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ORIGINAL ARTICLE

The strategies to DVI challenges in Typhoon Morakot

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Abstract Small village populations in which there is a high amount of kinship can cause complications in cases of disaster victim identification. This problem was highlighted by the loss of life after Typhoon Morakot struck Taiwan where over 500 people from small isolated communities lost their lives. Most of the victims were buried by landslides in the remote mountainous areas of southern Taiwan. Only 146 pieces of human remains were recovered after searching for 4 months. Most of the human remains were received for examination as severely damaged fragments prevented possible identification by morphological features. DNA testing using the traditional duo parent/child or sibling screening by STR data opens the possibility of including not only the actual victim but also false positives. Variable likelihood ratios were obtained when comparing DNA types from human remains to those from potential relatives; however, with the DNA typing of numerous members of the same living family, multiple matches to potential families were avoided. Of the 146 samples obtained and collapsed to 130 victims, they were linked to 124 individuals resulting in their identification when compared to a pool of 588 potential relatives. Six of the

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human remains could not be linked to any living relative and remain unknown.

Keywords Forensic science · Mass disaster · Victim identification · DNA typing · Typhoon Morakot

Introduction

DNA profiling is now a standard tool in victim identification from mass disasters including its use in airplane crashes [1, 2], the World Trade Center attack [3, 4], and natural phenomena such as the tsunami of 2004 [5–7] and hurricanes [8]. DNA typing has further been used to identify the perpetrator of terrorism-related atrocities [9]. With the rapid development and use of DNA in disaster victim identification (DVI), standard methods have been developed for the collection and storage of items, DNA extraction, and typing strategies, along with software for biostatistical interpretation [10–13]. Factors that affect the strategy of DNA typing in DVI include the extent of body decomposition and the available reference samples [14]. MiniSTR and single-nucleotide polymorphism typing have been developed to improve the success of DNA typing from highly degraded samples [15, 16]. The combination of Y-chromosome (STR) and MiniSTR was adopted in the identification of skeletal remains of Communist Armed Forces victims during and after World War II [17].

A 2007 DNA Commission of the International Society for Forensic Genetics (ISFG) on the role of forensic genetics in DVI provided a number of recommendations [10]. These recommendations are suitable for many DVI instances; however, additional challenges can be encountered when there are many genetically related victims of a natural disaster who live in an isolated area or belong to a small population. When linking a deceased to a putative relative for identification purposes, false inclusions must be limited [2], examples of such false inclusions arose in the DNA identification of victims of the 2004 tsunami and similar mass fatalities [6].

During Typhoon Morakot, more than 2,000 mm of rain have fallen in 48 h between August 8 and 10, 2009 in southern Taiwan. It was estimated that this storm killed over 1,000 people, although only 599 people were later reported as missing. Members of the forensic science community in Taiwan, particularly with specialties in pathology, anthropology, odontology, fingerprint, and DNA, were asked to assist in the identification of remains. The technical procedures of human forensic identification followed the guide of National Institute of Justice, USA [18], and DNA analysis followed the recommendations of ISFG [10]. Most of the human remains recovered were severely degraded, and little in the way of morphological features was present. Many of the victims were members of family groups with close genetic associations living in small villages within the valley where the flooding was at the greatest. We report on how traditional duo testing using STR loci from either pairs of parent/child or two siblings resulted in many potential false inclusions. Many victims of the flooding were identified with confidence after using a combination of Y-STR loci and/or mitochondrial DNA (mtDNA) along with testing of many combinations of relatives. The use of a rare allele assisted in the identification.

Materials and methods

Samples

Depending on the decomposition of the victim, samples ranged from buccal swabs and blood samples to bone fragments including phalanges, clavicles, and ribs. These samples were collected from August 14 to December 28, 2009. Buccal swabs or blood samples were collected from putative living relatives. These were received from August 14 to December 1 with the delay caused by reluctance by some possible relatives to donate a sample.

DNA extraction

Blood and buccal epithelial sample

DNA was extracted from blood and buccal epithelial samples by the QIAamp DNA Extraction Kit (QIAGENE, Germany) according to manufacturer's instructions.

Bone

Soft tissue attached to bone samples was cleaned with acetone and diethyl ether before pulverization to a fine powder under liquid nitrogen using a bone grinder. DNA was extracted from approximately 1 g of powder using the phenol/chloroform/isoamyl alcohol method [19]. If there was insufficient DNA or only a partial STR profile obtained from a bone sample, decalcification [20] and purification protocols of PureLink Genomic DNA Purification Kits (Invitrogen, USA) were added in the re-extraction.

DNA quantification

Extracted DNA was quantified by the Quantifiler Human DNA Quantification kit (Applied Biosystems, USA) using a 7500 Real-Time PCR System.

DNA analysis

Amplification of 15 autosomal STR loci was performed using the AmpFlSTR® Identifiler™ kit. If a partial STR profile was obtained using this kit, then Minifiler™ was used to complete the 15 STR loci. Samples that were from a male were typed using the 17 Y-chromosome STR loci in the Yfiler ™ kit. All DNA typing followed manufacturer's instructions (Applied Biosystems, USA), and the samples were amplified in a GeneAmp® PCR System 9700 (Applied Biosystems, USA) operating in 9600 emulation mode. All PCR products were separated on the ABI 3130*xl* Genetic Analyzer using POP-4™ polymer (Applied Biosystems, USA), and the data were analyzed using the GeneMapper® ID software v3.2.

DNA analysis of the mtDNA D-loop was amplified, purified, and sequenced according to Tsai LC et al. [21]. The cycle sequencing products were separated using a POP-6™ polymer (Applied Biosystems, USA) and detected using an ABI 3130*xl* Genetic Analyzer. The data were analyzed using the SeqScape software v2.6 according to the manufacturer's recommendations.

Data screening

Identical search

The forensic DNA data analysis system (FORDDAS) was used in the data screening; this was firstly developed and used in Taoyuan Airbus crash accident [1]. The purpose of this identity search was to re-associate remains to one individual, unless there are identical twins among the victims. A similarity search was performed to screen different allele sharing between samples. To have confidence of identification, there needed to be no inconsistent alleles and a random match probability of less than 10^{-14}.

Relationship search

Possible genetic relationships were identified within the population of the 146 samples from the victims. The likelihood ratio (LR) of any two samples between victims and relatives or among victims based on the duo combination of parent/child (PC) was extended at all 15 loci tested. The extension include multi-relative combinations such as a standard paternity test being two living parents and one alleged offspring (PPaC), one parent and one offspring compared to an alleged offspring (PCaC), two living offspring matching to one alleged parent (CCaP), and a parent and an offspring matching to the other alleged parent (PCaP). Under these kinship comparisons, a LR of 10^6 was set as the threshold for identity with confidence of identification reaching 99.9% (as there were 599 possible victims then $10^6 \times 1/599 = 10^3$, resulting in the posterior having a probability of 99.9%). The LR of multi-person kinship was calculated as the chance that the unknown sample is the genetic relative under consideration compared to the chance that the unknown sample comes from an unrelated person. Comparison of two siblings to one alleged sibling (SSaS) was also undertaken. An LR was calculated using the same logic as for the other possible genetic relationships.

Rare allele search

The presence of a microvariant rare allele allowed possible associations within an extended family group, when an undecided LR was observed between putative family members.

Confirmation of identity

The confidence of putative identifications that were based on a high LR of duo screening between victims and relatives was increased using multi-relative screening where possible. The use of Y STR loci and mtDNA was used where appropriate and if possible.

Results and discussion

Of the 599 people reported as missing, a total of 142 human remains where recovered from a landslide area and a further four samples from victims that were flushed along river to the Taiwan Strait and subsequently drifted to about 250 km north of Taiwan (Supplementary Table 1). Complete autosomal STR typing was obtained from all the 146 samples after multiple re-sampling and re-typing, with the exception of one locus (D5S818) in one sample even after two re-extractions.

There were 588 samples that were collected from living relatives, which came from 200 families. Full DNA profiles were obtained from the living relatives. Since most houses of the victims were destroyed in the typhoon, antemortem samples to identify the victims were not possible.

The STR data indicated that the 146 human remains originated from 130 profiles, there were 14 instances where two profiles had the same type and one case where three profiles came from the same individual. The largest random match probability of the shared profiles was 1.97×10^{-18}, and the average was 1.37×10^{-18}. Since no identical twins were claimed among the victims, there were 130 individuals waiting to be identified.

The timescale over which the human remains were transported to the laboratory impeded the identification as initially low LR values were obtained; this was due to only comparison with a small number of putative relatives to a few recovered remains. With increasing samples from both

living relatives and the human remains, the confidence in genetic association increased. By the end of December 2009, the duo parent/child screening resulted in each of 130 victims matching one of the 588 relatives in 177 pairs. A match was determined if there was at least one allele shared between the two samples at each of the 15 loci tested. Within the 130 victims there were 11 that matched with more than one family group and were examples of false associations. The LR values ranged from 4.00×10^9 to 7.59×10^9. Victims V007 and V008 shared alleles at all 15 loci with members of three different families, respectively (Supplementary Table 2). Relatives R007 and R020 were both matched to two different victims listed in Supplementary Table 2. In order to reduce the chance of a false inclusion, a combination of multi-relative screening and haplotype typing was undertaken. In Supplementary Table 2, there are 13 pairs which were later found to include false inclusions. This illustrated the potential complications of genetic association testing in a small village where there is high kinship. A total of 177 instances occurred where the DNA of a victim matched to one of those from the pool of relatives, 150 were what would be expected if parent and child, 21 if sibling pairs, 5 if uncle and niece, and 1 as grandparent to grandchild. In the case of 27 of the 177 pairs of parent offspring comparisons, the genetic evidence was contrary to putative visual identifications.

Supplementary Table 2 highlights how multi-relative screening either increased the confidence in genetic association or exclusion. After the search using multi-relative screening modes to investigate 177 duo parent/child pairs, 85 victims were identified with multiple relatives. Approximately 10^2- to 10^7-fold differences in LR values were obtained when using multi-relative screening than using only duo parent/child screening. There were 31 out of 177 pairs with LR values larger than 10^6. Although 23 victims were initially identified only by comparison to a single parent or child, they were confirmed by Y-STR and/or mtDNA data. A threshold of this type of value shows that two samples come from close genetic relatives, but not necessarily parent and child as supposed as three possible sibling pairs and one uncle niece pair were identified.

The use of two siblings to identify a third sibling (SSaS) is also a potentially powerful tool in searching the victims through relatives. In our simulation with 10,000 combinations of two siblings to identify a third sibling, about 98.91% of the population are excluded as being possible siblings (unpublished). In this case, duo sibling screening between 130 victims and 588 relatives showed 837 pairs with LR values larger than 10 and 38 pairs with LR values larger than 10^6. Identity could not be assigned directly based on LR values as multiple members of the same family were missing preventing identification of an unknown as either a parent, sibling, or uncle of a relative. SSaS screening was found to link 13 victims to pairs of siblings from the pool of relatives with high LR values. There were two victims who were identified by linkage to only one sibling, but as this was the only genetic relative available for testing, they were confirmed subsequently by mtDNA and Y-STR data. The SSaS model provided not only a powerful exclusion rate but resulted in linkage to living siblings with a high degree of confidence.

A search using a rare allele was able to find a possible link between a victim and their relatives. Only one victim carried FGA 25.2, and five relatives who were tested carried this same rare allele. This victim was identified as belonging to a family of two parents and three children all of whom perished (Fig. 1). The family was reported missing by siblings of the father. Although the human remain (V012) obtained a high LR value based on him being a sibling and an uncle to a niece to reference samples tested (Supplementary Table 3), this victim was linked to its relatives using the presence of a rare allele. Figure 1 shows that all five siblings of the father have allele 25.2, and although the sample of grandfather was not available, this allele can separate this family from the pool of relatives. Matching Y-STR on the paternal side and mtDNA data from the maternal relatives further confirmed identity of V012.

After genetic testing including linkage of matching samples, comparison to living relatives, and the use of rare alleles, 124 victims were identified. The screening modes with the highest LR value and the number of their missing relatives are shown in Supplementary Table 4. This table illustrates the complex nature of the identification process and how a multitude of comparisons was required to allow for the identification of the victims. Other forensic specialties aided with the identification. There were 42 fingerprints collected among 146 remains, and only seven hits with the national fingerprint database. These recon-

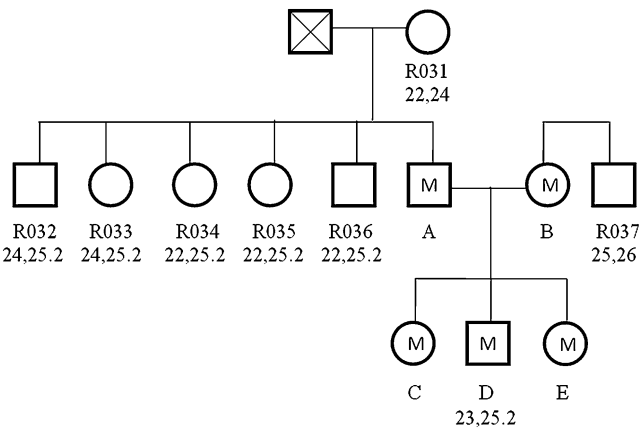

Fig. 1 Family carrying the rare allele 25.2 of the FGA locus. Human remain V012 was assigned to *D*, identified as the brother of *C* and *E* and child of *A* and *B*. *M* stands for missing, and the grandfather was not available for testing

firmed the DNA conclusion. Seven of the 124 victims were confirmed based on probable age of remains as grandparents or grandchildren. A particular tattoo and baldhead aided in the confirmation of identity of a victim. Three victims could be assigned to their families, but no specific identification could be made due to multiple members of the family missing and of the same age. No morphological features aided with these three victims. Dental X-rays taken from 33 of the victims proved to be of no assistance due to the lack of antemortem dental records for comparison. There are still six victims left to identify as their Y-STR and mtDNA data did not match any relative sampled as low or zero LR values for duo parent/child combinations with any of the DNA from the relatives and LR values of less than 20 for any possible sibling combinations were obtained. The use of X chromosome markers were not in use at the time of the investigation and could have aided in a small number of investigations.

Conclusion

This case illustrates the unexpected complication of using DNA testing for DVI in a highly related society of a small village. The strategies to identify as many of the victims as possible included using multi-relative screening and a rare allele search. The cross-comparisons among duo parent/child, PPaC, PCaC, PCaP, PaPaC, CCaP, CaPaP, and SSaS can provide a number of highly probable candidates. Confidence in putative identifications can be increased using Y-STR, mtDNA, and sex data, and/or ante- and postmortem information. Even when high LR values were obtained for parent child comparison or siblings, these were not always reliable when further testing was conducted. This work illustrates that the inclusion of more genetic relatives can greatly increase confidence in identity.

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