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Carmen Lanzillotti , John Charles Rotondo , Ilaria Bononi ,
Maria Rosa Iaquinta , Marco Manfrini , Fortunato Vesce ,
Mauro Tognon , Fernanda Martini

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Highlights

- BK (BKPyV), JC (JCPyV) and simian virus 40 (SV40) polyomaviruses were investigated for their vertical transmission.
- PyV footprints were analysed by innovative methods such as the digital droplet PCR and indirect E.L.I.S.As with viral mimotopes.
- PyV sequences and IgG antibodies were detected, with a different prevalence, in pregnant females and newborns.
- PyV vertical transmission from females to offspring was revealed suggesting a potential risk of diseases in newborns.

Mother-to-child transmission of oncogenic polyomaviruses BKPyV, JCPyV and SV40

Elisa Mazzoni^a, Elena Pellegrinelli^a, Chiara Mazziotta^a, Carmen Lanzillotti^a, John Charles Rotondo^a, Ilaria Bononi^a, Maria Rosa Iaquinta^a, Marco Manfrini^{a,b}, Fortunato Vesce^c, Mauro Tognon^{a1}, Fernanda Martini^{a1}

Affiliation

^aLaboratories of Cell Biology and Molecular Genetics, Section of Pathology, Oncology and Experimental Biology, Department of Morphology, Surgery and Experimental Medicine, University of Ferrara, Ferrara, Italy

^bBiostatistic Unit, Maria Cecilia Hospital, GVM Care & Research, Cotignola, Italy

^cSection of Gynecology and Obstetrics, Department of Morphology, Surgery and Experimental Medicine, School of Medicine, University of Ferrara, Ferrara, Italy

*Corresponding Author: Fernanda Martini, or Mauro Tognon, Department of Morphology, Surgery, and Experimental Medicine, Section of Pathology, Oncology and Experimental Biology, School of Medicine, University of Ferrara. 64/b, Fossato di Mortara Street. Ferrara 44121, Italy.

E-mail addresses: mrf@unife.it (F.Martini), tgm@unife.it (M.Tognon)

¹These authors contributed equally to this work

SUMMARY

Objectives: Polyomavirus (PyV) infections have been associated with different diseases. BK (BKPyV), JC (JCPyV) and simian virus 40 (SV40) are the three main PyVs whose primary infection occurs early in life. Their vertical transmission was investigated in this study.

Methods: PyV sequences were analyzed by the digital droplet PCR in blood, serum, placenta, amniotic fluid, vaginal smear from two independent cohorts of pregnant females and umbilical cord blood (UCB) samples. IgG antibodies against the three PyVs were investigated by indirect E.L.I.S.As with viral mimotopes.

Results: DNAs from blood, vaginal smear and placenta tested BKPyV-, JCPyV- and SV40-positive with a distinct prevalence, while amniotic fluids were all PyVs-negative. A prevalence of 3%, 7%, and 3% for BKPyV, JCPyV and SV40 DNA sequences, respectively, was obtained in UCBs. Serum IgG antibodies from pregnant females reached an overall prevalence of 62%, 42% and 17% for BKPyV, JCPyV and SV40, respectively. Sera from newborns (UCB) tested IgG-positive with a prevalence of 10% for BKPyV/JCPyV and 3 % for SV40.

Conclusions: In this investigation, PyV vertical transmission was revealed by detecting PyV DNA sequences and IgG antibodies in samples from females and their offspring suggesting a potential risk of diseases in newborns.

Keywords: PyV, vertical transmission, pregnancy, infection, antibody, prevalence

Introduction

BKPyV, JCPyV and SV40 are small DNA tumour viruses. Polyomavirus (PyV) primary infections occur during childhood. Subsequently, these PyVs remain latent, establishing life-long infections in the kidneys and B- and T lymphocytes of the host.¹ The onset of polyomavirus-related diseases may occur under certain patient conditions, including different immune-dysfunctions.²⁻⁴ Previous studies indicated that the prevalence of IgG antibodies against BKPyV and JCPyV in serum samples of healthy subjects is up to 80%.^{5,6} Simian Virus 40 (SV40) circulates with a lower prevalence, up to 20%, in the general population.⁷⁻¹⁰ Many studies indicated that BKPyV, JCPyV and SV40 can be transmitted horizontally in different ways, such as by oral-fecal and respiratory routes.^{11,12} However, few studies have been carried out on pregnant females and their newborns to verify mother-to-child PyV transmission.¹³⁻¹⁶

BKPyV is the main cause of polyomavirus-associated nephropathy (PVAN) that occurs in up to 10% of kidney transplant patients.¹⁷ Hemorrhagic cystitis, also caused by BKPyV, complicates between 6–30% of hematopoietic stem cell transplantations.¹⁸

JCPyV causes progressive multifocal leukoencephalopathy (PML), a potentially lethal, demyelinating brain disease, which mainly arises in HIV-infected/AIDS patients, immunosuppressed transplant recipients, in multiple sclerosis (MS) and other autoimmune diseases affected patients treated with immunomodulatory monoclonal antibodies, such as natalizumab.¹⁹ It should be recalled that JCPyV may reactivate from the kidney in pregnant females, probably because of their immune tolerance status, as reported in healthy subjects.²⁰

SV40 has been associated with kidney diseases, such as focal segmental glomerulosclerosis (FSGS). Indeed, SV40 sequences were detected in tubular epithelial cells from FSGS patients.²¹ PyVs have oncogenic potential.²² BKPyV, JCPyV and SV40 encode viral oncoproteins, known as large T (Tag) and small t (tag) antigens, which are responsible for cell transformation and oncogenic activities.²³ Indeed, their footprints have been detected in tumours of different histotypes.¹¹ BKPyV and JCPyV are classified as possibly carcinogenic infectious agents for

humans.¹¹ Indeed, the WHO classified BKPyV and JCPyV as 2B, i.e. potential oncogenic viruses.^{11,24} Cell transformation and tumor onset due to SV40 have been well established in vitro and in experimental animals, respectively.^{11,23} In this context, it worth recalling that DNA sequences of these three PyVs have been detected in rare brain tumors and osteosarcomas in children and adolescents²⁵⁻²⁹ Specifically, JCPyV was detected in medulloblastomas (Krynska 1999) and ependymomas (Del Valle, Cancer Research 2001), SV40 was found to be associated with ependymomas and choroid plexus papillomas (Bergsagel et al. 1991, Martini et al., 1995, 1996), whereas BKPyV footprints were revealed in neuroblastoma samples (Flaegstad Cancer Research, 1999). It has been suggested that PyV infections are transferred during pregnancy from females to their embryo/fetus/offspring.^{30,31} However, little is known about PyV vertical transmission.

Pregnant females, although recognising the paternal antigens expressed by the embryo/fetus, develop immune tolerance to them. Maternal immune tolerance dysregulation may result in recurrent spontaneous abortion or preeclampsia.³² On the other hand, pregnancy status/immune tolerance could be a risk factor for PyV infection/reactivation and a putative mechanism/route for vertical transmission. At present, it is not known whether pregnancy status alters immune surveillance for BKPyV, JCPyV and SV40.

In this study, mother-to-child transmission of BKPyV, JCPyV and SV40 was investigated. To this purpose, PyV footprints were analyzed in samples from two different female cohorts, known as A and B. Blood, serum, placenta, amniotic fluid, vaginal smear and umbilical cord blood (UCB) samples were collected from the two cohorts. Cohort A consisted of pregnant females at the 16th week of gestation. This cohort was chosen since at the 16th week of gestation pregnant females undergo amniocentesis for genetic testing. Cohort B was made up of females at the 39th week/end of pregnancy. Samples from their offspring were collected at delivery time.

Materials and methods

Study design and participants

Samples (n=153) were collected from two independent cohorts, A and B, at the University Hospital, Ferrara, Italy. (i) Maternal blood (n = 25), (ii) vaginal smears (n = 18) and (iii) amniotic fluid (n = 25) samples were obtained from cohort A, which was made up of pregnant females (n=53) who underwent amniocentesis for genetic testing at the 16th week of gestation; (i) maternal blood (n = 27), (ii) placental tissue (n = 29) and (iii) umbilical cord blood (n = 29) samples were collected from cohort B, which included females (n=27) who had delivered live births at the 39th week of gestation. There were 50% cesarean deliveries and 50% vaginal births during the study period. Samples were collected within 4 h of amniocentesis or at time of delivery. The age of the females was in the range of 27–46 years old, with a median age of 36 years old. The age distribution of the two independent cohorts, A and B, do not differ significantly ($p>0.05$).

All samples were collected after obtaining signed informed consent forms and ethics board approval from the County Ethics Committee of Ferrara, Ferrara, Italy, study number 151078. Written informed consent was obtained from all females and parents of newborns included in the study, at the time of hospital admission.

DNA extraction and purification

DNA was extracted from placenta samples (~50 mg each specimen) mechanically and minced with a sterile scalpel before the extraction process. Subsequently, the tissue was incubated overnight with proteinase K at 56°C to allow tissue digestion. DNA was extracted using a QIAmp DNA Blood and Tissue Extraction Kit (Qiagen, Milan, Italy), as reported.³³ DNA was eluted in 100 µl of buffer.

The commercial DNeasy Blood and Tissue (Qiagen, Milan Italy) kit was employed for DNA extraction from the mother's blood and the umbilical cord blood of newborns.³³ DNA was

extracted from amniotic fluid (400 µl) and vaginal smear samples using the ChargeSwitch™ Forensic DNA Purification Kit (Thermo Fisher Scientific, Milan, Italy).³⁴ Then, DNA was purified and eluted in 150-200 µl of buffer. It was assessed for concentration levels and the presence of contaminants using an optical density (OD) reading carried out with a NanoDrop 2000 spectrometer (Thermo Scientific, Milan, Italy).^{35,36} DNA was stored at -80°C until time of analysis. DNA was evaluated for its PCR suitability by amplifying the β-globin gene sequences, as reported before.^{28,37,38}

To verify whether cross-contamination had occurred during DNA extraction, purification and PCR procedures, each sample was processed simultaneously with negative controls, represented by salmon sperm DNA and a mock sample lacking DNA (distilled water).³⁶

Droplet-Digital PCR (ddPCR)

DNA samples were analysed using the high analytical PCR method known as droplet-digital PCR (ddPCR).^{33,36,39} ddPCR was performed using the QX200™ AutoDG™ Droplet Digital™ PCR System (Bio-Rad, Milan, Italy). The human eukaryotic translation initiation factor 2C1 (EIF2C1) gene, mapping to chromosome 1p34.3, was employed as the reference control/house-keeping gene (HKG) to quantify PyV copy numbers in DNA samples (Supplemental Table 1). In our experimental conditions, the ddPCR method does not require an internal positive control. Indeed, it detects unequivocally viral DNA load in an absolute manner. Automated droplet generation (Auto – DG) (Bio-Rad, Milan, Italy) was used to create the droplets. Emulsified droplets were heat sealed with the PX1™ PCR Plate Sealer (Bio-Rad, Milan, Italy), whereas the reactions were run on SimpliAmp™ Thermal Cycler, (Life Technologies, Milan Italy) with the initial cycle at 95 °C for 10 min, then 40 cycles of 94 °C for 30 s; 60 °C for 60 s, 98 °C for 1 min, and hold at 4 °C. The ddPCR reaction mixture consists of 11 µl of 2× Supermix for Probes (Bio-Rad, Milan, Italy), 0.5 µl of the target assay and 1 µl for the HKG and 10 µl of DNA (100 ng/reaction). Plates were transferred to the Bio-Rad QX200 droplet reader, then data analysis was

performed by using QuantaSoft v1.3.2.0 software (Bio-Rad, Milan, Italy). Viral DNA load values were reported as viral copies/ 10^4 human cell equivalents (copy/ 10^4 cells) of maternal blood, vaginal smears, placenta tissue and umbilical cord blood samples. Samples and controls were analysed in triplicate by three different operators in three replica experiments.

Indirect E.L.I.S.As

IgG antibodies against BKPyV/JCPyV/SV40 were investigated in sera/fluids from pregnant females, UCB and amniotic samples. BKPyV, JCPyV and SV40 are highly homologous, at up to 80%. In order to avoid cross-reactivity, specific BKPyV/JCPyV/SV40 synthetic peptides were employed in indirect ELISAs as reported in detail elsewhere.⁵⁻⁷ Two synthetic peptides for each PyV, mimicking epitopes/antigens, were from viral capsid proteins (VPs).⁵⁻⁷ Indirect E.L.I.S.As were carried out in different phases: (i) peptide coating, (ii) peptide blocking, (iii) primary and (iv) secondary antibody additions, (v) dye treatment and spectrophotometric reading at a wavelength (λ) of 405 nm (Thermo Electron Corp., model MultiskanEX, Vantaa, Finland).⁵⁻⁷ The cut-off was determined as reported. Briefly, the cut-off was identified in each assay using an OD mean reading of the three negative control sera, added to the SD three times (+3 SD). The three PyVs negative controls were selected from those below the cut-off value determined using second-degree polynomial regression by plotting the ranked net OD individual value for each peptide.⁵⁻⁷

Statistical Analysis

Viral DNA load data and PyV IgG antibody prevalence were analysed to assess whether there were statistical differences for PyVs. Data were statistically analysed using the Fischer exact test. Prevalence analyses were performed by Prism 6.0 (GraphPad software, La Jolla, CA).¹⁵ Linear regression models were employed to assay the statistical significance of linear relationships between antibodies titers of mothers and corresponding newborns. The R framework for statistical computing version 3.5.1. was used to calculate the OD linear relationship.^{5,6,15,40} The threshold

titers of the fourth quartile of positive samples were used as a cut-off for high antibody PyV titers. Data of serologic profile is presented as mean, standard deviation (SD), and range of values. Two-sided p values < 0.05 were considered statistically significant in all analyses.

Results

PyV DNA sequences coding for large T antigen (Tag), the viral oncoprotein, in pregnant females and their offspring

In this study, BKPyV, JCPyV, SV40 DNA sequences, encoding for large T antigen (Tag), the main viral oncoprotein, were investigated in pregnant females and their newborns. Specifically, maternal blood, vaginal smear, amniotic fluid, placental tissue and umbilical cord blood samples were investigated using the high analytical assay ddPCR.

DNA samples from cohorts A and B tested BKPyV-positive with an overall prevalence of 6% (9/153). Samples found to be BKPyV-positive from cohort A were taken from vaginal smears (1/18; 5%) and maternal blood (1/25; 4%), whereas amniotic fluids (n=25) were all BKPyV-negative (Table 1, Fig. 1).

DNAs from cohort B tested BKPyV-positive with a prevalence of 8.2% (7/85). DNAs were from maternal blood (6/27; 22%) and umbilical cord blood (UCB) (1/29; 3%) samples. Interestingly, two BKPyV-positive DNAs were paired, from the mother's blood and UCB samples. None of the DNAs from the placenta samples (n=29) were BKPyV-positive. Pregnant females (7/52; 13%. Table 1, Fig. 1) showed BKPyV DNAemia with a mean viral load of 19 copies/100,000 blood cells corresponding to approximately 1,000 μ l of fluid (Table 1, Fig. 1).

Samples from cohorts A and B tested JCPyV-positive with an overall prevalence of 13% (20/153; 13%). In cohort A, JCPyV-positive samples (9/68; 13%) were from vaginal smears (4/18; 22%) and blood samples (5/25; 20%). DNAs from amniotic fluids were all JCPyV-negative. Three

JCPyV-positive samples were paired, i.e. vaginal smear and maternal blood samples were from the same three females.

Cohort B samples tested JCPyV-positive with a prevalence of 13% (11/85). Maternal blood (5/27; 18%), placental tissue (4/29; 14%) and UCB (2/29; 7%) samples were found to be JCPyV-positive. The two UCBs tested JCPyV-positive paired JCPyV-positive DNA from the corresponding two maternal blood samples (Table 1). Pregnant females (10/52; 19%) showed JCPyV DNAemia with a mean viral load of 34 copies/100,000 blood cells (Table 1, Fig. 1) corresponding to 10 µl of peripheral blood sample. None of the samples from cohort A was SV40-positive, whereas DNAs from cohort B samples tested SV40-positive with a prevalence of 4% (3/85). These DNAs were from maternal blood (1/27; 4%), umbilical cord blood (1/29; 3%) and placenta (1/29; 3%) samples. The SV40-positive UCB sample paired SV40-positive DNAs from the maternal blood and placenta samples. One DNA from a female blood sample of cohort B (1/27, 4%) showed SV40 DNAemia with a mean viral load of 2 copies/100,000 blood cells corresponding to 10 µl of peripheral blood sample (Table 1, Fig. 1). PyV DNA loads analysed in samples from cohorts A and B were in the range of 3–50, 2–139 and 2–8 copies/100,000 cells for BKPyV, JCPyV and SV40, respectively (Table 1, Fig. 1). The different prevalence of PyV DNA sequences detected in maternal blood samples from the two groups, cohort A and cohort B, was not statistically significant (Fischer exact test, $p = 0.1013$). This result could be due to the low number of positive females.

DNAs from UCB samples, analysed by ddPCR, showed BKPyV, JCPyV and SV40 sequences with distinct viral loads (Table 1).

Different PyV DNA sequences, together with co-infections, detected in samples from cohorts A and B are summarized in Table 1.

IgG antibodies against BKPyV, JCPyV, SV40 in serum samples from pregnant females and newborns.

Serum samples from cohorts A and B were investigated by indirect ELISAs to reveal IgG antibodies against the three PyVs analysed herein. Sera (n=52) from pregnant females had a prevalence of IgG against BKPyV mimotopes L+M of 62% (32/52) (Table 2, Fig. 2). A correlation between BKPyV DNA copies in blood samples from females (stratified >40 copies/100,000 cells) and BKPyV-antibodies level detected in sera from the same females was revealed (Spearman correlation, $p<0.0001$).

Sera from UCB had a seroprevalence of 10% (3/29). Notably, the antibody against the BKPyV peptide L and peptide M had a similar prevalence ($p>0.05$). Three serum samples from UCBs, which tested BKPyV-positive, paired BKPyV-positive sera from their mothers (Table 2). Mothers (n=3) and their newborns (n=3), found to be BKPyV-positive, have high OD values (Fig. 3). The OD value threshold for the 75th percentile of samples, considered at high titer, was used as a cut-off for high antibody levels⁴¹. The correlation for BKPyV antibody levels (OD) between mothers and their newborns was not statistically significant ($p>0.05$), Fig. 3. None of the amniotic fluids reacted to BKPyV antigens.

JCPyV VP1, mimotopes K and N, were employed in indirect ELISAs to analyse sera from cohorts A and B. Samples from pregnant females reacted to both peptides VP1 K and N with a seroprevalence of 42% (22/52) (Table 2). The prevalence of antibodies against JCPyV in UCBs was 10% (3/29), while none of the amniotic fluids tested JCPyV-positive. Antibody prevalence against JCPyV peptide K and peptide N did not differ ($p>0.05$). No correlation between JCPyV DNA copies in blood of positive females and JCPyV-antibodies level (OD) in sera from the same females was detected (Spearman correlation, $p>0.05$).

The prevalence of antibodies against JCPyV in UCBs was 10% (3/29), while none of the amniotic fluids tested JCPyV-positive. The three sera from UCBs, found to be JCPyV-positive, paired the mothers' sera (Table 2). A direct correlation for JCPyV-IgG antibody levels (OD) between mothers and their newborns was revealed ($p<0.001$) (Fig. 3).

Sera, from cohorts A and B, were assayed using indirect ELISAs with SV40 VPs mimotopes B and C. SV40-seroprevalence in pregnant females was 17% (9/52). These 9 SV40-positive sera also tested positive for IgG against JCPyV and BKPyV. One serum sample, #18, from UCB and the mother's blood sample tested SV40-positive (Table 2). The blood sample from mother, which tested SV40 DNA-positive, was found to be negative for SV40-antibodies. Amniotic fluids were all SV40-negative (Table 2).

Sera from females of cohorts A+B showed PyV co-infections. Specifically, 9 sera tested positive for antibodies against the three PyVs, whereas 10 sera carried antibodies against BKPyV and JCPyV (Table 2, Fig. 2).

Discussion

In this study, BKPyV, JCPyV and SV40 vertical transmission was investigated by analysing PyV Tag DNA sequences and IgG antibodies in different samples from pregnant females, at two distinct stages of pregnancy, and their newborns. PyV vertical transmission was showed by the presence of BKPyV, JCPyV SV40 DNA sequences and IgG against BKPyV/JCPyV in umbilical cord blood (UCB) samples.

Pregnant females in cohort A, who had tested PyV-positive in blood samples for the three PyVs, showed BKPyV and JCPyV sequences in vaginal smears. None tested SV40-positive. It has been reported that BKPyV and JCPyV can be detected in samples from urogenital tracts.¹¹ Moreover, our data indicate a higher prevalence of JCPyV sequences and viral DNA loads than BKPyV in vaginal samples. The detection of PyV sequences in vaginal smears suggests that the sexual route could be one of the transmission ways for these viruses. Amniotic fluids from females of the cohort A tested negative for PyVs DNA sequences by ddPCR, indicating that these viral agents are not able to spread into the amniotic fluids at 16 weeks of pregnancy.

Placenta samples from females of the cohort B tested JCPyV- (14%) and SV40-positive (3%), while none was BKPyV-positive. This result suggests that JCPyV and SV40 are able to cross the placental barrier and could be transmitted to the fetus during pregnancy.⁴³

The different data obtained with the amniotic fluids, all PyV negative, and placenta samples found to be SV40 and JCPyV-positive with a prevalence of 3% and 14%, respectively are interesting. It is known that viruses do not pass directly from placental tissue into the amniotic fluid, Consequently, it is expected that PyVs are absent in the amniotic fluid sampled at the 16th week of pregnancy, when the placental barrier is too thick to allow the passage from maternal to foetal blood stream. On the other hand, the detection of PyVs DNA sequences and IgG, antibodies, along with the lack of IgM in the umbilical blood samples, indicate that the foetal contagion had occurred. Therefore, amniotic fluids may test PyV-positive or negative depending on the time elapsed from the viral infection.

BKPyV, JCPyV and SV40 sequences were detected in blood samples from females of the cohort B, at time of delivery, and in a fraction of UCBs. Interestingly, UCB samples tested positive for the three PyV-positive paired mothers. The detection of PyV DNA sequences in these samples indicates that in a fraction (ranging from 3-7%) of cases maternal-fetal transmission of JCPyV, BKPyV and SV40 may occur. Different prevalence of PyV DNA sequences reported in distinct studies could be due to differing techniques or investigated populations.^{30,31}

Indirect E.L.I.S.As carried out on female sera, showed an overall prevalence of 62%, 42% and 17% of IgG antibodies against BKPyV, JCPyV and SV40, respectively. These data confirm and extend recent results obtained using serum samples from healthy subjects analysed with indirect ELISAs with BKPyV, JCPyV and SV40 specific mimotopes.^{6,44,15} Overall, the IgG prevalence was not statistically different in pregnant females from cohorts A and B. In previous studies, IgG antibodies against SV40 in pregnant females, at different gestation times, gave similar data, i.e., approx. 13%.^{15,16,30} IgM antibodies against BKPyV, JCPyV, SV40 were not detected in sera from newborns, whereas the same sera tested IgG-positive for BKPyV, JCPyV, SV40.

IgG antibodies against PyVs detected in newborns with a prevalence of 10% for BKPyV/JCPyV and 3 % for SV40, paired to the mothers' results. UCBs tested IgG-positive against the three PyVs correspond the maternal IgG with higher antibodies levels. IgG titers in serum samples from mothers of the cohort B are higher than offspring. One may speculate that IgG antibodies against PyV detected in the UCBs from children have a lower prevalence since only IgG1 class is transmitted from mother to child. This IgG isotype is reported as being transferred efficiently from females to offspring⁴⁵ thus providing passive immunity at up to 15 weeks after birth.⁴⁶

The threshold titers of the fourth quartile of PyV-positive samples were used as a cut-off for high antibody titers. Our data indicate that all BKPyV-DNA-positive females have anti-BKPyV antibodies, whereas within females tested positive for JCPyV DNA sequences only 50% showed IgG antibodies against JCPyV. None of the SV40-DNA-positive females had anti-SV40 VP antibodies. The absence of IgG antibodies against SV40/JCPyV in subjects tested positive for SV40/JCPyV DNA sequences could be due to: (i) recent infections; (ii) PyVs infected subjects are not always responders; (iii) the immune tolerance status of pregnant females may affect differently the antibody response to PyV infections.

The detection of PyV DNA sequences in UCBs (ranging from 3-7%) suggests that these viruses may cross the placenta barrier thus infecting newborns. In addition, we showed that the IgG against BKPyV/JCPyV and SV40 (ranging from 3-10%) were transferred from mother to newborns.

Our study shows that PyV infection/reinfection or reactivation may occur in a fraction of pregnant females. An increased risk of PyV-associated diseases, including neuropathies/nephropathies/cancers in childhood have been reported.^{26,28,47,48} Taken together, these data suggest that child pathologies, associated to PyVs, are due to prenatal/perinatal infections acquired from PyV-positive mothers.^{49,50}

The detection of PyV footprints in pregnant females and their newborns provide an explanation for the presence of polyomaviral DNA sequences revealed in pediatric tumors. It is important to note that DNA tumor viruses, such as BKPyV, JCPyV and SV40, acquired during pregnancy or at the time of delivery from infected mothers need time to exert their oncogenic properties. It is possible that oncogenic polyomaviruses may trigger the tumor onset in a small fraction of PyV-infected children. The cancer development may occur in genetically predisposed subjects or in immune impaired conditions of the host.

In this context, it should be recalled that the vertical route of transmission has been proved to occur for human papillomaviruses (HPV), which are small DNA tumor viruses closely related to PyVs.⁵¹

Conflict of interest

Data of this work were enclosed, in part, in the Italian patents number 102016000083753 and number 10201600083859, February 4, 2019. Inventors: F.M., M.T., E.M., I.B. The two patents are related to the indirect ELISAs and BKPyV/JCPyV mimotopes.

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Figures' legends

Journal Pre-proof

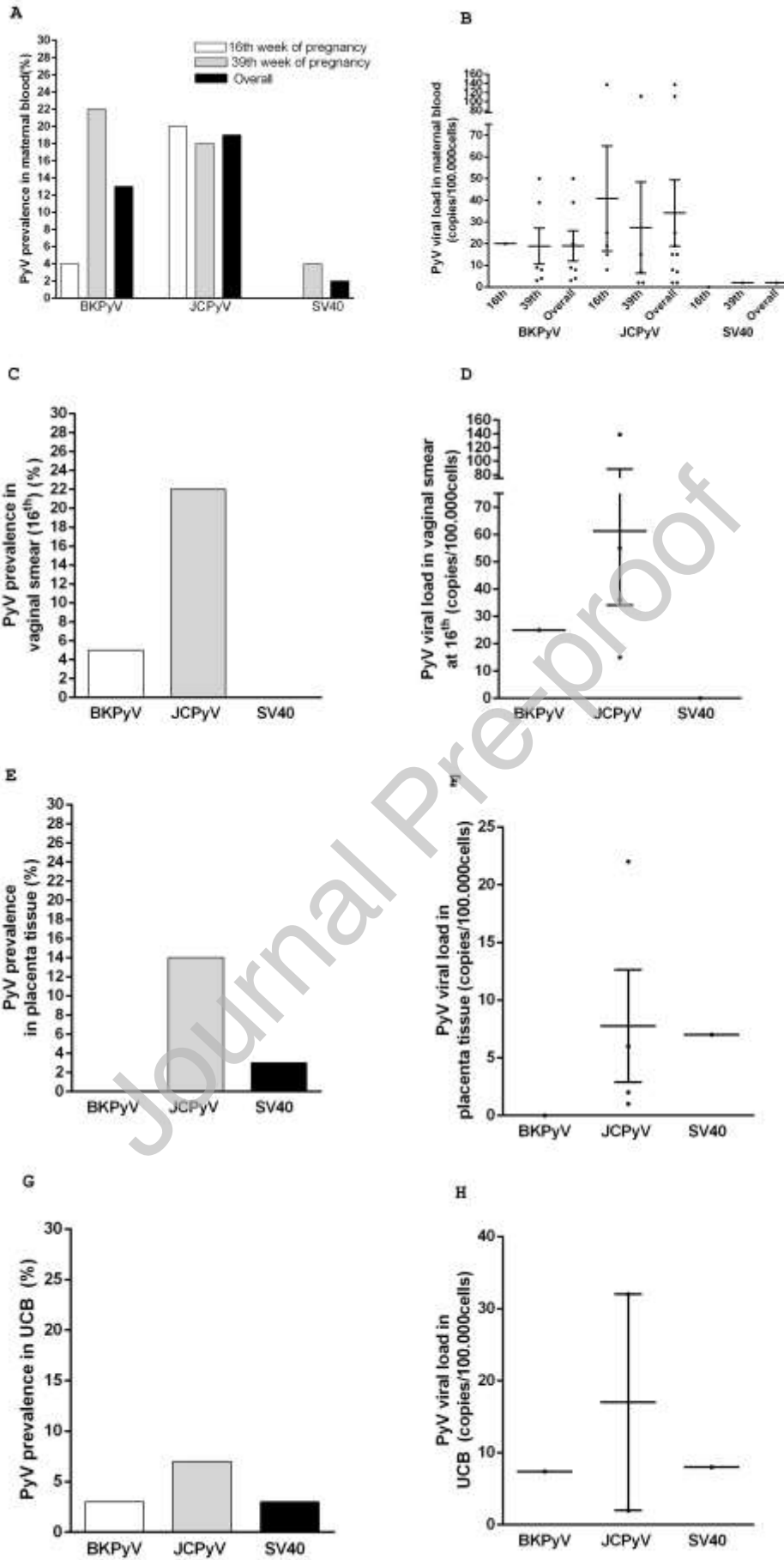


Fig. 1. Prevalence of PyV DNA sequences coding for the large T antigen (Tag), the viral oncoprotein, in pregnant females and offspring.

(A) Prevalence of BKPyV, JCPyV and SV40 DNA Tag sequences in the blood of pregnant women at 16th and 39th week of pregnancy and (B) their viral loads.

(C) Prevalence of PyV DNA sequences in vaginal smear sample and (D) their viral loads.

(E) Prevalence of PyV DNA sequences in placenta tissues and (F) their viral loads.

(G) Prevalence of PyV DNA sequences in umbilical cord blood (UCB) samples and (H) their viral loads.

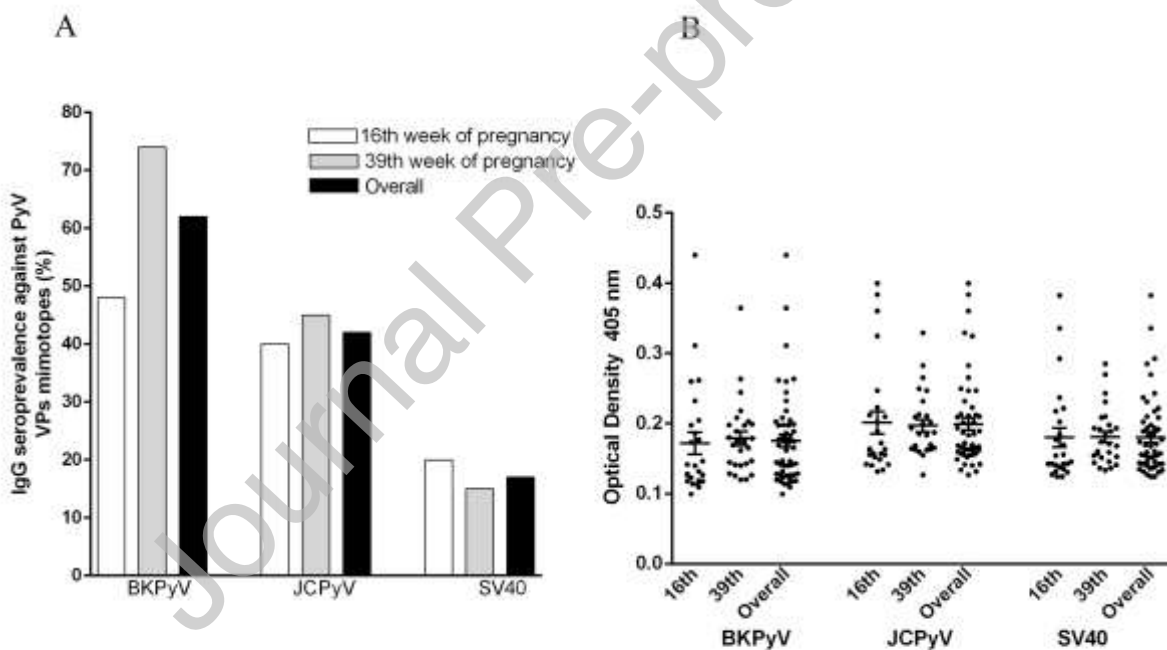


Fig. 2. Prevalence of antibodies reacting to BKPyV, JCPyV and SV40 VPs in sera from pregnant females.

(A) Prevalence of IgG antibodies reacting to BKPyV, JCPyV and SV40 VP mimotopes in sera from pregnant females at the 16th and 39th week of pregnancy and overall, 16th + 39th week of pregnancy.

(B) In the scatter dot plotting, each plot represents the dispersion of OD values to a mean level indicated by the line inside the scatter with Standard Error (SE) for each group of subjects analysed (Mean OD \pm SE). Results are presented as OD reading values at λ 405nm for serum samples diluted at 1:20 detected in indirect E.L.I.S.As. The difference in OD mean values were not statistically significant among groups $p>0.05$, Anova test).

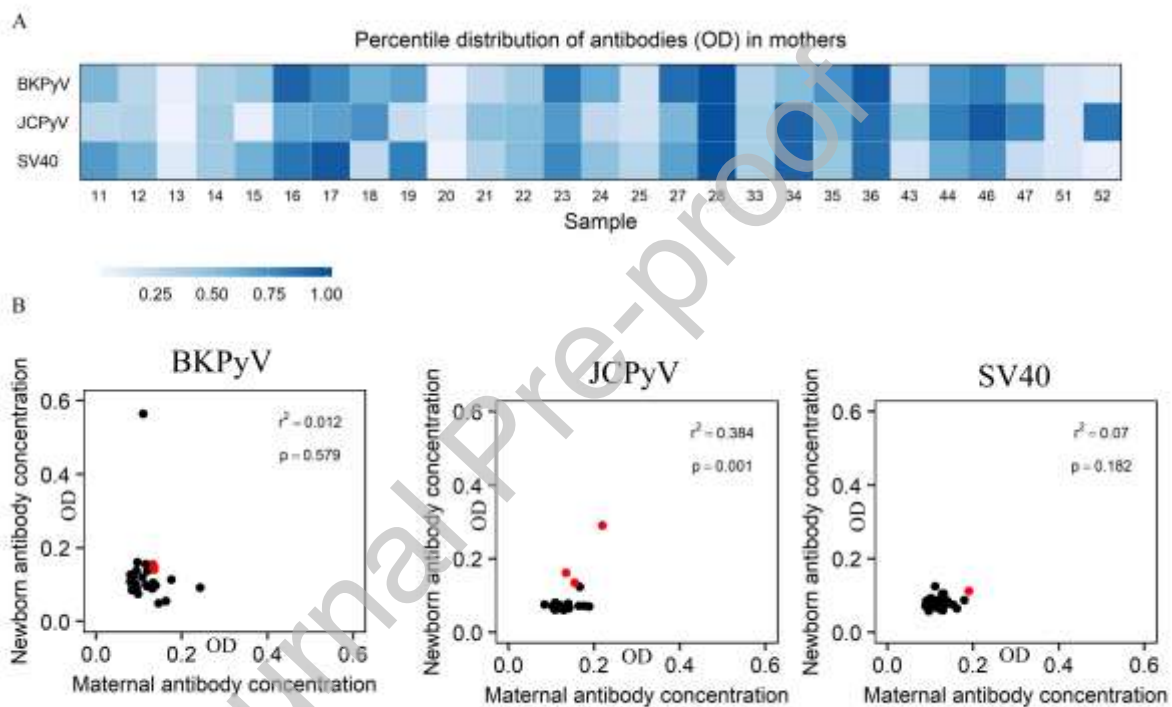


Fig. 3. Correlation between antibody levels against PyVs in sera from mothers of the cohort B and their newborns.

(A) Distribution of Optical Density (OD) in mothers measured by indirect E.L.I.S.A. expressed in percentile.

(B) Correlation analysis between maternal and newborn antibody levels against PyVs (OD). Positive samples from newborns are shown as red dots BKPyV and SV40 antibody levels (OD) detected in mothers and their newborns did not correlate ($p>0.05$), while the correlation between

mothers and their newborns for JCPyV antibody levels (OD) was statistically significant ($P=0.001$).

Graphical abstract

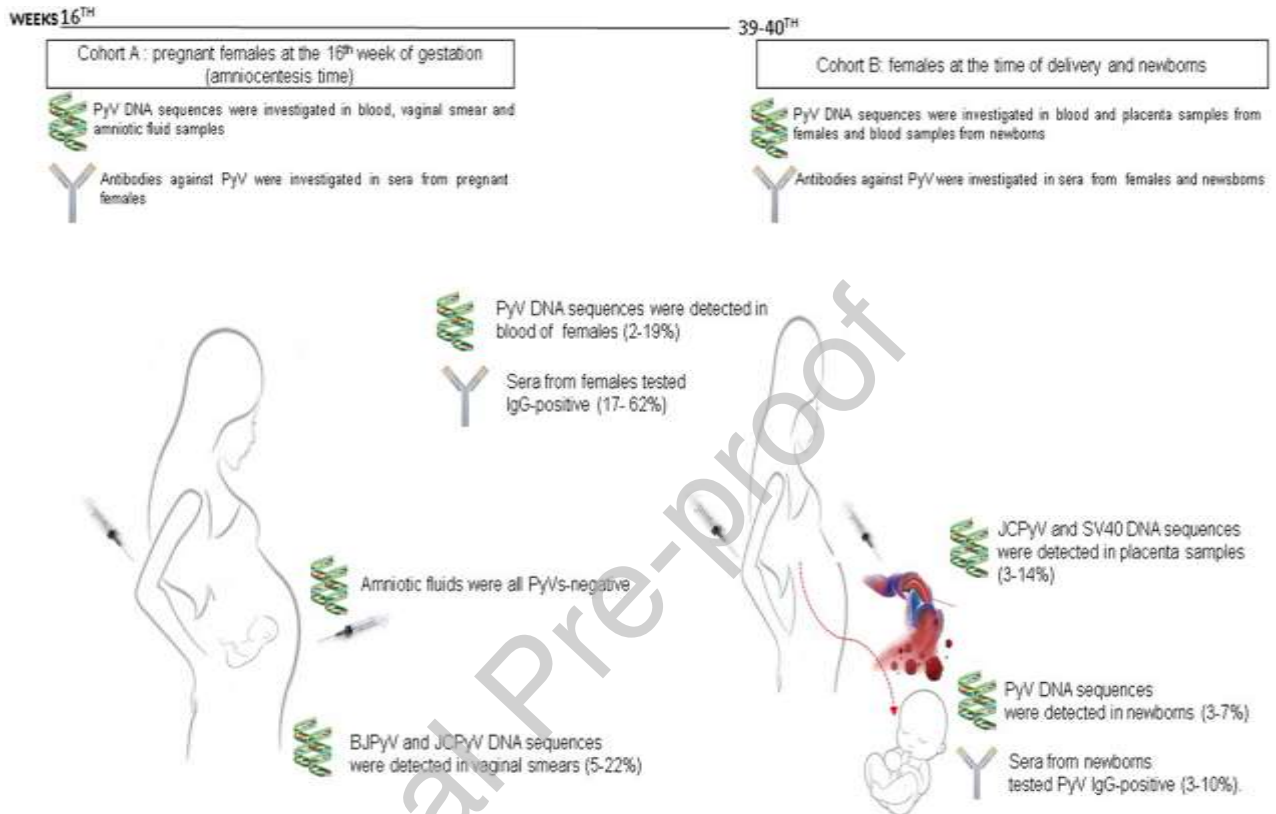


Table 1. Prevalence of PyV DNA sequences coding for the large T antigen (Tag), the viral oncoprotein, in pregnant females and offspring.

Specimen	BKPyV		JCPyV		SV40	
	number of positive samples/samples analysed (%)	viral load copy/ 10^4 cells	number of positive samples/samples analysed (%)	viral load copy/ 10^4 cells	number of positive samples/samples analysed (%)	viral load copy/ 10^4 cells
Pregnant female blood Cohort A	1/25 (4)	20	5/25 (20)	41	0/25	
Maternal blood Cohort B	6/27 (22)	19	5/27 (18)	28	1/27 (4)	2
Pregnant female/ Maternal blood Cohort A+B	7/52 (13)	19	10/52 (19)	34	1/52 (2)	2
Vaginal smear Cohort A	1/18 (5)	25	4/18 (22)	61	0/18	
Amniotic fluid Cohort A	0/25		0/25	61	0/25	
Placenta tissue Cohort B	0/29		4/29 (14)	8	1/29 (3)	7
Umbilical cord blood Cohort B	1/29 (3)	7	2/29 (7)	17	1/29 (3)	8

BKPyV-positive DNAs were detected in one vaginal smear sample (#2) and in one blood sample from a pregnant female (# 7) (cohort A).

BKPyV-positive DNAs were detected in two paired samples, from UCB (#27) and mother blood samples (#27) (cohort B).

One DNA from the blood sample of mother #14 (cohort B) tested positive BKPyV and JCPyV DNA sequences.

Three pregnant females #2, #32, and #40 (cohort A) tested JCPyV DNA positive in two paired samples, i.e. vaginal smear and maternal blood samples

Three mothers #11, #12, #47 (cohort B) tested JCPyV DNA positive in paired samples, i.e. maternal blood and placenta specimens

Two mothers and their newborns, #11, and #47, tested JCPyV DNA positive in 3 samples each, i.e. maternal blood, placenta and UCB specimens (cohort B)

Three samples, i.e. mother blood, placenta, UCB specimen from mother #18 (cohort B) tested SV40 DNA-positive.

Table 2

Prevalence of IgG antibodies reacting to BKPyV, JCPyV and SV40 VP mimotopes detected in serum and amniotic fluid samples from mothers, and umbilical cord blood (UCB) samples.

PyV	Sample				
	Mother (A) serum positive sample/ sample analyzed (%)	Mother (B) serum positive sample/ sample analyzed (%)	Mother (A+B) serum positive sample/ sample analyzed (%)	Mother (A) amniotic fluid	Newborn UCB positive sample/ sample analyzed (%) (IgG) (IgM)
BKPyV 0	12/25 (48)	20/27 (74)	32/52 (62)	0	3/29 (10)
JCPyV 0	10/25 (40)	12/27 (45)	22/52 (42)	0	3/29 (10)
SV40 0	5/25 (20)	4/27 (15)	9/52 (17)	0	1/29 (3)

(A) Samples analysed were sera and amniotic fluids from pregnant females at the 16th week of gestation, cohort A.

(B) Samples analysed were sera from pregnant females at the end of pregnancy, at 39 weeks, cohort B, and sera from umbilical cord blood (UCB).

The different prevalence of PyV antibodies in pregnant females at the 16th week of gestation and pregnant females at the end of pregnancy was not statistically significant ($p>0.05$). *P* values were determined using the Fischer exact test.

Antibody positive pair sera were taken from UCBs and mothers (cohort B); these samples were #11, #19 and #23 for BKPyV; #17, #44 and #28 for JCPyV; #28 for SV40.

Co-infections were revealed in sera from females (cohorts A+B); sera (n=9) were co-infected by three PyVs, whereas 10 sera were co-infected by BKPyV and JCPyV.