A34 Sex Estimation: A Novel Protein-Based Sex Assignment Technique Using Human Tooth Enamel and Mass Spectrometry

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After attending this presentation, attendees will better understand a powerful new methodology in which proteinaceous sample sources such as tooth enamel can be used as an alternative to DNA for the forensic analysis of humans. This presentation will inform attendees that compromised samples lacking DNA can still be used to determine information about the identity of a subject. Specifically, sex estimation can be achieved by proteomic analysis of human tooth enamel proteins.

This presentation will impact the forensic science community by offering an alternative science-based forensic technique using proteins as a biological sample source to achieve human sex estimation from human tooth enamel.

Human sex-estimation provides a fundamental metric essential for human forensic and bioarchaeological practice. Compromised samples that are influenced by fire, explosions, extreme environments, and long periods of time often do not meet the quality standards necessary for success of current sexing techniques. These techniques rely on anatomical sexual dimorphisms, which are particularly problematic in subadult skeletons' DNA-based analysis of sex chromosomes. Protein is chemically more stable than DNA and has an increased resistance to deterioration in the environment. Tooth enamel is the hardest tissue in the human body and is an ideal sample source in this context due to its preservation in harsh conditions. Probing the sex-linked protein amelogenin that is found in tooth enamel offers a novel way to sex compromised or subadult skeletons that lack DNA.

Using Mass Spectrometry (MS) -based proteomic techniques, the sex-linked isoforms of amelogenin can be detected directly by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) peptide sequencing. Approximately 20mg-30mg blocks of enamel were resected from each tooth. Protein extractions were performed by a milling, acid-etching, disulfide reduction and alkylation steps. Peptides were generated by proteolysis in the presence of the surfactant with trypsin. The resulting peptides were separated by high-pressure LC and analyzed by a Thermo™ Q-Exactive™ plus hybrid orbitrap/linear ion trap MS. Mass spectra were then matched to peptide sequences and a subset of peptides containing amino acid sequences peptides unique to the X- and Y-isoforms of amelogenin was generated. In this study, five male, five female, and one archaeological sample from a male and a female were tested for amelogenin X/Y isoform peptides in their tooth enamel. After the initial detection of unique isoform peptides was achieved, specific mass-to-charge ratios of interest were chosen for additional data acquisition targeted to collect MS/MS data correspondingly. Synthetic peptides were purchased for validation of mass-to-charge ratios and retention times of the peptides identified within experimental data. Optimization of protein extraction from tooth material was achieved by testing different extraction conditions (different chelating agents and

acid-etching time/temperatures) on a homogenized standard sample composed of eight different teeth. Dentin and cementum were also excised from teeth for amelogenin isoform detection.

X-isoform peptides were detected within every sample of the enamel dataset and peptides unique to the Y-isoform were consistently detected within all male samples of this group, yielding a positive predictive value of male and female sex-estimation of 100% in this cohort. These results reveal the potential of this technology for sexing of a given individual using a proteomic approach. By exploiting the durability of enamel in combination with the stability of protein, this technique provides a new pathway for analysis of compromised human remains in both forensic and bioarchaeological settings. Expansion of this research will likely establish that proteomic analysis of the isoforms of amelogenin in tooth enamel can be used for sex assignment when other established forensic and bioarchaeological techniques are ineffectual.

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Sex Estimation, Proteomics, Tooth Enamel