det.	1.84	93.7	3800	1.86	344.1	17,800
16	1.74	29.0	1.29×10^{6}	1.29	2.1	135	1.07	6.0	not det.	N/A	N/A	N/A	1.82	114.7	200	1.90	273.2	200
17	1.15	29.0	1.34×10^{5}	1.00	3.8	3950	1.28	2.4	135	2.81	39.2	not det.	1.49	36.8	not det.	1.88	96.4	3800
18	1.35	35.7	8.43×10^{5}	0.85	4.8	135	0.89	5.7	not det.	N/A	N/A	N/A	1.88	56.0	800	1.85	364.6	1000
19	1.66	12.3	2.68×10^{5}	0.39	54.6	28,750	1.38	3.6	not det.	2.54	35.9	not det.	1.61	77.0	600	1.88	93.4	11,800
20	1.67	8.8	2.48×10^{5}	2.06	3.8	29,981	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1.89	199.2	12
21	1.41	29.4	5.65×10^{5}	0.37	104.2	13,400	1.30	3.0	not det.	N/A	N/A	N/A	N/A	N/A	N/A	1.86	630.0	455
	1.66	26.6	7.88×10^{5} [3.18	0.94	4.6	2178	1.19	3.9	0.0	2.45	35.9	0 [0–0]	1.78	78.2	500	1.87	273.2	1000
	[1.14–1.92]	[6.0–45.5]	$\times10^{4}$–1.85×10^{6}]	[0.37–2.06]	[2.0–104.2]	[135–63,000]	[-0.89–1.91]	[2.4–10.6]	[0.0–135]	[1.88–2.81]	[24.4–61.2]		[1.40–1.88]	[13.1–114.7]	[0–3800]	[1.77–1.91]	[93.4–630.0]	[0–68,500]

* mean of triplicates
conc., concentration; F, female; M, male; mL, milliliter; N/A, not available; not det., not detected; ng, nanogram; SD, standard deviation; µL, microliter.

Table 3
JCV DNA quantification by qPCR and ddPCR.

Case	EDTA whole blood, QIAamp DNA Mini Kit, Qiagen		Urine, QIAamp DNA Mini Kit, Qiagen			Stool, QIAamp PowerFecal Pro-DNA Kit, Qiagen		
	JCV-DNA copies / mL (RT-PCR)	IC [Cp value] (RT-PCR)	JCV-DNA IU / mL (RT-PCR)	IC [Cp value] (RT-PCR)	JCV-DNA copies / mL (ddPCR)	JCV-DNA IU / mL (RT-PCR)	IC [Cp value] (RT-PCR)	JCV-DNA copies /mL(ddPCR)
Serum anti-JCV antibody index: <0.90								
1	not det.	26.34	not det.	26.85	not det.	not det.	22.43	not det.
2	not det.	26.51	not det.	26.92	not det.	not det.	22.87	not det.
3	not det.	26.47	not det.	27.11	not det.	not det.	22.46	not det.
4	not det.	26.52	not det.	27.09	not det.	not det.	22.30	not det.
5	not det.	26.49	not det.	26.10	not det.	not det.	21.55	not det.
6	not det.	26.30	not det.	27.06	not det.	not det.	22.22	not det.
7	not det.	26.49	not det.	26.72	not det.	not det.	22.35	not det.
Serum anti-JCV antibody index: 0.90–1.50								
8	not det.	26.43	not det.	27.14	not det.	not det.	22.49	not det.
9	not det.	26.44	not det.	27.04	not det.	not det.	26.50	not det.
Serum anti-JCV antibody index: >1.50								
10	not det.	26.85	not det.	26.85	not det.	not det.	26.47	not det.
11	not det.	25.53	6.20×10^{4}	29.61	1.04×10^{6}	not det.	26.47	not det.
12	not det.	26.70	not det.	26.70	not det.	not det.	22.11	not det.
13	not det.	not det.	not det.	not det.	not det.	not det.	not det.	not det.
14	not det.	27.03	not det.	27.03	not det.	not det.	26.10	not det.
15	not det.	27.04	not det.	27.04	not det.	not det.	26.53	not det.
16	not det.	23.85	1.07×10^{8}	23.85	1.81×10^{6}	not det.	26.65	not det.
17	not det.	26.50	1.71×10^{4}	26.80	4.62×10^{4}	not det.	26.29	not det.
18	not det.	26.84	not det.	26.84	not det.	not det.	26.11	not det.
19	not det.	26.34	not det.	26.34	not det.	not det.	26.54	not det.
20	not det.	23.59	3.20×10^{6}	23.59	9.85×10^{6}	not det.	25.87	not det.
21	not det.	26.60	not det.	26.60	not det.	not det.	22.60	not det.

ddPCR, droplet digital PCR; IC, internal control; mL, milliliter; not det., not detected; RT-PCR, real-time polymerase chain reaction.

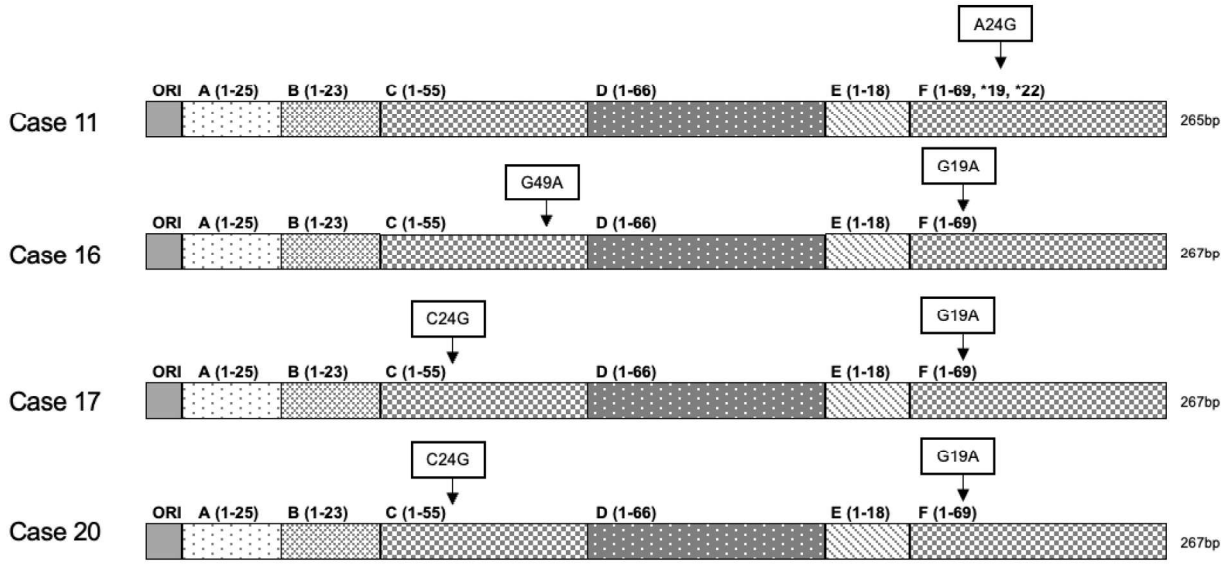

Fig. 1. Graphic presentation of JCV NCCR isolates.
Patient-derived isolates were aligned to the NCCR region of a wild-type JCV isolate (CY) and a PML-associated viral strain (Mad-1) to identify potential PML-associated rearrangements. All isolates highly resembled the organization of the wild-type (Yogo et al., 1990). Asterisks represent deletions.

challenges. While stool can be sampled non-invasively and is easily accessible, it might not fully represent the gut as a potential reservoir. Therefore, we cannot exclude from this study that the gut is a relevant reservoir from where symptomatic JCV reactivation originates. As an alternative study approach, future studies could focus on gut biopsies to evaluate gut epithelial and lymphatic tissue to explore further the putative role of the gut in PML development. Such studies are of high clinical relevance: if the PML-relevant reservoir for JCV could be identified, the development of viral genetic changes at the niche of the persistent infection could be monitored, and cases of PML could be prevented, e.g., by early termination or modification of an immune suppressive disease-modifying therapy.

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CRediT authorship contribution statement

Finja Schweitzer: Conceptualization, Investigation, Methodology, Writing – original draft. **Anne Ladwig:** Conceptualization, Resources, Writing – review & editing. **Sarah Opala:** Investigation. **Sarah Laurent:** Resources. **Michael Schroeter:** Resources, Writing – review & editing. **Susan Goelz:** Conceptualization, Funding acquisition, Validation, Writing – review & editing. **Gereon R. Fink:** Writing – review & editing. **Ulrike Wieland:** Resources, Writing – review & editing. **Steffi Silling:** Methodology, Resources, Validation, Writing – review & editing. **Clemens Warnke:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – original draft.

Declaration of competing interest

C.W. has received institutional honoraria or grant support from Novartis, Sanofi-Genzyme, Alexion, Janssen, Merck, Biogen, Hexal and Roche.

S.G. was working for Biogen as a consultant.

M.S. received honoraria from Alexion, Argenx, Bayer, Biogen, Datamed, Genzyme, Gilead, Grifols, Merck, Novartis, Roche, Teva.

The remaining authors have nothing to declare.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.msard.2024.105664.

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