CASE REPORT

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Identification of victims of the 1998 Taoyuan Airbus crash accident using DNA analysis

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Abstract In February 1998 a civilian aeroplane carrying 196 individuals crashed in Taiwan and killed another 6 people on the ground. Although there were dental and medical records, fingerprints, photographic evidence and personal effects to identify some of the victims, DNA analysis was required to further identify severely damaged remains. From the 202 people known to have perished in the plane crash, a total of 685 fragments of human remains were subjected to DNA analysis. The analysis was carried out using nine microsatellite loci, plus amelogenin to cluster the 685 fragments into 202 groups, accounting for all the victims. To establish genetic relatedness of the victims to other victims and living relatives, additional DNA loci were used. In this case the paternity index was increased by using HLA DQA1 plus Polymarker. The same 16 DNA loci were used to test blood samples from 201 relatives to establish parent/child and sibling relationships. With the exception of 19 victims identified by non-genetic evidence, 183 victims were successfully identified by DNA typing with relatively high values of paternity index by the direct or indirect comparison of relatives. The 202 victims were from 37 different families, ranging in size from 2 to 13 members and 74 individuals known to be unrelated to any other victim. The DNA from living relatives was used to identify one member of a family group, from which other victims of the

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Forensic Science Unit, Department of Pure & Applied Chemistry, University of Strathclyde, Glasgow G1 1XW, United Kingdom family could be identified. ABO blood group information was further used to confirm genetic relatedness within families. A comparison of the DNA profiling results to the ABO blood group of the victims showed no discrepancies with the exception of two mutations in the FGA locus. In cases of severely damaged victims from a plane crash, DNA analysis proved to be the best choice to identify victims.

Key words Disaster victim identification \cdot Short tandem repeat (STR) \cdot HLA DQA1+PM \cdot Paternity index

Introduction

On February 16, 1998, a twin-engine Airbus 300-600R jet with 182 passengers and 14 crew members flying in from Bali, Indonesia, crashed into the Provincial Highway No. 15 and a row of apartment buildings off the north side of Chiang Kai-Shek Airport in Taoyuan, Taiwan. All 196 people on board the plane, plus 4 people who were driving on the highway and 2 people in the apartments were killed in this disaster. The remains from the 202 victims were distributed over an area of approximately 2 km². Thousands of fragmented remains, visually identified as human tissue, were recovered from the wreckage. Of the 202 victims, dental records, pathology evidence and personal affects identified only 19 bodies. A total of 685 tissue samples from the crash site, which could be visually identified as being of human origin, was collected and sent to the forensic laboratories of the Criminal Investigation Bureau, Taiwan Province Police Administration, Investigation Bureau and Central Police University for identity testing by DNA analysis.

From the passenger list information, it was known that the 202 victims comprised 37 family groups with only 74 individuals known to be unrelated to any other victim and 13 members of the same family were all tragically killed. For further identification, 201 blood samples were submitted by the relatives of these unrecognised victims to establish parent/child and sibling relationships. The relaMass disasters, such as this aeroplane crash, require the positive identification of the victims. Traditional methods of disaster victim identification (DVI) have been based on ante mortem (AM) data such as dental records, dermal ridge patterns, or the identification of personal affects. DNA typing has recently become a major tool in DVI when such AM data is not available [1–6].

We report on the DNA typing of all 202 individuals by analysing nine STR loci. The STR DNA profiles were further confirmed by DQA1+PM typing and ABO blood grouping. Comparison of the genotypes from the 202 individuals to genotypes from living relatives was performed. This report further confirms the value of DNA in mass DVI studies.

Materials and methods

DNA extraction

DNA from muscle tissue and blood samples was extracted by QI-Aamp Tissue Kit (QIAGEN, USA) and quantified by the Quantiblot method (PE Applied Biosystems, USA) according to the manufacturers instructions.

DNA analysis

ABO genotypes were tested by the PCR-RFLP method described by Lee and Chang [7]. Short tandem repeat (STR) loci were amplified by use of the Perkin-Elmer (USA) Profiler Kit which amplifies nine microsatellite loci, or STR DNA loci, (D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317 and D7S820) and the amelogenin sex test. Between 1 ng and 2.5 ng of DNA was added to each PCR and amplification was performed according to manufacturer's instructions using either a PE Applied Biosystems 9600 or 2400 thermalcycler. The STR loci were separated and detected by either a PE Applied Biosystems PRISM 377 DNA sequencer or PE Applied Biosystems PRISM 310 Genetic Analyzer. The data were collected and analysed by the GeneScan computer software. Amplitype PM+DQA1 kit from Perkin-Elmer (USA) was used to type the HLA DQA1, LDLR, GYPA, HBGG, D7S8 and GC loci and performed according to the manufacturer's instructions.

Statistical tests

The nine STR loci, DQA1 and Polymarker kit have a combined probability of a random match of 1.2×10^{-12} and a power of exclusion (PE) of about 0.9998 for trios and 0.9936 for duos in parentage testing in the Chinese population from Taiwan (Lee and Chang unpublished data). The statistical comparison was done by single-allele shared rule for parent/child comparison on an Excel spreadsheet and kinship determination of any two persons by the method described by Wenk et al.[8] and computed by a software program written by the DNA laboratory of the Central Police University. Due to the high number of family members who were victims of the disaster, paternity index (PI) calculations were considered the most appropriate means to compare the genotypes.

Results and discussion

All of the samples from the collected remains of the crash victims and samples donated by the relatives were analysed by AmpFISTR Profiler and HLA DQA1+PM. The results of nine STR loci and amelogenin established that the 685 samples of human remains comprised 202 groups. The number of samples within each group varied from 1 to 7 and the 202 groups accounted for all the known victims.

Having established the genotype for the nine STR loci for all of the relatives and victims, every one of the 201 relative's DNA profile was compared to all victim's DNA profiles. The strategy chosen was to calculate the PI for each cross pair using the method of Wenk et al [8]. These PI values would range from zero when one of the alleles from the 16 loci indicated a mismatch, to a positive number when at least one allele from each of the 16 loci indicated a match. For the nine STR loci, HLA DQA1 and Polymarker the PI ranged from 0 to over 0.5 billion.

The identification of all 201 victims proceeded in two separate stages. This was due to the large number (37) of different families travelling on the plane. Initially, 137 victims were identified by comparison to living relatives and from these identified victims, other members of the same family could be identified. Table 1 shows the way in which the identification proceeded. The first identified victim had a monozygotic twin to whom a comparison could be made, 14 children were identified from living parents and 67 parents were identified by reference to 51 samples from children. Further examples are shown in Table 1. From these direct comparisons, victims were identified and these individuals could be used to identify other members of the same family. This is illustrated in

Table 1 Illustrating the approach used to identify all 202victims

Note: Initially victims were identified by comparison to known living relatives. This identified 137 victims. Identified victims were then used to further identify members of the same family. It should be noted that 19 victims were identified by non-genetic evidence and were not included in this Table

Direct comparison	Comparison from identified victims			
Living relatives	Victims	Identified victims	Victims	
64 parents (32 couples)	35 children	22 parents	32 children	
45 single parents	14 children	7 children	7 single parent	
51 children	67 parents	7 single parents	7 children	
20 children and a single parent	20 single parent			
1 monozygotic twin	1 monozygotic twin			
201 relatives	137 victims		46 victims	

Table 2 An example of DNA match from a mother and father to identify a missing child

	XY	THOI	TPOX	CSF1P0	D3S1358	vWA	FGA	D5S818	D13S317	D7S820
R189	Х, Ү	7, 9	11, 11	9, 12	16, 16	14, 18	22, 24	8, 13	9, 11	11, 12
R190	Χ, Υ	8,11	8,11	9, 11	15, 16	17, 19	21, 27	10, 11	8, 9	10, 11
V100	Х, Ү	7, 9	11, 11	11, 12	16, 16	18, 19	21, 24	10, 13	8, 9	10, 12

Note: R189 is from the father, R190 is from the mother and V100 is a child victim. The PI value for this parent/child relationship was calculated to be 3.4×10^6

Table 3 Mutations of otherwise matching DNA in two families There are two families in this Table designated by A and B. F represents father, M mother,	Sample	CSF1PO	D13S317	D3S1358	D5S818	D7S82	20 FGA	A T	HO1	TPOX
	V96AF	10, 11	10, 14	15, 16	12, 12	10, 10	23,	24 6,	6	8, 8
	V198AM	10, 12	11, 11	15, 17	10, 13	10, 10	21,	23 6,	7	8, 8
	V179AS	11, 12	11, 14	15, 17	10, 12	10, 10	21,	25* 6,	7	8, 8
	V34AD	11, 12	10, 11	15, 15	10, 12	10, 10	23,	23 6,	7	8, 8
	V169BF	10, 13	8,9	15, 17	10, 12	10, 12	22,	27 7,	9	10, 11
	V160BM	11, 12	12, 12	17, 17	11, 12	9, 11	23,	25 6,	9	8, 11
	R12BS	12, 13	8, 12	17, 17	12, 12	10, 11	25,	26* 9,	9	10, 11
	Sample	vWA	XY	HLA D	QA1	LDLR	GYPA	HBGG	D7S8	GC
	V96AF	17, 19	XY	1.2, 1.3		AB	AA	AB	AB	AB
	V198AM	17, 18	XX	1.3, 4.2	/4.3	AB	AB	AB	AA	AC
	V179AS	17, 19	XY	1.3, 4.2	/4.3	AB	AB	AB	AA	AC
	V34AD	17, 18	XX	1.2, 4.2	/4.3	AB	AA	AB	AB	BC
	V169BF	10, 13	XY	1.3, 3		BB	AB	BB	BB	BC
	V160BM	11, 12	XX	1.2, 3		AB	BB	AB	AA	AC
S son, and D daughter	R12BS	12, 13	XY	3, 3		BB	AB	BB	AB	AC

* Mutant allele

columns 3 and 4 of Table 1 where 32 children were identified once their mother and father had been identified, 7 children were identified from a single parent and 7 single parents were identified once at least one of their children were identified. When two or more victim children matched a mother/father pair, amelogenin and the ABO blood types were used to further identify the children. The use of nine STR loci resulted in high PI values when mother/father combinations were used to compare to victim children. An example of this is shown in Table 2.

The 16 loci used could not genetically link 2 victims to any family group. Examination of the genotype from one of these individuals established a high PI value to one parent/child pair when the FGA locus was removed from the equation. Only the FGA locus prevented an inclusion being called. When 15 out of 16 loci match there are two possibilities; either there has been a mutation at this locus or there is an exclusion. It is known that STR loci can have a high mutation rate, for example HUMvWA has a mutation rate of 0.2% [9]. Due to the high PI value for the other 15 loci $(3.1 \times 10^8 \text{ and } 2.4 \times 10^7 \text{ for victims V96 and})$ V169 and families, respectively), the high mutation rates at STR loci and the defined number of victims, the most likely explanation was that a mutation had occurred at the FGA locus (Table 3).

In this aeroplane accident, the captain's family was also on board. The captain was recognised by his uniform and a blood sample had previously been taken from the body to test for the presence of alcohol. His wife was identified by her parent's DNA profiles. Using this couple's DNA profiles the daughter and son were identified. In identification work, allele C from the HBGG locus has never been recorded in the Chinese population. The victims with this allele were later found to be of African origin. Within a closed population such as this plane crash, the HBGG C allele may assist in identifying individuals of African populations within an otherwise Chinese population.

The identification of all the victims was completed within 3 weeks of the crash. This was due in part to the use of PCR-based commercial kit, such as AmpFISTR Profiler, automatic fluorescent detection and dedicated computer software. In this case the nine STR loci could differentiate every one of 202 victims. Ante mortem samples would have been better samples to work from, but in this instance no such samples were available. In Taiwan, the ABO blood group of almost every citizen is typed after birth for blood transfusion purposes. ABO genotypes were determined in this case for direct comparison with the AM record of victims. The ABO and sex matching can also be used to confirm the results of DNA identification. DNA analysis in victim identification will play an important role in DVI, particularly in the absence of AM data.

References

^{1.} Whitaker JP, Clayton TM, Urquhart AJ, Millican ES, Downes TJ, Kimpton CP, Gill P (1995) Short tandem repeat typing of bodies from a mass disaster: high success rate and characteristic amplification patterns in highly degraded samples. Biotechniques 18:670-677

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- Clayton TM, Whitaker JP, Maguire CN (1995) Forensic identification of bodies from the scene of a mass disaster using DNA amplification of short tandem repeat (STR) loci. Forensic Sci Int 30:7–15
- 3. Corach D, Sala A, Penacino G, Sotelo A (1995) Mass disasters rapid molecular screening of human remains by means of short tandem repeats typing. Electrophoresis 16:1617–1623
- 4. Clayton TM, Whitaker JP, Fisher DL, Lee DA, Holland MM, Weedn VW, Maguire CN, DiZinno JA, Kimpton CP, Gill P (1995) Further validation of a quadruplex STR DNA typing system: a collaborative effort to identify victims of a mass disaster. Forensic Sci Int 30:17–25
- Brkic H, Strinovic D, Slaus M, Skavic J, Zecevic D, Milicevic M (1997) Dental identification of war victims from Petrinja in Croatia. Int J Legal Med 110:47–51
- Olaisen B, Stenersen M, Mevag B (1997) Identification by DNA analysis of the victims of the August 1996 Spitsbergen civil aircraft disaster. Nat Genet 15:402–405
- Lee JCI, Chang JG (1992) ABO genotyping by polymerase chain reaction. J Forensic Sci 37:1269–1275
- Wenk RE, Traver M, Chiafari FA (1996) Determination of sibship in any two persons. Transfusion 36:259–262
- 9. Brinkmann B, Möller A, Wiegand P (1995) Structure of new mutations in 2 STR systems. Int J Legal Med 107:201–203