B124 Proteomic Genotyping: Increased Sensitivity Using Targeted Mass Spectrometry Platforms

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Learning Overview: This presentation will provide attendees with information regarding the efficacy and sensitivity of proteomic genotyping. Attendees will learn that there are other genetically identifying information types that are available to investigators when DNA-based information is partial, incomplete, or missing from an evidentiary sample. Attendees will learn more about this novel genotyping approach, where it would be useful to investigators, and the potential for this method in extracting genetic information from difficult samples. Attendees will also learn about the potential of forensic proteomics.

Impact on the Forensic Science Community: Protein is a component of all biological evidence. Protein is more stable than DNA and recent advances in proteomic mass spectrometry allows protein to be analyzed with increasing depth and precision. Protein also carries genetic information in the form of genetically variant peptides. This information is novel and can be accessed when DNA is compromised or degraded. In this way investigators may be able to increase the discriminatory power of degraded evidentiary samples.

Proteomic genotyping is a mass spectrometry-based method that infers the genotype of non-synonymous Single Nucleotide Polymorphism (SNP) alleles by detecting the resulting single amino acid polymorphisms found in genetically variant peptides in proteomic datasets from evidentiary samples. Like any genotype, these can be used to statistically associate an individual to forensic evidence. The utility of the inferred genotype increases as the detection of genetically variant peptides increases and as the technology is transferred to mass spectrometry platforms available to forensic practitioners.

Digests of single (2cm) human hair shafts from three European and two African subjects were analyzed using data-dependent acquisition on a Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ system, data independent acquisition and a variant of parallel reaction monitoring on a Orbitrap Fusion™ Lumos™ Tribrid™ system, and multiple reaction monitoring on an Agilent® 6495 triple quadrupole system. Average genetically variant peptide detection from a selected 24 genetically variant peptide panel increased from 6.5 ± 1.1 and 3.1 ± 0.8 using data dependent and independent acquisition to 9.5 ± 0.7 and 11.7 ± 1.7 using parallel reaction and multiple reaction monitoring ($p < 0.05$). Targeted methods of analysis resulted in a 1.3-fold to 1.6-fold increase in detection sensitivity compared to the standard shotgun proteomic methodology. This increase in biomarker detection has a functional impact on the statistical association of a protein sample and an individual. Increased biomarker sensitivity, using Markov Chain Monte Carlo modeling, produced a median estimated random match probability of over 1 in 10 billion for parallel reaction monitoring methodologies and 1 in 10 trillion for multiple reaction monitoring methodologies from a single hair using targeted proteomics. Detected genetically variant peptides were validated by the inclusion of stable isotope labeled peptides in each sample as well as independent confirmation of inferred genotypes by direct exome sequencing.

This research accomplishes two aims: the demonstration of utility for alternative analytical platforms in proteomic genotyping and the establishment of validation methods for the evaluation of inferred genotypes by forensic practitioners. Importantly, the targeted proteomic platform of multiple reaction monitoring is readily available to forensic toxicologists and investigators.

Proteomic Genotyping, Genetically Variant Peptides, Forensic Proteomics

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