A60 What Can Forensic Proteomics Tell Us About Biological Age and Postmortem Interval (PMI) Estimation?

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Learning Overview: After attending this presentation, attendees will understand both the state of the art in forensic proteomics, with its applications to biological age and PMI estimation, and a novel finding of potential protein biomarkers for Age At Death (AAD) and PMI estimation from skeletonized remains, an innovative and ground-breaking application in the forensic field.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by showcasing a novel application of proteomics to estimate PMI and AAD with increased success.

One of the most debated themes in forensic anthropology is the estimation of PMI as well as the approximation of the AAD from skeletal remains; despite the presence of distinct analytical and morphological methods to address these aims, their applicability on heavily decomposed bodies or skeletonized remains is strongly limited and suffers from poor accuracy.[1-3] Due to the well-known use of proteomic methods to evaluate aging and decay phenomena for archaeological purposes, this study applies proteomics to forensic applications, looking for new biomarkers in pig skeletal remains that could address AAD and PMI from a previously unexplored perspective.[4]

To achieve these goals, two separate studies were performed. The first compared porcine skeletal remains from five different-aged animals to look for intra-bone and inter- and intra-skeletal proteomic differences, to evaluate potential biomarkers for AAD estimation. Bones were sampled, their proteomes extracted using a protocol that minimizes laboratory-induced decay and were submitted to Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-Orbitrap-MS/MS) analysis. For the second part of this study, four piglets of similar age were experimentally buried, and their bones collected at selected time points (one to six months PMI). Samples were then subjected to the same treatment mentioned above but focused on the evaluation of proteins leaching into the surrounding soil, as well as proteome postmortem decay via the study of post-translational modifications as a means to estimate PMI.

Results demonstrated greater intra-bone rather than inter-bone and intra-individual proteomic differences, and overall higher data reproducibility was obtained sampling the midshaft of long bones (tibiae) than their epiphyses. When using tibiae midshaft to compare proteomes among different-aged pigs, a bone protein, fetuin-A, was observed to be significantly different in terms of its relative abundance, which was negatively correlated with the biological age of the individual; this phenomenon is one example by which forensic proteomics could provide alternative means to evaluate the AAD from bone samples. When investigating proteomic differences between carcasses with different PMIs, it was possible to observe a gradual leach of several groups of proteins from bones as a function of PMI, starting with serum and muscle proteins that were the first to decrease in abundance with prolonged burials. Looking specifically at post-translational protein modifications, biglycan deamidation ratios appeared to increase with protracted PMIs in a statistically significant way. Further studies may validate the use of this protein as new potential biomarker for PMI estimation ranging from one to six months and beyond.

Overall, these studies set the baseline for increasing the awareness of the potential application of proteomics to forensic science, allowing the development of future works to extend the dataset to a wider range of biological ages and also to prolonged PMIs. Further studies on human samples will be required to corroborate these results, to allow their application to real forensic casework.

Reference(s):

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3. L. Konigsberg et al. Estimation and Evidence in Forensic Anthropology: Age-At-Death. In: *Journal of Forensic Sciences*. 53(3):541-57·June 2008.
4. R. Sawafuji et al. Proteomic Profiling of Archaeological Human Bone. *Royal Society Open Science*. Published 7 June 2017.DOI: 10.1098/rsos.161004.

AAD Estimation, PMI Estimation, Proteomics