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ADVANCED REVIEW

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An interdisciplinary forensic approach for human remains identification and missing persons investigations

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Abstract

The resolution of unidentified human remains (UHR) and long-term missing persons (LTMP) cases is paramount for administrative, legal, and humanitarian reasons. There are various forensic profiling methods for human identification; however, their utility is dependent on several factors. First, UHR can be found in different stages of decomposition, so the availability and diversity of post-mortem (PM) information will differ. Second, the availability and totality of the LTMP's ante-mortem (AM) information will differ. Therefore, the suitability of existing methods will be dependent on the quality and quantity of PM and/or AM data available for comparison. Visual recognition is the simplest and quickest method, but typically not practiced or possible, owing to the altered, fragmented, or skeletonized state of UHR. Primary forensic profiling methods involve the comparison of fingerprint, dental, DNA, and medical data. Secondary forensic profiling methods from anthropology, radiology, geochemistry, and anatomy disciplines can provide supplementary evidence to support comparative identification approaches. Emerging forensic molecular technologies such as genomics, microbiomics, epigenetics, and proteomics, together with individual digital footprints from personal devices, offer new investigative leads for establishing identity. However, despite the success of these individual methods, their limitations must be considered when used in isolation. Through the development of a guiding forensic examination framework, this review endorses an interdisciplinary response to unidentified and missing persons investigations, where various forensic specialists collaboratively examine UHR using a suite of contemporary forensic profiling methods to produce multiple and/or different lines of evidence to link them effectively, efficiently, comprehensively, and systematically to LTMP.

This article is categorized under:

Forensic Science in Action/Crime Scene Investigation > Special Situations and Investigations

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forensic profiling, human identification, interdisciplinary forensic investigation, missing persons, unidentified human remains

1 | INTRODUCTION

The forensic examination of unidentified human remains (UHR), whether it be for long-term missing persons (LTMP) cases, disaster victim identification (DVI), or humanitarian reasons, is crucial to provide the forensic evidence required to establish the identity, and cause and manner of death, of an unknown deceased person where possible. The ability to be able to scientifically link UHR and LTMP cases is influenced by three main factors: the preservation of UHR [e.g., decomposition stage and peri- or post-mortem (PM) trauma], the quality and quantity of ante-mortem (AM) information, and the availability of human identification techniques (Blau et al., 2021). The advantages and disadvantages of forensic profiling methods, and their use in isolation or in combination, are considerations when police and forensic investigators are determining the most appropriate examination strategy in light of the case context and complexity.

International guidelines devised by the International Committee of the Red Cross (ICRC), International Criminal Police Organization (INTERPOL), and International Organization of Migration (IOM) recommend the use of one or more of the primary forensic profiling methods to identify deceased individuals; specifically the comparison of fingerprint, dental, and DNA data (ICRC, 2013; INTERPOL, 2018a; Robins, 2019). This data should be generated by fingerprint examiners, forensic odontologists, and forensic DNA specialists, respectively, as part of the PM examination conducted by a forensic pathologist. The comparison of unique and/or rare medical features observed in the PM examination can also be used as a primary method of identification (ICRC, 2022; Ubelaker et al., 2019). Depending on the state of the unidentified body, a forensic radiologist and forensic anthropologist may assist with this component of the PM examination. Published standards and best practice procedures are available to inform the conduct of these scientific analyses in specific operational contexts; however, the focus has been on mass fatality incidents to date [e.g., various American National Standards Institute (ANSI)/American Standards Board (ASB) best practice recommendations].

If identification is not possible using these comparative approaches, either because the forensic evidence is not present on the body or body part located (e.g., no or insufficient fingerprints, teeth, DNA, or medical devices with unique serial numbers) or the required AM data is not available (e.g., no existing fingerprint, dental, DNA, or medical records), other forensic profiling methods can be applied to offer new investigative leads. These may include radiocarbon dating, isotope analysis, craniofacial reconstruction (CFR), DNA-based ancestry and appearance estimation, forensic microbiomics, forensic epigenetics, forensic proteomics, and forensic investigative genetic genealogy (FIGG). Alternatively, some of these secondary forensic profiling methods may be used early in an investigation to assist with establishing the coronial significance of the remains or developing a biological or physical profile of the unknown individual. The diversity of forensic human identification techniques available therefore enables the relevant legal authority to consider different lines of evidence produced using different profiling techniques by forensic experts from different disciplines.

There is a need for a shift from a multidisciplinary approach, where forensic experts independently examine UHR through their own discipline's lens, providing supplementary (but not necessarily complementary) clues to the identity of UHR, to an interdisciplinary approach, where forensic experts collaboratively examine UHR, integrating findings from multiple forensic disciplines to arrive at a holistic identification that is able to be presented to the relevant legal authority such as a coroner, medical examiner, or identification board/committee/commission. The implementation of such an approach would facilitate the effective, efficient, and systematic identification of UHR that will aid death investigations in a variety of case contexts involving one or more deceased persons. This review presents the current and emerging forensic profiling methods available for forensic human identification and highlights how their interdisciplinary use will ensure accurate and comprehensive conclusions are reached regarding identity and the timely resolution of LTMP cases.

2 | VISUAL RECOGNITION

The human face and body is unique and holds information about an individual's identity, as well as age, sex, and ancestry (Caplova et al., 2018). Distinguishing physical features, such as the location and appearance of birthmarks, scars, moles, deformities, and body modifications like tattoos and piercings may provide information to individualize UHR (Prahlow & Byard, 2011). Tattoo pigments especially are able to withstand severe burns (Lee et al., 2008) and experience minimal morphological changes during decomposition, with most changes occurring in the bloat stage (Probert et al., 2021). Rapid alteration to physical features with advancing decomposition, or the length of time a person has been missing, will provide challenges for identification (Wilkinson, 2014). Visual recognition is also implausible for human remains that have been skeletonized or subject to trauma.

Visual recognition is generally performed by family members or a recognizant person (Caplova et al., 2018; Hanzlick & Smith, 2006; New South Wales Coroners Court, 2020; Uzün et al., 2012). As such, it is not considered scientific (Caplova et al., 2018) and carries a risk of misidentification, suggesting that it should be considered only when there is other scientific evidence supporting identity. Visual recognition supported by circumstantial evidence found with human remains such as identification documents, other personal effects, and/or clothing can also be unreliable. The International Commission on Missing Persons (ICMP) have recently reported numerous cases of victim misidentification following the conflicts in the Western Balkans in the 1990s attributed to the past use of visual recognition or personal effects which are now inconsistent with the genetic identification results (Parsons et al., 2019).

3 | PRIMARY FORENSIC PROFILING METHODS FOR IDENTIFICATION

The most reliable methods of identification involve scientific approaches where known information (i.e., AM data) about a LTMP and scientific information (i.e., PM data) about a set of UHR can be compared and a "match" is made (ICRC, 2013). The three primary scientific methods traditionally used by forensic practitioners for identification purposes include comparative fingerprint, dental, and DNA data analysis (INTERPOL, 2018b; Prahlow, 2010), with medical data increasingly recognized as important AM and PM data to collect and compare as part of the identification process (ICRC, 2022; INTERPOL, 2018b; Ubelaker et al., 2019). During the PM examination, the relevant forensic specialists will examine the remains, estimate post-mortem interval (PMI), record the fingerprints, chart teeth and dental restorations, collect biological samples for DNA profiling, detect pathological conditions (e.g., diseases) and signs of trauma (e.g., fractures), and document any unique or rare skeletal, dental, or medical features, if this evidence is present (ICRC, 2013). This biometric information can then be compared with AM data collected from the LTMP's home, family, friends, colleagues, medical or dental practitioners, or other authorities, including any existing fingerprint, dental, medical, and/or DNA profile records in local, national, and international systems.

3.1 | Fingerprint analysis

Human identification using friction ridge skin such as fingerprints relies on two fundamental principles: (1) fingerprints are unique to an individual, and (2) fingerprints are a permanent characteristic of that individual (Fieldhouse & Stow, 2016). In cases of UHR investigations, a forensic fingerprint expert analyses the UHR's fingerprints and compares them with existing fingerprint records (ANSI/ASB Best Practice Recommendation 007, 2018). The records could be available through national (e.g., National Automated Fingerprint Identification System in Australia, IDENT1 in the United Kingdom (UK)) and international fingerprint (and palm print) databases (e.g., Automated Fingerprint Identification System (AFIS)), or fingerprint data from criminal and noncriminal persons stored by relevant authorities for other purposes.

However, there are a number of limitations that restrict the use of fingerprint analysis for human remains identification. This technique is not possible for skeletonized remains or those subjected to severe trauma such as burning or mutilation. In addition, the condition of the friction ridge skin at the time the body is discovered can affect the ability to recover quality PM fingerprint impressions (Fieldhouse & Stow, 2016). Most importantly, recovered fingerprints will only aid identification if there are relevant AM fingerprint records available for comparison. The holdings of law enforcement databases like AFIS are often limited to arrested persons, police applicants, and a short list of other occupations, so they may not always be useful for LTMP investigations. Developments in friction ridge analysis, including

the examination of palm prints (Ungureanu et al., 2020; Zhong et al., 2019), finger texture patterns (Al-Nima et al., 2020), and palm vein patterns (Wu et al., 2019), will expand the types of comparative data available from unidentified bodies when the soft tissues of the hand are present.

3.2 | Dental analysis

Teeth are generally recovered with UHR due to their durable composition and ability to withstand trauma (Krishan et al., 2015). Dental analysis is performed by a forensic odontologist who examines the UHR to chart the position and condition of the teeth and to establish tooth class characteristics (ANSI/ASB Best Practice Recommendation 108, 2021). Other features such as dental restorations, pathology, and anomalies are also recorded. These are then compared with AM dental records, including dental charts on LTMP databases [e.g., National Missing Persons and Victim System (NMPVS) in Australia and the National Missing and Unidentified Persons System (NamUs) in the United States (US)], dental radiographs, and dental implant registers such as private (e.g., Dental Implant Registry in Australia), national (e.g., Finnish Dental Implant Register), or local (e.g., universities, hospitals, etc.) collections of records (Naemi et al., 2021). Facial photographs (including those from social media) can serve as a comparison for anterior teeth (Krishan et al., 2015). Radiological analysis of x-rays and three-dimensional (3D) computed tomography (CT) scans are extremely useful for dental analysis as they can be captured using a mobile scanner without disturbing the human remains and allow 3D visualization of the dental data that can be segmented and reformatted to produce different views (Forrest, 2019). Imaging techniques are further able to detect unique or rare bone and sinus features which can be used to perform radiographic comparisons with dental records (Viner & Robson, 2017).

Dental identification is dependent on the availability, adequacy, and accuracy of AM dental records (Hinchliffe, 2011). Under Australian law, dental records must be retained for at least 7 years for an adult patient or in the case of a minor, until the age of 25 (Australian Dental Association, 2021). Record retention in the US follows a similar timeline with the American Dental Association suggesting a minimum of 6 years following a patient's last date of service or until the patient reaches the legal age (American Dental Association, 2023). Unless dental records are collected soon after a person is reported missing, they are often unavailable for LTMP who may have received dental treatment decades earlier. There are also other problems associated with dental records, including lack of international standardization regarding the information to collect and/or upload to databases, poor quality of radiographs, and incomplete or illegible records (Hinchliffe, 2011). In such cases where AM records are not available or suitable, PM dental profiling can be performed by forensic odontologists to infer other biological or biographical information about the deceased individual (Smitha et al., 2019). This includes estimation of age, sex, ancestry, socioeconomic status, personal habits, systemic health, occupation, and dietary status (Vodanović & Brkić, 2012).

3.3 | DNA analysis

DNA analysis is often considered the gold standard for human identification due to its high evidentiary value, presence in various sample types, and ability to reassociate body parts or skeletal elements, even for UHR which are compromised by a vast range of environmental insults or the passage of time (de Boer et al., 2018; Watherston et al., 2018). However, DNA profiling of compromised skeletal remains can often be challenging using routine autosomal short tandem repeat (STR) profiling procedures, or additional genetic information is required to infer kinship, so a number of alternative genetic markers such as lineage markers or panels of single nucleotide polymorphisms (SNPs) should be available for DNA identification casework (Ward, 2017). The DNA analysis process has continued to evolve, becoming progressively faster and more sensitive as an increased number of genetic markers are able to be analyzed in a single run for a variety of sample types contributing to a robust identification (Jordan & Mills, 2021). Newer rapid DNA technologies have also expedited identification with several instruments showing increased utility for human identification with PM sample types including nail, tissue, teeth, and bone (Turingan et al., 2020; Watherston et al., 2021). These technologies are suitable for portable field use and are generally able to produce STR profiles within a few hours.

Best practice recommendations for implementing a DNA-led identification program for missing persons investigations have been published by several parties including INTERPOL (INTERPOL, 2015), the Scientific Working Group on DNA Analysis Methods (SWGDAM; SWGDAM, 2014), Parsons et al. (Parsons et al., 2019), and Ward (Ward, 2017). DNA identification involves the analysis of a set of genetic markers recovered from UHR to develop a DNA profile for

direct comparison to direct reference samples (DRS) or indirect comparison to family reference samples (FRS) if available. A DRS is a DNA sample collected from a LTMP's stored medical specimens (e.g., newborn screening card, blood sample, or biopsy sample) or personal items (e.g., toothbrush, razor, or hairbrush). A FRS is a volunteer DNA sample collected from close genetic relatives of the LTMP. The DNA profile recovered from the UHR is either compared to a nominated DRS and/or FRS DNA profile, or DNA profiles housed in all relevant indexes of national and/or international law enforcement DNA databases, including DNA profiles from other unknown deceased persons, LTMP, and LTMP's relatives. Databases for direct matching of STR profiles with DRS include the National Criminal Investigation DNA Database (NCIDD) in Australia, the Combined DNA Index System (CODIS) in the US, and the INTERPOL DNA Database. Databases for kinship matching with FRS include the NCIDD-Integrated Forensic Analysis (NIFA) in Australia, CODIS in the US, and INTERPOL's I-Familia.

3.3.1 | Autosomal markers

Autosomal markers are inherited equally (but segregated) from both parents and autosomal STR loci are favored for DNA analysis as they are highly polymorphic and offer a high power of discrimination for identification purposes (Watherston et al., 2018). Identity informative SNPs (II SNPs) can be used for the same purpose when DNA is degraded, as is often the case for UHR, but more of them are required for the same level of discrimination. A direct "match" made from the comparison of autosomal genotypes will provide probabilistic support for the proposition that the UHR and the donor of the DRS are the same person. Autosomal markers are also used for familial/kinship analysis to identify related individuals in instances where DRS are not available (Bieber et al., 2006; Maguire et al., 2014). Profiles from UHR can be compared with FRS in either a pairwise (one-to-one) or pedigree (one-to-many) search (SWGDAM, 2014). A familial/kinship "match" can provide probabilistic support for propositions about genetic relationships between the UHR and donors of any FRS. Autosomal markers are preferred over lineage markers for familial/kinship analysis because of their higher discrimination power (Li et al., 2019); however, best practice guidelines promote the use of more than one DNA modality for establishing a genetic identification (Hartman et al., 2015; SWGDAM, 2014).

In cases where the DNA is degraded and DNA fragment lengths are shorter than required for STR profiling, SNPs, insertions/deletions (indels), or microhaplotypes may be preferred as genetic markers (Watherston et al., 2018). Other limitations to consider with autosomal markers for comparative DNA analysis include availability and provenance of AM samples and availability of close relatives as the weight of support for identification decreases with increasing genetic distance (Parsons et al., 2019). While STRs are suitable for estimating short-range genetic relationships (i.e., siblings, parents, and offspring), they offer limited utility when applied to medium or longer range relationships as there are not enough STR markers to provide strong probabilistic support and they are more prone to mutations than SNPs (Grandell et al., 2016). Large panels of kinship informative SNPs (KI SNPs) are required for estimating medium- to long-range genetic relationships. KI SNP panels such as the FORensic Capture Enrichment (FORCE) panel (Tillmar et al., 2021) and the ForenSeq Kintelligence Kit (Verogen, 2021) are extended panels of II SNPs with a sufficient number to provide probabilistic support for propositions about medium or longer range genetic relationships.

3.3.2 | Lineage markers

Lineage markers are uni-parentally inherited and often targeted for identification purposes because they can provide additional genetic information to support a biological relationship between UHR and a putative relative (Alvarez-Cubero et al., 2012). For LTMP cases, lineage markers may be the only appropriate DNA analysis method if there are only distant genetic relatives available for comparison. Y chromosome DNA (Y-DNA) markers are inherited only by males from their biological fathers and mitochondrial DNA (mtDNA) markers are inherited by both males and females from their biological mothers. In addition, mtDNA is more abundant than nuclear DNA (i.e., autosomal DNA, Y-DNA, and X-DNA) and may produce DNA profiling results for old and degraded samples when routine autosomal testing methods fail. X chromosome DNA (X-DNA) markers can provide additional information in certain kinship scenarios because of their unique inheritance pattern such that males inherit only one copy from their biological mothers, while females inherit one copy from each of their biological parents (Gomes et al., 2020). Most modern STR genotyping assays now include a small panel of Y-STRs and X-STRs, together with autosomal loci, to aid genetic identification (Kayser, 2017). If a UHR's lineage marker haplotype matches that derived from a DRS or FRS, then there is

probabilistic support for the proposition that the UHR and the donor of the DRS or FRS have the same paternal or maternal lineage. Reference population databases such as the European DNA Profiling Group (EDNAP) mtDNA Population Database (EMPOP) and Y-STR Haplotype Reference Database (YHRD) can be used for lineage inference.

3.4 | Medical analysis

The analysis of medical data for human remains identification is now recommended as an additional primary method (ICRC, 2022). During a PM examination, the presence of individualizing soft-tissue or skeletal traits that might be known to family members or available in medical and/or radiological records will be catalogued by a forensic pathologist, or accompanying forensic specialists, for comparison using observation and imaging techniques (ANSI/ASB Best Practice Recommendation 009, 2019; Prahlow & Byard, 2011). These include healed fractures, amputations, skeletal deformities, surgical modifications, and other atypical conditions of the bones and teeth (Austin & King, 2016; Ubelaker et al., 2019). Further, when artificial body parts such as orthopedic implants (e.g., prosthetic joints and surgical plates), pacemakers, breast implants, and dental implants are present, manufacturer information such as serial or batch numbers can serve as unique identifiers (Berketa et al., 2010; Wilson et al., 2011). The use of these medical implants for identification through comparison of serial numbers with patient records is considered a rapid and definitive method (Blessing & Lin, 2018; Khartade et al., 2021; Mansour et al., 2019). However, the utility of medical data comparisons is dependent on the centralization and/or availability of AM medical records, which will differ between countries, and individual medical and imaging practices. In Australia, the Australian Orthopedic Association National Joint Replacement Registry (AOANJRR) and Australian Breast Device Registry collects and stores information about joint and breast patients, while in the US, the American Joint Replacement Registry and the National Breast Implant Registry records the same. Similar orthopedic registers exist in the UK, Canada, Europe, and New Zealand (AOANJRR, 2023), and the Netherlands, UK, and Sweden also have well-populated breast implant registries (Song et al., 2020).

4 | SECONDARY FORENSIC PROFILING METHODS FOR IDENTIFICATION

Secondary forensic profiling methods can be used in combination to generate sufficient information for human identification in selected cases where data for primary identification is not available or there is a lack of access to primary scientific techniques (INTERPOL, 2018a). Recently, the ICRC advised that identification resulting from the comparison of individualizing traits should only be concluded when all of the supplementary information is also concordant and lacks inconsistencies that cannot be reasonably explained (ICRC, 2022). This highlights the need for broader AM data collection to include the equivalent information that can be discovered from secondary forensic profiling methods. For example, information about the LTMP's biological profile (e.g., sex, age, ancestry, and height), notable physical features (e.g., marks, morphological traits, and eye, hair, and skin pigmentation), medical and dental history (including accessing available treatment records and radiographs), residence history (e.g., region/s born, lived, and traveled across lifespan), dietary preferences (e.g., main food types and water sources consumed), lifestyle or environmental factors (e.g., occupation, activity levels, and personal habits), facial photographs, and genealogy records. Some common secondary forensic profiling methods include forensic anthropological, radiological (also used in dental and medical analysis), and isotopic analysis, CFR, and a number of novel forensic genomics techniques.

4.1 | Forensic anthropology

Forensic anthropology involves the examination and analysis of skeletal remains recovered from the surface or sub-surface. When suitably informative skeletal elements are present, forensic anthropology can assist to develop a biological profile of the UHR, including sex, ancestry, age, and stature (ANSI/ASB Best Practice Recommendation 010, 2018). This profile can be supplemented by assessment of lifestyle indicators that can provide insights into cultural practices and occupation (ANZPAA, 2020b). Additional information such as pathological conditions, trauma, and estimated PMI can be documented to aid cause and manner of death determinations (Ubelaker et al., 2019). Furthermore, a forensic anthropologist can assist to identify UHR of coronial significance, by distinguishing between human and nonhuman remains (Donlon et al., 2020; Garvin et al., 2021) and contemporary or historical remains (Donlon, 2016). This is

especially important in countries like the US, South America, and Australia, where Indigenous remains pre-dating European settlement are frequently recovered.

Both metric and nonmetric methods are used to evaluate age, sex, ancestry, and stature relying on both cranial and postcranial data (Blau, 2010; Dirkmaat et al., 2008; Hackman, 2016; Spradley & Jantz, 2011; Swift et al., 2022). Using a metric approach, the forensic anthropologist will take landmark measurements from the skeletonized remains and conduct univariate and/or multivariate discriminant function analysis, or use software such as Fordisc (Jantz & Ousley, 2005) and CranID (Wright, 2010) to compare them to population specific reference data (Austin & King, 2016; Spradley & Weisensee, 2017). However, prediction accuracy is dependent on understanding the capabilities and limitations of the matching algorithms, assumptions made regarding the patterning of human populations, and population specific reference data. Furthermore, metric methods are reliant on a complete set of measurement data from the UHR, which is not always possible owing to trauma and/or taphonomic changes (Spradley & Jantz, 2011). Nonmetric methods require the forensic anthropologist to make a subjective assessment of skeletal variation (i.e., shape and size). They may also consult a forensic odontologist to aid their biological profile assessment, considering aspects such as teeth condition, shape, wear patterns, and eruption.

The preferred skeletal elements for biological profile estimation using metric and nonmetric methods according to the *Guidelines for Forensic Anthropology Practitioners* prepared by the Medical Sciences Specialist Advisory Group for the Australia New Zealand Policing Advisory Agency (ANZPAA) have been summarized in Table 1. This list is in general agreement with guidelines published by other relevant agencies such as the ANSI/ASB (ANSI/ASB Best Practice Recommendation 010, 2018) and the Scientific Working Group for Forensic Anthropology (SWGANTH; SWGANTH, 2010a, 2010b, 2012, 2013a, 2013b).

Sex and ancestry estimation can be performed using either nonmetric or metric approaches, while age estimation is a nonmetric process and stature estimation is a metric process (Spradley, 2016). There are several factors that can affect the accuracy of these estimation methods, such as interobserver and interpopulation variation, pathological and taphonomic changes, the experience level of the forensic anthropologist, and the presence or absence of informative morphological features. Using the pelvis as an example, the accuracy rates of nonmetric sex inference when applying the Phenice (1969) technique have been recorded to range from 83% to 96% (Lovell, 1989; McFadden & Oxenham, 2016; Sutherland & Suchey, 1991; Ubelaker & Volk, 2002). The nonmetric assessment of both pelvis and crania resulted in correct estimation of male sex by experienced forensic anthropologists in 100% of 180 male individuals from two mass graves in Serbia (Đurić et al., 2005). The success rate dropped to 70% when only crania were assessed (26 were incorrectly inferred to be female and 27 were considered ambiguous). Similarly, macroscopic sex estimates of 66 individuals from the 13th to 16th century Hospital of St John the Evangelist, Cambridge, were concordant with DNA-based inferences for 98% of cases where both pelvis and crania were assessed, 96% of pelvis-only cases, and 90% of cranium-only cases (Inskip et al., 2019). Thomas et al. (2016) found that sex estimates by forensic anthropologists were more concordant with DNA-based inferences as more skeletal material was available for analysis and as the education level and certification of the examiner increased. Half of incorrect assessments resulted from cases in which only one skeletal element was available. In contrast, sex was correctly inferred in 100% of 164 individuals from the CAL Milano Cemetery Skeletal Collection using only a pelvic morphological method (Selliah et al., 2020). Similarly, metric sex estimation from long bones, such as the femur, show a range of accuracy rates across different populations (Curate et al., 2016; Kranioti et al., 2017; Monum et al., 2017; Selliah et al., 2020). Ancestry estimation can be affected by the age of the individual, as well as the selection of appropriate reference groups and statistical methods of classification (SWGANTH, 2013b). Further, cultural indicators specific to the context of countries (e.g., significant dental attrition and lack of caries in Australian Aboriginal ancestral remains) may be present that can assist with ancestry assessment and inform the subsequent testing and/or management of the remains (ANZPAA, 2020b).

Age estimation generally requires the application of different techniques based on the maturity of the recovered dental and skeletal elements. The choice of optimal technique can be further impacted by the preservation state of the remains, especially the fragility and availability of the skeletal elements required for age assessment (Ubelaker & Khosrowshahi, 2019). Therefore, age estimates are typically presented as a broad age range to account for these issues and also individual variation, lifestyles, and personal habits (Shirley et al., 2013). Age ranges estimated for younger individuals are typically narrower than for older individuals (Priya, 2017). These wide age-at-death estimates are more accurate but arguably less informative for UHR investigations when searching LTMP lists, with age ranges sometimes spanning 29–89 years (e.g., using the Osborne et al. (2004) auricular surface method). Alternative methods for age estimation exist, such as those using bone histology, but are often limited due to the specialized training required for specimen preparation and features interpretation (Ubelaker & Khosrowshahi, 2019). This highlights the need for a more

TABLE 1 Preferred skeletal elements (in order) for the estimation of sex, ancestry, age, and stature using nonmetric and metric methods to develop a biological profile for skeletonized human remains (ANSI/ASB Best Practice Recommendation 010, 2018; ANZPAA, 2020b; SWGANTH, 2010a, 2010b, 2012, 2013a, 2013b).

	Skeletal elements	
Feature	**Nonmetric**	**Metric**
Sex	Pelvis Skull	Long bones
Ancestry	Skull Dentition	Skull
Age	Juvenile: • Skull • Teeth • Long bones Early adult and adult: • Skull • Pelvis—pubic symphysis and auricular surface • Ribs • Vertebra	N/A
Stature	N/A	Long bones (alone and in combination)

precise method for age estimation, which could be achieved by combining radiology, anthropology, dental, radiocarbon dating, epigenetic, and proteomic analyses.

PMI estimations for UHR investigations can provide a time frame between death and the recovery of remains, which may assist to refine LTMP lists. A forensic anthropologist and other relevant experts (e.g., forensic pathologist or taphonomist) can collaboratively review both the scene context and findings from the PM examination, such as gross morphological changes, regional variation, intrinsic and extrinsic influences, grave soil ecology, vegetation, and effects of scavengers to guide PMI evaluations (Wescott, 2018). Recently, there have been attempts to correlate PMI with degradation of biomolecules extracted from the UHR such as DNA, RNA, and proteins (Choi et al., 2019; Tozzo et al., 2020; van den Berge et al., 2016). However, PMI estimation is fundamentally a subjective process and has proven difficult to quantify due to the many variables involved in influencing the rate of decomposition. There have been numerous reported problems with the accuracy and precision of PMI estimation methods owing to the differences in both exogenous and individual endogenous factors (Cockle & Bell, 2015; Ferreira & Cunha, 2013; Suckling et al., 2016). However, as for biological profile estimation, the adoption of a multidisciplinary approach may assist to improve the accuracy of PMI information gleaned.

4.2 | Forensic radiology

The increasing application of forensic radiology in clinical practice is evidenced by the widespread use of both conventional radiography (e.g., x-rays) and advanced radiographic techniques such as CT (Carew & Errickson, 2019). Forensic radiology is a core component of both dental and medical data analysis for the purpose of identification. The comparison of AM and PM radiographic data is usually focused on the skull, teeth, chest, and limbs, although other regions of the body have been reported to be informative (Ciaffi et al., 2011). Dental age estimation is frequently performed from radiographs recording the development of third molars using Cameriere's third molar maturation index method (Çakan et al., 2021; Silva et al., 2013). Head radiographs provide information on gross anatomic structures, along with unique morphological features such as size and configuration of the frontal (Christensen, 2005), paranasal (Ruder et al., 2012), and sphenoid sinuses (Wen et al., 2022). Chest radiographs, in particular the thorax (Kuehn et al., 2002), and other vertebral features observable in x-rays (Kahana et al., 2002) can also be useful for forensic identification (Ciaffi et al., 2011). Rarer skeletal features such as surgical interventions, healed fractures, amputations, or cancer lesions can also be used as individualizing traits (Cappella et al., 2019). However, the usefulness of the radiographic comparison depends on the availability of AM data that can be obtained from medical records and LTMP databases. Record keeping

practices in medical facilities and private practices are variable and the main sources of error in imaging concerns the quality of the radiographs and the PM position of the UHR during imaging (Brogdon, 1998). Further, the comparisons of anatomical features lack standardization. For example, De Angelis et al. (2020) summarize the issue of quantification; specifically regarding the number of concordant traits required for identification. PM imaging also depends on the availability of imaging equipment in forensic medicine facilities, although the development of portable and mobile scanners has facilitated sharing of equipment.

Radiographic imaging techniques are also used to digitize and reconstruct forensic osteological samples and "virtual forensic anthropology" can provide an alternative to physical biological profiling of UHR (Aalders et al., 2017; Carew & Errickson, 2019). Modern imaging software like CT is able to visualize and mathematically analyze cranial and other landmarks to produce accurate measurements for statistical analysis of sex and ancestry (Ramsthaler et al., 2010). Age estimation for the remains of young adults or late adolescents can be assessed through the hand-wrist complex and medial clavicle from radiographic scans (Franklin et al., 2016), with a recent preliminary study also highlighting the value of the medial clavicle for age estimation in older adults as well (Toutin et al., 2022). The radiographs are further able to detect taphonomic alternation (i.e., both ante- and peri-mortem pathology or trauma) or PM modifications (Franklin & Marks, 2021). Studies have also validated the use of post-mortem CT (PMCT) for "virtopsy" as a substitute or triaging tool for traditional autopsy for forensic casework applications (Chatzaraki et al., 2018; Le Blanc-Louvry et al., 2013), with PMCT becoming an integral part of the identification process in some Australian facilities (Blau et al., 2021). A range of information can be extracted from PMCT data including osteological and dental reconstructions (Brough et al., 2015), with a recent study showing the usefulness of volumetric analysis of mastoid air cells extracted from PMCT images to characterize individuals (Oura et al., 2022). Developments in portable and digital approaches (i.e., handheld x-ray and mobile CT scanners) have significantly facilitated the inclusion of radiography in the identification process (Viner & Robson, 2017). The ease of sharing data between practitioners, the ability to re-assess material, and the printing of images and models can all facilitate effective interpretation of radiographic data for identification (Franklin & Marks, 2021). However, more research is required to assess the accuracy of the technique and standardize the technical parameters for casework (Uldin, 2017).

4.3 | Forensic isotope analysis

The utility of isotope analysis to estimate a UHR's date of birth and death, geographical region of origin, residence and travel history, and dietary choices is predicated on the ability to accurately measure differences in isotope signatures (Bartelink & Chesson, 2019). This is possible as the isotope ratio of different elements will vary depending on a person's exposure to environmental factors such as drinking water and food nutrition (Chesson et al., 2017). Isotopic analysis of different tissue types can reveal traces of an individual's life history because they have different rates of tissue turnover (Ubelaker & Francescutti, 2020). Both stable isotope analysis (SIA) and unstable (radioactive) isotope decay can contribute to a range of information regarding a deceased individual's movements from the time of birth to death.

4.3.1 | Stable isotope analysis

The stable isotopes that are of particular interest in human remains identification include oxygen (O), strontium (Sr), hydrogen (H), carbon (C), nitrogen, (N), lead (Pb), and sulfur (S; Franklin & Marks, 2021). They exist in particular ratios and deviations from those ratios indicate physical and biological processes that fractionate or discriminate against the lighter or heavier isotope. The isotopes of an element are distinguished by differences in the number of neutrons. Stable isotopes of lighter elements (e.g., H, C, N, and O) are generally measured by a forensic geochemist using isotope ratio mass spectrometry, while trace metals (e.g., Sr and Pb) are measured using thermal ionization mass spectrometry or multi-collector inductively coupled plasma mass spectrometry (Chesson & Berg, 2021).

The use of SIA for predicting the life history of UHR involves two main factors: (1) assessing natural composition of stable isotopes in various ecological and geological systems, and (2) understanding 'fractionation' processes to create predictive models (Bartelink et al., 2016). The relative amounts of water, plant biomass, ocean produce, and so forth, consumed by an individual while they are alive will dictate various isotope ratios in their remains after they die (Chesson et al., 2018). This is supported by the development of a variety of geological maps of isotope distributions (termed isoscapes) which reveal variations in vegetation and organic tissues (Meier-Augenstein, 2019). Isoscapes are

TABLE 2 Stable isotope ratios used to aid the identification of human remains (Bartelink et al., 2018; Bartelink & Chesson, 2019; Chesson & Berg, 2021; Gutiérrez et al., 2020).

Information	Element	Isotope ratio
Geographic provenance (birthplace)	Hydrogen	$^{1}H/^{2}H$
	Oxygen	$^{16}O/^{18}O$
	Strontium	$^{86}Sr/^{87}Sr$
Geographic mobility (residence/travel history)	Hydrogen	$^{1}H/^{2}H$
	Oxygen	$^{16}O/^{18}O$
	Strontium	$^{86}Sr/^{87}Sr$
	Lead	$^{204}Pb/^{206}Pb$
		$^{206}Pb/^{207}Pb$
		$^{206}Pb/^{208}Pb$
Dietary patterns	Hydrogen	$^{1}H/^{2}H$
	Carbon	$^{12}C/^{13}C$
	Oxygen	$^{16}O/^{18}O$
	Nitrogen	$^{14}N/^{15}N$
	Sulfur	$^{32}S/^{34}S$

particularly useful for multi-isotopic profiling where a single isotope does not provide sufficient information about the UHR to aid identification (Bartelink & Chesson, 2019) (Table 2).

There are some challenges related to the availability of detailed isoscapes, degree of isotopic variation between different regions, and the impact of global food consumption on dietary differences that may make it difficult to definitely pinpoint a geographic region of origin from measured isotopic ratios (Holobinko, 2012). The availability and accuracy of this information is often dependent on the UHR tissue type analyzed; with bone, teeth, nail, and hair often providing varying estimates based on their differing tissue turnover rates (Chesson & Berg, 2021).

4.3.2 | Radiocarbon dating

Unstable radioisotopes have a distinct radioactive decay rate and half-life. They can occur naturally, or as a result of artificially altered atoms from nuclear testing or accidents (Brock & Cook, 2017). Traditionally, forensic geochemists have performed radiocarbon dating analysis of the unstable ^{14}C isotope using accelerator mass spectrometry due to its precision in dating organic archeological materials up to approximately 50,000 years of age (Korlević et al., 2018). Bomb curve radiocarbon (^{14}C) dating is used to determine the age of modern remains by comparing the ^{14}C level to the artificial carbon level created as a result of above ground testing of nuclear weapons during the 1950s and 1960s (Alkass et al., 2011; Johnstone-Belford & Blau, 2020). Estimation of year of birth relies on the low carbon turnover in tooth enamel and petrous temporal bone during an individual's lifetime (Brock & Cook, 2017; Pilli et al., 2018), with error rates particularly low for tooth enamel (Alkass et al., 2011, 2013; Kondo-Nakamura et al., 2011; Spalding et al., 2005). For the estimation of date of death, the trabecular bone is considered more dependable due to its faster rate of collagen turnover and lower lag time in bones, especially for individuals who died at an advanced age (Ubelaker et al., 2015; Ubelaker & Parra, 2011). Recent research has shown promising results for estimation of date of death when analyzing hair, nail, and puparia, which can be valuable for UHR investigations when these biological materials are present (Johnstone-Belford et al., 2022).

4.4 | Craniofacial reconstruction

CFR involves the estimation of a UHR's facial appearance in life from the underlying bony structure of the skull using knowledge of facial anatomy, relationship between hard and soft tissues, and ancestry and sex indicators (Evison

et al., 2016; Wilkinson, 2014). The approaches for CFR are either anatomical (i.e., Russian method), which uses musculature to define the face shape, or anthropometrical (i.e., American method), focusing on the average tissue depth of the face, or a combination (i.e., Manchester method) (Bonda, 2018; Gupta et al., 2015). The decision regarding the CFR approach employed varies between forensic artists depending on their background, experience, and context of the CFR; however, the Manchester method is generally preferred (Johnson, 2016). It is suitable for UHR examinations due to its focus on an individual's facial structure and lower reliance on soft-tissue thickness data as tissue depth data are correlated with sex and single ancestral populations of origin such as European, Asian, or African (Wilkinson, 2004). However, the value of this approach has been questioned and the application of average skin thickness can be used instead to accommodate multiple ancestries, as well as individuals with mixed parentage (Stephan & Simpson, 2008). Some limitations of CFR include the subjective nature of assessing resemblance, particularly assessment of ancestry and the shape of some facial features such as ears, mouth, and nose (Evison et al., 2016). The complementary use of new forensic genomics techniques could enhance CFR methods by considering the biomolecular-based estimations of age, sex, biogeographical ancestry (BGA), and externally visible characteristics (EVCs) when adding relevant surface details. Other recent advancements include computerized 3D CFRs, facilitated by handheld 3D scanning and haptic feedback systems which allow for more detailed, objective, time efficient, and cost effective rendering (Gupta et al., 2015).

5 | EMERGING FORENSIC PROFILING METHODS FOR IDENTIFICATION

Emerging forensic methods for UHR identification can provide supplementary or new investigative leads, beyond those able to be extracted using conventional primary and secondary forensic profiling approaches. The emergence of forensic "omics" (i.e., forensic genomics, microbiomics, epigenomics, and proteomics) and digital forensics tools present new opportunities for coronial investigations, by providing intelligence information about a deceased individual, without the necessity for detailed AM data for comparison.

5.1 | Forensic genomics

In instances where there are no FRS or a UHR sample does not 'match' with a DNA database record, the value of a STR profile diminishes. However, the widespread adoption of massively parallel sequencing (MPS), and improvements to the cost and labor requirements for analyzing large numbers of SNPs, has rapidly expanded the type and number of genetic markers available for forensic investigations. Together with more readily available reference population databases, forensic genomics makes it feasible to draw a range of genetic inferences about biological samples including BGA, EVCs, and lineage information (Kayser & Parson, 2018; Plesivkova et al., 2019; Scudder et al., 2018b). However, there are some ethical and privacy considerations associated with BGA and EVC inference, including the potential for stigmatization in forensic contexts (Michael et al., 2021; Scudder et al., 2018b; Toom et al., 2016; Williams & Wienroth, 2014).

5.1.1 | Biogeographical ancestry

Ancestry estimation relates to patterns of human genetic variation, such that individuals with ancestors from the same geographical locations will share more portions of DNA. BGA inference uses common autosomal genetic variations, such as SNPs and more recently microhaplotypes (Kidd et al., 2013, 2018), that are linked with specific ancestral populations due to geographical and cultural isolation coupled with random genetic drift and natural selection (Bulbul et al., 2016). The BGA analysis of forensic samples requires three main elements: (1) a panel of ancestry informative markers (AIMs), (2) reference genotypes for those AIMs in indicative populations, and (3) prediction algorithms (McNevin, 2020). There has been a continuous development of AIM panels to detect genetic diversity patterns amongst and within continental populations (Pereira et al., 2017; Phillips et al., 2014, 2019; Xavier et al., 2020). The genetic markers genuinely associated with BGA exhibit allele frequency differences between populations and can be selected from increasingly available, publicly accessible reference human genotypes (e.g., FROG-kb web portal: https://frog.med.yale.edu/FrogKB/). There are a number of algorithms for inferring BGA from autosomal genotypes both with and without genetic admixture (Cheung et al., 2017, 2018).

Inferred BGA can aid in estimation of the biological profile of UHR and complement EVC estimations for CFR. However, inferring the most likely population of origin of the ancestors of UHR does not always provide direct information about the geographical origins of UHR themselves owing to several factors such as migration, admixture, variation in allele frequency differences, small or undetectable genetic distances between some populations, and ambiguous definitions of reference populations (Pereira et al., 2020).

5.1.2 | Externally visible characteristics

Phenotype informative markers (PIMs) can be used to estimate the physical appearance of an individual, including hair color, eye color, skin color, and facial features (Kayser, 2015). This method relies on the principle that genetic variations can alter the functional properties of proteins which are expressed in distinct phenotypes (Marano & Fridman, 2019). The associated genes and markers (e.g., phenotype informative SNPs and indels) are identified and verified using suitable statistical predictive models and public reference databases (e.g., HIrisPlex-S web portal: https://hirisplex.erasmusmc.nl). Currently, human pigmentation traits are the most accurately predicted characteristics from PIMs, including eye, hair, and skin color (Kayser, 2015); with some categories able to be more accurately determined than others. For example, blue and brown eyes have been shown to be more accurately predicted than intermediates such as green-hazel eyes (Schneider et al., 2019).

Despite the heritability of pigmentation traits being high, they are not entirely predictable from DNA, due to contributions from environmental influences such as age and exposure to solar radiation. Additionally, genetically influenced EVCs can be deliberately altered (e.g., hair dyeing and skin tanning) (Angers et al., 2021; Schneider et al., 2019). There have been genome wide association studies performed for other physical traits such as height; however, there are no current reliable prediction assays owing to the greater genetic complexity of these traits (Bush & Moore, 2012; Marouli et al., 2017; Watanabe et al., 2019). Some studies have also found genetic associations with facial features, which have the potential to produce a 'DNA facial composite' (Claes et al., 2018; Marano & Fridman, 2019; White et al., 2021). However, the studies are preliminary and will require greater understanding of the factors that determine facial variation (e.g., shape of the chin, cheeks, eyes, forehead, lips, and nose) prior to casework implementation (Kayser, 2015).

5.1.3 | Long-range familial/kinship analysis of autosomal markers

While short-range familial/kinship analysis of STR profiles is preferred for identifying first degree genetic relatives (siblings, parents, and offspring) of UHR, medium- to long-range familial/kinship analysis generally requires medium- to high-density autosomal genotypes consisting of up to one million KI SNPs across the genome to infer genetic relationships beyond first degree relatives (Kennett, 2019; Phillips, 2018). This method has been greatly assisted by the rise in popularity of consumer genomics and the publicly accessible genealogy databases that contain an ever-expanding number of KI SNP profiles from consumers conducting their own genealogy research. There are over 40 million KI SNP profiles currently housed in the four major consumer genomics company databases (in order: AncestryDNA®, 23andMe®, MyHeritage™, and FamilyTreeDNA; International Society of Genetic Genealogy, 2022); however, only FamilyTreeDNA, GEDmatch (through the GEDmatch PRO™ portal), and DNASolves allow law enforcement access for defined investigative purposes (Kling et al., 2021; Skeva et al., 2020). These three databases permit consenting individuals to upload their KI SNP profile (downloaded from their consumer genomics company of choice) and make it available for law enforcement searching and matching.

The use of long-range familial/kinship analysis (termed FIGG) has assisted to resolve a number of UHR cases in the US (Rodriguez et al., 2022), with more countries beginning to evaluate its use for unidentified and missing persons investigations (Tillmar et al., 2020; Ward, 2022). By combining the use of advanced sequencing technologies such as microarrays or whole genome sequencing (WGS) to generate dense KI SNP profiles with long-range familial/kinship searching of these profiles on private and public genealogy databases, there is now the opportunity to identify distant genetic relatives of UHR beyond what is possible using law enforcement DNA databases alone. While microarrays are cost effective, a large quantity of high quality DNA is required which is not always present in forensic samples like bones (Davawala et al., 2022). Therefore, WGS approaches are generally preferred for UHR, while microarrays are suitable for FRS (Tillmar et al., 2020). Following database searching, a genetic genealogist will use the available genetic data, public records, and other lawful means to obtain information about (deceased and living) genetically related

individuals to build family trees and narrow the search to a region, a family, or an individual. Probabilistic support for identity can then be obtained if at least one of the identified close genetic relatives consents to provide a comparative FRS for short-range familial/kinship analysis (Greytak et al., 2019).

Scudder et al. (2020) assessed the feasibility of FIGG in operational forensic laboratories from an Australian perspective and found several limitations related to sourcing external expertise and service provision, and the absence of validation criteria and guidelines when dealing with a private consumer or forensic genomics company. Proprietary familial/kinship searching and matching algorithms can also vary between different companies with minimal peer review of the applied methodology and techniques (Kennett, 2019). Some of these concerns can be alleviated if law enforcement agencies make a strategic choice about the genotyping methodology they use for FIGG. For example, the recent development of commercial targeted amplicon sequencing panels which target a limited number of KI SNPs (e.g., ForenSeq Kintelligence Kit) now enable forensic laboratories to implement an insourced end-to-end FIGG workflow.

5.2 | Forensic microbiomics

Microbial forensics for human identification refers to the analysis of the microbial cohabitants that live on and within humans. This microbial community is influenced by geographic, ethnic, lifestyle, and environmental factors (Cho & Eom, 2021). The variability of the human microbiome, both within and between individuals, could be exploited to provide forensically relevant information such as tissue/body fluid identification (Hanssen et al., 2017; López et al., 2019, 2020) and PMI estimations (Belk et al., 2018; Metcalf et al., 2016; Tozzo et al., 2022), or for personal identification (Woerner et al., 2019; Yang et al., 2019). Several studies have identified and categorized human bone microbial decomposer communities (Emmons et al., 2020, 2022) to determine microbial succession as a marker for PMI with promising results (Deel et al., 2021). For personal identification, it is possible to target the stable personal microbiome signature of humans (Park et al., 2017; Schmedes et al., 2017, 2018), with most research up to now focused on microbiomes successfully collected from the skin (e.g., hands), body fluids, and personal belongings of living individuals. Metcalf (2019) has summarized the various knowledge gaps in forensic microbiomics, including assessment of the PM timeframe in which an individual's personal signature is altered by the decomposer microbes to determine the validity of microbiomic analysis for remains in the later stages of decomposition. Early research suggests that this microbial succession could occur within 48–60 h of death for indoor scenes (Kodama et al., 2019; Pechal et al., 2018). Therefore, application for human remains identification will require further assessment of the PM stability and diversity of the skin microbiome of deceased individuals in different environments, the microbiome profiling success of hard tissue samples, and the ability to link an individual's unique microbiome in life and death. While making the microbiome a potentially useful instrument for estimation of PMI, microbial succession is likely to limit the potential of the microbiome to be useful for forensic identity.

Some studies have also revealed variation in the human microbial ecology of different populations around the world, highlighting the potential for detecting microbial signatures that are specific to geographical regions (Brinkac et al., 2018; Cho & Eom, 2021; Grantham et al., 2019; Haarkötter et al., 2021; Lax et al., 2015). This requires the creation of a robust and reliable international microbiome database containing an adequate sample size and associated metadata (e.g., geographic origin, ethnic group) for geolocation purposes, such as the Forensic Microbiome Database which currently contains microbiomes from 35 countries (139 cities; Singh et al., 2021). As for forensic identity, microbial succession is also likely to limit the usefulness of the microbiome for inferring an individual's geographical history. The use of microbial forensics to aid the identification of skeletonized remains is therefore still in its infancy; however, targeted studies reflective of these scenarios will assist to determine its utility for human remains identification.

5.3 | Forensic epigenetics

Epigenetics refers to heritable alterations in gene expression as a response to various short- or long-term environmental influences (Vidaki & Kayser, 2018). Forensic epigenetics is a promising method for age prediction of biological material relying on the change in DNA methylation levels during an individual's lifespan (Montesanto et al., 2020; Parson, 2018; Shabani et al., 2018). DNA methylation is the addition of a methyl group to a cytosine that precedes a guanine nucleotide (CpG; Freire-Aradas et al., 2017; Tammen et al., 2013) and the methylation proportion at some CpG clusters (termed CpG islands) has been found to increase or decrease linearly with chronological age in human samples

(Freire-Aradas et al., 2016; Naue et al., 2017; Vidaki et al., 2017). A complicating factor is that different tissues have different methylation signatures. Most forensic age predictors have been built for human blood, including a recent model by Aliferi et al. (2022), but studies investigating sample types relevant to UHR cases are increasing, such as teeth (Bekaert et al., 2015; Correia Dias et al., 2021; Giuliani et al., 2016; Márquez-Ruiz et al., 2020), bones (Correia Dias et al., 2021; Lee et al., 2020), and nails (Fokias et al., 2021).

For UHR investigations, DNA methylation levels from a set of CpG islands known to be associated with age are analyzed in an appropriate tissue type to determine biological age as a proxy for chronological age. Compared to non-genetic age estimation methods, which suffer from low precision estimates for adult remains, methylation-based age estimation could offer a more objective and reliable method. This is important in order to provide an accurate and complete biological profile of the UHR. It is also relevant for EVCs prediction because several appearance traits are age-dependent (Vidaki & Kayser, 2018). Because methylation and demethylation of CpG islands can be triggered by environmental stimuli, forensic epigenetics can also be used to infer other lifestyle information about a deceased individual, including their smoking habits, activity levels, and diet (Ballard et al., 2020; Vidaki & Kayser, 2017). Current limitations of forensic epigenetics include lack of examination of intergroup variability (i.e., sex, population, and disease) and analyses of life events and their influence on age-correlated methylation levels. These include environmental factors like diet, toxins, lifestyle, and hormones.

5.4 | Forensic proteomics

Forensics proteomics, the analysis of proteins and peptides, has produced new biological markers to aid identification in forensic investigations (Parker et al., 2021). Proteins contain both genotype and phenotype information (Merkley et al., 2019) and are highly resistant to degradation (Díaz Martín et al., 2019). The success of mass spectrometry (MS)-based proteomics, combined with the increasing availability of protein sequence databases, has enabled inference of age, PMI, BGA, and sex. However, the science of forensic proteomics is still emerging and researchers are seeking to define similar standards and methodologies for protein analysis as have been established for DNA analysis. These include methods for sample preparation and protein extraction for various sample types, analytical methods, and statistical tools and algorithms to identify variations (Parker et al., 2021).

5.4.1 | Age and post-mortem interval

Proteomic studies of bone samples have revealed new prospective marker proteins for biological age and PMI estimation in simulated forensic contexts (Choi et al., 2019; Procopio et al., 2017; Procopio, Chamberlain, & Buckley, 2018; Procopio, Williams, et al., 2018; Sawafuji et al., 2017). Several marker proteins for skeletal samples have been correlated with age including alpha-2-HS-glycoprotein, albumin, kininogen-1, vimentin, and osteopontin (Duong et al., 2021; Mickleburgh et al., 2021). For PMI estimation of skeletonized remains, collagen alpha-1 chain, collagen alpha-2 chain, decorin, and matrix Gla protein appear to be good candidates (Mickleburgh et al., 2021), along with hemoglobin, serum transferrin, and biglycan (Díaz Martín et al., 2019). Although there are consistent reports of the utility of these markers in animal and human studies (Duong et al., 2021; Mickleburgh et al., 2021; Procopio, Williams, et al., 2018), replication studies using a greater number of human samples are required to create a panel of robust biomarkers and account for differences amongst and within individuals and biological samples.

5.4.2 | Biogeographical ancestry

Recent research has demonstrated the possibility of obtaining ancestral information from genetically variant peptide (GVP) profiles through inference of the corresponding SNP alleles in human bone and hair samples (Franklin et al., 2020; Mason et al., 2018; Parker, Goecker, et al., 2019). This is possible because proteins contain genetic variation in the form of single amino acid polymorphisms resulting from SNPs, that can be aggregated to create a genetic profile for an individual (Parker et al., 2016). This means that BGA can be inferred from genotype frequencies in reference populations, as for DNA profiles. The inferred SNP profile can be used to complement information obtained from nuclear or mtDNA (Mason et al., 2018). Currently, almost 500 SNP alleles have been accurately inferred from protein

sequences (Parker et al., 2021) and panels of alleles are being considered for different sample types based on a range of factors such as ease of detection via MS, population frequency information, uniqueness, and statistical independence from other GVPs (Chu et al., 2019).

5.4.3 | Sex

The most advanced application of proteomics in human identification has been the determination of sex-specific amelogenin peptides in tooth enamel (Parker, Yip, et al., 2019; Stewart et al., 2017). Amelogenin is an enamel forming protein encoded in both X and Y chromosomes with differences in the amino acid sequences, allowing for differentiation between male and female samples. Proteomic sex determination shows promising utility in forensic contexts, with recent comparative research by Buonasera et al. (2020) finding proteomic sex estimation using teeth enamel to be more sensitive than osteological and genomic sex estimation. The proteomic signal was also found to be better preserved over time than for DNA (Buonasera et al., 2020).

5.5 | Digital forensics

The evolution of digital technology has greatly influenced people's interactions with the online world, leaving a digital trail behind them. The use of digital forensic applications to recover information about a LTMP found on their personal digital devices or online accounts can potentially provide additional AM information to assist an investigation. Collection of images from family members or smart phones (e.g., 'selfie' or smiling photographs) have been used in several cases as supplementary dental evidence (Fakher et al., 2020; Miranda et al., 2016; Silva et al., 2015). A forensic odontologist may be able to interpret features observed in AM photographs, including various dental variations and characteristics such as diastema, teeth rotations, missing teeth, and lip anomalies, and compare with equivalent dental features observed during the forensic odontological examination of the UHR. Such facial imagery can also be used to compare to UHR physical appearance descriptions based on the PM examination or DNA-based estimation of EVCs. However, complicating factors include the cosmetic and environmental alteration of pigmentation related traits, and the use of specific features related to social media applications, like filters and mirror imaging (Robinson et al., 2020). Social media data from websites and applications like Facebook, Instagram, LinkedIn, and Twitter can further provide personal information useful for AM-PM data comparisons. Mobile health (mHealth) and electronic health (eHealth) data available through mobile applications and wearable devices such as smart watches and fitness trackers may also provide identifying information, including the wearer's movements, physical activity, and health and wellbeing status at various times (Paglialonga et al., 2018). Investigators have already started to use this type of digital evidence in criminal cases (Hauser, 2018; Moriarty, 2018; Watts, 2017), highlighting a potential investigative avenue for LTMP cases.

6 | THE FORENSIC IDENTIFICATION PROCESS FOR MISSING PERSONS INVESTIGATIONS

Due to the complex nature of investigating and identifying missing and deceased persons, a forensic examination framework that summarizes the type, number, and sequencing of lines of evidence could assist stakeholders to request and/or apply an interdisciplinary death investigation process dependent on their role in the identification process. This type of framework is contingent upon the state and condition of the UHR and the accessibility and affordability of forensic techniques. For example, a recently deceased individual, typically encountered in mass disasters, will potentially reveal more identifying information in the early phases of an investigation than skeletonized remains, typically encountered in LTMP cases. Additionally, more human, physical, and financial resources are commonly deployed for victim identification in the event of a high-profile mass disaster than historically afforded to backlogged, domestic cases of unidentified and missing persons. Table 3 outlines a guiding framework for the scientific examination of skeletonized remains, which incorporates some general prioritization principles discussed in this section.

The first general principle is the use of less destructive and invasive methods before more destructive and invasive methods, in order to preserve the human remains for repatriation to family members and/or future forensic analysis. The least destructive and minimally invasive methods are typically physical assessments conducted as part of a routine

TABLE 3 An example framework for the sequential forensic examination of skeletonized human remains.

Step	Forensic profiling method	Line of evidence	Example post-mortem information recovered	Example ante-mortem information for comparisons
1	Physical analysis	Personal effects	Clothing, jewelry, identification documents, smart phones/watches	LTMP reports, photos, government records, other (e.g., bank, phone, health) records, online accounts
		Distinguishing features	Tattoos, piercings, marks, prosthetic devices, morphological traits	LTMP reports, photos, medical records
	Radiological analysis	Medical findings	Medical conditions and diseases, skeletal injuries, surgical interventions, medical implants	Medical records, medical registers, LTMP reports, photos, medical information on LTMP databases
			Individual identity (based on individualizing skeletal features or medical implants)	Medical records, medical registers, medical information on LTMP databases
		Dental findings	Dental charts, dental restorations, dental diseases, dental anatomy	Dental records, dental registers, LTMP reports, photos, dental information on LTMP databases
			Individual identity (based on individualizing dental features, sinus patterns or dental implants)	Dental records, dental registers, dental information on LTMP databases
		Biological profile	Age	LTMP reports, government records, photos
	Anthropological analysis	Medical findings	Medical conditions and diseases, skeletal injuries, surgical interventions, medical implants	Medical records, medical registers, LTMP reports, photos, medical information on LTMP databases
			Individual identity (based on individualizing skeletal features or medical implants)	Medical records, medical registers, medical information on LTMP databases
		Date of death	PMI	LTMP reports
		Biological profile	Age, sex, ancestry, stature	LTMP reports, photos, government records
		Life history/ lifestyle	Personal habits, physical or sporting activities, occupation	LTMP reports
	Odontological/dental analysis	Dental findings	Dental charts, dental restorations, dental diseases, dental anatomy	Dental records, dental registers, photos, dental information on LTMP databases
			Individual identity (based on individualizing dental features or dental implants)	Dental records, dental registers, photos, dental information on LTMP databases
		Biological profile	Age, sex, ancestry	LTMP reports, photos
		Life history/ lifestyle	Socioeconomic status, personal habits, occupation, dietary patterns	LTMP reports
2	Radiocarbon dating	Date of birth/ death	Age, PMI	LTMP reports, government records
	Isotope analysis	Life history/ lifestyle	Region of origin, residence/travel history, dietary patterns	Isoscapes, LTMP reports, government records
3	DNA analysis—direct DNA searching and matching	Genetic findings	Individual identity (based on individualizing genetic profiles using autosomal markers)	DRS, DNA profiles on law enforcement DNA databases
		Biological profile	Sex	LTMP reports, government records, photos

TABLE 3 (Continued)

Step	Forensic profiling method	Line of evidence	Example post-mortem information recovered	Example ante-mortem information for comparisons
4	DNA analysis—short-range familial/kinship DNA searching and matching	Genetic findings	Close genetic relationships (e.g., parents, siblings, children)	FRS, DNA profiles on law enforcement DNA databases
		Biological profile	Sex	LTMP reports, government records, photos
5	DNA analysis—lineage estimation	Genetic findings	Support for individual identity or close genetic relationships inferred in Steps 3 and 4	DRS, FRS, DNA profiles on law enforcement DNA databases
			Paternal/maternal lineage	DRS, FRS, DNA profiles on reference DNA databases, government records
6	Genomics—phenotype estimation	Physical appearance	EVCs	DNA profiles on reference DNA databases, LTMP reports, photos
	Genomics—ancestry estimation	Biological profile	BGA	DNA profiles on reference DNA databases, LTMP reports, photos, government records
	Genomics—medium-range familial/kinship DNA searching and matching	Genetic findings	Medium-range genetic relationships (e.g., uncles, aunts, grandparents, grandchildren)	FRS, DNA profiles on local/internal DNA databases
7	Microbiomic analysis	Date of death	PMI	LTMP reports
		Biological profile	BGA	Microbiome databases, LTMP reports, photos, government records
	Epigenetic analysis	Biological profile	Age	LTMP reports, photos, government records
		Life history/lifestyle	Personal habits, dietary patterns, activity levels	LTMP reports
	Proteomic analysis	Date of death	PMI	LTMP reports
		Biological profile	Age, sex, BGA	LTMP reports, photos, government records
8	Craniofacial reconstruction	Physical appearance	Facial approximation	LTMP reports, photos, facial recognition databases, government records
9	Genomics—long-range familial/kinship searching and matching (FIGG)	Genetic findings	Distant genetic relationships (e.g., cousins)	DNA profiles on private and public genealogy databases, government records, public records

Note: Progression to a subsequent step would typically only occur if the evidence recovered in the previous step was not sufficient for identification (by the relevant legal authority) or the forensic profiling method/s were not available. Within each step, individual forensic profiling methods may be applied simultaneously or only as required based on the case context. The available post-mortem information is compared with equivalent ante-mortem information sourced from relevant databases, public and private records, and families to support a conclusion of identification, exclusion, or an inconclusive finding. In support of an interdisciplinary approach, use of multiple forensic profiling methods from multiple steps should be considered to produce multiple and different lines of evidence for identification.

Abbreviations: BGA, biogeographical ancestry; DRS, direct reference samples; EVCs, externally visible characteristics; FIGG, forensic investigative genetic genealogy; FRS, family references samples; LTMP, long-term missing persons; PMI, post-mortem interval.

PM examination in either a field or mortuary setting (*Step 1*, Table 3). Following *Step 1* (Table 3) analyses, the resulting PM data should be searched against AM dental, medical, and other case-related information housed on national databases (e.g., NMPVS in Australia and NamUs in the US) prior to more costly, time-consuming, destructive, and invasive methods such as radiocarbon dating and isotope analysis (*Step 2*, Table 3) if required, or DNA analysis (*Step 3*, Table 3). However, it may be necessary to employ methods from *Step 2* (Table 3) immediately following *Step 1* (Table 3) to assess

the need for the identification process to proceed further when the coronial significance of the UHR is not evident. The utilization of forensic profiling methods from *Step 3* (Table 3) onwards should firstly consider the choice of sampling technique, the quality and quantity of material available to sample, and the likelihood of analytical success.

This leads to a second general principle which is the use of accepted primary identification methods (i.e., fingerprint, dental, DNA, and medical analysis as specified by international bodies such as the ICRC, INTERPOL, and IOM) before secondary identification methods, or consideration of other nonscientific information (ICRC, 2022; INTERPOL, 2018a; Robins, 2019). This ensures the investigation and identification process employed adheres to current internationally recognized best practices (Puerto et al., 2021). Additionally, authorities should also be considerate of any relevant state or national policies and procedures, such as the *Australia New Zealand Policy for Missing Persons Investigations* (ANZPAA, 2020a), Brazil's *National Policy on the Search for Missing Persons* (Calmon Silva et al., 2022), and the Argentine Forensic Anthropology Team's (EAAF) *Forensic Guide to the Investigation, Recovery and Analysis of Human Skeletal Remains* (EAAF, 2020). This may lead to conflict with the first principle where, for example, it is deemed necessary to advance directly to (more destructive and invasive) DNA analysis (*Step 3*, Table 3) before other (less destructive and invasive) dental or medical analyses (*Step 1*, Table 3) are completed. This could be because the nature of the incident and/or the state of the remains warrants it (e.g., high fragmentation or no teeth present), or the lead authority requests it.

A third general principle is the use of rapid, simple, and accessible methods before more laborious, complex, and expensive methods. Anthropological and dental analyses (*Step 1*, Table 3) are relatively rapid and simple, and can be performed as part of the initial PM examination when forensic anthropologists and odontologists are involved. The forensic anthropologist may subsequently request supplementary testing (*Step 2*, Table 3) to help determine the age and origin of the remains. This is particularly practiced in countries that have comprehensive and accurate isotope maps. This supplementary testing will add time and cost to the initial investigation; however, if the remains are determined to not be of coronial significance, unnecessary forensic testing would not follow. This would result in resource savings being realized at this point, as well as preventing inappropriate testing of human remains of archeological or ancestral origin. DNA analysis (*Step 3*, Table 3) would then typically follow, with a biological sample collected routinely for both well-preserved and skeletonized remains. DNA analysis is now largely automated and relatively inexpensive in most countries and turnaround times are in the order of days, reducing to hours if rapid DNA instrumentation is deployed. In cases of large-scale UHR identification efforts, or fragmented and commingled remains, DNA might also be the only effective and efficient means of identifying and/or reassociating large numbers of complete or incomplete remains (Goodwin, 2017). If the DNA result is not useable due to sample condition or the lack of comparative records, a suite of more complex, expensive, and boutique genomic, microbiomic, proteomic, and/or epigenetic analyses (*Step 6* and beyond in Table 3) might follow for investigative lead generation (depending on their availability).

A fourth general principle is the use of all other forensic profiling methods before potentially privacy invasive ones. There should be a sequential unmasking of genetic information (Table 4) when implementing forensic genomics for operational use (Scudder et al., 2018a) so that fewer and smaller genetic targets are analyzed (beginning with *Step 3* in Table 3) before more and longer genetic targets (up to *Step 9* in Table 3). Despite privacy protections [e.g., *Privacy Act 1988* (Cth) in Australia] not typically being triggered for information relating to UHR, these actions would serve to minimize unnecessary genetic privacy risks to living relatives of UHR or LTMP found alive. However, in cases of limited UHR samples, it might be necessary to consider sample conservation, operational benefit, and even costs of individual tests over privacy risk. For example, FIGG (*Step 9*, Table 3) could be considered for finite samples before other specialized methods such as microbiomic, epigenetic, and proteomic analysis (*Step 7*, Table 3) which are not universally available and are potentially not as informative. Furthermore, the privacy considerations should also extend to information from other non-DNA profiling methods. For example, both anthropological and proteomic analysis can reveal information about an individual's ancestry that is shared with genetic relatives.

Another privacy enhancing step is to ensure law enforcement DNA databases, which are not publicly available, are searched prior to the use of online genealogy databases, which are generally searchable by the public. Law enforcement use of genealogy databases for conducting FIGG has initiated major privacy debates, with calls for regulation regarding informed consent for the sharing of an individual's genetic information (McEwen et al., 2021; Ram et al., 2021). Suggested solutions include the establishment of institution-wide ethics committees to provide ethical oversight of business practices for law enforcement agencies and consumer/forensic genomics companies (Roffey & Scudder, 2021), routine use of privacy impact assessments before implementing new genetic technologies (Scudder et al., 2018b), and/or development of agency or national guidelines which stipulate suitable case criteria and conditions for the use of FIGG. Some examples of FIGG policy frameworks and guidelines include the US Department of Justice (DOJ) *Interim Policy*

TABLE 4 An example of the principle of sequential unmasking of genetic information used in unidentified and missing persons investigations.

Step	Genetic targets	Example kits	Test samples	Example comparisons with ante-mortem information
1	Identity informative markers: STRs	GlobalFiler™ PCR Amplification Kit, PowerPlex® 21 System, Investigator® 24Plex QS Kit	UHR DRS FRS	Direct searching of law enforcement databases (e.g., NCIDD in Australia, CODIS in the US, and INTERPOL DNA Database) Short-range familial/kinship searching of law enforcement databases (e.g., NIFA in Australia, CODIS in the US, and I-Familia)
2	Lineage informative markers: Y-DNA and mtDNA	YFiler™ Plus PCR Amplification Kit, PowerPlex® Y23 System, Investigator® Argus Y-28 QS Kit, ForenSeq mtDNA Whole Genome Kit, Precision ID mtDNA Whole Genome Panel	UHR DRS FRS	Direct searching of law enforcement databases (e.g., NIFA in Australia and CODIS in the US) Lineage estimation using public reference databases (e.g., EMPOP and YHRD databases for maternal and paternal lineages, respectively)
3	Low-density SNPs for identity: II SNPs	ForenSeq DNA Signature Prep Kit (DPMA), Precision ID Identity Panel	UHR DRS	Direct comparison between UHR and DRS SNP profiles within a case or direct searching of local databases
4	Low-density SNPs for ancestry and phenotype: AI SNPs and PI SNPs	ForenSeq DNA Signature Prep Kit (DPMB), Precision ID Ancestry Panel	UHR	BGA and EVCs estimation using public reference databases (e.g., FROG-kb and HIrisPlex-S databases for BGA and EVCs, respectively)
5	Medium-density SNPs for kinship: KI SNPs	FORCE panel, ForenSeq Kintelligence Kit	UHR FRS	Comparison between UHR and FRS SNP profiles within a case or medium-range familial/kinship searching of local databases
6	High-density SNPs for FIGG: KI SNPs	ForenSeq Kintelligence Kit, microarrays, whole genome sequencing	UHR FRS	Long-range familial/kinship searching of private and public genealogy databases (e.g., FamilyTreeDNA, GEDmatch PRO™, and DNASolves)

Note: Progression to a subsequent step would typically only occur if the previous step was not sufficient for identification (by the relevant legal authority). Some of the steps can be applied simultaneously or only as required based on the case context. In support of best practice guidelines, more than one DNA modality should be used to establish a genetic identification.

Abbreviations: AI SNPs, ancestry informative SNPs; BGA, biogeographical ancestry; DRS, direct reference samples; EVCs, externally visible characteristics; FIGG, forensic investigative genetic genealogy; FRS, family reference samples; II SNPs, identity informative SNPs; KI SNPs, kinship informative SNPs; mtDNA, mitochondrial DNA; PI SNPs, phenotype informative SNPs; SNPs, single nucleotide polymorphisms; STRs, short tandem repeats; UHR, unidentified human remains; Y-DNA, Y chromosome DNA.

on Forensic Genetic Genealogical DNA Analysis and Searching (U.S. DOJ, 2019), the *Forensic DNA Traces and Genealogy* report from Sweden (The Swedish Police Authority, 2021), SWGDAM's *Overview of Investigative Genetic Genealogy* document (SWGDAM, 2020), and the UK's FIGG feasibility report (Biometrics and Forensics Ethics Group, 2020). Some states in the US, specifically Maryland and Montana, have taken this one step further and elected to introduce relevant legislation to govern the use of the technology (Taylor, 2021), with other states considering similar bills. For most countries, FIGG will likely be applied as a last resort identification method when all other forensic profiling methods (*Steps 1–8*, Table 3) fail to yield a result, in order to minimize the privacy impacts for genetic relatives of UHR. However, in cases where UHR samples, DNA extracts, and/or investigative resources are limited, FIGG may be considered earlier in order to retrieve as much valuable (genetic) information as possible from a finite sample or expedite identification, as long as its use complies with relevant policies and procedures.

The registration of high quality and quantity unidentified and missing persons information (i.e., AM and PM data) in centralized national and/or international databases is a fifth general principle that is crucial for the effective and efficient searching and matching of relevant biometric information across state and country borders. In addition, countries

TABLE 5 A summary of the various forensic profiling methods currently available to support the interdisciplinary examination of skeletonized human remains in order to establish multiple and different lines of evidence for identification.

Evidential category	Forensic profiling methods
Identity	• Physical analysis • Radiological analysis • Anthropological analysis • Dental analysis • Direct DNA analysis (autosomal markers)
Genetic (familial/kinship) relationships	• Short-range familial/kinship DNA analysis (autosomal markers) • Lineage DNA analysis (Y-DNA and mtDNA markers) • Medium-range familial/kinship DNA analysis (autosomal markers) • Long-range familial/kinship DNA analysis (autosomal markers)
Age	• Radiological analysis • Anthropological analysis • Dental analysis • Radiocarbon dating • Epigenetic analysis • Proteomic analysis
Sex	• Radiological analysis • Anthropological analysis • Dental analysis • DNA analysis (autosomal and Y-DNA markers) • Proteomic analysis
Ancestry/BGA/region of origin	• Anthropological analysis • Dental analysis • Isotope analysis • Lineage DNA analysis (Y-DNA and mtDNA markers) • BGA DNA analysis (BGA markers) • Microbiomic analysis • Proteomic analysis
Physical appearance	• Physical analysis • Anthropological analysis • EVCs DNA analysis (EVC markers) • Craniofacial reconstruction
Life history/lifestyle	• Anthropological analysis • Dental analysis • Isotope analysis • Epigenetic analysis
PMI	• Anthropological analysis • Radiocarbon dating • Microbiomic analysis • Proteomic analysis

Note: For each evidential category, more than one analysis method should be used to infer concordant investigative and identifying information about the unidentified human remains.

Abbreviations: BGA, biogeographical ancestry; EVCs, externally visible characteristics; mtDNA, mitochondrial DNA; PMI, post-mortem interval; Y-DNA, Y chromosome DNA.

should evaluate the advantages and disadvantages of developing and utilizing public-facing databases, or restricting use to law enforcement only. The majority of the forensic profiling methods in Table 3 require a LTMP database and/or DNA database for searching and comparison purposes. For example, national databases containing searchable information such as locations, clothing, age, sex, ancestry, medical and dental records, and DNA profiles related to both unidentified and missing persons could help narrow a pool of potential candidates once the relevant comparative profiling information becomes available. Many authorities are advocating for the use of centralized databases to create robust information management systems and biometric data comparison platforms for identification efforts (ICRC, 2022;

INTERPOL, 2015; Rodriguez et al., 2022; Ward, 2018). Furthermore, they provide the means to reassociate body parts or skeletal elements when components of incomplete UHR are discovered over time. The NMPVS and NCIDD/NIFA in Australia, the NamUs and CODIS in the US, and Canada's Missing database and National DNA Data Bank in Canada, are examples of both national law enforcement and public databases being increasingly used for humanitarian purposes with demonstrated success. For example, since its inception in 2007, NamUs has aided the resolution of over 2500 unidentified persons cases (NamUs, 2022). However, countries without existing database infrastructure can seek to use databases like the ICRC's AM/PM Database (Hofmeister et al., 2017) or ICMP's Identification Data Management System (Parsons et al., 2019) to manage their AM and PM data and/or if an INTERPOL member country, they can access INTERPOL DNA databases like I-Familia (Laurent et al., 2022). Such databases help to eliminate problems caused by the lack of information sharing between the different jurisdictions, agencies, and stakeholders often involved in unidentified and missing persons investigations.

A sixth general principle is that the identification process requires forensic experts from different fields working together to conduct a holistic and exhaustive investigation in order to confirm or refute a hypothesis of identity (ICRC, 2022). This is especially vital as best practice recommendations advise employing multiple forensic profiling methods for achieving identification, rather than relying on a single forensic profiling method (EAAF, 2020; Goodwin, 2017; ICRC, 2022; Puerto et al., 2021; Ward, 2018); even for primarily DNA-led approaches. This is due to the possibility of coincidental or adventitious matches, human error, contamination, and use of incorrect statistical calculations or reference databases. This principle shifts the focus from the results of individual identification techniques or experts, to the consolidation of multiple lines of complementary evidence which exhibit comparable AM and PM data for the identification process. Table 5 summarizes the various forensic profiling methods that are currently available to assist authorities to establish multiple and different lines of evidence for UHR identification. The ICMP's current efforts to identify over 70% of the 40,000 individuals missing following the conflicts in the Western Balkans region in the 1990s, including approximately 90% of the 8000 men and boys who were killed in the 1995 Srebrenica Genocide, demonstrates how successful the application of a large-scale, multidisciplinary (i.e., DNA-led not DNA-only) identification strategy can be (ICMP, 2021; Parsons et al., 2019). This approach has involved acknowledging and utilizing the expertise of forensic archeologists, anthropologists, and odontologists as an important component of the DNA-led identification process. The same lesson was learned following the World Trade Center attack, with many forensic science subspecialities including forensic medical examiners, anthropologists, fingerprint examiners, odontologists, radiologists, and DNA specialists needing to collaborate to achieve the effective and efficient identification of a large number of disaster victims (Budimlija et al., 2003; de Boer et al., 2020). Similarly, the recent identification of a decedent in a 103-year-old UHR case highlights the value of employing a combined identification effort involving forensic anthropologists, genetic genealogists, and law enforcement (Michael et al., 2022). Finally, the European Council of Legal Medicine has recently devised an UHR inspection form to guide and harmonize the collection of relevant data from forensic pathologists, anthropologists, odontologists, geneticists, entomologists, and toxicologists, thus supporting a collaborative framework for death investigations (Cecchi et al., 2022).

The careful and considered understanding and implementation of the general principles described here are necessary to strike a balance between the cost, speed, and accuracy of human remains identification and managing expectations of all stakeholders involved in LTMP investigations, including law enforcement, coroners and medical examiners, families and friends, and the general public.

7 | NATIONAL DNA PROGRAM FOR UNIDENTIFIED AND MISSING PERSONS

The Australian Federal Police (AFP) National DNA Program for Unidentified and Missing Persons (Program) commenced in July 2020 and is funded by proceeds of crime until December 2023 (Ward, 2022). This Australian-first initiative is using modern forensic techniques and databases to assist Australian law enforcement to identify UHR, connect them with LTMP cases, and provide answers to their families. The nationally coordinated program provides stakeholders with a centralized and contemporary capability that adheres to the best practice principles described above and models aspects of other internationally recognized DNA-led identification efforts researched as part of author Ward's 2015 Churchill Fellowship (2018), such as that of the ICMP, University of North Texas Center for Human Identification, Armed Forces DNA Identification Laboratory, and EAAF Genetics Laboratory.

Some of the common elements of these successful identification programs include:

- Centralization and maintenance of specialized expertise at a national level;
- Creation and utilization of fit-for-purpose database/s for national searching and matching of case and biometric data;
- Adoption of a suite of contemporary DNA testing methods optimized for compromised and skeletonized UHR;
- Implementation of an extensive multi-media public outreach program to facilitate collection of AM information, records, and samples;
- Application of a comprehensive and multidisciplinary approach to forensic human identification.

The initial phase of the Program involved State and Territory police, in consultation with forensic laboratories and mortuaries, conducting an audit of all unidentified and missing persons cases in their jurisdiction. The nation-wide case audit recorded approximately 750 UHR and 2500 LTMP cases in Australia (as of December 2022), some dating back to the mid-1900s; figures which reflect similar "silent mass disasters" being experienced in other countries (Ritter, 2007). Following completion of a privacy impact assessment, Program specialists introduced multiple new forensic human identification technologies to the AFP and partnered with external forensic service providers offering specialized methods in a dedicated effort to scientifically link these UHR and LTMP across the country.

The primary forensic profiling methods being applied in the Program include forensic odontology and forensic anthropology examinations (including forensic radiology, radiocarbon dating, and isotope analysis as required) of the UHR, followed by autosomal, Y-chromosome, and mtDNA analysis, if this testing has not been previously conducted. Following testing, relevant forensic specialists enter the anthropological, medical, dental, and DNA data into national law enforcement databases for national searching. For those UHR that are not able to be identified using medical, dental, or DNA data comparisons at the national level, the biometric data is searched against equivalent data in international law enforcement databases where relevant and/or secondary forensic profiling methods are applied to offer new investigative leads. These currently include BGA and EVC estimation, CFR, and extended kinship analysis including FIGG.

The program is supported by a number of national and international law enforcement databases hosted by the Australian Criminal Intelligence Commission and INTERPOL respectively, including the following points:

•NMPVS—a database for conducting national searches of case and dental records to link UHR and LTMP via matching of circumstantial (e.g., dates and locations), demographic, physical, medical, or dental data;

•NCIDD—a database for conducting national searches of DNA profiles to link UHR and LTMP via direct matching of DNA data;

•NIFA—a database for conducting national searches of DNA profiles to link UHR and relatives of LTMP via kinship matching of DNA data;

•INTERPOL DNA Database—a database for conducting international searches of DNA profiles to link UHR and LTMP via direct matching of DNA data;

•I-Familia—a database for conducting international searches of DNA profiles to link UHR and relatives of LTMP via kinship matching of DNA data.Family members of LTMP have been integral to the Program and various physical and virtual outreach activities have been employed to encourage participation, including a dedicated and accessible website (https://www.missingpersons.gov.au/support/national-dna-program-unidentified-and-missing-persons). Noting the potential psychological and/or emotional impact on participating families, the Program collaborated with an Australian missing persons and ambiguous loss expert to understand how the FRS collection procedure could be improved to minimize augmenting their trauma (Wayland & Ward, 2022).

To aid the UHR identification process, the following forensic information, records, and samples (or consent for collection and use of this information by law enforcement) was requested from LTMP families:

•FRS from multiple close biological relatives;

•Stored personal items, biological samples, or medical samples of the LTMP from which a DRS may be recovered (e.g., toothbrush, razor, baby/wisdom tooth, lock of hair, newborn screening card, and blood/biopsy sample);

•Contact details of the dentist(s) and doctor(s) used by the LTMP;

•Dental and medical records of the LTMP (e.g., treatment records, specialist reports, and x-rays/CT scans);

•Circumstantial, biographical, and physical information about the LTMP (e.g., date/location last seen, clothing/shoes/jewelry last worn, sex, age, ancestry, eye/hair/skin color, height, tattoos, birthplace, and lifestyle choices);

•Photographs of the LTMP, including facial photographs with and without teeth showing (e.g., portrait, passport/license, and "selfie" photographs);

- Genealogical/family history records and charts.

To date, this unique Program has assisted to resolve both historical and contemporary cases of unidentified and missing Australians using a combination of genetic and nongenetic techniques, and local and national databases. The success of this multifaceted, multijurisdictional, multiagency, and multidisciplinary Program has centered on the AFP working collaboratively with (1) police, coronial, and forensic agencies across Australia; (2) national and international forensic experts in government, university, and private laboratories; and (3) families of LTMP.

However, more identifications will be achieved if the following occurs:

- Increased participation from authorities and families;
- Continued population of relevant national and international databases;
- Greater use of international law enforcement, private, and public DNA databases;
- Championing of new forensic capabilities such as FIGG;
- Exhumations of previously interred UHR with no stored PM samples;
- Reform of legislation hampering national and international biometric data searching;
- Development of policies and procedures to achieve national standardization (based on international best practice) of forensic practices for UHR-LTMP investigations.

The program's legacy could be a future state where forensic human identification experts and specializations are encouraged and supported to form partnerships and intertwine their knowledge, skills, and opinions to provide stakeholders with a dedicated, comprehensive, and integrated human remains identification service. However, similar to other national identification capabilities globally, this will only be possible and sustainable if this vital resource is supported by recurrent government funding, rather than be reliant on funding derived from one-off or ad hoc sources such as grants, sponsorships, or donations.

8 | CONCLUSION

Human remains identification is an important process for the justice system, society, and families of the missing. It is essential to restore the identity of an unknown deceased person, so they can receive a proper and dignified burial, families suffering ambiguous loss can get answers, death certificates can be issued for practical reasons, and justice for victims can be served. The process of forensic human identification should, at a minimum, involve a multifaceted, multijurisdictional, multiagency, and multidisciplinary approach, with the integral actors being investigators and/or practitioners from police, forensic, and coronial agencies, and relatives of LTMP. The progression to an interdisciplinary examination and identification strategy will require forensic experts to cooperatively and collaboratively synthesize their disciplinary findings to arrive at a comprehensive and consistent presentation of a scientifically determined identification to the relevant legal authority. This review provides information about the utility of individual forensic profiling methods, how they can be combined to achieve robust and reliable scientific identifications, and best practice recommendations for implementing an interdisciplinary forensic approach to identify unknown and missing individuals within death investigation contexts. Technological advancements in the various forensic disciplines and enhanced national and international databases, together with the establishment of national forensic human identification capabilities with the centralized expertise, technology, and resources to conduct this type of unique and challenging casework, is proving to be the most effective and efficient way to generate investigative leads, identify UHR, and resolve LTMP cases in Australia and elsewhere.

AUTHOR CONTRIBUTIONS

Ayusha Dahal: Writing – original draft (lead); writing – review and editing (supporting). **Dennis McNevin:** Conceptualization (supporting); supervision (equal); visualization (lead); writing – review and editing (supporting). **Madelen Chikhani:** Writing – original draft (supporting); writing – review and editing (supporting). **Jodie Ward:** Conceptualization (lead); funding acquisition (lead); supervision (equal); writing – original draft (supporting); writing – review and editing (lead).

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Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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