B188 Obtaining Significant Powers of Individual Discrimination From Hair Shaft Proteins

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After attending this presentation, attendees will better understand the potential role of protein in obtaining quantifiable statistically based powers of discrimination from biological samples.

This presentation will impact the forensic science community by explaining how the past few decades have seen the impact of nuclear and mitochondrial DNA typing on forensic science. This presentation introduces a third identifying method based on protein typing. Genetically variant peptides from hair shafts can be used to infer the status of **Single Nucleotide Polymorphism** (SNP) loci in a subject's genome.

If DNA is not present in biological samples collected as evidence, the options for the forensic investigator are limited. Protein is considerably more stable and abundant than DNA by many orders of magnitude. Genetic variation found in protein encoding genes, in the form of non-synonymous SNPs (nsSNPs), results in changes in protein amino acid sequence. Identifying and detecting these single amino acid polymorphisms in proteins therefore allows genetic content of a subject's DNA to be inferred, allowing peptides to be a surrogate for absent or unusable DNA.

To demonstrate this approach, a thorough examination of the protein population in hair shafts was conducted. Hair shafts are a poor source of nuclear DNA, yet are present in many forensic and bioarchaeological contexts. Methods were developed to identify and maximally detect peptides in hair shafts that retain genetic information. First, hair is milled and extracted with high levels of reductant, urea, and mass spectrometry-compatible detergent. The proteins are then alkylated and digested with trypsin to produce a highly complex mixture of peptides. These peptides are then resolved by high-pressure liquid chromatography and analyzed by tandem mass spectrometry. The resulting mass spectra are then matched to peptide sequences and a subset of peptides containing single amino acid polymorphisms are identified.

Genetically Variant Peptides (GVPs) have been characterized that correspond to 43 genetic nsSNP loci. In applying the product rule, dependency between loci within the gene boundary and full independence beyond it were assumed for now. When the product rule is applied, these GVP profiles result in powers of discrimination up to 1 in 5.4 million in the European population. The efficacy of each GVP in inferring the genetic status of corresponding genomic SNPs can be directly measured when compared to direct Sanger sequencing of subjects' DNA. Out of 608 inferences made, only 12 were false positive assignments, a greater than 98% true positive rate. Importantly, false positives clustered around a limited number of less-predictive GVPs. When poorly performing GVPs are eliminated from the analysis, the false positive rate decreases to less than 1%. When using SNP allelic frequencies from the African population, the GVP profiles were considerably less common by a factor of up to 29,000. The resulting likelihood scores, including those from European and African subjects, are a source of biogeographic information that range more than seven orders of magnitude across the samples tested. This indicates that peptides have the potential to provide information about the genetic background of the hair donor. These numbers have been obtained by applying the equivalent of less than 2mm of hair shaft to mass spectrometric analysis.

Application of this methodology to forensic fieldwork samples requires two milestones to be achieved: information needs to be obtained from a single hair, and dependence patterns of hair shaft proteins need to be fully elucidated. Both of these projects are under active investigation. Expansion of these methodologies beyond hair proteins to include alternative tissue types such as teeth, bone, and skin cells is critical to expanding the scope and application space for this novel approach to human forensics.

Protein Typing, Hair, Proteomics

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