Preliminary DNA Identification for the Tsunami Victims in Thailand

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The 2004 Southeast Asia Tsunami killed nearly 5,400 people in Southern Thailand, including foreign tourists and local residents. To recover DNA evidence as much as possible from the seriously decomposed bodies, we explored procedures of sample preparation from both bone and tooth samples as well as both mitochondrial and nuclear markers. Despite having failed to recover enough DNA for nuclear marker typing, we succeeded in obtaining fully informative results for mitochondrial markers (HV1 and HV2) from 258 tooth samples with a success rate of 51% (258/507). Using an organic DNA extraction method coupled with an ultrafiltration step, we obtained 16 STR (including 13 CODIS loci, one sex discrimination locus, and two Identifiler loci) profiles for 834 samples with a success rate of 79% (834/1,062). In addition, by comparing the allelic frequencies between the typed samples as a group and other index populations, we conclude that the Thai tsunami victims are a combined group of several populations. Our results provide valuable evidence and protocols for the future forensic practice.

Key words: DNA identification, decomposed body, tsunami victims, mitochondrial marker

Introduction

Brought by an Indian Ocean earthquake, the notorious 2004 Southeast Asia Tsunami killed nearly 5,400 people in Southern Thailand. The victims include a large number of foreign tourists from Europe, Asia, and other regions of the world, in addition to Thai nationals. As part of an international relief effort, we participated in a scientific and humane endeavor to reveal the identity of these victims based on current forensic methods with important modifications.

The Chinese scientists arrived in Phuket, Thailand on December 31, 2004, and joined immediately the multi-national task force to collect samples from the remains of victims. Due to the scale of the disaster, the climate, and the process to initiate an adequate rescue effort, by the time when large-scale sample collection initiated, the remains of most tsunami victims had been seriously decomposed, not only making the sample collection process very difficult but also posing questions about the success of DNA identification. After in-depth literature studies and discussions with experts in this field, on January 1, 2005, we made a critical proposal that the samples collected for forensic tests should definitely include thick bones in addition to teeth, although the latter are the preferred specimens by standard forensic and anthropological studies for DNA-based identification. We have three basic arguments. First, the decomposing process in a humid and high-temperature environment may be significantly accelerated, especially when plentiful ocean-borne microbial species are stirred up by tsunami waves from oceanic sediments. Second, teeth should be among the first body parts being exposed to the microbe-rich seawater and the situation should be worsened when the floating bodies, often facedown, are soaked for days. Third, DNA-rich bone marrows are covered by the skin, muscle tissues, and calcified compact bones so that it takes much longer time for

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microbes to penetrate through. Upon the acceptance of our proposal by the task force, 507 tooth samples and 1,062 bone (a portion of femur) samples were collected on site by certified forensic dentists and forensic anthropologists, respectively, and were received by our laboratory in Beijing, from January 15 to June 11, 2005.

In this report, we describe our experience in DNA identification and results from a preliminary analysis on two types of data from nuclear and mitochondrial markers, which were acquired from two different specimens, teeth and bones. These results and analyses are believed to be highly beneficial for forensic scientists who may handle samples from specific environmental conditions, not limited to tsunami victims.

**Results**

**Genotyping of the HV1 and HV2 loci with mitochondrial DNA from tooth specimens**

Since most of the specimens from teeth had failed in STR typing due to low abundance and degradation of the nuclear DNA, we used these samples for mitochondrial DNA (mt-DNA) sequence analysis. We have succeeded in obtaining results from 258 tooth samples (258/507, success rate 51%) in both HV1 and HV2 sequences. A comparison to the Anderson sequences and results are summarized in Tables 1 and 2.

### Table 1 Statistics of 258 Tooth Samples from HV1 and HV2

| Locus* S°(Ts/Tv) | No. | Ins. | No. Del. | No. Freqency |
|-----------------|-----|------|----------|--------------|
| 16004 C-T       | 10  | 1.13%|          |              |
| 16019 C-T       | 16  | 1.81%|          |              |
| 16021 C-T       | 4   | 0.45%|          |              |
| 16026 C-T       | 23  | 2.60%|          |              |
| 16030 C-T       | 2   | 0.23%|          |              |
| 16032 T-A       | 3   | 0.34%|          |              |
| 16032 T-G       | 2   | 0.23%|          |              |
| 16042 G-A       | 1   | 0.11%|          |              |
| 16051 A-G       | 6   | 0.68%|          |              |
| 16053 C         | 2   | 0.23%|          |              |
| 16067 C-T       | 1   | 0.11%|          |              |
| 16069 C-T       | 21  | 2.38%|          |              |
| 16070 A-G       | 1   | 0.11%|          |              |
| 16085 C-G       | 3   | 0.34%|          |              |
| 16085 C-A       | 1   | 0.11%|          |              |
| 16086 T-C       | 4   | 0.45%|          |              |
| 16092 T-C       | 7   | 0.79%|          |              |
| 16093 T-C       | 7   | 0.79%|          |              |
| 16095 C-T       | 1   | 0.11%|          |              |
| 16095 C-G       | 2   | 0.23%|          |              |
| 16104 C-T       | 1   | 0.11%|          |              |
| 16104 C-A       | 1   | 0.11%|          |              |
| 16108 C-T       | 5   | 0.57%|          |              |
| 16111 C-T       | 3   | 0.34%|          |              |
| 16114 C-A       | 4   | 0.45%|          |              |
| 16124 T-C       | 2   | 0.23%|          |              |
| 16126 T-C       | 46  | 5.21%|          |              |
| 16127 A-C       | 1   | 0.11%|          |              |
| 16129 G-A       | 28  | 3.17%|          |              |
| 16129 G-C       | 2   | 0.23%|          |              |

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Table 1  Continued

| Locus* S* (Ts/Tv) | No. Ins. | No. Del. | No. Freqency |
|------------------|----------|----------|--------------|
| 16192 C-T        | 15       |          | 1.70%        |
| 16193 C-T        | 2 C 3    |          | 0.57%        |
| 16195 T-C        | 1        |          | 0.11%        |
| 16203 A-G        | 2        |          | 0.23%        |
| 16207 A-G        | 2        |          | 0.23%        |
| 16209 T-C        | 4        |          | 0.45%        |
| 16213 G-A        | 5        |          | 0.57%        |
| 16217 T-C        | 5        |          | 0.57%        |
| 16218 C-T        | 2        |          | 0.23%        |
| 16219 A-G        | 1        |          | 0.11%        |
| 16221 T-C        | 4        |          | 0.45%        |
| 16222 C-T        | 4        |          | 0.45%        |
| 16223 T-C        | 53       |          | 6.00%        |
| 16224 T-C        | 10       |          | 1.13%        |
| 16227 A-G        | 1        |          | 0.11%        |
| 16230 A-G        | 1        |          | 0.11%        |
| 16231 T-C        | 8        |          | 0.91%        |
| 16232 C-T        | 1        |          | 0.11%        |
| 16232 C-A        | 3        |          | 0.34%        |
| 16233 A-G        | 2        |          | 0.23%        |
| 16234 C-T        | 3        |          | 0.34%        |
| 16235 A-G        | 2        |          | 0.23%        |
| 16239 T-C        | 8        |          | 0.91%        |
| 16240 A-G        | 2        |          | 0.23%        |
| 16242 C-T        | 1        |          | 0.11%        |
| 16243 T-C        | 2        |          | 0.23%        |
| 16247 A-G        | 1        |          | 0.11%        |
| 16249 T-C        | 4        |          | 0.45%        |
| 16255 G-A        | 1        |          | 0.11%        |
| 16256 C-T        | 13       |          | 1.47%        |
| 16257 C-T        | 1        |          | 0.11%        |
| 16258 A-G        | 1        |          | 0.11%        |
| 16260 C-T        | 5        |          | 0.57%        |
| 16261 C-T        | 16       |          | 1.81%        |
| 16263 T-C        | 5        |          | 0.57%        |
| 16265 A-G        | 1        |          | 0.11%        |
| 16266 C-T        | 4        |          | 0.45%        |
| 16266 C-A        | 1        |          | 0.11%        |
| 16269 A-C        | 1        |          | 0.11%        |
| 16270 C-T        | 21       |          | 2.38%        |
| 16271 T-C        | 1        |          | 0.11%        |
| 16278 C-T        | 13       |          | 1.47%        |
| 16286 C-T        | 2        |          | 0.23%        |
| 16286 C-A        | 1        |          | 0.11%        |
| Total            | 871      | 9        | 3 100%(883)  |

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Table 1  **Continued**

| Locus*  | S(Ts/Tv) | No. Ins. | No. Del. | No. Frequency |
|---------|----------|----------|----------|---------------|
| 46      | T-A      | 1        |          | 0.08%         |
| 61      | C-A      | 1        |          | 0.08%         |
| 61      | C-T      | 12       |          | 1.00%         |
| 64      | C-T      | 20       |          | 1.67%         |
| 72      | T-C      | 7        |          | 0.58%         |
| 72      | T-G      | 5        |          | 0.42%         |
| 73      | A-G      | 118      |          | 9.86%         |
| 93      | A-G      | 2        |          | 0.17%         |
| 94      | G-A      | 2        |          | 0.17%         |
| 114     | C-T      | 3        | C 3      | 0.33%         |
| 132     | C-T      | 3        |          | 0.25%         |
| 140     | C-T      | 1        |          | 0.08%         |
| 143     | G-A      | 3        |          | 0.25%         |
| 146     | T-C      | 20       |          | 1.67%         |
| 150     | C-T      | 27       |          | 2.26%         |
| 151     | C-T      | 2        |          | 0.17%         |
| 152     | T-C      | 46       |          | 3.85%         |
| 153     | A-G      | 3        |          | 0.25%         |
| 173     | T-C      | 1        |          | 0.08%         |
| 182     | A-G      | 7        |          | 0.58%         |
| 185     | G-A      | 7        |          | 0.58%         |
| 188     | A-G      | 3        |          | 0.25%         |
| 189     | A-G      | 13       |          | 1.09%         |
| 192     | T-C      | 1        |          | 0.08%         |
| 194     | C-T      | 9        |          | 0.76%         |
| 195     | T-C      | 47       |          | 3.93%         |
| 196     | T-C      | 1        |          | 0.08%         |
| 198     | C-T      | 2        |          | 0.17%         |
| 199     | T-C      | 8        |          | 0.67%         |
| 200     | A-G      | 2        |          | 0.17%         |
| 204     | T-C      | 17       |          | 1.42%         |
| 207     | G-A      | 16       |          | 1.34%         |
| 210     | A-G      | 1        |          | 0.08%         |
| 214     | A-G      | 4        |          | 0.34%         |
| 215     | A-G      | 7        |          | 0.58%         |
| 215     | A-C      | 1        |          | 0.08%         |
| 217     | T-C      | 4        |          | 0.34%         |
| 222     | C-T      | 1        |          | 0.08%         |
| 225     | G-A      | 5        |          | 0.42%         |
| 226     | T-C      | 2        |          | 0.17%         |
| 227     | A-G      | 2        |          | 0.17%         |
| 228     | G-A      | 11       |          | 0.92%         |
| 234     | A-G      | 2        |          | 0.17%         |
| 239     | T-C      | 4        |          | 0.34%         |

| Statistics of 258 tooth samples from HV2 | Locus*  | S(Ts/Tv) | No. Ins. | No. Del. | No. Frequency |
|-----------------------------------------|---------|----------|----------|----------|---------------|
| 242                                    | C-T     | 2        |          |          | 0.17%         |
| 249                                    | A       | 19       |          |          | 1.59%         |
| 250                                    | T-C     | 3        |          |          | 0.25%         |
| 253                                    | C-T     | 1        |          |          | 0.08%         |
| 253                                    | C-G     | 1        |          |          | 0.08%         |
| 257                                    | A-G     | 1        |          |          | 0.08%         |
| 259                                    | A-G     | 1        |          |          | 0.08%         |
| 262                                    | C-T     | 1        |          |          | 0.08%         |
| 263                                    | A-G     | 249      |          | 816      | 100.00%       |
| 264                                    | C-T     | 1        |          |          | 0.08%         |
| 282                                    | T-C     | 1        |          |          | 0.08%         |
| 285                                    | C-T     | 2        |          |          | 0.17%         |
| 290                                    | AA      | 2        |          |          | 0.17%         |
| 291                                    | A-T     | 1        |          |          | 0.08%         |
| 295                                    | C-T     | 22       |          |          | 1.84%         |
| 297                                    | A-G     | 1        |          |          | 0.08%         |
| 309                                    | C       | 101      |          |          | 8.44%         |
| 309                                    | CC      | 24       |          |          | 2.01%         |
| 310                                    | T-C     | 4        |          |          | 0.34%         |
| 310                                    | C       | 21       |          |          | 1.75%         |
| 310                                    | TC      | 6        |          |          | 0.50%         |
| 315                                    | C       | 200      |          |          | 16.79%        |
| 315                                    | CCC     | 1        |          |          | 0.08%         |
| 316                                    | G-C     | 1        |          |          | 0.08%         |
| 317                                    | C-T     | 1        |          |          | 0.08%         |
| 317                                    | C-G     | 1        |          |          | 0.08%         |
| 319                                    | T-C     | 6        |          |          | 0.50%         |
| 323                                    | G-A     | 1        |          |          | 0.08%         |
| 345                                    | C-T     | 1        |          |          | 0.08%         |
| 356                                    | C       | 2        |          |          | 0.17%         |
| 362                                    | C-A     | 22       |          |          | 1.84%         |
| 366                                    | G-A     | 11       |          |          | 0.92%         |
| 376                                    | A-C     | 1        |          |          | 0.08%         |
| 379                                    | A-C     | 2        |          |          | 0.17%         |
| 380                                    | G-C     | 3        |          |          | 0.25%         |
| 385                                    | A-G     | 1        |          |          | 0.08%         |
| 389                                    | G-A     | 1        |          |          | 1.01%         |
| 402                                    | A-T     | 1        |          |          | 0.17%         |
| 404                                    | C-T     | 2        |          |          | 0.17%         |
| 408                                    | T-G     | 3        |          |          | 0.25%         |
| 411                                    | C-G     | 4        |          |          | 0.33%         |
| 463                                    | C       | 1        |          |          | 0.08%         |
| 816                                    | 356     | 25       |          |          | 100.00%       |
| (1,197)                                |          |          |          |          |               |

* Locus lists the base location in the human mitochondrial DNA (D-loop region). # S stands for substitutions. The detailed base changes, transition (Ts) or transversion (Tv) (underlined), are listed.
**Table 2 Alleles and Their Frequencies in 15 Autosomal STR Loci**

| Locus | Allele and Frequency |
|-------|----------------------|
| CSF1PO | 7 0.2635% 8 0.3953% 9 2.5692% 10 25.2964% 11 30.3689% 12 34.8485% 13 5.4677% 14 0.7246% 15 0.0659% |
| FGA    | 16 0.3157% 17 0.3157% 18 1.5152% 19 6.6919% 20 6.2500% 21 0.2525% |
| TH01   | 4 0.1238% 6 12.0050% 7 25.8045% 8 8.4777% 9 37.0050% |
| D3S1358| 4 0.1230% 6 0.1845% 7 56.3961% 8 10.6396% 9 3.1365% 10 27.9213% 11 1.5990% |
| D5S818 | 7 1.6049% 8 0.0617% 9 15.1292% 10 27.4293% 11 9.4711% 12 2.0295% 13 0.1230% |
| D7S820 | 7 1.3871% 8 19.5509% 9 8.3884% 10 15.9181% 11 34.2140% 12 17.3052% |
| D8S1179| 8 0.4305% 9 0.1845% 10 13.6531% 11 8.0566% 12 11.9311% 13 18.5732% |
| D13S317| 6 0.0634% 7 0.1901% 8 28.7072% 9 12.4842% 10 9.8859% 11 23.0672% |
| D16S539| 8 0.9963% 9 18.8045% 10 10.3362% 11 31.9427% 12 25.1557% 13 11.0834% |
| D18S51 | 7 0.0650% 9 0.2601% 10 0.4551% 11 1.1704% 12 5.9818% 13 14.3043% |
| D21S11 | 27 0.4969% 28 8.0745% 29 0.1863% 30 24.6584% 31 29.21 3.1066% 30 24.9068% |
| TH01   | 4 0.1238% 6 12.0050% 7 25.8045% 8 8.4777% 9 37.0050% |
| TPOX   | 6 0.1481% 7 0.0617% 8 12.4514% 9 26.2369% 10 8.7546% 11 14.3033% 12 1.7879% |

In the HV1 region, we detected 147 loci from base 16004 to 16391 in a total of 883 variants, including 871 SNPs and 12 Indels (Insertions and Deletions). There are eight loci with frequencies exceeding 3% (16126, 16129, 16183, 16189, 16223, 16294, 16311, and 16362). In the HV2 region, we found 85 loci from base 46 to 463 with a total of 1,197 variants. Among them, we have 816 SNPs and 381 Indels. There are four high-frequency loci in this region (73, 263, 309, and 315). We notice the uneven distribution of the variants between the two regions among the loci in their distribution, allelic frequencies, and variation types (base substitutions and Indels).

**Genotyping of nuclear STR loci with nuclear DNA from bone specimens**

After encountering difficulty in extracting high-quality and adequate DNA from tooth specimens, we tried three different methods to extract DNA from bone specimens. The IQ™ system from Promega...
and the QIAamp Micro kit from Qiagen did not give rise to consistent results but the organic method coupled with an ultrafiltration step with the Microcon YM-100 concentrator (1) showed a promising STR profiling. For genotyping, we used a commercial kit from Applied BioSystems, the Identifier, with 16 STR loci that include 13 CODIS (the FBI Laboratory’s Combined DNA Index System) loci, one AMEL locus (amelogenin genes that are found on both the X and Y chromosomes; ref. 2), and two others (D2S1338 and D19S433). We succeeded in obtaining qualified STR profiles from 834 samples (Tables 3 and 4) and three examples are shown in Figure 1. At the TPOX locus (Figure 1, A and B), the allele 8 has an extremely high frequency and so do the 8 and 8/11 genotypes. TPOX shows the lowest discrimination power (DP), with the DP value of only 56.7%. The D3S1358 locus (Figure 1, C and D) has a more balanced allele frequency with a DP value of 74.35% but is lower than the FGA locus (Figure 1, E and F), which has a DP value of 84.97%. We also compared our data from the 13 CODIS loci to the references at the STRBase (www.cstl.nist.gov/div831/strbase) (Table 5).

| Locus | Genotype and Frequency |
|-------|------------------------|
| CSF1PO | 7/10 0.1318% 7/12 0.2635% 7/13 0.1318% 8/11 0.3953% 8/12 0.2635% 8/13 0.1318% 9/10 1.0540% 9/11 2.2398% 9/12 1.5810% 9/13 0.2635% 10 8.8274% 10/11 13.5705% 10/12 15.8103% 10/13 2.2398% 10/14 0.1318% 11 10.6719% 11/12 20.4216% 11/13 2.6350% 11/14 0.1318% 12 13.3070% 12/13 3.8208% 12/14 0.7905% 12/15 0.1318% 13 0.6588% 13/14 0.3953% |
| FGA | 16 0.1263% 17/19 0.1263% 16/19 0.1263% 17/21 0.2525% 16/22 0.1263% 17/22 0.7576% 17/23 0.1263% 18/20 0.5051% 18/21 0.7576% 18/22 0.1263% 18/23 0.1263% 19/20 1.0540% 19/21 2.2398% 19/22 0.7576% 19/23 0.1263% 20 8.8274% 20/21 13.5705% 20/22 15.8103% 20/23 2.2398% 20/24 0.1318% 21 10.6719% 21/22 20.4216% 21/23 2.6350% 21/24 0.1318% 22 5.3030% 22/23 6.8182% 22/24 0.1263% 23 2.6515% 23/24 4.6717% 23/25 0.6313% 24 1.7677% 24/25 4.2929% 24/26 0.5051% 25 0.8838% 25/26 0.8838% 26 0.3788% |
| TH01 | 4/7 0.2475% 5/7 1.7327% 6/7 1.8564% 6/8 0.3788% 6/9 5.5693% 6/10 5.3030% 7/8 3.3416% 7/9 4.4554% 7/10 3.4416% 8/9 2.5830% 8/10 2.5830% 8/11 2.5830% 8/12 2.5830% 9/10 2.5830% 9/11 2.5830% 9/12 2.5830% 9/13 2.5830% 9/14 2.5830% 10/10 2.5830% 10/11 2.5830% 10/12 2.5830% 10/13 2.5830% 11/11 2.5830% |
| TPOX | 6/11 0.3690% 7/11 32.9643% 8/11 29.8893% 8/12 19.9808% 8/13 11.6851% 8/14 9.0347% 8/15 5.4121% 9/10 0.7905% 9/11 0.3788% 9/12 0.3788% 10/10 0.3788% 10/11 0.3788% 10/12 0.3788% 10/13 0.3788% 10/14 0.3788% 11/10 0.3788% 11/11 0.3788% 11/12 0.3788% 11/13 0.3788% 11/14 0.3788% 11/15 0.3788% 12/11 0.3788% 12/12 0.3788% 12/13 0.3788% 12/14 0.3788% 12/15 0.3788% 13/11 0.3788% 13/12 0.3788% 13/13 0.3788% 13/14 0.3788% 13/15 0.3788% 14/12 0.3788% 14/13 0.3788% 14/14 0.3788% 14/15 0.3788% 15/12 0.3788% 15/13 0.3788% 15/14 0.3788% 15/15 0.3788% 16/13 0.3788% 16/14 0.3788% 16/15 0.3788% 17/14 0.3788% 17/15 0.3788% 18/15 0.3788% |
| Locus | Genotype and Frequency |
|-------|------------------------|
| vWA   |                        |
| 13/19 | 0.2460% 14 4.1820% 14/15 1.9680% 14/16 6.5191% 14/17 12.4231% |
| 14/18 | 9.1021% 14/19 3.3210% 14/20 1.2300% 15 0.1230% 15/16 1.4760% |
| 15/17 | 2.9520% 15/19 0.7380% 15/18 4.0691% 15/19 0.2466% 16 1.8450% |
| 16/17 | 7.2571% 16/18 7.6261% 16/19 2.8290% 16/20 0.8610% 17 7.3801% |
| 17/18 | 9.7171% 17/19 6.5191% 17/20 0.8610% 17/21 2.4600% 17/22 0.1230% |
| 18    | 4.4280% 18/19 2.8290% 18/20 0.7380% 19 0.8610% 19/20 0.2460% |
| D3S1358 | 12/15 0.2466% 12/17 0.2466% 13/14 0.3699% 13/17 0.2466% 14 0.4932% |
| 14/15 | 3.6991% 14/16 3.6991% 14/17 2.2195% 14/18 0.8631% 15 6.9051% |
| 15/16 | 19.2355% 15/17 16.2762% 15/18 4.0691% 15/19 0.2466% 16 12.7004% |
| 16/17 | 13.6868% 16/18 4.3157% 16/19 0.3699% 16/20 0.2466% 17 4.8089% |
| 17/18 | 4.0691% 17/19 0.1233% 18 0.6165% 18/19 0.1233% 19 0.1233% |
| D5S818 | 7/10 0.9877% 7/11 1.1111% 7/12 0.8642% 7/13 0.2469% 8/10 0.1235% |
| 9     | 0.6173% 9/10 2.8395% 9/11 1.8519% 9/12 0.7380% 9/13 2.9741% 9/14 0.7380% |
| 10    | 5.6790% 10/11 9.6290% 10/12 10.8642% 10/13 8.6420% 10/14 0.2466% |
| 11    | 9.3827% 11/12 11.9753% 11/13 9.0123% 11/14 0.9877% 12 6.9136% |
| 12/13 | 8.1481% 12/14 0.2469% 12/15 0.2469% 13 3.3333% 13/14 0.2469% |
| 14    | 0.1235% |
| D7S820 | 7/10 0.7926% 7/11 1.0568% 7/12 0.3963% 7/8 0.2642% 7/9 0.2642% |
| 8     | 4.6235% 8/10 6.4729% 8/11 12.2853% 8/12 6.6050% 8/13 0.1235% |
| 9     | 0.1231% 9/10 1.0568% 9/11 3.6988% 9/12 0.2466% 9/13 0.2466% |
| 10    | 1.8494% 10/11 9.6290% 10/12 10.8642% 10/13 8.6420% 10/14 0.2466% |
| D8S1179 | 10/11 2.2140% 10/12 3.1980% 10/13 5.1661% 10/14 4.9200% |
| 11/12 | 8.1481% 11/13 12.2853% 11/14 6.6050% 11/15 1.0568% 11/16 0.2466% |
| 12    | 1.4760% 12/13 1.4760% |
| D13S317 | 6/8 0.1267% 7/11 0.2535% 7/9 0.1267% 8/10 9.2522% 8/11 4.4360% |
| 8/14 | 0.1267% 8/10 9.2522% 8/11 4.4360% 8/12 7.9759% 8/13 2.4081% 8/14 1.0139% |
| 9/12 | 1.1070% 9/10 3.1980% 9/11 5.1661% 9/12 1.4760% 9/13 1.4760% |
| 7/13 | 5.421% 7/12 4.3093% 7/13 1.1407% 7/14 0.2491% 7/15 0.1245% |
| 12/13 | 3.6900% 12/14 3.3210% 12/15 1.3210% 12/16 0.1245% 12/17 0.1245% |
| D16S539 | 7/12 2.2814% 7/13 2.4707% 7/14 1.6905% 7/15 1.5605% 7/16 0.7802% |
| 8/11 | 0.7472% 8/12 0.4981% 8/13 0.7472% 8/14 0.5070% 8/15 0.3802% |
| 9/11 | 12.3288% 9/10 6.7248% 9/11 2.8643% 9/14 0.1245% 9/15 0.2491% |
| 10/11 | 6.8493% 10/10 5.4795% 10/12 1.1407% 10/13 9.3400% 10/14 2.4707% |
| 11/12 | 15.6912% 11/13 8.4682% 11/14 2.4707% 12/13 5.6040% |
| 12/14 | 1.1208% 12/15 1.1208% 12/16 1.1208% 12/17 1.1208% 12/18 0.2491% |
| D18S51 | 7/16 0.1300% 9/10 0.1300% 9/11 0.1300% 9/12 0.1300% 9/13 0.1300% |
| 10/13 | 0.2601% 10/14 0.1300% 10/15 0.1300% 10/16 0.1300% 10/17 0.1300% |
| 11/14 | 0.6502% 11/15 0.1300% 11/16 0.1300% 11/17 0.1300% 11/18 0.1300% |
| 12/14 | 1.6905% 12/15 2.6008% 12/16 1.5605% 12/17 0.7802% 12/18 0.5202% |
### Table 3

| Locus     | Genotype and Frequency |
|-----------|------------------------|
| **D18S51** |                        |
| 12/19     | 0.520% 12/23 0.3901%   |
| 13/14     | 4.681% 13/15 6.5020%   |
| 13/21     | 13/22 0.2601% 13/23 0.6502% |
| 13/24     | 3.4611% 14/12 0.1300% |
| 14/15     | 14/16 8.3225% 14/17 5.8518% |
| 14/21     | 14/22 0.7802% 14/23 0.1300% |
| 15/18     | 2.0806% 15/20 6.6320% |
| 16/19     | 3.6411% 16/20 0.1300% |
| 17/19     | 0.9864% 17/20 1.2330% |
| 18/20     | 0.2466% 18/21 0.1233% |
| 19/21     | 0.3901% 19/22 0.2466% |
| 20/21     | 0.1300% 21/22 0.1300% |
| **D21S11** |                        |
| 27/28     | 0.2484% 27/29 0.2484% |
| 28/30     | 0.7453% 28/31 1.1180% |
| 29/30     | 1.4907% 29/31 7.6087% |
| 29/32     | 2.2360% 29/34 0.3727% |
| 30/32     | 0.3727% 30/33 0.7453% |
| 31/32     | 0.6211% 31/33 0.6211% |
| 32/32     | 0.7453% 32/33 0.7453% |
| 33/32     | 0.6211% 33/34 0.1242% |
| 34/32     | 0.2484% 34/33 0.2484% |
| **D2S1338** |                      |
| 9/13      | 0.2460% 9/14 0.6165% |
| 11/14     | 0.1233% 11/15 0.3699% |
| 12/15     | 4.266% 12/16 3.0826% |
| 12/16     | 0.1233% 12/17 1.4797% |
| 13/14     | 0.2466% 13/15 2.5894% |
| 13/15     | 0.2466% 13/16 6.4118% |
| 13/16     | 0.1300% 13/17 0.9864% |
| 13/17     | 0.2466% 13/18 6.0419% |
| 14/15     | 2.3428% 14/16 0.8964% |
| 15/16     | 0.1233% 15/17 0.1233% |
| 16/17     | 0.1233% 16/18 0.1233% |

DNA Identification for Tsunami Victims
Table 4 Statistics of 15 Identifier Loci Common Index

| Locus  | Allele No. | Genotype No. | PIC  | PICe | Pm  | Pme | DP  | DPe | EP  |
|--------|------------|--------------|------|------|-----|-----|-----|-----|-----|
| CSF1PO | 9          | 25           | 66.53% | 66.47% | 33.47% | 28.14% | 66.53% | 71.86% | 49.56% |
| FGA    | 20         | 99           | 84.97% | 86.10% | 15.02% | 12.63% | 84.97% | 87.37% | 75.73% |
| TH01   | 9          | 25           | 74.88% | 72.67% | 25.12% | 23.95% | 74.88% | 76.05% | 57.23% |
| TPOX   | 7          | 17           | 56.70% | 53.18% | 8.39%  | 6.76%  | 56.70% | 59.14% | 36.15% |
| vWA    | 10         | 30           | 81.18% | 77.73% | 18.82% | 19.47% | 81.18% | 80.53% | 63.42% |
| D8S1179| 11         | 41           | 81.67% | 82.85% | 18.33% | 15.27% | 81.67% | 84.73% | 70.60% |
| D13S317| 9          | 31           | 78.45% | 77.38% | 21.55% | 19.80% | 78.45% | 80.19% | 63.01% |
| D16S539| 9          | 26           | 75.03% | 75.03% | 24.97% | 21.87% | 75.03% | 78.13% | 59.44% |
| D18S51 | 20         | 82           | 80.88% | 83.88% | 19.12% | 14.51% | 80.88% | 85.49% | 72.37% |
| D21S11 | 15         | 59           | 80.62% | 81.52% | 19.38% | 16.55% | 80.62% | 83.45% | 69.04% |
| D2S1338| 11         | 60           | 81.06% | 86.04% | 18.94% | 12.64% | 81.06% | 87.36% | 75.49% |
| D19S433| 16         | 58           | 75.03% | 75.03% | 24.97% | 21.87% | 75.03% | 78.13% | 60.00% |

Fig. 1 Allele and genotype frequencies from three representative loci: TPOX (A and B), D3S1358 (C and D), and FGA (E and F).
### Table 5 Comparison of Allele Numbers from 13 CODIS Loci Between the Thai Tsunami Victims and the STR Base Reported Population

| Locus    | Detected allele | Reported allele | Detected/Reported |
|----------|-----------------|-----------------|-------------------|
| CSF1PO   | 9               | 12              | 75.0%             |
| FGA      | 20              | 67              | 29.9%             |
| TH01     | 9               | 20              | 45.0%             |
| TPOX     | 7               | 10              | 70.0%             |
| vWA      | 10              | 26              | 38.5%             |
| D3S1358  | 9               | 20              | 45.0%             |
| D5S818   | 9               | 10              | 90.0%             |
| D7S820   | 8               | 22              | 36.4%             |
| D8S1179  | 11              | 13              | 84.6%             |
| D13S317  | 9               | 14              | 64.3%             |
| D16S539  | 9               | 10              | 90.0%             |
| D18S51   | 20              | 42              | 47.6%             |
| D21S11   | 15              | 72              | 20.8%             |

### Discussion

**Nuclear DNA is degraded in tooth specimens but mt-DNA from them is recoverable**

We had quite a struggle in extracting enough DNA for nuclear markers from tooth specimens initially due to the variable amount of tooth samples (decomposed bodies often have missing teeth to different extents). Despite various controls, careful planning for the experiments, and DNA enrichment procedures, the amount of DNA purified from an entire tooth or pooled from multiple extraction procedures was not enough to give rise to satisfactory results for all nuclear loci; some of the samples did not show any evidence of remaining DNA. When some of the specimens did give positive results, the quality was often poor and unusable. Having worked at the sites of specimen collection, we observed that the victim bodies stored in the local morgue were not maintained in low temperature and most of them had been washed in badly contaminated sea water for days before sample retrieval. It was reported by numerous investigators that DNA degrades rather quickly when exposed to high temperature \(^3\); salt and bacterial contaminations are also inhibitory factors for appropriate DNA preparation and amplification \(^4, 5\). Furthermore, the amount of recoverable DNA is also critical since low DNA concentration often causes false positive results. Finally, the procedure to collect teeth, though done by forensic professionals, is different from anthropologists’ procedures in which the teeth are often collected with the skull. When teeth are removed, the cavity is exposed to air and also becomes accessible to microbes. As a result, we encountered tremendous difficulty in recovering DNA in an adequate amount (often lower than 3 pg in total pooled extracts) to perform our experiments that insist information as complete as possible.

Since these specimens are very precious and important for the victims’ family to identify their beloved ones lost in the tragedy, we attempted to rescue mt-DNA in case of request. We chose to genotype loci in the non-coding region of mitochondrial sequences, the displacement loop (D-loop, sometimes referred as the control region) that is approximately 1,100 bp in length. The forensic value of mt-DNA relies on the sequence variability of the D-loop between individuals, and the maternally inherited nature of mitochondria makes it suitable for matching blood relatives of maternal inheritance. In addition to skin, blood, semen, saliva, and the usual body remains for forensic DNA identification, mt-DNA has also been extracted from teeth and used for such purpose \(^6, 7\). Because the tooth specimens of the tsunami victims were badly degraded and could not be used to obtain satisfactory nuclear STR profiling, we decided to sequence mt-DNA from the tooth specimens to salvage as much information as we can. The complete sequences from the multiple hypervariable regions often enable investigators to identify remains of war casu-
alties and individuals involved in mass disasters or criminal case (8). We indeed achieved a 51% success rate in recovering mt-DNA information, ready to serve any relatives who step up for DNA matching in searching for missing family members in the disaster.

Bones are better specimens for typing nuclear marks but an enrichment step is of essence

Realizing that similar situation may happen in preparing DNA from the bones, we designed a series of experiments to work out a standard protocol for tsunami victims. We first investigated commercial kits from companies whose DNA purification kits are widely used in forensics. The IQTM system from Promega and the QIAamp Micro kit from Qiagen are two examples for DNA extraction, which are successfully used to extract DNA from blood stains, buccal swabs, hair follicles, sperms, teeth, and bones. We also tested in parallel several classical methods. Among them, a classical organic method used to prepare DNA from calcified tissues (4), coupled with an sample concentration step with the Microcon YM-100 concentrator, an ultrafiltration unit, gave us the most satisfactory result. Among those complicated reasons, the most decisive factors are the relative purity of the resulted DNA preparations and the higher yield of the procedure. The yields of this protocol is often ten times higher than the commercial kits when start with the same amount raw bone samples. Another important notion from our experience is that the bone specimen is not limited by size, and can be readily grinded into a fine powder for DNA extraction, yielding a higher concentration of DNA with minimal degradation. Relatively pure and high concentration of the DNA samples gave us consistent results for the nuclear STR typing.

The Thai tsunami victims are ethnic diverse group based on STR profiles

The Thai tsunami victims are an admixture of foreign tourists and local residents, including not only Thai nationals, but also other Asians, Europeans, Americans, and so on. As we anticipated, the results show a great diversity among the typed loci from the victims when compared to any of the reference population-based data. Comparing the values of the observed heterozygosity (Ho) and expected heterozygosity (He) to those of U.S. Caucasian, African American, Hispanic, Chamorro, or Filipino populations, we found that the Thai tsunami victims generally have lower Ho and He values than those of the U.S. Caucasian and African American populations (Tables 6 and 7).

### Table 6 Comparison of Allele Frequencies from 15 STR Loci Between the Thai Tsunami Victims and Other Reference Populations

| Locus | Allele | Thai tsunami victim (N=834) | U.S. Caucasian (N=302) | African American (N=258) |
|-------|--------|-----------------------------|------------------------|---------------------------|
| CSF1PO | 7      | 0.003 – 0.053               | –                      |                           |
|       | 8      | 0.004 0.005 0.060           | –                      |                           |
|       | 9      | 0.026 0.012 0.037           | –                      |                           |
|       | 9.3    | – 0.257                    | –                      |                           |
|       | 10     | 0.253 0.217 –               | –                      |                           |
|       | 11     | 0.304 0.301 0.249           | –                      |                           |
|       | 12     | 0.348 0.361 0.298           | –                      |                           |
|       | 13     | 0.055 0.096 0.037           | –                      |                           |
|       | 14     | 0.007 0.008 0.010           | –                      |                           |
|       | 15     | 0.001 – –                   | –                      |                           |
|       | Ho     | 0.665 0.725 0.759           | –                      |                           |
|       | He     | 0.667 0.724 0.776           | –                      |                           |
| FGA   | 16     | 0.003 – –                   | –                      |                           |
|       | 16.2   | – – 0.022                  | –                      |                           |
|       | 17     | 0.003 – –                   | –                      |                           |
|       | 18     | 0.015 0.026 0.002           | –                      |                           |
|       | 18.2   | – – 0.012                  | –                      |                           |

| Locus | Allele | Thai tsunami victim (N=834) | U.S. Caucasian (N=302) | African American (N=258) |
|-------|--------|-----------------------------|------------------------|---------------------------|
| FGA   | 19     | 0.067 0.053 0.062           | –                      |                           |
|       | 20     | 0.063 0.127 0.056           | –                      |                           |
|       | 20.2   | 0.003 – –                   | –                      |                           |
|       | 21     | 0.149 0.185 0.116           | –                      |                           |
|       | 21.2   | 0.018 0.005 –               | –                      |                           |
|       | 22     | 0.205 0.219 0.196           | –                      |                           |
|       | 22.2   | 0.012 0.012 0.004           | –                      |                           |
|       | 22.3   | – – 0.002                  | –                      |                           |
|       | 23     | 0.165 0.134 0.171           | –                      |                           |
|       | 23.2   | 0.010 0.003 0.002           | –                      |                           |
|       | 24     | 0.125 0.136 0.122           | –                      |                           |
|       | 24.2   | 0.009 0.002 –               | –                      |                           |
|       | 25     | 0.092 0.071 0.124           | –                      |                           |
|       | 25.2   | 0.006 – –                   | –                      |                           |
|       | 26     | 0.037 0.023 0.081           | –                      |                           |
|       | 26.2   | 0.002 – –                   | –                      |                           |
| Locus   | Allele | Thai tsunami victim (N=834) | U.S. Caucasian (N=302) | African American (N=258) |
|---------|--------|-----------------------------|------------------------|--------------------------|
| D3S1358 | 11     | 0.002                       | 0.002                  | 0.002                    |
|         | 12     | 0.002                       | 0.002                  |                          |
|         | 13     | 0.002                       | 0.002                  |                          |
|         | 14     | 0.059                       | 0.103                  | 0.089                    |

**Table 6 Continued**

| Locus   | Allele | Thai tsunami victim (N=834) | U.S. Caucasian (N=302) | African American (N=258) |
|---------|--------|-----------------------------|------------------------|--------------------------|
| D3S1358 | 15     | 0.288                       | 0.262                  | 0.302                    |
|         | 15.2   | 0.288                       | 0.262                  | 0.302                    |
|         | 16     | 0.335                       | 0.253                  | 0.335                    |
|         | 17     | 0.232                       | 0.215                  | 0.205                    |
|         | 18     | 0.073                       | 0.152                  | 0.060                    |
|         | 19     | 0.006                       | 0.012                  | 0.004                    |
|         | 20     | 0.001                       | 0.002                  |                          |
|         | 21     | 0.001                       | 0.002                  |                          |
|         | 22     | 0.001                       | 0.002                  |                          |

**TH01**

| Locus   | Allele | Thai tsunami victim (N=834) | U.S. Caucasian (N=302) | African American (N=258) |
|---------|--------|-----------------------------|------------------------|--------------------------|
| 4       | 0.001  | 0.000                       | 0.004                  |                          |
| 5       | 0.002  | 0.002                       | 0.004                  |                          |
| 6       | 0.120  | 0.232                       | 0.124                  |                          |
| 7       | 0.258  | 0.190                       | 0.421                  |                          |
| 8       | 0.085  | 0.184                       | 0.194                  |                          |
| 8.3     | 0.001  | 0.001                       | 0.000                  |                          |
| 9       | 0.370  | 0.114                       | 0.151                  |                          |
| 9.3     | 0.103  | 0.368                       | 0.105                  |                          |
| 10      | 0.061  | 0.008                       | 0.006                  |                          |
| 11      | 0.001  | 0.002                       | 0.002                  |                          |
| 12      | 0.749  | 0.719                       | 0.760                  |                          |
| 13      | 0.727  | 0.756                       | 0.738                  |                          |

**TPOX**

| Locus   | Allele | Thai tsunami victim (N=834) | U.S. Caucasian (N=302) | African American (N=258) |
|---------|--------|-----------------------------|------------------------|--------------------------|
| 5       | 0.002  | 0.002                       | 0.101                  |                          |
| 6       | 0.002  | 0.002                       | 0.017                  |                          |
| 7       | 0.564  | 0.535                       | 0.372                  |                          |
| 8       | 0.106  | 0.119                       | 0.178                  |                          |
| 9       | 0.031  | 0.056                       | 0.089                  |                          |
| 10      | 0.279  | 0.243                       | 0.219                  |                          |
| 11      | 0.016  | 0.041                       | 0.021                  |                          |
| 12      | 0.001  | 0.002                       | 0.002                  |                          |
| 13      | 0.567  | 0.656                       | 0.764                  |                          |
| 14      | 0.532  | 0.637                       | 0.764                  |                          |

**vWA**

| Locus   | Allele | Thai tsunami victim (N=834) | U.S. Caucasian (N=302) | African American (N=258) |
|---------|--------|-----------------------------|------------------------|--------------------------|
| 12      | 0.001  | 0.002                       | 0.008                  |                          |
| 13      | 0.001  | 0.002                       | 0.008                  |                          |
| 14      | 0.215  | 0.094                       | 0.078                  |                          |
| 15      | 0.444  | 0.111                       | 0.186                  |                          |
| 16      | 0.151  | 0.200                       | 0.248                  |                          |
| 17      | 0.274  | 0.281                       | 0.242                  |                          |
| 18      | 0.198  | 0.200                       | 0.155                  |                          |
| 19      | 0.095  | 0.104                       | 0.062                  |                          |
| 20      | 0.020  | 0.005                       | 0.016                  |                          |
| 21      | 0.001  | 0.002                       | 0.004                  |                          |
| 22      | 0.001  | 0.002                       | 0.002                  |                          |
| 23      | 0.812  | 0.841                       | 0.802                  |                          |
| 24      | 0.777  | 0.810                       | 0.813                  |                          |
| Locus  | Allele | Thai tsunami victim (N=834) | Caucasian (N=302) | African (N=258) |
|--------|--------|-----------------------------|-------------------|-----------------|
| D13S317 | 8      | 0.287                       | 0.113             | 0.033           |
|        | 9      | 0.125                       | 0.075             | 0.033           |
|        | 10     | 0.099                       | 0.051             | 0.023           |
|        | 11     | 0.231                       | 0.339             | 0.306           |
|        | 12     | 0.183                       | 0.248             | 0.424           |
|        | 13     | 0.057                       | 0.124             | 0.145           |
|        | 14     | 0.016                       | 0.048             | 0.035           |
|        | 15     | –                            | 0.002             | –               |
|        | Ho     | 0.785                       | 0.745             | 0.690           |
|        | He     | 0.774                       | 0.786             | 0.702           |
| D16S539 | 8      | 0.010                       | 0.018             | 0.039           |
|        | 9      | 0.188                       | 0.113             | 0.196           |
|        | 10     | 0.103                       | 0.056             | 0.116           |
|        | 11     | 0.319                       | 0.321             | 0.318           |
|        | 12     | 0.252                       | 0.326             | 0.196           |
|        | 13     | 0.111                       | 0.146             | 0.118           |
|        | 14     | 0.016                       | 0.020             | 0.017           |
|        | 15     | 0.001                       | –                 | –               |
|        | 16     | 0.001                       | –                 | –               |
|        | Ho     | 0.750                       | 0.735             | 0.783           |
|        | He     | 0.742                       | 0.754             | 0.795           |
| D18S51 | 7      | 0.001                       | –                 | –               |
|        | 9      | 0.003                       | –                 | 0.004           |
|        | 10     | 0.005                       | 0.008             | 0.006           |
|        | 11     | 0.012                       | 0.017             | 0.002           |
|        | 12     | 0.060                       | 0.127             | 0.078           |
|        | 13     | 0.143                       | 0.132             | 0.053           |
|        | 13.2   | 0.003                       | –                 | 0.006           |
|        | 14     | 0.185                       | 0.137             | 0.072           |
|        | 14.2   | 0.005                       | 0.002             | –               |
|        | 15     | 0.230                       | 0.159             | 0.161           |
|        | 15.2   | –                            | –                 | 0.002           |
|        | 16     | 0.156                       | 0.139             | 0.158           |
|        | 17     | 0.080                       | 0.126             | 0.152           |
|        | 18     | 0.045                       | 0.076             | 0.123           |
|        | 19     | 0.029                       | 0.038             | 0.099           |
|        | 20     | 0.009                       | 0.022             | 0.064           |
|        | 21     | 0.017                       | 0.008             | 0.010           |
|        | 21.2   | –                            | –                 | 0.002           |
|        | 22     | 0.013                       | 0.008             | 0.006           |
|        | 23     | 0.004                       | –                 | 0.002           |
|        | 24     | 0.003                       | –                 | 0.002           |
|        | 25     | 0.001                       | –                 | –               |
|        | Ho     | 0.809                       | 0.881             | 0.860           |
|        | He     | 0.839                       | 0.880             | 0.885           |
| D21S11 | 25.2   | –                            | 0.002             | –               |
|        | 26     | –                            | –                 | 0.002           |
|        | 27     | 0.005                       | 0.026             | 0.078           |
Table 6 Continued

| Locus   | Allele | Thai tsunami victim (N=834) | U.S. Caucasian (N=302) | African American (N=258) |
|---------|--------|----------------------------|------------------------|--------------------------|
| D19S433 | 15     | 0.088                      | 0.152                  | 0.078                    |
|         | 15.2   | 0.143                      | 0.035                  | 0.060                    |
|         | 16     | 0.018                      | 0.050                  | 0.004                    |
|         | 16.2   | 0.025                      | 0.015                  | 0.027                    |
|         | 17     | 0.001                      | 0.008                  | –                        |

Table 7 Comparison of Allele Frequencies from Two STR Loci Between the Thai Tsunami Victims and Other Five Reference Populations

| Locus   | Allele | Thai tsunami victim (N=834) | African American (N=167) | U.S. Caucasian (N=152) | Hispanic (N=142) | Chamorro (N=72) | Filipino (N=71) |
|---------|--------|----------------------------|--------------------------|------------------------|------------------|----------------|----------------|
|         | 16     | 0.0149                     | 0.0449                   | 0.0296                 | 0.0176           | 0.0278         | 0.0282         |
| D2S1338 | 17     | 0.1291                     | 0.1018                   | 0.1941                 | 0.2218           | 0.1042         | 0.0775         |
|         | 18     | 0.1005                     | 0.0659                   | 0.0526                 | 0.0423           | 0.0833         | 0.0563         |
|         | 19     | 0.1868                     | 0.1377                   | 0.1447                 | 0.2601           | 0.1875         | 0.2183         |
|         | 20     | 0.1128                     | 0.0630                   | 0.1546                 | 0.1409           | 0.1111         | 0.0775         |
|         | 21     | 0.0396                     | 0.1527                   | 0.1974                 | 0.0106           | 0.0139         | 0.0423         |
|         | 22     | 0.0584                     | 0.1377                   | 0.0296                 | 0.0704           | 0.0972         | 0.0634         |
|         | 23     | 0.1667                     | 0.9880                   | 0.1349                 | 0.1232           | 0.1736         | 0.1338         |
|         | 24     | 0.1245                     | 0.0928                   | 0.1217                 | 0.0609           | 0.1319         | 0.2606         |
|         | 25     | 0.0590                     | 0.0838                   | 0.0954                 | 0.0387           | 0.0556         | 0.0352         |
|         | 26     | 0.0078                     | 0.0210                   | 0.0230                 | 0.0070           | 0.0069         | 0.0070         |
|         | 27     | –                          | –                        | –                      | 0.0069           | –              | –              |
| D19S433 | 9      | 0.0105                     | –                        | 0.0035                 | –                | –              | –              |
|         | 10     | –                          | 0.0150                   | –                      | –                | –              | –              |
|         | 11     | 0.0006                     | 0.0689                   | –                      | 0.0035           | –              | –              |
|         | 11.2   | 0.0025                     | –                        | –                      | –                | –              | –              |
|         | 12     | 0.0524                     | 0.1138                   | 0.1086                 | 0.0563           | 0.0347         | 0.0282         |
|         | 12.2   | 0.0049                     | 0.0808                   | 0.0066                 | 0.0211           | 0.0139         | –              |
|         | 13     | 0.2546                     | 0.2964                   | 0.2828                 | 0.1620           | 0.3542         | 0.2887         |
|         | 13.2   | 0.0456                     | 0.0509                   | 0.0263                 | 0.1092           | 0.0417         | 0.0423         |
|         | 14     | 0.2626                     | 0.1976                   | 0.3355                 | 0.3204           | 0.2292         | 0.1549         |
|         | 14.2   | 0.0875                     | 0.0539                   | 0.0033                 | 0.0458           | 0.0972         | 0.0493         |
|         | 15     | 0.0881                     | 0.0389                   | 0.1349                 | 0.1197           | 0.0903         | 0.1056         |
|         | 15.2   | 0.1430                     | 0.0389                   | 0.0263                 | 0.0810           | 0.0972         | 0.2465         |
|         | 16     | 0.0179                     | 0.0210                   | 0.0428                 | 0.0423           | –              | 0.0141         |
|         | 16.2   | 0.0253                     | 0.0180                   | 0.0263                 | 0.0352           | 0.0139         | 0.0634         |
|         | 17     | 0.0066                     | –                        | –                      | –                | –              | –              |
|         | 17.2   | 0.0024                     | 0.0030                   | 0.0033                 | –                | 0.0278         | 0.0070         |
|         | 18.2   | 0.0012                     | 0.0030                   | 0.0033                 | –                | –              | –              |

Conclusion

As the final note to this report, we are happy to announce the completion of the project after handing all our data to the Thai Tsunami Victims Identification Center (TTVI center), since we are only responsible for the postmortem DNA profiling and the victim identification process. We have learnt recently that
over 200 relatives of the tsunami victims have already found the bodies of their family members, who are indeed identified by the DNA method, including both Thai nationals and foreign tourists from several nations.

Materials and Methods

Sample collection and processing

Bone and tooth specimens were collected by certified forensic scientists and shipped on dry ice to our forensic laboratory. After data entry, each sample was decontaminated and processed according to standard forensic DNA extraction protocols. Tooth marrow tissues were collected and DNA was extracted with commercial kits (DNA IQ™ System, Promega Corp., Madison, USA; QIAamp DNA Micro Kit, Qiagen, Inc., Hilden, Germany).

The bone specimen of approximately 2 g was cut into 5×5×5 mm pieces and washed in 50-mL Falcon tubes with Terg-A-Zyme (an enzyme-active powdered detergent made by Alconox, Inc., New York, USA) just enough to cover the samples. The mixture was sonicated for 30 min, rinsed thoroughly with distilled water, and dried at 56°C in an incubator for more than 2 h. The dried bone pieces were milled in a SPEX CertiPrep 6750 freezer mill (SPEX CertiPrep, Inc., Metuchen, USA). DNA samples extracted from the fine bone powders were concentrated with Microcon YM-100 concentrators (Millipore Corp., Billerica, USA) (1).

DNA amplification and genotyping

For STR analysis, the AmpFLSTR® Identifier® PCR amplification kit (Applied Biosystems, Foster City, USA) was used for PCR amplification and the results were analyzed with the 3730XL DNA Analyzer (Applied Biosystems) and the Genemapper software. For mt-DNA amplification, two pairs of primers specific to the human mt-DNA in the hyper-variable regions were used (HV1 primers: 1F/ctcagggagctcttcgtc and 1R/gggttgatgccatttttaaacag; HV2 primers: 2F/ccacccattgacccaaagc and 2R/tccttgacccacatcct). The mitochondrial data were analyzed with the DNAStar package.

DNA was amplified in a total volume of 25 μL, containing 4.5 μL template DNA, 2.5 μL primers (1 pmol/μL), 2.5 μL dNTP (2.5 mM), 2 μL Mg²⁺ (25 mM), 2.5 μL 10× LA Buffer, 1 μL Taq polymerase (5 U/μL), and 10 μL ddH₂O. The PCR cycles were set as: denaturation at 95°C for 2 min, amplification at 95°C/60°C/72°C for 30 s respectively in a total of 35 cycles, and final extension at 72°C for 10 min. The PCR products were purified with a Millipore 96-well purification plate. Mitochondrial samples were sequenced on the 3730XL DNA Analyzer with Dye Terminator kits (GE Healthcare, USA).

Acknowledgements

We are extremely grateful to Drs. Theresa Caragine and Sheila Estacio Dennis from the Office of the Chief Medical Examiner of the City of New York for their instructive help. We thank the Chinese Academy of Sciences, Ministry of Foreign Affairs, and Chinese Embassy in Thailand for their support on this effort. We also thank the Chinese Academy of Sciences and the Ministry of Commerce for funding this work.

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