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Evolutionary analysis of JC polyomavirus in Misiones' population yields insight into the population dynamics of the early human dispersal in the Americas

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ABSTRACT

Background: JC polyomavirus (JCV) has an ethno-geographical distribution across human populations.
Objective: Study the origins of the population of Misiones (Argentina) by using JCV as genetic marker.
Methods: Viral detection and characterization was conducted by PCR amplification and evolutionary analysis of the intergenic region sequences.
Results: 22 out of 121 samples were positive for JCV, including 5 viral lineages: MY (n = 8), Eu-a (n = 7), B1-c (n = 4), B1-b (n = 2) and Af2 (n = 1). MY sequences clustered within a branch of Native American origin that diverged from its Asian counterpart about 21,914 years ago (HPD 95% interval 15,383–30,177), followed by a sustained demographic expansion around 5000 years ago.
Conclusions: JCV in Misiones reflects the multiethnic origin of the current population, with an important Amerindian contribution. Analysis of the MY viral lineage shows a pattern consistent with the arrival of early human migrations to the Americas and a population expansion by the pre-Columbian native societies.

Credit Author Statement

Pereson MJ: Conceptualization, Methodology, Validation, Investigation, Data curation, Writing – original draft, Writing – review & editing, Review & Editing. Sanabria DJ: Conceptualization, Methodology, Validation, Investigation, Data curation, Writing – original draft. Schurr TG, Resources; Writing – review & editing. Liotta JD Resources; Writing – review & editing. and Campos RH: Resources; Writing – review & editing. Torres C: Data curation, Writing – review & editing, Review & Editing. Di Lello FA: Writing – review & editing, Review & Editing. Badano Ines: Conceptualization, Methodology, Formal analysis, Data curation, Resources; Project administration; Funding acquisition; Writing – review & editing

1. Introduction

The JC polyomavirus (JCV, family *Polyomaviridae*, genus *Betapolyomavirus,* species *Betapolyomavirus secuhominis*) is a double strand DNA virus of approximately 5.1 kb length which is widely distributed among human populations. The molecular epidemiology of JCV and its evolutionary characteristics have been interpreted as resulting from co-evolution with its human host, thereby making it a useful anthropological genetic marker (Agostini et al., 1997; Sugimoto et al., 2002a; Holmes, 2004; Yogo et al., 2004; Torres, 2020; Forni et al., 2020).

JCV genetic variants can be classified through the analysis of the viral intergenic region (VT-Intergenic region). Based on this kind of analysis, three main super-lineages designated A, B and C and several

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lineages have been characterized (Sugimoto et al., 1997; Torres, 2020). Of these, Super-Lineage A contains mainly European and Mediterranean lineages (Eu-a, Eu-b, Eu-c) and Super-Lineage C contains an African lineage characteristic of this continent (Af-1). Super-Lineage B is more diverse and contains lineages originating in Africa (Af2 and Af3), Europe (B1-c) Asia and the Americas (B1-a, B1-b, B1-d, B2, CY, MY, MX) and Asia and Remote Oceania (B3-a, SC, 2 E, 8 A, 8 B) (Sugimoto et al., 1997, 2002b; Torres, 2020). Interestingly, the MY lineage is present in Northeast Asians and Native Americans (such as Na-Dene Indians and Guarani), leading the hypothesis that the distribution of this lineage reflects the first arrival of humans to the Americas (Stoner et al., 2000; Fernandez-Cobo et al., 2002; Zheng et al., 2003; Cayres-Vallinoto et al., 2012; Torres, 2020).

Misiones is a province located in the extreme northeast of Argentina and shares 90% of its borders with Brazil and Paraguay. The historical settlement of this region involves many waves of migrations and admixture between different ethnic populations (Bartolomé, 1975; Gallero and Krautstofl, 2010). The original people subsisted as small, nomadic hunter-gatherer groups (Humaitá and Umbú cultures) that arrived approximately 10,000 years before present (ybp) (Bauni and Homberg, 2015). Later, about 1500–2000 ybp, Guarani societies established themselves in the region, expanding from their homeland in Paraguay and Brazil (Poujade, 1992; Marrero et al., 2007). Europeans arrived in the America and colonized what is now Argentina in the 16th century, bringing with them enslaved Africans (Luisi et al., 2020). More recently, Argentina experienced a wave of Europeans during the First World War, with these immigrants settling in small semi-isolated colonies through the territory. Thus, Misiones has been historically described as being mainly European from socio-cultural perspectives. However, studies of human genetic markers have begun to unravel the complex admixture of its population (Corach et al., 2010; Catelli et al., 2011; Badano et al., 2013).

The human migration history of Misiones has also been explored using different viruses which infect humans, including human papillomavirus type 16 (HPV16), hepatitis B virus (HBV) and JCV. These studies have shown that local populations have a significant component of geographically attributed European viral lineages for HPV 16 (93%) and HBV (65.4%) but not for JCV (33%), for which Amerindian lineages were more frequent (47.6%.) (Badano et al., 2015; Mojsiejczuk et al., 2016; Sanabria et al., 2019). While helping to delineate the relative frequency of JCV lineages in Misiones, our previous study did not explore the evolutionary history of the MY lineage.

Thus, in this new study, we have enlarged our study area to include multiple localities within Misiones and analyzed a more phylogenetic informative sequence region (VT-Intergenic region) of JCV. As a result of this work, we have genetically characterized JCV sequences from Misiones and used the resulting data to elucidate the phylogeography history of the Native American MY lineage circulating in the modern-day population of the province.

2. Materials and methods

2.1. Study population

One hundred twenty-one individuals from different locations within Misiones were invited to participate when they were attending local health centers. After obtaining informed consent, we collected urine samples from these individuals at three locations in the province, including the North (26%), Center (56%) and South (18%). Participants also completed a questionnaire with general personal information (age, place of birth, current place of residence, and nationality). We excluded members of the same family or related persons to avoid any bias in the genetic characterization of viral DNAs due to intra-family transmission of JCV (Suzuki et al., 2002; Zheng et al., 2004).

2.2. Viral sequence detection and analysis

DNA extraction from urine samples was conducted as described in previous work (Sanabria et al., 2019). JCV detection was conducted through PCR amplification of the V-T Intergenic region (IG region) using primers Fw (5′-TTTTGGGACACTAACAGGAGG-3′) and Rv (5′AGCAGAAGACTCTGGACATGG-3′) (Kunitake et al., 1995). The IG region is a 610 bp. fragment that contains 12% of the entire JCV genome, and consists of the 3′ end of VP1 gene (400 bp.) a short intergenic region (75 bp.), and the 3″ end of the T antigen-coding gene (135 bp. in the complementary strand) (Ault and Stoner, 1992). The amplified products were sequenced in both directions using Sanger sequencing (Macrogen Inc.). The resulting sequence data were read and analyzed using CodonCode aligner software v. 3.0.1 (CodonCode Corporation, USA).

2.3. JCV phylogenetic characterization

The phylogenetic classification of Misiones samples was assessed by using a dataset with sequences from all known lineages (Dataset 1, n = 140). The dataset included IG sequences randomly selected from the National Center for Biotechnology Information (NCBI) repository, including 17 lineages Eu-a, Eu-b, Eu-c, Af1, Af2, Af3, B1-a, B1-b, B1-c, B1-d, B2, B3-a, CY, MY, MX, SC, 2 E (Torres, 2020), and two viral types (8a and 8 b), according to the nomenclature proposed by Stoner et al. (2000). Details concerning the accession numbers, geographic location of samples and lineage assignment are provided in Supplementary Table 1. Sequence alignments were performed on MAFFT online version (https://mafft.cbrc.jp/alignment/server/) against the NCBI reference sequence (NC_001699). Phylogenetic analysis was conducting using Bayesian (BP) inference in MrBayes v3.2.7 (Ronquist et al., 2012). The best nucleotide substitution model (HKY + F + I + G4) was selected according to those suggested by Bayesian Information Criterion (BIC) obtained with the IQ-TREE (Kalyaanamoorthy et al., 2017). We applied Bayesian Markov Chain Monte Carlo (MCMC) of 3×10^6 length and sampled every 3×10^5 steps. BP analysis was run up to convergence of parameters: effective sample size (ESS) $\geq$ 200, with a 10% burn-in, verified with Tracer v1.7.1 software (Rambaut et al., 2018).

2.4. Molecular dating of JCV

To better understand the phylogenetic history of JCV we performed the molecular dating of Dataset 1 (time to the most recent common ancestor, tMRCA) by using Beast v1.10.4 software (Suchard et al., 2018). The best nucleotide substitution model (HKY + F + I + G4) was selected according to the BIC obtained with the IQ-TREE (Kalyaanamoorthy et al., 2017). The temporal calibration was performed using the following known human migration events: (i) 60,000–100,000 years for the root; (ii) 40,000–52,000 for migration into Europe; and (iii) 12,000–18,000 years for the human arrival in the Americas (Bolnick et al., 2016; Demenocal and Stringer, 2016; Posth et al., 2018). In addition, we assessed different tree topologies to resolve the reported polytomies at the basal level of the JCV tree. These hypotheses included a non-constrained (NC) analysis (null hypothesis, described above) and three alternative roots: Model 1 (A, (C,B)): Super-lineage A “European” Basal Clade; Model 2 (C, (A,B)): Super-lineage C “African” Basal Clade and Model 3 ((A, C), B): Super-lineages A and C as sister clades. It is important to note that Model 2 resemble the Out of Africa model of human evolution (Cann et al., 1987). A graphic summary of the different tree topologies obtained is presented in Fig. 1. The analyzes were performed under an uncorrelated lognormal clock (UCLN) and Bayesian Skyline demographic model (BSK). A MCMC with 200×10^6 generations and sampling every 200×10^5 steps was performed and the convergence of the parameters (ESS $\geq$200, burn-in 10%) were verified with Tracer v1.7.1 (Rambaut et al., 2018). The best-fit model was tested through Bayes Factor (log10 B F) using the Marginal Likelihood values estimated

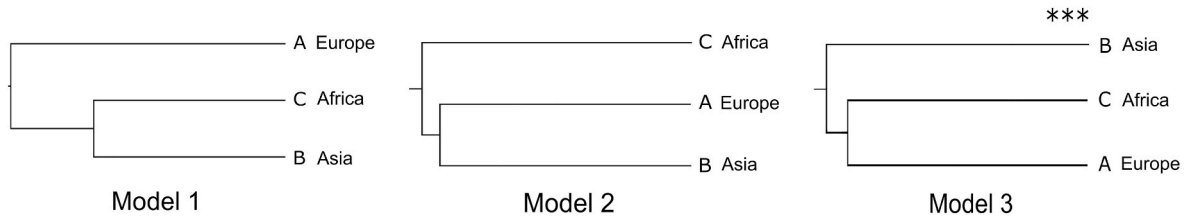

Fig. 1. Graphic summary of the different evolutionary hypothesis tested in this study. The most probable hypothesis is marked with asterisks ***.

by the Path sampling and Stepping-Stone methods (Baele et al., 2013). The strength of the evidence against the null hypothesis (H0) was evaluated as follows: log10 B F 0–0.5 = weak evidence; 0.5–1 = substantial evidence; 1–2 = strong evidence, and >2 = decisive evidence (Kass and Raftery, 1995). The number of path steps were set in 100 and the length of the chain at each step was 2×10^6, defined to produce a total number of iterations equal to the MCMC run used to estimate parameters, for which convergence was confirmed, according to Baele et al. (2013) (Baele et al., 2013).

2.5. Time-dependent substitution rate

Considering that the substitution rate estimates may varies according to the time scale of their measurement (Aiewsakun and Katzourakis, 2015; Membrebe et al., 2019), we performed a Time Dependent Rate molecular clock (TDR) in order to obtain a better approximate of the evolution of JCV in more recent times. The analysis was performed with Dataset 1 with the same temporal calibration as described previously. We considered a time scale in units of thousands of years and established three different epoch structures. These were 0–16,000 ybp, between 16,000 and 44,000 ybp and from 44,000 ybp up to infinity. Because the last epoch extends to infinity, we assumed an alternative finite time midpoint of ≈50,000 years (i.e., assuming a JCV root of 100,000 ybp). The analyzes were performed with Beast v1.10.4 software (Suchard et al., 2018), using a BSK demographic model. The best nucleotide substitution model (HKY + F + I + G4) was selected according to the BIC obtained with the IQ-TREE (Kalyaanamoorthy et al., 2017). A MCMC with 200×10^6 generations and sampled every 200×10^5 steps was performed. The convergence of the parameters (ESS ≥200, burn-in 10%) were verified with Tracer v1.7.1 (Rambaut et al., 2018).

2.6. Evolution of MY lineage

The evolutionary history of the MY lineage was estimated by using a second Dataset (Dataset 2) containing 222 MY sequences from the IG region selected from the NCBI repository with available data about geographic sampling. Details concerning the accession numbers, and geographic location of samples are provided in Supplementary Table 2. Alignments were performed on MAFFT online version (https://mafft.cbrc.jp/alignment/server/) against the NCBI reference sequence (NC_001699). Phylogenetic analysis was conducting using Bayesian (BP) inference in MrBayes v3.2.7 (Ronquist et al., 2012). The best nucleotide substitution model (HKY + F + G4) was selected according to those suggested by Bayesian Information Criterion (BIC) obtained with the IQ-TREE (Kalyaanamoorthy et al., 2017). We applied Bayesian Markov Chain Monte Carlo (MCMC) of 3×10^6 length and sampled every 3×10^5 steps. BP analysis was run up to convergence of parameters: effective sample size (ESS) ≥ 200, with a 10% burn-in, verified with Tracer v1.7.1 software (Rambaut et al., 2018).

2.7. Viral demographic growth in America

In addition, the demographic profile for Misiones samples was run with a sub-sample of sequences from Dataset 2 (n = 119) that were identified as Branch II. This group of sequences represented a monophyletic cluster of isolates from the American continent (including Peru, Mexico, Guatemala and Brazil). The analysis was calibrated with a uniform mutation rate prior: (2.00×10^{-7}-3.74×10^{-7} substitution/sites/years) corresponding to the 95% Highest Posterior Density interval (HPD 95% interval) obtained in the *Molecular Dating* section. An alternative demographic profile was constructed by applying the HPD 95% interval of the substitution rate obtained for the first epoch structure of the TDR clock (from present up to 16.000 ybp) (1.04×10^{-6}-1.62×10^{-6} substitution/sites/years). The best nucleotide substitution model (HKY + F + G4) was selected according to those suggested by BIC obtained with the IQ-TREE (Kalyaanamoorthy et al., 2017). An UCLN under and a BSK model were applied. A MCMC with 100×10^6 generations and sampled every 100×10^5 steps was performed. The convergence of the parameters [effective sample size (ESS) ≥ 200, burn-in 10%] was verified with Tracer v1.7.1 (Rambaut et al., 2018).

2.8. Nucleotide sequences accession numbers

Nucleotide sequences for the JCV have been deposited in GenBank under accession numbers OQ615748-OQ615769.

2.9. Ethical aspects

The Ethics Committee of the Hospital Dr. Ramón Madariaga (Posadas, Misiones, Argentina) approved this study (no code number, date: June 29, 2012) and conducted under approved Grant ANPCyT 2014–2018 (Res.468–14). The study was designed and performed according to the Helsinki declaration and all subjects gave their written informed consent to participate.

3. Results

3.1. Characteristics of the study population and JCV detection

Samples from 121 individuals living in different locations within Misiones were analyzed. The median age of participants was 44 years old (range 22–81), 64% were women, and 13% were born in foreign countries (Brazil n = 7, Paraguay n = 2, and others n = 3). Twenty-two samples (18.2%) were found to be positive for JCV through PCR amplification and direct sequencing. For those individuals with JCV, their median age was 54.5 years old (range 18–79), with 45% of them being women. Of the Argentina individuals, 13.6% lived in the North, 77.3% in the Center and 9.1% in the South of the province, with 25% being born in neighboring countries (20% Brazil and 5% Paraguay).

3.2. Phylogenetic characterization of JCV

The phylogenetic analysis of global JCV sequences is shown in Fig. 2. Sequences from Misiones were classified as 32% Super-lineage A [European Eu-a (n = 7)] and 68% Super-lineage B [Amerindian MY (n = 8), Eurasian B1-c (n = 4), Asian B1-b (n = 2) and African Af2 (n = 1)]. JCV sequences were further geographically classified as 36% Native American (MY), 49.8% European (Eu-a, B1-c), 9% Asian (B1-b) and 4.5% African (Af2). This classification was based on the prevalence of the various genotypes in these populations.

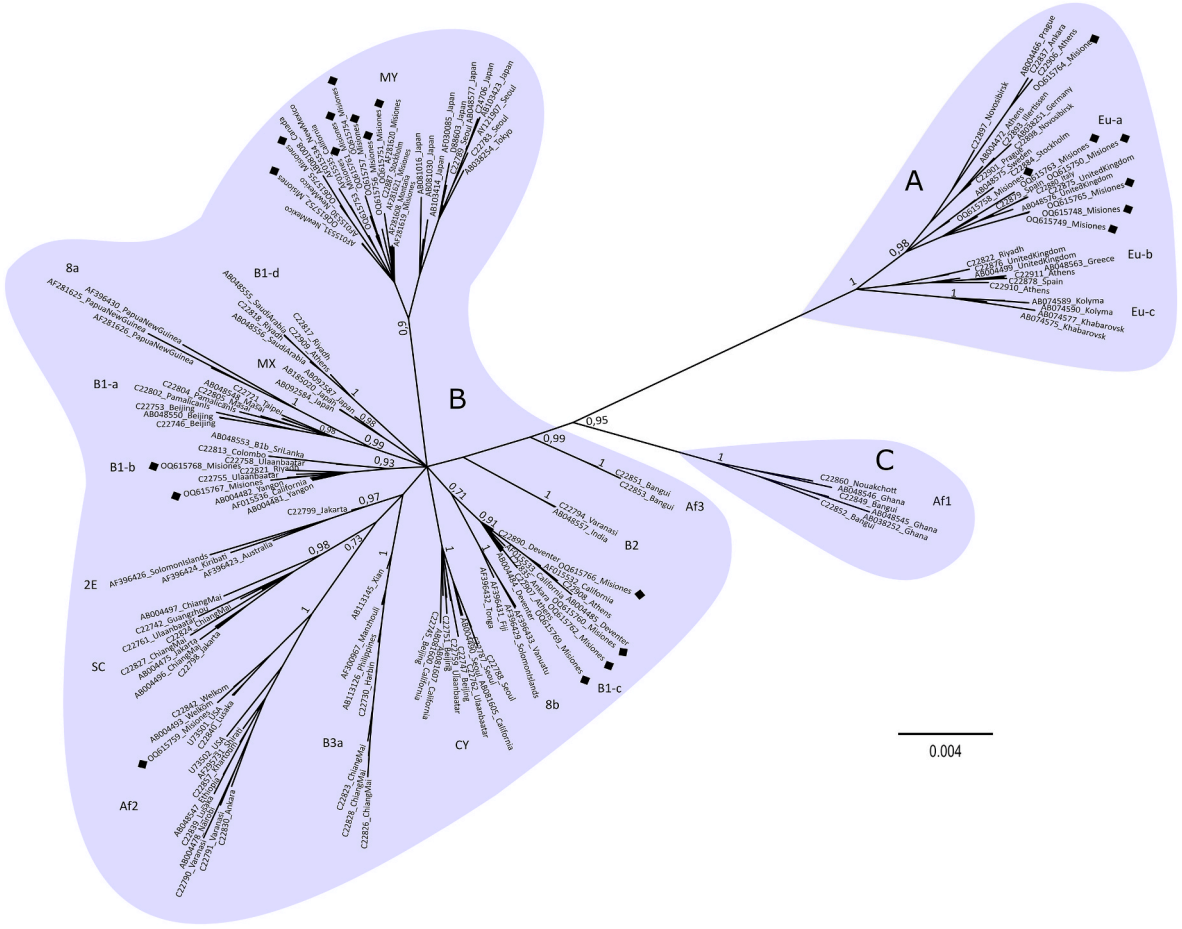

Fig. 2. An unrooted-radial JCV Bayesian phylogenetic tree (HKY + F + I + G4 model for nucleotide substitutions). The numbers of each node represent the posterior support for the main groups (values lower than 0.7 are not shown). Sequences obtained in this study are indicated with diamond symbols. This result was repeated in the Maximum Likelihood analysis.

3.3. Molecular dating

Bayesian estimates for the tMRCA of JCV sequences are shown in Table 1. The analysis of the different tree hypothesis for the earliest diversifications of JCV showed that Model 3 was better supported (log10 B F > 1.1 against the other models). This topology was characterized by A and C (Af1 and European clusters) being sister groups. The age of the tMRCA was about 87,506 ybp (HPD 95% interval

Table 1
Estimates of the tMRCA principal groups considered in this study of JCV for the IG region by Bayesian coalescent methods under a UCLN molecular clock and a Skyline population model. Different evolutionary hypothesis and its respective substitution rate estimates are shown.

Coalescence	Non-constrained analysis		*MODEL 1 (A (B,C))*		*MODEL 2 (C (A,B))*		*MODEL 3* [a] *((C,A) B)*	
	mean	*95% HPD interval*	*mean*	*95% HPD interval*	*mean*	*95% HPD interval*	*mean*	*95% HPD interval*
Mutation rate	$2.87E^{-7}$	$2.00E^{-7}$-$3.74E^{-7}$	$2.88E^{-7}$	$2.07E^{-7}$-$3.69E^{-7}$	$2.65E^{-7}$	$1.97E^{-7}$-$3.33E^{-7}$	$2.64E^{-7}$	$2.05E^{-7}$-$3.36E^{-7}$
Cluster tMRCA								
MY Amerind	15.355	12.626–17.999	15.257	12.443–17.988	15.670	12.806–17.990	15.677	12.716–17.998
MY Asian	15.704	9.761–21.457	15.724	9.987–20.872	16.881	9.830–23.381	17.093	11.217–25.006
MY lineage	20.134	14.345–27.111	20.208	14.582–27.708	21.778	14.922–29.753	21.914	15.383–30.177
Lineage Eu-a	22.651	14.390–34.227	22.753	13.620–32.371	23.742	13.937–32.140	23.736	14.909–34.004
Lineage B1-b	19.821	9.947–30.866	19.476	9.380–29.288	20.568	10.026–31.413	20.584	10.423–31.156
Lineage Af2	25.156	13.181–39.695	24.926	13.810–39.312	27.794	15.238–43.186	28.697	15.503–44.446
Lineage B1-c	14.543	8.378–22.458	14.606	7.929–21.041	15.685	12.187–36.343	15.777	8.265–23.660
Super Lineage A	43.606	40.005–49.626	43.739	40.003–49.900	43.314	40.012–48.607	43.451	40.019–49.518
Super Lineage B	54.403	33.077–66.807	NA	NA	62.293	42.913–83.080	64.497	45.471–87.208
Super Lineage C	20.356	9.687–34.133	20.295	9.811–32.469	24.377	10.993–41.753	24.208	10.590–40.023
A/B	NA	NA	NA	NA	81.960	63.032–97.744	NA	NA
B/C	61.287	42.070–82.317	60.440	41.511–81.171	NA	NA	NA	NA
C/A	NA	NA	NA	NA	NA	NA	81.144	64.939–97.255
Tree Root	88.000	71.725–99.914	87.606	70.824–99.993	87.776	72.017–99.938	87.506	71.692–99.929

*NA = not applicable.
*tMRCAs are expressed in years.
[a] More favored model.

71,692–99,929), and for the MY lineage 21,914 ybp (HPD 95% interval 15,383–30,177), with the Amerindian branch dating to 15,677 ybp (HPD 95% interval 12,716–17,998). The evolutionary rate was estimated as 2.64×10^{-7} s/s/y (HPD 95% interval 2.05×10^{-7}-3.36×10^{-7}). Calculations of BF for the different models are presented in Supplementary Table 3. Maximum clade credibility trees for all models are presented in Supplementary Figs. 1 and 4.

3.4. Time Dependent Rate

The tMRCA of JCPyV phylogenetic events under TDR clock are shown in Table 2. The corresponding mean substitution rates were 1.31×10^{-6} (HPD 95% interval 1.04×10^{-6}-1.62×10^{-6} s/s/y), 1.52×10^{-7} (HPD 95% interval 1.16×10^{-7}-1.96×10^{-7} s/s/y) and 2.24×10^{-8} (HPD 95% interval 1.64×10^{-8}-2.92×10^{-8} s/s/y), for the epoch 1 (up to 16,000 ybp), 2 (between 16,000 and 44,000 ybp) and 3 (from 44,000 ybp up to ∞), respectively. Moreover, the age of the tMRCA for JCV was about 95,851 ybp (HPD 95% interval 88,218–99,976), and for the MY lineage 14,491 ybp (HPD 95% interval 12,265–17,234), with the American branch dating to 12,782 ybp (HPD 95% interval 12,000–14,296). A maximum clade credibility tree is presented in Supplementary Fig. 5.

3.5. Evolution of MY lineage

The phylogenetic tree for MY lineages is presented in Fig. 3. The tree topology showed a clear division of the IG sequences into two main groups (named here as Branch I and Branch II) with high support values. In particular, Branch II contained most sequences circulating in the American continent [n = 119 out of 222 (53,6%)], whereas Branch I encompassed only those from Asia (n = 103). All of the sequences obtained in this study belonged to Branch II. With regard to the Misiones samples, they were not monophyletic and intermingled with other South American samples.

3.6. Viral demographic growth in America

The Bayesian Skyline plot for MY Branch II estimated at a slow rate is shown in Fig. 4. The tMRCA for the MY Amerind cluster was 19,364 ybp (HPD 95% interval 9319–30,522) with a demographic expansion starting close to 5000 ybp (Fig. 4a). Phylogenetic analysis showed that Misiones samples grouped into three supported clusters (posterior >0.9), with the following ages: 3701 ybp (612–4832); 3919 ybp (1254–7144) and 4968 ybp (1736–8191) (Supplementary Fig. 6). By

Table 2
Estimates of the tMRCA principal groups considered in this study of JC virus for the IG region by Bayesian coalescent methods under a TDR molecular clock. The obtained substitution rate estimates are also shown.

Coalescence	Time dependent rate	
	mean	***95% HPD interval***
Substitution rate	1.31×10^{-6}	1.04×10^{-6}-1.62×10^{-6}
	1.52×10^{-7}	1.16×10^{-7}-1.93×10^{-7}
	2.24×10^{-8}	1.64×10^{-8}-2.92×10^{-8}
Cluster tMRCA		
MY Amerind	12,782	12,000–14,296
MY Asian	4695	2374–6864
MY lineage	14,491	12,265–17,234
Lineage Eu-a	5181	2889–7284
Lineage B1-b	4927	2277–6636
Lineage Af2	4988	3066–6685
Lineage B1-c	3275	1822–4906
Super Lineage A	44,116	40,011–50,258
Super Lineage B	45,034	19,639–65,093
Super Lineage C	4035	2091–5935
Tree Root	95,851	88,218–99,976

*tMRCAs are expressed in years.

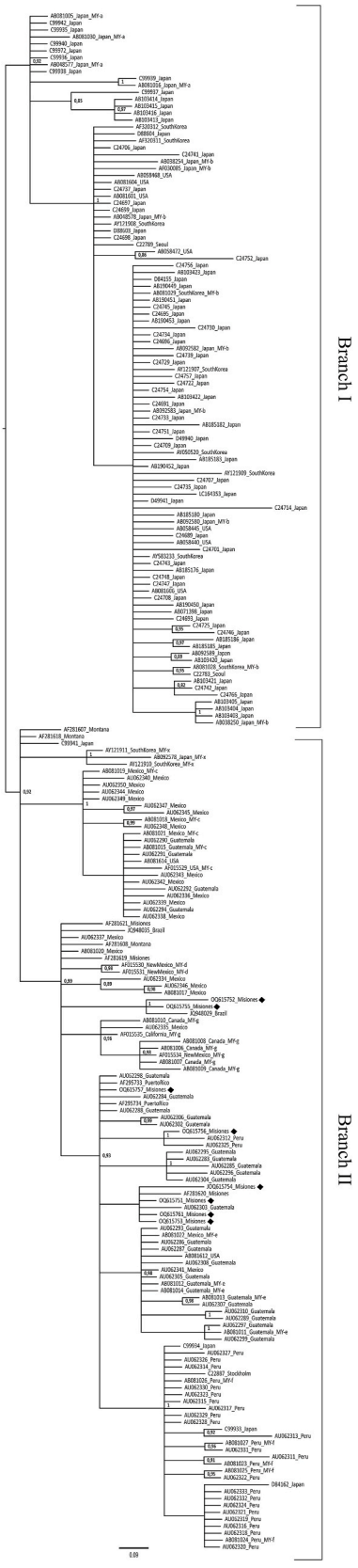

Fig. 3. Mid-point rooted JCV-MY lineage Bayesian phylogenetic tree (HKY + F + G4 model for nucleotide substitutions). Two hundred twenty-two sequences comprising the IG region with geographic location. Numbers of each node represent the posterior support (values lower than 0.7 are not shown). Sequences obtained in this study are indicated with diamond symbols. This result was repeated in the Maximum Likelihood analysis.

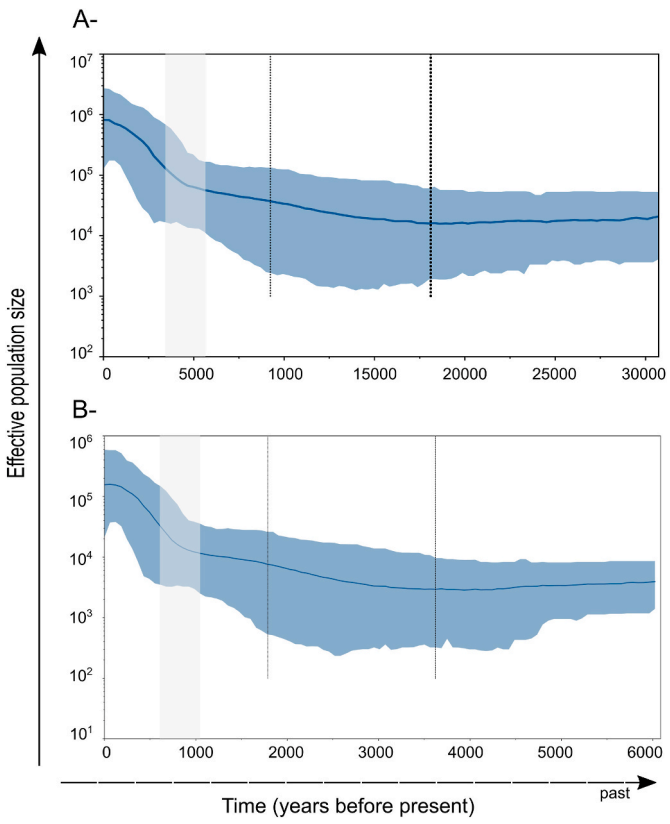

Fig. 4. Past population dynamics of the JCV-MY lineage Branch II. Molecular Clock of: a) Slow, range of 2.05 × 10^{-7}-3.36 × 10^{-7} s/s/y; b) Fast, 1.04 × 10^{-6}-1.61 × 10^{-6} s/s/y The analysis was carried out under a Skyline population model and a UCLN molecular clock. The shaded portion is the 95% Bayesian credibility interval and the solid line indicates the posterior median. The vertical dotted lines represent the mean estimate for the time to the root of the tree and to its 95% HPD interval (the highest overlapping with the Y-axis). The x-axis is the time in years and the y-axis is the effective population size in a log-scale. Grey shading bars indicates the beginning of the major population expansion.

contrast, the use of the TDR molecular clock indicated that the root dated to 3766 ybp (HPD 95% interval 1969–5961) and a demographic expansion started close to 1000 ybp (Fig. 4b), with Misiones clusters having an age range of 725–987 ybp (Supplementary Fig. 7).

4. Discussion

The people of Misiones have been described as having European ancestry based on genetic (autosomal SNPs and Y-chromosome data) and sociocultural studies (Corach et al., 2010; Catelli et al., 2011). However, these approaches have underestimated the contribution of Native Americans to the population. According to mitochondrial DNA (mtDNA) analysis, the Amerindian contribution could be as high as 68%, highlighting the fact that more than half of the current population has an Amerindian ancestral grandmother (Corach et al., 2010; Catelli et al., 2011; Badano et al., 2018). In this context, the study of viral molecular markers was necessary to gain a broader perspective on the genetic diversity of this region of Argentina.

The use of the JCV as a means of tracing human migration and admixture is well documented. Published studies have involved Koreans and Japanese (Zheng et al., 2003), Northeastern Siberians and Canadian Inuit (Sugimoto et al., 2002b), modern Puerto Ricans (Fernandez-Cobo et al., 2001), African Americans (Chima et al., 2000), among others.

In our study, we found that several JCV lineages are circulating in the Misiones's population (Eu-a, MY, B1-c, B1-b and Af2) with these sequences being classified as 63.7% Asian-American, 31.8% European and 4.5% African in origin. Overall, the presence of these three ethnogeographic components has been reported in all mixed populations in the Americas, but with some differences in their composition. These discrepancies are associated with the processes of ethnic background/ancestry of each population being evaluated (Chima et al., 2000; Zheng et al., 2003; Fernandez-Cobo et al., 2001; Cayres-Vallinoto et al., 2012). In agreement, the frequencies were quite similar to those reported for the countryside of Misiones (Sanabria et al., 2019).

Interestingly, both studies have revealed a non-trivial contribution of African ancestry to the population, which is higher than the one reported by the 2010 census (0.3%) (INDEC, 2010). This African contribution can be attributed to recent population movements, since 20% of the participants were born in Brazil, a country with a significant African genetic background in its population (Alves-Silva et al., 2000). Other sources may go back to the Africans that moved into the territory from Brazil to escape from slavery during colonial times (18th-19th centuries) and after the Triple Alliance war (1865–1870) (Lamborghini et al., 2017).

Although the strong ethno-geographical classification of JCV in Misiones is consistent with its population history, the overall phylogeny of worldwide viral strains did not resemble the Out of Africa Model of human evolution. Indeed, the general topology showed a 'trichotomy' at the basal super-lineages, in concordance with what is widely documented in the literature (Sugimoto et al., 1997; Yogo et al., 2004; Shackelton et al., 2006; Kitchen et al., 2008; Torres, 2020). Most studies have attributed this pattern to inadequate sampling, viral extinction, or a more complex scenario of human-viral dispersal (for example, a two migration model of the human dispersal out of Africa) (Pavesi, 2004).

In this study, we analyzed several phylogenetic parameters to test the best-fit model for different rooting hypotheses for the JCV tree. Conspicuously, we found that the Out-of-Africa model was less supported whereas super lineages A and C were possibly involved in the earliest phase of JCV divergence. Similarly, other non-Out of Africa topology has been reported for Merkel-cell and BK polyomavirus, both viruses closely related to JCV (Torres et al., 2018). These findings may require us to reconceptualize the origin and dispersal of these viruses. In this regard, there is growing evidence for hybridization between modern humans and archaic hominins that may be pertinent to studies of viral evolution and dispersion (Sankararaman et al., 2012; Pimenoff et al., 2017; Smith et al., 2017; Chen et al., 2018). For example, Chen et al. (2006) and Nishimoto et al. (2007) hypothesized a transmission of subtype IV of the BK polyomavirus from an archaic hominin population in Asia to an ancestral population that generated modern Asians (Chen et al., 2006; Nishimoto et al., 2007). Collectively, these studies suggest a much more dynamic past in terms of gene flow and virus host jumps in ancient populations that will deserve future consideration.

Regarding MY-Amerindian evolutionary history of Branch II (Zheng et al., 2003), the molecular dating of the JCV tree shows that the split from the sister Asian branch (Branch I) occurred some 21,914 ybp, a date consistent with the migration of ancestral Native American groups into Beringia at the beginning of the Last Glacial Maximum (16,000–25,000 ybp) (Schurr and Sherry, 2004; Posth et al., 2018). Locally, Misiones samples were not monophyletic and belonged to different clusters whose ages ranged from 3532 to 4680 ybp. This period encompasses the origin and dispersion of the Tupian linguistic family (5000 ybp) in South America and the later expansion of Guarani Indians into the Misiones region approximately 1500–2000 ybp (Poujade, 1992; Marrero et al., 2007).

Moreover, our demographic analysis showed that the viral demographic expansion of MY lineage in the Americas started around 5000 ybp. In terms of population size, it is estimated that the Andean region was inhabited by nearly 15, 600, 000 people by the time of the Inca Empire (1000–500 ybp) and 28, 950, 000 people in Mesoamérica during the period encompassing the Mayan (3500–500 ybp) and Aztec (1100–1500 ybp) empires (Koch et al., 2019). In the Amazon region, up

to 4,000,000 people with ~900,000 Tupi family speakers were living along the Brazilian coast nearly 2500 ybp (Koch et al., 2019; Castro E Silva et al., 2020; Bolnick et al., 2016). The growth of large settlements and cities in these regions may have increased the number of susceptible hosts and enlarged the transmission networks of many agents, including JCV in the continent.

Lastly, we use the TDR clock as an alternative approach for the molecular dating of JCV evolution. This was necessary because the substitution rate for JCV is still a matter of debate (Shackelton et al., 2006; Kitchen et al., 2008; Sharp and Simmonds, 2011). In this sense, there is increasing evidence that evolutionary rate estimates are dependent on their measurement timescales (i.e., the shorter the timescale, the higher the estimated value) (Aiewsakun and Katzourakis, 2015; Membrebe et al., 2019). Thus the rationale for conducting a TDR analysis was to gain a clearer understanding of the evolutionary parameters of the leaves of the tree. This approach allowed us to analyze a time-frame consistent with and traceable to Misiones population history as an alternative hypothesis to classic co-divergence rates. Not surprisingly, we estimated a substitution rate of 1.32×10^{-6} s/s/y for the last 16,000 years of evolution, which is one logarithm faster than predicted with the traditional UCLN model mentioned above (2.64×10^{-7} s/s/y).

Based on this TDR estimation, the demographic expansion of the Amerindian MY lineage started more recently, approximately 1000 ybp with the effective sample size rising at 500 ybp. Although this demographic model is a less plausible explanation of MY evolution in expanding Amerindian populations, including the Tupi-Guarani speaking tribes, it deserves consideration for future studies of large datasets. Interestingly, neither model (i.e., slow versus fast substitution rate) reflects the bottlenecks suffered by Native Americans after the arrival of Europeans (530 ybp) when their populations drastically declined, mainly due to epidemics and slavery (O'Fallon and Fehren-Schmitz, 2011).

Therefore, JCV survival in admixed populations suggest the occurrence of host switching events between populations. This hypothesis relies on the fact that viral transmission can be horizontal through close interpersonal contact, vertical from mother to newborn, and vertical-like by intra-family long-term cohabitation (Kitamura et al., 1994; Kunitake et al., 1995; Boldorini et al., 2011; Mazzoni et al., 2020). Thus, JCV infection may not be governed by population size per se as much as transmission networks, which facilitated their survival for up to 400 years after the arrival of Europeans in the resulting admixed population.

5. Study limitations and perspectives

The use of human migration as a calibration point in JCV evolution is controversial (Shackelton et al., 2006; Kitchen et al., 2008). However, other approaches such as the analysis of stamped-date samples have proven unsuccessful in retrieving the phylogenetic history of this and other slow mutation rate viruses (Firth et al., 2010; Sharp and Simmonds, 2011). Moreover, in a recent study, Forni et al. (2020) use JCV and human mtDNA reconciliation trees to provide complementary evidence for the codivergence hypothesis (Forni et al., 2020). Meanwhile, the exploration of parameters and mathematical models with larger datasets and genome region will remain as an available tool for exploring alternative hypothesis for JCV evolution.

6. Conclusions

Admixed populations offer an informative model for evolutionary analysis of the origin and spread of viruses within them. Our results confirm the significant presence of JCV lineages attributed to the Amerindian component in the current population of the Misiones Province. Phylogenetic analysis shows that the Amerindian JCV viral population has not decreased over the last 1000 years. We explained this finding by an initial population expansion with the pre-Columbian native societies, and a host switch events between populations after European arrival 500 years ago. New data from human and viral markers and larger genome coverage are needed to increase the sensitivity of our analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virol.2023.05.009.

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