SEX ESTIMATION BASED ON ANALYSIS OF THE ENAMEL PROTEOME

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Sex estimation is necessary to place skeletal remains in forensic context. The current field of forensic anthropology relies on two basic methods to estimate sex in skeletal remains: analysis of sex-specific osteologial markers and detection of DNA markers specific to the X- and Y-chromosomes. However, sexually dimorphic osteological markers may be missing when not developed in sub-adult skeletons, or degraded by environmental processes. Detection of DNA markers from X- and Y-chromosomes is more direct and predictive. However, the DNA backbone contains phosphodiester bonds and can easily degrade below the point where it can be amplified. Therefore, this analysis is also not often available.

Amelogenin genes on the X- and Y-chromosomes are expressed as protein in teeth and play a major in the biosynthesis of enamel. Protein is more stable than DNA and can persist in skeletal elements well after DNA degrades. Enamel is also the most robust and archaeologically persistent tissue in the body. Detection of peptides unique to the Y-chromosome form of amelogenin protein (AMELY_ Human) in the enamel proteome is an unambiguous signal for the presence of Y-chromosome in the sample.

We processed an upper right molar from an archaeological skeleton with male osteological markers (1175 ± 28 BP, CA-ALA-554-85) into 4 enamel blocks and 2 powder aliquots. The enamel was milled in the presence of 1.2 M hydrochloric acid, incubated for 1 hour at 56°C, reduced, neutralized, alkylated, and digested with trypsin overnight with the presence of mass-spectrometry compatible detergent. The peptides in the filtrate were purified and applied to a proteomic tandem mass spectrometer. The resulting data collected from the mass spectrometer was analyzed using PEAKS ™ analytical software (Bioinformatics Inc.). The Y-Chromosome AMELY_HUMAN protein signal was detected in all samples ($1.54 \pm 0.53 \times 10^9$ ions), accounting for 2.11 ± 0.66% of the total peptide signal in each sample. Out of the sexually dimorphic peptides, WYQ(d)SM(ox)IRPPY was the most abundant, accounting for 53 ± 6% of the sexually dimorphic AMELY peptide signal. This is the first example of using this technique to predict the sex of an archaeological sample. Enamel proteome analysis has the potential to be used as an alternative method to sex skeletal remains in the event that DNA is degraded in the sample.