EXPLORING THE GENETIC IDENTITIES OF THE MEDIEVAL SPANISH ROYAL HOUSE OF ARAGON THROUGH MITOCHONDRIAL LINEAGES AND NUCLEAR SNPs DETECTED BY A NOVEL ENRICHMENT APPROACH

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The Medieval Royal House of Aragon was one of the most powerful and influential dynasties of the 11th and 12th centuries. Their reign over the kingdom united the territories and led to the return of the rich domains of the northern Iberian Peninsula (Ebro Valley and Valencia). Through collaborative efforts of archaeologists/anthropologists, historians, and analytical chemists, the putative identities of the members of the royal dynasty were able to be attributed to the skeletal remains discovered within specified Spanish pantheons. Previous efforts investigated the use of radiocarbon dating and stable isotope testing as well as historical accounts of the time period in an attempt to identify the remains believed to belong to the Medieval Royal House of Aragon. Currently, genetic analyses are underway exploring both the mitochondrial and nuclear DNA contained within these ancient bones (estimated to be approximately 850-950 years old). Mitochondrial DNA (mtDNA) testing is often initially performed on skeletal remains due to its relatively high copy number and capacity to resist degradation. Whole mitochondrial genome testing was performed via the Precision ID mtDNA Whole Genome Panel (Thermo Fisher Scientific; Waltham, MA). The nuclear genome of ancient remains typically is highly degraded and damaged, and thus genetic information often cannot be obtained. Current methodologies are incapable of typing such low quality samples. A novel one-step PCR technology, Reverse Complement PCR (RC-PCR), designed to target 27 human identity single nucleotide polymorphisms (SNPs) was explored. The EasySeq™ NGS Reverse Complement-PCR kit (NimaGen B.V.; Nijmegen, Netherlands) was used to amplify these target SNPs requiring DNA fragments to be approximately 50 basepairs in length. The RC-PCR assay is highly sensitive (tested down to 60 pg of input DNA) and has added features in that it is a single, closed system which reduces chances of contamination and improves workflow. Genetic profiles from both mitochondrial testing and RC-PCR were obtained from the highly degraded DNA of the Royal House of Aragon skeletal remains. The impact of these assays on forensic and historical investigations, to be able to obtain genetic profiles from compromised samples and potential identification of the remains of the Medieval Royal House of Aragon, will be discussed.