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Contents

Review Articles

Original Research Papers

Case Reports

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Sequence, Haplotype, and Ancestry: Using the Mitochondrial DNA Hypervariable Region to Predict Forensic "Race"

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Introduction

In forensic casework, DNA is primarily used for the individuation of evidence, or the identification of remains. This is particularly true for autosomal DNA (auDNA), which can be used in a positive identification, but is generally the case for Y-chromosome (yDNA) or mitochondrial DNA (mtDNA) as well (Edson and Christensen this volume). While variation in yDNA and mtDNA actually only places an individual within one or more particular groups of paternally or maternally related individuals, it is used in conjunction with other circumstantial evidence (such as a passenger manifest) to determine individual identity. But what about when individuation is not possible, either because references are not available for comparison or because no victim names are known? How well can DNA be used to assign an individual to a population group?

Several recent studies have evaluated the degree to which genetic data correlates with ancestry and therefore its predictive value for "race" and/or "ethnicity" (e.g., Lao et al. 2010; Royal et al. 2010). This chapter focuses on mtDNA, as it is the type most frequently sequenced from skeletal remains, due to its high copy number and good preservation. Because mtDNA is a nonrecombining, uniparentally inherited genome, correlating it with individual ancestry raises a different, if related, set of issues from those posed by autosomal DNA.

A given mtDNA haplotype can be assigned to a particular ancestral group using either a phylogenetic approach (e.g., Lao et al. 2010) or a computational approach (e.g., Egeland et al. 2004). The first classifies each sequence into a broad haplogroup and follows a simple chart or map to determine with which continental population that haplogroup is associated. This approach is often used in studies of population history and admixture (e.g., Stefflova at al. 2009). The second is a statistical model, independent of phylogeny: How similar is this sequence to each different sequence in the reference populations and to which population does it show the greatest affinity? One such study achieved close to 90% accuracy in prediction of what was referred to as "coarse ethnic group"—that is, Caucasian, African, Asian, and Hispanic (Lee, Măndoiu, and Nelson 2011).

This chapter takes a step back from these approaches and breaks the problem down into three independent questions that must be addressed in any forensic case:

- How accurately can a given sequence be assigned to a specific haplogroup?
- How tightly defined is the ancestral population with which that haplogroup is associated?
- What is the correlation between that ancestral population and the circumstances of the particular forensic case?

Sometimes, these questions will allow a given case to be placed within a forensically useful ancestral category with a fair degree of certainty; sometimes they will not. This chapter will consider the three questions in order, and then specifically examine

mtDNA haplogroups A, B, C, and D, which are of particular interest for the casework of the Joint POW/MIA Accounting Command—Central Identification Laboratory (JPAC-CIL). Each of these haplogroups can be found in both Native Americans and East Asians, and the distinction between these two groups is often of forensic relevance in CIL cases. Finally, two case studies will be considered.

Illustrative examples are drawn from CIL casework, which consists primarily of skeletal remains believed to be associated with US casualties from World War II, the Korean War, and the Vietnam War. For CIL military casework, mtDNA analyses are conducted by the Armed Forces DNA Identification Laboratory (AFDIL), and the frequency of each mtDNA sequence is reported against its casework population database (CPD). The CPD supplements the original Scientific Working Group for DNA Methods reference database of 4,839 individuals (Monson et al. 2002) with additional sequences from AFDIL's own analyses for a total of 10,428, sorted into forensically relevant subsamples. For identification purposes, AFDIL compares evidence sequences to conflict-specific databases of family reference samples (FRSs)—that is, samples taken from maternal relatives of casualties that provide the mtDNA sequences that those casualties can be expected to have had.

Throughout these examples, matches to casualty references will be listed under the racial classifications used in those service members' original files. Individuals who might now be classed as Hispanic, for instance, might be listed as Mexican, Puerto Rican, or White; in the latter case, Hispanic surnames are noted. The terms used in the records vary over time and between individuals, and include some that are inappropriate at best to modern ears, including several casualties from the Korean War classified as "Mongolian," whose actual ancestors came from China, Japan, and Korea, and others classified as "Malayan" who appear to be of Filipino, Chamorro, or Native Hawaiian ancestry.

Hypervariable Region Sequence and Phylogeny

Because mtDNA mutates without recombination, haplotypes can all be placed within a single phylogeny based upon their evolutionary relationships (Figure 15.1). Clades within this phylogeny are called haplogroups. Early studies of mtDNA variation at the population level (e.g., Torroni et al. 1993) defined haplogroups based on restriction fragment length polymorphisms (RFLPs) and assigned them letter designations. As work progressed, it was discovered that some of these lettered haplogroups nested within others, and an increasing use was made of sequence data rather than RFLPs (Macaulay et al. 1999; Ingman and Gyllensten 2001). In the current system, the letter designations are preserved for historical continuity, but new clades are designated with a series of numbers and letters within the original haplogroups, such as B4a1a1a or D4b1a2a1b (van Oven and Kayser 2009). The current phylogeny is based upon extensive sequencing of entire mtDNA genomes, and its broad outlines are unlikely to change (Behar et al. 2012).

While haplogroups have been the focus of population genetics studies, forensic analyses have individuation as their focus. As a result, forensic laboratories generally sequence the hypervariable region (HVR) and, occasionally, the broader control region (CR). The high mutation rate across the HVR makes it ideal for individuation, but also means that a particular mutation may well have occurred multiple times and therefore be of limited phylogenetic significance (Behar et al. 2007). Individuals belonging to different haplogroups and exhibiting different polymorphisms elsewhere in their mitochondrial genome may bear the same sequence in the HVR. Some polymorphisms in the HVR are more stable and are associated with particular haplogroups, but most haplogroups are defined on the basis of coding region polymorphisms that are not sequenced in standard forensic casework. In some cases, forensic individuation may require analysis of such single-nucleotide polymorphisms (SNPs) in the CR to differentiate between otherwise identical HVR sequences (e.g., Asari et al. 2007; Just et al. 2009).

Table 1 lists the basic HVR motifs associated with each lettered haplogroup. To determine in more detail what haplogroup a particular HVR sequence might belong to, several references are available. The current master phylogeny is available at http://www.phylotree.org (van Oven and Kayser 2009; references in this chapter are to build 14, dated April 5, 2012) and http://www.mtdnacommunity.org (Behar et al. 2012). Individual polymorphisms may be searched against these charts to locate where a given sequence might fall. mtDNAmanager (http://mtmanager.yonsei.ac.kr; Lee et al. 2008) provides a web-based application to compare sequence data to published sequences and predict haplogroup assignment, as well as a reference chart of HVR mutation motifs for each haplogroup (http://mtmanager.yonsei.ac.kr/help/MutationMotifs.pdf; references in this chapter are to the version dated October 2–3, 2011). Haplogroup assignment can be double-checked by comparing the sequence data to the set of complete mtDNA haplotypes made available by

mtDNA Community (12,813 as of the April 6, 2012, release).

As a general rule, sequences that exhibit few polymorphisms in comparison to the Cambridge reference sequence (CRS) will be from European twigs of the phylogeny, in H, V, and U. This is because the CRS belongs to haplogroup H2a2a1. In particular, any sequence that does not exhibit a 73G almost certainly belongs either to R0 (including HV, H, and V) or L0, as those are the primary positions on the phylogeny where the original 73A has mutated to G. (The same G–A transition also appears in minor branches L3h1a1 and C4c2, but they are very rare.) Further, the absence of 73G combined with the presence of 72C places a sequence securely in HV0 or its daughter, V. Sequences with 263A are more common, occurring in multiple twigs of the tree, but without much phylogenetic significance. As a result, throughout this chapter, all sequences are listed with an assumed 73G-263G unless stated otherwise.

Macrohaplogroup R is marked by the loss of 16223T. While this mutation does occur in other branches, the presence of 16223T is a good indicator that the sequence falls within L, M, or N (although Behar et al. 2007 report that 2.5% of all pre-R genomes exhibit 16223C, while 1.1% of R genomes have mutated back to 16223T). Within these macrohaplogroups, some of the branches, particularly those found in Europeans, East Asians, and Native Americans, are quite well defined in the phylogeny. Others are still very poorly delineated, as they are found in parts of the world where the number of samples to date is very low in comparison to the amount of phylogenetic variation. Recent work in South and Southeast Asia, for instance, has defined a large number of new haplogroups within M, some (but not all) of which have distinctive HVR profiles (see Chandrasekar et al. 2009; Peng et al. 2010). A large number of sequences from African populations are available (Behar et al. 2008), but it is likely that much diversity remains unsampled there as well.

Throughout the process of haplogroup assignment, care should be taken to avoid overspecificity, especially if the evidence sequence lacks rare polymorphisms. Sometimes, a given HVR sequence might be indicative only of a general area of the tree, or even of multiple, distantly separated branches. As an example, consider the sequence 16223T-16278T-

Table 1. Mitochondrial DNA Haplogroups

Haplogroup	Defining HVR Polymorphisms[a]	Simplified Macroclade Structure
L0	16129A, 16187T, 16189C, 16223T, 16230G, 16311C, 146C, 152C, 195C, 247A, 263A	
L1	16187T, 16189C, 16223T, 16278A, 16311C, 152C, 182T, 185T, 195C, 247A	>L1′2′3′4′5′6
L5	16129A, 16148T, 16166G, 16187T, 16189C, 16223T, 16278A, 16311C, 182T, 195C, 247A	>L2′3′4′5′6 > L1′2′3′4′5′6
L2	16223T, 16278A, 16390A, 146C, 150T, 152C, 182T	>L2′3′4′6 > L2′3′4′5′6 > L′2′3′4′5′6
L6	16048A, 16223T, 16224C, 16278A, 16311C, 146C, 152C, 182T, 185C	>L3′4′6 > L2′3′4′6 > L2′3′4′5′6 > L1′2′3′4′5′6
L4	16223T, 16311C, 16362C, 195C	>L3′4 > L3′4′6 > L2′3′4′6 > L2′3′4′5′6 > L1′2′3′4′5′6
L3	16223T	>L3′4>L3′4′6 > L2′3′4′6 > L2′3′4′5′6 > L1′2′3′4′5′6
M	16223T, 489C (control region)	>L3
C	16223T, 16298C, 16327T, 249del, 489C	>CZ > M8 > M
Z	16185T, 16223T, 16260T, 16298C, 249del, 489C	>CZ > M8 > M
E	16223T, 16362C, 16390A, 489C	>M9 > M
G	16223T, 16362C, 489C	>M12′G > M
Q	16129A, 16223T, 16241G, 16311C, 16362C, 489C	>M29′Q > M
D	16223T, 16362C, 489C	>M80′D > M
N	16223T	>L3
I	16129A, 16223T, 16391A, 199C, 204C, 250C	>N1a′c′d′e′I > N1 > N1′5 > N
W	16223T, 16292T, 189G, 195C, 204C, 207A	>N2 > N
Y	16126C, 16231C	>N9 > N
A	16223T, 16290T, 16319A, 235G	>N
O	16213A, 16223T	>N
S	16223T	>N
X	16189C, 16223T, 16278T	>N
R	None	>N
HV	73A	>R0 > R
V	16298C, 72C, 73A	>HV0a > HV0 > HV > R0 > R
H	73A	>HV > R0 > R
J	16069T, 16126C, 295T	>JT > R2′JT > R
T	16126C, 16294T	>JT > R2′JT > R
F	16304C, 249del	>R9c > R9 > R
B	16183C, 16189C	>R
P	None	>R
U	None	>R
K	16224C, 16311C	>U8b > U8 > U2′3′4′7′8′9 > U > R

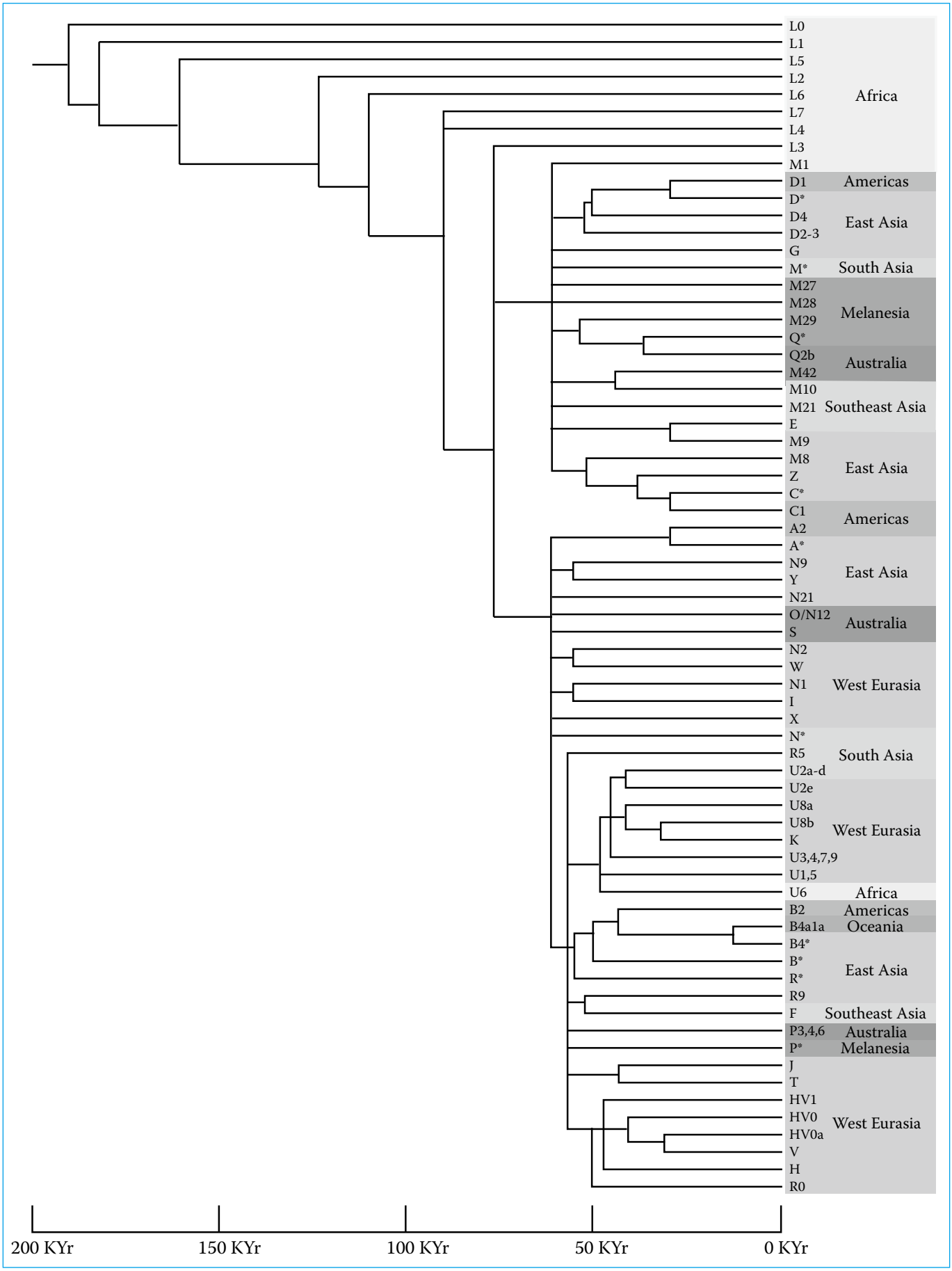

Fig.1 Simplified phylogeography of human mtDNA. Dates are approximate. An asterisk after haplogroup designations indicates "members of this haplogroup not specified on other branches."

16362C (here and elsewhere in this chapter, C-stretch insertions are ignored). In CIL casework, this sequence has been found in multiple sets of remains from both North and South Korea, as well as World War II contexts on Pacific islands. The mtDNAmanager reference list shows this as the profile for haplogroup D4g1, which is consistent with Northeast Asian ancestry, such as Korean or Japanese. In fact, this sequence is found in over 2% of modern Japanese and almost 1% of Koreans (Sekiguchi et al. 2008; Jin et al. 2006). Does this mean that all of those individuals actually belong to haplogroup D4g1? Not necessarily. With the addition of 16189C, this sequence is also consistent with G2a; since 16189 has a fairly high mutation rate, it is possible that some haplotypes within this haplogroup would show the same HVR sequence. Given that D and G are related clades within macrohaplogroup M and show similar distributions, it might be safer to state that the sequence probably falls within D or G, and might specifically belong to D4g1.

However, this same sequence is found in 11/10,428 individuals in AFDIL's CPD: two Koreans, four Turkmen, one Asian American, three African Americans, and one Hispanic. It also matches family references for two casualties from the Korean War: one African American and one Korean American. Are the African American and Hispanic matches due to Asian gene flow? Probably not. Instead, it turns out that the same HVR profile can be found in some haplotypes within L3b1a (Soares et al. 2012). So without additional sequencing of other regions of the mtDNA genome, all we can say about this particular HVR sequence is that it belongs either to D/G or to L3b1a. Because one of the mutations that define macrohaplogroup M (including D and G) is 489C, sequencing of the full CR would differentiate D/G and L3b1 sequences.

Phylogeny and Ancestry

Once a haplotype is located within the phylogeny, what can we say about its ancestry? On a local level, population genetic studies have shown a weak correlation between mtDNA and geography because of the movement of wives between communities (e.g., Besaggio et al. 2007). On a broader, continental level, it does have a high correlation with geography, as haplogroups branched off from each other with the spread of modern humans across the world (Fig.1; Maca-Meyer et al. 2001). The very diverse macrohaplogroup L, aside from its descendant clades M and N, is restricted in distribution to African populations, which exhibit great diversity and time depth (Behar et al. 2008). Macrohaplogroups M and N branched off from L3 around the time of our ancestors' departure from Africa and subsequently diversified across Eurasia. A few branches, such as M1 and U6, indicate back-migrations into Africa after this date (Olivieri et al. 2006). Within M, several clades have received their own letter designations (C, D, E, G, Q, and Z), while dozens of others have simply been numbered. Some of these clades are both very distinctive and very restricted in distribution (e.g., Q in Papua and Australia), while others spread across much of Asia and even (in the case of C and D) the Americas. Within N, there are two broad clades: R and N*, with the latter also including the haplogroups A, I, O, S, W, X, and Y. R is in turn divided into R* (including B, F, J, P, and T), R0 (including HV, H, and V), and U (including K). The R0 and U clades are restricted to western Eurasia, while R* and N* include some branches restricted to Asia (and in the case of A and B, the Americas) and others found in Europe (such as T).

Beyond these broad patterns, however, there is a fair amount of variation. Within any one designated haplogroup, smaller branches may have much more restricted distributions. Some of these distributions may be widely disjunctive, as well, as a result of historical migrations (Table 2). Thus, while haplogroups L1, L2, and L3 are restricted to sub-Saharan Africans and their descendant populations, specific branches within them have been documented from Slavic groups and likely date to prehistoric small-scale population movements (Malyarchuk, Derenko, et al. 2008).

Ancestry and Forensic Relevance

Sequence 1 exhibits an extensive series of polymorphisms that allow it to be securely placed in the phylogeny: 16223T-204C-199C, in that order; place it in the N1a′d′e′I branch, with 152C-16248T-16355T narrowing it to N1a. N1a1a is marked by 16147A and then 16320T. Finally, 207A and the reversal of a previous 16172C mutation mark N1a1a2. This subclade has only been reported from Europe and has actually been sequenced from Neolithic farmers from Germany (Palanichamy et al. 2010). Sequence 2 exhibits the same 16223T-204C-199C, followed by the 250C-16391A that marks haplogroup I (a subclade of N1, and similarly distributed in Europe). Its placement within I1a1 is determined by the successive mutations 16311C-16172C-203A. In the Pacific islands, European maternal ancestry indicates that the remains are likely to come from a US or Australian casualty who can eventually be identified by the CIL.

Sequences 3 and 4 both exhibit the 16223T-16362C that characterizes haplogroups D, G, and some other, minor branches of M. G3 is marked

by 16274A, and G3a2 by 143A-152C-16189C-16265C, placing sequence 3 securely. Sequence 4 is more difficult, however, as it also exhibits the 16390A that characterizes haplogroup E. However, no sequence within E in the reference phylogeny exhibits 16260T, which characterizes G2b2b. By contrast, one of the reference sequences for G2b2b exhibits 16093C-16189C-16390A as private mutations (that is, differences present in just one sequence and therefore not used in the current phylogeny) and thus matches sequence 4 perfectly. Haplogroup G is generally restricted to Northeast Asia, and both sequences have been reported from Japanese individuals (Sekiguchi et al. 2008; Nohira, Maruyama, and Minaguchi 2010). On a Pacific island, such a sequence is most consistent with the remains belonging to an Imperial Japanese service member.

Sequence 5 is phylogenetically the most distinctive of all. Haplogroup Q is characterized by 16129A-16223T-16241G-16311C, and 16144C-16148T-16265C-16343G-89C-92A-146C marks Q1. This is an ancient branch (perhaps as much as 50,000 years old) only found in the indigenous population of Papua New Guinea and Island Melanesia (Friedlaender et al. 2005). Such a sequence indicates that the remains are almost certainly of indigenous Melanesian origin.

Table 2. Selection of mtDNA Subhaplogroups with Disjunctive Distributions

Haplogroup	Subhaplogroup	N/12813[a]	n[b]	Primary Region
L1		200		Africa
	L1b1a8[c]		0	Europe
L2		232		Africa
	L2a1k		2	Eastern Europe
M		1386		Eurasia
	M1		80	North Africa
C		423		Northeast Asia
	C1b-d		211	Americas
	C4c		4	Americas
	C5c1		11	Eastern Europe
D				East Asia
	D1		50	Americas
	D2a		48	Americas
	D3		7	Americas
	D4b1a2a1b		4	Eastern Europe
	D4e1c		2	Americas
	D4e4b		2	Eastern Europe
	D4h3a		45	Americas
	D5a3a		4	Northeast Europe
A		426		Northeast Asia
	A2		277	Americas
X		161		Circumpolar
	X2a		24	Americas
	X2g		1	Americas
B		452		East Asia
	B2		122	Americas
	B4a1a1a		67	Polynesia
U		1208		Europe
	U2a,b,c,d		28	South Asia
	U6		119	North Africa, Mediterranean
Z		62		Northeast Asia, South Asia
	Z1a1a		22	Scandinavia

How Far Back Is That Asian Ancestry? Distinguishing Asian and Native American Sequences

Traditional triracial categorizations in physical anthropology lumped Native American and Asian populations together as "Mongoloid" (e.g., Brues 1990). In fact, the founding gene pool of Native Americans is a reduced subset of northeast Asian variation, as the Americas were colonized by small groups crossing the Bering Strait who were largely isolated over subsequent millennia. This is particularly clear in the mitochondrial genome. Initial studies of mtDNA phylogeography defined haplogroups A, B, C, and D as the four founding Native American types and recognized that they also occurred in Asia (Torroni et al. 1993). As the phylogenetic details were refined, it became clear that the American branches A2, B2, C1, and D1 were distinct from the Asian branches of the same haplogroups, and that haplogroup X should also be added as a founder. More recently, full genome sequencing has revealed that extant Native American mitochondrial lineages trace back to at least 15

Table 3. Cases from Pacific Islands

Individual	1	2	3	4	5
Recovery location	Papua New Guinea	Philippines	Philippines	Papua New Guinea	Papua New Guinea
Sequence	16086C, 16147A, 16223T, 16248T, 16320T, 16355T, 152C, 199C, 204C, 207A	16172C, 16223T, 16311C, 16362C, 16391A, 189G, 199C, 203A, 204C, 250C	16189C, 16223T, 16265C, 16274A, 16362C, 143A, 152C	16093C, 16189C, 16223T, 16260T, 16362C, 16390A, 195C	16144C, 16148T, 16188T, 16223T, 16241G, 16265C, 16311C, 16343G, 89C, 92A, 146C, 208C
Haplogroup	N1a1a2	I1a1	G3a2	G2b2b	Q1
Ancestry	European	European	East Asian	East Asian	Melanesian
N/10428[a]	0	0	0	0	0
Probable origin	United States	United States	Japanese	Japanese	Papuan

distinct branches, each with Asian cousins: A2*, A2a, A2b, B2, C1b, C1c, C1d*, C1d1, C4c, D1, D2a, D3, D4h3a, X2a, and X2g (Perego et al. 2010). Furthermore, at least one additional haplogroup, an otherwise undocumented branch of M, has been found in a 5,000-year-old burial from British Columbia (Malhi et al. 2007), indicating that it is possible or even likely that other as of yet unknown lineages are, or were, present in the New World, if at very low frequencies. Nonetheless, the vast majority of Native American sequences belong to one of the four originally identified branches.

In CIL casework, recognizing whether a given sequence is of Asian or Native American origin is frequently probative, as the majority of the remains received by the CIL come from Asian or Pacific contexts. While there are US casualties of Asian ancestry missing in Korea and Southeast Asia, AFDIL can compare unknown sequences from those areas to their family references. A sequence of Asian origin that does not match a known Asian American casualty almost certainly represents a non-US individual, and the remains are therefore not likely to be identified by the CIL. Any sequence of Native American origin found in the region, by contrast, has a very high likelihood of representing a US casualty who is (hopefully) identifiable.

Haplogroup A

Haplogroup A is a subclade of macrohaplogroup M and is distinguished by the HVR profile 16223T-16290T-16319A-235G. It has a restricted distribution in northern Asia and is found throughout the Americas (Achilli et al. 2008; Derenko et al. 2007). Within haplogroup A, there are three successive splits marked by HVR polymorphisms: 152C differentiates A3, A4, A7, A9, and A11 from A5, A8, and A10; 16362C differentiates A4; and within A4, 16111T-146C-152T-153G differentiates A2. Within A2, A2a and A2b are found in both northeast Asia and the Americas, while all the other clades, most of which share also 64T, are restricted to the New World. For forensic purposes, it is safe to consider any haplogroup A sequence exhibiting 16111T and/or 64T to be almost certainly of Native American ancestry, however distant.

Five cases provide examples from haplogroup A (Table 4). Individual 1 was recovered from the island of Tarawa, where thousands of US and Japanese service members were buried in 1943. He exhibits the A4 profile without any of the distinctive A2 polymorphisms and also has a 200G, which marks the A4c′d branch. Since A4d also has 151T, while A4c has no further HVR mutations, he most likely falls within the latter clade. While no exact match to this sequence could be located in the literature, A4c is a North Asian, and particularly Siberian, clade (Derenko et al. 2007), and a similar sequence has been reported from Korea (Jin et al. 2006). It thus appears more consistent with Japanese ancestry than American, although the recently discovered New World branches of haplogroups C and D (Table 2) show that other related but long isolated branches may still turn up.

Individuals 2, 3, and 4 were recovered from North Korea. The first bears a 16187T that is characteristic of A5a; this sequence is common in both Korean and Japanese populations. The second and third both exhibit the markers of A2 (except for 152T, which has mutated back to 152C in multiple branches within A2). They cannot be placed more precisely than that, which is not surprising, as A2 contains a broad range of variation, consistent with a small founding population spreading across a wide area and growing exponentially (Kumar et al. 2011). This haplogroup is indicative of Native American ancestry, which is consistent with their FRS. Individual 5, recovered from Vietnam, does not match any sequences in the CPD or FRS database. However, despite the lack of database matches, this sequence can also be securely placed in haplogroup A2. All three sequences almost certainly belong to US service members.

Haplogroup B

Haplogroup B is a subclade of macrohaplogroup R and is characterized

Table 4. Haplogroup A Examples

Individual	1	2	3	4	5
Recovery location	Tarawa	North Korea	North Korea	North Korea	Vietnam
Sequence	16093C, 16223T, 16290T, 16319A, 16362C, 152C, 200G, 235G	16187T, 16223T, 16290T, 16319A, 235G	16111T, 16223T, 16290T, 16319A, 16362C, 16378T, 64T, 146C, 153G	16051G, 16111T, 16223T, 16249C, 16290T, 16319A, 16362C, 146C, 153G, 235G	16111T, 16223T, 16290T, 16319A, 16362C, 146C, 153G, 159C, 235G
Haplogroup	A4c	A5a	A2	A2	A2
Ancestry	Northeast Asian	Northeast Asian	Native American	Native American	Native American
N/10428[a]	0	1 Kazakh	0	1 Caucasian	0
FRS matches[a]	None	None	1 White (Hispanic surname)	1 White, 1 American Indian	None

in the HVR by (a) the change at 16223 to the CRS that typifies R, and (b) 16183C-16189C. Given that both 16183 and 16189 exhibit high mutation rates (the former site is not even considered in the formal phylogeny), it can be difficult to distinguish B if no other mutations are present. Branches are found throughout East Asia, the Pacific, and the Americas (Achilli et al. 2008; Derenko et al. 2012; Li et al. 2007; Melton et al. 1995). Within B, the primary division is between B4, marked by 16217C, and B5, marked by 16140C and four other mutations outside the HVR. Within B4, 16261T divides B4a, g, h, and i from B4b, d, and e (the last a minor branch marked by a slew of other polymorphisms as well). A simple sequence of 16189C-16217C could belong to either B4b or d. The difficulty here is that B4b includes haplogroup B2, the haplogroup present in the New World, which is differentiated from B4b by five mutations outside the HVR. Therefore, HVR data alone cannot always discriminate between Asian and American Bs. Fortunately, B4b1, the largest clade within B4b, is marked by 16136C, usually with multiple other polymorphisms as well, which does allow its separation from B2.

Table 5 provides some examples of the difficulties raised by haplogroup B in evidence cases. Individual 1 was recovered from North Korea and exhibits the basic B4 profile plus 16390A. Unfortunately, this polymorphism is not significant within the current B4 phylogeny. The sequence does match four Hispanics in the CPD, as well as two casualties who both appear likely to fall within that same modern category. It therefore appears likely that these remains will be identifiable as those of a US casualty. Similarly, individual 2 has the basic B4 profile plus 150T. This sequence matches 10 Navajo (out of the 146 in the CPD), but it is certainly possible that it might also match indigenous Southeast Asians, given how poorly that region is represented in databases and the fact that B4c is present in the region (Peng et al. 2010). Without additional information, either from elsewhere in the genome or from the archaeological context, these remains cannot be securely assigned to either American or Asian ancestry.

Individual 3, by contrast, presents a set of polymorphisms distinctive to B2g1. The transversion of C to G at 114 is particularly significant, as most mutations are transitions (between either C and T or A and G). This is a haplotype that appears to be of native Mesoamerican origin. The remains were recovered from a World War II aircraft crash site in Germany, and in that context, US ancestry is the most plausible explanation.

Individual 4 exhibits the HVR motif of B5a, including another distinctive transversion from C to A at 16266. This is a haplogroup that is quite common in mainland Southeast Asia (Peng et al. 2010). Given that these remains were recovered in Vietnam, they almost certainly represent an indigenous individual.

The last three sequences present a different conundrum. They exhibit a set of polymorphisms distinctive to B4a1a: 16189C-16217C-16261T-146C. This branch appears to have arisen in early Austronesian populations spreading out across islands of Southeast Asia (Melton et al. 1995). Individual 5 was recovered from North Korea, but the same sequence has also been found in remains recovered from a World War II context in Papua New Guinea. Individuals 6 and 7 come from Saipan and exhibit the 16247G that marks B4a1a1a. The combination of 1627C-16247G-16261T has been labeled the "Polynesian motif" (Redd et al. 1995), and it is one of a handful of lineages carried by the Austronesian populations that expanded into Polynesia (Kayser 2010).

At first glance, the population database matches of these three sequences do not match what might

Table 5. Haplogroup B Examples

Individual	1	2	3	4	5	6	7
Recovery location	North Korea	Cambodia	Germany	Vietnam	North Korea	Saipan	Saipan
Sequence	16182C, 16183C, 16189C, 16217C, 16390A	16183C, 16189C, 16217C, 150T	16183C, 16189C, 16217C, 16298C, 114G, 146C	16140C, 16183C, 16189C, 16266A, 210G	16182C, 16183C, 16189C, 16217C, 16261T, 146C	16182C, 16183C, 16189C, 16217C, 16247G, 16261T, 146C	16182C, 16183C, 16189C, 16217C, 16247G, 16261T, 146C, 151T
Haplogroup	B4/B2	B4/B2	B2g1	B5a	B4a1a	B4a1a1a	B4a1a1a
Ancestry	East Asian/Native American	East Asian/Native American	Native American	Southeast Asian	Island Southeast Asia	Polynesian	Polynesian
N/10428	4 Hispanic	10 Navajo	5 Hispanic	0	2 Chinese, 1 African American	4 Asian, 4 African American, 1 Hispanic, 1 other	2 Asians, 1 Hispanic, 1 other
FRS matches	1 White (Hispanic surname), 1 Mexican	None	1 White (Hispanic surname)	None	2 Filipino	1 Black, 2 Hawaiian, 1 Chinese Hawaiian, 1 Asian American	1 White, 3 Hawaiian, 1 Chinese, 1 "Mongolian"

be expected from their phylogenetic position. The "Asian" samples are all either Taiwan Chinese, who could be of indigenous Austronesian ancestry, or Asian Americans, a category that often includes Pacific Islanders as well. But how are distinctly Pacific sequences appearing within the African American and Hispanic populations? Some insight is provided by the list of matching casualties from within the FRS database, for whom some individual biographical data are available. Individual 5 matches two Filipino American casualties, as expected given the distribution of B4a1a. The group of casualties matching individual 7, despite the variety of racial categories, are all of Native Hawaiian maternal ancestry. Given the population history of Hawaii, it is perfectly possible that the CPD Hispanic who matches this sequence is in fact also of Native Hawaiian maternal descent. Individual 6 also matches three casualties of Native Hawaiian maternal ancestry and one of uncertain Asian ancestry. However, the last match is to an African American whose maternal lineage can be traced to the late nineteenth century in Tennessee and is very unlikely to have any Hawaiian ancestry.

So what accounts for the "Polynesian motif" appearing in an African American? In fact, several African American data sets include sequences belonging to B4a1a, as well as the Southeast Asian haplogroup F3b (Allard et al. 2005; Diegoli et al. 2009; Stefflova et al. 2009). These sequences are usually attributed to recent non-African admixtures. However, it is more likely that they trace back to Madagascar, one source of the Atlantic slave trade (Lee et al. 2009; see Razafindrazaka et al. 2010 for a recent analysis of Malagasy mtDNA).

So what is the significance of a B4a1a or B4a1a1a sequence in CIL casework? It depends upon the recovery area. For remains recovered in Saipan or elsewhere in the Pacific, it is possible that they belong to a Filipino American or, less likely, African American service member. It is much more likely, however, that they belong to an indigenous individual. By contrast, remains recovered on the mainland of Asia or in another combat theater probably belong to a US service member of uncertain ancestry.

Haplogroup C

Haplogroup C is a branch of haplogroup M distinguished by 16223T-16298C-249del (which it shares with haplogroup Z), and 16327T. C1, the primary Native American branch, is further distinguished by 16325C-290del-291del. C and its sister Z are largely restricted to northeast Asia, although some branches reach further afield (Table 2; Derenko et al. 2010; Ebenesersdóttir et al. 2011). Five cases from the sister haplogroups serve as examples (Table 6).

Individuals 1 and 2 exhibit sequences that can be securely placed within the phylogeny. The first has the standard C1 motif plus 16086C-16189C-16278T-143A, which together mark C1b4. The second exhibits the C1 motif minus 16223T (an example of the parallel occurrence of the T–C transition at this locus). The 215G matches the profile expected for C1c1. Both of these sequences are present in CPD Hispanics, indicating likely Native American ancestry, which in turn, when recovered from North Korea and Papua New Guinea, indicates that the remains most likely belong to US service members.

However, we can actually draw even more specific conclusions from a careful examination of the literature. In the FRS database, sequence 1 matches three Puerto Ricans, and Martínez-Cruzado (2010) has identified it as one of the founding Native American lineages in Puerto Rico. As many US troops of Puerto Rican ancestry did serve in Korea, it is likely that these remains represent one of them. Sequence 2 is consistent with Mexican American examples (Kumar et al. 2011), suggesting that that origin is more likely.

Individual 3 presents a very unusual sequence. It bears the C1 motif, but the other five polymorphisms are absent from all published examples. Given the number of polymorphisms,

Table 6. Haplogroup C Examples

Individual	1	2	3	4	5	6
Recovery location	North Korea	Papua New Guinea	Saipan	North Korea	North Korea	Hawaii
Sequence	16086C, 16183C, 16189C, 16223T, 16278T, 16298C, 16325C, 16327T, 143A, 249del, 290-291del	16298C, 16325C, 16327T, 215G, 249del, 290-291del	16131C, 16164G, 16207C, 16223T, 16224A, 16234T, 16298C, 16325C, 16327T, 249del, 290-291del	16093C, 16223T, 16234T, 16288C, 16298C, 16327T, 249del	16223T, 16298C, 16327T, 16357C, 204Y, 207A, 249del	16185T, 16223T, 16260T, 16298C, 16301Y, 16362C, 151T, 249del
Haplogroup	C1b4	C1c1	C1	C5c1	C4c1?	Z
Ancestry	Native American	Native American	Northeast Asian/ Native American?	East European	Asian/Native American?	East Asian
N/10428	7 Hispanic	2 Hispanic	0	1 Caucasian	0	0
FRS matches	3 Puerto Rican	1 White (Hispanic surname)	None	1 White	1 American Indian	None

this sequence appears to represent an old lineage within C1 that has not been sampled in published studies. It is certainly possible that it is indeed Native American; it is also possible that it is Northeast Asian, belonging either to C1a with a subsequent loss of the 16356C that marks that haplogroup or to a yet undescribed clade. As these remains are from a Pacific island, the former option would be consistent with US ancestry, the latter with Japanese ancestry.

Individual 4 is placed within C5 by the 16288C and then C5c1 by 16093C-16234T. Given C5's distribution in Northeast Asia and the recovery location of these remains in North Korea, it would be logical to assume a Korean or Chinese ancestry. However, the sequence also happens to match one White US service member. Further investigation reveals that C5c1 is indeed an Eastern European branch, first defined from Polish individuals, and owes its Asian maternal ancestry to a settler from the steppes millennia ago (Derenko et al. 2010). Without the FRS match, it would have been easy to set these remains to the side as not from the United States and therefore not identifiable by the CIL.

Individual 5 provides a similar case. The sequence lacks the diagnostic markers of C1 and C5, which leaves C4 and C7 as phylogenetic possibilities, since neither of these clades is defined by HVR mutations. The 16357C indicates a possible placement within C4a, best documented from South Asia (Chandrasekhar et al. 2009). However, the sequence also matches a casualty of Native American ancestry. Given this match, it is possible that the sequence actually falls within the recently defined C4c, which has been documented from multiple Native Americans and represents an additional founding lineage in the hemisphere (Hooshiar Kashani et al. 2012). Several of the sequences that fall within this haplogroup lack any distinctive HVR mutations.

Individual 6 was recovered on the island of Oahu and is included here to show the difference between haplogroups C and Z. Although recovered from a context where a prehistoric Native Hawaiian interment was a possibility, the mtDNA sequence obtained is inconsistent with that origin, since haplogroup B4a1a1a would be expected in that case. Haplogroup Z is quite rare and therefore poorly documented, so no identical sequences were encountered in the literature. However, very similar examples have been reported from Guangdong, China (Chen et al. 2008; Wang et al. 2010). The remains likely represent a nineteenth- or twentieth-century descendant of East Asian immigrants; depending upon the recovery context, they could be from either a historical cemetery or a more recent missing person case.

Haplogroup D

Haplogroup D is one of the largest clades within haplogroup M and is found across Asia and the Americas (Achilli et al. 2008; Derenko et al. 2010). Its basic HVR profile is 16223T-16362C, which unfortunately is also the root profile for multiple other haplogroups, such as M6, M9 (which includes E), and G. As illustrated previously, some HVR profiles within D also match even more distantly related phylogenetic twigs. As a result, it is often not possible to locate a related sequence precisely within the phylogeny. In some cases the sequence may be useful even without a more precise cladistic position. For instance, the basic D profile is found in approximately 4% of both the Japanese and Korean populations (Jin et al. 2006; Sekiguchi et al. 2008). In a forensic situation where there is a high prior probability of encountering remains of either of those ancestries, such as a recovery on a Pacific island, this profile may be sufficient to determine the disposition of remains without a more exact phylogeny.

Table 7 provides six casework examples of haplogroup D sequences. The first three were all recovered in North Korea. Individuals 1 and 2 each exhibit the 16325C that marks D1. Like A2, D1 has a fairly broad and shallow phylogeny, and it can be difficult to place sequences more precisely. Individual 1's 16274A-16368C

Table 7. Haplogroup D Examples

Individual	1	2	3	4	5	6
Recovery location	North Korea	North Korea	North Korea	Vietnam	Papua New Guinea	Papua New Guinea
Sequence	16223T, 16274A, 16325C, 16362C, 16368C	16147T, 16223T, 16325C, 150T, 152C, 185A, 489C	16093C, 16209C, 16223T, 16274A, 16295T, 16325C, 16362C, 189G, 195C, 203A, 204C, 228A, 298T, 325T	16129A, 16223T, 16362C, 152C	16129A, 16223T, 16243C, 16362C, 151T, 152C	16164G, 16172C, 16182C, 16183C, 16189C, 16223T, 16266T, 16362C, 150T
Haplogroup	D1i	D1?	D4g2a	D4a	D4a	D5a2a1
Ancestry	Native American	Native American?	East Asian	East Asian	East Asian	East Asian
N/10428	4 Hispanic	0	0	10 Asian	0	8 Asian
FRS matches	2 White (1 also called Mexican)	1 White (with Spanish surname)	None	None	None	None

does not match any specific branch; however, one sequence that is placed in D1i exhibits the same motif. Individual 2 lacks the 16362C of D, but because his full CR was sequenced, we know that he has the 489C that marks macrohaplogroup M, and within this, the presence of 16325C and match to a casualty of apparent Hispanic ancestry suggest D1. The other polymorphisms, 16147T-150T-152C-185A, do not match any documented sequence. Individual 3 has a 16274A-298T that places him within D4g2a; the other polymorphisms match a published Japanese sequence from this clade (Tanaka et al. 2004). In North Korea, the first two individuals are most likely US service members, while the third is either Korean or Chinese.

Individuals 4 and 5 both show the 16129A-152C that marks D4a, in the latter case with an additional 16243C-151T not shown in the phylogeny. This haplogroup has a different significance in the two different recovery locations. In Southeast Asia, D is present, albeit at low frequencies (Peng et al. 2010). In Melanesia, it is absent in the indigenous populations (Friedlaender et al. 2007). Therefore, the former case is most likely an indigenous Vietnamese individual, while the latter is most likely an Imperial Japanese service member. Finally, individual 6 bears a profile distinct to haplogroup D5a2a1. This haplotype was first described from Siberia, but is also present in China (Starikovskaya et al. 2005; Yu et al. 2010). In Vietnam, it most likely indicates Sino-Vietnamese ancestry.

Skeletal Ancestry versus Mitochondrial Lineage: A Korean War Case

From 1996 through 2005, recovery teams led by CIL anthropologists excavated numerous sites in North Korea. From one of these sites, a foxhole on a battlefield from November 1950, a team recovered the skeletons of three US service members with fragments of their uniforms but without any identification media. The remains were well preserved, allowing a detailed anthropological analysis as well as mtDNA testing (Table 8). Determinations of ancestry were based upon cranial morphology and discriminant function analyses conducted in FORDISC 3.0 (Jantz and Ousley 2005), which yielded consistent results. Subsequently, two of the three individuals have been identified, allowing verification of the ancestry determinations.

For individual 1, skeletal ancestry appeared consistent with the mtDNA haplogroup and database matches, indicating that this service member was most likely classified as Negro in 1950, although the individual has not yet been identified to confirm this. For individuals 2 and 3, the results are less clear. In the former case, the skeleton appears to be of African ancestry, while the mtDNA haplotype belongs to the European haplogroup U, and the database matches are predominantly Hispanic. In the latter case, the skeleton appears to be of European ancestry, while the mtDNA haplotype is very strongly African.

Sequence 2 presents an example of how the details of the mtDNA phylogeny do not necessarily fit our "racial" categories particularly well. Malyarchuk et al. (2010) have argued that U5 (defined by 16192T-16270T) is the oldest European haplogroup, and that it evolved over the last 30,000 years in southwestern Europe and the adjoining southern rim of the Mediterranean. U5b1b1b in particular (defined by 16320T) is documented from both US Hispanics and Senegalese. Martínez-Cruzado et al. (2005) found this haplotype (which they labeled U5b2) in 9/800 Puerto Ricans and considered it to be of West African origin; however, it also appears plausible that it reached US Hispanics from a North African or Iberian source. In this particular case, it is likely of West African origin, as the remains were identified as those of the Black casualty whose family reference matched.

Sequence 3 belongs to one of the deeper branches on the African mitochondrial phylogeny, L1, which is found at low frequencies in both West and East Africa (Gonder et al. 2007). While this exact sequence is not found in the literature, the L1b1a clade is documented in African Americans and West Africans and also in individuals from elsewhere in Africa, Portugal, Cyprus, Jordan, and Israel (Behar et al. 2008). This lineage almost certainly entered the US population through

Table 8. Skeletal Group from North Korea

Individual	1	2	3
Sequence	16223T, 16278T, 16294T, 16309G, 146C, 152C, 195C	16189 C, 16192T, 16270T, 16320T, 150T	16114T, 16126C, 16187T, 16189C, 16215C, 16223T, 16264T, 16270T, 16278T, 16293G, 16311C, 152C, 182T, 185T, 195C, 247A
Haplogroup	L2a1a	U5b1b1b	L1b1a1
Ancestry	African	African?	African
N/10428	33 African-American, 1 Hispanic	8 Hispanics	0
FRS matches	9 Black, 1 White	2 Puerto Rican Whites, 1 Black	1 White
Skeletal ancestry	Negroid	Negroid	Caucasoid
Casualty race	Unknown	Black	White

the slave trade, but the one casualty whose reference matched was White. In fact, the remains were subsequently identified as that individual, who may or may not have been aware that he had a distant African ancestor.

In these latter two cases, if ancestry had been assigned to the remains based purely upon the mitochondrial haplogroup, it would have been incorrect. The skeletal determination, however, matched the bureaucratic one.

Ours or Theirs? A South Asian Case Study

During World War II, 594 US and Allied aircraft carrying 1,659 personnel were lost flying "over the hump," carrying supplies from British India to China. These crash sites have proven to be some of the most difficult in the world to locate and recover, given their remoteness. In several cases, hikers and other private citizens have picked up remains at remote sites and subsequently turned them over to JPAC. One such individual provided a selection of bones that he said he had obtained from a US crash site in the mountains along the India–Burma border. However, without archaeological provenience, the CIL was unable to verify whether the remains actually did represent US casualties. Samples were taken from all of the bones for mtDNA analysis, and AFDIL reported five sequences (Table 9). The first three sequences matched multiple individuals of European ancestry in the CPD and therefore appear fully consistent with the alleged provenience. Sequence 1 exhibits the 16356C-195C that defines U4, with a 146C that marks U4b1a3, sequence 2 the basic K profile of 16224C-16311C, and sequence 3 the 16126C that marks JT with the additional 16069T-295T of J. The absence of 150T-152C, which defines J2, indicates that sequence 3 belongs to J1.

Looking at the two sequences that do not match any references in the CPD, it is easy to hypothesize that sequence 4, with only three polymorphisms, is likely to be European, especially as 16304C is a fairly common variant. However, the combination of 199C and 16304C, along with the 16223T that excludes the sequence from macrohaplogroup R, is in fact very distinctive and places this sequence within haplogroup M35b (Chandrasekhar 2009). M35 itself has no HVR polymorphisms, but 199C marks the M35a′b branch, and 16304C in turn marks a clade of M35b1 and M35b2. Like many South Asian haplogroups, it is clear that M35 is very diverse, particularly in comparison to the limited data so far available on it, and is present in individuals from throughout the subcontinent. M35b2 is in fact best documented from Eastern European populations, where it appears to have been carried by the ancestors of the Vlax Roma (Salihović et al. 2011; Mendizabal et al. 2011). It is now present in a high percentage of Vlax, but also in the non-Romany population of Slovakia (Malyarchuk, Perkova, et al. 2008).

However, all European M35b sequences known to date exhibit the string of polymorphisms 16129A-16230G-16233G-16344T in addition to the 199C-16304C, suggesting that sequence 4 is more likely to be from an indigenous South Asian than an Eastern European. Furthermore, the two sequences in the whole genome database that match this HVR profile exactly are from a Thai "sea gypsy" and a dental patient from Andhra Pradesh, India (Behar et al. 2012), while other related examples come from the Tharu of Nepal (Fornarino et al. 2009). Given this distribution, it appears most likely that the bone in question does not belong to a US casualty and was instead improperly associated with the other remains, either by the individual who turned the remains over to US authorities or by a third party who possessed them previously.

Conclusions

Because race is a cultural category, albeit one based upon perceived biological differentiation, it will never perfectly correspond with determinations of ancestry based upon any form of biological variation. Uniparental lineage markers such as YDNA and mtDNA need to be used with particular care. Nonetheless, mtDNA variation can help us to hypothesize the ancestry of a given set of remains, and what race their owner might have been classified in, if we are clear about its limitations. The three questions outlined in this chapter provide a guide to these limitations in any given case. At one

Table 9. Skeletal Group from South Asia

Individual	1	2	3	4	5
Sequence	16356C, 146C, 195C	16224C, 16311C	16069T, 16126C, 295T	16223T, 16304C, 199C	16069T, 16126C, 16145A, 16231C, 16261T, 16355T, 150T, 152C, 195C, 215G, 295T, 310.1T
Haplogroup	U4b1a3	K	J1	M35b	J2a1a1a
Ancestry	European	European	European	South Asian	European
N/10428	4 Caucasian	17 Caucasian, 1 Hispanic, 13 "other"	7 Caucasian	0	0
Probable origin	United Stat	United States	United States	Indigenous	United States

end are those cases where a distinctive HVR sequence can be firmly placed in the phylogeny, on a branch with a restricted geographic distribution, and that maternal geographic origin is of high forensic significance; at the other end are those where a nondescript HVR sequence can only be placed in one or more broad areas of the phylogeny with little geographic restriction, and where minimal forensic significance can be placed on that maternal origin. An example of the former is provided by haplogroup Q1 remains recovered from Papua New Guinea; an example of the latter by remains that could be placed in D, G, or L3b1a recovered from Southeast Asia.

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History of Death Investigation

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1 Introduction

Forensics, derived from the word "forum," refers to the application of various fields of science and medicine in the resolution of legal proceedings. The beginnings of forensic science were crude and rudimentary but formed the basis of areas of research and progress to modern-day use of lasers, sophisticated laboratory equipment to identify particles and drug identification, and computerization to improve response time to locate the answers to questions asked by investigators, some in near real time. This chapter reviews the historical beginnings of forensic medicine and its parallel in criminalistics, contrasts the coroner and medical examiner investigation systems, as well as provides insight into governing for accreditation, certification, and licensure.

2 Mandates, Jurisdiction and Laws

Forensic science has its origins in early China and was documented in an early transcript of text, Washing Away of Wrongs by Sung Tz'u written in 1248. He was a criminal affairs officer who wrote the book based on personal experiences. Within the text he described a scenario of a local village murder by a sickle used to harvest grain. The murderer was unknown and the investigator had each farmer bring their tools to the village to be examined. It was noted that flies were attracted to one particular sickle. This was apparently due to adherent tissue and blood on the tool and ended with the farmer admitting to the crime. The story has roots for the basis of forensic entomology with its observation of the relevance of insects and their relationship to the cycle of death. He described handling of male corpses by local men of low social standing and the female corpses were managed by local midwives.

The early Greeks performed anatomical dissections in an attempt to understand the workings of the body and organ relationships. However, it wasn't until the late 18th century when a book written by Giovanni Morgagni that described autopsy dissections with descriptions of disease processes that they gained acceptance in the West. This served as a framework in the late 19th century for Dr. William Osler, the acclaimed physician and educator, supporting the autopsy as a great teaching method for physicians to learn about their patient's disease and to see for oneself the disease process. His work and influence served as the basis for medical training that still is in existence today. The period after World War II showed extensive interest in autopsies, and most were done in the hospital setting to gain knowledge about the effectiveness of new treatments, as well as learn about the disease itself. Hospital autopsies were done for approximately 50% of deaths.

In 1954 the United States passed the Model PostMortem Act, which outlined general classes of deaths that need to be further investigated and certified by a government body rather than a hospital pathologist or treating physician. This act was used as a framework for each state to develop its own particular laws regarding death investigation. The act outlines reporting of all violent deaths; unusual, unnatural, or suspicious deaths; all prison deaths; and any death thought to represent a public health hazard. Over the years, each state has modified their laws to adapt to advances in the medicolegal

system, but for the most part they read as they were originally written and reflect these guidelines.

Today, it is estimated that hospital autopsies are done in less than 10% of hospital deaths. The decline is related to multiple influences, including the deleted requirement by the Joint Commission of American Hospitals (their accrediting

body) for a minimum autopsy rate and reimbursement to the hospital for this service, as well as improved radiologic methods for patient evaluation [1]. The interesting finding, however, is autopsies discover 22–33% findings that were not previously known even with the current technology [1]. They provide answers to families and further understanding to medical science, but unfortunately continue to decline in the hospital community.

Caseloads for medical examiners and coroner offices continue to increase as the population increases. With shortages of forensic pathologists and limited tax-based funds, death investigation offices must limit autopsies performed to those mandated by law. This creates a void for hospitals and families wishing for answers. Hospital pathologists rarely perform them, and in modern hospitals, morgues are no longer included as an essential area of the laboratory department. Those autopsies not falling under jurisdiction of the state's death investigation laws require signed family permission to proceed. State laws even outline the family members who may give this permission, usually following the order of spouse, adult children, parents of the deceased, adult siblings, a legal guardian, and then the individual charged with the disposition of the remains.

In the situation of religious objections and deaths falling under the jurisdiction of the death investigator, an autopsy may proceed without family permission. However, it is best for public relations to work with the family and try to abide by their wishes or perform the autopsy within their religious constraints if at all possible. This may require a rabbi to be present during the procedure, collection of all body fluids to return with the body, or particular religious practices to be performed before or after the procedure. Muslim and Jewish religions request burials prior to sundown of the day of death if at all possible. Some religions forbid embalming. Some families request no autopsy because they wish to have an open casket and viewing of the decedent. With education about the procedure, they can be reassured that the incisions will be done in locations that will not preclude viewing, embalming, and open-casket funerals if the body was not damaged extensively by trauma prior to the autopsy. Objections can be overcome with meaningful conversations between the family and the death investigator or pathologist.

There is no universal body governing the death investigation system at a national level, and each state performs death investigations differently from its neighboring state. Many deaths reported to a death investigation office involve sudden death due to unknown mechanism. They represent natural diseases not previously or well documented prior to death, or the treating physician may be unavailable to sign for a patient with a well-documented history. Generally, there is a time limit in which a death certificate needs to be filed with a local health department after death. For this reason, the medical examiner serves as a resource to fill these gaps. The pathologist can issue meaningful causes of death based on medical records and external examination of the body without the need for an internal examination and full autopsy.

The local health department is the governing body that filters all death certificates to the National Bureau of Vital Statistics. They review and numerically code the causes of death into categories so that trends in causes may be recognized to adjust surveillance, prevention, and treatment practices. The local health department also issues burial or cremation permits to funeral homes after a valid death certificate is filed. They supply copies of death certificates, which are usually public record and can be obtained by anyone. The 1992 Model State Vital Statistics Act and Regulations serves as a template for each state to model their vital records practices and can serve as a reference to answer unusual questions when completing a death certificate [2].

The coroner system has been in existence since organized colonization began in 1492 when the concept was imported with the settlers from England. The first medical examiner office was established in New York

City in 1918, and it was the first government division of its kind in the United States [3]. They were also responsible for the first toxicology laboratory in 1918 [3]. The first chief medical examiner in the New York office was Dr. Charles Norris. This was followed by New York University establishing the first department of forensic medicine in 1933.

The Center for Disease Control maintains a list of the medical examiner and coroner jurisdictions within the United States [4].

3 History of Criminalistics

Parallel to the development of forensic medicine and autopsies, the world of criminalistics was also developing and spurring forward the science of evaluating evidence and tracking criminals. Alphonse Bertillon was a French law enforcement officer in the late 19th century who performed research in anthropometry, which is a study of physical characteristics of a person that make him or her unique. His study involved recording measurements of various body regions, such as forearms, trunks, ears, fingers, and faces, to differentiate one person from another. This had applications for differentiating criminals from each other, because the usual method had been for station police officers at the entrance to the jail to make visual identifications, which were sometimes inaccurate. The main purpose was to separate repeat-offender prisoners from first offenders. At the time, it was a huge scientific advancement and was thought to be reliable until the early 20th century when it failed to differentiate a case of twins. Although these particular measurements were eventually found to be unreliable, they formed the basis for the science of biometrics that utilizes a similar idea of individualizing characteristics but includes more detailed, patterned relationships, such as iris scans, fingerprints, and facial-recognition software used in security programs.

Another notable hallmark in criminalistics was the work of Edmond Locard in the early 20th century. He too was a Frenchman but with a background in medicine and law. He became interested in forensics during his studies and eventually formed the first criminalistics laboratory in Lyon, France, in 1910. He is best known for Locard's exchange principle, which states that whenever two items come into contact they exchange material between them. This is the principle used to recover evidence and particular trace evidence on the body or other items in a crime scene and link it to the individual who left it. The importance of this principle is that no scene is without trace evidence; it is the job of the investigator to locate it and collect it. Locard was also extremely interested in fingerprints, and through his work in microscopy, he detailed characteristics of them that are used today in fingerprint identification. He is generally considered the first criminalist.

In 1923, August Vollmer, chief of the Los Angeles, CA, Police Department, established the first American crime laboratory. The second crime laboratory was established in 1929 by Calvin Goddard (well known for his work in ballistics) at Northwestern University in Chicago, IL.

3.1 Coroners

Coroners are elected officials and in most jurisdictions the only credentials required for being placed on the ballot are a high school diploma and a voter registration card. Medical, science, or law enforcement background is not required. In some jurisdictions, the position has been combined with the sheriff position to decrease administrative overheads. In this case, sworn law enforcement deputies may rotate through the positions of death investigator and not be specifically trained in death-scene evaluations. There can be a public question of conflict of interest when investigations cross into departmental operations. Other jurisdictions utilize a separate department with a chief coroner and deputy coroners. Coroner positions may also be linked to local funeral homes as experienced personnel in dealing with the dead.

The history of the word "coroner" originates in England from the word "crowner," a tax collector for the dead. In old England, coroners were given the task of investigating any local event that might result in revenue for the Crown. Death was a potential source of money, and suicides, fires, shipwrecks, and others all were levied taxes or goods were confiscated by the Crown as fines. After the conquest of the Normans, the countryside continued to kill Normans. To discourage this, a heavy fine was levied against a community in which a Norman was found dead. It became

the job of the coroner to determine the origin of the dead person, and they largely assumed the person was a Norman and levied the tax unless someone could prove the decedent was English. This tax levy was known as "murdrum"' and became the origin of the word "murder" [5]. The concept of coroners and sheriffs was brought to the United States with the colonists when it was initially settled. It spread throughout the United States as colonies developed and the need for investigation and management of the dead became a necessity.

The United States varies from state to state in a mixture of medical examiner and coroner jurisdictions and combinations thereof [4]. Because coroners are not physicians, they are unable to perform autopsies. This is contracted to forensic pathologists who supply a cause of death to the coroner. The coroner then has the legal ability to certify the cause and manner of death on the death certificate. Most times, the coroner will agree with the cause of death, but if not, he or she may place any cause on the certificate or rule the manner as he or she wishes. Being a political position can have some bearing on this decision and is a potential downfall of the coroner system. Most times the job is performed justly and accurately in conjunction with the science of the autopsy in mind. In some jurisdictions, mainly ones where the position is combined with the sheriff department, the coroner is also a law enforcement officer with the ability to carry a weapon. Strict coroner office personnel are generally unarmed.

The chief coroner serves as an administrator and leader of the office for death investigations. Most times, he or she hires additional lay persons, many with a medical or law enforcement background, as deputy coroners who are responsible for day-to-day investigations. Because these positions are based on the county divisions of a community, a coroner's office can be a small operation. The deputy coroner may have a varied job description, including crime scene investigation, preparation of death certificates, body transport, and autopsy assistance.

3.2 Medical examiners

Medical examiners are forensic pathologists with education as a medical doctor (either an M.D. or D.O.), completion of at least 4 years of anatomic pathology (5 years if also trained as a clinical pathologist), and at least 1–2 years of subspecialty training in forensic medicine. In total, approximately 13–15 years of training after high school is needed to become a forensic pathologist.

Medical school includes the study of basic medical science, including pharmacology, pathology, biochemistry, human physiology, and anatomy, as well as patient care and skills necessary for the practice of medicine. Following medical school, medical students enter a residency program where they specialize in an area of interest. The residency begins with a general year that previously was known and perceived by the public as an internship with continued responsibility and in-depth learning of the specialty over at least 3 years (family medicine, internal medicine) to surgery and pathology (5 years). Because the study of medicine has become so complicated, large branches of medicine have subspecialized into even smaller groups, such as cardiology (internal medicine), head and neck (surgery), and forensic medicine (pathology).

There are a couple of clinical forensic medicine programs in the United States that are areas of subspecialization within emergency medicine but these are not common. Currently in the United States, there are approximately 500–600 board-certified forensic pathologists and 30–40 in training each year [6]. Unfortunately, the number training is less than the greater number of pathologists who are retiring, and there is a projected severe shortage as the number of pathologists continues to age and retire. Even though the number of hospital pathologists performing autopsies has greatly declined, the number of medicolegal autopsies continues to increase as the population increases.

A medical examiner office is typically under the direction of a chief medical examiner who reports to a board of supervisors, state legislature, or the department head of the public health department. Under the chief may be an additional forensic pathologist. Current recommendations are the performance of 250 autopsies per year and no more than 350 per year per forensic pathologist. In large population cities, more than 7,000 deaths are reported to a medical examiner office with at least 4,000–4,500 cases accepted for jurisdiction. Not all cases accepted are necessarily

autopsied and may be certified by history or external examination. The autopsy rate is generally at least 60% and more commonly greater than 70–75% depending on staffing and workload. At 4,000 cases, more than 10 pathologists are needed to comply with the accreditation standards. It is clear that even by present-day availability there is a shortage of forensic pathologists and current training will be inadequate to meet future society's needs.

3.3 Notable forensic pathologists

Milton Helpern was the second chief medical examiner of the New York City medical examiner office. His support of research and teaching led to many forensic pathologists to later become chief forensic pathologists in other locations, spreading the concept of forensic medicine throughout the United States.

Dr. Thomas Noguchi is a modern-day pathologist who is best known as the "coroner of the stars" and formed the basis of the TV show "Quincy." This notoriety and positive portrayal of death investigations improved the public's perception of the science of forensic medicine. Present-day TV shows similarly dramatize the work of crime scene investigators (CSIs), criminalistics, autopsies, and courtrooms. These shows are not totally realistic but have raised an awareness of the science to most households. The negative side is the "CSI effect," in which juries expect similar results on cases presented to them even though the real forensic science does not support many of the concepts dramatized nor operate under the same time and money constraints as TV laboratories.

3.4 Medicolegal death investigators

Medicolegal death investigators serve as observation personnel and key assistants to the forensic pathologist at scenes. It is not possible for the forensic pathologist to visit all scenes and observe the body as it was found. It would be ideal, but just not possible. Death investigators are trained personnel with skills in observation, photography, and social skills to deal with the public during difficult situations. Their backgrounds are diverse and a cross-section of skills is helpful in any office. Some investigators have a funeral home background as funeral directors, others are emergency medical personnel, nurses, retired law enforcement officers, or physician assistants.

The ability to speak to physicians and understand medical terminology is an essential skill. Rudimentary photography skills are also very useful, as well as basics in evidence recovery and management. The ability to explain medical findings to families in lay language and serve as an intermediary between the pathologist and family is essential. Report writing skills, including grammar, are also necessary. Formal education can vary from a high school education to graduate school. Most offices now will not hire an investigator without a college degree. They are also not eligible for board certification via the American Board of Medicolegal Death Investigators (ABMDI) without at least an associate's degree. The ability to become a good death investigator resides more on the personality of the person than a particular degree or level of education. Many of the skills are learned on the job or through various continuing-education and certification courses. They generally work on a shift basis and are available to receive death calls 24 hours a day. In some offices, the death investigator also serves as a body transport team. In others, removals from the scene are contracted to local removal services or funeral homes. Typically, medicolegal death investigators are unarmed and do not serve as law enforcement officers unless they are part of a sheriff–coroner system.

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Exploration of New Indexes of Event-Related Potentials in Polygraph

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Abstract The research adopts the method of the simulation of crime situation, takes waveform deviation and wave area as the indexes, explores the EEG response of the guilty individuals and innocent individuals to target pictures, as well as familiar and unfamiliar pictures. The results show that there is no significant difference in the waveform deviation and wave area of the EEG response of the individuals of different genders to the three types of pictures; the response of the guilty individuals to the target pictures and familiar pictures is more similar; the response of the innocent individuals to target pictures and unfamiliar pictures is more similar. In the future research and practice, waveform deviation and wave area can be acted as the effective new indexes by which to examine whether an individual is guilty.

Keywords: Forensic science, Polygraph, EEG, Waveform Deviation, Wave Area, New Indexes.

1 Introduction

Legal case (including civil cases and criminal cases) related brain potential polygraph (lie detection) is based on event-related potentials (ERP) technology and is the brain cognitive process scalp potentials in which identification or recognition and judgment are conducted on the strange and familiar, case-related (probing stimulus) pictures and statements with different cognitive attributes through the recording-comparing the tested brain, so as to analyze the relationship between the tested individual and the investigated cases[1]. Because ERP test is mainly conducted on the suspicious criminal suspect that policemen find, it can make unique contributions to the case detection in the stage of criminal interrogation[2].

Miyake et al. (1986) first reported the use of the physiological parameter ERP in deception detection and found that the P300 amplitude of the key project (name of the subject) was significantly higher than that of the non-critical projects (the name of the other)[3]. Rosenfeld (1989) used ERP to conduct lie detection study successfully in the laboratory for the first time, taking the related words as the target stimulus and P300 amplitude as the index, found that the P300 of the experimental group (subjects pretend to steal items) was significantly greater than that of the control group (innocent subjects), and proved that ERP was feasible for lie detection[4].

It is Yang Wenjun (1992) who first conducted the preliminary researches on the possibility of ERP for lie detection in China; in the experiment, the memorized pictures of people were considered as target stimulus and pictures of strangers without memorization were regarded as non-target stimulus; the results showed that after the subjects saw strangers' pictures, no matter whether they admitted that, P300 amplitude and wave area could provide the basis for determining the true situations (95% of positive rate)[5]. Chen Xingshi et al. (1994) also conducted ERP test on subjects with strangers' pictures as the target stimulus, and again proved the feasibility of P300 for lie detection[6]. In 1999, Zhou Liang et al. conducted the experimental research of simulation of theft cases for the first time, made the lie detection research with the P300 wave as indexes, and found that under the experiment condition, P300, as an objective index was feasible for lie detection and the subjects who were familiar with the scene did not produce false positive results in the lie detector[7].

As for Guilty Knowledge Test (GKT) technology in the traditional criminal polygraph, whether the subjects have specific plot memory of the cases is determined on the of basis

of the difference of the physiological changes such as skin conductivity, respiration, heart rate and blood pressure on the related questions and unrelated questions to the cases and the purpose of distinguishing between perpetrators and the innocent ones is achieved[8]. In the lie detection of ERP, the combination of Oddball model and GKT technology forms the P300-GKT paradigm. The study tries to explore the subjects' EEG response by adopting more intuitive and simpler waveform deviation and wave area so as to obtain simpler solutions of distinguishing the criminals and the innocent.

2 Research Methods

2.1 Subjects

102 subjects were randomly selected, including 62 simulating perpetrators and 40 simulating innocents; the statistics showed that only 102 subjects were valid, including 74 men and 28 women, at the age from 18 to 32, with the average age 22.36; all of them had no physical or mental illness, had normal vision or corrected visual acuity, and got adequate compensation after the experiment.

2.2 Experimental Materials

The pictures that the tests rendered were divided into three groups; each group contains three types of pictures, that is, target pictures, familiar pictures and unfamiliar pictures.

2.2.1 The Stolen Item

The target picture of the test is one picture of the stealer, the familiar picture is one picture of one famous actor; the unfamiliar pictures are a total of seven other strangers' pictures.

2.2.2 Theft Place

The target picture of the test is one picture of the lab; the familiar picture is one picture of the work learning environment of the subjects; the unfamiliar pictures are a total of seven space pictures picture that subjects have never seen.

2.2.3 Packaging of the Stolen Item

The target picture of the test is one picture of the insurance company envelope; the familiar picture is one picture of the envelope of the subjects' unit; the unfamiliar pictures are a total of seven envelopes of other units that the subjects have never seen.

2.3 Experiment Design

The research simulates a burglary case in the laboratory environment and the articles in Room 808 include cabinets, tables, chairs, etc. The host forgets to lock the door before going out; after coming back 30 minutes later, the host finds a picture of the important personnel in the envelope inked with future insurance company in the cabinet is stolen and the envelope is thrown to the ground.

2.4 Experiment Program

2.4.1Theft Simulation

Simulation of the perpetrators: the subjects read instructions and complete the burglary personally: first enter the specified simulation scene Room 808, carefully observe its internal environment and articles agreement inside, and then rummage through the pictures; observe the characteristics of the pictures and packing materials, and then remove the pictures and throw the packing materials away, next leave the room. The simulating perpetrators don't admit theft during the test.

Simulation of the innocent: Inform an important item in Room 808 is stolen and now the suspects have been listed by the public security organs, so we will conduct the polygraph.

2.4.2 EEG Test

In accordance with the order of the stolen item, the theft place and the packing of the stolen item, the subjects are required to observe the pictures carefully and then answer the problems of the experimenter. Each group test is repeated for three times; the target pictures, familiar pictures, and unfamiliar pictures are randomly presented in the tests of each group; the target pictures appear for four times; familiar pictures appear for three time and the seven unfamiliar pictures respectively present once; each picture appears as long as 500 milliseconds and appears once every other 2000 milliseconds; the test records the data after the picture is shown for 1000 milliseconds. The test equipment is TH-B polygraph (lie detection) machine prototype researched and developed by Beijing Tongfang Shenhuo Joint Technology Development Co., Ltd.

Statistical analysis is conduced on all the experimental data by using SPSS16.0 software.

3 Results and Analysis

The EEG test mainly inspects EEG data at CZ point. According to the previous results of the researches on the effectiveness and practicability of ERP polygraph patterns, the study investigates the data within 1000 milliseconds in CZ point after the subjects' stimulus presentation, and tries to investigate indexes waveform deviation and wave area.

3.1 Difference Test of Waveform Deviation of Three Kinds of Pictures

The research conducts the analysis of variance on the subjects' waveform deviation of the three kinds of pictures, namely the picture of the stolen item, picture of the theft place and picture of the packing of the stolen item; the results show that as for the stolen picture, there is no significant interaction to the three kinds of pictures among gender, guilt and waveform deviation ($F = 0.501$, $p = .608$; $F = .968$, $p = .383$; $F = .070$, $p = .933$); there is significant interaction between guilt and the waveform deviation of

three kinds of pictures ($F = 182.654$, $p < .001$; $F = 182.698$, $p < .001$; $F = 153.403$, $p < .001$); the back testing shows that guilty subjects and innocent subjects are significantly different in the waveform deviation of the three kinds of pictures.

3.2 Differences Test of Wave Area of Three Kinds of Pictures

The research conducts the analysis of variance on the subjects' wave area of the three kinds of pictures on the picture of the stolen item and the results show that there is significant interaction between three kinds of pictures and the guilt ($F = 83.26$, $p < .001$; $F = 111.45$, $p< .001$; $F = 55.68$, $p < .001$). The back testing shows that the guilty subjects are not significantly different in the wave area of the response to the target pictures and familiar pictures; the guilty subjects are significantly different in wave area of the response to target pictures and unfamiliar pictures; the innocent subjects are significantly different in the wave area of the response to the target pictures and familiar pictures; the innocent subjects are not significantly different in wave area of the response to target pictures and unfamiliar pictures.

4 Discussions

4.1 Waveform Deviation Variance Analysis of Three Kinds of Pictures

The waveform deviation adopted by the research refers to the average of amplitude difference of three types of pictures at various time points within 1000 milliseconds of stimulation. The degree of similarity between different types of pictures is determined by investigating the pair-wise waveform deviation of the three kinds of pictures.

Statistical tests show that individuals of different genders, whether guilty subjects or innocent ones, show no significant difference in the picture of people, the picture of the theft place and the picture the stolen item packaging; there is no significant difference in male and female's judgment of pictures; therefore, in the future research and practical use, special stimulus materials for the individuals with different genders are not needed to be set.

The research results show that as for picture of people, picture of the theft place and picture of the stolen item packaging, the waveform deviation of guilty individuals to the target pictures and familiar pictures is significantly lower than the that of the target pictures and unfamiliar pictures, namely, guilty individuals have more similar reactions to the target pictures and familiar pictures; the waveform deviation of innocent individuals to target pictures and unfamiliar pictures is significantly lower than the that of the target pictures and familiar pictures, namely, innocent individuals have more similar reactions to the target pictures and unfamiliar pictures. The result is consistent with the research results of Zhou Let al. Because the guilty individuals contacted the information relating to the case in the past, the stimuli is familiar ones for guilty individual and there are more similar reactions; for innocent individuals, the information relating to the case is also strange stimulus, so the innocent individuals have more similar reactions to the target pictures and unfamiliar pictures. The results validate the assumptions. Therefore, in the future researches and practice, comprehensive consideration can be conducted with waveform deviation as an effective analysis index.

4.2 Wave Area Variance Analysis of Three kinds of Pictures

The study investigates the wave area of the subjects within 1000 milliseconds of stimulus presentation and the results show the individuals of different genders, whether guilty ones or innocent ones show no significant difference in wave area of the response to the three kinds of pictures; thus, in the future research and practice, special stimulus materials for the individuals with different genders are not needed to be set.

Research results show that the wave area of the guilty subjects shows no significant differences in the reaction to the target pictures and familiar pictures, and shows significant differences in the reaction to the target pictures and unfamiliar pictures; the results indicate that the guilty subjects have more similar reactions to the target pictures and familiar pictures, namely, for them, the target picture also belongs to the familiar one; while they have significantly different reactions to the target pictures and unfamiliar pictures; wave area of innocent subjects shows significant differences in the reaction to the target pictures and familiar pictures, and shows no significant differences in the reaction to the target pictures and unfamiliar pictures; the results indicate that the innocent subjects have more similar reactions to the target pictures and unfamiliar pictures, namely, for them, the target picture also belongs to the unfamiliar one, and they have more significantly different response to the target pictures and familiar pictures, namely, they belong to different types of pictures. Thus, in practical use, preliminary investigation on wave area of the three types of pictures can be more directly made through the bar chart so as to distinguish criminal individuals and the innocent ones.

4.3 Variance Analysis of the Response of Criminals and Innocent of Different Genders to Three Types of Pictures

The research results show that there is no significant difference in the

response of the individuals of different genders to the three types of pictures. The previous researches showed that male and female had different response to picture stimulus, but there is no such difference in the study because what the research inspects is ERP differences rather than non-behavior data; male and female have bigger differences in behavior, but no great differences in EEG. Therefore, in the future practice, the impact of gender factor doesn't need to be considered and different stimulus materials do not need to be set for subjects of different genders.

Research results show that as for the factors waveform deviation and wave area, guilt subjects have more similar response to the target pictures and familiar pictures; innocent individuals have more similar response to the target pictures and unfamiliar pictures. By investigating the ERP reaction of the individuals to the target pictures, familiar pictures and unfamiliar pictures, criminals and innocent can be effectively identified, thus, it can provide directions for detection work.

The case-related information the study investigates including the stolen item, the theft place and the packaging of the stolen item is related to the typical elements of the case and has strong stimulus for criminals; thus, when hey are presented to the individuals, the stimulus will be stronger than the other two types of stimuli for the real culprits. Besides, since the stimulus is the information that criminals know and is familiar stimulus, the reaction is consistent with the familiar stimulation. It is converse for the innocent subjects.

4.4 Problems and Prospects

Endogenous component P300 is the commonly-used identification index in ERP lie detection; it is incurred by extraneous stimulus of small probability; its amplitude is determined by the amount of the allocated attention resources and it reflects the context updating level in working memory. It is generally believed that P300 is mainly related with attention, episodic memory, working memory and other psychological factors [9]. The existing researches that involve the plots of cases basically adopt the form of case simulation and so the study does; the psychological pressure it makes the subjects produce and the psychological stress that the actual cases bring to criminal suspects are different, so it brings some difficulty to the promotion of the conclusions. Besides, in the experiments in the laboratory, the relevant conditions of the experiments are strictly controlled and the results of the study are relatively ideal; however, the actual case investigations may be affected by various factors; thus, relatively ideal target stimulus can't be collected and deviation emerges. Therefore, in actual practice, the researchers should try to protect scene information, especially the important related information, so as to ensure more accurate results can be obtained in the tests.

Although the existing researches have compared different types of stimulation EEG indexes and have found the differences, they do not give feasible test procedures and marking criteria; thus, further analysis can be conducted in the future researches. Furthermore, there are not enough researches on special group, especially people with psychopathy and antisocial personality disorder; whether the lie detection of the special group has the same effectiveness as the general population remains to be thoroughly discussed [10].

5 Conclusions

5.1 There is no significant difference in the response of individuals with different genders to the three types of pictures.

5.2 The response of the guilty individuals to the target pictures and familiar pictures is closer; the response of the innocent individuals to the target pictures and unfamiliar pictures is closer.

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Genetic Analysis of Y-Chromosome 17 STR in Four Indigenous Populations from Bandarban

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Abstract Despite rapidly growing understandings and dependency on single nucleotide polymorphisms (SNPs), highly variable autosomal 17 Y-chromosome short tandem repeats (STR) are still regarded as the most established method to differentiate individuals. Ethnic and cultural diversity of Bandarban area throughout the Chittagong Hill Tract (CHT) suggests that this hilly range play vital role in genetic evolution of the region. Our previous study suggests that this mountain area acted as a corridor to gene flow across the Indian midland to CHT of Bangladesh. In the present study, we analyzed 17 Ychromosomal short tandem repeat (Y-STR) haplotypes to investigate the Y-STR diversity of four indigenous populations from Bandarban. This study included 55 unrelated male samples from four ethnic populations (Tanchangya, Khumi, Khyang and Mro) were analyzed, among which 41 were unique and 14 Y-STR profiles are shared across the four populations. Khumi and Khyang exhibit relatively high degree of genetic homogeneity lower than 0.5, whereas Tanchangya and Mro represent the other extreme with all loci registering values above 0.5 for the same parameter.

Keywords: Forensic science, CHT indigenous Population, Bandarban, 17 Y-STR, Allelic frequencies, Gene diversity.

1 Introduction

Chittagong Hill Tracts (CHT) is located Southeastern part of Bangladesh and is surrounded by Mizoram state of India and Arakan of Myanmar in the East. The CHT area covers approximately 13.3 thousand km2 of three hill districts (Rangamati, Khagrachori and Bandarban) which indicates about 10% of land area in Bangladesh. Of these three areas, Bandarban is hilly but smaller in land area. There are 11 indigenous populations living in Bandarban (Tanchangya, Khumi, Khyang, Mro, Chak, Baum, Lusai, Pankhua, Chakma, Marma and Tripura).[1] The largest group is Chakma, Tripura, Marma, Tanchangya, and Mro which together make up to 90 percent of the indigenous population of the region.[2] Main tribal populations of Bangladesh Chakma, Marma, and Tripura, Khumi, Mro and Khyang are Tibeto-Burman speakers except Tanchangya which is Indo-European speaking tribe. Rest of the groups is less in percentage which indicates that the smaller groups are, overall, more vulnerable than the larger groups. Therefore it is essential to understand the origin and genetic diversity of these male population using uniparental (Ychromosome STR) markers.

A Y-STR is a short tandem repeat on the Ychromosome. Y-STR are often use for forensic, paternity and genealogical DNA testing. Unlike other chromosomes, Y-chromosomes do not come in pairs. Every human male has only one copy of that chromosome and there is no chance of variations of which copy is inherited, and also for not any shuffling between copies by recombination; so unlike autosomal haplotype, there is effectively not any randomization of the Y-chromosome haplotype between generations.[3] The human male should largely share the same Ychromosome as his father; gives or take few mutations. Thus Y-chromosome tends to pass largely intact from father to son, with limited but accumulated number of mutations that can serve to differentiate male lineage.[4-5]

Arlequin v3.2 and PowerStatV12 were used for calculating haplotype frequencies, matching probabilities,

and performing comparative population genetic analyses.[6-8] The global repository Y-Chromosome Haplotype Reference Database (YHRD) currently supports most frequently used haplotype formats, namely, nine-locus minimal (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, and DYS385a/b), SWGDAM-recommended 11-locus extended (minimal + DYS438 and DYS439), 12-locus PowerPlex (SWGDAM + DYS437), and 17-locus Yfiler haplotypes for comparison study.[9]

Our previous studies of Y-chromosomal biallelic[10] and autosomal STR polymorphisms of Tibeto-Burman revealed that these groups arrived in the CHT area during the Neolithic time and strong affinity to Northeast Indian Tibeto-Burman group.[11]

In this study, we have typed four (4) indigenous populations collected from Bandarban of CHT. Tanchangya (19), Khumi (10), Khyang (11), Mro (15), total of fifty five (55) samples were analyzed for 17 Ychromosome short tandem repeat (STR) loci.

2 Materials and Methods

Sample Collection

Blood samples were collected from those healthy male individuals, following procedure Helsinki revised declaration of 1983.[12]

DNA Extraction and PCR Amplification

DNA was extracted[13] and quantified by NanoDrop-100 (Thermo Fisher Scientific, USA). Amplification of the 17 Y-STR loci was performed using AmpFlSTR® Y filer ™ PCR amplification kit. PCR amplification was performed in Bio-Rad C1000 thermal Cycler (Life Science Research, 2000 Alfred Nobel Drive Hercules, CA 94547) according to the manufacturer's recommendations. The PCR amplified products were separated by capillary electrophoresis on ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Data analysis

The results were analyzed using GeneMapper v3.2.1. Alleles were designated according to the recommendations for the DNA commission of the International Society of Forensic Genetics (ISFG) guidelines for forensic STR analysis.[14] Arlequin v3.2 and PowerStat v12 were used for calculating allelic frequency, genetic diversity, haplotype diversity, and discrimination capacity, Neighbor joining (NJ) phylogenetic tree was constructed by Phylip 3.69 by using allelic frequency of studied population and five Indian tribal populations.[15]

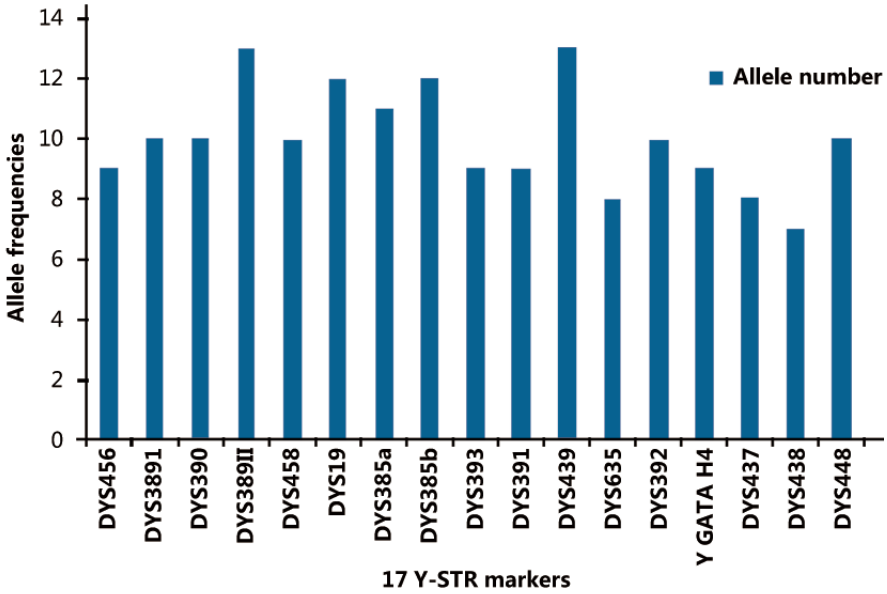

Figure 1. Allele information in four indigenous populations of Bandarban.

3 Results

Gene diversity and allelic frequencies for the 17 YSTR loci analyzed for 4 different Bandarban CHT collections are listed in Supplementary 1 (S1-S4). Markers DSY389II and DYS439 both are equally the most informative loci. The least discriminating and informative locus is the DSY438 (Figure 1). As expected, Mro possesses the highest average gene diversity (0.6325) followed by Tanchangya (0.5890), Khyang (0.2103) and Khumi (0.1266) respectively.

A total of 48 haplotypes for the

Table 1. Parameters of forensic interest in Bandarban populations using 17 Y filer Haplotypes.

Haplotypes	Tanchangya	Mro	Khyang	Khumi	Total
Sample size	19	15	11	10	55
No. of unique haplotypes (n=1)	13	15	7	6	41
No. of different haplotypes (n=2)	3	0	2	2	7
Genetic Diversity	0.5890	0.6325	0.2102	0.1266	0.3895
Discrimination Capacity	0.5692	0.5733	0.2281	0.2101	0.8727
Haplotype Diversity	0.9625	0.00	0.9166	0.8928	0.99069
Matching probability	0.0833	0.00	0.0375	0.1071	0.009309

17 Y-STR markers were identified in 55 unrelated four Bandarban tribal populations – Tanchangya, Mro, Khyang and Khumi. Among 41 (75.53%) haplotypes were unique and 7 were found in 2 individuals (Table 1). Two null alleles were detected at loci DYS439, one in Khumi and other in Khyang.

The haplotype diversity determined in Bandarban collections at 17 Y-STR loci was 0.99069 while the corresponding higher and minimal haplotypes were 0.9625 and 0.8928 in Tanchangya and Khumi respectively. The genetic homogeneity in Khumi is also reflected in their reduced average gene diversity (0.1266). In addition discrimination capacities in Khumi and Khyang are lower than that of Tanchangya and Mro (Table 1). The haplotype matching probability in all four groups determined was 0.009309. The Khumi shows the highest maximum match probability (0.1071) followed by Khyang 0.0833 and Tanchangya 0.0375, whereas Mro shows the unique match probability respectively.

Phylogenetic relationship between the four Bandarban populations and other neighboring populations were assessed using NJ tree (Figure 2). The genetic similarities observed in three populations Mro, Khyang and Khumi based on their Y-STR loci and are reflected in the high frequencies of Y haplogroup (O3a3c) as observed in our previous study.[11]

4 Discussion

DNA samples from 55 healthy unrelated male individuals of four indigenous community of Bandarban were analyzed. A total of 41 unique haplotypes were identified among 55 individuals. From this study it is found that in Bandarban populations' marker DSY389II and DYS439 both are equally the most informative loci. The least discriminating and informative locus is the DSY438. This finding is different from other study done with mainstream Bengali population[16] of Bangladesh. Therefore, this data reveals that 17 Y-STR might differentiate the indigenous people from mainstream population. The values of combined Matching Probability (MP), probability of discrimination (PD) and exclusion indicates that these results have enriched the databases of 17 Y-STR loci for four indigenous populations of Bandarban and exposed as an excellent tool for male human identification tests and population genetic analysis.

The significance increase in the proportion of unique haplotypes using 17 Y-STR markers compared to the minimal haplotype reflects the power of discrimination at different loci. The overall diversity of Bandarban population was 0.9906 while the corresponding values for the extended

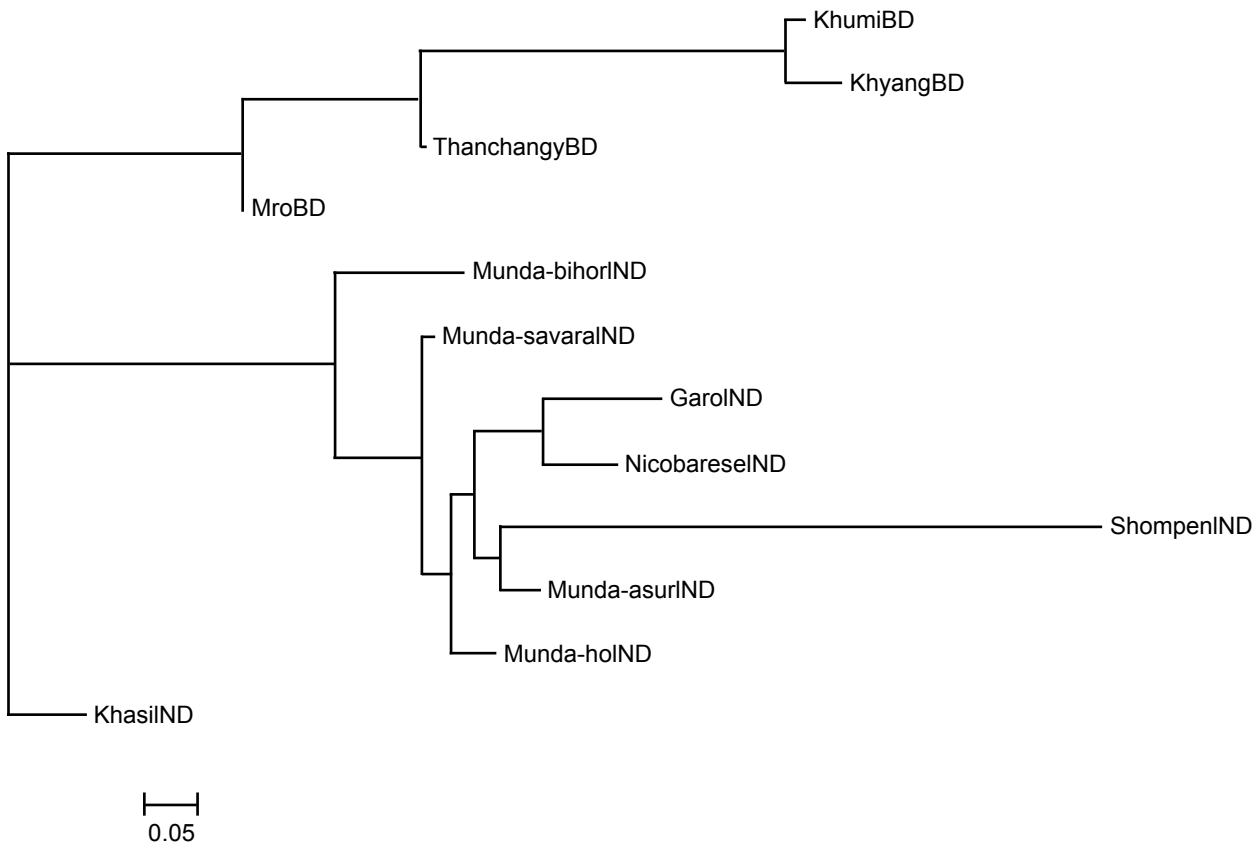

Figure 2. The NJ tree of four Bandarban collection compared with Indian population..

and minimal haplotypes were 0.9625 for Tanchangya and 0.8928 for khumi respectively (Table 1). The relatively lower diversity for Khyang (0.9166) and Khumi (0.8928) may be attributed to the reduced heterogeneity observed in these populations, which in turn may be the results of founder effects in this case. The only Indo-European speaking tribe Tanchangya has higher haplotype diversity than the Tibeto-Burman collection due to higher heterogeneity. The haplotype diversity for Mro is found zero (0.000) due to unique haplotype for each studied individual.

The NJ tree revealed that the Bandarban tribes (Khyang and Khumi) are more close within the populations but distant from Mro and Tanchangya but are distant from South and North Indian tribes[17] (Munda, Khasi, Shompen, Garo and Nicobarese). The unpublished data on autosomal STR depicted that these tribes are more related to East and Southeast Asian population and it is supported by their phenotypic traits thus implying their recent dispersal from Southeast Asia followed by admixture with local Tibeto-Burman populations. However Tanchangya is especially different in terms of language and autosomal STR reported in previous study.

5 Conclusion

In conclusion this study, 17 Y-STR database along with other database have been enlarged for population of Bangladesh and this can contribute considerably for individual identification and further research in population genetics. This is so far the first report on Bandarban indigenous population based on Y-STR analysis.

From the result of this study it can be concluded that, 17 Y-STR might be capable of differentiate tribal male from mainstream male of Bangladeshi populations. This 17 Y-STR also differentiate Indo-European speaking tribes from Tibeto-Burman tribes, which was previously found by autosomal STR data. Therefore, it can be concluded that this 17 Y-STR might play an important role to understand the genetic structure of indigenous population of Bandarban as well as CHT of Bangladesh. To confirm this hypothesis, increasing substantial number of samples and huge information needs to be gathered.

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Detection of Nanoscale Soil Organic Matter by Middle Infrared Spectrum for Forensic Science

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Abstract Soil is useful as a kind of trace evidence for forensic science. Thus it is very crucial to identify sources of soil. The nanoscale soil organic matter (NSOMs) can be used to differentiate soil sources because their constituents and contents are relatively stable with time but variant by location. In this study, NSOMs fromeighteen regions of Shandong Province in China were examined by middle infrared spectrum (4000–400 cm^{-1}). The results showed that the constituents and contents of NSOMs in eighteen samples were dramatically different; a NSOMfingerprint for each sample was drawn based on these characteristics. This suggests that a national or global NSOM fingerprint database could be rapidly established by the one-step middle infrared spectrum analysis for different soil samples, which will be helpful to determine crime scenes by comparing the middle infrared spectrum of forensic soil with the NSOMs fingerprint database.

Keywords: Forensic science, Middle Infrared Spectrum, Nanoscale Soil Organic Matter.

1 Introduction

Soil as important trace evidence can provide meaningful information for criminal investigation. The constituents of soil consist of organic matter, inorganic mineral grain, chemical precipitates (such as calcium carbonate and salt crystal), dead plants, animal matter, insect carapace, bacteria, soil algae, fungi, roots of higher plants, and so forth. Forensic detection of soil evidence has been performed by comparing soil components like organic matter, heavy and light minerals, oxides, stable and radioactive isotopes, pollens, diatoms, and microorganisms, meanwhile determining physical properties, such as color, distribution of particle sizes, and density [1]. Of these characteristics, the nanoscale soil organicmatter (NSOMs) are the most easily affected by environmental factors, for instance, wreathing, microorganisms present in the soil, plants in nearby soil, animal residues, and artifacts of human beings [2]. This means soil from different places was discrepant.However, NSOMs are relatively stable in a period, which may give actual information from crime scenes.

The main constituents of NSOMs were composed of lignin, lipid or fatty acid, carbohydrate, protein, cellulose, hopane, and so forth [3]. The main functional groups of these compounds can be rapidly identified and quantified by middle infrared spectrum (4000–400 cm^{-1}), which has become a powerful rapid assessment tool for determining soil properties [4]. In this study, eighteen soil samples from different regions in Shandong Province in China were selected and applied to Fourier transform infrared spectroscopy. Their NSOMs constituents (the main functional groups) and contents were rapidly and accurately determined. The results showed that there was a one-to-one relationship between the constituents and contents of NSOMs and the soil sources. Thus, a NSOM fingerprint was easily drawn, which suggests that it is possible to perform the rapid batch detections for a large number of soil samples to establish a national or global NSOM fingerprint database for forensic science.

2 Materials and Methods

2.1. Sample Collection. Eighteen soil samples were collected from different regions in Shandong Province in China. Samples were named as S1 to S18, representing Heze, Qufu, Zibo, Zhuangjia (Yantai), Penglai (Yantai), Zouping County (Bingzhou), Jiaxiang County (Jining), Kenli County (Dongying), Wulian County (Rizhao), Zaozhuang, Laiwu, Liaocheng, Rongcheng (Weihai), Jiaozhou (Qingdao), South Mountainous Area (Jinan), Taian, Changyi (Weifang),

Xiajin (Dezhou), and Jining, respectively.

2.2. Sample Preparation. Soil sampleswere grounddown into powder and dried at 120°C for 12 hours. Then 2.5 mg of the dried soil sample was mixed with 500mg of dry potassium bromide (KBr).The mixture was pressed into a mold (8 mm in diameter and 0.05 nm in thickness) with a pressure of 1×10^8 kg/m^2.

2.3. Infrared Spectra. Infrared spectra were recorded in frequency ranges from4000 to 400 cm^{-1} by a Fourier transform infrared spectroscopy (Bruker AXS VERTEX 70, Germany). The quantitative analysis of NSOMs was performed using KBr tablet method according to JJG-1996.

3 Results and Discussion

3.1. The Organic Spectra Peak Assignment. According to the previous investigation about the infrared spectra of soil organic matter (SOMs) [4–7], the peak assignments from S1 to S18 were analyzed. As shown in Figure 1, the peak assignment was varied with the soil source. The wide absorption peaks at 3591–3626 cm^{-1} were the characteristics of hydroxyl groups (–OH). The small peaks at 3107–3448 cm^{-1} and 2322–2360 cm^{-1} were the characteristics of –NH and NH$^+$, respectively. The peaks at 1612–1616 cm^{-1} belonged to C=C stretching. The peaks at 1429–1635 cm^{-1} were attributed to O–N=O/C=C stretching. The peaks at 1379–1436 cm^{-1} were assigned to C–N=O/C=O stretching. The broad and intense stretching peaks at 1379–1384 cm^{-1} belonged to C–H stretching. The peaks at 1006–1122 cm^{-1} were assigned to C–O stretching. The peaks at 1000–1100 cm^{-1} and 1014–1024 cm^{-1} belonged to the characteristics of P–O=R and C–O–C/C–O, respectively.The peaks at 740–779cm^{-1} were associated with of C–O–C or C–H stretching vibrations. The strong stretching peaks at 484–532 cm^{-1} and 469–457 cm^{-1} were attributed to P–Cl and C–X (halogen) stretching,

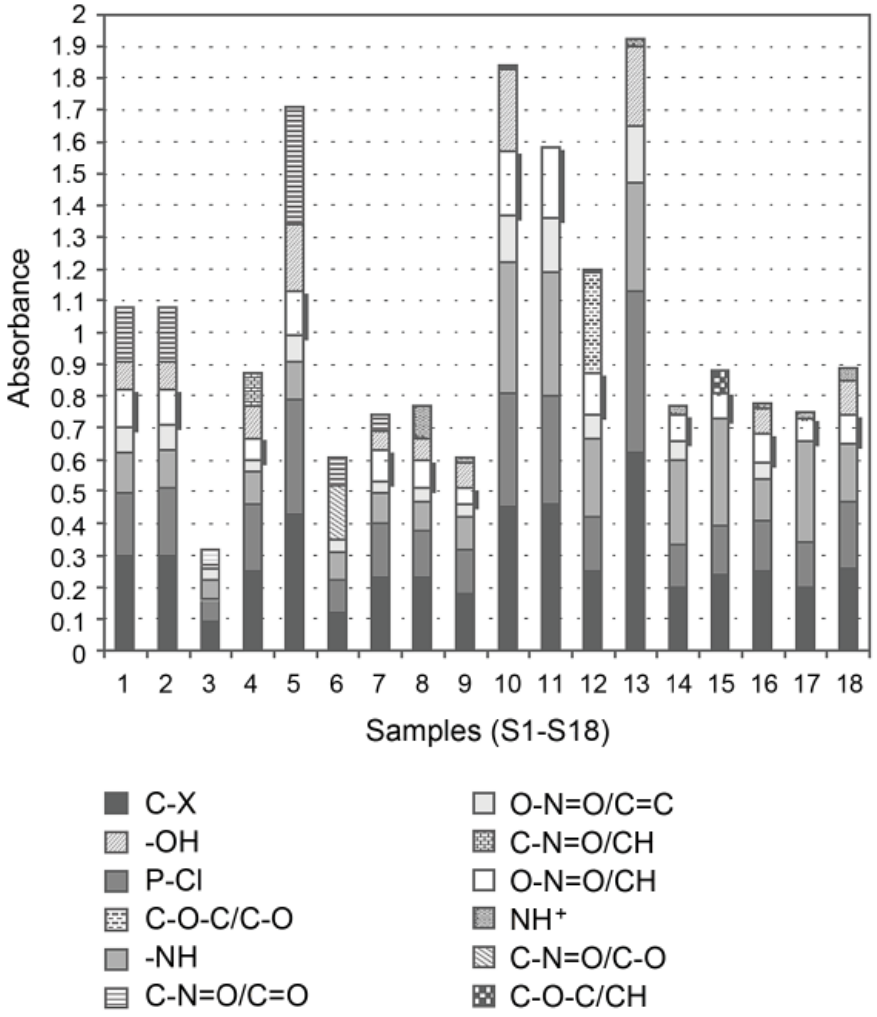

Figure 1. Infrared spectra of eighteen soil samples (S1 to S18).

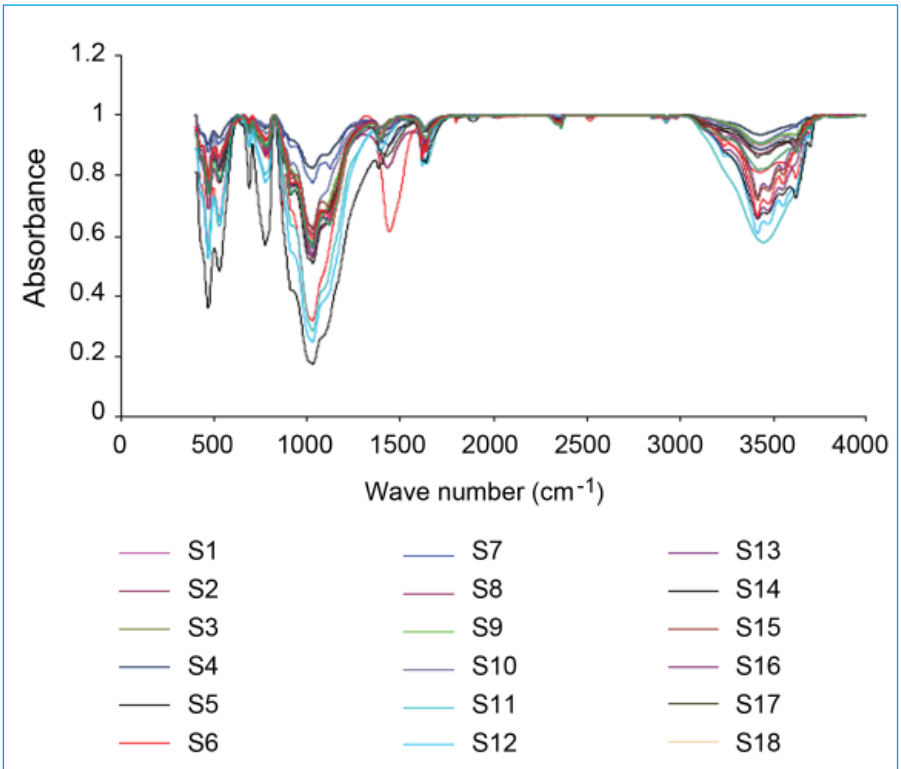

Figure 2. The number, type, and absorbance value of the main functional groups in NSOMs.

respectively.

3.2. Quantitative Analysis. The numbers and types of the main functional groups in NSOMs of S1–S18 were analyzed according to the infrared data. As shown in Figure 2, the number, type, and absorbance value of the main functional groups in NSOMs varied with the source of the soil sample due to a variety of geological environments in different regions. The samples S1, S2, S4, S5, S7, S8, S9, S10, and S12 were composed of seven functional groups. S6, S13, and S18 consisted of six functional groups. S3, S11, S14, S15, and S17 contained five functional groups.

The hydroxyl group (–OH) was present in all of 18 samples, but it was infinitesimal in S6, S15, S16, and S17. There were eleven types of functional groups in S18,which indicated that this sample contained abundant organic matter [8]. By contrast, S11 and S15 only contained seven types of functional groups, which suggested that the organic matter were poor in these soil samples. The relative contents of C–X and P–Cl groups in S13 were highest in all of the samples, which were probably associated with the situated chemical plants in the sampling locations [9]. However, there were scarcely any C–X and P–Cl groups in S3, which meant that the soil of the sampling location was hardly contaminated [10].

The types of the main functional groups were only halfway similar for each sample.The absorbance value of the same functional group was different as well.These differences will be a favorable reference for the analysis of the soil source using a computer program.

3.3. NSOM Fingerprint Database. The SOM fingerprint database of tested soil samples (S1–S18) could be established according to the difference of the main functional groups in SOMs identified by the middle infrared spectra. The scheme of SOMfingerprint database was designed as follows: the database was created and named as A. Then a table, namely B, was created in this directory, which contained some fields including number, type, and absorbance value of themain functional groups of NSOMs in each soil sample. Taking the table of S1 sample as an example, the procedure of the database table design was described (Table 1). Thus, the source of unknown soil sample may be rapidly identified using computer program through an SQL main organic functional groups query (SELECT * FROM TABLE B WHERE B.NumberOfKind = sample's number of kind AND B.Absorbance valueOftype1 = sample's concent of type1 AND. . .). This suggests that the establishment of the forensic SOM fingerprint database was feasible based on the infrared spectra analysis of SOMs, and the crime scenes will be rapidly locked using the forensic SOM fingerprint database.

4 Conclusions

In this study, Fourier transformmiddle infrared spectroscopy was applied to analyze eighteen soil samples from different regions of Shandong Province in China. The resulting infrared spectra showed that the constituents and contents of nanoscale soil organic matter (NSOMs) were varied with their sources. ThemainNSOMconstituents of tested samples were composed of –OH, –NH,NH^+, C=C,O–N=O/C=O, C–N=O/C=O, C–H, C–O, P–O=R, C–O–C, O–N=O/CH, P–Cl, and C–X with different contents. Based on these characteristics, the NSOM fingerprints of tested soil samples were established, which could be useful for the rapid analysis of crime scenes by comparing the forensic soil with the NSOM fingerprint database.

Table 1. The database table design of S1 sample.

Number of functional groups	Type of functional groups	Absorbance value of functional groups
7	C–X	0.31
	P–Cl	0.19
	–NH	0.13
	O–N=O/C=C	0.09
	O–N=O/CH	0.12
	C–N=O/C–O	0.08
	–OH	0.17

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Homicide-Suicide of an Older Couple Involving Complex Suicide: An Unusual Case

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Abstract Homicide-suicide is defined by a homicide committed by someone who subsequently commits suicide. "Dyadic death" term has been also used for these deaths as these incidents often involve a pair of persons. Homicidesuicides usually involve a male perpetrator, who commits one or more homicides and then commits suicide. They generally occur within a family and may involve children. There is a love or spousal relationship between the victim and perpetrator in most cases. Three types of homicide-suicides have been classified; familial, spousal/consortial, and extrafamilial types. They have different sub-classifications defined by age of individuals and relationship. Homicide-suicides in older couples were interpreted as dyadic deaths, dual suicide pacts, or homicide-suicides with altruistic motives which are attributed to the perpetrator.

We present a homicide-suicide of an older couple involving a complex suicide. A 77-year-old retired man, who lived with his 72-year-old wife, was described as hot tempered by his relatives. On the incident day, his wife reported to her neighbors that he was acting strangely, and she was worried. Later that evening, he killed his wife by hitting her on the head with an iron bar. He then killed himself by cutting his abdominal region and by hanging himself. A detailed death scene investigation and autopsies of the couple were performed.

The case presented is unique because it involves both homicide-suicide and complex suicide which is defined as using more than one method to induce death. In addition, a homicide-suicide case involving complex suicide has not been reported in the literature before.

Keywords: Forensic science, Homicide-suicide, Dyadic death, Death.

Introduction

Homicide-suicides are defined as events where a perpetrator kills one or more victims before taking their own life. "Dyadic death" term is also used for these incidents. Homicide-suicides usually involve a male perpetrator who commits homicide/s and then commits suicide. They normally occur within a family context. Children may be involved, but in most cases between the perpetrator and victim, there is a spousal or love relationship. The incidence of murder-suicide was found to be 0.2 to 0.3 per 100.000, in a review of the literature [1]. While the reported data show that homicide-suicide happens around the world, it also demonstrates the complications involved in prevention and the rarity of homicidesuicides.

Homicide-suicides are classified into three types, as spousal/consortial, familial, and extrafamilial types, with different sub-classifications defined by relationship and age of individuals involved [2]. Older couples' homicide-suicides were interpreted as dyadic deaths, dual suicide pacts, or homicide-suicides with altruistic motives attributed to the perpetrator.

In this case report, we present a homicide-suicide case involving an older couple and complex suicide.

Case Report

A 77-year-old retired man, who lived with his 72-year-old wife, was described as hot tempered by his relatives. In addition, he had undergone a craniotomy operation to drain a subdural hematoma after a traffic accident two months previously.

After this operation he had depressive symptoms.

On the incident day, his wife reported to her neighbors that he was acting strangely, and she was worried. Later that evening, he killed his wife by hitting on the head with an iron bar. He then killed himself by cutting his abdominal region and by hanging himself. A detailed death scene investigation and autopsies of the couple were performed.

At scene investigation there was a 20 cm long iron bar on the bed and a bloodstained knife on the carpet. The victim was found dead lying on the floor (Figure 1). In another room, the perpetrator was lying on the floor as the hanging material was cut by his relatives before the scene investigation (Figure 2).

At autopsy of the homicide victim there was a 4 cm long laceration on left temporal region (Figure 3). Temporal bone fracture (Figure 4), subarachnoidal hemorrhage and brain contusion were observed at internal examination. The death was due to brain contusion and subarachnoidal hemorrhage.

At autopsy of the perpetrator; on the upper left quadrant of abdominal region there were multiple superficial incised wounds (Figure 5). There was

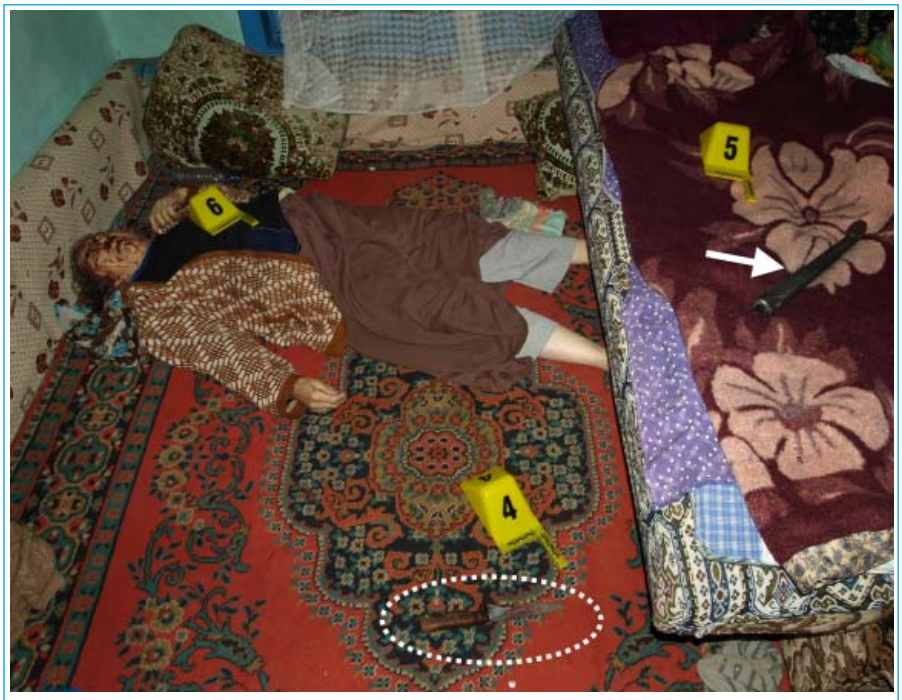

Figure 1. The homicide victim (72-year-old woman) is lying on the floor. There is a bloodstained knife on the carpet and an iron bar on the bed at the scene.

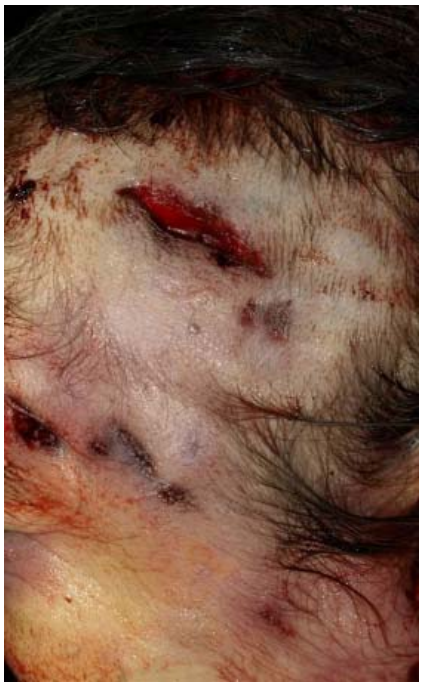

Figure 3. A 4 cm long laceration on left temporal region of the homicide victim.

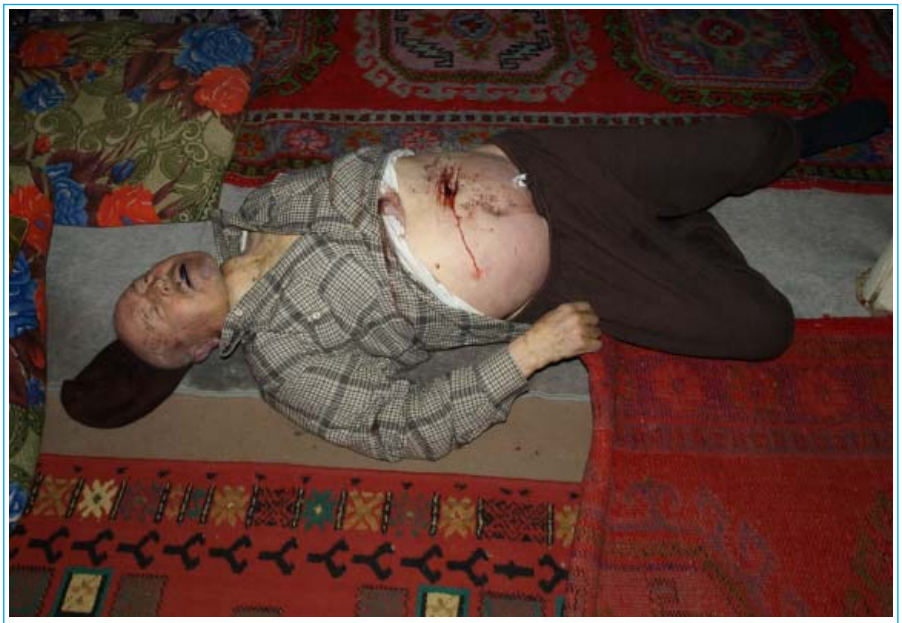

Figure 2. The perpetrator is lying on the floor as his relatives cut the hanging ligature. Incised wounds are observed at abdominal region.

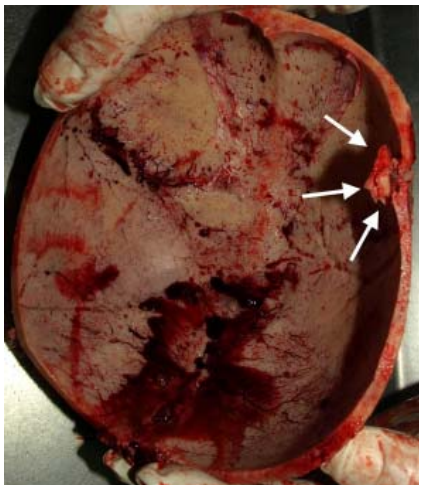

Figure 4. Left temporal bone fracture of the homicide victim.

a clothesline and a ligature mark on the neck (Figure 6). General asphyxia findings such as petechial hemorrhages, congestion and cyanosis were observed both at external and internal examinations. The death was due to mechanical asphyxia by hanging.

Discussion

Homicide-suicides are generally associated with the additive effects of many circumstances which lead to the emergence of homicidal-suicidal behavior. Schizophrenia, depression, and other mental disorders are implicated with homicide-suicides.

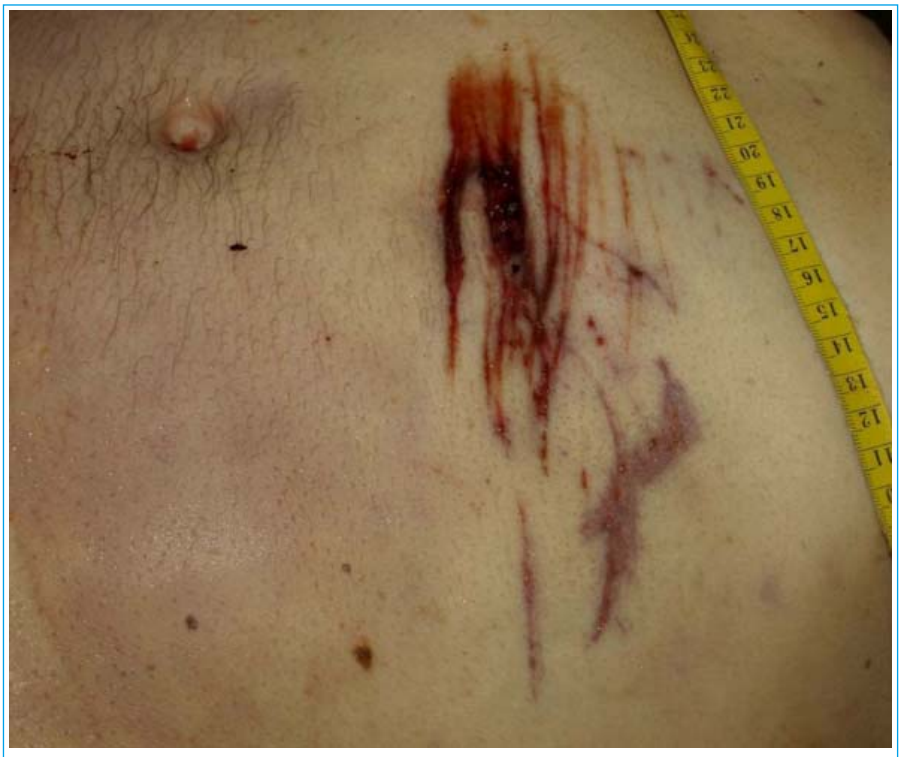

Figure 5. Multiple superficial incised wounds at upper left quadrant of abdominal region of the perpetrator.

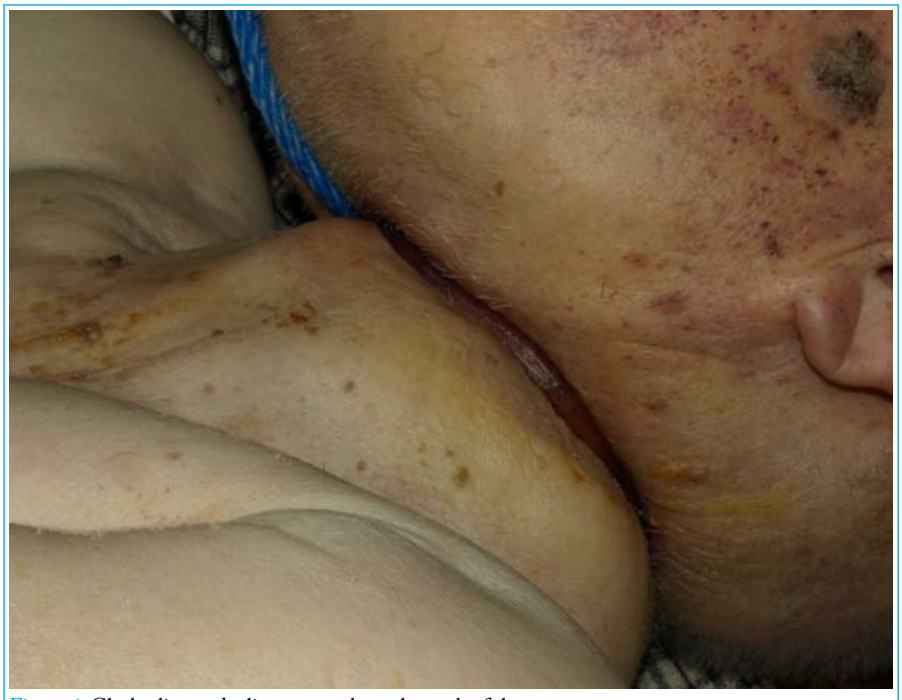

Figure 6. Clothesline and a ligature mark on the neck of the perpetrator.

They are also violent events with multiple cultural, environmental, health, and psychosocial antecedents [2–5]. In the presented case, male perpetrator had depressive symptoms after a craniotomy operation to drain a subdural hematoma.

In a study conducted in the same city (Konya, Turkey) which the case presented from, 10 homicide-suicides were determined out of total of 3940 death examinations and autopsies between 2000 and 2007. The victims' mean age was reported as 26 years (ranging from 2 to 50 years) and the mean age of the offenders was 32 years (ranging from 21 to 48 years) [6]. In the case presented, the perpetrator was 77-year-old and the victim was 72-year-old and they were significantly older than the homicide-suicide victims in this area.

There are commonalities in the details of homicide-suicides of spouses/couples. The risk factors usually include the relationship between homicide-suicide perpetrators (generally intimate) and homicide victims, homicide victim's sex (generally female), homicide-suicide perpetrator's sex (generally male), the victim's age (generally younger than the perpetrator), a weapon usage (generally a gun), and mental illness history in the homicide-suicide perpetrator (most often a depressed male). The resemblances are universal and similar findings were reported in many countries. In Scandinavia [7], South Africa [8,9], southern Europe [10], China [11], and the United States [12], most homicide-suicide victims are female, with most of their perpetrators being male intimate partners who killed them violently with weapons. The characteristics of our case are similar to the cases reported in the literature the perpetrator and the victim were intimate partners, the homicide victim was female, the perpetrator was

male, the victim was younger than the perpetrator, and a history of mental illness in the perpetrator was a male who had depressive symptoms.

Previous studies reported consistency of homicide-suicide rates in adult populations and this has been attributed to the constancy of depression and other psychiatric problems. Depression has been implicated in homicide-suicides committed by older men and those that involve couples [13–15]. Spouse caregivers will be at high risk of depression and other psychiatric disorders, as people continue to live longer, with the greater likelihood of frailty, comorbidity, and disability, including Alzheimer's disease and some related disorders [16].

In future research, the definition and types of homicide-suicide are important methodological considerations since homicide-suicides are sophisticated cases. It was reported that the homicide-suicide continuum, includes ideation, gestures, nonfatal attempts at homicide and/or suicide, and completed events at least for spousal/consortial events. It is likely that within each of these categories there are also variations and that antecedent variables are different, since these thoughts and behaviors are different from one another. For this reason, definitions of the dependent variable to include subtypes are required for accurate assessment and prediction of spousal/consortial homicidal-suicidal behaviors. For such rare events, this will be a methodological challenge [2].

The case presented in this paper is unique because it involves both homicide-suicide and complex suicide. A homicidesuicide case involving complex suicide which is defined as using more than one method to induce death has not been reported in the literature. The most frequently used suicide methods in Turkey are hanging, firearms and insecticide ingestion[17]. Before opting to use more lethal techniques by victims, methods of lesser lethality may be used. Pain, frustration, and anguish experienced by the suicidal individual are most likely associated with the conversion from lesser methods of lethality to greater ones [18]. As first suicide method, flexor surface of elbow and/or wrist cutting method was chosen in a study about complex suicides, as this method both gives pain and acute ache and takes too much time, second method was applied. In other words, the more lethal and second method was chosen by the victim due to pain, ache, and taking too much time [17]. The homicide-suicide perpetrator in the presented case has chosen incised wounds on the abdominal region, but he preferred hanging as the more lethal and second method.

It is concluded that similar cases which will be presented in the literature will contribute to assessment of homicidesuicide and complex suicide entities.

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An Autopsy Study on 68 Cases of Murder Suicides

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Abstract The present prospective study was carried from 2009 to 2012. During this period a Total of 3907 Homicides were Autopsied, of which 68 cases were recorded due to Murder Suicide Incidents, this contributed to 1.74% of Homicides Cases. A total of 174 victims died as a result of Murder. 89.72%(n-61) of the perpetrators were Male. The Most of the perpetrators belonged to the age group 31-40 years, contributing to 66.18% of the cases , and the least age group involved was between 11-20 and 41-50,contributing to 2.94% of cases. None of the Perpetrators above the age of 50 years were involved. Majority of the Victims were Females contributing to 78.74% (n-137) of cases. The Maximum number of Victims belonged to 31-40 year Age group contributing to 40.08%, the least age group involved was between 11-20 and 41-50,contributing to 7.47% and 12.64% respectively. There were no elderly victims recorded. Majority (30.88%;n-21) of the perpetrators involved were policemen and the least type of individuals involved were Soldiers contributing to 2.94%(n-02) of cases. The Relationship of the Victims revealed Divorced Spouse to be the Major Victims contributing to 29.31%(n-51) of the Victims, followed closely by Girlfriends and Children's contributing to 16.09%(n-28) each. The least affected were Housewives contributing to 11.49%(n-20) of the Victims. The Extramarital relationship (27.94%;n-19) and Jealousy(25%;n-17) were the Two Major Motives behind the Murder Suicide. The least provoking factors were Work stress(4.41%;n-03) and Disease conditions(2.94%;n-02). 91.18%(n-62) of the Perpetrators committed suicide by Gunshots. The least method adopted to commit suicide was Hanging contributing to 1.47% (n-01). 90.80%(n-158) of the Victims died as a result of Gunshot wounds. The least method adopted to Kill was by Ligature Suspension/ Hanging contributing to 1.72% (n-03) each. The perpetrator preferred in 30.88%(n-21)of Incidents Girlfriends House for Murder, followed closely by acts in Home in 25%(n-17) of cases. The commonest weapon of Choice was Firearm, which contributed to 91.18%(n-62) of the Cases, of which Handguns contributed to 75%(n-51) of cases. The least method adopted was by Ligature Suspension recorded in 1.47%(n-01) of cases. None of the incidents reported Murder and Suicide in different premises. The maximum distance between Suicide and Murder reported was 20 meters.

Keywords: Forensic science, Firearm, Death, Motive, Occupation, Psyche, Murder, Suicide, Sharp force, Hanging.

Introduction

A murder–suicide (or murdercide) is an act in which an individual kills one or more other persons before, or at the same time as, killing him- or herself. The combination of Murder and Suicide can take various forms. Homicide-suicides are rare but catastrophic events mainly occurs in intimate relationships and families. Though there is no national tracking system for murder–suicides in the United States, medical studies into the phenomenon estimate between 1,000 to 1,500 deaths per year in the US[1] with the majority occurring between spouses or intimate partners, males were the vast majority of the perpetrators, and over 90% of murder suicides involved a firearm. Depression, marital or/and financial problems, and other problems are generally motivators.

Violent deaths form a major menace in the urban society. the worst form of violent deaths are those of Murder Suicides(dyadic Deaths). With the onset of Urbanization and industrialization has created immense opportunities both in the form of employment, investment and Business. This has also contributed to unemployment, Jealousy, Divorces, Sexual partner, work stress, diseases physical and mental, financial stress, domestic stress. All this factors have contributed to crimes in society. The present study is focused on those crimes involving Murder Suicides during the period of 2009 to 2012. Though Public health organizations have recommended restricted access

and safe storage practices as means to reduce firearm injuries and deaths despite this precaution and legislation the firearm is always a weapon of choice for majority of Deaths.

Aims and Objectives

- To study the age and sex distribution of victim and perpetrator in murder suicides.
- To study the occupation of the perpetrator.
- To study the relationship of the victim with the perpetrator.
- To study the circumstances and motive leading to murder suicide.
- To study the causes of deaths in murder suicide.

Material and Methods

All the cases referred to the Legal medicine unit of the Ministry were Materials for study, Many of the cases were referred as Murders only proper Crime scene examination, Ballistic and Autopsy examination Concluded as Murder Suicide.

Standard procedures adopted in studying circumstances, crime scene evaluation and autopsy examination. Circumstances were analyzed from witnesses statement, past history of Marital problems, Relation crisis. Crime scene examination by collecting all the Exhibits from the Ammunition, Weapon and analyzing the Weapon involved in the Crime and the number of bullets discharged from it, Besides Gunshot shot residues from all the Victims and Perpetrators.

Perpetrator identified.

Suicide confirmed. By the Number of Gunshots,Acessability,Gunshot shot residue, Location and type of wound, Weapon and Bullets found in the body.

Standard ballistic examination procedures followed.

Radiological examination of the body prior to autopsy is conducted to identify the Bullets or its fragments location in the body to facilitate retrieval and also to know the possible track taken by the Trajectory.

Intrusion by third party ruled out by carefully analyzing the Ballistic report, Crime scene, Relationship of the weapon and Bullet, exclusion of injuries due to other weapon.

The data in relation to circumstances were collected from the Police in few cases directly from the witness, Crime scene examination was conducted by the Author in majority of the cases except Few which was analyzed by Photographs and Vediographs of the scene. Autopsy details were very vital besides the Ballistic report to conclude the Incident.

Observations

(Table 1-9)

Results

1. A total of 3907 cases of Homicides reported during the said period of Study, of which 68 cases were due to Murder Suicide, contributing to 1.74% of Homicides incidents. A total of 174 victims died as a result of the murder suicide incident.

2. Of the Total 68 Perpetrators, 61(89.71%) were Males and 07(10.29%). were Females involved in the Murder Suicide. The Maximum number of perpetrators belonged to 30-40 year Age group contributing to 66.18%,followed by individual belonging to 20-30year age group,27.94% and the least age group involved was between 10-20 and 41-50,contributing to 2.94% of cases. None of the Perpetrators above the age of 50 years were involved.

3. Of the Total 174 Victims, 37(21.26%) were Males and 137(78.74%). were Females Victims . The Maximum number of Victims belonged to 31-40 year Age group contributing to 40.08%,followed by Victims belonging to 21-30year age group and children's less than 10years with each contributing to 22.99% and 16.09% respectively. the least age group involved was between 11-20 and 41-50,contributing to 7.47% and 12.64% respectively. There were no elderly victims recorded.

4. Majority(30.88%; n-21) of the perpetrators involved were policemen, This was followed by Perpetrators belonging to Business Community (20.59%; n-14), and the Unemployed(17.65%; n-12). However Four House wife (5.88%) were perpetrators and Chronic Drug Abuser(4.41%; n-03) ,Soldiers(2.94%; n-02) and Security Staff(4.41%; n-03) were involved in the Case.

5. Divorcee contributed to 29.31%(n-51) of the Victims, followed closely by Girlfriends and Children's contributing to 16.09%(n-28) each. The least affected were Housewives contributing to 11.49%(n-20) of the Victims. The other category of victims were Paramours, contributing to 14.37% (n-25) of the Victims.

6. The Extramarital relationship (27.94%; n-19) and Jealousy (25%; n-17) were the Two Major Motives behind the Murder Suicide. Other Factors like Infidelity(16.18%; n-11) and Domestic(17.65%; n-12) issues were the provoking factors. The Financial owes (5.88%; n-04),Work stress(4.41%;n-03) and Disease(2.94%; n-02) related issues also acted as triggering factors.

7. Indicates the different Causes of Death of the Perpetratorand Victim.

Majority of the Perpetrator ,91.18%(n-62) committed suicide by Gunshots. The least method adopted to commit suicide was Hanging contributing to 1.47% (n-01). The other methods adopted to commit Suicide were Poisoning in 2.94%(n-2) and Sharp Weapons in 4.41% (n-03) of Cases. Majority of the Victims ,90.80%(n-158) died as a result of Gunshots. The least method adopted to Kill was by Ligature Suspension/ Hanging contributing to 1.72% (n-03). The other means of killing the Victims were Poisoning in 4.02%(n-07) and by Sharp Weapons in 3.45% (n-06)of Cases.

8. The most preferred place (30.88%; n-21)of Incidence is Girlfriends House, followed closely by Incident in Home(25%; n-17). The least preferred place was in Public Place and Place of Work which contributed to only 2.94%(n-02) each. Other places preferred for the act were Paramours House(16.18%; n-11), at the residence of the Divorced spouse(14.71%; n-10) and outside the house in 7.35% (n-05) of Incidents.

9. The common weapon of Choice was Firearm, which contributed to 91.18%(n-62) of the Cases, of which Handguns contributed to 75%(n-51) of cases, other weapons like M16 (11.76%; n-08) and MP5 (4.41%; n-03) were also used for the act. The other means of Killing adopted were by Machete (Sharp weapon) contributing to 4.41%(n-03) of cases, Organophosporus poison in 2.94%(n-02) and by Ligature Suspension in 1.47%(n-01) of cases.

10. The majority of the Suicidal injuries involving firearm were single Shot to the Head over the Temple in 94% and over the Chin in 4% and Left side chest in 2% incidences.

11. All the perpetrators died immediately after the Murder and the longest interval reported was suicide involving sharp force fatalities wherein the perpetrator committed suicide within 30minutes after the act.

Discussion

The present prospective study carried out for over a period of Four years(2009-2012),A total of 68 cases of Murder Suicide were reported(1.74%) claiming over 242 lives(174 +68). In a similar study conducted by Roma. P et al(2012)[2] in Italy, Between 1985 and 2008, 662 cases of homicide-suicide were identified, with 1776 deaths. The

Table 1. Total Number of Homicides and Murder Homicides.

Year	Total Homicides	Number of Murder -Suicides	Percentage
2009	877	14	1.60%
2010	976	27	2.77%
2011	1112	15	1.35%
2012	942	12	1.27%
TOTAL	3907	68	1.74%

Table 2. Perpetrators Age and Sex Distribution.

Perpetrator	10-20	20-30	30-40	41-50	51-60
Male	02	18	39	02	00
Female	00	01	06	00	00
Total	02	19	45	02	00
Percentage	2.94%	27.94%	66.18%	2.94%	00

Table 3. Victims Age and Sex Distribution.

Victims	<10	11-20	21-30	31-40	41-50
Male	11	03	03	19	04
Female	17	10	37	62	18
Total	28	13	40	71	22
Percentage	16.09%	7.47%	22.99%	40.80%	12.64%

Table 4. Occupation of the Perpetrators.

Sl No	Occupation	Total No	Percentage
01	Buisnessmen	14	20.59%
02	Policemen	21	30.88%
03	House Wife	04	5.88%
04	Unemployed	12	17.65%
05	Drug Abuser	03	4.41%
06	Farmer	09	13.24%
07	Soldier	02	2.94%
08	Security Staff	03	4.41%

Table 5. Victim'S Relationship with the Perpetrators.

Sl No	Relationship	Total No	Percentage
01	House Wives	20	11.49%
02	Girlfriends	28	16.09%
03	Divorcee	51	29.31%
04	Children	28	16.09%
05	Paramour	25	16.09%
06	Inlaws	22	12.64%
Total		174	

Table 6. Motives Behind the Murder Suicide.

Sl No	Relationship	Total No	Percentage
01	Infidelity	11	16.18%
02	Jealosy	17	25%
03	Domestic	12	17.65%
04	Economical/ Finance	04	5.88%
05	Extramarital	19	27.94%
06	Work Stress	03	4.41%
07	Disease	02	2.94%

Table 7. Causes of Death.

Sl No	Causes of Death	Total No Perpetrators	Percentage	Total No.Of Victims	Percentage
01	Gunshot Wounds	62	91.18%	158	90.80%
02	Hanging	01	1.47%	03	1.72%
03	Poisoning	02	2.94%	07	4.02%
04	Sharp Weapons	03	4.41%	06	3.45%

Table 8. Place of Incident.

Sl No	Place Of Incidence	Total No	Percentage
01	Home	17	25%
02	Girlfriend House	21	30.88%
03	Divorcee's Residence	10	14.71%
04	Outside House	05	7.35%
05	Publice Place	02	2.94%
06	Paramours House	11	16.18%
07	Work Place	02	2.94%

Table 9. Types of Weapon of Assault.

Sl No	Weapon	Total No	Percentage
1	Handguns	51	75%
2	M16	8	11.76%
3	Mp5	3	4.41%
Total	Firearms Used	62	91.18%
	Other Means To Death		
1	Organophosporus Insecticide Poison	2	2.94%
2	Sharp Weapons-Machette	3	4.41%
3	Work Place	1	1.47%

present study was in a population of 30,00,000/ i.e. on average one incidence per 100000 population per year, in a similar study conducted by Roberts. K. et al(2010) [3] in South Africa, for the years 2000 to 2001, The incidence was 0.89 per 100,000, higher than the international average. In the present study Majority of the Perpetrators were Male, comprising 89.71%(n-61) of the incidences and Majority of the victims were Females comprising 78.74%(n-137) cases, similar were the views of Adinkrah M,(2014)[4] and the observations made from Galta K,(2010) [5] ,who concluded 90% of the perpetrators were Male and 80% of the Victims were Females. Toygar M (2013) [6] , De Koning E, Piette MH(2014 [7] and Cengija M(201)[8] made similar observations ,who concluded that Males were the major perpetrators with97%, 86% and 82% of incidences respectively. In the present study the Maximum incidences,66.18%(n-45) involving Perpetrators belonged to 31-40years,followed by individuals belonging to 21-30years(27.94%; n-19),similarly the Majority of the Victims belonged to the aged group 32-40(40.80%;n-71),the results are close to the observations made by Roberts K(2010) [3] but this observations are contrary to the claims made by Verzeletti A (2014) [9] who concluded that the Victims were usually young (30% was in the 21-30 years class) and males (64%).Majority of the perpetrators were men in uniform, Policemen (30.88%; n-21),followed closely by the individuals from the Business group(20.59%;n-14) and Unemployed (17.65%;n-12).The least involved were Soldiers(2.94%;n-02) and Security Staff(4.41%;n-03). All this individuals had one common factor , i.e. easy access to the weapon. Hence the Accessibility of the weapon is another important provoking factor which cannot be neglected. In the present study all the Suicides were committed immediately after the Murder with a delay of Maximum 30minutes in one case, similar are the views of Bourget D(2010) [10] and Shiferaw K(2010) [11] . In the present study the Extramarital relationship and Jealousy were the major Motive behind the Incidence comprising 27.94%(n-19) and 25%(17) respectively, this were closely followed by Infidelity and Domestic Factors with 16.18%(n-11) and 17.65%(n-12) respectively. in a study conducted by Hellen F(2014) [12] ,he identified Breakdown of the marital relationship and social descent as probable leading motives. De Koning E, Piette MH(2014) [7] in his study made closer observation with the present study wherein, he concluded that the main motive for offenders to execute M-S is amorous jealousy (56%), followed by familial, financial, or social stressors (27%),In the present study financial and stress factors contributed to 5.88%(n-04) and 4.41%(n-03) respectively. The observations made by Roma. P.(2014)[2] were close to the present study wherein, The most common motivation was romantic jealousy, followed by socio-economic stress. Over all the Family related factors were the major motivating factors for the incidence similar were the views of Gupta BD, Gambhir Singh O(2008) [13] . It is obvious that ,all the motivating factors have a longstanding impending factors on the Psyche of the perpetrators which has manifested into Murder Suicide Incident Galta K(2010) [5] . This Instability in the minds of the Perpetrator like fear of losing the family, reputation, sexual jealousy, infidelity has provoked him to puruse this act. The major cause of death were as a result of Firearm related deaths ,90.80%(n-158) followed by Sharp weapon injuries in 4.41%(n-03) of cases ,similar were the observations made by Grabherr S(2010) [14], Shiferaw K(2010)[11] and du Plessis M, Hlaise KK(2012) [15] . In the present study an incidence of Hanging formed the mode of Murder Suicide incident .The common weapon of assault was Firearm in 91.18%(n-62) of the incidences, with handgun contributing to major part of firearm ,75%(n-51),similar are the views of Shiferaw K(2010) [11] and Roma

P(2012) [2] ,In the present study the Majority of the incidence,30.88%(n-21) occurred in Girlfriends house, follow closely by the incidences occurring in Home of the Perpetrators, 25%(n-17) and the seperated partners(divorcee) residence in 14.71%(n-10) of the incidence, the least place preferred by the perpetrators were the Public place and Place of Work. All this clearly indicates the Family related factors, Jealousy and fear of loosing the partner to others and reputation culminating into a triggering factor for Murder Suicide incident. Roma P(2012) [2] De Koning E, Piette MH(2014) [7] . In the present study the psychiatric evaluation of the Perpetrators were not included and all the Standard procedures were followed to establish the Perpetrator and Suicide. The majority of the Suicidal injuries involving firearm were single Shot to the Head over the Temple in 94% and over the Chin in 4% and Left side chest in 2% incidences.

Conclusions

- Perpetrator had a fair access to firearm make.
- Firearm is the preferred weapon in murder suicide.
- Males form the major group of perpetrator.
- Family (spouse/girlfriend/ separated partner) related issues are the major motivating factor in murder suicide
- Majority of the crime committed at the place of spouse/girlfriend/separated wife.
- Suicide after murder at the place of crime are the commonest and usual pattern.
- Standard procedures to be followed in studying the circumstances, crime scene evaluation and autopsy examination to confirm the suicide and crime.

Recommendation

Psychiatric evaluation /counseling essential in family related issues.

Firearm legislation to be renewed.

Acknowledgements

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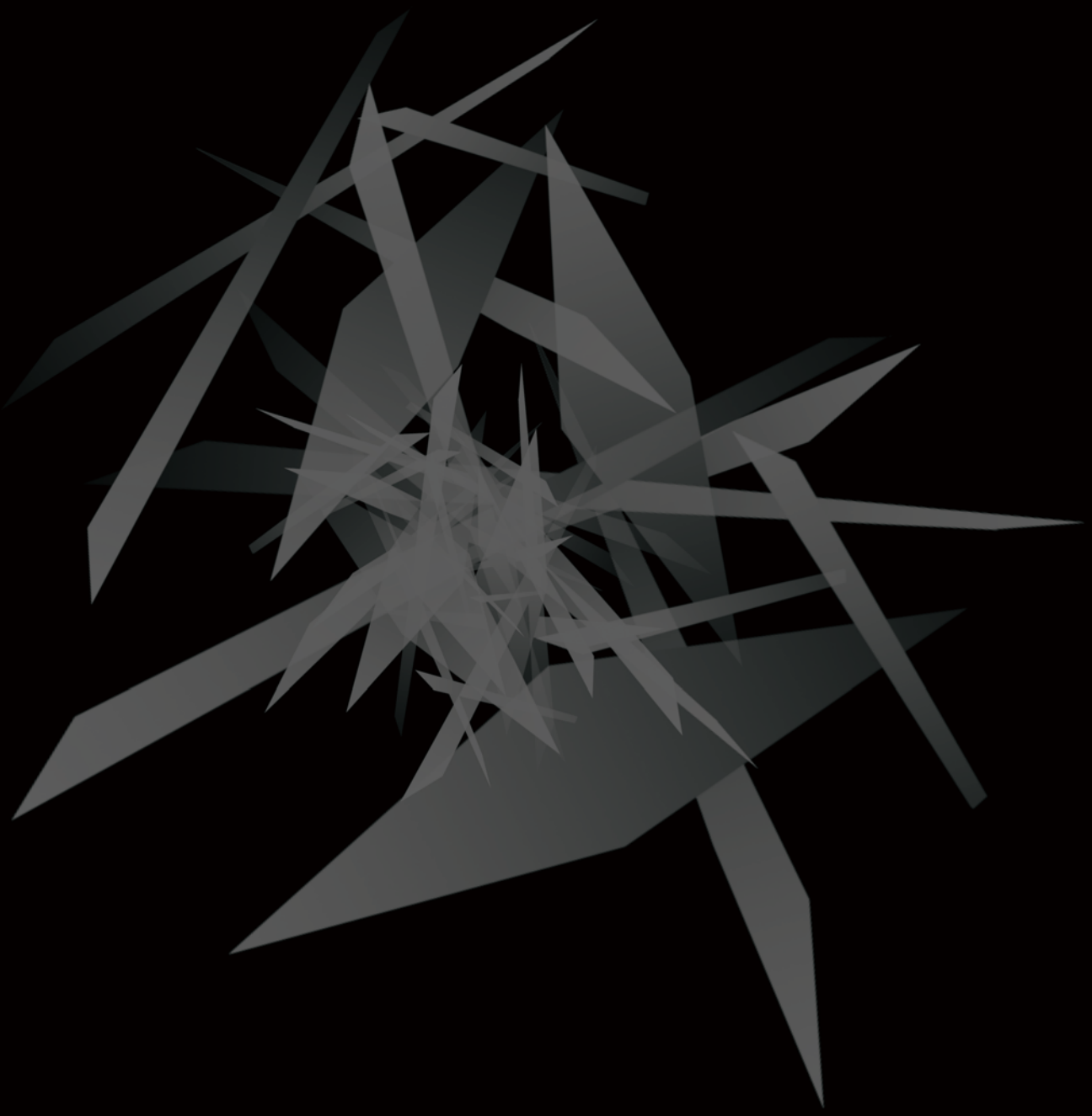

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