IDENTIFICATION OF WAR VICTIMS FROM MASS GRAVES IN CROATIA, BOSNIA, AND HERZEGOVINA BY USE OF STANDARD FORENSIC METHODS AND DNA TYPING

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CASE REPORT

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Identification of War Victims from Mass Graves in Croatia, Bosnia, and Herzegovina by the Use of Standard Forensic Methods and DNA Typing

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ABSTRACT: The postmortem remains of sixty-one war victims were excavated from 6 mass graves in Bosnia and Herzegovina one and a half years after interment. Using standard identification methods, including the matching of medical and dental records, the recognition of distinguishing characteristics such as the use of clothing and belongings, and video superimposition, 35 persons were identified. For the remaining 26 persons identification efforts continue. DNA typing was performed at the HLA DQA1 locus and five PM system loci. Results from DNA typing were confirmed by other methods. DNA profiles of family members of 150 missing persons are now being developed using the 6 loci. These DNA profiles will then be compared with those generated from the bone and teeth remains of the unidentified victims.

KEYWORDS: forensic science, pathology and biology, human identification, DNA, genetic typing, war, mass graves, PCR

This report describes our work on the identification of the postmortem remains of 61 persons using a variety of applicable methods. During 1993 through 1994, the postmortem remains of 61 persons who died on the Kupres battlefield of Bosnia and Herzegovina (1) were transported to the Department of Pathology and Forensic Medicine, Clinical Hospital in Split, Croatia. Excavated one and half years after death, the remains from six common graves were in an advanced stage of decomposition. At the same time, data on missing persons from that region (totaling 150), who had either disappeared or had been killed during the April 1992 Serbian aggression were collected and registered. In all cases, forensic examiners performed a detailed examination of the clothing and belongings of the dead, described special features, analyzed skeletal remains to estimate sex and height, and compared premortem dental records with postmortem dental records. In addition, X-ray comparisons were performed for bone morphology. Identification of several persons by the superimposition of skull and photographic images was performed. The compiled, premortem data were compared with the autopsy findings.

Because of the advanced decomposition of many bodies, we considered identifying war victims partly by DNA typing from bones and teeth (2). It was reported by Lee et al. (3) that the recovery of DNA can be up to 10 μg from spongy bone or up to 0.5 μg from compact bone per milligram of starting tissue. The best way to obtain genetic information from samples with degraded DNA is a PCR-based test. We therefore analyzed the HLA DQA1 locus and the five loci of the AmpliType PM typing kit.

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Material and Methods

DNA Sources

Samples of long bones and teeth were collected for DNA analysis at the time of the autopsies. Bone and teeth specimens were collected from the following individuals: No. 4, femur; No. 14, tibia and tooth; and No. 44, femur. The amount of bone collected for DNA analysis varied from 5 to 20 g. Teeth for DNA extraction were prepared as described (4). From the bone samples, an average of 100 ng of human DNA per gram of powdered bone were recovered. The bone and teeth samples were stored at −20°C. At the same time, blood samples of 5 mL each and hair samples were collected for DNA typing from members of each family.

DNA Extraction

After cleaning the contaminated surfaces of the bone and tooth samples, the specimens were pulverized into a fine powder. Approximately 1.0 g of bone powder was added to 450 μL of extraction buffer (10m*M* Tris-hydrochloric acid [HCl]) pH 7.5, 1 m*M* ethylenediaminetetraacetate [EDTA] pH 8, 100m*M* sodium chloride [NaCl], and 2% sodium dodecyl sulfate [SDS]) in the presence of proteinase K (200 μg/mL) (Gibco BRL). This solution was incubated at 56°C for 18 h, and DNA was isolated by organic extraction (3,4).

DNA Quantification

The quantity of human DNA was determined by slot blot analysis using the Quanti BlotTM Human DNA Quantitation Kit (Roche Molecular Systems, Inc., Branchburg, NJ).

DNA Amplification and Typing

PCR amplification was performed on the Perkin Elmer GeneAmp PCR System 9600, using the original Amplitype HLA DQA1 PCR Amplification and Typing Kit, as well as the original AmpliType PM PCR Amplification and Typing Kit. Hybridization and detection of amplified HLA DQA1 and PM DNA were performed according to the manufacturer's instructions.

Analysis of Typing Results—DNA types from bone and teeth were analyzed and compared to the DNA types from living relatives. The relative population frequency of the matches were calculated using the methods described by Schanfield and Stern (5). From these values, the likelihood in favor of parentage, or relatedness, and Bayesian probability of parentage or relatedness as calculated using the formula $P = 1 / 1 +$ relative population frequency, assuming a prior probability of 0.5. Allele frequencies used were a pooled sample of Croatians.[8]

Results

From the information given during the exchange of remains and from eye witnesses, it was determined that the 61 of the remains came from 6 mass graves. All remains were in an advanced state of decomposition; some were only skeletal. Fifty eight had full or partial military uniforms, and two were clothed as civilians. In one case, only the clothes, without any part of the body, were recovered. Seven bodies were partially or completely saponified, and one body was carbonized. Since military uniforms are identical, they provided no distinguishing clues. It was established that all remains were male. Thirty-five persons were successfully identified using combined techniques of identification. In every case a positive identification was based on the data from two or more techniques. However, details on the clothes, as well as civilian underwear and personal belongings, aided in the identification of 31 cases. In 32 cases, the statures were estimated from the length of long bones and other bone remains. Because of the lack of long bones, height could not be established in almost half the cases. Dental records confirmed the identity of 15 persons. Special marks (such as old scars and old fractures) were useful in seven cases. In addition to fingerprints and dental examinations, in some circumstances, radiographic examination of the bone was also helpful in identification of the remains (6).

[8]Keys et al., manuscript submitted for publication, 1995.

In our work, the identifies of four individuals were confirmed by X-ray comparison of bone shape, size, or indications of old fractures. Only 19 persons were identified by dental and medical records. The two main reasons for that were: (1) the lack of adequate antemortem records and (2) a great number of extremely damaged skulls, without upper or lower jaws, or both. Identity determination was established by means of the image superimposition technique in five cases. Video superimposition was done comparing skulls of victims and their photographs (7,8). In skull identification, by taking soft tissue thickness into consideration and with the help of modern photographic or electronic super projection processes, identification is possible as a result of numerous reference point matches in comparable material (9). Other methods of identification included seven cases in which we were aided by data indicated the circumstances of death (type and location of wound, eyewitnesses accounts, and place of burial) and eight cases in which hair color and length have been useful. Wound types helped clarify conditions of death in some cases. Gunshot wounds were found on five skulls in this category.

The DNA was successfully amplified and typed at the HLA DQA1 and PM loci. Figure 1 shows the DNA typing results of matches between living relatives and three missing persons. Using this method, three identifies were consistent in conjunction with additional antemortem and postmortem data.

Discussion

In the absence of antemortem data for comparison identification of human remains is exceedingly difficult (10). Because of advanced decomposition and extensive postmortem changes, even the cause of death was often not apparent. Since the bodies were buried and excavated carelessly, identification was further impaired. The circumstances of burying and excavation varied. DNA typing offered the possibility of obtaining information from human skeletal remains as an additional method for successful identification.

Many authors emphasize the difficulties in forensic DNA analysis from bone specimens (10,11). A large number of specimens contained insufficient quantities of DNA, or the DNA from these specimens was degraded or contaminated. Since the use of restriction length fragment polymorphisms (RFLPs) is not applicable in cases involving extensive degradation of DNA (12), PCR permits the amplification of a relatively small DNA target sequence and subsequent DNA typing from bone exposed to extreme environmental conditions (13). In our work, results of DNA typing are consistent with those of the offspring of known parents or relatives. Using allele frequencies recently obtained from the Croatian population at loci LDLR, GYPA, HBGG, D7S8, Gc, and HLA-DQA1,[8] we calculated the probability of parenthood in the following cases: 99.617% for Case 4, 99.446% for Case 14, and a probability of relatedness of 98.393% for Case 44.

Both phenol and Chelex extraction methods have been used for the processing of bone samples. In this work, the phenol extraction method was found to be more effective. No results were obtained when DNA was extracted from bone in the presence of Chelex resin (8). In contrast, Chelex extraction of blood samples was useful. We also found that the decalcification step was not required for sufficient DNA yield from skeletal remains confirming the findings of Fisher et al. (12). In addition, we found Centricon® micropurification to be useful for purifying the extract, particularly in cases in which nuclear DNA was highly degraded.

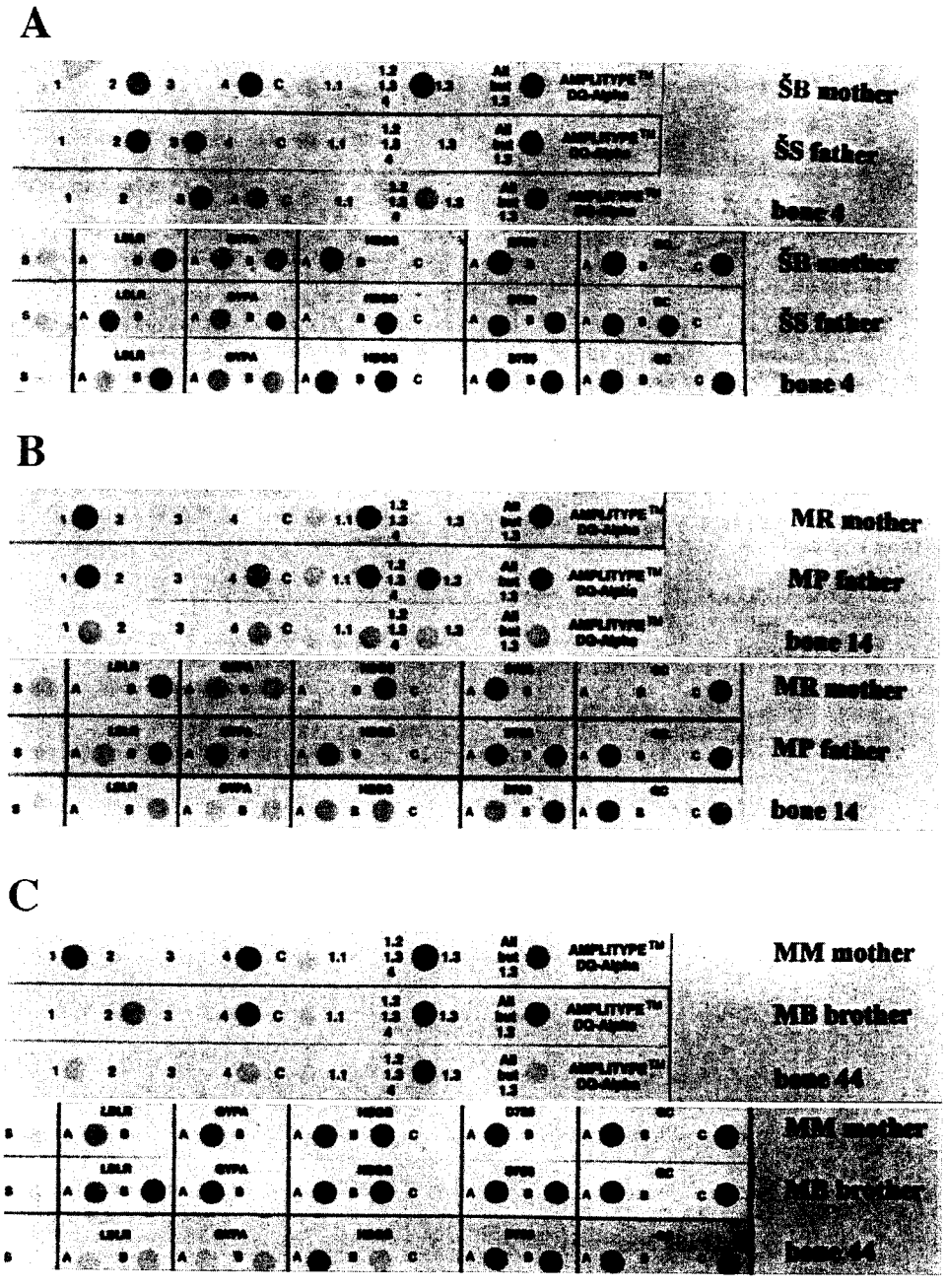

FIG. 1—*Summarizes the results of DNA typing from missing persons and presumptive relatives (mother, father, or brother). Panel A shows typing results from compact bone 4 genotype (AB, AB, AB, AB, AC; 3; 4) compared with DNA typing results from mother's genotype (BB, AB, AA, AA, AC; 2; 4) and father's genotype (AA, AB, BB, AB, AB; 2; 3). Panel B shows typing results from compact bone 14 genotype (BB, AB, AB, AB, AC; 1.1; 4) compared with DNA typing results from mother's genotype (BB, AB, BB, AA, CC; 1.1; 1.1) and father's genotype (AB, AA, AA, AB, AC; 1.1; 4). Panel C shows results of DNA typing from compact bone 44 genotype (AB, AB, AB, AB, AC; 1.2, 4) compared with DNA typing results from brother's genotype (AB, AA, AB, AB, AC; 2, 4) and from mother's genotype (AA, AA, AB, AA, AC; 1.2; 4).*

Currently, we are the only DNA forensic laboratory in the area in which war victims from Croatia and Bosnia and Herzegovina totaled more than 150,000 between 1991 and 1992 (14). The laboratory has the responsibility for accurate identification for many of these victims. We are in the process of testing new methods such as mitochondrial DNA typing for identification of human remains (15).

The importance of identifying victims from a war situation involves humanitarian as well as medical and legal concerns. Establishing postmortem identity can be very difficult. DNA typing, therefore, represents a potentially useful method in cases in which forensic pathologists have too little data for identification by other means.

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