Looking Beyond HV1 and HV2: The Application of VR1/VR2 Testing of Mitochondrial DNA to Further Discriminate Ancient Skeletal Remains of American War Casualties

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The Control Region of the mitochondrial DNA (mtDNA) genome, which is approximately 16,600 bases, contains two hypervariable regions, HV1 and HV2, which are useful in distinguishing among maternal lineages. The Armed Forces DNA Identification Laboratory (AFDIL) has employed mtDNA sequencing of the HV1 and HV2 regions on a large scale since March, 1994 for assistance in the identification of American service members lost in the Vietnam and Korean conflicts, as well as World War II. Because mtDNA is maternally inherited, blood from a maternal relative of the missing service member has been required as the reference source. On occasion, there are multiple families associated with the skeletal remains that contain the same mtDNA sequence or that differ by only one polymorphism in the HV1 and HV2 regions. It then becomes necessary to look elsewhere in the mtDNA Control Region to distinguish among these individuals. In addition to the HV1 and HV2 regions, there are two areas within the mtDNA Control Region, which have shown to be variable between maternal lineages. VR1, Variable Region 1, is located between HV1 and HV2 from position 16366 through position 72, and encompasses the origin of replication. VR2, Variable Region 2, is located downstream of HV2 from position 341 to position 578. When there is at least one difference in VR1 and/or VR2 between the references, PCR amplification of VR1 and/or VR2 from the questioned skeletal remain(s) can be attempted.

Three CILHI cases, each comparing mtDNA sequence information from skeletal remains to the mtDNA sequence information from multiple families, were investigated. In each of these three cases, the mtDNA sequence of the HV1 and HV2 regions were insufficient to support the association of the skeletal remains to a single family. In order to further distinguish between the family reference samples, the entire mtDNA Control Region of the family reference sample was amplified and sequenced. When a difference between family references was identified within VR1 and/or VR2, amplification of a portion of that VR1 and/or VR2 was attempted on the skeletal remains. Due to the highly degraded nature of the skeletal elements associated with these cases, smaller primer sets were developed. The skeletal remains from each of these cases was then amplified using the appropriate primer set. A discussion of the results of amplification and sequencing of the mtDNA from the skeletal remains of these three CILHI cases will be discussed.

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