



Mitochondrial lineage A2ah found in a pre-Hispanic individual from the Andean region

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Abstract

Objectives: The aim of this study was to contribute to the knowledge of pre-Hispanic Andean mitochondrial diversity by analyzing an individual from the archaeological site Pukara de La Cueva (North-western Argentina). The date of the discovery context (540 ± 60 BP) corresponds to the Regional Developments II period.

Methods: Two separate DNA extractions were performed from dentin powder of one tooth. HVR I was amplified by PCR from each extract in three overlapping fragments and the haplotype was determined by consensus among all obtained sequences. The procedures were carried out under strict protocols developed for working with ancient DNA.

Results: The individual belonged to the A2ah lineage due to the presence of the 16097C and 16098G transitions, which constitute its distinctive motif. This lineage is very rare in Native American populations and was described in four individuals from current groups inhabiting the Bolivian Llanos, two from South-eastern Brazil, and one from the Gran Chaco region. In addition, two other mutations (16260T and 16286T) were shared with one of the individuals from the Bolivian Llanos region.

Conclusions: Considering that the origin of this lineage was postulated for the South American lowlands, the present pre-Hispanic discovery in the Andean area could be taken as a new evidence of gene flow between these regions. Also, it allows the questioning of the geographical origin of this mitochondrial lineage.

1 | INTRODUCTION

Genetics can be a valuable tool for unraveling pre-Hispanic population dynamics complementing archaeological studies. Among the many advantages of analyzing mitochondrial DNA in ancient samples, mainly related to its large copy number (Pakendorf & Stoneking, 2005), its usefulness increases when particular haplotypes are studied.

With the aim of contributing to the knowledge of pre-Hispanic mitochondrial diversity, we analyzed one individual from Pukara de La Cueva as a first approximation to the genetic study of this archaeological site. It is located in the northern sector of the Quebrada de Humahuaca valley, in an adjacent ravine called Quebrada de La Cueva (Jujuy province, northwestern [NW] Argentina) (Figure 1). The study of communication routes and the presence of varied material

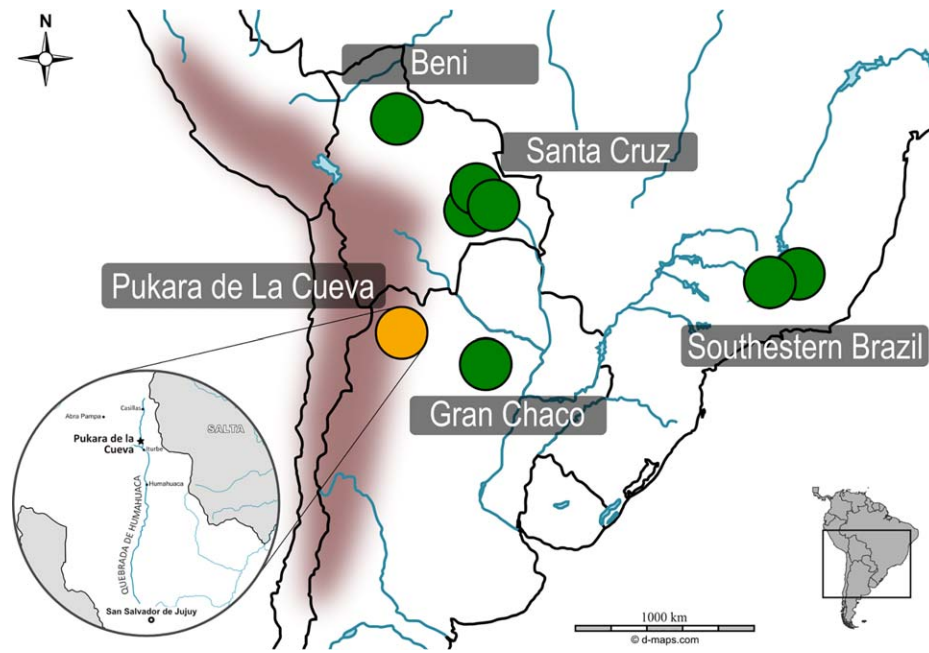


FIGURE 1 Location of the individuals carrying the A2ah mitochondrial lineage. The inset zooms in on a more precise location of the Pukara de La Cueva site, in the Andean region of the Jujuy province. Map constructed from the map available in <https://d-maps.com>

culture in this ravine allowed inferring the important role it played in the interaction networks among diverse regions such as northern and central Quebrada de Humahuaca, the Yungas region, the Puna of Jujuy province, northern Chile, and Bolivia (Ramundo, 2015).

The Pukara de La Cueva is a 1 hectare-extension site located at 3500 m.a.s.l. It was a permanent residence settlement inhabited during the Regional Developments II (700–520 BP) and Inca (520–414 BP) periods (Ramundo, 2012). The architectural and cultural features highlight the similarity between Pukara de La Cueva population and the remaining sites of Quebrada de Humahuaca, particularly those inhabited during the Regional Developments II period, to which the analyzed individual belonged according to the dating obtained from the discovery context (540 ± 60 BP; LP-2268) (Ramundo, 2012).

During this period, pre-Hispanic Andean populations from NW Argentina experienced strong socio-cultural changes. These included the agglomeration into bigger settlements concentrated in the main valleys and ravines, and the intensification of goods interchanged among different regions across all the South-central Andes area and even with non-Andean populations (Nielsen, 2001). The *pukara*-like sites are characteristic of this period. They were large settlements located in higher places such as small hills, which conferred them with a better visibility, probably serving as a defensive strategy (Nielsen, 2001) but also possibly allowing for communication among different communities. For example, from Pukara de La Cueva it was possible to see both the entrance of the ravine through the Angosto de

La Cueva and the closest settlement called Pukara Morado (Ramundo, 2012).

Here, we present evidence that might contribute to a better understanding of the genetic pool of pre-Hispanic populations from Quebrada de Humahuaca and their relationship with other groups from diverse regions.

2 | METHODS

2.1 | Archaeological sample and context

The human remains analyzed in this study correspond to a probable secondary burial since they were found completely disarticulated in a confined sector inside the habitational enclosure n° 45 of the site (Aranda, Luna, & Ramundo, 2012). Bioarcheological analyses determined that at least six individuals of diverse ages (from 1–2 to 45–55 years old) and both sexes were buried there (Aranda et al., 2012). Burial of individuals in domestic areas was a common practice during the Regional Developments period (Nielsen, 2001; Seldes, 2014). Along with the re-utilization of graves and secondary burying, these mortuary practices draw attention to the social importance of maintaining a daily connection between the living and the dead for these pre-Hispanic populations (Aranda et al., 2012; Zaburlin, Seldes, & Mercolli, 2006). Moreover, individuals buried in the same grave were most likely genetically related (Russo et al., 2016).

As a first approach for genetic analyses, we used the first lower right molar of an adult male (sample ID: 269) found in situ on the jaw.

2.2 | Laboratory procedures

The tooth was washed with bleach solution (10% vol/vol), rinsed with deionized water, and UV irradiated for 45 minutes each side in order to remove surface contaminants. Dentin powder was obtained through internal drilling of the dental crown. DNA extraction was performed from approximately 150 mg of powder. Two independent extractions were carried out with different protocols. First, we used QIAamp DNA Investigator Kit (Qiagen) following the manufacturer’s specifications. Second, we applied an organic extraction method with phenol–chloroform following a previously published protocol (Russo, Gheggi, Avena, Dejean, & Cremonte 2017).

HVR I amplification was performed in three overlapping fragments comprising the mitochondrial positions 15984 to 16411. Primers used, PCR amplification, and sequencing conditions were described in a previous study (Russo et al., 2017). For each DNA extract, several amplifications were carried out systematically. Haplogroup determination was performed with Haplogrep 2 (Weissensteiner et al., 2016).

All laboratory procedures were conducted under strict protocols previously described (Russo et al., 2017) developed for preventing contamination when working with ancient DNA (eg, Kemp & Smith, 2010).

2.3 | Phylogenetic analysis

The obtained HVR I sequence was aligned with others belonging to the same mitochondrial lineage and for which geographical and/or ethnical information was available (Alves-Silva et al., 2000; Cabana, Merriwether, Hunley, & Demarchi, 2006; Taboada-Echalar et al., 2013). Also, we included the reference sequences rCSR (Andrews et al., 1999) and RSRS (Behar et al., 2012), along with a representative of the nodal A2 haplogroup. Alignment was performed using ClustalW in MEGA6 (Tamura, Stecher, Peterson, Filipowski, & Kumar, 2013). MEGA was also used for phylogenetic reconstructions. We applied two different methods. First, using the Maximum Parsimony method, a consensus tree was inferred from the 9 most parsimonious trees. Second, the Maximum Likelihood method was employed based on the HKY model (Hasegawa, Kishino, & Yano, 1985) and a consensus tree was inferred from 1000 replicates (Felsenstein, 1985).

3 | RESULTS

The analyzed individual presented the following mitochondrial HVR I mutations: 16097C, 16098G, 16111T, 16223T, 16260T, 16286T, 16290T, 16319A, and 16362C. By searching this haplotype in Haplogrep, we found that it belonged to

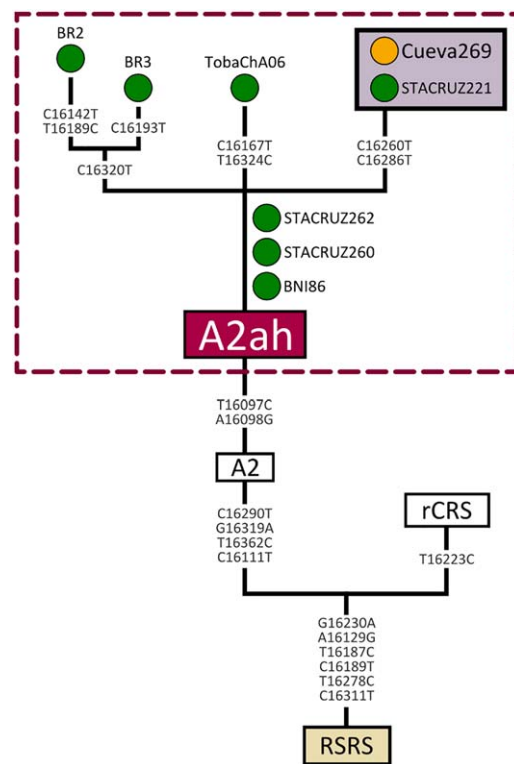


FIGURE 2 Phylogeny of the A2ah mitochondrial lineage based on HVR I sequences. Both Maximum Parsimony and Maximum Likelihood reconstructions led to the same typology. The tree was rooted with the RSRS. Sample ID: *Cueva*: Pukara de La Cueva, *STACRUZ*: Santa Cruz, *BN*: Beni, *BR*: South-eastern Brazil, *TobaChA*: Toba Gran Chaco

the lineage A2ah due to the presence of transitions 16097C and 16098G, its defining motif (Taboada-Echalar et al., 2013).

This lineage is very rare among Native American populations. To date, it has only been reported in seven current individuals from the South American lowlands (Figure 1). Two individuals carrying the A2ah motif were from Minas Gerais state, southeastern Brazil (Alves-Silva et al., 2000); one from a Toba group of the Argentinean territory included in the Gran Chaco region (Cabana et al., 2006); and four from two departments (Beni and Santa Cruz) in the Bolivian Llanos (Taboada-Echalar et al., 2013).

The reconstructed phylogeny of the A2ah lineage based on all those available HVR I sequences (Figure 2) showed that the analyzed individual from Pukara de La Cueva shared a haplotype with one of the individuals from the Bolivian Llanos (Taboada-Echalar et al., 2013).

4 | DISCUSSION

In this study, we report for the first time the presence in the Andean area of a mitochondrial lineage previously described only for the South American lowlands. Moreover, the A2ah



lineage was reported for current populations, while we have found it in a pre-Hispanic individual.

This finding might have considerable implications. Could the presence of this mitochondrial lineage in the Andean area, more specifically in Quebrada de Humahuaca (NW Argentina), be the consequence of a pre-Hispanic gene flow between the Andean and lowlands regions? Moreover, might it be due to an Andean origin of this mitochondrial lineage?

Archaeological evidence supports the existence of interchange among diverse populations across eco-regions, and its increase during the Regional Developments II period (Nielsen, 2001). Moreover, the Quebrada de La Cueva ravine, where Pukara de La Cueva is located, was linked by diverse footpaths to many other regions, including the eastern forests and lowlands (Ramundo, 2012). Also, several archaeological sites across this ravine have rupestrian art with the characteristic caravan trade motifs (Ramundo, 2015) indicating possible participation in the pre-Hispanic goods interchange networks.

Nevertheless, whether this network, in which many pre-Hispanic populations were involved, could have also included gene flow remains uncertain. Regarding this, ancient DNA studies can contribute deeply to our understanding. If gene flow was being regularly maintained among highland and lowland pre-Hispanic populations, some particular genetic variants of one region could eventually be found in the other. Such could be the case for this study in which an infrequent mitochondrial haplotype presumably of the South American lowlands, was found in the Andean area. Considering our results, it would be interesting to extend the genetic analyses to other individuals recovered from Pukara de La Cueva in order to test this hypothesis at the population level. Also, isotopic analyses (such as strontium and oxygen) could shed light on the region in which the analyzed individual lived during the first years of his life.

Finally, ancient DNA studies remain the best way to unravel past population dynamics. Considering that the A2ah place of origin was proposed to be the lowlands region based on studies in current individuals (Taboada-Echalar et al., 2013), our finding highlights the advantages of including ancient samples in understanding past events. The genetic pool of current Native American groups most probably does not reflect the pre-European situation due mainly to the microevolutionary and demographic processes triggered by the conquest. In this context, the fact that one pre-Hispanic Andean individual had the A2ah lineage (and even that the haplotype was shared with a current inhabitant of the South American lowlands, at least considering HVR I) could become a starting point for future studies seeking to increase knowledge about the evolutionary history of this mitochondrial lineage and the populations in which it was present. For example, mitogenome sequencing of this individual could help to refine the haplotype definition and confirm its

presence in a current lowland population. Also, incorporating genetic analyses of the archaeological sites located in the eastern lowlands would be very helpful, not only to elucidate the relationships established with the Andean groups, but also to confirm the origin of the A2ah lineage.

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AUTHORS CONTRIBUTION

CBD, PR, and MGR designed the study. MGR obtained and analyzed the data, and drafted the manuscript. CBD, SAA, VS, and PR edited the manuscript for intellectual content and provided critical comments.

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