Case report

Identification of bodies from the scene of a mass disaster using DNA amplification of short tandem repeat (STR) loci

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Abstract

The accompanying paper in this issue describes work conducted during a collaborative effort to identify the victims of a mass disaster that occurred on the 19th of April 1993 near Waco, Texas. The DNA identification programme was also used partly as an exercise to further investigate the robustness and reliability of a recently developed STR quadruplex. The preceding paper provides details of the loci used and also deals with efforts to assess the applicability of STR profiling and its suitability for forensic investigations of this nature. In this paper, we present the results obtained from 61 Waco bodies. Using reference blood samples and family trees 26 positive identifications were made using a 'paternity style' analytical approach. Worked examples, representing a range of casework situations, are used to illustrate the kind of approach taken in interpretation of the data and highlight factors which affected its success. Additionally, we report on the successful application of a PCR-based gender test to 24 of the Waco bodies.

Keywords: DNA; PCR; STR profiling; Body identification; Mass disaster; Case history

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1. Introduction

The Waco disaster was a particularly destructive incident, not only because of the raging fire, but also as a result of the presence of a large stockpile of ordinance and armaments kept within the compound. The fire within the compound triggered the explosion of these munitions and, as a result, prevented the access of emergency services to the immediate area of the fire. The blaze continued unabated until its fuel load was consumed and it eventually subsided. The recovery of the remains was then further hampered and delayed due to the risk of more explosions from the smouldering embers. The net result of this was that many of the bodies in the fire were not recovered for several days after the event. When recovered, the remains were extensively charred or partially incinerated and very badly fragmented. The recovery of remains was further complicated because after the onset of the fire, many of the occupants of the complex had apparently been congregated in the same, very small, area of the compound. This meant that many of the remains were concentrated in a relatively small area and were mingled together.

The DNA identification programme involved the analysis of pathological specimens from 61 bodies. These included soft tissues such as deep muscle, skin and internal organs, and skeletal samples, such as intact bones and bone fragments. Some specimens were in an advanced state of putrefaction and others badly charred. Although some bodies had been identified by dental comparison, fingerprints, radiography or a combination of these, approximately 40 still remained to be positively identified. During the DNA identification programme all the submitted samples were tested using the STR quadruplex and, in addition, a PCR-based sex test was employed on some samples. This was, firstly, to help confirm a proposed identification and secondly, to provide gender information where none was available from existing pathology results. The sex test relies upon amplification of part of the XY-homologous gene, amelogenin [1,2]. This was performed using a single pair of primers spanning part of the first intron of the amelogenin gene and generates products of 106 bp and 112 bp from the X and Y homologues, respectively. The major advantage of this type of approach, as compared to tests utilising Y-specific probes, is that a female result is manifested by the presence of amplified product of 106 bp in size as opposed to simply obtaining a negative result. This, therefore, means that a female result is easily distinguished from a truly negative result.

2. Material and methods

A full account of the material and methods used is provided in the preceding paper in this issue. The amelogenin sex test was conducted as described in [1].

3. Results and discussion

Table 1 shows the results obtained when the quadruplex and sex-test systems were applied to each of the pathological specimens. Full STR profiles (i.e. results

Table 1

Summary of STR quadruplex and amelogenin sex-test results obtained from 61 body samples recovered from the aftermath of the fire at the Branch Davidian headquarters, Waco Texas

Serial number	Sample type	Allelic designations				Sex test
		HUMVWFA3	HUMTHO1	HUMF13A1	HUMFES/FPS	
1	Left fourth rib	14,18	6,7	3,6	11,12	
2	Psoas muscle	16,19	7,8	5,6	10,11	
3	Femoral muscle	17,18	6,7	6,6	11,13	
4	Right psoas muscle	15,17	7,8	6,7	10,11	
5	Heart	15,16	9,10	5,6	10,10	Male
6	Psoas muscle	19,19	7,10	7,7	NR	Male
7	Femur	15,16	6,9	3,6	11,11	Female
8	Psoas muscle	15,17	6,10	5,6	10,11	
9	R. innominate	19,21	7,8	5,6	10,12	Female
10	R. psoas muscle	16,20	8,9	3,6	11,11	
11	Psoas muscle	15,18	6,6	4,6	9,10	
12	Fibula	15,16	7,7	5,8	11,11	Male
13	R. psoas muscle	14,19	6,8	5,15	12,12	Female
14	Psoas muscle	17,18	6,6	7,7	8,10	Female
15	Rib fragment	14,15	7,8	5,6	11,11	Female
16	Muscle	15,18	6,10	4,7	8,12	
17	Heart	17,18	6,6	7,7	8,10	
18	Gluteus muscle	16,18	7,9	5,6	9,12	Female
19	Psoas muscle	17,17	8,8	6,14	11,11	
20	Femoral muscle	14,16	7,9	5,5	11,11	Female
21	Liver	15,19	6,7	3,7	10,12	Female
22	Sacrum	14,18	6,10	4,7	8,9	Female
23	Ilium	16,17	7,7	3,5	12,12	Female
24	L. third rib	17,18	6,9	6,6	10,12	
25	Rib	18,19	6,9	6,7	11,11	
26	Femur	15,18	6,9	3,7	9,12	Male
27	Femoral muscle	15,17	6,10	6,14	10,11	
28	Thigh muscle	17,19	9,9	3,6	12,13	
29	Psoas muscle	14,16	9,10	3,16	10,11	
30	Rib	16,17	6,8	7,7	11,11	
31	Rib	17,17	6,10	4,14	11,11	
32	Muscle	16,18	6,8	5,7	12,12	
33	Fibula	16,18	6,9	3,7	10,12	
34	L. fourth rib	17,18	6,8	5,7	NR	
35	Rib	NR	NR	NR	NR	
36	Muscle	18,19	6,9	5,7	11,11	
37	Humerus	NR	NR	NR	NR	
38	Rib	NR	NR	NR	NR	
39	Muscle	18,19	7,8	5,7	10,11	Female
40	Femur	16,18	6,9	6,6	10,10	
41	Muscle	15,18	10,10	3,6	10,12	Female
42	Radius	18,19	6,9	6,7	11,11	
43	Rib	17,18	8,10	6,7	10,10	
44	L. fourth rib	16,18	9,10	NR	NR	
45	Ulna	15,18	8,9	5,7	10,10	
46	Femur	15,17	9,10	3,6	10,11	Female

Table 1 (contd.)

Serial number	Sample type	Allelic designations				Sex test
		HUMVWFA3	HUMTHO1	HUMF13A1	HUMFES/FPS	
47	Os coxa	14,17	7,10	6,7	10,10	
48	Heart	15,17	9,10	3,6	10,11	Female
49	Humerus	15,16	NR	NR	NR	
50	L. fourth rib	17,17	6,10	NR	NR	
51	Tibia	NR	NR	NR	NR	
52	Femur	15,18	10,10	3,6	10,12	Female
53	Leg muscle	16,20	7,7	3,8	9,11	Female
54	Rib	16,20	7,7	6,8	11,11	Female
55	Psoas muscle	16,17	9,10	6,6	11,12	
56	L. humerus	15,19	9,9	6,6	NR	
57	L. ulna	15,17	6,10	6,14	10,11	
58	Femur	16,17	10,10	6,14	10,11	
59	Femoral muscle	15,18	9,10	3,6	10,11	Male
60	Muscle	NR	NR	NR	NR	
61	Muscle	15,18	9,10	3,6	10,11	Male

NR, No result.

from four loci) were obtained from 50 of the 61 body samples submitted. A further six gave partial profiles (onc, two or three loci) and five body samples gave no results at all. The partial profiles exhibited a characteristic loss of higher molecular weight in preference to lower molecular weight loci (HUMVWA < HUMTHO1 < HUMF13A1 < HUMFES). Amelogenin sex tests were carried out on 23 of the bodies. These were successful in every case. This probably reflects the fact that amplification from the amelogenin locus requires only a relatively short target fragment of DNA to remain intact (112 bp for a female). Since the smallest quadruplex allele known to date is the HUMVWA 11 allele (126 bp), this means that a sex-test result can generally be obtained even when typing from higher molecular weight loci is unsuccessful.

Body identifications were made by using the results from relatives' blood samples and information gathered from family trees, to predict the genotype of the deceased family member, in a paternity-style analysis (see below). A total of 26 positive identifications were made on this basis. Identification of the other bodies, for which results were obtained, was limited simply because of the shortage of relatives. The identification programme results indicated that six, purportedly different bodies formed three pairs of results that were the same across all four loci (bodies 27 and 57, 46 and 48, 59 and 61 respectively). These could have been fortuitous four loci random matches, but the probability of this is quite low. Alternatively, one of the pairs of profiles could represent a set of twins. Two sets of twins were in fact present in the complex, of which, one pair remain unidentified. However, given the fragmentary nature of many of the recovered remains the most likely explanation for these results is that these samples actually represent different parts of the same,

Table 2
Relatives' profiles and possible genotypes of deceased individuals in three specific cases[a]

	STR Allele designations			
	HUMVWA31	HUMTHO1	HUMF13A1	HUMFES/FPS
Case 1				
Mother	16,17	9,10	4,6	11,11
Father	16,17	6,10	6,14	11,12
Possible genotypes of	16,16	6,9	4,6	11,11
children	16,17	6,10	4,14	11,12
	17,17	9,10	6,6	
		10,10	6,14	
Case 2				
Daughter	15,16	6,9	3,7	11,11
Daughter	14,20	7,9	3,5	11,13
Possible genotypes of	14,15	6,7	3,?	11,?
mother	14,16	9,?	5,7	
	15,20			
	16,20			
Case 3				
Spouse	15,17	6,8	6,7	10,12
Son 1	17,18	6,6	7,7	8,10
Son 2	15,18	6,10	4,7	8,12
Sister 1	14,18	6,10	5,7	8,9
Sister 2	14,18	7,7	5,7	11,11
Sister 3	16,18	6,10	5,7	8,11
Possible genotypes of	14,18	6,10	4,7	8,9
mother	16,18			8,11
	18,18			

[a]See Results and discussion, Sections 3.1, 3.2, 3.3.
?, All possible alleles at that locus.

fragmented body. Case examples outlining the process by which the genotypes of some of the deceased individuals were predicted and their bodies identified are presented below. These are used to demonstrate the kind of approach taken and to highlight factors which can affect the success of that approach.

3.1. Case 1

The most straightforward situation, other than where the deceased has a surviving monozygotic twin, is where both mother and father have provided reference samples. In this situation the deceased's genotype is predicted simply by considering all the possible genetic combinations, according to Mendelian principles, that could arise from a mating between the two individuals. In Case I, profiles were obtained from the mother and father of the deceased (Table 2). The range of possible genotypes of their children could then be predicted (96 possible combinations). Despite the large number of combinations, of the DNA genotypes obtained from

the body samples submitted to the laboratory, two matched the DNA profiles possible for the children of this couple (Table 1, bodies 31 and 55. N.B. Body 50 was also a partial match but was excluded on the basis of other evidence). In this particular case, the parents had lost both a son and daughter (Fig. 1). Pathology results indicated that both bodies were of the correct age to be the individuals concerned and that body 55 was that of a male whilst body 31 was that of a female. It was possible to then use the identification of the daughter (body 31) and the results from the identification her partner (body 5), to locate two of her children, who had also perished in the fire (Table 1, bodies 27/57 and 58).

Commonly, only a single parent was surviving. This increased the number of possibilities for the offspring. However, the discriminating power of the quadruplex
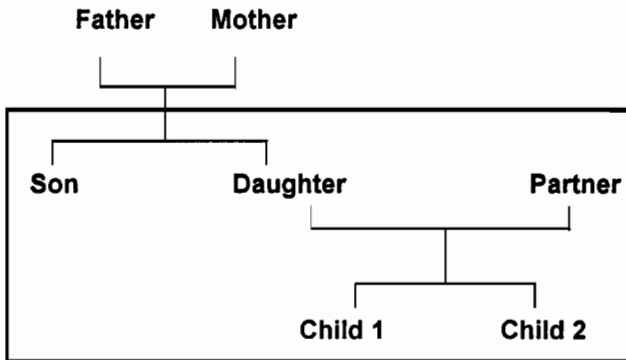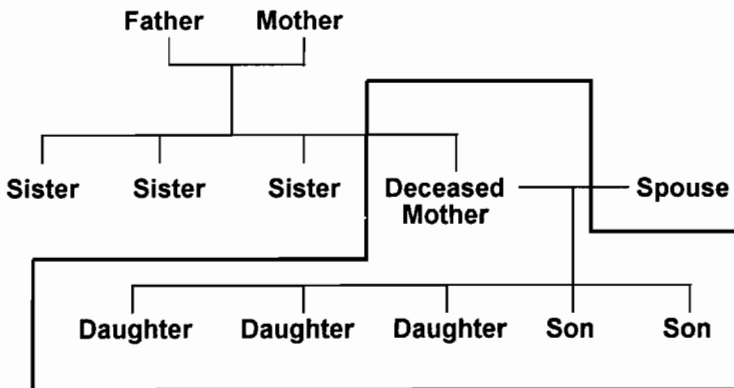
Fig. 1. Family trees pertaining to cases 1 and 3.

system was still sufficiently good, even with the relatively large number of remains received, to enable the vast majority of body samples to be eliminated and, on occasion, for an identification to be made.

3.2. Case 2

A situation that arose frequently was where the deceased had no surviving parents. In such circumstances, children of the deceased often provided sufficient information to enable an identification to be made. In case 2, the deceased lady had two children (Table 2). From their results, it was possible to predict the possible alternative genotypes of their deceased mother since each child must have inherited one of their mothers' alleles. This yielded a large array of possible combinations. However, of the DNA results obtained from the body samples submitted to the laboratory, only two bodies matched with any of those predicted DNA profiles (Table 1, bodies 10 and 29) Pathology results confirmed that body 10 was female and of the correct age, whilst body 29 was excluded on the basis of other evidence. On a number of occasions when parental samples were unavailable, the number of possible genotypes for a deceased could be reduced by referring to samples from surviving sibs. This is illustrated in case 3 (Table 2, Fig. 1).

3.3. Case 3

In this case, the deceased woman had five children (all of whom had died with her in the disaster). Two of the children had been identified by pathology and, critically, their father (the deceased's spouse) was alive and had supplied a blood sample. Successful profiling of the samples from the deceased's two positively identified children was achieved (Table 1, bodies 16 and 17) From these results and their father's genotype, the possible genotypes of the deceased lady could be predicted by subtracting the paternal alleles from the childrens' genotypes. At this stage, the genotype of the deceased could be unambiguously determined at the HUMTHO1 and HUMF13A1 loci. Since samples from the deceased's parents were unavailable and in order to determine more precisely her alleles at the HUMVWA31 and HUMFES loci, the deceased's profile was compared with those obtained from her three sisters (Table 2).

At the HUMFES locus, sister 1 was 8,9, whilst sister 2 was 11,11. This meant that the deceased's parents must have been 8,11 and 9,11, respectively. Since the deceased had inherited an allele 8 the second allele must, therefore, have been either a 9 or an 11.

At the HUMVWA31 locus, analysis of the genotypes of the three sisters revealed two possible alternatives for the deceased's parents. Either the deceased's parents must have been 14,16 and 16,18, respectively, or 14,16 and 18,?, respectively (where the ? represents anyone of all the possible alleles at the locus other than 16). Although both these possibilities led to a large array of possible genotypes at this locus for offspring born to these parents, in this case it could be narrowed down considerably. This is because the deceased was known, by reference to her sons' profiles, to possess an 18 allele. Hence, of all the possible combinations resulting from the parental genotypes only those combinations including an 18 allele were

relevant. This left three alternatives 14,18; 16,18 and 18,18 and the number of possible genotypes for the deceased was consequently reduced to a total of six (Table 2). Of the DNA genotypes obtained from the body samples submitted to the laboratory, only one matched one of the predicted DNA profiles (Table 1, body 22). A sex test confirmed the body to be that of a female and pathology information indicated it to be of the correct age. Using this result and the genotype of the deceased's spouse, it was then possible to identify another two of the deceased's three female children (Table 1, bodies 11 and 14.

A statistical appraisal, in the form of a 'likelihood ratio', for each of the cases was undertaken. A likelihood ratio is a numeric expression of the 'weight' of the DNA evidence. It can be used to establish how likely it is that the remains in question, have originated from an individual related to the family in question, compared to the likelihood of observing the genotype at random in the population. The analysis was based upon allele frequencies drawn from the UK population as information pertaining to USA populations was not available at that time. To ensure conservatism, the highest allelic frequency from the three broad ethnic databases (Caucasian, Afro-Caribbean and Asian) was used in the calculations irrespective of the race code of the deceased person. Furthermore, for alleles with an observed frequency of less than 0.05 a default value of 0.05 was used to add extra conservatism into the calculated figures. For homozygous and heterozygous loci, p^2 and $2pq$ respectively, were used in the calculation of the match probability.

Whilst data pertaining to allelic mutation rates for these STR loci is still in the process of being collected, the broad consensus from the literature is that in general, for tetrameric STRs, mutation rates are in the region of 10^{-2} to 10^{-4} [3–5]. Although the possibility of mutations were borne in mind during this study, no occurrences were uncovered in the families tested.

Our analyses provided values in the range 35 to greater than one million. Higher likelihood ratios were generally obtained for those cases in which there were fewer possibilities in the profile predicted for the deceased. Since the number of possibilities are generally reduced when reference can be made to samples supplied by close family (i.e. sibs, children spouses/partners and grandparents) this highlights the advantages of analysing these additional samples if at all possible.

For the proposed identifications, entirely concordant results were obtained by the US AFDIL laboratory and so provided further, independent, corroboration of our findings.

This study has demonstrated the utility and future potential of PCR amplification of STR loci when applied to forensic investigations, especially those involving samples in an advanced state of decay. The discriminating power of the quadruplex has been demonstrated practically. Both the quadruplex and amelogenin typing systems proved to be accurate reliable and robust. Consequently, a high success rate was achieved and a large number of identifications made possible. These data reinforce those previously obtained by Lygo et al. [6] in confirming the potential of this type of human identification for use in routine forensic casework.

Acknowledgements

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