MUMMY DNA

Characterization of Genetic Markers of a Kawésqar Body and the Last Descendants of the Same Ethnic Group

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Using nuclear and mitochondrial DNA analysis, we determined that the mummy was a Kawésqar Indian. His mitochondrial DNA was identical to an ancient Aonikenk, an indigenous people in Chile. The Y-chromosome haplotype clearly showed the mummy to be an Amerindian.

INTRODUCTION

The Kawésqara or Alacalufes were nomadic seafaring people. Historically, their territory comprised the southern channels between Golfo de Penas and the Magellan Strait in the Brecknock Peninsula. They lived on calm, 300-mile-long navigable waterways. Their modern descendants live mainly in the cities of Puerto Eden, Puerto Natales and Punta Arenas.

In 2004, a partially mummified body from a Kawésqar Indian (Figure 1) was found in a cave by the shores of Capitan Aracena Island, located 62 miles southwest of the city of Punta Arenas at the southernmost end of Chile (Figure 2). The mummified remains were estimated to be 100 to 150 years old. This finding led the Kawésqar community of Punta Arenas to request the support of the Investigative Police in Chile to protect the remains and provide scientific knowledge to the Magellanica community and indigenous people. With the support of the Council of National Monuments and the Indian Peoples Development Corporation, a working project was set up to i) provide scientific information about the possible causes of death of the individual concerned, ii) reconstruct the face using forensic techniques; iii) record fingerprints and iv) verify the existence of living descendants in the Kawésqar community of Punta Arenas using mitochondrial and nuclear DNA analysis. In this article, we describe the genetic analysis results of these mummified remains.

INSPECTION OF THE BODY

In January of 2008, a multidisciplinary working group consisting of genetic forensic analysts from the Investigative Police in Chile, a molecular geneticist from the University of Chile Medical School and anthropologists from the Indian Peoples Development Corporation and the Council of National Monuments inspected the mummified remains. The objective was to find samples that could be used for genetic analysis, to take measurements and pictures of the body, and perform anthropological examination. These activities were performed in situ to avoid removal or excessive manipulation of the remains.

The body was found at the bottom of a cave in a fetal position within a sarcophagus made of wood and seal skin. An anthropologic examination could not establish the gender of the native because the body was impossible to move, the pelvis was not visible and the scalp still had remnants of soft tissues and hair. Genetic analysis was therefore required.

MATERIAL AND METHODS

Sample Collection and DNA Extraction

Thirteen members of the Kawésqar community of Punta Arenas donated biological samples, which were collected as buccal swabs. DNA was extracted from these buccal swabs using the DNA IQ™ Casework Sample Kit for Maxwell® 16[(a)].

Samples of soft mummified tissue (muscle) from the thorax and forearm of the body, as well as a bone fragment from the superior right end of an exposed carpus, were taken for genetic examination. Tissue remains were rehydrated with sterile distilled water, and DNA was extracted using the DNA IQ™ Casework Sample Kit for Maxwell® 16. For bone samples,

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Figure 1. Partially mummified body of an Indian from the Kawésqar ethnic group.

approximately 1 g of bone was decontaminated by successive washes with sodium hypochlorite and distilled water. The bone was decalcified with EDTA and digested with proteinase K prior to organic extraction with phenol:chloroform:isoamyl alcohol and chloroform:isoamyl alcohol. DNA was recovered by isopropanol and ammonium acetate precipitation, washed with 75% ethanol and resuspended in sterile distilled water. DNA was further purified using the Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281) as per the manufacturer's instructions.

DNA Quantitation and Amplification

DNA quantitation was performed by real-time PCR using the Quantifiler® Human DNA Quantification Kit and Quantifiler® Y Human Male DNA Quantification Kit (Applied Biosystems) on an Applied Biosystems 7300 Real-Time PCR System.

Nuclear DNA (0.5 ng) was amplified using an Applied Biosystems 9700 thermal cycler and the PowerPlex® 16 System[(b–f)] (Promega) and AmpF*l*STR® MiniFiler™ and Identifiler® PCR amplification kits (Applied Biosystems) as directed by the manufacturers. Amplified products were analyzed by capillary electrophoresis using an ABI PRISM® 3100-*Avant* Genetic Analyzer and MegaBACE® 1000 DNA Analysis System (GE HealthCare).

Mitochondrial DNA (0.5 ng) was amplified using an MJ Research PT100 thermal cycler and four primer sets. DNA fragments associated with haplotypes A, B, C and D were amplified, then analyzed by RFLP and agarose gel electrophoresis. Additionally, eight primer sets were used to amplify the HV1 and HV2 mtDNA regions. Each fragment was sequenced from both ends by Macrogen, Inc.

RESULTS

The mummified body found on Capitan Aracena Island and members of the Kawésqar community of Punta Arenas, who are the last descendants of this ethnic lineage, were characterized by mitochondrial markers (RFLP haplotyping and HV1 and HV2 sequencing), Y chromosome STRs (16 S TR loci) and nuclear markers used routinely in forensic genetics (PowerPlex® 16, MiniFiler™ and Identifiler® kits).

The MiniFiler™ results and partial DNA profiles obtained using Identifiler® and PowerPlex® 16 from bone samples confirmed that the body was male (Figure 3). For DNA obtained from muscle, only the amelogenin locus was amplified, with the same result.

We found that, when using Identifiler®, 14 of 16 loci were amplified (no amplification of the CSF1PO and D2S1338 loci was observed). Using PowerPlex® 16, 15 of 16 loci were amplified, with no amplification of the Penta E locus. Additionally, we observed differences at the D18S51 locus between the kits: PowerPlex® 16 produced a heterozygous 14,18 profile, while Identifiler® produced only a very weak allele 14 peak. The discrepancy can be explained by the dropout of allele 18 when using Identifiler® due to the degraded nature of the material used. However, these results need to be confirmed.

Results of the mitochondrial DNA analysis showed the mummified body to be of haplotype C. The body had almost all of the polymorphisms found in living Kawésqar individuals of this same haplotype. His mitochondrial DNA was identical to an ancient Aonikenk with whom he shared a very uncommon point mutation (16318G). With the exception of this polymorphism, the other differences are common in other populations originating from Chile and South America.

The Y-chromosome haplotype clearly showed the mummy to be an Amerindian. Comparison with available databases allowed grouping of the mummy with populations from Ecuador and Peru. However this is probably due to a bias in the database caused by the scarce information available for Chilean and Argentinean aborigines.

Figure 2. Location of Capitan Diego Aracena Island.

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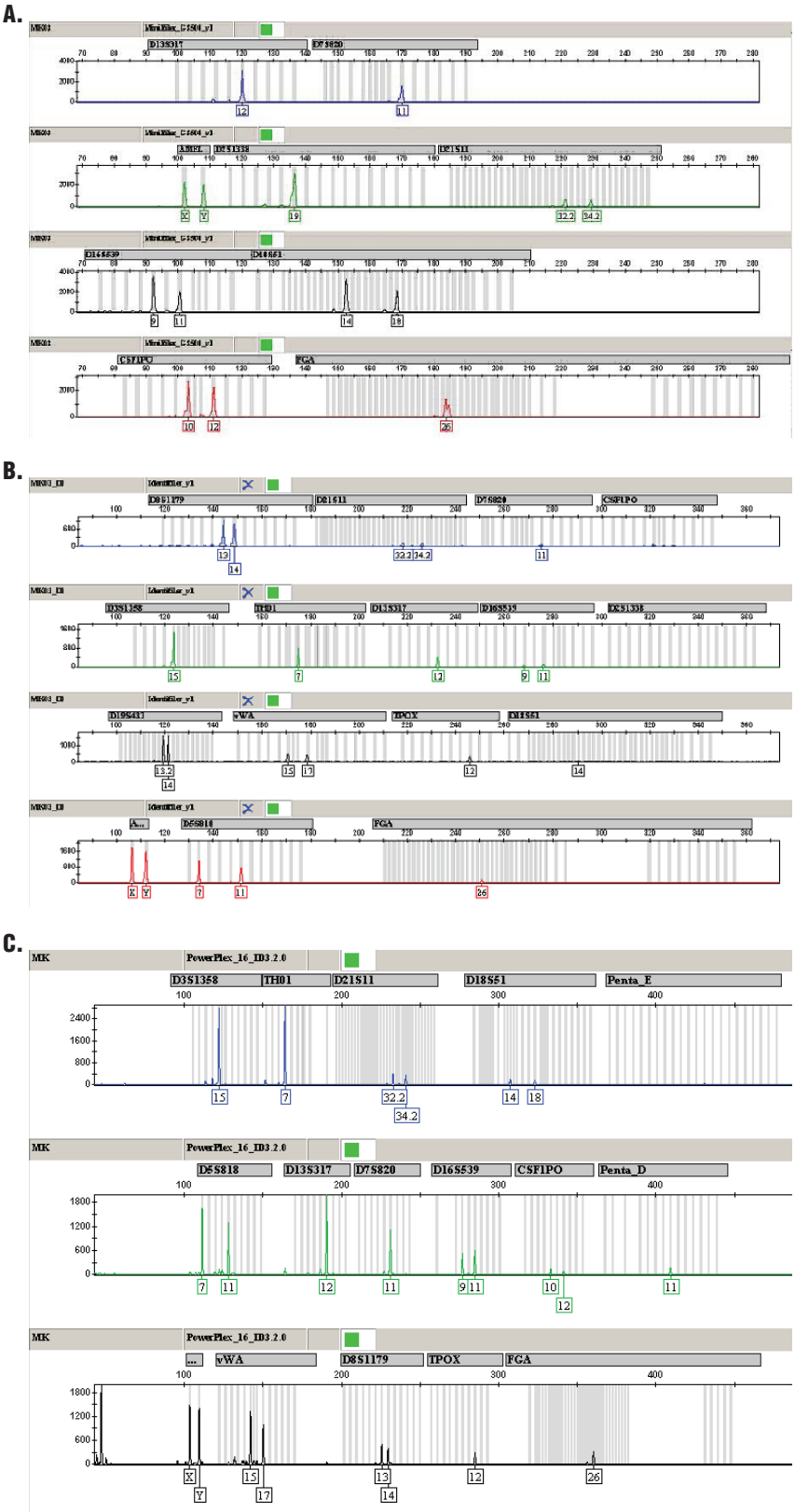

For the most part, members of the existing Kawésqar community belong to the C and D mitochondrial DNA haplotypes. The sequences of HV1 and HV2 showed a high frequency of the D4h3 haplotype, a founder lineage that is extremely rare in America. Originally described in Cayapas population, this haplotype contains two mutations (16241G and 16342C) in addition to the characteristic D mutations. Their Y-chromosome haplotypes are unique and therefore differ from that of the mummified body.

The comparative study performed with samples from the mummified body and 13 members of the Kawésqar community did not allow direct kinship to be established between these members and the mummy. There is a need for further analysis of genetic markers from a larger number of individuals in the community.

CONCLUSIONS

One of the most notable aspects of this work was its multidisciplinary nature, with specialists from diverse academic disciplines and institutions working together on a key project that contributed to the knowledge, preservation and diffusion of the cultural patrimony of the Kawésqar population.

Although comparative studies could not establish the existence of living descendants of the mummy in the existing Kawésqar community, genetic analysis of the body revealed interesting information, especially regarding the historical mitochondrial and Y haplotypes in this region.

The mitochondrial data showed an irrefutable proximity between the Kawésqar individuals and the previously studied population of aborigines from Patagonia and Tierra del Fuego. The high frequency of the D4h3 haplotype strongly indicated that the individuals studied corresponded to descendants of the seafaring populations and that the Fuego-Patagonia population is a relic of a hunter-gatherer population that arrived at these latitudes during the early Holocene period.

Furthermore, these results motivated 14 other members of the Kawésqar community to donate samples for a future genetic study.

Figure 3. (at left) Genotype of the mummified Kawésqar indian body. DNA was purified from bone, then amplified using the MiniFiler™ and Identifiler® kits and PowerPlex® 16 System. **Panel A.** The MiniFiler™ profile. **Panel B.** The Identifiler® profile. **Panel C.** The PowerPlex® 16 profile.