Tohoku J. Exp. Med., 2021, **255**, 275-281

Evaluation of the Utility of Mitochondrial DNA Testing in Personal Identification Work in the Great East Japan Earthquake of 2011

Tsukasa Ohuchi,[1] Xueting Guan[1] and Masato Funayama[1]

[1]Department of Forensic Medicine, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan

Although the Great East Japan Earthquake occurred on March 11, 2011, identification of victims is still ongoing. Typically, mitochondrial DNA (mtDNA) is performed when it is difficult to identify an individual using nuclear DNA. In Japan, samples from criminal investigations are subjected to nuclear DNA testing at the Scientific Research Institute belonging to each prefectural police headquarters, while all mtDNA tests were originally conducted at the National Research Institute of Police Science. However, the appraisal work using mtDNA became more time-consuming as the number of target samples increased. Because our department is capable of performing mtDNA testing, the Miyagi Prefectural Police requested that our department perform mtDNA testing. Specifically, we focused on 16 individuals as putative candidates for 11 unidentified human remains; efforts to identify these remains were performed using samples from 20 relatives. These efforts positively identified six victims. This included confirmation that one corpse had originally been identified incorrectly. Although disasters of a similar scale can strike Japan again, there are limited facilities that can consistently perform mtDNA testing. Expensive sequencing machines and properly trained operators are essential for mtDNA testing, but they cannot be established at the forensic departments of all medical schools. There is thus an urgent need to establish core facilities at appropriate sites, such as Tohoku University in the Tohoku Region, to build a mtDNA testing system suitable for the aftermath of any disaster.

Keywords: disaster victim identification; forensic DNA sample; Great East Japan Earthquake; mitochondrial DNA testing; tsunami victim
Tohoku J. Exp. Med., 2021 December, **255** (4), 275-281.

Introduction

The Great East Japan Earthquake struck on March 11, 2011, with the resulting tsunami causing enormous damage and loss of life. More than 15,000 people were killed and over 2,500 people were still missing as of March 2021, 10 years later (National Police Agency of Japan 2021). The condition of human remains found in disaster-hit areas, including in the sea, varies depending on the postmortem interval and physical damage. Human remains can be identified using information such as belongings, facial features, dental records, fingerprints, and DNA (National Police Agency of Japan 2012a; Kuroda et al. 2017; Numata et al. 2017). DNA is particularly useful as a sample for identification purposes that can be obtained from human remains that have undergone substantial deterioration postmortem. If typical individual sources of DNA, such as a toothbrush, have been lost because of the tsunami, identification can be achieved using DNA by finding matches between victims and their surviving family members. Forensic DNA analysis is divided into the testing of nuclear DNA and mitochondrial DNA (mtDNA). In general, nuclear DNA has higher discriminatory power and is therefore prioritized, while mtDNA is used when nuclear DNA is significantly degraded or when the maternal line is being analyzed.

In Japan, samples related to criminal cases are usually subjected to nuclear DNA testing at the specific Scientific Research Institute belonging to each prefectural police headquarters, but all mtDNA testing has only been performed at the National Research Institute of Police Science. Nuclear DNA tests for personal identification are also performed at the Scientific Research Institute, even if they are

Received June 29, 2021; revised and accepted August 31, 2021. Published online December 9, 2021; doi: 10.1620/tjem.255.275.
Correspondence: Tsukasa Ohuchi, Department of Forensic Medicine, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8575, Japan.
e-mail: ouchi@forensic.med.tohoku.ac.jp

not associated with a criminal case. This was also the case for DNA testing aimed at identifying the victims of the 2011 earthquake. However, as time passed after the earthquake, the amount of unidentified putrefactive human remains with little to no identifying information increased, as did the number of samples that could not be identified by nuclear DNA testing. As mentioned above, in Japan, the National Research Institute of Police Science conducts mtDNA tests, with priority given to criminal cases. Initially, efforts to identify human remains from the earthquake using mtDNA were performed without delay, but as the number of mtDNA samples increased, it took longer to obtain the test results. Under these circumstances, the Miyagi Prefectural Police requested that our department, the Department of Forensic Medicine, Tohoku University Graduate School of Medicine, perform mtDNA testing, which we agreed to do free of charge. To date, we have tested 11 unidentified human remains along with 20 samples from bereaved family members that are associated with 16 putative victims. In this report, we analyze and summarize the results of these mtDNA tests, and consider how to construct an optimal mtDNA testing system in Japan that would function in the event of a similar disaster striking again.

Materials and Methods

In this section, we describe the status of the 11 unidentified samples in storage, the list of candidates for these samples (16 in total, since there are several candidates for 4 samples), and the analysis method. Table 1 provides brief information on the materials of the unidentified victims. Table 2 presents missing individuals who were putative candidate sources of the human remains and relatives of these individuals.

Materials of unidentified victims

Between August 2014 and February 2021, mtDNA tests were performed on samples (blood, muscle, nails, and bones) collected from 11 unidentified sets of human remains (Table 1). The most common samples collected from the human remains were bones (five cases: No. 1-5), followed by nails (four cases: No. 6-9), blood (one case: No. 10), and muscle (one case: No. 11). The latter two samples (No. 10 and No. 11) were obtained from victims found shortly after the disaster and were stored as is, and these two were used to confirm that the bodies had been mishandled.

Putative victims

A “putative victim” here refers to an individual who remains missing and is yet to be identified within a particular geographical area including sites where human remains had been found, in accordance with comments from the bereaved family about where the individuals were last seen alive and other physical characteristics such as physique, hair color, and moles. Sixteen people were selected as putative victims (Table 2). Twelve were older women over the age of 60.

Relatives of the putative victims

Twenty samples were collected from people related to the 16 putative victims (Table 2). In terms of their relationships with the putative victims, they were most commonly their nephews and nieces, followed by their children and siblings. All samples, other than those from two relatives who were already deceased, were provided as mucosal cells from the oral cavity. One of the two samples obtained from an already deceased person was blood, which was taken shortly after the disaster and stored by the police. This sample is indicated as the child of “N” in Table 2 and was used to re-examine previously examined human remains, given that incorrect identification had been shown to have occurred.

The other case in which saliva could not be collected from a living person is as follows. The parents of the putative victim (“C” in Table 2) had already died, and she had no children or living matrilineal relatives. An additional sample from a deceased relative was obtained from a camera strap. Specifically, this strap used by this person’s great aunt’s son (already deceased) was provided as a reference. Unfortunately, the sample appeared to contain a mixture of mtDNAs, so it was difficult to compare maternal kinship using this sample.

Initial personal identification error and subsequent correct judgment: two special cases

Two months after the earthquake, charred human remains (No. 11) were found near a burned car. Because of the burning and postmortem changes, blood could not be collected. Exposed muscle was collected instead. In addition, the corpses of one adult male and one adult female were found in the car. According to the testimony of two survivors who had been in the car but were thrown out of it during the tsunami, six people were initially in this car attempting to escape the tsunami. At the time victim No. 11 was found, among the six people associated with the car, there were these two survivors, the two adult victims who were identified, and two others who were missing. The relationship between these two is as follows: a woman in her 50s (“N” in Table 2) and a woman in her teens (“O” in Table 2), who are grandmother and granddaughter. The police originally certified that the burned remains (No. 11) were of putative person “N” (see Table 2) without performing DNA testing because of the observed physique, and they were returned to the bereaved family. The two corpses found in the car were identified only by their faces, although blood samples were collected from them and stored.

However, a middle-aged female corpse (No. 10) remained unidentified for 6 years, even though she was found shortly after the tsunami. After the police re-examined the case in the above context, the following possibilities emerged: There may have been an error in determining the identity of victim No. 11 and victim No. 10 may be "N." Thus, further verification work was carried out using the stored blood sample of victim No. 10. "N" had one child who had died in the car, a blood sample of whom had been stored as mentioned above. Because only a few short tandem repeat (STR) loci were detected in nuclear DNA testing because of postmortem changes, mtDNA testing was performed and victim No. 10 was confirmed to be person "N" (see Fig. 1).

Analysis

DNA was extracted from blood, muscle, and nail samples using the QIAamp DNA Investigator Kit (Qiagen, Venlo, the Netherlands). The bone was washed and pulverized using a multi-bead shocker (Yasui Instruments, Osaka, Japan), decalcified overnight at 56°C, and then DNA was extracted using this kit. For hypervariable region 1 (HV1, positions 16,024 to 16,365) of mtDNA, the primers described by Hashiyada et al. (2002) were used. For hypervariable region 2 (HV2, positions 73 to 340), the primer sequences of Gabriel et al. (2001) were added with the same universal sequence as those of the hypervariable region 1 primers.

PCR amplification was performed using AmpliTaq Gold™ 360 Master Mix and a Veriti® Thermal Cycler (both Thermo Fisher Scientific, Waltham, MA, USA). The PCR conditions were as follows: one cycle of 95C for 5 min, followed by 40 cycles of 95°C for 15 s, 50°C for 30 s, and 72°C for 30 s, and ending with a final step at 72°C for 7 min. The amplified product was confirmed by electrophoresis on a 1.2% agarose gel and purified with the MinElute® PCR Purification Kit (Qiagen).

A cycle sequencing reaction was performed using the primers of Hashiyada et al. (2002), the purified amplification product as a template, and the BigDye™ Terminator v1.1 Cycle Sequencing Kit (Thermo Fisher Scientific). Then, the product was purified using BigDye™, XTerminator™ Purification Kit (Thermo Fisher Scientific), and electrophoresed on a 3500 Genetic Analyzer (Thermo Fisher Scientific). The nucleotide sequence was obtained with Sequencing Analysis Software v5.4 (Thermo Fisher Scientific).

If the sequences of the victim and bereaved family member(s) matched, it was defined as positive identification.

Ethical approval

This study was approved by the Ethics Committee of Tohoku University Graduate School of Medicine (No. 2020-1-1202).

Results

Positive identification

The mtDNA sequences could not be detected in one bone sample (No. 5) because it had been severely burned (Table 1). Of the 10 unidentified samples, excluding victim No. 5, six were compared with each potential family member, leading to the positive identification of three victims (No. 2, No. 6, and No. 7) (Table 2). In the other three samples, mtDNA from two family members was tested and identified as having the same maternal lineage as one of the unidentified individuals. Of these, two samples (No. 10 and No. 11) were found to have initially been incorrectly identified (as discussed in the next section) (Fig. 1). For the remaining sample (No. 9), three people were considered as candidate putative victims, but none of them was identified upon comparison with the mtDNA test results from a matrilineal bereaved family member (Table 2). Therefore, overall, six victims were positively identified.

Confirmation of the two special cases

A mother and her child were in the burned car mentioned above (six people got in it and tried to flee). The child was found dead and has already been identified. The mother, in contrast, was considered victim #11. However, the results of the testing on victim No. 10 revealed that both were of the same maternal lineage, and that "N" was not victim No. 11 but rather victim No. 10 (Fig. 1). In this case, mtDNA testing was also performed on the relatives (brother and two aunts) of another missing person (a woman, "M") at the same time, for which the existence of a maternal relationship was ruled out.

A question thus remained about the identity of victim No. 11. In this context, a middle-aged woman ("P") who was still missing and had a surviving child was proposed as a candidate. DNA testing was carried out, but postmortem changes meant that only a few STR types could be detected by nuclear DNA testing in the muscle samples of victim No. 11. Therefore, mtDNA testing was performed. The results revealed that the woman and child were of the same maternal lineage, resulting in the positive identification of victim No. 11 as "P" (Fig. 1 and Table 2).

Additionally, the police analyzed another sample when performing mtDNA testing of victim No. 11. This sample was from the mother of "O" (a survivor), which helped completely rule out that victim No. 11 was "O." It was clear that the human remains of victim No. 11 were not those of a teenage girl, but the police wanted to confirm this using a DNA test. The results of mtDNA testing showed a clear difference in maternal lineage. Fig. 1 indicates the case involving initially incorrect identification between victims No. 10 and No. 11.

Discussion

Earthquakes and tsunamis are classified as "open disasters," in which the number and identity of all victims are unclear, and it is difficult to compile a complete list of missing persons shortly after the disaster (Utsuno 2019). In addition, the difficulty of identification increases as postmortem changes progress. After the Great East Japan Earthquake and associated tsunami, blood samples were

Table 1. Materials of victims and results of mtDNA typing.

Victim No.	Material	Period from disaster date to request date	Substitutions in HV1 and HV2
1	Bone	3y 8m	16187 C > T*1, 16223 C > T, 16290 C > T, 16319 G > A, 73 A > G, 235 A > G, 263 A > G, 309.1 C*2, 315.1 C
2	Bone	3y 8m	16223 C > T, 16325 T > C, 16362 T > C, 73A > G, 150 C > T, 263 A > G, 315.1 C
3	Bone	8y 2m	16182 A > C, 16183 A > C, 16189 T > C, 16223 C > T, 16398 G > A, 73 A > G, 185 G > A, 263 A > G, 309.1 C, 309.2 C, 315.1 C
4	Bone	9y 10m	16223 C > T, 16311 T > C, 16362 T > C, 73 A > G, 263 A > G, 309.1 C, 315.1 C
5	Bone	9y 11m	Not detected because it was severely burnt
6	Nail	3y 11m	16209 T > C, 16223 C > T, 16291 C > T, 16324 T > C, 73 A > G, 263 A > G, 315.1 C
7	Nail	9y 1m	16111 C > T, 16140 T > C, 16173 C > T, 16182 A > C, 16183 A > C, 16189 T > C, 16234 C > T, 16243 T > C, 16291 C > T, 73 A > G, 131 T > C, 204 T > C, 263 A > G, 309.1 C, 315.1 C
8	Nail	9y 4m	16136 T > C, 16183 A > C, 16189 T > C, 16217 T > C, 16284 A > G, 73 A > G, 199 T > C, 202 A > G, 207 G > A, 263 A > G, 309.1 C, 309.2 C, 315.1 C
9	Nail	9y 9m	16223 C > T, 16278 C > T, 16362 T > C, 73 A > G, 263 A > G, 315.1 C
10*3	Blood	6y 6m	16129 G > A, 16153 G >A, 16172 T > C, 16223 C > T, 16362 T > C, 73 A > G, 146 T > C, 152 T > C, 263 A > G, 309.1 C, 315.1 C
11*4	Muscle	6y 7m	16209 T > C, 16223 C > T, 16291 C > T, 16324 T > C, 73 A > G, 263 A > G, 315.1 C

*1Cytosine, the 16,187th base in the Revised Cambridge Reference Sequence (rCRS) (Andrews et al. 1999), was replaced with thymine.
*2Insertion of cytosine between the 309th base and the 310th base in rCRS (Andrews et al. 1999).
*3Despite its early discovery, it remained unidentified for 6 years.
*4Victim No. 11 was initially errorneously identified as person N (see Fig. 1 and Table 2) without DNA typing, but it turned out to be a mistake.

collected from human remains, such as from corpses discovered soon after the disaster, for the purpose of DNA testing. However, it became increasingly difficult over time to collect blood, so nails or bones were targeted for collection. These samples have been retained by the police.

When DNA was used to identify disaster victims, analyses targeting nuclear DNA (including the Y chromosome) were initially carried out at the Scientific Research Institute belonging to each prefectural police headquarters. However, in some cases, it was difficult to achieve reliable verification by comparison with living relatives because all family members had died. In other cases, it became impossible to collect DNA from personal items such as toothbrushes because the individuals' houses had been washed away by the tsunami. Another persistent problem was how nuclear DNA decays and degrades over time. When that occurs, mtDNA testing is often carried out instead of nuclear DNA testing, but the only police organization responsible for this type of testing is the National Research Institute of Police Science. However, this Institute is mainly involved in analyzing samples from criminal investigations, in principle, not confirming the identities of disaster victims. This unprecedented catastrophe led to this Institute being involved in the identification of tsunami victims. However, such identification work became increasingly time-consuming as the number of target samples increased. From this background, Miyagi Prefectural Police requested our group, the Department of Forensic Medicine, Graduate School of Medicine at Tohoku University, to carry out mtDNA testing.

Because there are hundreds to thousands of copies of mtDNA in a single cell, it can be detected even in samples that have undergone significant deterioration. This is in strong contrast to nuclear DNA, which only has two copies per cell. Therefore, in forensic practice, mtDNA testing is generally reserved for samples (e.g., bones and teeth) that are so highly degraded that nuclear DNA cannot be detected. Among the five bone samples described here, in one case (No. 5), the sequencing was unsuccessful. Here, the sample was obtained from a corpse found in a burned car, and the sample itself was severely burned.

In this appraisal work to identify human remains from the disaster, 12 out of 16 putative victims (75%) were over 60 years old, which is reasonable because about 70% of the tsunami victims were in this age group (National Police Agency of Japan 2012b). Of these 12 elderly victims, 10 were women. In the three prefectures of Iwate, Miyagi, and Fukushima, which suffered severe tsunami damage, the death toll included 8,363 women and 7,360 men (National Police Agency of Japan 2012b). As such, many women were clearly victims, but this is not the only reason for the sex observed here. For males, comparisons with paternal relatives were performed targeting the Y chromosome (17 types of Y-STR polymorphisms). The discriminatory power for men is believed to be increased by adding some Y-STR types. However, in certain cases, male victims may not have living paternal relatives. For the two male victims who had undergone mtDNA testing (No. 1 and No. 4), their

Table 2. Putative victims, information on expected family members, and results of mtDNA typing.

Victim No.	Putative victim as source of the material (age, sex)	Relationship of putative victim with the expected family members	Substitutions in HV1 and HV2	Identification	Remarks
1	A (early 90s, M)	Nephew (sister's child)	16223 C > T[*1], 16324 C > T, 16316 A > G, 16362 T > C, 73A > G, 263 A > G, 309.1 C[*2], 315.1 C	Negative	Putative victim's parents were dead and his child was adopted.
2	B (early 60s, F)	Son	16223 C > T, 16325 T > C, 16362 T > C, 73A > G, 150 C > T, 263 A > G, 315.1 C	Positive	In bone material, only a few short tandam repeat types were detected.
3	C (late 60s, F)	Great aunt's son[*3]	Multiple types of mtDNA were detected from the strap of a camera.	Difficult to match	Putative victim's parents were dead. She had no children and no living matrilineal relatives.
4	D (late 80s, M)	Two nephews (sister's children)	16245 C > T, 16362 T > C, 73 A > G, 191.1 C, 194 C > T, 199 T > C, 207 G > A, 309.1 C, 315.1 C	Negative	Putative victim's parents were dead and his child was adopted.
5	E (early 70s, F)	Daughter	16223 C > T, 16355 C > A, 16362 T > C, 73 A > G, 263 A > G, 315.1 C	Difficult to match[*4]	Bone material was severely burned.
6	F (late 50s, F)	Brother	16209 T > C, 16223 C > T, 16291 C > T, 16324 T > C, 73 A > G, 263 A > G, 315.1 C	Positive	Putative victim's father is missing and her mother was dead. She had no children.
7	G (late 90s, F)	Nephew (younger sister's child)	16111 C > T, 16140 T > C, 16173 C > T, 16182 A > C, 16183 A > C, 16189 T > C, 16234 C > T, 16243 T > C, 16291 C > T, 73 A > G, 131 T > C, 204 T > C, 263 A > G, 309.1 C, 315.1 C	Positive	Putative victim's parents were dead and her child was adopted.
8	H (late 80s, F)	Two nephews (sister's children)	16136 T > C, 16183 A > C, 16189 T > C, 16217 T > C, 16284 A > G, 73 A > G, 199 T > C, 202 A > G, 207 G > A, 263 A > G, 309.1 C, 309.2 C, 315.1 C	Positive	Putative victim's father is missing and her mother was dead. She had no children.
	I (early 90s, F)	Nephew (sister's son)	16092 T > C, 16102 T > C, 16164 A > G, 16182 A > C, 16183 A > C, 16189 T > C, 16223 C > T, 16266 C > T, 16362 T > C, 73 A > G, 150 C > T, 263 A > G, 309.1 C, 309.2 C, 315.1 C, 328 A > G	Negative	Putative victim's parents were dead and his child was adopted.
9	J (late 80s, F)	Cousin	16048 G > A, 16187 C > T, 16223 C > T, 16290 C > T, 16319 G > A, 73 A > G, 235 A > G, 263 A > G, 309.1 C, 309.2 C, 315.1 C	Negative	Putative victim's parents were dead. She had no children.
	K (late 70s, F)	Brother (half-brother)	16136 T > C, 16183 A > C, 16189 T > C, 16217 T > C, 16284 A > G, 73 A > G, 146 T > C, 199 T > C, 202 A > G, 207 G > A, 263 A > G, 309.1 C, 309.2 C, 315.1 C	Negative	Putative victim's parents were dead. She had no children. Three younger brothers (half-brothers) are living and mtDNA was compared with one of them.
	L (late 70s, F)	Sister (half-sister)	16189 T > C, 16223 C > T, 16254 A > G, 16362 T > C, 73 A > G, 227 A > G, 263 A > G, 315.1 C	Negative	Putative victim's parents were dead. She had no children. Biological sister was dead. Biological brother died in the earthquake and his sample was not collected.
10	M (early 30s, F)	Brother and two aunts	16223 C > T, 16227 A > G, 16234 C > T, 16278 C > T, 16362 T > C, 73 A > G, 152 T > C, 200 A > G, 263 A > G, 315.1 C	Negative	Case related to incorrect identification (see results).
	N (late 50s, F)[*5]	Child[*6]	16129 G > A, 16153 G > A, 16172 T > C, 16223 C > T, 16362 T > C, 73 A > G, 146 T > C, 152 T > C, 263 A > G, 309.1 C, 315.1 C	Positive	
11	O (early 10s, F)[*5]	Mother	16129 G > A, 16223 C > T, 16362 T > C, 73 A > G, 263 A > G, 315.1 C	Negative	Case related to incorrect identification (see results). Initially, the police had determined victim No. 11 to be person N, but this was not accurate. In the muscle material, only a few STR types were detected.
	P (early 60s, F)[*7]	Child	16209 T > C, 16223 C > T, 16291 C > T, 16324 T > C, 73 A > G, 263 A > G, 315.1 C	Positive	

[*1]Cytosine, the 16,223rd base in the Revised Cambridge Reference Sequence (rCRS) (Andrews et al. 1999), was replaced with thymine.
[*2]Insertion of cytosine between the 309th base and the 310th base in rCRS (Andrews et al. 1999).
[*3]All expected family members had already died. A possession that victim No. 3's putative relative used when he was alive was tested.
[*4]No mtDNA was detected from the burned bone material.
[*5]Person N and person O had been in the same car attempting to escape the tsunami. They were still missing.
[*6]A blood sample was collected from the corpse found in a burned car after the tsunami and stored.
[*7]It was later discovered that victim No. 11 was not person N, she emerged as a new putative victim.

parents had already passed away and they had no children. In addition, there were some cases in which there was no suitable bereaved family members, mainly because the victims were elderly. Case No. 3 was such an example, and a camera strap belonging to the son of the potential bereaved great aunt had to be used as a test sample. However, the results suggested that the sample contained a mixture of mtDNAs, so the identification failed. This demonstrates the difficulty in obtaining meaningful DNA analysis results from an object because the item may not necessarily have been used exclusively by the person just because it was that person's property. Although mtDNA testing has less discriminatory power than nuclear DNA testing, the list of missing persons from the area surrounding the location where the corpses had been found helped narrow down their identities. In addition, nuclear DNA types were taken into account if partial results on them had been obtained. If the sample's mtDNA matched the putative victim's mtDNA and there were no other putative victims, then the case was defined as having been positively identified and the remains were handed over to the bereaved family.

The ability of mtDNA testing to rule out maternal kinship is important in that it prevents incorrect identification of human remains. Even if the face has not suffered severe damage and multiple bereaved family members recognize the corpse as a member of their family, this identification can be wrong. In addition to a lack of lighting in the morgue, such an error may arise from the emotional state brought on by the sudden death of a relative or the strong desire to leave the morgue and return home immediately.

The mtDNA testing conducted in our laboratory revealed one misidentification. Details are given in the results section above. mtDNA testing revealed that one of the allegedly identified victims was actually someone else.

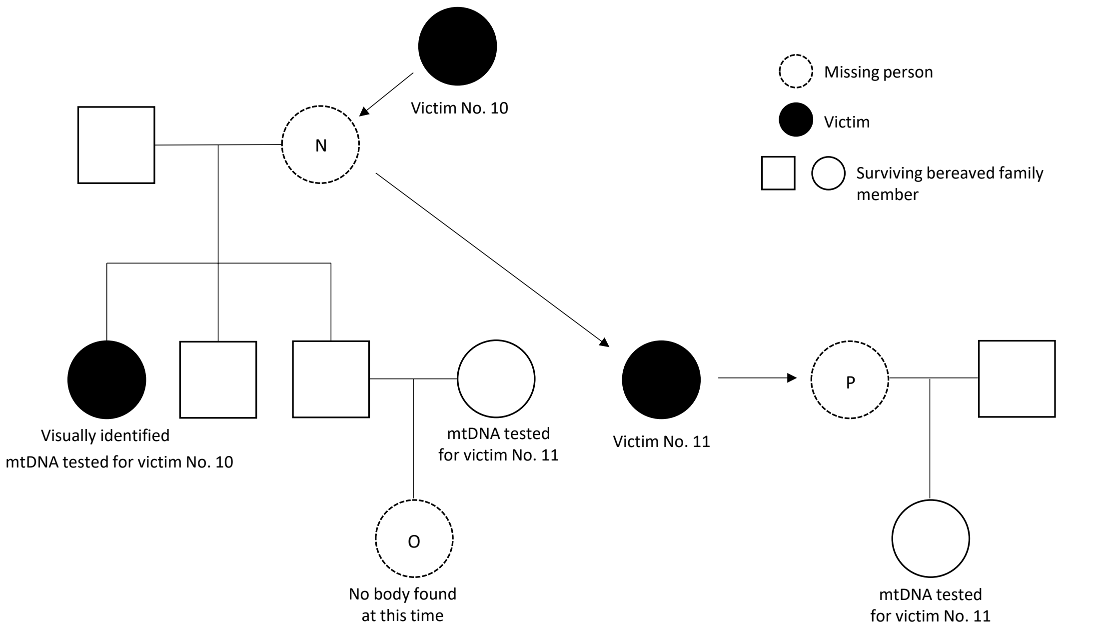

Fig. 1. Relationship between victims No. 10 and No. 11.
Squares and circles show males and females, respectively. Victim No. 11 was erroneously identified as person N before DNA testing, but mtDNA testing revealed that victim No. 11 was person P and victim No. 10 was person N (see Table 2).

As a result, it was determined that an individual who had remained unidentified for a long time was actually the first person to be misidentified. A stored blood sample of victim No. 10 and muscle of victim No. 11 were used for the testing that revealed this identification process. In general, for a corpse found shortly after the disaster, even if the testimonies of multiple bereaved family members have confirmed the identity of the corpse, DNA samples may be used later to identify the corpse of another relative. Such samples are also useful in the unlikely event of an error in personal identification.

Because the tsunami damaged houses and killed many related individuals, reference samples could often only be provided by distant relatives. Information on biological connections, including potentially sensitive issues such as adoption and half-siblings, was provided for assistance. In particular, adoption is often known only to certain relatives within a family. In addition, when the candidates were elderly, it was often difficult to reach them or obtain relevant material. It is crucial to obtain accurate information from the relatives of missing persons to aid in identification. Therefore, police involved with collecting such information should have at least a basic knowledge of DNA testing, including appropriate sample sites and sizes of sample materials suitable for testing. This should include a knowledge of mtDNA testing so that samples can be properly selected for comparison with living or deceased relatives if needed.

As mentioned above, Miyagi Prefectural Police asked our institute to perform mtDNA tests to reduce the burden on the police. All inspection work including mtDNA testing related to identifying earthquake/tsunami victims was performed at the university free of charge, as we believe it is the mission of the university in the disaster-stricken prefecture to correctly identify and return the bodies to the bereaved families as soon as possible.

Currently, forensic schools at many universities in Japan do not have a system in place for performing mtDNA tests. Although a disaster on a scale similar to the Great East Japan Earthquake could occur in the future, facilities that can consistently perform mtDNA testing are limited. In eastern Japan, only one facility in Hokkaido, two facilities in Tohoku, and a few universities in Kanto can perform this analysis. Because police agencies in each prefecture cannot carry out mtDNA testing, there is an urgent need to build an mtDNA testing system that would be ready to perform personal identification work within a short period of time in Japan. Expensive sequencing machines and properly trained operators are essential for mtDNA testing, but they are not currently available in the forensic departments of all Japanese medical schools. Therefore, there appears to be an urgent need to establish core facilities within particular areas, such as Tohoku University in the Tohoku Region, to build a mtDNA testing system suitable for the aftermath of any disaster.

Acknowledgments

We thank Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

Conflict of Interest

The authors declare no conflict of interest.

References

Andrews, R.M., Kubacka, I., Chinnery, P.F., Lightowlers, R.N., Turnbull, D.M. & Howell, N. (1999) Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nat. Genet.*, **23**, 147.

Gabriel, M.N., Huffine, E.F., Ryan, J.H., Holland, M.M. & Parsons, T.J. (2001) Improved MtDNA sequence analysis of forensic remains using a "mini-primer set" amplification strategy. *J. Forensic Sci.*, **46**, 247-253.

Hashiyada, M., Funayama, M., Nata, M., Mimasaka, S. & Adachi, N. (2002) Discrimination of skeletal remains using mitochondrial DNA. *Acta Crim. Japon.*, **68**, 129-134.

Kuroda, H., Inoue, K., Takayama, S. & Ishii, T. (2017) A victim of the Great East Japan Earthquake identified with the preserved medical samples of her deceased mother. *Tohoku J. Exp. Med.*, **242**, 247-249.

National Police Agency of Japan (2012a) Identification status of unidentified bodies in the Great East Japan Earthquake May 11, 2012.
https://www.npa.go.jp/hakusyo/h24/toukei/00/0-05.xls
[*Accessed*: March 24, 2021].

National Police Agency of Japan (2012b) Causes of death of the dead in the Great East Japan Earthquake March 11, 2012.
https://www.npa.go.jp/hakusyo/h24/toukei/00/0-04.xls
[*Accessed*: March 24, 2021].

National Police Agency of Japan (2021) Damage situation and police countermeasures associated with 2011 Tohoku district - off the Pacific Ocean earthquake March 10, 2021.
https://www.npa.go.jp/news/other/earthquake2011/pdf/higai-jokyo.pdf
[*Accessed*: March 24, 2021].

Numata, N., Makinae, H., Yoshida, W., Daimon, M. & Murakami, H. (2017) Disaster victim identification using orthopedic implants in the 2011 East-Japan Earthquake and Tsunami. *Tohoku J. Exp. Med.*, **241**, 219-223.

Utsuno, H. (2019) Victim identification in large-scale disasters using dental findings. *IATSS Res.,* **43**, 90-96.