Molecular characterisation of human JC polyomavirus and risk of developing prostate adenocarcinoma at an early age.

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Research Article

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Abstract

Background: The putative functions of *JC polyomavirus* (*JC PyV)* infections and *Simian Virus 40* (*SV40*) in the initiation of prostate tumors have recently been discovered.

Objective: The objective of our study is to determine the frequency and the genotype of *JCPyV* and *SV40* infections and the clinical correlation of tumor characteristics.

Material and methods: 80 samples were analyzed 50 prostate cancer patients and 30 control cases. DNA extracts were obtained from 50 men treated at the University Hospital of the city of Rabat (Morocco) between June 2021 and February 2022. These samples were then genotyped and analyzed using Sanger.

Results: Ten *JC PyV* infections were detected, and 50% of patients had a Gleason score of 6. A significant association between the following variables was noted: age at diagnosis and *JC PyV* infection (P=0.004); background medical (P=0.034); alcohol (P=0.035); BMI (P<0.001). However, no significant differences were observed between men infected and uninfected concerning other settings pathological, including pathological Gleason score, antigen prostate-specific (PSA), history surgical, stage histology of the tumor (P =0.172, P=0.107, P=1.00, P=0.258, and P=0.884, respectively). So, we don't have could detect *SV40* infection in PCa samples, suggesting this virus is unrelated to the development of this type of tumor. The evolution structure of the *JC PyV* according to the sequences previously aligned was discussed.

Conclusion: Our results reveal the correlations between the parameters of the Clinico-pathology of prostate tumors and *JC PyV* infections. Research prospects with a wide size sample are essential to elucidate statistically the relationship between viral infections and parameters of prostate tumors**.**

Introduction

Prostate cancer (PCa) is the second most diagnosed malignancy after lung cancer worldwide in men, corresponding to 1,276,106 new cases and resulting in 358,989 deaths (3.8% of all deaths induced by cancer for men) in 2018 [1]. Major risk factors that are likely to promote PCa risk for men include age, smoking, obesity, and inheritance of risk alleles. Notably, the incidence and mortality of PCa are variable from various ethnic sources. Among the various risk factors for prostate cancer, some research shows that viral infections can be very important risk factors [2]. The fact that the *JC PyV* and Simian Virus 40 (*SV40*) contribute to malignancies, especially PCa, *JC PyV* has been detected in prostate cells; therefore, prostate cancer cells might be susceptible to infection with *JC PyV*, *JC PyV* is a human DNA virus. Its virus-like particles (VLPs) have a tropism identical to that of native virions and are capable of transmitting genes into target cells to be expressed there [3]. SV40 has significant tumorigenic potential and cell transformation potential in some species, but its presence in human cancer remains a problem. Published data support both the arguments for and against the role of *SV40* as a human carcinogen [4]. The correlation between the presence of *JC PyV* and *SV40* in prostate cancer blood samples and tumor clinical criteria of Moroccan men has not been studied before. The present study aimed to detect viral infections of *JC PyV* and *SV40* in PCa blood samples and to find correlations between these viral infections and the clinical criteria of the tumor. The present research aims to determine the frequency of *JC PyV* with *SV40* infections that may contribute to the risk of PCa, as well as the correlations between these variants and the characteristics of prostate tumors.

Materials and methods

Prostate blood samples

All clinical and pathological parameters were recorded by the physicians in the medical records register of the surgery department of Mohammed V *Military Hospital* of Instruction between June 2021 and February 2022 **(**Table 1**).**

Table I: Clinical characteristics of patients with prostate cancer (n=50).

Tumor features	n = 50 (%)
Pathological Gleason score	
=6	16 (32%)
>6	34 (68%)
PSA ng / mL	
PSA<10	11 (22%)
>=10 <20	3 (6%)
>=20	39 (78%)
Age did diagnosis	
<60	15 (30%)
>=60	35(70%)
Medical background	
Yes	25 (50%)
No	25 (50%)
Surgical history	
Yes	10 (20%)
No	40 (80%)
Smoking	
Yes	36 (72%)
No	9 (18%)
weaned	5 (10%)
Alcohol	
Yes	30 (60%)
No	10 (20%)
weaned	10 (20%)
BMI	
<20	2 (4%)
>=20 <25	30 (60%)
>=25	18 (36%)
Pathological T-stage	
T1	30 (60%)
T2 x	2 (4%)
T3 x	2 (4%)
T4	16(32%)

The study was approved by the Moroccan Biomedical Research Ethics Committee (No. 3/2018/ April 30, 2018). Each subject signed an informed consent form. Clinicopathological criteria for all the patients are presented in (Table 1). Thirty-four patients (68%) had a pathological Gleason score > 6, ie a high grade. And 16 patients (32%) had a score = 6, indicating that cancer may be slow growing. Thirty-nine patients (78%) had an elevated blood level of prostate antigen (PSA), 3 patients (6%) had a PSA (>=10 <20), and 11 patients (22%) had a PSA <10. Most patients were aged 60 or over (70%) and (30%) of patients were under 60 years old. Twenty-five (50%) patients had a PCa history, and the same proportion did not. Forty patients (80%) had a surgical history, and 10 patients (20%) had no surgical history. Among the patients who smoked, we had 36 (72%) and 9 (18%) non-smokers, and 5 (10%) patients who had started smoking. Thirty men (60%) were frequent drinkers, 10 (20%) were nondrinkers, and 10 (20%) had quit drinking. We had 30 patients (60%) with a body mass index (BMI) (of >=20 <25), 18 patients (36%) with a BMI (of >=25), and finally 2 patients (2%) and a BMI (<20). In addition, 30 patients (60%) were classified as having stage T1 tumors, meaning that the tumor involved one side or half of both sides of the prostate, while two patients (4%) with stage T3 indicated that the tumor was in the prostate. or both sides or in the seminal vesicles, the last eight patients (16%) had stage T4 tumors, indicating that cancer had spread to adjacent structures beyond the seminal vesicles (Table 1).

Viral DNA Extraction and genotyping

The DNA was extracted using the mini kit (Invitrogen Genomic DNA Mini Extraction Kit, Thermo Scientific, US), according to the manufacturer's instructions. The quantity and quality of the DNA obtained were evaluated using the NanoDrop 2000 (Technologies, Wilmington, DE, USA). USA).

Viral DNA amplification by a polymerase chain reaction

JC human polyomavirus detection

DNA extracts were amplified by polymerase chain reaction (PCR). and then tested with β-globin before starting amplification for *JC PyV* and *SV40* polyomavirus using the specific forward and reverse GH20/PCO4 primers (5'-CAACTT=CATCCACGTTCACC-3'**;** 5'-GAAGAGCCAAGGACAGGTAC- 3') (Table S1) [5]**,** and the remaining negative samples were discarded from subsequent testing. Using a positive control (JCPyV DNA) was performed during manipulation**.** The PCR for β-globin was carried out according to the following steps: initial primary denaturation for 10 min at 94°C, 35 cycles of denaturation at 94°C for 45 s, 94°C, 35 cycles of denaturation at 94°C for 45 s, annealing at 54°C for 45 s, extension at 72°C for 1 min. after a final extension at 72°C for 10 min. Positive β-globin gene PCR products were subjected to confirmatory PCR for *JC PyV* to perform the polymerase chain reaction. The specific *JC PyV* gene was identified by a polymerase chain reaction from specific primers (5'-AGTCTTTAGGGTCTTCTACC-3';5'-GGTGCCAACCTATGGAACAG-3') which have been described elsewhere (Table S2) [6]. Briefly, the PCR reaction was performed in a 50 µl reaction mix including genomic DNA (8 ng), 2 × Qiagen USA Taq PCR master mix kit, and 20 µmol forward and reverse primers. PCR amplification was performed by a Thermocycler PerkinElmer 2400 GeneAmp PCR System 2400, CA, USA. Using the primers listed in the table. The cycling conditions were as follows: denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 3 minutes. Denaturation at 94 ◦C for 1 minute, annealing at the specific temperature. Extension at 72◦C for 1 min and the reaction was completed with a 10 min extension at 72◦C.

The sizes of these PCR products were checked on a 2% agarose gel and directly sequenced. The amplicons obtained were 173 bp and 300 bp respectively as previously described [6,7].

SV40 human polyomavirus detection

The *SV40* virus above was detected by PCR using specific primers: forward and reverse (5'-AGCCCTGTCCTCCTGCAGGAT-3'; 5'-GGCCAGCCTCACGGGGTTCA-3') (Table S3) [7]. The PCR programs were as follows: initial denaturation at 94˚C for 3 min, then 35 cycles of denaturation at 94˚C for 1 min, annealing at 62˚C for 1 min, and extension at 72˚C for 1 min, and extension final at 72˚C for 10 min. Primer sequences and expected amplicon size of *SV40* amplification.

Analysis of sequence identification and molecular evolution

PCR products were analyzed by gel electrophoresis for 1.5 h at 70 V on 2% agarose to determine and validate the size of our amplicons. Subsequently, the PCR products were purified by applying the ExoSAP- IT™ Express PCR Product Cleanup method (Thermo Fisher Scientific, Inc.). Final purification was performed using the BigDye purification kit XTerminator® (Thermo Fisher Scientific, Inc.). Bidirectional Sanger sequencing was performed using the BigDye Sequencing Kit Terminator v1.1 Cycle (Thermo Fisher Scientific, Inc.). Forward and reverse PCR primers were used for Sanger sequencing performed by a 3130 Genetic Analyzer (Applied Biosystems). Purification and sequencing were performed at the National Center for Scientific and Technical Research (CNRST) in Rabat, Morocco. Searching for identical sequences was performed using NCBI analysis tools (BLAST): https://blast.ncbi.nlm.nih.gov/moleblast/moleblast.cgi. In parallel, as part of the analysis of the phylogeny of the different viral genotypes, an MSA analysis was also carried out using the viewer integrated into the NCBI-Blast tools.

Statistical analysis

The statistics were made by applying the jamovi software (V2.2, 2021) https://www.jamovi.org. A T-test was performed to analyze continuous variables while Fisher's exact and $\chi 2$ tests were performed for categorical data. Positive and negative cases were analyzed according to all clinical and pathological parameters. In all analyses, a P value < 0.05 was considered statistically significant.

Results

The presence of viral infections in the tissues of prostate cancer was determined in the 50 patients with this cancer. 10 (20%) were infected with *JC PyV* and no patient was infected with SV40. As shown in Table 2, the pathological Gleason score was 6 for 50% of these patients. PSA concentration also varied significantly (6–26 ng /ml). The age of diagnosis varied among individuals infected with *JC PyV* (60–80 years). And 8 patients had a medical and surgical history. While 8 patients were smokers, and 9 patients drank alcohol, and 10 patients had a BMI (> = 25). Regarding tumor stage, 6 men had stage 1 tumor and 4 men had histological stage 4 tumors (Table 2).

Table II Clinicopathological settings in 10 men infected with *JC polyomavirus*.

ID	Patients infected by JCPyv	pathological Gleason score	PSA ng / mL	age at diagnosis	medical background	surgical history	tuxedo	Alcohol	BMI	Pathological T-stage
ID-1	Yes	6	7	60	Yes	Yes	Yes	Yes	25	T1
ID-3	Yes	6	6	62	No	No	No	Yes	27	T4
ID-5	Yes	6	7.5	71	Yes	Yes	Yes	Yes	36	T1
ID-8	Yes	7	10	65	No	No	Yes	Yes	28	T1
ID-22	Yes	7	20	63	No	No	Weaned	Weaned	30	T1
ID-34	Yes	6	12	66	No	No	Yes	Yes	29	T1
ID-56	Yes	6	22	70	No	No	Yes	Yes	27	T4
ID-78	Yes	7	8	80	No	No	Yes	Yes	28	T1
ID-89	Yes	7	24	71	No	No	Yes	Yes	25	T4
ID-96	Yes	7	26	69	No	No	Yes	Yes	24	T4

To assess the correlation between tumor parameters and *JC PyV* infection; we performed statistical significance tests (Table 3). Five patients infected with *JC PyV* had a Gleason score of 6. In contrast, 11 uninfected patients had the same Gleason score. There was no significant difference between the presence of viral infection and the Gleason score concentration (P = 0.172).

Regarding PSA, 4 infected patients had a low PSA concentration below 10 ng /ml, 2 had a moderate PSA concentration and 4 patients had a high PSA concentration (> 20 ng /ml). In contrast, 4 of 40 men with prostate cancer and not infected with *JC PyV* had low PSA, and 36 of 50 men had PSA greater than 10 ng /ml. There was no significant difference between viral infection and PSA concentration (P = 0.107).

In terms of age, all individuals infected with JC PyV were > = 60 years old and 25 of the 40 men who were not infected with *JC PyV* were also aged > = 60 and 25 years. There was a significant difference between the Gleason score and infection with one of the two viruses tested (P = 0.004). Similarly, a significant difference was observed for medical history (P = 0.034), alcohol (P = 0.035), and BMI (P < 0.001).

Table III Correlation between prostate cancer tumor parameters and patients infected or not with *JC polyomavirus* and *SV40*.

Tumor characteristics	Number of cases tested	Number of cases Infected by JC Polyomavirus (n = 10)	Number of cases not infected with JC Polyomavirus (n = 40)	P-value	OR (95% CI)
Pathological Gleason score					
= 6	16 (32%)	5	11	0.172	2.64 (0.637–10.9)
> 6	34 (68%)	5	29		
PSA ng / mL					
PSA < 10	8 (16%)	4	4	0.107	-
>=10 < 20	3 (6%)	2	1		
>=20	39 (78%)	4	35		
Age at diagnosed _					
< 60	15 (30%)	0	15	0.004	10 (2-0.004)
>=60	35(70%)	10	25		
Medical background					
Yes	25 (50%)	2	23	0.034	0.185 (0.034–0.983)
Nope	25 (50%)	8	17		
Surgical history					
Yes	10 (20%)	2	8	1.00	1(0.177–5.65)
No	40 (80%)	8	32		
Smoking					
Yes	36 (72%)	8	28	0.258	-
No	9 (18%)	1	8		
weaned	5 (10%)	1	4		
Alcohol					
Yes	30 (60%)	9	21	0.035	-
No	10 (20%)	0	10		
weaned	10 (20%)	1	9		
BMI					
< 20	2 (4%)	0	2	< 0.001	-
>=20 < 25	30 (60%)	0	30		
>=25	18 (36%)	10	8		
Pathological T-stage.					
T1	30 (60%)	6	24	0.884	-
T2 x	2 (4%)	0	2		
T3 x	2 (4%)	0	2		
T4	16(32%)	4	12		

We constructed a phylogenetic tree for JC PyV by comparing sequence data already recorded in NCBI databases with sequences from our study. We have presented the evolutionary molecular homology to other *JC PyV* genotypes in (Fig. 1). Some homologies of our sequence are indicated with genotypes registered in the NCBI database. The original taxonomic classification of *Polyomaviridae* according to nucleotide similarity.

Discussion

Prostate cancer is the result of many factors, including geographical location, age, genetic mutations and viral infections [8, 9]. Over the past three decades, several studies have been conducted on the role of viral infections in the development and growth of cancer [10]. *JC PYV* and SV40 are considered to be one of the main infections linked to prostate cancer [11].

In Morocco, there is very little research on the prevalence of these two viruses, both in prostate cancer and in healthy men. The presence of *JC PyV* infections in blood samples from healthy and cancerous prostates has been reported previously, however, the frequency of this type of infection remains variable in patients with prostate cancer, and there is no absolute confirmation of the predisposing role of *JC PyV* infections in the development of prostate tumors [12]. Furthermore, only a small number of studies have examined *JC PyV* in relation to CPa and have produced conflicting data [13, 14, 15, 16].

Therefore, we conduct this study to investigate a possible causal link between *JC PyV* and CPa. The results of the present study, which focused on *JC PyV*, well-described as a high-risk genotype, reported 10 *JC PyV* infections (among 50 prostate cancer patients) in prostate cancer blood samples. And no SV40 infection was found in the same subset of subjects.

The correlation between tumor parameters and viral infections was studied and revealed a significant association between viral infections and age (P = 0.004), medical history (P = 0.034), alcohol (P = 0.035), and BMI (P = < 0.001). Most of the infected men had a score of 6. Depending on the pathology, a score of 6 or less describes cancer cells that show similarity to normal cells and indicates that the cancer is likely to grow slowly. In addition to these results, medical history, alcohol, and BMI were significantly deferential. This means that the *JC PyV* virus infection occurred at its variables, while the tumors were growing. We, therefore, concluded that there is a potential role of *JC PyV* genotypes in the early development of prostate tumors. This result should be interpreted with caution, however, as other data on tumor criteria, including pathological Gleason score, pathological T-stage, PSA ng / mL, surgical history, smoking status, pathological T-stage at diagnosis or treatment, were not significantly different between men infected with *JC PyV* and uninfected men (P = 0.172, P = 0.107, P = 1.00, P = 0.258, P = 0.884 respectively). Also, the Gleason pathological score, age, and surgical history were considered risk factors for the development of prostate cancer because the odds ratio value was greater than 1. Thus, the result of *JC PyV* infections in men who have undergone radical prostatectomy should be considered with specific attention to viral infections at clinical stages.

These results are in agreement with the data reported by Zambrano et al. In prostate tissue or urine samples, 50% of prostate cancer patients were detected by prostate cancer patients [17], Furthermore, no significant difference was observed in the two separate studies on *JC PyV* detection between cancerous samples and clear prostate tissue [18, 19]. In addition, Mexican researchers did not detect any *JC PyV* sequence [20] in prostate cancer biopsies. The differences observed between these studies may be explained by technical differences and the sample size of the virus detection method. In addition, due to the small number of samples taken in this study and the small number of previous samples, difficulties in obtaining new surgical structures limited the statistical significance of our data.

Research in 2015 also showed that the risk of prostate cancer infected by 58.3% of cancerous and clear prostate tissue samples was significantly increased. Haemophilic viruria and JC virus were observed in 62.5% and 8.3% of prostate cancer patients. In addition to BK virus, replication is evident in the urine pool. Therefore, in the prostate cancer patients included in this study, JC virus is more important than BK virus. Even with regard to the average number of BK virus genomes in the prostate sample, the average number of BK virus genomes is higher. JC virus (P = 0.002) [21].

In another study conducted in 2021, the prevalence of JCPyV/BKPyV DNA was significantly different between PCa and BPH tissues (27/76 [35.52%] and 2/30 [6.7%], respectively, p = 0.003). LT and VP1 proteins were identified in 27 (35.52%) and 29 (38.2%) PCa samples, respectively, compared to no protein in BPH samples (p < 0.001). PCa cells were more susceptible to

JCPyV infection than BPH tissues [odds ratio (OR) 7.71, 95% CI: 1.71–34.09, p = 0.003]. Prostate cancer patients with elevated PSA levels and high Gleason scores were also estimated to have an elevated risk of viral infection (OR 1.1, 95% CI 1.000-1.003; p = 0.045 and OR 6.18, 95% CI 1.26–30.33, p = 0.025, respectively). Expressed LT protein associated with risk of prostate cancer was 2923.39-fold (95% CI 51.19-166963.62, p < 0.001) [22].

In previous studies that examined mRNA expression to identify disease severity, patients diagnosed with high-grade PCa (GS > 7) were shown to have high mRNA expression of specific genes [23, 24, 25, 26]. They demonstrated that when *JCPyV* attacks PCa cells, its oncoprotein LT may increase cellular mRNA expression of specific genes and may affect the GS score and PCa progression. However it remains to be studied whether the changes after the viral infection are related to the opinion [27].

Conclusion

Viral infections of the prostate have been the subject of intense debate for several years. As the link between viral infections and prostate tumors, in general, has not been fully studied, the transmission of these viruses between men and women should be considered, and further research into this possible circulation is therefore needed. The results of the present study revealed that *JC PyV* infections were observed in the prostate at the early stage of tumorigenesis. A causal relationship is suggested in a subset of cases. Therefore, further studies are needed to clarify the clinical implications of these findings. To confirm our conclusion, further studies with a large sample size are needed to examine the correlation between viral infections and other tumor parameters.

Declarations

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Author contributions

IT: conceived and designed the experiments, optimized the experimental approach, realized the experiments, and analyzed the sequencing data. Write the manuscript, and all authors approved the final version. **AL** and **KE**: managed sample collection and processing and the place in which all sample was stored. **MM** : provide DATA collection. **YE** and **BA**: analyzed the sequencing data. **MNB, IS**, **KAT**, **NT** and **SAS**: Approved the final version of the Manuscript. **MME**: conceived and designed the experiments, optimized the experimental approach, realized the experiments and analyzed the sequencing data. Write the manuscript, all authors approved the final version, and coordinate the project.

Ethics approval and consent to participate

Agreement of the Ethics Committee of Biomedical Research in Morocco code: (n°3/2018/April 30/2018- Morocco). All of the patients consented to take participate in this research study data were considered anonymous by ethical standards.

Competing interests

The authors declare that they have no competing interests.

Consent to Participate

All patients consented to participate in the study according to the ethical standards.

Consent for publication All authors approved and consented to publish the presented results.

Availability of data and materials

The data generated in this study are available from the corresponding author.

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Figures

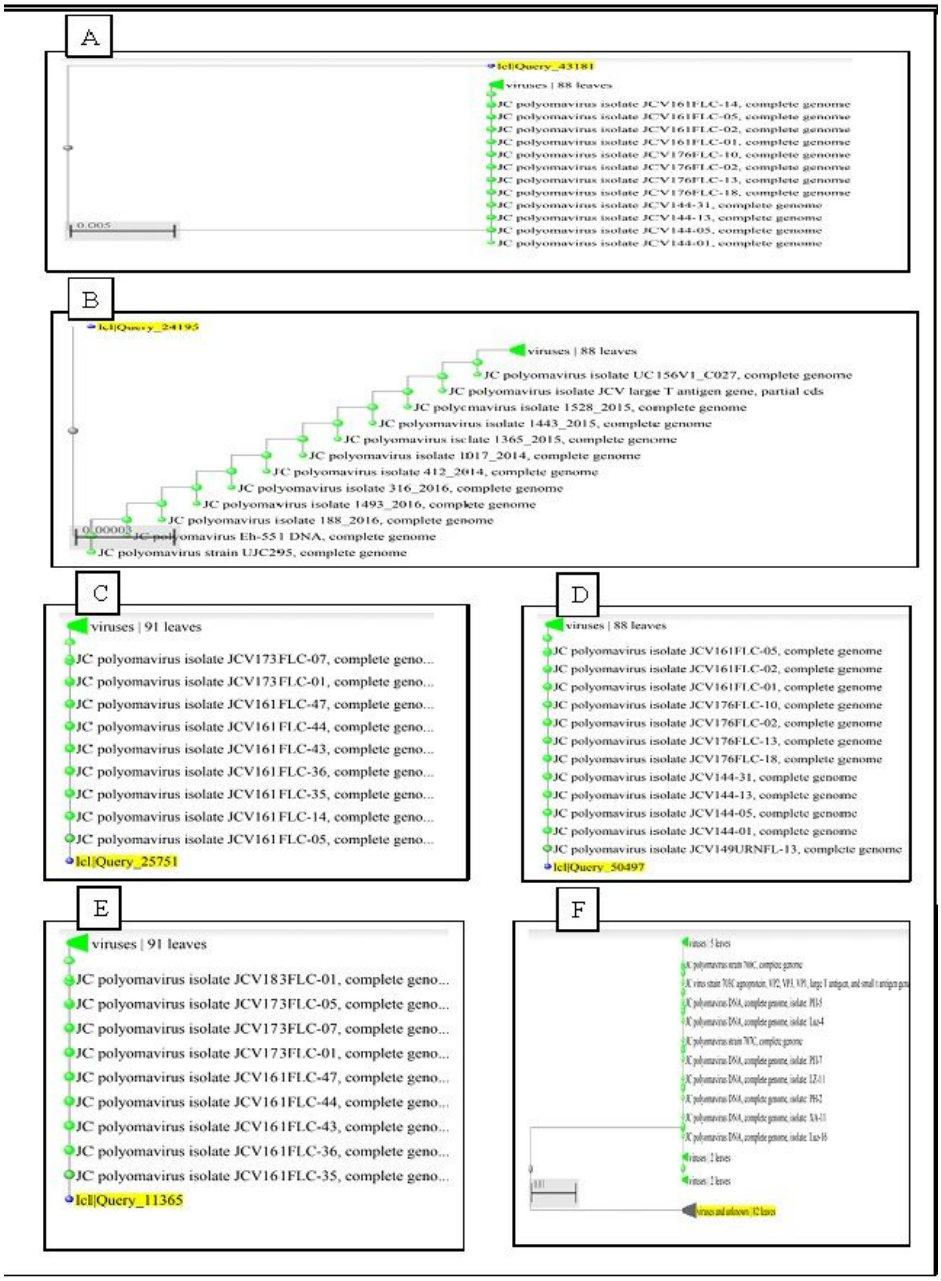

Figure 1

Alignment of the *JC polyomavirus* sequences obtained in this study with the long-range three pairs of the database sequences stored in the NCBI GenBank. The sequences obtained in this study were analyzed using the NCBI-Blast tools, method: minimum rapid evolution, maximum sequence differences=0.75; (A, B, C, D, E, and F): Variants of the *JC PyV).*

Supplementary Files

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- SupplementaryMaterial.docx