B180 Is Specific Melanin Content in Human Hairs Related to Mitochondrial DNA Typing Success?

Jarrod R. Champagne, BS, Virginia Commonwealth University, Department of Forensic Science, 1000 West Cary Street, PO Box 842012, Richmond, VA 23284; Charles A. Linch, BS, Virginia Department of Forensic Science, Trace Evidence Section, 700 North Fifth Street, Richmond, VA 23219; and Michelle D. Bonnette, BS, and Tracey Dawson Cruz, PhD, Virginia Commonwealth University, Department of Forensic Science, 1000 West Cary Street, PO Box 842012, Richmond, VA 23284*

After attending this presentation, attendees will understand the difference between the types of melanin within human hair, the potential role of melanin in the ability to successfully obtain mitochondrial DNA (mtDNA) sequence data, and the advantages that a preliminary microscopic examination of a hair may provide.

This presentation will impact the forensic community and/or humanity by demonstrating how this data may help determine if a preliminary microscopic examination of a hair prior to mtDNA analysis would serve as a way to gauge the potential for mtDNA success. This type of screening procedure based upon the biological features of a hair could potentially save time and money for the forensic DNA laboratory.

Hairs are the only structure in the human body that completely replace themselves by continuously cycling through three phases. Anagen is the growing phase of the hair that lasts several years. Catagen is the regression phase of the hair marked by termination of melanogenesis, mitosis, and growth. Telogen is the resting phase of the hair that precedes exogen during which apoptosis is complete and the root stem is fully keratinized. Most human head hairs found at crime scenes are in the telogen phase, often lacking follicular tissue, which makes them unsuitable for highly discriminating nuclear DNA (nDNA) analysis. Thus, it is often necessary for forensic analysts to turn to mitochondrial DNA extraction and analysis for these types of samples. Most hairs contain melanin – an organic polymer built from oxidative tyrosine derivates. Melanin is synthesized in the elliptical or oval melanosome organelle. There are two types of melanin in hairs and skin: eumelanin and pheomelanin. Pheomelanin is the cysteine-rich red- yellow form while eumelanin is a less-soluble black-brown form. Eumelanin grains are the larger, denser particles and have been shown to protect nDNA from UV radiation through their supranuclear distribution in dermal keratinocytes. Eumelanosomes in hair shafts are wedged between keratin macrofibrils, next to the mitochondria, thus possibly affording photoprotection for mtDNA. Pheomelanin has been shown to be photosensitive and contribute to the degradation of nDNA and mtDNA in living cells after UV-visible light exposure. Eumelanin is predominately found in darker hairs (dark brown, black) while pheomelanin is predominately found in lighter hairs (red, blonde). This study will aim to determine if eumelanin protects mtDNA in the hair shaft from photodegradation as it does in skin. If this hypothesis is true, the predominate eumelanin dark brown hairs' mtDNA sequence would be expected to be more easily typed than mtDNA from photosensitive pheomelanin red hairs or gray hairs that lack pigmentation altogether.

Hairs were collected from three Caucasian groups: predominate eumelanin (dark brown), predominate pheomelanin (red), and no melanin (gray). From each donor, a hair was taken from the left and right sides of the scalp. The prescreened hairs that were selected for this experiment met a baseline diameter and length size for inclusion in the study, making the type of melanin present in the hair the only significant variable factor in determining mtDNA typing success. Reference donor samples were collected and used to compare to donor hair samples for sequence confirmation. Washed hair fragments that were 2 cm long, and cut at a location 5 cm away from the root, were ground using a microtissue grinder and DNA was extracted organically. HV1 primers F16140 and R16420 were used in amplification, product gels were run to gauge amplification success, and Rapid PCR Purification Systems were used for product cleanup. ABI BigDye® Terminator v.3.1 Cycle Sequencing Kit was used for cycle sequencing and DTR Gel Filtration Cartridges were used for cycle sequencing cleanup. Samples will be sequenced using the ABI 3100-Avant Genetic Analyzer with ABI Sequencing Software v.5.1.1 and Sequencher v. 4.7 used for analysis. Sequencing success will be measured by contiguous mtDNA sequence length in base pairs.

Product gels showed bands at the expected size of 281 bp for each hair sample and the positive control upon mtDNA PCR amplification. All reagent blanks were clear. Sequence data is being accumulated for analysis and will be presented and discussed. This data may help determine if a preliminary microscopic examination of a hair prior to mtDNA analysis serves as a way to gauge the potential for mtDNA success. This type of screening procedure based upon the biological features of a hair could potentially save time and money for the forensic DNA laboratory.

Hair Biology, Melanin, Mitochondrial DNA

 ** Presenting Author*