CASE REPORT

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DNA Identification of "Earthquake McGoon" 50 Years Postmortem

ABSTRACT: This report describes the genetic identification of James "Earthquake McGoon" McGovern, a WWII fighter ace who perished in Laos while providing supplies to French troops during the French Indochina war. Because reference samples were unavailable for all of the potential casualties, testing of the entire mitochondrial genome, autosomal STRs and Y-chromosomal STRs was performed to increase the genetic information available for analysis. Kinship analyses performed on the evidentiary data and numerous indirect family references for McGovern excluded other possible casualties and definitively established McGovern's identity. This particular case demonstrates the practical utility of novel research technologies and aggressive genetic typing protocols in the identification of aged, degraded remains.

KEYWORDS: forensic science, DNA typing, low copy number, Y chromosome, single nucleotide polymorphism, mitochondrial DNA, short tandem repeat, degraded skeletal remains

Mitochondrial DNA (mtDNA) testing is regularly employed in forensic identifications involving aged, degraded specimens, due to the relative abundance of intact mtDNA when compared with nuclear DNA (nucDNA) in these types of remains (1,2). There are cases, however, for which data from the mtDNA control region do not provide enough information to resolve the identification question. Examples include those cases for which no maternal references are available to include/exclude remains, or the presence of common control region types that limit the discriminatory power of the mtDNA data. For these situations, assays that target mtDNA coding region variation, as well as aggressive typing protocols designed to improve recovery of nuclear DNA from low copy number (LCN) specimens have been developed (3–6). These methods increase the molecular information recovered from degraded remains and thus improve the discriminatory power and evidentiary value of the genetic data. We report here on the successful application of a suite of genetic markers and new technologies to a set of degraded skeletal remains, which resulted in an identification that would otherwise not have been made. Data from multiple marker systems, combined with reference data from numerous distant kin, resulted in the identification of a well-known US military pilot, James B. McGovern.

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History

On May 6, 1954, James B. McGovern, a 32-year-old World War II fighter ace, was killed in Laos after his aircraft was hit by ground fire over northern Vietnam. McGovern was piloting a Civil Air Transport (CAT) plane that was supplying ammunition to beleaguered French troops at Dien Bien Phu during the last few days of the French Indochina War. On board with McGovern were his American co-pilot, 28-year-old Wallace Buford, as well as four French servicemen: a Lieutenant, two Corporal Chiefs and a Legionnaire. According to various reports, McGovern attempted to land the crippled C-119 Flying Boxcar on a low ridge 75 miles from the battlefield, but a wing tip clipped a tree and the plane cart wheeled. The French Lieutenant and Legionnaire survived the crash, but McGovern, Buford and the two French Corporal Chiefs were all killed instantly.

The death of James McGovern received major media coverage in 1954. He had become a household name in the 1940s and 1950s for his combat aviation exploits in both China during World War II, and in Indochina after the war. By all accounts, McGovern was the material of which legends are made. He weighed over 260 pounds and was so large that he required a specially constructed pilot's chair to accommodate his bulk (7). His size and general presence prompted a saloon owner in China to nickname him "Earthquake McGoon"—after the fierce and primitively charismatic wrestler from the popular *Li'l Abner* comic strip (7). McGovern was reportedly quite a character in real life, as well; a happy-go-lucky fellow who tackled perilous situations with humor. One report describes McGovern as talking his way out of a 6-month captivity in China, after capture by communist forces (http://freerepublic.com/focus/f-vetscor/1344974/posts). During World War II, McGovern was credited with destroying at least nine enemy planes. After the war, he flew for CAT and prior to his death on May 6, 1954, had flown 44 successful missions over Dien Bien Phu in support of the French garrison (7).

Anthropological Findings

Multiple surveys of the crash site area between October 1997 and July 2002 revealed small fragments of aircraft wreckage, but did not reveal the burial sites described by local informants who had relayed knowledge of a C-119 crash in 1954. Excavations of both the crash site area and the suspected burial sites in September of 2002 again yielded crew-related equipment and aircraft wreckage, but still no human remains. Finally, in November of 2002, the remains of a single individual were discovered.

The recovered skeletal material comprised cranial and postcranial elements. Anthropological analyses indicated that the elements were consistent with an adult Caucasoid male of approximately 69 inches in height. Unfortunately, no official form containing medical information was available for McGovern, but historical descriptions suggest that he was a Caucasoid male of approximately 70 in.

Dental remains recovered at the burial site consisted of 27 teeth, fragments of maxilla and mandible and a gold-fixed partial denture. Although the available dental records for Wallace Buford were inconsistent with the recovered remains, no antemortem dental records were available for James B. McGovern or either of the missing French soldiers. Thus, no dental comparisons could be made for the remaining three men. The bridge was, however, consistent in appearance with a smiling photograph of James McGovern Jr. that shows the relevant teeth.

Although this evidence indicated that the remains were consistent with McGovern, the data were insufficient to exclude other possibilities. Thus, no definitive identification could be established on the basis of the circumstantial and anthropological data. As a result, in May 2003, the left femur was selected for mitochondrial DNA typing (8).

Materials and Methods

Osseous material was extracted with either a standard phenol/chloroform protocol as described in Edson et al. (2), or a modified extraction protocol that includes a complete demineralization step (O. Loreille, personal communication). Mitochondrial control region sequence data were acquired as described (2) and mtDNA single nucleotide polymorphisms (SNPs) were typed (4), with minor modifications described here. Reference samples were typed in a 15 μL total volume reaction with 2.5 U AmpliTaq™ Gold DNA polymerase (Applied Biosystems, Foster City, CA). Skeletal extracts were amplified in a 25 μL total volume reaction with 6.25 U AmpliTaq Gold. PCR reaction cleanup was performed using exonuclease I (EXO) and shrimp alkaline phosphatase (SAP; USB Corporation, Cleveland, OH) in a ratio of 3 U EXO and 0.17 U SAP for each 1 μL PCR product. Unincorporated dideoxynucleotides were removed with 2 U SAP per reaction. Extension products were separated and detected on either an Applied Biosystems 3100 or 3130*xl*. SNP profiles were evaluated in Genemapper v3.2, using custom bins and panels. Control region data were compared with the SWGDAM mitochondrial control region database of 4839 unrelated individuals, including 1655 Caucasians (10). The combined control region plus SNP haplotype was compared with a database of 284 U.S. Caucasians typed for both control region and SNP data. Low copy number amplifications were conducted using the PowerPlex 16 system (Promega Corporation, Madison, WI) and the AmpF*l*STR® YFiler system (Applied Biosystems). Thermal cycling temperatures and times were performed according to the manufacturer's recommendations. However, for each multiplex, twice the recommended *Taq* concentration and six additional PCR cycles were used. PCR products were separated on either a Prism® 3100 or 3130*xl*, and analyzed using Genescan® v3.7 or Genemapper® v3.2 (Applied Biosystems). Genotyper® v3.7 and Genemapper® v3.2 were used to assign allele calls to electropherograms, using the allelic ladders provided in the respective kits as references. Final STR profiles were generated using a consensus approach, wherein alleles were confirmed only if duplicated in two or three amplifications (5). Y haplotypes were compared with a database of 3561 individuals typed for YFiler. The likelihood ratio for autosomal STRs was calculated using DNAView™ (C. Brenner, Berkeley, CA) through a Progeny (Progeny Software LLC, South Bend, IN, http://www.progeny2000.com) interface that has been implemented in a custom application, LISA (Laboratory Information Systems Application, FTI Inc. Fairfax, VA).

All mitochondrial, Y-chromosome and autosomal data were confirmed in multiple amplifications from independent bone submissions and independent extractions. All experimental negative controls were clean.

Results

The mtDNA control region data, generated from multiple short amplicons, resulted in an 884 base profile consistent with the maternal cousin of James B. McGovern. Furthermore, the mtDNA control region data excluded the remains as being those of Wallace Buford because the control region sequence from the skeletal element differed from the Buford maternal reference at more than two nucleotide positions. Although helpful, the exclusion of Wallace Buford was still insufficient to conclude with certainty that the remains were McGovern primarily because the mtDNA control region type shared by the evidence and the casualty's cousin is the single most common control region haplotype observed in the SWGDAM "Caucasian" database. It occurs in 7.7% (128 observations in 1655 individuals) of that sample (3,9). Furthermore, maternal references for the missing French personnel were not available for testing. Thus, the incomplete set of references and the common evidentiary mtDNA profile left investigators with a tenuous genetic link to McGovern, but without a confident exclusion of all other possibilities.

Mitochondrial DNA single nucleotide polymorphisms were subsequently typed in order to address the evidentiary limitations. The SNPs targeted in this particular case have been shown to resolve unrelated individuals sharing the most common hypervariable region I and II haplotype (2,3,10). The SNP data were consistent with the sequence data at position 16519, but also revealed a coding region polymorphism shared by the bone specimen and the McGovern family reference in the mtDNA coding region (Fig. 1). When compared with the common control region sequence alone, the combined control region + SNP mitotype was found in only 1.4% of a Caucasian SNP + control region database (four observations in 285 individuals; the upper 95% confidence limit for the frequency of this mitotype in the Caucasian population is 3%). The SNP information supported the mtDNA evidence or relationship between the skeletal element and the cousin of James B. McGovern and significantly increased the discriminatory power of the mtDNA data. However, confirmatory data were sought to establish a final identification.

Low copy number nuclear DNA amplifications were conducted on the skeletal remains in an attempt to acquire additional, informative genetic data. Multiple amplifications from two independent extractions of the femur sample resulted in concordant profiles and replicated alleles at all PowerPlex 16 loci (Fig. 2). However, to further authenticate the LCN autosomal STR results, the Central Identification Laboratory re-sampled the left femur and submitted the sample for replicate analysis. Again, multiple amplifications from two independent extractions of the second sample yielded concordant data that confirmed results from the initial sample submission. In addition, a Y-STR profile not previously reported, as evaluated with

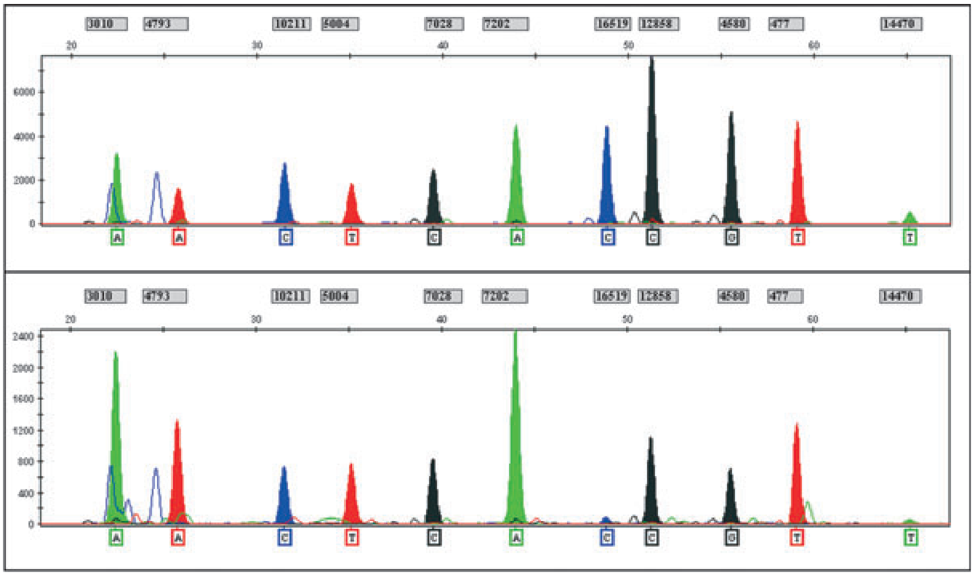

FIG. 1—*MtDNA SNP profiles from reference (upper panel) and evidence (lower panel) specimens indicate consistency at all 11 sites.*

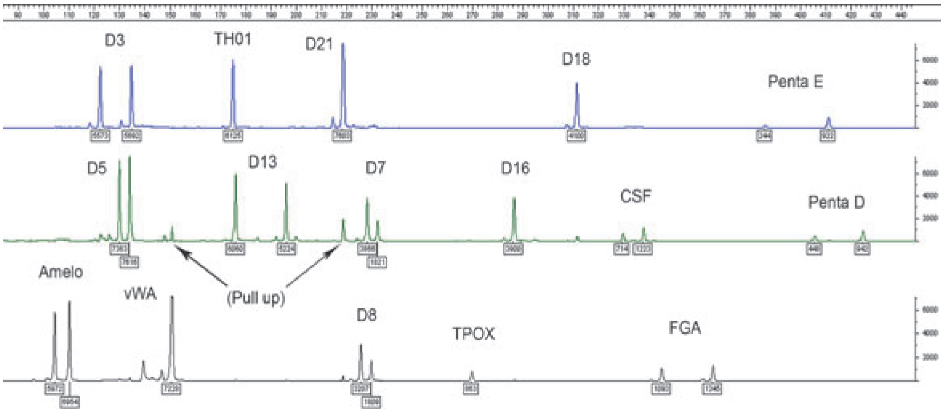

FIG. 2—*Example low copy number PowerPlex 16 amplification from skeletal element of James B. McGovern. Skeleton recovered at crash site of C-119 supply plane, 50 years post-mortem. Labels indicate RFU values of alleles.*

the Applied Biosystems YFiler database (3561 profiles), was obtained from the re-submitted sample. Following the successful recovery of nuclear DNA markers from the skeletal material, reference material from additional relatives of James B. McGovern were sought and acquired. Using a cousin, a sister-in-law and five nieces and nephews as references, the autosomal and Y-STR evidence were evaluated. The unique Y-STR profile was consistent between the evidence material and the paternal nephew of McGovern. Kinship analysis of the autosomal STR data from the skeletal remains and all the extended family reference individuals produced a likelihood ratio of 96,900 in support of the hypothesis that the bone is from an individual related to the family references as indicated in Fig. 3, rather than from an unrelated individual.

In this particular case, somewhat distant, but numerous, family references provided sufficient data to conclusively identify the missing serviceman through combined autosomal, Y-chromosomal, and mitochondrial DNA evidence.

Discussion

The identification of James McGovern demonstrates the utility of new markers/technologies and aggressive molecular protocols in cases for which some of the basic limitations of mtDNA testing are encountered. With the McGovern case, the mtDNA evidence was restricted by the lack of maternal references available to make inclusions/exclusions and presence of the most common control region type. The mtDNA SNPs, which complemented the control region sequence data, would have likely provided enough information to associate the remains with McGovern if family references for the French soldiers had been available. The benefit of mtDNA SNPs will be strongest in those cases for which references for all casualties are available because SNP typing can often discriminate clearly amongst reference samples.

Fortunately, the quality of the autosomal STR profiles recovered from the McGovern remains combined with the number of relatives available as references made identification via standard STRs feasible. In routine practice, however, the utility of low copy number typing may well be limited by the quality of the profile obtained from the evidence, as well as the references available for comparison. With degraded skeletal remains, the STR profiles obtained will often be incomplete and, in many cases, may be insufficient to generate strong statistics. Even when the recovered profiles are complete, a lack of suitable references may limit the potential for identification. For the identification of James McGovern, the references were not the most ideal for kinship analysis (i.e., immediate kin would have been preferable). However, the quality of the STR

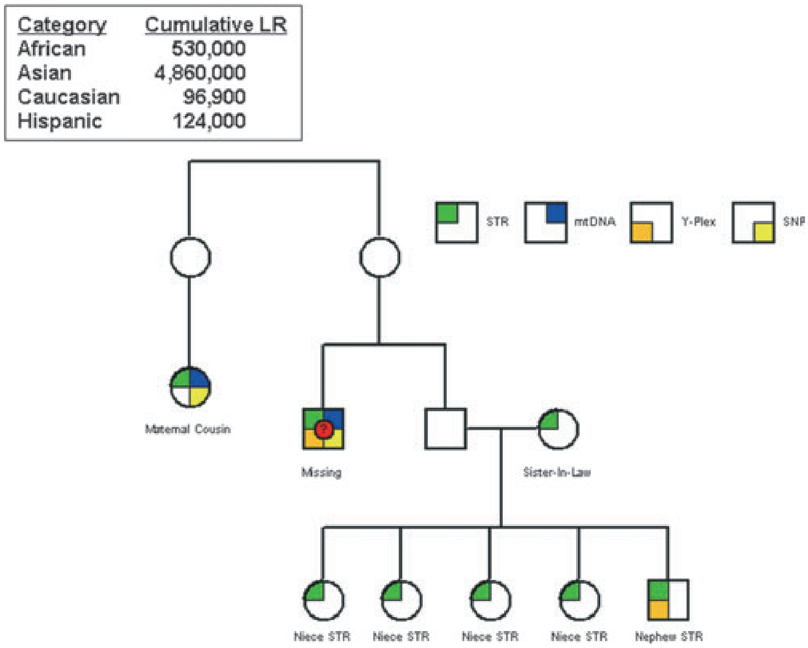

FIG. 3—*Pedigree used in the identification of James B. McGovern. The missing soldier is highlighted with the question mark. A maternal cousin served as the sole reference for mtDNA. A nephew served as the sole reference for Y-STRs. All references were used in the likelihood ratio calculation generated from autosomal STRs.*

profiles recovered from the remains and the large number of family references available for comparison ultimately provided sufficient information to establish identity. Thus, while it is desirable, it is not necessary to obtain a complete set of exclusionary references when undertaking mtDNA analyses of degraded skeletal remains. Additional, independent genetic markers can now substantiate or overturn presumptive findings derived from limited mtDNA data. Similarly, this case demonstrates that, although it would have been simpler to address the STR evidence using reference samples obtained from a first degree relative of the casualty (parent, child or sibling), perfectly adequate conclusions can be drawn using a combination of markers and more distant relatives.

The identification of James B. McGovern demonstrates both the potential for LCN STR typing protocols in the identification of aged, degraded skeletal elements and the array of technologies now available to address a variety of casework scenarios. Many historic, missing persons and criminal cases may benefit from similar applications of these techniques.

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