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Research paper

JC polyomavirus circulation in one-year surveillance in wastewater in Santiago, Chile

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A B S T R A C T

Human polyomavirus JC (JCPyV) is a widely distributed viral agent and because it high resistance against environmental conditions it is frequently recovered from diverse sources of water and is considered a good marker for human pollution. Phylogenetic analysis of JCPyV isolated in different part of the world has revealed 7 genotypes, which have been associated with specific populations or ethnics groups. This feature has been used to trace pre-historic and historic human migration patterns across the world. Although there are many reports describing genotypes distribution around the world, data on JCPyV genotypes in the southernmost areas of South America are scarce. The goal of this study is to detect and characterize the JCPyV that circulates in Santiago, Chile using sewage samples from wastewater treatment plants (WWTP). Sewage samples were obtained monthly during 1 year from three WWTPs which together process about 80% of wastewater generated in the city of Santiago, Chile. Our results show that JCPyV profusely circulates in Santiago, Chile, because it was detected in 80.56% of the samples, reinforcing the use of JCPyV as a feasible marker to assess human environmental pollution. JCPyV was detected in high frequency in influents and effluents samples, with the largest WWTPs showing the highest percentage of detection and viral loads. In the phylogenetic analysis the Chilean sequences clustered mainly with genotype 2A (Asian genotype). This is similar to that previously reported from Buenos Aires, Argentina and divergent to data from Brazil, where the circulation of European subtypes 1 and 4 and African subtypes 3 and 6 has been described.

1. Introduction

The JC polyomavirus (JCPyV) is a non-enveloped, small icosahedral particle (~45 nm), with a closed circular, supercoiled, double-stranded DNA genome of approximately 5000 base pairs (Frisque et al., 1984; Pinto and Dobson, 2014). Like other polyomaviruses, the JCPyV genome is organized in two protein-coding regions (early and late) that are transcribed in opposite directions starting from a common non-coding control region (NCCR). The early region encodes the large T and small t antigens as well as several splice variants of the T antigen, while the late coding region encodes VP1, VP2, VP3 and the agnoprotein (Frisque et al., 1984; White et al., 2009; Decaprio and Garcea, 2013).

JCPyV was originally described from degenerated brain tissue of a deceased patient who had progressive multifocal leukoencephalopathy (PML) (Zu Rhein and Chou, 1965), and 6 years later it was isolated in primary cultures of human fetal glial (PHFG) cells by inoculation of a similar brain lesion extract obtained from a patient with Hodgkin's disease (Padgett et al., 1971). Current knowledge indicates that JCPyV is widespread in the human population and it only causes PML or other diseases in immunosuppressed individuals such as patients with AIDS or in treatment with immunosuppressive drugs (Ferenczy et al., 2012). Seroepidemiological studies have shown that JCPyV infection is acquired very early in life, likely before 6 months of age, and a sizeable percentage of infants will become seropositive within 2 years of age (Elia et al., 2017). Seroprevalence increases with age, reaching about 50–80% in healthy adults throughout the world (Padgett and Walker, 1973; Taguchi et al., 1982; Kitamura et al., 1990; Knowles et al., 2003; Egli et al., 2009; Antonsson et al., 2010; Decaprio and Garcea, 2013; Sroller et al., 2014; Gossai et al., 2016; Elia et al., 2017).

It has been described that after primary asymptomatic infection JCPyV can persist in renal tissues, and in adults the virus can replicate to generate progeny, being profusely released through the urine (Chesters et al., 1983; Kitamura et al., 1994; Kitamura et al., 1990). Between 40 and 70% of healthy adults eliminate virus through the urine in concentrations ranging from 10^2 to 10^7 genome copies (GC) per mL (Agostini et al., 1996; Ling et al., 2003; Polo et al., 2004; Berger et al., 2006; Rodrigues et al., 2007; Rossi et al., 2007; Egli et al., 2009). JCPyV is frequently detected in urban wastewater from widely diverse geographical areas around the world (Bofill-Mas et al., 2000, 2001; Bofill-Mas and Girones, 2003; Albinana-Gimenez et al., 2006; Fumian et al., 2010a), as well as in river water and in drinking water treatment plants (DWTP). Molecular assays to detect JCPyV have proven useful to detect anthropogenic pollution in different water environmental sources (Albinana-Gimenez et al., 2006, 2009).

Currently, there are 7 JCPyV genotypes (1, 2, 3, 4, 6, 7 and 8) circulating in the world, each with one to five subtypes (Stoner et al.,

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2000; Cubitt et al., 2001; Cui et al., 2004; Calvignac-Spencer et al., 2016). Interestingly, the presence of the different subtypes has been associated with defined geographic areas and also with different ethnic groups; therefore, it is being used to trace pre-historic and historic human migration patterns across the world (Hatwell and Sharp, 2000; Sugimoto et al., 2002; Yogo et al., 2004; Pavesi, 2005; Kitchen et al., 2008). Phylogenetic analyses have shown that JCPyV strains of genotypes 1 and 4 are found primarily in persons of European descent, genotypes 2, 7, and 8 in Asians and Pacific Islanders, and genotypes 3 and 6 in Africans (Agostini et al., 1996; Agostini et al., 1997; Jobe et al., 2001; Fernandez-Cobo et al., 2002; Sugimoto et al., 2002; Yanagihara et al., 2002). The genetic characterization of JCPyV in closed Amerindian populations from North, Central and South America supports the theory of the settlement of the Americas through Beringia during the last ice age, because a marked presence was observed of genotype 2, subtype 2A, which was originated in northeast Asia (Stoner et al., 2000; Fernandez-Cobo et al., 2002; Nielsen et al., 2017). The genetic characterization of JCPyV strains circulating in modern Ameridian populations has shown some variability, possibly introduced by the historic post-Columbian migrations. Meaningful differences have been observed in the circulating genotypes in Rio de Janeiro and Porto Alegre, Brazil (Fumian et al., 2010a; Comerlato et al., 2017) in comparison to Buenos Aires, Argentina (Torres et al., 2016), suggesting that the current distribution of the JCPyV types is heterogeneous in the southernmost regions of America.

Based on the wide acceptance that the presence of virus in wastewater from an urban site reflects the current prevalence of this virus of the human population which lives in this site, independently of the symptomatology (Bofill-Mas et al., 2000, 2001; Bofill-Mas and Girones, 2003; Sinclair et al., 2008), we analyzed wastewater generated in the city of Santiago during 1 year period in order to approach the molecular epidemiology of JCPyV. The generated data together with previous reports allowed us to contribute to complete the molecular epidemiological map of JCPyV in the southernmost region of the Americas.

2. Materials and methods

2.1. Sewage samples

Raw sewage samples were collected from three Wastewater Treatment Plants (WWTPs) which together process about 80% of wastewater generated in the city of Santiago, Chile (about 5 million inhabitants), i.e. La Farfana, with a flow of 8800 L/s (~ 50% of wastewater from Santiago), El Trebal, with a flow of 4400 L/s (~28%), and Los Trapenses with a flow of 40 L/s (~0.22%). The samples were obtained monthly from January to December in 2010, from the influent and effluent of each WWTP in sterile propylene bottles, and processed the same day of sampling.

Thirty-six mL of each sample were concentrated by utracentrifugation and the final pellet was re-suspended in 200 µL of phosphate buffered saline (PBS) pH 7.4 and stored at −80 °C until use (concentration factor = x180). According to previous studies, the recovery rate of this procedure in sewage samples with similar conditions was 47%. (Fumian et al., 2010b).

2.2. Nucleic acid extraction

Total viral nucleic acid was extracted from concentrated sewage using the High Pure Viral Nucleic Acid Kit (Roche Diagnostics GmbH, Germany) as described by the manufacturer. In order to avoid block the extraction column with excessive impurity, 50 µL of each sample were diluted up to 200 µL using PBS before processing (dilution factor = 1:4). The eluates obtained from each sample were stored at 20 °C until use. The final concentration factor was calculated to be 45 × (180:4). The presence of PCR inhibitors might lead to underestimation of the viral load and the frequency of positive sample. Hence, to get more information concerning the level of inhibition in PCR, PP7 phage was used as an internal control to identify the occurrence of PCR inhibition (Fumian et al., 2010b). No inhibitory effect was observed when comparing the Ct value of internal control of the sewage samples to the negative control, (data not shown). The negative samples were analyzed directly and also diluted 1:10 to discard the eventually effects of inhibitors.

2.3. JCPyV detection and quantification

JCPyV detection and quantification were carried out using quantitative real-time polymerase chain reaction (qPCR) according to the protocol described by Pal et al. (2006). Briefly, an 89 bp fragment from the large T antigen (LTAg) coding region of JCPyV (positions 4251 to 4339 in reference sequence NC_001699.1) was amplified using the forward and reverse JE3 (Mad-1) primers and 6-FAM/BHQ1 JE3 (Mad-1) hydrolysis probe (Integrated DNA Technologies IDT, USA). The reaction mix contained 1 × TaqMan Universal PCR Master Mix (Applied Biosystems, USA), 0.5 µM forward and reverse primers, 150 nM probe plus 2 µL of DNA sample in a final volume of 25 µL. The amplification was carried out in a Rotor-Gene real time thermal cycler (Qiagen, Germany) using the following thermal parameters: 50 °C for 2 min, 95 °C for 10 min followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min, with green channel (510 nm) fluorescence measured at the end of each elongation step. For quantification purposes, a standard curve was made using the cycle threshold value (CT) of tenfold serial dilutions of quantitated plasmid containing the LTAg coding region and the sample CT were interpolated in this curve. The analytic sensitivity and limit of quantification of the qPCR assay was determined to be 5 genome copies (GC) per reaction (5 GC in 25 µL reaction volume, equivalent to 200 GC/mL, CI 95%). Results were adjusted to the initial volume (concentration factor = x45) and expressed as genome copies per mL (GC/mL). Therefore, the final limit of quantification was calculated in 4.44 GC/mL.

2.4. Phylogenetic analysis

JCPyV-positive samples were amplified with specific primers for a 610 base pair intergenic region (IG region) as described by Bofill-Mas et al. (Bofill-Mas et al., 2000) with modifications. In brief, a 666 bp fragment of this region was amplified using P1A and P2A primers (positions 2100 to 2766 in reference sequence NC_001699.1). Five µL of DNA sample were added to a PCR mix that consisted of 1 × GoTaq Flexi Buffer, 10 µM dNTPs, 2 µM $MgCl_2$, 0.5 µM of each primer and 1 U GoTaq Flexi DNA polymerase (Promega, USA) in a final volume of 50 µL. The amplification program consisted of initial denaturation at 94 °C for 10min, followed by 50 cycles of 94 °C for 1.5 min, 55 °C for 1.5 min and 72 °C for 2.5 min in a 2720 Thermal Cycler (Applied Biosystems, USA). The amplified PCR products were cloned in pGEMt easy vector (Promega, USA) and then transformed in *Escherichia coli* JM109 competent cells (Promega, USA). To estimate the circulating genotypes in Santiago, 12 clones (1 per month) were arbitrarily chosen. To perform a deeper phylogenetic analysis 30 more clones were selected from samples collected in January, July and December (10 per month). DNA was extracted from each clone using the PureYield Plasmid Miniprep System (Promega, USA), sequenced by Macrogen Inc. (Seoul, South Korea) using T7 and SP6 promoter universal primers and deposited in GenBank (Accession numbers in Supplementary Table 1).

The nucleotide sequences obtained excluding primer sequences were aligned with 69 GenBank sequences representative of currently known genotypes and subtypes (Accession numbers indicated in Figs. 2A and 3) using Clustal W software (Larkin et al., 2007). The phylogenetic analysis and calculation of genetic distances were performed using Mega 6.0 software (Tamura et al., 2013) with the Neighbor-Joining method (bootstrap 1000 replicates) (Saitou and Nei, 1987) and the evolutionary distances were computed using the Kimura

Table 1
JCPyV in influents and effluents of WWTPs in Santiago, Chile.

WWTP	Influents			Effluents		
	Samples	JCPyV positive	Percentage	Samples	JCPyV positive	Percentage
A	12	8	66.7%	12	3	25.0%
B	12	10	83.3%	12	7	58.3%
C	12	11	91.7%	12	6	50.0%
Total	36	29	80.6%	36	16	50.0%

A: Los Trapenses; B: La Farfana; C: El Trebal.

Table 2
JCPyV genome copy quantified in WWTP in Santiago, Chile.

Sampling point	WWTP			Total average (GC/mL)
	A range (GC/mL)*	B range (GC/mL)*	C range (GC/mL)*	
Influents	4.44–660	5.3–23,400	5.8–8910	1820
Effluents	< 4.44	< 4.44–27.3	< 4.44–378	< 4.44

* Quantification limit of qPCR were 5 GC/mL equivalent to 4.44 GC/mL of wastewater (concentration factor = 45).

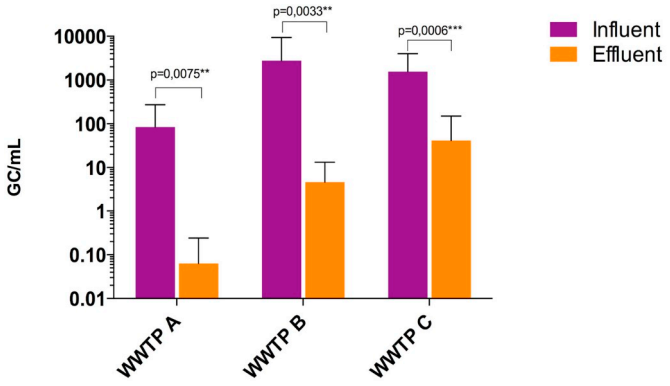

Fig. 1. JCPyV quantification in sewage from three WWTPs. JCPyV was quantified by qPCR and expressed as genome copy/mL (GC/ml). Statistical analyses were made with GraphPad Prism version 6.04 for Windows using a non-parametric test.

2-parameter method (Kimura, 1980). All PCR reactions were performed taking all precautions to avoid cross contamination and negative and positive controls were included in each run.

2.5. Statistical analysis

The Kruskal Wallis and Mann-Whitney non-parametric tests were computed using Prism version 6.04 for MacOs, GraphPad Software, La Jolla California USA, www.graphpad.comGraphPad Prism.

3. Results

3.1. Detection and quantification of JCPyV in sewage samples

Seventy-two samples (36 raw influents and 36 processed effluents) belonging to three WWTP of Santiago, Chile, were analyzed for the presence of JCPyV by qPCR. Of these, 29 (80.56%) influent and 16 (50%) effluent samples were positive for this virus. The percentage of detection varied among plants, with WWTP A showing lower values than plants B and C, which showed similar detection percentages (Table 1 and Table S2).

The quantification analysis showed wide variability in the concentration of GC among positive samples; they ranged from < 4.44 GC/mL (under the quantification limit) to 23,400 GC/mL (Table 2). Independently of the WWTP, as we expected, the global average of GC/mL was higher at the influents than at the effluents (1820 and < 4.44 GC/mL, respectively); and this difference was statistically significant using the Mann-Whitney non-parametric test (Table 2).

Likewise, the JCPyV GC concentration at the influents showed considerable variation among the studied WWTPs (Kruskal Wallis test $P < .05$*). WWTP A showed the lowest value of JCPyV GC and it varied significantly from plant B (Mann Whitney $P < .05$*) and from plant C ($P < .05$**) while there was no statistically significant variation between plants B and C ($P = .4771$). In contraposition, the GC levels between the effluents from the 3 WWTP showed no statistically significant difference ($P = .4771$) (Fig. 1).

3.2. Molecular characterization of JCPyV in sewage

In order to determine the main JCPyV genotypes that circulate in Santiago, Chile during 2010, a 610pb IG region was amplified from 12 JCPyV-positive samples (one every month) and the PCR products were cloned and sequenced. In addition, another 30 sequences were obtained from clones isolated in January (10), July (10) and December (10) and included in the analyses to get a better understanding of the diversity of the circulating JCPyV strains. The divergence matrix of the total data set (42 sequences) showed a general mean distance of 1.3%, ranging from 0.00 to 5.5%. The resulting phylogenetic tree showed that the Chilean isolates segregated into three well-differentiated clusters. The first cluster included 37 isolates (88.09%) and grouped with sequences belonging to JCPyV subtype 2A. The second cluster, with 3 isolates (7.14%), grouped with JCPyV type 1. Finally, the last cluster was composed of two isolates (4.76%), which grouped with JCPyV type 3 reference sequences (Fig. 2A, B). All three groups showed high within-group identity (98.6–99.5%) and high divergence among them (3.3–4.9%).

3.3. Comparison of Chilean sequences regarding previously reported 2A subtypes

To obtain a detailed characterization of the subtype most frequently detected (subtype 2A), 18 Chilean sequences representative of the 2A cluster obtained in this study, were compared with 23 GenBank sequences belonging to several American and East Asian countries, where this subtype has been frequently isolated. The phylogenetic tree showed three defined clusters, the first cluster grouped isolates obtained from continental Asia (China), the second, Korean and Japanese isolates and the third grouped all Amerindian isolates, including the Chilean isolates. Within this cluster, the Chilean isolates showed a dispersed pattern of distribution with the exception of isolate JCt-MARCH col. 8, which associated with Andean Peruvian clones (AB081024, AB081023, AB081026) and JC-E-2, JC-D-2 and JC-D-7 isolate which clusters with the Guarani Argentinian clone AF281620 (Fig. 3).

4. Discussion

This is the first study to detect and characterize the JCPyV that circulates in Santiago, Chile using sewage samples from WWTP. Previous reported presence of JCPyV in Chile has been limited to the clinical experience in patients with HIV infection, where this virus represents 1.1% of all opportunistic diseases that define AIDS (Lasso and Ceron, 2012; Martinez et al., 2016), while data on the distribution of JCPyV genotypes in the southernmost areas of South America are scarce. In the present study we observed that JCPyV circulates profusely in Santiago, Chile, since it was detected in 80.93% of the raw influents of three WWTP. This result is consistent with the polyomavirus prevalence reported around the world (Bofill-Mas et al., 2000, 2001; McQuaig et al., 2009; Fumian et al., 2010a; Staley et al., 2012; Torres

A.

(caption on next page)

Fig. 2. (A) Phylogenetic analysis of JCPyV in sewage from Santiago, Chile. Forty-five IG610 sequences belonging to Chilean positive samples (red squares) along with 69 Genbank prototype sequences (Accession numbers indicated), representing all known genotypes were analyzed. The evolutionary history was inferred using the Neighbor-joining method. The bootstrap consensus tree was inferred from 1000 replicates. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches and partitions reproduced in < 40% bootstrap replicates are collapsed. The evolutionary distances were computed using the Kimura 2-parameter method. Evolutionary analyses were conducted in MEGA5 software. (B) Frequency of JCPyV genotypes obtained from sewage in Santiago, Chile. The genotype distribution of 45 sequences obtained from wastewater as shown in 2A. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

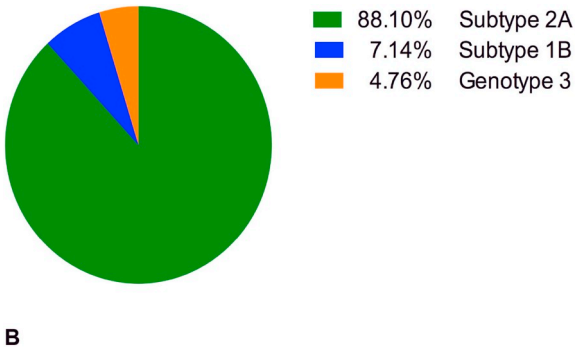

Fig. 2. (*continued*)

et al., 2016). Previous studies conducted in South America obtained similar results, for instance, Fumian et al. (2010a) detected JCPyV in 96% of the raw sewage from Rio de Janeiro, Brazil, while Torres et al., 2016) found JCPyV in 83.3% of wastewater in Buenos Aires, Argentina. In contrast, Comerlato et al. (2017) reported JCPyV detection from sewage in only 40% of samples from Porto Alegre, Brazil. In those studies, the observed differences in detection were attributed to biological aspects such as the magnitude of viral excretion..

We included three WWTP from Santiago and found important differences in JCPyV detection between them. WWTP A showed the lowest frequency of detection and the viral load was at least one order of magnitude less than B and C plants. These differences are probably due to the wastewater flow received by each WWTP, because plant B and C represents the largest WWTP in Chile and South America and together processes the wastewater generated by about 80% of the Santiago population. On the contrary, the WWTP A only covers a small population in the same city. In addition, there are other factors that can affect the

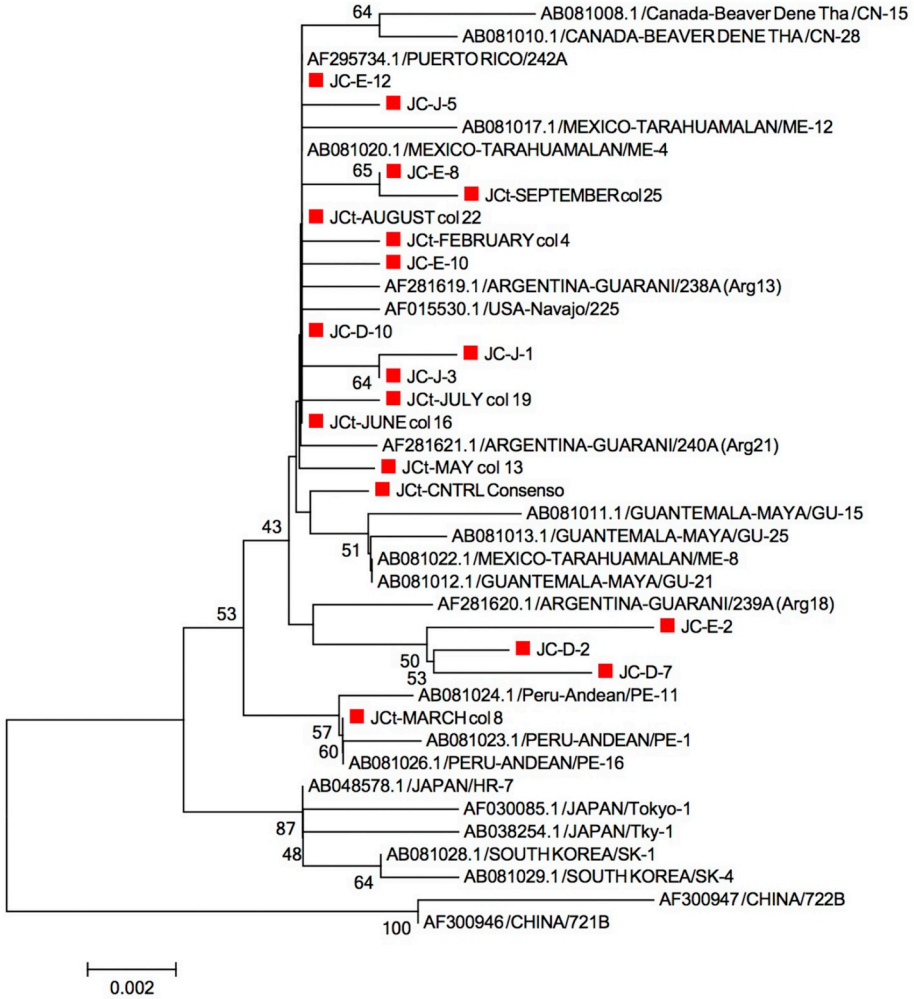

Fig. 3. Phylogenetic analysis of 2A subtypes of JCPyV obtained from sewage in Santiago, Chile. 18 Chilean IG610 sequences (red squares) along with 23 GenBank sequences of 2A subtype belonging to diverse geographical areas and ethnic groups were analyzed. The evolutionary analyses were conducted in MEGA5 software using the same parameters as in Fig. 2A. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

JCPyV concentration in samples, such as the socio-economic status of the populations served, or geographic location of the WWTPs. However, this is out of our scope and new studies are needed to evaluate these factors.

In the present study JCPyV was detected throughout the year from raw sewage in a wide range of GC/mL without seasonal distribution, and these results are in the same line of previous studies (Kitajima et al., 2014; Di Bonito et al., 2015; Jurzik et al., 2015).

Regarding JCPyV detection after treatment in WWTPs, we observed a significant reduction in the mean percentage of detection at the influents (80.56%) vs. the effluents (about 50%), with a significant decrease in GC/mL quantification (1820 v/s < 4.44 GC/mL). Other authors have also observed that JCPyV is not completely removed by wastewater treatment; for this reason, this virus is considered as a good marker for tracking contamination of human origin (Bofill-Mas et al., 2000; Bofill-Mas and Girones, 2003).

The phylogenetic analysis of a 610 pb IG region allowed us to differentiate variants of the virus circulating in sewage from Santiago, Chile. The Asian-originated subtype 2A was the major component of the JCPyV types (88.09%) followed by European subtype 1B (7.14%) and African genotype 3 (4.76%). This data is consistent with those reported by Torres et al., 2016 who showed coexistence of subtypes 2A and 1B in sewage samples from Buenos Aires, Argentina. However, the European subtypes 1 and 4 and African subtypes 3 and 6 were the predominant types detected from sewage in the Brazilian population (Fumian et al., 2010a; Comerlato et al., 2017). These diferences could be explained by the differences in the composition between Brazilian population and other LatinAmerican countries.

There is an emerging consensus in recent years that people arrived at or before 15,000 years ago from Asia to North America across Beringia and then dispersed to Central and South America, bringing with them JCPyV subtype 2A from northeast Asia (Agostini et al., 1996; Nielsen et al., 2017; Yogo et al., 2004). Successive and more recent migrations have made up the structure of the modern Hispanic-American population described by a tri-hybrid genetic model (Sans, 2000). Genetic contributions in varying proportions come from Europe (mainly Spanish), from various African countries that supplied the slave trade and from the Amerindians. Genetic analysis of Chilean individuals using single nucleotide polymorphisms (SNP's) in several ancestry-informative markers (AIM's) estimated the average contribution of the Amerindian (40.55% ± 15.02%), European (54.91% ± 15.69%) and African (4.53% ± 7.32%) to the modern population of Santiago, Chile (Fuentes et al., 2014). Therefore, the strains found in the present study represent all of the groups thought to have contributed genetically to the population process, i.e. type 1 from Europe, type 3 from Africa, and type 2A from Asia. However, it should be noted that the frequency of the 2A subtype (88.09%) was much higher than that expected on the basis of genetic studies of human polymorphisms. This phenomenon has been previously observed in the modern population of Puerto Rico and could reflect a replicative advantage of the Asiatic subtype over the European and African subtypes (Agostini et al., 1996; Stoner et al., 2000; Fernandez Cobo et al., 2001; Fernandez-Cobo et al., 2002). Evidences on this direction are the reports describing a higher frequency of JCPyV excretion in native Amerindians (subtype 2A, 65–80%) than in European (subtypes 1 and 4, 40%) and African natives (subtypes 3 and 6, 20%) (Agostini et al., 1997; Stoner et al., 2000; Chima et al., 1998). However, it is unclear whether the Asian subtype can replicate faster, at a higher viral load or it can reactivate more frequently than the European and African subtypes. A consequence of this replicative advantage could be the overrepresentation of the Asian subtype in wastewater because, on one hand, the higher frequency of detection of this subtype would reflect the overlaping of a greater number of individuals infected and a greater viral loads excreted by the infected individuals, or on the other hand, it is plausible that during 500 years of direct competition between these three viral subtypes, a replicative advantage along with additionals host factors could have led a predominance of the Asian subtype over the others in modern Chilean populations. We believe that in order to unveil this phenomenon, longitudinal studies of excretion frequency are required along with molecular studies to explain these differences at the level of viral replication.

JCPyV subtype 2A is widely dispersed in East Asia and America, and has been described in many Amerindian populations. In order to investigate the phylogenetic relationship between Chilean 2A isolates and those belonging to the above-mentioned populations we compared representative Chilean sequences from this study with available sequences obtained in previous studies from Asian populations (China, Korea, Japan), Flathead (Montana, USA), Navajo (New Mexico, USA), Guaraní (Misiones Province, Argentina), Beaver Dene Tha' (Alberta, Canada), Tarahuamara (Chihuahua, Mexico) and Andean (Peru) (Agostini et al., 1996; Fernandez-Cobo et al., 2002; Yogo et al., 2004). We observed that 2A sequences were segregated into 3 defined clusters, where the isolates from China were clearly distinguishable from those from Japan and Korea, and the American isolates formed another cluster. As previously observed by Shackelton et al. (2006), the American 2A subtype showed a high genetic diversity and it was clearly distinguishable from the ancestral Asian 2A subtype.

5. Conclusions

In this report we demostrate that JCPyV profusely circulates in Santiago, Chile reflecting high excretion rate and prolonged persistence in the environment. Furthermore, JCPyV genotypes present in sewage were consistent with population genetic studies indicating that the Chilean population has a genetic contribution, in different proportions, from European (type 1), Africans (type 3) and Amerindians (type 2A) genotypes.

Finally, the present report indicates some grade of diversity between the genotypes circulating in South American countries and contributes to obtain a more detailed picture of phylogeographic distribution of JCPyV in the southern most part of the continent.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.03.017.

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