B79 Proteomic Genotyping of Human Head Hair

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Learning Overview: After attending this presentation, attendees will understand about hair shaft proteomics and the impact of chemical processing in obtaining significant powers of discrimination for sample individualization. Attendees will also learn about mass spectrometry, and its use for inferring genotype.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by shifting human identification into a dual DNA-proteomics perspective. This research will also bolster the reliability of hair analysis as a means of identification, as well as establish reliable methods in forensic proteomics.

This research explores a proteomic approach to hair analysis. Forensic hair evidence, historically analyzed by microscopic morphological comparison, has been criticized as being subjective, unreliable, and not reproducible. Mitochondrial DNA analysis has been able to remedy the reliability of hair analysis, but DNA can degrade substantially under environmental insults, particularly at the distal end of the hair shaft. Unlike DNA, protein has peptide bonds that are more resistant to cleavage. Single amino acid polymorphisms in a protein sequence may be inherited genetically, as they are a result of nonsynonymous single nucleotide polymorphisms (SNPs). These genetically-variant peptides (GVPs) offer the prospect of more discriminating analysis.[1] Genetic information such as SNPs can be predicted via the protein sequence and confirmed with parallel DNA sequencing. Instrumental detection can further be improved with the use of isotope-labeled peptide standards in conjunction with a targeted approach of mass spectrometry, termed Parallel Reaction Monitoring (PRM). Instrumental detection can also be improved using Data Independent Acquisition (DIA). With these analytical improvements, more GVPs can be used to help identify an individual or even classify ancestral origin given that the GVPs have different genotypic frequencies among ancestral populations. The authors hypothesize that the use of a targeted strategy for mass spectrometry in conjunction with an optimized chemical processing method will improve GVP yield and detection and therefore increase the overall power of discrimination for hair digests to over 1 in 1 billion with multiple hair shafts and over 1 in 1 million with a single hair shaft (2cm).

This research focuses on increasing both peptide yield and instrumental sensitivity by optimizing hair chemical processing and characterizing targeted standards. Hair is chemically stable and therefore physically robust due to covalent linkages such as disulfide and isopeptide bonds. Data have been obtained to optimize conditions for disulfide reduction, alkylation, and peptide digestion. Temperature, time, agitation types, and concentrations of the reagents have been tested. The three metrics used to determine the best parameters for processing are yield of peptides in the soluble fraction, unique peptide number, and the number of GVPs. Results testing 2cm of hair indicate that disulfide reduction is improved with lower temperatures, higher concentrations of DTT, and agitation by stirring. Trypsinization for 6 hours solubilizes most of the hair by mass and results in detection of more peptides. The optimized hair processing procedure, with shorter times for both reduction and digestion, has yielded improvements in detectable GVPs, and results in a similar number of GVPs compared to other approaches that rely on urea denaturation and a mass spectrometry compatible detergent. However, these other hair procedures rely on large quantities of hair. When isotope-labeled GVP standards are applied to hair digests, spectral patterns are compared between the standard and endogenous peptide to serve as a confirmation of identity. Spectral features are then used for PRM. Results from hair digests analyzed using PRM and DIA with 24 standard peptides indicate an increase in the detection sensitivity of endogenous peptides and may provide enough sensitivity to identify peptides not detected in standard modes of mass spectrometry without the use of internal standards. The PRM method in conjunction with labeled internal standards may greatly benefit detection of low-abundant GVPs. Overall, the data show that yield and detection of GVPs increases with optimized chemistry and application of a PRM or DIA strategy. Further objectives of this research include developing methods for objectively classifying human hair by ancestral group and deriving likelihood ratios to compare ancestral groups using calculated powers of discrimination.

Reference(s):

1. Parker, Glendon J., Tami Leppert, Deon S. Anex, Jonathan K. Hilmer, Nori Matsunami, Lisa Baird, Jeffery Stevens et al. "Demonstration of protein-based human identification using the hair shaft proteome." *PloS one* 11, no. 9 (2016): e0160653.

Proteomics, Hair, Genetically Variant Peptides